**Scientific Association of Geneticists and Breeders of the Republic of Moldova**
**Institute of Genetics, Physiology and Plant Protection**
**Moldova State University**
**Academy of Sciences of Moldova**
**Romanian Society of Bioengineering and Biotechnology**

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# THE HERITAGE OF A.E. KOVARSKY'S SCHOOL IN THE FIELD OF BREEDING AND GENETICS

**Yevgeny V. KOVARSKY**

In the life of an academician of the Academy of Sciences of the Republic of Moldova, a professor of the Chisinau Agricultural Institute named M. V. Frunze, a doctor of agricultural sciences Anatoly Efimovich Kovarsky (1904-1974) three periods of his activity are distinguished, related to the study of field crops in the Republic of Moldova, in the steppe part of Ukraine, in the mountainous part of Kyrgyzstan - in these three regions, which differ from each other by their climate, soils and gene pool of field crops that were of scientific interest to A. E. Kovarsky.

The core of A.E. Kovarsky's legacy is the School of A. E. Kovarsky, which includes theoretical and practical experience that Anatoly Yefimovich left to his students in the Republic of Moldova. It is well known that the students of A.E. Kovarsky grew up to become important scientists, academicians and corresponding members of the Academy of Sciences of the Republic of Moldova, headed scientific schools and institutes. To the heritage of A.E. Kovarsky belong scientific approaches of A.E. Kovarsky to the knowledge of Nature, based on the provisions of C. Darwin, practical recommendations of I.V. Michurin and N.I. Vavilova.

Among them are the influence of growing conditions on plants, morphogenetic processes, self-pollination, the effect of inter-varietal and foreign mentor pollen, cross-pollination in self-pollinating plants, mentor lines, the method of distant-geographical hybridization, cytoplasmic male sterility (CMS), hybridization, intergeneric and interline hybridization, experimental mutagenesis, and recombinational variability.

Born in 1904, in the countryside (S. Popovka, Sumy region) of Ukraine, in a large family of farmers engaged in sugar beet, Anatoly Yefimovich, like his older brother Mikhail, followed his father's footsteps and became an agronomist, founding Kharkov Agricultural Institute. Since 1924, first in the state reserve of Ascania Nova, and then in Kharkov and Kherson agricultural institutes and in the Kherson labor colony. The fate of his older brother[1], whom he loved very much, could not help but affect Anatoly Efimovich, but he continued to work, solving food problems.

During this early period of his scientific and creative life, A.E. Kovarsky read extensively, both professional and literary works. He became deeply interested in the teachings of Charles Darwin and I.V. Michurin, published numerous independent studies, and authored books on peanuts and sesame, which he wrote in

[1] Mikhail Kovarsky, was one of the founders of the Machine-Tractor Stations (MTS), was awarded for this by the Order of Lenin, worked as deputy head of the Tractor Center USSR, but was arrested by the Soviet authorities and shot in 1933 on a false report about counter-revolutionary rebel activities in Ukraine, rehabilitated in 1957.

his native Ukrainian language. These books were published in large print runs for distribution to collective and state farms (*kolkhozes and sovkhozes*). At a young age, while heading the phyto-breeding station at the Askania-Nova Nature Reserve, A.E. Kovarsky gained valuable experience in organizational and managerial work. He studied the geographic sowing strategies of the Vavilov Institute (VIR), introduced new field crops, and worked with cotton, sesame, peanuts, and legumes. He also acquired practical experience during the difficult years of famine and drought from 1926 to 1928.

As a result of his expedition to the mountainous region of Crimea, he collected and described varieties of winter and spring wheat, as well as wild einkorn wheats.

A.E. Kovarsky received his first official academic title while working as a staff member at the All-Union Plant Breeding Institute (VIR, named after N.I. Vavilov). Later, in 1939, he defended his doctoral dissertation on the topic "Introduction of Peanuts in the USSR."

The second period of Anatoly Yefimovich Kovarsky's scientific career took place during the evacuation of the Kherson Agricultural Institute to Southern Kazakhstan and Kyrgyzstan. Between 1941 and 1942, he served as Deputy Director and Head of Department at the Kaplanbek Republican Agricultural College (Kazakhstan). From 1942 to 1944, he was Head of the Department of Breeding and Seed Production, Head of the Experimental Field, and Chief Agronomist of the Training Farm at the Kyrgyz Agricultural Institute.

In 1943, he was awarded an Honorary Diploma by the Presidium of the Supreme Soviet of the Kyrgyz SSR. During this period, A.E. Kovarsky carried out research on leguminous crops, in which his wife, agronomist D.A. Kovarskaya, also took part.

The third and longest period of A.E. Kovarsky's professional activity, lasting thirty years, took place in the Republic of Moldova. His main work was centered in Chișinău, where he was engaged in both scientific and teaching activities at the Agricultural Institute and the Academy of Sciences. He also managed the operations of the Experimental Breeding Base / Trial Station of the Agricultural Institute, located in the suburb of Costiujeni. In addition, he was active in public outreach, civic, and governmental work, which included founding the Society of Geneticists and Breeders of the Republic of Moldova, editing well-known journals and academic collections, participating in sessions of the Supreme Soviet, holding meetings with the leadership of the Republic of Moldova and the Ministry of Agriculture, and visiting collective farms throughout the territory of the Republic of Moldova.

The practical heritage includes A.E. Kovarsky varieties and hybrids, as well as A.E. Kovarsky field crop collections, field crop farming experience and seed production.

A. E. Kovarsky and S. L. Pynzar, along with their colleagues, collected and studied a large collection of samples of chickpea, bean, vetch, soybean, and cowpea.

For soybean and especially for cowpea and mung bean, they used the introduction of initial material from outside, from the center of its diversity (according to N. I. Vavilov, 1935) and distant-geographical and distant-genetic hybridization. By methods of distant-geographical, distant-genetic hybridization and experimental mutagenesis, they developed wheat varieties — Kishinevskaya 9, Kishinevskaya 24, Kishinevskaya 101, Kishinevskaya 102, Eritrospermum 103, Kishinevskaya Early, Kishinevskaya Large-Grained, new 56- and 42-chromosome triticale forms; soybean varieties — Kishinevskaya 1 and Kishinevskaya 5; bean varieties — Kishinevskaya Stambovaya and Delicates; chickpea varieties — Favorit and others.

The collection of 709 local Moldovan maize samples, initiated by A. E. Kovarsky together with V. G. Uchkovsky in 1945 — the vast majority of which belong to the flint and semi-dent subspecies of maize — served as the source for obtaining male pollen sterility, known as the "Moldovan type of cytoplasmic male sterility" or "Moldovan CMS." Based on the Moldovan type of CMS, several maize hybrids zoned in the Republic of Moldova were developed and converted to a sterile cytoplasmic base, including VIR 42 MV, Kishinevsky 109 M, Kishinevsky 150 MV, Kishinevsky 161 MV, Kishinevsky 167 M, Doina, Vstrecha, Kishinevsky 183, Kishinevsky VL 115, and others.

A.E. Kovarsky were collected new collections of samples of maize from different countries; attracted to the crossing with maize its closest relatives from triba maize; created a collection of spontaneous mutations, formed due to the action of natural factors of external environment; a collection of induced mutations resulting from the use of chemical and physical mutagenesis methods; a collection of so-called menthar lines resulting from the mutating effect of pollen decay products. The collection includes a large number of samples from Central Asia and Kazakhstan. The collection contains more than 1,000 samples from 45 foreign countries, which are mainly obtained through ERA. The maize collection is periodically (after 4-5 years) updated by pollinating inside a sample of 5-10 pollen plants with other 5-10 plants. This method aims to keep the samples in their original state.

In order to preserve the heritage of A.E. Kovarsky School in the field of breeding and genetics, it is necessary to preserve, maintain and develop the collections of the gene pool created by A.E. Kovarsky, study of the works of A.E. Kovarsky and his students, continue research, Discussion of results at scientific conferences, preservation of the memory of Anatoly Efimovich Kovarsky.

# Session I

# GENERAL AND MOLECULAR GENETICS

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# MOLECULAR DIAGNOSTICS OF SOME FUNGAL PATHOGENS IN GREENHOUSE GROWN TOMATOES

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***Abstract.*** *Tomato production in protected environments such as greenhouses has become a popular trend in agriculture. The main advantage of greenhouse crop production over open field systems is the ability to control the environment, limiting the impact of abiotic and biotic factors. However, under greenhouse conditions tomatoes are still susceptible to certain soil-borne fungal diseases. Early detection of fungal pathogens is paramount for controlling the diseases and their spread. The aim of the study was to monitor fungal pathogens affecting tomato plants grown in protected conditions. Molecular diagnostics of pathogens Alternaria spp., Fusarium spp., Penicillium spp. and Aspergillus spp. was carried out during the vegetative season in various organs of tomato plants planting in greenhouse using nested-PCR assay. A. alternata was the predominant pathogen among all tested tomato genotypes. Fusarium and Aspergillus species were not detected in greenhouse tomato plantings during vegetative season.*

***Keywords: fungi greenhouse, pathogens PCR Tomato.***

## INTRODUCTION

Global temperature rise is a trigger for climate change and, as a result, a multitude of risks to organisms all over the world. Climate change has a major impact on the agricultural sector, decreasing crop productivity. High temperatures and extreme fluctuations in precipitation quantities distort plant growth cycle, facilitate the emergence of new pests and diseases, their propagation and widening the host range [1, 2]. Threats of epiphytoties caused by various pathogens are

currently increasing in agroecosystems in temperate regions [3]. The increasing impact of pathogens has led to the excessive use of chemicals, which have negative consequences for the environment, human and animal health [4, 5]. Plant breeding programs and long-term use of pesticides have provoked the emergence of strains with increased virulence [6]. Numerous studies on the impact of climate change on biological processes demonstrate the need for effective measures to reduce the severity of diseases, using more environmentally friendly methods of plant protection. Mitigation methods include phytosanitary control of seed and planting material, the use of resistant varieties and microclimate modification. In the last decade, tomato production in protected environments such as greenhouses has become a new trend in agriculture. The main advantage of greenhouse crop production over open field systems is the ability to control the environment, limiting the impact of abiotic and biotic factors [7]. Greenhouse tomatoes have better quality and are uniform in shape, size and color compared to those grown in the open field. However, under greenhouse conditions tomatoes are still susceptible to certain microbial diseases, as the closed environment can promote the development of infections. Soil-borne fungi are the main limiting factor for tomato production and cause significant economic losses [8]. Early detection of fungal pathogens is paramount for controlling the diseases and their spread.

The aim of the study was to monitor fungal pathogens affecting tomato plants grown in protected conditions. Molecular diagnostics of pathogens *Alternaria* spp., *Fusarium* spp., *Penicillium* spp. and *Aspergillus* spp. was carried out during the vegetative season in various organs of tomato plants grown in greenhouse using nested-PCR assay.

## MATERIALS AND METHODS

Monitoring of phytopathogenic fungi was carried out during the 2024 growing season. The following tomato genotypes were studied: the wild tomato species Solanum pimpinellifolium, Elvira and Rufina varieties (selection of IGFPP). Asymptomatic tomato plants at different phases of development were tested: seeds before sowing (2023 harvest), leaves (phase of 4-5 leaves, flowering phase), fruits, “new” seeds. Total DNA from plant material were isolated by a modified CTAB method [9]. At all stages of the analysis, DNA extraction from tomato plants was performed in triplicate to ensure reproducibility of the results.

Molecular analysis by nested-PCR was performed using primers for the detection of *Alternaria* spp., *Fusarium* spp., *Penicillium* spp. and *Aspergillus* spp. Primer design was done based on species-specific sequences of each pathogen from NCBI nucleotide collection presented in GenBank database using Primer-BLAST designing tool: glyceraldehyde 3-phosphate dehydrogenase gene (GPD) of *A. alternata* and *A. solani*; translation elongation factor 1-alpha (*tef1*) gene of *F.*

*verticillioides, F. oxysporum, F. solani, F. avenaceum;* beta-tubulin (*tub2*) gene sequences of *F. equiseti, F. sporotrichioides, Penicillium citrinum;* oxygenase (*fum6*) gene sequences of *F. proliferatum;* o-methyltransferase A (*aflP*) gene of *Aspergillus flavus*.

The PCR mixture, in a volume of 25 µl, contained: 66 mM tris-HCl (pH-8,4), 16 mM $(NH_4)_2SO_4$, 2,5 mM $MgCl_2$, 0,1% Tween 20, 7 % glicerol, 100 µg $ml^{-1}$ *Bovin Serum Albumin*, 0,2 mM dNTPs of each, 1,25 U *Taq DNA* polymerase, 5 pM of each primer, and 5-10 ng DNA.

The first round of the *nested*-PCR included 1 cycle: 95 $^0$C – 4 min, 60 $^0$C – 1 min, 72 $^0$C – 1 min; followed by 29 cycles: 95 $^0$C –1 min, 60 $^0$C – 1 min, 72 $^0$C – 1 min. The second-round conditions of the *nested*-PCR were: 35 cycles: 95 $^0$C – 1 min, 60 $^0$C – 1 min, 72 $^0$C – 1 min.

The amplification products were separated in 1,5 % agarose gel by electrophoresis (5-8 V/cm) and compared with molecular marker (100 bp DNA ladder, Thermo Fisher Scientific) in a migration buffer of Tris/borate/EDTA (pH 8.0) with ethidium bromide, viewed in the UV (302 nm) and photographed.

## RESULTS AND DISCUSSION

Prior to molecular diagnostics of pathogens, total DNA extracted from plant material was tested for the presence of PCR inhibitors. DNA quality analysis was performed using primers designed based on plant 18S ribosomal RNA gene sequences [10]. The results of amplification selectively demonstrated in Figure 1.

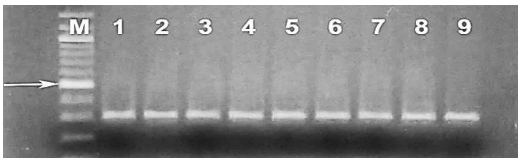


Figure 1. Electropherogram of PCR products obtained on total DNA extracted from tomato seeds using primers to the 18SrDNA gene.

*1-3. Solanum pimpinellifolium, 4-6. Elvira, 7-9. Rufina. M - 100 bp DNA ladder. The arrow points at a 500 bp marker fragment.*

Specific fragment of expected size (315 bp) was revealed for all tomato DNA samples. The analysis indicated that DNA was free of PCR inhibitors and was suitable for downstream pathogen testing.

Molecular analysis of pre-planting seeds using species-specific primers to *Alternaria* spp. revealed the presence of *Alternaria alternata,* but absence of *Alternaria solani* in all genotypes of tomato. *Penicillium citrinum* was detected only in the seeds of Solanum pimpinellifolium (Figure 2).

The positive signals in the second round of *nested*-PCR for *A. alternata*

were marked with the presence of a 288 bp amplicon on electrophoresis (Figure 2A.) and 250 bp – for *P. citrinum* (Figure 2B.). It is known that infected seeds can serve as a source of primary infection, increasing the pathogenic load in plants during the growing season.

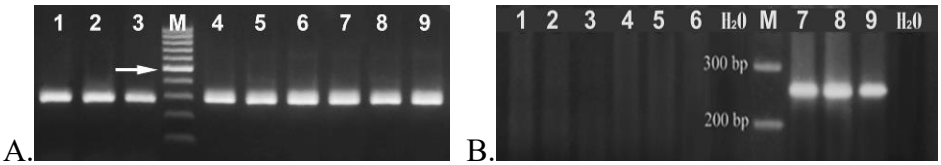


Figure 2. Electropherogram of nested-PCR products obtained using primers for detection of *A. alternata* (A.) and *P. citrinum* (B.) and total DNA extracted from the seeds.

*1-3. Elvira, 4-6. Rufina, 7-9. Solanum pimpinellifolium. M - 100 bp DNA ladder. The arrow points at a 500 bp marker fragment.*

Thus, in order to obtain healthy planting material, a phytosanitary assessment of their quality is necessary before storing and compliance with storage conditions. *A. alternata* was revealed in the tomato plants at the phase of 4-5 leaves in all studied tomato genotypes (Figure 3).

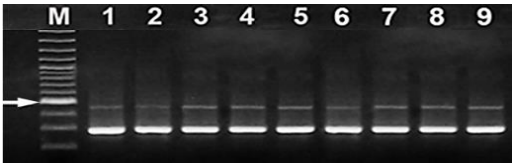


Figure 3. Electropherogram of nested-PCR products obtained using primers for detection of *A. alternata* in total DNA extracted from the tomato plants at the of 4-5 leaves.

*1-3. Elvira, 4-6. Rufina, 7-9. Solanum pimpinellifolium. M - 100 bp DNA ladder. The arrow points at a 500 bp marker fragment.*

*Alternaria* spp. was not found in leaves collected at the flowering phase. The single incidence of *A. alternata* infection was revealed in 'Rufina' fruits, but absent in "new" seeds of all tomato genotypes. We have previously shown that fungi of the genus Alternaria infect tomato plants in open field during all phases of development [11]. Therefore, in greenhouse conditions it is possible to contain the development of this pathogen.

During the vegetative season, *Fusarium*, *Aspergillus* and *Penicillium* species (*F. verticillioides, F. oxysporum, F. solani, F. avenaceum, F. equiseti, F. sporotrichioides, F. proliferatum, A. flavus, P. citrinum*) were not detected in greenhouse grown tomatoes. In previous studies we detected these fungal species

at different stages of tomato development in open field conditions [12]. It is known, that *Alternaria* can inhibit the growth and development of *Fusarium* [13]. However, the effectiveness of suppression can be influenced by many factors, including environmental conditions and the genotype of the host plant [14]. Thus, greenhouse growing conditions and the presence of *Alternaria* spp. probably limited the development of the named species of *Fusarium, Aspergillus* and *Penicillium*.

As shown, *A. alternata* was the predominant pathogen in tomato plants during some phases of development. So, it can be used as a representative pathogen for presymptomatic assessment of phytosanitary status of tomato plantations to optimize fungal disease control under greenhouse conditions.

The probability of tomato infection by soil-borne fungal pathogens exists at all stages of cultivation. To overcome this risk, it is necessary to use healthy seeds, genotypes with low susceptibility to pathogens and to optimize the planting conditions. Success in combating pathogens largely depends on the correct application of plant protection measures to obtain high-quality and healthy products. Of great importance is the detection of potential foci of infection before the appearance of external signs of disease in affected plants. Seasonal monitoring of pathogens using PCR-based diagnostic methods is necessary. Presymptomatic detection of tomato pathogens allows optimizing the use of chemical plant protection products and obtaining a more environmentally friendly and healthy product and seed material.

## CONCLUSIONS

As a result of monitoring some fungal infections of tomatoes in greenhouse conditions, a decrease in the pathogenic load in plants was found, compared to open field systems. *A. alternata* was the predominant pathogen among all tested tomato genotypes. *Fusarium* and *Aspergillus* species were not detected in greenhouse tomato plantings during vegetative season. None of the pathogen species declared in the study were detected in the fresh seeds, which is favorable for their subsequent reproduction.

**Acknowledgments:** This study was supported by the research projects 011101 “Genetic and biotechnological approaches of management of agroecosystems under climate change conditions”, funded by the Ministry of Education and Research; UNDP-IRH-00181 „Introduction and application of new genomic techniques in agricultural sector to face climate challenges” funded by UNDP and Czech Republic Development Cooperation.

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# POOL AND DIVERSITY OF PROKARYOTES OF CARBONATE CHERNOZEM OF LONG-TERM FIELD EXPERIENCE OF MOLDOVA

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**Abstract.** For the first time in Moldova, changes in microbiological indicators obtained using polymerase chain reaction (PCR) in carbonate chernozem were studied depending on the type of land use under different anthropogenic loads. The work used soil samples from winter wheat crop rotation of long-term field experiments of different types of use: without fertilizers, mineral and organic background, as well as 75-year-old fallow land . A high diversity of genetic information with a spectrum consisting of 17 phyla was established. According to the International Committee on Prokaryotic Taxonomy (2021), they are new, identified and / or confirmed by metagenomic analysis. The prokaryotic pool varied from 0.01% to 46.53%: Actinomycetota (38.17-46.53%), Pseudomonadota (19.60-27.02%), Bacillota (7.32-22.40%), Bacteroidota (5.31-8.55%), Acidobacteriota (1.94-3.32%), Verrucomicrobiota (1.37-2.09%), Myxococcota (1.09-1.71%), Nitrospirota (0.15-0.65%), Planctomycetota (0.58-0.65%), Gemmatimonadota (0.36-0.58%), Patescibacteria (0.08-0.17%), Cyanobacteriota (0.05-0.08%), Chloroflexota (0.03-0.07%), Fibrobacterota (0.01-0.05%), Abditibacteriota (0.01-0.07%), Bdellovibrionota(0.01-0.011%), and Nitrososphaerota (7.32-22.4%). The first 16 are representatives of the Bacteria domain, and the Nitrososphaerota phylum belongs to the Archaea domain. Based on the results of multiple observations of the content of conserved DNA regions of different phylogenetic groups in the soil, it can be concluded that the prokaryotic community is susceptible to the type of chernozem use. As an alternative to soil conditions, bacteria act in the following sequence: soil without fertilizers → organic background → mineral background → fallow land, and archaea - vice versa.

***Keywords: prokaryotes, bacteria and archaea, 16S rAPH gene, metagenomic analysis.***

## INTRODUCTION

Prokaryotes, whose representatives include two domains – Bacteria and Archaea, are “the basic unit and universal basis of life” [6]. Approximately 5% of the total number of prokaryotes on Earth are concentrated in the soil [4], characterizing the soil environment as the largest reservoir of microbial diversity on Earth. The majority belongs to bacteria, while archaea account for 0.5 to 3.8% of the total number of all prokaryotes. However, the high level of genetic diversity of the soil microworld remained unknown for many years [1]. Only the advent of new methods of molecular ecology of microorganisms

revealed that at present, only about 0.1% of the total microbial diversity of the biosphere is taken into account by the cultivation of microorganisms [2].

With the introduction of methods of molecular ecology of microorganisms, science has gained the opportunity to take into account not only cultivated but also uncultivated forms of microorganisms [8, 12]. It is believed that the microbial community is a kind of "mirror" that reflects the features of the habitat, and its diversity represents the hidden potential of the soil microworld [1, 5] in connection with which the metagenomic characteristics of soils are in demand for assessing the sustainability of soil ecosystems under the influence of natural and anthropogenic factors, and interest in such studies is constantly increasing. The soils of Moldova have not been studied from this point of view.

**The aim** of this study was to study the pool and diversity of prokaryotes of the carbonate chernozem of the Republic of Moldova using quantitative PCR and high-throughput sequencing of 16S rRNA gene libraries.

## MATERIALS AND METHODS

The object of the study was microbial communities of carbonate chernozem: light loam with a humus content of 2.5-3.0%, mobile phosphate of 0.8-1.5 mg / 100 g, exchangeable potassium of 18-22 mg / 100 g and carbonates of 1.8-2.2% in the 0-20 cm layer. The studies were carried out in 2022 in a long-term stationary field experiment (founded in 1950) of the Ketrosy scientific and educational farm, district Anenii Noi . Soil samples were collected in the spring from variants with winter wheatof eight-field of crop rotation : 1 - without fertilizers, 2 - with the application of mineral fertilizers, 3 - with the application of organic fertilizers and 4 - fallow land. Fertilizer application rates: total for crop rotation N675P480K480 (N90P60K60 for corn, N120P60K60 for winter wheat before corn and before sunflower, N45P60K60 for sunflower and peas); total for crop rotation 144 t/ha of semi-rotted manure (40 t for corn, 22 t for winter wheat, 20 t for sunflower).

Metagenomic analysis was carried out using modern methods [3, 4] and equipment of the Collective Use Center “Genomic Technologies, Proteomics and Cell Biology” of the All-Russian Research Institute of Agricultural Microbiology, St. Petersburg, Russia. Taxonomic identification of OTUs was performed using the RDP (SILVA) database, the classification was refined using the online database https://www.ncbi.nlm.nih.gov/taxonomy) [9, 10, 11].

## RESULTS AND DISCUSSION

A study of long-term used carbonate chernozem soil revealed that its prokaryotic community consists of 17 phylums: *Actinomycetota,*

*Pseudomonadota, Bacteroidota, Bacillota, Acidobacteriota, Verrucomicrobiota, Planctomycetota, Myxococcota, Nitrospirota, Gemmatimonadota, Cyanobacteriota, Patescibacteria, Chloroflexota, Fibrobacterota, Abditibacteriota, Bdellovibrionota,* и *Nitrososphaerota* **(Fig. 1).** *The first 16 are representatives of the Bacteria domain, and Nitrososphaerota* belonged to the domain *Archaea*. According to the quantitative assessment of the content of conservative DNA regions of different phylogenetic groups in the soil, it can be concluded that the bacterial community is most responsive to the type of use of chernozems. As an alternative to soil conditions, bacteria act in the following sequence: soil without fertilizers → organic background → mineral background → fallow land, and archaea - vice versa.

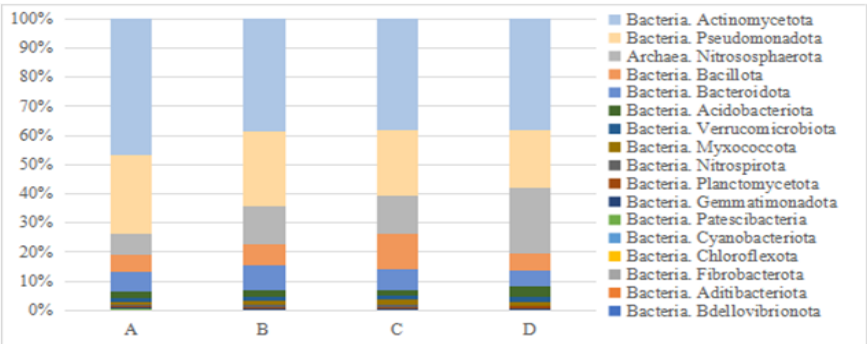



Figure 1. Taxonomic structure of prokaryotic communities of carbonate chernozem at the phylum level, %:

*A – without fertilizers. B – mineral background, C – organic background, D – fallow land*

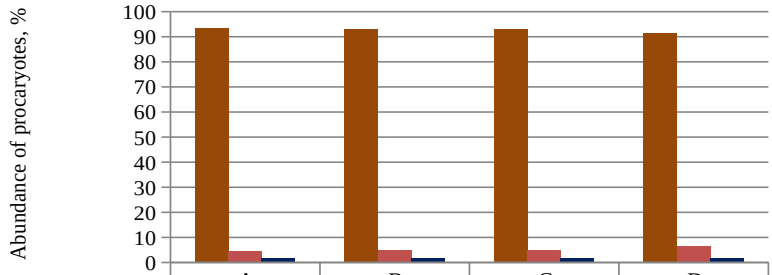
The identified microbiome was similar in the composition of its components during the study, but the presence of phyla in the variants was different. All phyla were simultaneously present only in the soil of the crop rotation without fertilizers (control). In the fertilized variants, 15 phyla were identified, and in the fallow soil – only 13. The phyla also differed in their proportion of representation in the community. Among the identified prokaryotes, the highest abundance was recorded in microorganisms of the Bacteria domain (77.60–92.68%). The leaders among them were representatives of the Actinomycetota phylum (38.17–46.53%), reaching the highest value in soil without fertilizers and the lowest in soil fertilized with organic fertilizers for a long time and in the fallow land. Next, among the majority of components of the prokaryotic community, representatives of Pseudomonadota (19.60-27.02%) were widely encountered, which in general were almost 2 times inferior to actinobacteria in numbers, recording a similar development with actinobacteria in variants. The third in terms of representativeness was the archaeal phylum Nitrososphaerota (7.32-22.40%).

despite the fact that it was represented almost twice less than the previous one, it recorded the highest ratios in the fallow and the lowest in the unfertilized soil, while the fertilized variants occupied an intermediate position. In addition, it should be emphasized that the contribution of archaea in the prokaryotic community doubled in fertilized variants (13.04-13.25%) in relation to unfertilized soil (7.32%), while at the same time noting a doubled representation in the fallow land soil (22.40%) in relation to fertilized variants (13.04-13.25%) and an almost 4-fold excess in relation to unfertilized crop rotation soil (7.32%). The phylum Bacillota was also characterized by high rates (6.08.-12.42%), distinguished by the highest values of abundance in the organic background soil and the lowest in unfertilized soil and fallow land.

The Bacteroidota phylum had a taxa abundance of 5.31-8.55% with the highest indicators in the fertilized variants (6.90-8.55%).Further, attention is drawn to the phyla that did not differ in either large fluctuations or large values of abundance in the community: Acidobacteriota (1.94-3.32%) Verrucomicrobiota (1.37-2.09%) and Myxococcota (1.09-1.71%). They recorded the highest indicators in the soil of the organic background or in the fallow soil, and the lowest - in the unfertilized soil. Of these, the highest values were recorded by the phylum Acidobacteriota (3.32-1.94%) and the lowest - by the phylum Myxococcota, and Verrucomicrobiota (1.37-2.09%) occupied an intermediate position among them. The following 9 phyla had very small contributions to abundance prokaryotic community. Moreover, the first 5 phyla: Nitrospirota (0.15-0.65%), Planctomycetota (0.58-0.65%), Gemmatimonadota (0.36-0.58%) Patescibacteria (0.08-0.19%) Fibrobacterota (0.01-0.05%) were found comparatively more often and showed 0.03-0.65% contribution, in comparison with the rest. And the phyla Aditibacteriota and Bdellovibrionota with a contribution of only 0.01-0.07% were not identified in the soil of fertilized variants and in the fallow land soil.

Ranking of phyla by individual “significance” abundence revealed that long-term agricultural use of carbonate chernozem provoked a strong restructuring of the prokaryotic community. The same composition of components hides a distinctive hierarchical role of taxa in the community (Fig. 2). Based on this, all phyla can be divided into 3 groups, which, being constant in composition, differ in their relative abundance throughout the study period. The first group with a dominant ecological role and an occurrence frequency of ≥5% had the highest representation among these 3 groups, individually differing significantly both in the variant and among them, accounting for about 91.56–93.63% of 16S rRNA genes from all sequences without significant differences among variants themselves.It consisted of 5 phyla, which in order of decreasing abundance were as follows: Actinomycetota (38.17–46.53%), Pseudomonadota (19.60–27.02%), Nitrososphaerota (7.32–22.40%), Bacillota (6.08–12.42%) and Bacteroidota (5.31–8.55%). The second group, commonly found commonly encountered, was formed

from 3 phyla with a secondary ecological role and abundance ≤ 5% and averaged about 4.40–6.60%, recording an increase of 25% in the organic background soil and 50% in the fallow soil, compared to the rest. Moreover, the greatest contribution of Acidobacteriota (1.94–3.32%) and Verrucomicrobiota (1.37-2.09%) was recorded in the fallow soil, and Myxococcota (1.09-1.71%) in the organic background. The smallest contribution of this group of microorganisms was noted in the unfertilized soil of the crop rotation. Representatives of the second group of prokaryotes formed the following descending sequence: Acidobacteriota (1.94–3.32%) → Verrucomicrobiota (1.37–2.09%) → and → Myxococcota (1.09–1.71%) – in the soilorganic background. The third group included rarely seen representatives of prokaryotes with an abundance of ≤ 1%, having an insignificant ecological role in the community and amounted to only 1.84-1.97%, differing slightly in variants and at the same time constituting the smallest group of prokaryotes in terms of contribution and the largest group among them in terms of the number of phyla (9).




| | A | B | C | D |
|---|---|---|---|---|
| Group ≥5%. | 93,63 | 93,13 | 92,93 | 91,56 |
| Group 1-5% | 4,4 | 4,93 | 5,17 | 6,6 |
| Group ≤ 1%. | 1,97 | 1,94 | 1,9 | 1,84 |

Figure 2. Redistribution of the relative participation of prokaryotes under winter wheat in carbonate chernozem, according to the degree of dominance (d.d.), %:
*A – unfertilized, B – mineral background, C – organic background, G – fallow land*

They were arranged in descending order of abundance as follows: Nitrospirota (0.15–0.65%) → Planctomycetota (0.58–0.65%) →

Gemmatimonadota (0.36–0.58%) → Patescibacteria (0.08–0.19%) → Cyanobacteriota (0.05–0.08%) → Chloroflexota (0.03–0.07%) → Fibrobacterota (0.01–0.05%) → Abditibacteriota (0.01–0.011%) and → Bdellovibrionota (0.01–0.07%).

Consequently, under the influence of long-term agricultural use of carbonate chernozem, physicochemical and climatic factors from year to year, since the beginning of time, a significant restructuring and redistribution of the prokaryotic community occurs. Microorganisms, changing the degree of their dominance in the

community, losing or acquiring a new ecological status, survive and remain an important component of the microbiome of carbonate chernozem [7, 12]. It is these properties that characterize prokaryotes as structural elements of living matter in the soil, which interact with each other in the struggle for nutrients, thereby ensuring the role of the molecular alphabet of living matter.

## CONCLUSION

1. Carbonate chernozem is characterized by high genetic diversity of the most ancient representatives of the living world - prokaryotes, the spectrum of which consists of 17 phyla of both domains, similar to soils of other regions. But at the same time, all were present only in the unfertilized soil of the crop rotation.

2. The pool of prokaryotes ranged from 0.01% to 46.53% with the highest values in the unfertilized soil and the lowest in the organic background and fallow soil. The mineral background occupied an intermediate position among them.

3. According to the estimates of the International Committee on Prokaryotic Taxonomy (2021), the phyla are new, identified and / or confirmed by metagenomic analysis.

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# METHOD OF ACTIVATING ENTOMOPHAGES AND POLLINATORS HYMENOPTERA ORDER NATURAL POPULATIONS WITH THE AIM OF INCREASING THE AGROSYSTEM PRODUCTIVITY

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***Abstract.*** *Insects provide important ecosystem services of great economic value. We investigated a method for activating of entomophages and pollinators of Hymenoptera order natural populations ("field breeding"), consisting of a combination (synergy) of overseeding spice plants and placing Fabre hives nearby the fruit garden. The objects of the study were entomophages and pollinators of the Hymenoptera order. The experiment was conducted in three different biotopes: an apple orchard near a forest belt, a collection of spice plants near a pear orchard, and a field of Miscanthus sinensis. The maximum number of Megachilidae bee nests were found in artificial dark-colored shelters: made of cylindrical dark plastic (70,8%), and deltoid dark cardboard (20,8%). The bees preferred to build nests in the agrocenosis of the pear orchard nearby a plot of spice plants (91,7%). It has been established that the optimal strategy for the method of entomophage's activating consists of combining a variety of different family's spicy plants and ensuring continuous flowering (conveyor).*

***Keywords: entomophages, field breeding, artificial shelters, Hymenoptera, aromatic plants.***

## INTRODUCTION

Insects provide important ecosystem services of great economic value. Proper management of agro-landscape resources can contribute to the biodiversity conservation. In this context, agri-environmental schemes (AES) were included in the EU agricultural policy in 1985 and have become the main instrument for biodiversity conservation in Europe [1]. We investigated a method for activating of entomophages and pollinators of Hymenoptera order natural populations ("field breeding"), consisting of a combination (synergy) of overseeding spice plants and placing Fabre hives nearby the fruit garden, which contributes to an increase in the beneficial insect's survival and activity, and supports the formation of stable self-developing and self-regulating agrobiogeocenoses [2]. Since many predators are also anthophiles, their survival, productivity and performance are enhanced, when

flowering herbaceous plants are present within the agrocenosis. Research by scientists has shown, that strips of flowering plants near gardens and fields increase the biodiversity of pollinators and entomophages [3]. Flowering plants of the Apiaceae and Lamiaceae families significantly increase the number of beneficial insects in agroecosystems [4]. Wild bees (e.g. *Osmia* spp.) are more effective in pollinating fruit crops than honey bees. The placement of Fabre hives for *Osmia* increases the yield of fruit orchards several times [5]. *Osmia* bees prefer high-lipid pollen with a high content of sterols (fam. Asteraceae) [6]. Analysis of the nectar of essential oil plants showed that maltose and sucrose dominate in plants, pollinated by bees [4]. The key amino acid for the energy metabolism of solitary wasps (Sphecidae) and bees is proline, and the amino acids tryptophan and phenylalanine increase the survival and fertility of parasitoid wasps (Braconidae) [7, 8]. Terpenoids (linalool, geraniol) in the nectar of lavender and mint attract bees and repel unwanted insects [9]. To adapt the method to a specific garden, it was important to study local bee species and the optimal set of partner plants.

The aim of our work was to study the method of entomophage's natural populations activating, which involves the inclusion of a spice plant's plot in the industrial garden array, in combination with the use of artificial shelters to attract of Hymenoptera entomophages and pollinators to the agrocenosis.

## MATERIALS AND METHODS

The place and time of the research are laboratory and field conditions of the Institute of Genetics, Physiology and Plant Protection of the Republic of Moldova, 2024. The development of technological elements of artificial shelters for attracting economically important Hymenoptera order entomophages was carried out on the basis of certain modifications of Fabre hives. The objects of the research were entomophages and pollinators of the Hymenoptera order. In order to evaluate the most attractive filling materials, we used hollow tubes (reed and plastic) in each shelter. Four variants of the enclosure were considered: 1) white cardboard; 2) dark cardboard; 3) transparent plastic; 4) dark plastic. The experiment was conducted in three different biotopes: an apple orchard near a forest belt, a collection of spice plants near a pear orchard, and a field of *Miscanthus sinensis* (provides insects with natural tubular stems for nesting).

The sowing of spicy plants, which are widely used in medicine, culinary and perfumery, contributed to the increase in the economic efficiency of the method. The diversity of species that make up the collection of spicy-aromatic plants differs not only in botanical origin, but also in the period of flowering and secretion of nectar and pollen. The attractiveness of artificial shelter options and agrocenoses was assessed by comparing the density of the tubes with Hymenoptera. The insects

were counted by sequentially inspecting of artificial shelter's sections, determining the taxonomic affiliation of individuals and counting them.

## RESULTS AND DISCUSSION

A dependence of the Hymenoptera nests number on the artificial shelter structure and the type of agrocenosis was established. The maximum number of solitary bees (Megachilidae) nests were found in cylindrical shelters made of dark plastic (70,8%), delta-shaped shelters made of dark cardboard (20,8%) and 8,4% in delta-shaped shelters made of white cardboard. Tubular reed stems with a diameter of 0,7–0,9 mm were populated, and bee nests were not found in plastic tubes. Thus, bees prefer dark-colored shelters and tubular reed stems (Figure 1).

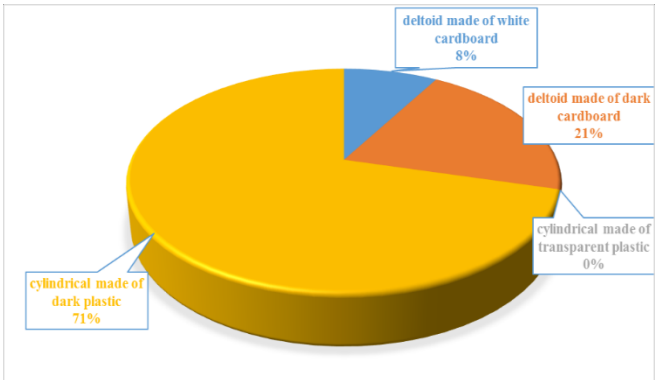


Figure 1. Population of artificial shelters by wild solitary bees depending on the type of body and material

In the agrocenosis of the pear orchard near the spice plot nested 91,7 % of bees, in the agrocenosis of the apple orchard - 4,2 %, and 4,1 % - in the agrocenosis of the *Miscanthus sinensis* field. In our studies in 2024, we obtained imagoes and nests of solitary bees from two genera (Megachilidae): one of the largest genera of bees in the family, the genus *Osmia* Panzer, 1806 (mason bees) and the genus Stelis Panzer, 1806 (cuckoo bees) (Figure 2).

Inside the cavity of the reed stems, *Osmia* bees divided the passage into brood chambers. Each chamber was supplied with enough pollen to feed one larva. The bee laid an egg on each pollen mass and sealed the chamber. The end of the tunnel was sealed with mud, plant resins, pieces of leaves or flower petals. *Osmia* bees pollinate an extremely wide range of flowering plants. *Stelis* bees lay eggs in the nests of other bees (Figure 3).

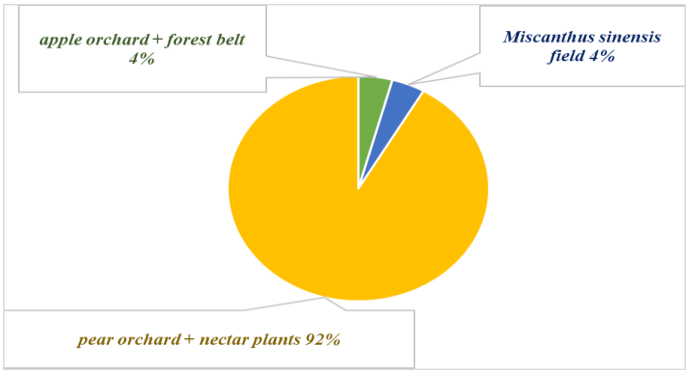


Figure 2. Population of artificial shelters and agrocenosis types by Megachilid family bees in various agrocenoses

Figure 3. Nesting of solitary bees (Megachilidae).
*Bee nests in tubular reed stems with multi-colored plugs (1), open bee nests (2), imago of the genus Osmia bees (3), imago of Stelis (4).*

The hosts of *Stelis* are other Megachilinae. In most cases, the parasite, having discovered the host's nest, returns to it repeatedly to lay an egg in each of several host cells before they close. The ability of bees to populate anthropogenic cavities of suitable sizes and the wide polytrophism of species allows us to consider Megachilidae bees as very promising species for field breeding in artificial shelters in order to increase the economic efficiency of agroecosystems.

In order to maintain and activate the population of Hymenoptera order entomophages and pollinators in the agrocenosis, a food base was provided from the continuous flowering of spice plants throughout the season. The flowering period in the collection of spice and aromatic plants in 2024 began on April 17 with the flowering of *Bergénia crassifólia* L. (Saxifragaceae) and *Adonis vernalis* L. (Ranunculaceae), which lasted for 18-20 days, respectively, and the visit (mainly

by bumblebees) was average. Then, from April 24 (flowering for 25 days), the flowering of *Nepeta transcaucazica* L., (Lamiaceae) and *Veronica officinalis* L. (Plantaginaceae) began. Their flowering is shorter 13-15 days, as well as *Rumex acetosa* L., *Rheum officinale* Baill. visits by insects, which are more numerous and varied.

Starting from April 25 and for 25-30 days, species, such as *Thymus vulgaris* L., *Thymus serpilium* L., *Thymus longicaulis* L., *Salvia officinalis* L. (Lamiaceae) bloomed, and at the end of April, beginning of May – *Rosa gallica* L. and *Sanguisorba officinalis* L. (Rosaceae). From the beginning of May and for 20-25 days *Thymus x citriodora* Schreb., *Asparagus officinalis* L., *Ruta graveolens* L. (with inconspicuous flowers, but very much visited by insects), *Veronica chamaedrys* L., *Matricaria chamomilla* L., *Plantago lanceolata* L., *Linum usitatisimum* L., *Centaurea cianus* L., *Allium shoenoprasum* L. bloomed. In early June and for 25-30 days, most species of the Lamiaceae family bloom and secrete nectar, which are well visited by insects. Among them are *Lavandula angustifolia* Mill., *Melissa officinalis* L., *Hyssopus oficinalis* L., *Dracocepalum moldavica* L.

The month of July is rich in flowering of the Apiaceae family species, which are visited by insects from the moment of the budding phase of plants (lasts from 5 to 13 days for different species). During this period, from July 2 to 5, insects "check" the umbrellas for nectar. The most frequent visits by insects are observed during the period of the flowering beginning and until the flowers in the umbrella have completely faded. This period begins on July 5 and lasts from 25 days for the annual species *Anethum graveolens* L. to 25 days and up to 30-35 days for the biennial species *Passtinaca sativa* L., *Apium graveolens* L., *Foeniculum vulgare* Mill., *Ammi visnaga* L., *Anhtriscus cerifolium* L. – equal to 25-35 days. In August-September, such species as *Allium ramosum* L., *Satureja montana* L., *Inula helenium* L. bloom. Thus, the site saw continuous flowering (conveyor) of 34 species of spice plants throughout the growing season (April-September).

## CONCLUSIONS

1. The degree of artificial shelters attractiveness for Hymenoptera order entomophages was established, depending on the design and materials from which they are made. The maximum number of bee's nests of the *Osmia* and *Stelis* (Megachilidae) genera were found in artificial shelters of a dark color: from cylindrical dark plastic (70,8%), from deltoid dark cardboard (20,8%). Bees preferred to build nests in the agrocenosis of a pear garden near a plot of spice plants (91,7%);

2. It was established that the optimal strategy for the activating entomophages method consists in combining a variety of spice plants of different families and ensuring continuous flowering (conveyor). This composition of the

collection has a long flowering of species and provides sufficient food for insects living nearby, an eco-friendly environment, and is also important for creating favorable conditions for pollination and increasing seed productivity of spicy-aromatic and garden crops.

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# ASSESSING GENETIC DIVERSITY IN TRITICALE POPULATIONS USING iPBS MARKERS

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***Abstract.*** *This article aimed to evaluate the efficacy of the iPBS (inter-Primer Binding Site) molecular marker system, leveraging mobile genetic elements, for genotyping diverse triticale accessions sourced from the collection of the Institute of Genetics, Physiology and Plant Protection. The objectives included the isolation and purification of genomic DNA from 7-day-old triticale seedlings of 'Ingen-54' and 'Ingen-93' genotypes, the empirical determination of optimal iPBS primer efficiency through a series of experimental amplifications, and the subsequent selection of highly informative primers for triticale genotyping. Standard polymerase chain reaction (PCR) amplifications were conducted utilizing specific iPBS primers (Pb13, Pb20, Pb21), followed by electrophoretic separation of amplification products on agarose gel. Amplification produced 33 clear, 100% polymorphic loci. POPGENE analysis revealed high informativeness (Nei's gene diversity h=0.1290, Shannon index I=0.2403) despite low genetic distance (0.0074–0.0169) between the closely related varieties. Primers Pb20 and Pb21 generated the most polymorphic loci. These results confirm the high utility of iPBS markers for genetic diversity assessment and cultivar identification in triticale breeding, even for closely related genotypes.*

***Keywords: genotyping, polymorphisms, retrotransposons molecular markers, triticale.***

## INTRODUCTION

Triticale (× Triticosecale Wittmack) is an artificial amphidiploid species derived from a cross between wheat (*Triticum aestivum*) and rye (Secale cereale). This hybrid was developed to combine the agronomically valuable traits of both progenitors: the high yield and grain quality of wheat with the cold tolerance, disease resistance, and environmental adaptability typical of rye.[1, 2] In terms of nutritional and feed value, triticale holds an intermediate position between wheat and rye. Triticale has high antioxidant activity due to its rich content of polyphenolic compounds and flavonoids, particularly phenolic acids such as 4-hydroxybenzoic acid, vanillic acid, caffeic acid, chlorogenic acid, p-coumaric acid, and rosmarinic acid [3]. Due to its combined traits, triticale isonsidered a promising cereal crop, especially in regions with harsh climatic conditions and marginal soils.

The world production of triticale currently exceeds 20 million tons per year and is mainly concentrated in Europe, accounting for over 80% of the global output. In the countries of Central and Eastern Europe, including Romania and Ukraine,

triticale is actively introduced into crop rotations as an alternative to winter wheat on low-productivity soils.[4] Its adaptability, resistance to abiotic stress, and relatively low input requirements make it an important candidate for sustainable agriculture and food security in the context of climate change. In Moldova, national agricultural research institutions have prioritized the development of high-performing triticale varieties. [2, 5]

Because of long-term efforts by the Institute of Genetics, Physiology and Plant Protection of the Republic of Moldova, was created a diverse collection of autochthonous triticale varieties, which has been developed to improve agronomic performance under Southeastern European conditions. Notable examples include Ingen 93 (1998), widely used as a reference standard in local research, as well as Ingen 33, Ingen 35, Ingen 40 (2015), Ingen 54 (2020), and Costel (2022) [2]. The existence of such a diverse gene pool within a relatively limited geographic area underscores the necessity for precise identification and differentiation of genetic variability among these cultivars.

Genetic diversity analysis plays a fundamental role in crop breeding, conservation, and genotype selection. Various marker systems have been employed to evaluate genetic polymorphisms, including phenotypic, biochemical, cytogenetic, and molecular markers. Among these, molecular markers are considered the most reliable due to their high reproducibility, independence from environmental factors, and the potential for automation and scalability. Techniques such as RFLP, SSR, RAPD, AFLP, SNP, and iPBS have been used to assess genetic variation in cereal crops [6]. The iPBS (inter-Primer Binding Site) marker system, based on the conserved regions of long terminal repeat (LTR) retrotransposons, offers several advantages, including high sensitivity to polymorphisms, especially among closely related genotypes. This makes it a powerful tool for cultivar identification and genetic diversity studies in breeding programs [7]. Current work focuses on using iPBS markers for triticale genotyping as well as assessing molecular diversity within populations with different genetic background.

## MATERIALS AND METHODS

**Plant material.** Two triticale genotypes – Ingen-54 and Ingen-93 were used in this study. The Laboratory of genetic resources of Institute of Genetics, Physiology and Plant Protection provided plant material. Seeds of 2022 yield were germinated on Petri dish and filter paper under 27°C during 7 days. Eight seedling of each genotype were selected for population analysis. Approximately 2 cm of apical meristem were cut off and used for DNA extraction.

**DNA extraction**. DNA extraction was performed using the ISO 21571 method [8]. A sample of extracted DNA was subjected to a series of dilutions (1:4, 1:14). From the extracted samples, we randomly selected 16 genotypes, 8 genotypes

each of the Ingen-54 and Ingen-93 varieties, for PCR testing. Purified and diluted DNA was subsequently used for PCR tests.

**Primers.** Several primers (Pb13, Pb20, and Pb21) designed to universal iPBS markers and described in (Kalendar R. et al., 2010) were used for genotyping and population analysis (Tab.1).

Table 1. Primers used for qPCR analysis

| Primers | Sequence | Tm (°C) |
|---|---|---|
| Pb13 | CCATTGGGTCCA | 45.7 |
| Pb20 | GGCTCAGATGCCA | 50.5 |
| Pb21 | GCTCATCATGCCA | 47.6 |

**Amplification.** PCR was performed in 25 µl of mix containing: Commercial buffer x10 (DreamTaq Buffer) – 2.5 µl, primer (forward + reverse) – 4.5 µl, dNTP mix – 0.5 µl, Taq polymerase – 0.3 µl, diluted DNA template – 10 µl, water – up to 25 µl volume. The amplification protocol was set up as follows: initial denaturation: 95°C, 3 minutes, followed by 45 cycles of denaturation at 95°C, 30 seconds, primer annealing at 55°C, 30 seconds and elongation at 72°C, 1 minute. Amplification concluded with final elongation: 72°C, 7 minutes.

**Product analysis.** PCR results was visualized on a 1.5% agarose gel stained with ethidium bromide. Molecular weight of the amplicons was assessed using 100 bp DNA Ladder (Thermo Fisher Scientific).

Data processing was carried out using the following software: GelAnalyzer, MS Excel, POPGENE.

## RESULTS AND DISCUSSIONS

Total DNA quality test via agarose gel electrophoresis showed crisp and intense bands of high molecular weight without smears and low RNA content (Fig.1). This presumes high yield of integral DNA suitable for molecular genotyping.

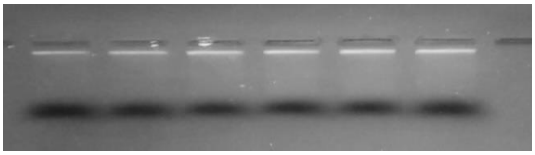

Figure 1. Electrophoregram of total triticale DNA

The amplification profiles obtained using three iPBS primers (Pb13, Pb20, and Pb21) are shown in Figures 2–4. The banding patterns reveal the presence of polymorphic loci and demonstrate the capacity of the chosen primers to

discriminate between triticale genotypes.

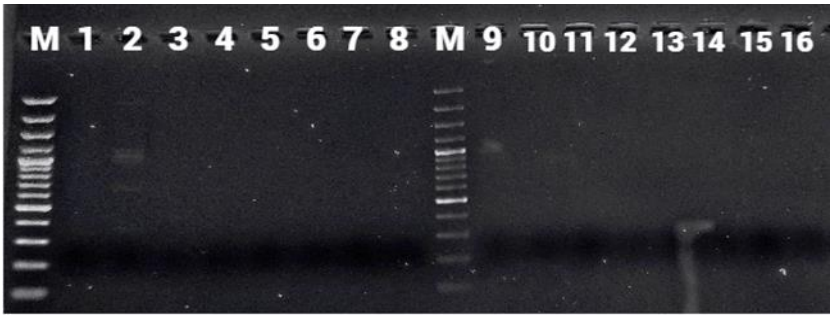


Figure 2. Electrophoregram of PCR products amplified with iPBS primer Pb13.
*1-8 – Ingen-54; 9-16 – Ingen-93; M – 100 Plus Ladder (Thermo Fisher Scientific)*

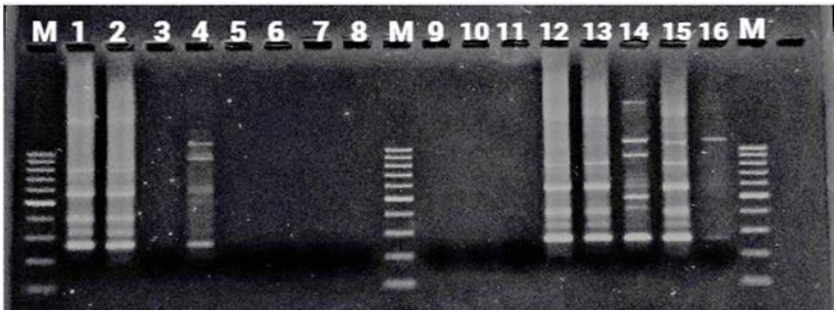


Figure 3. Electrophoregram of PCR products amplified with iPBS primer Pb20.
*1-8 – Ingen-54; 9-16 – Ingen-93; M – 100 Plus Ladder (Thermo Fisher Scientific)*

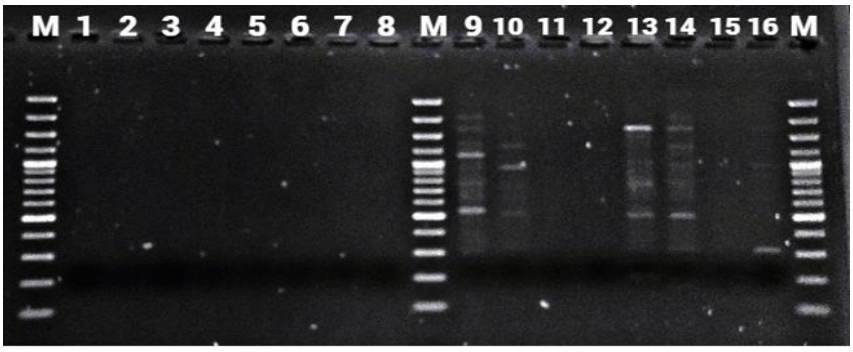


Figure 4. Electrophoregram of PCR products amplified with iPBS primer Pb21.
*1-8 – Ingen-54; 9-16 – Ingen-93; M – 100 Plus Ladder (Thermo Fisher Scientific)*

Amplification with these primers produced a total of 33 clear and reproducible loci (attested in three amplifications), ranging in size from approximately 243 to 2912 bp.

According to POPGENE statistical analysis, all 33 loci were polymorphic (100%), indicating the high informativeness of the chosen primer set. Nei's gene diversity (h) ranged from 0.0625 to 0.4188 with a mean of 0.1290, while Shannon's Information Index (I) varied from 0.1426 to 0.6096 (mean = 0.2403). The highest polymorphism was observed at locus 257 (h = 0.4188), suggesting it as a key marker for genotypic discrimination, which was detected when amplifying with Pb20 primer.

Nei's genetic distance between the two triticale varieties was low (0.0074–0.0169), with genetic identity ranging from 0.9832 to 0.9926, indicating a relatively close genetic relationship. Gst values (genetic differentiation) averaged 0.0642, and gene flow (Nm) was high (mean Nm = 7.29), reflecting limited genetic subdivision.

Primers Pb20 and Pb21 generated the greatest number of polymorphic loci and showed higher values of gene diversity (h), making them more suitable for distinguishing closely related triticale genotypes. Consequently, these specific primers are highly recommended as potent tools for prospective genotyping and breeding programs.

## CONCLUSIONS

iPBS markers proved to be effective for both discriminating between triticale cultivars with different genetic background, as well was evaluating population diversity for this species. It has potential application in triticale breeding programs and supplying national Gene bank information on triticale.

PCR amplification using primers Pb13, Pb20, and Pb21 yielded a total of 33 clear and reproducible loci. All 33 loci demonstrated 100% polymorphism, confirming the high informativeness of the selected iPBS primer set.

The highest level of polymorphism (h=0.4188) was recorded for locus 257. Nei's genetic distance between the two triticale varieties ('Ingen-54' and 'Ingen-95') is low, while genetic identity is high.

Primers Pb20 and Pb21 generated the greatest number of polymorphic loci and exhibited higher gene diversity (h) values compared to Pb13, making them more suitable for distinguishing closely related triticale genotypes.

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# PARASITIC NEMATOFAUNA IN PEA CROPS (*Pisum sativum* L.) UNDER THE IMPACT OF THE UNSTABLE ENVIRONMENTAL CONDITIONS OF THE REPUBLIC OF MOLDOVA

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***Abstract.*** *The results of the present research estimated the efficiency of the phytosanitary helmintological control in peas grown in open field and elucidated the helmintological parasitic impact, establishing the range of the invasive nematofauna, the frequency and the abundance of the associations. As a result of the phytosanitary research and the analysis of the helmintological impact on pea plants,it was found that the parasitic nematode complexes consisted of 14 species included in 4 families: Aphelenchidae, Hoplolaimidae, Tylenchidae, Heteroderidae, of the order Tylenchida, class Nematoda, distributed according to the investigated areas and classified according to the spectrum of trophic specialization in 5 groups. A larger number of species was detected in the Center area 15 species from 8 genera. It was found that the values varied from 7 to 30%, the damage being caused mainly by invasive associations of parasite nematodes of the genera: Heterodera, Meloidogyne, Ditylenchus, Pratylenchus, Helicotylenchus, Aphelenchus. The results of the phytosanitary monitoring contributed to elucidating the degree of nematological damage and brought new evidence in favor of applying sustainable pest control measures suitable for fabaceae croops.*

***Keywords: helmintological control, nematodes, parasitic impact, pea sectors, trophic specialization.***

## INTRODUCTION

Cultivating peas for grains, seed material and fodder solves three strategic problems: increasing seed production, increasing vegetable protein production and improving soil fertility, being 2-3 times richer in vegetable protein and having the advantage that it can be sown and harvested extra early [1, 2, 3, 4]. They are sown as a first priority under the environmental conditions of the Republic of Moldova, when the soil has sufficient moisture and allows sowing, usually in early March, but delayed sowing leads to significant production losses. Pea plantations for grain and fodder represent 8% of the arable land in the Republic of Moldova. They are

annually invaded by over 40 harmful species, which are also associated with invasive nematode complexes that trigger helminthiasis, which motivates specific annual monitoring to control the populations and their parasitic impact in the cultivation process of this crop [4, 5, 6]. Annually, according to the new institutional project 2024-2027, we conduct nematological research, including biodiversity estimates, morpho-ecological assessments and analyses of the structure of complexes of parasitic fauna, affecting various field crops, including peas [5, 6, 7, 8].

In the Republic of Moldova, helminthological research and monitoring of the biodiversity and structure of parasitic nematode complexes, detected in the agrocenoses of field crops cultivated in state-owned and private enterprises, were initiated and conducted by specialists in phytohelminthology and parasitology, renowned at the national level and recognized internationally, such as: Petru Nesterov, (1979-1980); Nicolai Ocopnnîi, (1982-1999); Melnic Maria et al., (1970-2024); Poiras Larisa et al., (1996-2016) [4, 5, 6, 7, 9]. Currently, taking into account the specifics of areas with contrasting and unstable climates, we aim at investigating invasive nematode complexes from the families *Heteroderidae Hoplolaimidae, Tylenchidae*, affecting pea plants (*Pisum sativum* L), in the context of applying new modern cultivation technologies, comparatively by areas, production associations, plantations and purpose of production. Based on current events and the estimated purpose, the goal of our research has been to establish the structure and diversity of invasive helminth species from the families: *Heteroderidae, Hoplolaimidae* and *Tylenchidae,* associated with parasitic forms affecting pea crops (*Pisum sativum* L.), determining the parasitic impact by comparative analyses of frequency and abundance indices, in various production and experimental sectors, in the dynamics of phenological stages [5, 6, 7, 8].

## MATERIALS AND METHODS

The proposed program included specific, helminthological investigations in field crop plantations (legumes, winter cereals, technical crops), where samples of soil and plants affected by helminths were taken and surveys and periodic records were made comparatively across various investigated sectors, mainly in the Central area as compared with the Northern area.

To establish the areas affected by helminthiasis, over 6 field trips, in 10 localities, 12 sectors, from 4 administrative districts in the Central area, were undertaken, where over 200 samples of soil and diseased plant organs were collected and analyzed (Fig. 1). Subsequently, indices of parasitic impact were determined, such as: species diversity, population density, frequency of attack (F%), intensity of attack (I%), estimation scales were used following phytosanitary control, field and laboratory records were made.

A B C

Figure 1. A, B, C. Surveys with soil sampling and analysis of plants collected during the growing season (Criuleni district, March-June 2024-2025)

A B C

Figure 2. A, B, C. Logistics and helminthological extraction analyses and microscopic establishment of morpho-taxonomic indices of nematodes extracted from analyzed samples.

The collected samples were subsequently analysed in the laboratory, according to the classical and new methods, with some modifications depending on the specifics of the nematode genera. Through specific techniques, nematodes were extracted from the soil and affected organs, applying the classic flotation-decantation method (*Baermann funnel)*, with some methodological adjustments, with subsequent fixation in 4% formalin, at temperature of 60°C, for morpho-taxonomic studies, reflected in figure 2. The fixed material was analysed under a microscope, establishing the population size, trophic groups, as well as other parasitic indices in the investigated soil and plants. Methodological and logistical support was offered by the Parasitology and Helminthology Laboratory, with methodology according to; Парамонов А. А. 1970; Nesterov P.I. 1978-1988; Melnic M., Erhan D., Rusu Ș2014.; Poiras L. et al., 2016, Iurcu-Straistaru E. at al. 2018-2023. The taxonomic units were determined using nematological identification guidelines according to: Decker H. 1972, Baldwin, J.G, Nadler, S.A., Adams, B.J. 2004; Perry, R.N., Moens, M.M. 2006 Decramer, W., & Hunt D. J. 2006; Siddiqi, M.R. 2010; Sasanelli, N. et al. 2018 [3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14].

## RESULTS AND DISCUSSIONS

Biological control of pea plantations affected by helminths was verified in various private households and production associations in the Center of the Republic of Moldova and in the administrative districts of Criuleni, Anenii Noi, Nisporeni, located in the South-East and South-West, where winter cereals, corn, sunflower, fodder crops, etc. are also intensively cultivated. Peas are cultivated on large and significant areas, in the sectors of the Central area, but in the North the areas are limited, instead, soybean plantations for grains and fodder predominate. For this reason, our research was focused mainly on sectors of the Central area, making detailed surveys and analyses.

Phytosanitary surveys were conducted monthly, in the period March-June, 2024-2025, which were characterized by relatively warm and humid weather conditions, which had an advantageous influence on the development of plants including but also of parasitic nematode complexes. The average frequency and intensity of helminthic attack were established, reaching values up to 25-30%. Abundant precipitation and higher temperatures have determined the severity of helminthiasis in association with various disastrous diseases such as: ascochytosis, fusariosis, which appeared early on the roots and stems during the vegetative phase. Simultaneously, on the preventively marked samples the presence of helminthic diseases was detected, the values of intensity and frequency of attack were analyzed comparatively, and the structure and predominance of certain genera and species with specific pathogenic invasive impact were highlighted. In all the investigated sectors, helminthic diseases and infestations were reported through local outbreaks, with retarded plants, in association with wilted, partially necrotic ones in variegated colors, presented in Figure 3.

The periods with abundant rainfall and unstable daytime temperatures, in the months of April-June, caused an increase in the damage severity and in the numerical density of the pest populations, reaching average values in the Central area 50-250 individuals/100 gr./soil. It was found that, in all the investigated sectors, there were extensive nematode complexes that produce helminthiasis, in certain sectors affecting the plants severely. This is due to the adaptive resistance capacities in the soil. Meanwhile, nematodes form specialized associations in these crops, caused by non-compliance with maintenance techniques. Such conditions favored the increase in the degree of parasitic helminthological impact, affecting plants in the stages of leaf formation, until the formation of pods.

A B C

Figure 3. A, B, C. Plants affected by mixed helminthic diseases associated with downy mildew and powdery mildew, on all plant organs, in the pod formation phase; A-productive sector May-June, Criuleni district, 2025

During this period, the reported helminth symptoms remain clearly accentuated and extensive, the visual symptoms including chlorosis, a reduced number of mature leaves, dwarf leaves, roots seriously affected by necrosis and rot caused by helminths. The species of pathogenic fungi in the soil estimated in ascending comparative values, by phenological stages, vary from 5% in the stage of 2-5 leaves up to 30% in the fruit development stage, presented in table 1.

Table 1. The estimation of phytosanitary indices by comparative values of helminthic parasitic impact in various phenological stages of pea plants grown in the districts of the Central area, (March-June, 2025).

| Central area and pilot districts | 26 March, stage of 2-5 developed leaves | | | 25 April, stage of 10-15 developed leaves | | | 27 May, flowering stage | | | 26 June, fruit development – ripening stage | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | D. (100 g soil) | F.(%) | I.(%) | D. (100 g soil) | F.(%) | I.(%) | D. (100g soil) | F.(%) | I.(%) | D. (100 g soil) | F.(%) | I.(%) |
| Nisporeni, Vărzărești village | 5-17 | 7-10 | 5-7 | 17-23 | 9-12 | 7-10 | 25-35 | 12-15 | 8-11 | 180 | 25-28 | 22-25 |
| Criuleni district, Pașcani village | 7-15 | 5-9 | 3-5 | 14-18 | 8-9 | 6-8 | 20-28 | 10-13 | 9-12 | 250 | 27-30 | 24-27 |
| Criuleni, Bălăbănești village | 8-16 | 8-12 | 5-7 | 10-15 | 7-10 | 6-9 | 28-33 | 13-18 | 7-10 | 220 | 25-28 | 22-25 |
| Anenii Noi, Mereni village | 6-14 | 5-8 | 2-5 | 13-16 | 10-14 | 8-12 | 23-30 | 9-14 | 7-9 | 190 | 20-25 | 18-22 |

*Legend: D. – density of nematode per 100 grams of soil; F (%) – frequency of attack; I (%) – intensity of attack.*

A B C

Figure 4. A, B, C. Pea plantations in the budding-fruit development stages, poly-invasively affected by helminthiasis and other diseases associated with specific pathogens, May - June, Criuleni district.

Such investigations were carried out for the first time in the Republic of Moldova on legume crops, including peas, as this research was conducted in another technologically intensive format and in the new private system, related to establishing the diversity and structure of nematode complexes, aspects of parasitic impact, trophic specialization, strategically adapted specialization, with the estimation of the most invasive forms in this early-spring crop. The investigations carried out consistently reflect in the seasonal dynamics, during growth stages, of the parasitic helminthic impact associated with specific diseases affecting peas, such as: downy mildew – pathogen *Peronospora pisi* Syd., powdery mildew – pathogen *Erysiphe pisi* Diet., blight – pathogen *Ascochyta pisi* Sacc., wilt – pathogen *Fusarium* spp., triggered by some problems in the cultivation techniques and environmental factors. Pea plantations affected by the above-mentioned diseases, in the budding-fruit development stages, are presented in Fig. 4 A, B, C.

These analyses and results are useful for adjusting short and long-term forecasts and are useful for applying an efficient integrated protection management for peas, namely by reducing the damage caused by pest invasions, the degree of infestation, the resistance in critical phases etc., aimed at production associations, experimental research sectors and private households. In this regard, by analyzing the samples collected from soil and affected plants in the laboratory, the structure and taxonomic diversity of invasive nematode complexes was determined in the dynamics of plant growth and development stages, in the presence of helminth associations accumulated in the soil planted with peas, the growing season March-June 2025, presented in Table 2. The analyses presented in Table 2 estimate the structure, diversity and density of the nematode populations, established at genus level, and the level of parasitic impact and infestation, up to 3 points (250 individuals per 100 grams/soil), detected from the first stages of plant development and increasing each month, according to the biological development cycle. In the detected complexes, the individuals belonged to 8 genera, namely: *Ditylenchus* spp., *Helycotilechus* spp., *Tylenchus* spp., *Fylenchus* spp, *Nothotylenchus* spp., *Heterodera* spp., of the order *Tylenchida*, with diverse trophic

specialization (endo-ecto-semi-endoparasites), migratory and sedentary.

Table 2. Diversity and structure of nematode complexes, accumulated in the soil planted with peas (March-June, 2025) in the dynamics of phenological stages

| Detected genera | Trophic group | Date of collecting and analyzing soil samples (100 g) from the plant's rhizosphere | | | |
|---|---|---|---|---|---|
| | | 25.03 | 25.04 | 25.05 | 30.06 |
| 1. *Ditylenchus sp.,* | Migratory endoparasites | + | + | ++ | ++ |
| *2. Prathylenchus sp.,* | | + | + | ++ | +++ |
| 3. *Helycotilechus sp.,* | Ectoparasites | - | + | + | ++ |
| *4. Tylenchus sp.,* | | - | + | + | ++ |
| *5. Fylenchus sp.,* | | - | + | ++ | ++ |
| *6. Nothotylenchus sp.* | | - | + | + | ++ |
| 7. *Meloidogyne sp.,* | *Cyst* and gall-inducing sedentare | + | + | ++ | +++ |
| *8. Heterodera sp.,* | | - | + | ++ | +++ |

*Legend: - no individuals; + from 30 to 100 individuals are present; ++ from 100 to 200 individuals are present; +++ over 200 individuals are present.*

All the estimated genera were found practically in all the investigated sectors, but the most invasive, with high frequency and abundance, were the forms belonging to the genera *Meloidogyne* and *Prathylenchus*, from the families *Tylenchidae* and *Heteroderidae*. These highlighted complexes represent a significant danger to pea plants, because they are associated with various species of insects and diseases, and are also vectors of microbial infections in the soil.

## CONCLUSIONS

As a results of the helminthological monitoring, carried out in 4 production associations from 4 districts, mainly belonging to the Central area of the Republic of Moldova, in plantations of peas grown for grains (*Pisum sativum* L.), the diversity and structure of invasive nematode complexes in the dynamics of phenological stages were established for the first time, detecting the presence of the most dangerous forms associated in 8 genera, such as: *Ditylenchus spp, Pratylenchus spp., Meloidogyne spp., Helycotilechus spp., Tylenchus spp., Fylenchus spp, Nothotylenchus spp., Heterodera spp.*, which belong to the families *Heteroderydae, Aphelenchidae Tylenchidae, Hoplolaimidae, Neotylenchidae,* order Tylenchida, with diverse trophic specialization (endo- ecto- and semi-endoparasites), migratory and sedentary.

The more abundant and frequent pests were the forms of migratory endoparasitic nematodes, the *Tylenchidae* family and the gall-inducing forms belonging to the *Heteroderidae* family, class Nematoda. After establishing the indices of the population density and frequency and intensity of attack, it was found that the nematodes that appear early, at the seed germination stage, the complexes belonging to the genera *Pratylenchus*

and *Meloidogyne*, detected in the rhizosphere of young pea plants, had an abundance of 50 to 250 individuals (adult forms, eggs, invasive larvae).

Helminthic diseases were reported as local outbreaks, with a frequency of 5 to 25% with an average attack intensity of 10-30%. These highlighted complexes represent a significant danger to pea plants, because they are also associated with species of lepidopteran and coleopteran insects in the soil, various diseases, causing unfavorable conditions for obtaining biological and agricultural harvests.

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# DETERMINATION OF THE PHYLOGENETIC LINEAGE OF THE SPECIES *MICROTUS ARVALIS* FROM THE REPUBLIC OF MOLDOVA BASED ON *CYTB* GENE SEQUENCING

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***Abstract.*** Resolving phylogenetic structure in cryptic rodent species is essential for understanding evolutionary history, population connectivity, and informing conservation strategies. In the Republic of Moldova, *Microtus arvalis* co-occurs with the morphologically similar *M. levis*, complicating species-level identification based on external traits alone. To determine the phylogenetic affiliation of local *M. arvalis* populations, we sequenced the mitochondrial *cytochrome b* (*CYTB*) gene (1106 bp) from five individuals identified molecularly from a total of 13 vole samples collected in central Moldova. These sequences were analyzed alongside 26 reference sequences retrieved from GenBank, representing the major phylogenetic lineages of *M. arvalis* across Europe. Phylogenetic reconstruction, performed using the Neighbor-Joining method in R software environment, revealed that the samples from Moldova form a highly homogeneous cluster and group with sequences from Poland, Slovakia, Finland, and Hungary. This pattern indicates a clear affiliation with the eastern phylogenetic lineage. The results expand the phylogeographic understanding of *M. arvalis* in Eastern Europe and underscore the utility of mitochondrial markers in distinguishing closely related species and supporting regional biodiversity assessments.

## INTRODUCTION

The *CYTB* gene of mitochondrial origin, encodes for *cytochrome b*, an integral component of the mitochondrial electron transport chain responsible for driving oxidative phosphorylation and *adenosine triphosphate* (*ATP*) production.

Its sequence exhibits low intraspecific variability and pronounced interspecific divergence, making it a reliable molecular marker for phylogenetic reconstruction. C*ytochrome b* DNA sequences from a phylogenetically and geographically diverse array of species are systematically archived in reference databases, such as GenBank and BOLD, enabling high-resolution species identification based on sequence homology, including cases where morphological traits are ambiguous due to convergence, development stage, or cryptic speciation.

Although small in size, voles exert a significant influence on the habitats they occupy, shaping trophic interactions, vegetation dynamics, and nutrient cycling. Monitoring voles populations is therefore critical, as their abundance and distribution often serve as sensitive indicators of ecosystem stability. Analysis of intraspecific genetic variation provides critical insights into the evolutionary trajectory and ecological differentiation of vole populations resolving fine-scale phylogeographic structure and demographic history. By reconstructing patterns of lineage divergence, gene flow, and population connectivity, such studies may inform the design of targeted population control strategies and resolve phylogenetic relationships among geographically or ecologically distinct populations. In the Republic of Moldova, the genus *Microtus* is represented by 2 species: *M. arvalis* (common vole) and *M. levis* (East European vole). These taxa are morphologically cryptic and often inhabit overlapping ecological niches, rendering species-level identification based solely on external characteristics unreliable during field sampling. *Microtus arvalis* is paraticularly abundant occupying open grassland habitats across a broad geographic range that extends from the Atlantic coast of Europe to Central Siberia [1]. Previous research [1] suggests that there are several phylogenetic lineages of the common vole across Europe and Asia, including western (United Kingdom, France, Spain), Italic (Italy), central (Germany, Netherlands, Denmark), eastern (Hungary, Slovakia, Poland, Ukraine, Finland) and a distinct lineage (Ukraine, Georgia, Armenia), which corresponds to the distribution of the *obscurus* form. In the present study, we sequenced and phylogenetically analyzed the mitochondrial *CYTB* gene from *M. arvalis* specimens from Moldova, with the aim of determining their lineage affiliation. The phylogenetic inference was performed through comparative analysis of *CYTB* sequences of the same species, extracted from GenBank [2,3].

## MATERIALS AND METHODS

Species-level identification of Microtus arvalis and M. levis in the Republic of Moldova was not feasible during sampling due to the lack of diagnostic morphological traits and the potential for sympatric distribution across habitats. A total of 13 samples were collected, and after sequencing 5 were affiliated to *M. arvalis*. The samples were taken from the suburbs of Chisinau and from several

other localities in the central region of the country (Table 1).

Table 1. Localities and geographical coordinates where the voles included in the study were captured

| **Localities** | **Latitude** | **Longitude** | **Identified specimens** |
|---|---|---|---|
| Piatra Alba | 46.926 | 28.825 | 1 x M. arvalis |
| Horesti | 46.806 | 28.929 | 3 x M. arvalis<br>3 x M. levis |
| Malcoci | 47.016 | 28.665 | 5 x M. levis |
| Chisinau | 47.021 | 28.718 | 1 x M. arvalis |

Tissue samples were immersed in 96% ethyl alcohol for preservation and subsequently stored in a freezer until DNA extraction. DNA was extracted using the *GeneJET Genomic DNA Purification Kit* [4] using column purification technology and according to the *Thermo Fisher Scientific* manufacturer's protocol. The sequencing of the 13 samples was performed at the Institute of Ecology and Evolution of the University of Bern using the *Sanger* technology. The 5 sequences of interest with a size of 1106 bp were compared with 26 other *CYTB* sequences (extracted from *GenBank*) that represent the species *M. arvalis* and are associated with all the evolutionary lineages mentioned above. The phylogenetic analysis of 31 sequences was performed in the R programming environment [5] using the *NJ (Neighbor Joining)* method [6] the pipeline being adapted based on several sources [7, 8]. The analyzed sequences comprise all 5 mentioned phylogenetic lineages and originate from specimens from the following countries: Armenia, Denmark, Finland, France, Georgia, Germany, Hungary, Italy, Moldova, Netherlands, Poland, Slovakia, Spain, Ukraine, United Kingdom (Orkney).

## RESULTS AND DISCUSSIONS

Analysis of 31 mitochondrial *CYTB* sequences revealed relevant phylogeographic structure and patterns of genetic similarity between *Microtus arvalis* specimens across different regions of Europe. The resulting genetic distance matrix delineated distinct sequence clusters, with Moldova samples exhibiting minimal pairwise divergence, indicative of high genetic homogeneity within the local population. These sequences together with sequences from Poland, Finland, Slovakia and Hungary form a well-defined cluster, suggesting a high genetic similarity between the Eastern European populations of this species (Fig. 1). Consistently low inter-sample distances, approaching zero in the heatmap, further underscore the close genetic affinity among these Eastern populations.

The presence of significant genetic homogeneity between populations from Moldova and neighboring Eastern European countries suggests a long-standing

ecological connectivity that has allowed for the maintenance of gene flow among these populations. This pattern has important implications for understanding the population dynamics of *M. arvalis* in eastern Europe and contributes to a better understanding of its phylogeographic map. The absence of major geographic barriers in this region may be a key factor in maintaining this uniform genetic structure.

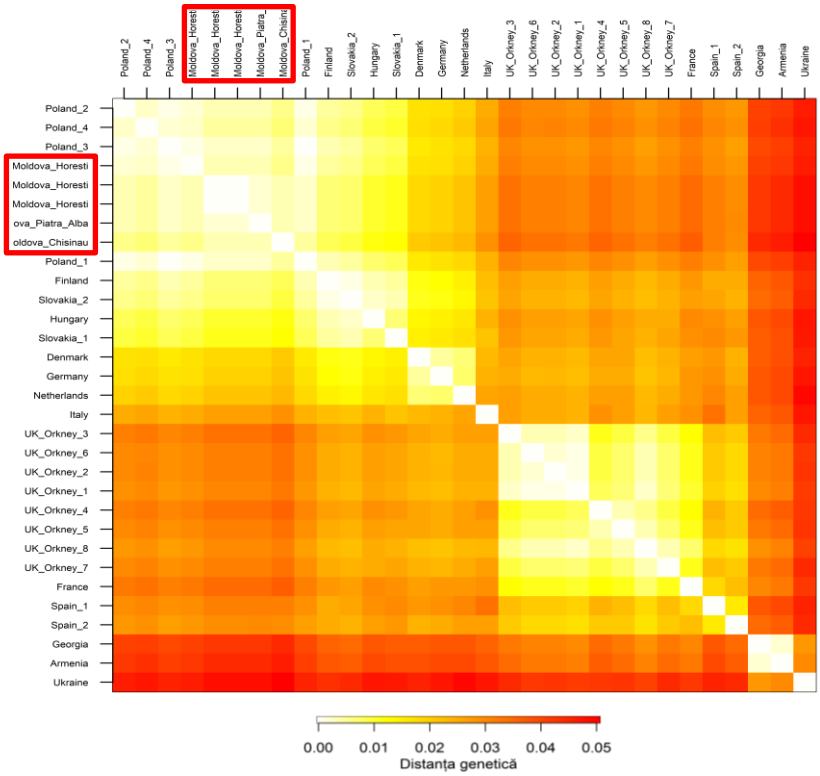


Figure 1. Genetic distance between 31 CYTB gene sequences in the species M. arvalis

Sequences from western Europe, such as those from the United Kingdom (UK), France, Spain and Italy, show some degree of genetic differentiation from eastern populations. Notably, sequences from the UK samples form a relatively homogeneous cluster, but distinct from the rest of continental Europe, confirming their geographic isolation and reinforcing the idea that oceanic barriers have a crucial role in shaping their unique genetic signature. The minimum genetic distance observed between any two sequences was 0.001 (0.1%), indicating a very close relationship, likely within the same local population or closely related individuals. The average genetic distance across all comparisons was 0.024 (2.4%), providing a measure of overall variability within the species across the studied

range. The maximum genetic distance recorded was 0.05 (5%), highlighting the most divergent evolutionary relationships found among the *M. arvalis* samples. In total, 5 clusters associated with the 5 previously described phylogenetic lineages were obtained, with the analyzed specimens from Moldova grouping with those from the Eeastern phylogenetic lineage (Fig. 2).

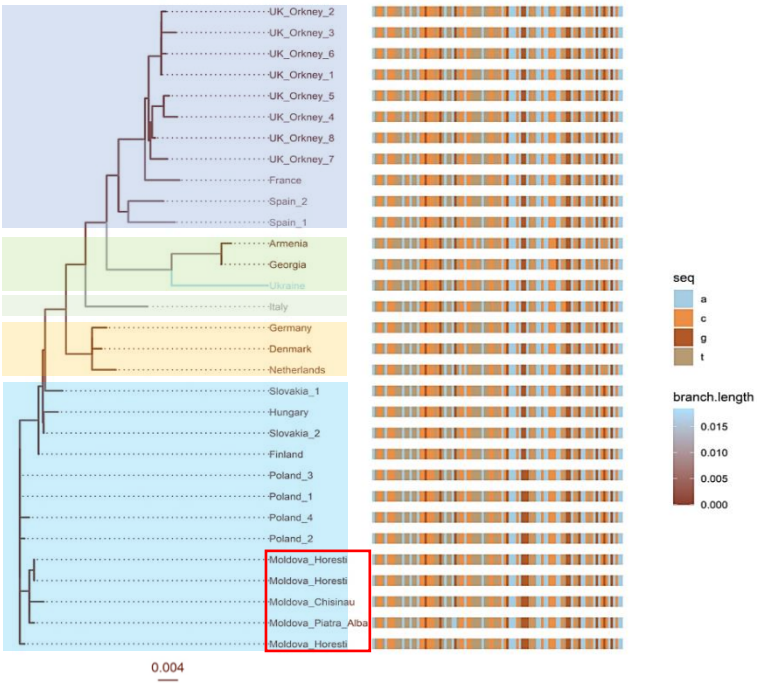


Figure 2. Phylogenetic tree obtained from sequences of the CYTB gene in the species M. arvalis

*Phylogenetic lines that can be observed: 1. Western; 2. Line associated with the obscurus form; 3. Italic; 4. Central; 5. Eastern, which also includes the voles from the Republic of Moldova; a, c, g, t – adenine, cytosine, guanine, thymine.*

The color scale of the tree branches represents the genetic distance and the color changes of the aligned sequences show the nucleotide differences. A relatively large difference is observed between the lineages, especially between the eastern and western clusters on the one hand and the eastern and the one associated with the *obscurus* lineage on the other. At the same time, there is no large variation between the sequences within the eastern phylogenetic lineage. The presented phylogenetic tree has 29 internal nodes and 31 top branches. The analyzed sequences have a size of 1106 bp and present 113 sites of variation. Nucleotide substitutions per site (unit of branch length) have a value of 0.004 which constitutes 0.4% genetic variation per 100 nucleotide sites. To assess the robustness of the

phylogenetic inference, we evaluated the congruence between the tree topology and the original genetic distance matrix (Fig. 3). The cophenetic correlation coefficient was 0.99, indicating an excellent fit between the tree structure and the pairwise genetic distance.

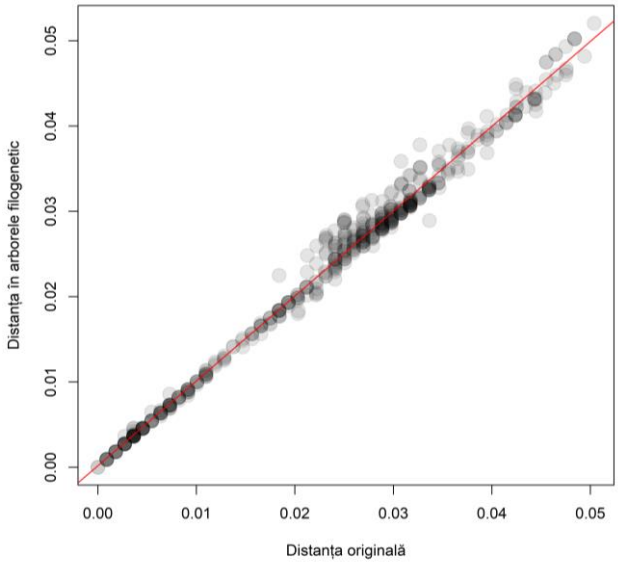


Figure 3. Correlation between the distance shown in the phylogenetic tree and the original distance

Mitochondrial DNA analysis of *M. arvalis* offers critical insights into the species evolutionary history, ecological adaptability and response to environmental change. Such data not only elucidates historical population dynamics but also informs predictive models of future evolutionary trajectories under shifting ecological conditions. These insights are essential for developing evidence-based conservation and management strategies aimed at safeguarding genetic diversity and ensuring population resilience. Furthermore, sequencing the *CYTB* gene provides valuable taxonomic resolution and enables molecular discrimination of morphologically cryptic species, such as *Microtus arvalis* and *Microtus levis*, within the Moldova fauna.

## CONCLUSIONS

The study provides a phylogeographic assessment of *M. arvalis* in the Republic of Moldova based on mitochondrial *CYTB* sequences, offering a

comparative perspective within the broader European context. The samples collected in the Republic of Moldova show a high genetic homogeneity and cluster together with those from Poland, Finland, Slovakia, and Hungary. These findings confirm the affiliation of the local samples with the **eastern phylogenetic lineage** of *M. arvalis*, one of the major phylogenetic clades previously described in the literature.

All sequenced samples were registered on the global platform *Barcode of Life Data Systems* as part of the *TERVM* project – *Terrestrial Vertebrates of the Republic of Moldova* [9]. The work was conducted under the MSU subprogram no. 010701 and contract no. 01-23p- 096/03-05-2024, financed by the National Environmental Fund.

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DOI: https://doi.org/10.53040/cga12.07 UDC: 632.4:576.385.5:632.937

# MOLECULAR-GENETIC IDENTIFICATION OF CERTAIN FRUIT TREE MYCOPATHOGENS TRANSMITTED BY APHID VECTORS

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***Abstract.*** This study focuses on the molecular-genetic identification of phytopathogenic fungi transmitted by aphids in fruit orchards. Specimens of *Dysaphis* and *Hyalopterus* aphids were collected from apple and plum trees respectively, and DNA was extracted using the SDS protocol. A two-step PCR method with species-specific primers targeting fungal pathogens (*Monilinia* spp., *Penicillium* spp., and *Botrytis cinerea*) was applied. Amplification products revealed the presence of *P. chrysogenum*, *M. fructigena*, and *B. cinerea* in samples from *Hyalopterus*, while *Dysaphis* samples tested negative for all target fungi. These results confirm that *Hyalopterus* aphids can act as vectors for both generalist and specialist phytopathogens at early stages of the growing season. The findings highlight the complex multi-level interactions between insect vectors, pathogens, and host plants in agroecosystems. Continuous monitoring and early detection of secondary fungal infections are essential for maintaining phytosanitary stability.

***Keywords: phytopathogenic fungi, aphids, vectors, PCR, Monilinia spp., Penicillium spp., B. cinerea.***

## INTRODUCTION

The formation of specific pathologies in tree species and their populations—both in natural forest communities and in cultivated orchards, parks, and urban plantings—is largely influenced by biogenic factors. These factors include various living organisms and their associations, encompassing biological systems at different levels of organization. Representatives of different kingdoms, phyla, classes, and numerous lower taxonomic groups are involved: higher and lower plants, fungi, insects, mites, nematodes, protozoa, bacteria, mycoplasmas, phytoplasmas, spiroplasmas, and viruses.

To date, it has been established that most significant infectious diseases of woody plants and mycoses in particular, are in one way or another associated with the activity of insects [10]. Entomochory in the pathology of woody plants is a complex and multifaceted phenomenon that encompasses processes such as

transmission, reservation, reproduction, persistence, and penetration of phytopathogenic infections into plant tissues and organs, including fruits and seeds. The joint colonization of the same plant organs by phytopathogens and dendrophilous insects promotes their interaction and the formation of various forms of contact. These organisms may simultaneously act as both pests and vectors of infection.

Among the dendrophilous insects capable of transmitting phytopathogenic fungi are representatives of various orders: Coleoptera, Lepidoptera, Hymenoptera, Heteroptera, Homoptera, Diptera [8]. Some aphid (order Hemiptera) species, such as the green apple aphid (*Aphis pomi*), are the most widespread pests of apple trees, affecting them throughout the Northern Hemisphere. When aphids multiply en masse, the affected leaves and shoots curl and wilt, leading to a decline in fruit tree productivity. Plum trees are colonized throughout June and sometimes into early July. Under high air temperatures and moderate humidity, due to their high reproductive potential, the number of plum aphid larvae on young plum trees can reach 7–8 thousand per 100 leaves, completely covering the underside of the leaf blades [1]. Additionally, apple aphids are known to play a role in the maintenance and spread of phytopathogenic bacteria such as *Erwinia amylovora*, *E. aphidicola*, *Pseudomonas syringae*, *Dickeya dadantii*, and *Pantoea stewartii* [4]. It is known that microorganisms, including phytopathogens, may enter the aphid body from the surface and internal tissues of host plants. Many of these microbes are capable of adapting to life in the aphid's digestive tract and may even reproduce there. However, scientific researches focusing on aphids (order Hemiptera) as vectors for pathogenic fungi and outbreaks of certain diseases is scarce. Therefore, this topic requires a thorough investigation for its future exploitation in environment-friendly pest control management.

Current work aims to elucidate the role of aphids in transmitting certain pathogenic fungi in plum orchards in the Republic of Moldova. The identification of phytopathogenic fungi transmitted by aphids plays a crucial role in ensuring the phytosanitary stability of agroecosystems. Identifying specific fungal species carried by aphids not only enables effective pest control but also prevents secondary fungal infections. This makes it possible to include not only aphid monitoring and control but also additional fungal infection prevention in the Integrated Pest Management (IPM) system.

## MATERIALS AND METHODS

At the beginning of the fruit ripening season, specimens of two aphid genera parasitizing apple (*Dysaphis sp.*) and plum (*Hyalopterus sp.*) trees growing in the IGFPP orchard were collected (Fig. 1). DNA extraction from the insects was performed using the ISO 21571 protocol [2].

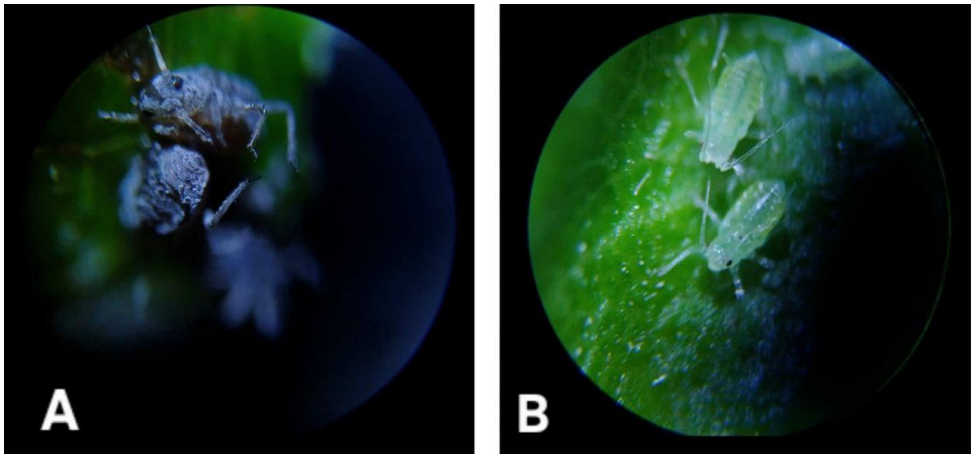


Figure 1. Visualization of aphid specimens collected in the IGFPP fruit orchard under a photon microscope.
*A – Dysaphis, B – Hyalopterus*

Table 1. Primer sequences for PCR analysis

| Primer | Taxon | Primer orientation | Primer sequence |
|---|---|---|---|
| pchbt1 | *P. chrysogenum* | Forward primer | GTTGCTAATCGATTTACAGGCAAAC |
| pchbt2 | | Reverse primer | CACCGCTGGGCCTAGATTTGTC |
| pchbt3 | | Forward primer | TGATGGGGATTCGTGTGATCA |
| pchbt4 | | Reverse primer | CCGCTGCGCTAGATTTGTCAA |
| pex1 | *P. expansum* | Forward primer | CAACCTCCACCCCGTGTTT |
| pex2 | | Forward primer | ACTCGTCCTGAGAGATTTGTGT |
| pex3 | | Reverse primer | CGGAATCGGGGGGAGGGG |
| pex4 | | Reverse primer | ATTTGGGTTGTATCGGGAGC |
| mfg1 | *M. fructigena* | Forward primer | CAATTATTGCTGATTGGGTGACA |
| mfg4 | | Reverse primer | AGGTGGCGTAATTTTGGAATTAACTT |
| mfg2 | | Forward primer | GATTGGGGTGACAACTTCTATAATCCG |
| mfg3 | | Reverse primer | TCTAAACTTCTTCGCTGACCAATC |
| mla1 | *M. laxa* | Forward primer | AGCAATTACTGCTGACTGGG |
| mla4 | | Reverse primer | AGGGCACTTATGCGGAATGTT |
| mla2 | | Forward primer | GTTCCATCACTCGCGAAGTCA |
| mla3 | | Reverse primer | AAAGTGGCGAAATCTTGGAATC |
| bc1 | *B. cinerea* | Forward primer | TCACCCGCACCTAATTCGTC |
| bc2 | | Reverse primer | AGGAGCCTTCTCCCTTGGTT |
| bc3 | | Forward primer | TGTGCGAGGGTGAAGTAGTG |
| bc4 | | Reverse primer | GCACTCGGCGATCCAGATAA |

Molecular identification of pathogens was carried out using a two-step PCR method with primers specific to genomic regions of various fungal pathogens: *Monilinia spp*., which cause significant spoilage of the harvest, leading to fruit rot;

as well as *Penicillium spp*. and *B. cinerea*, which are producers of mycotoxins. The primers were developed by staff of the Molecular Genetics Laboratory (IGFPP) based on species-specific sequences stored in the GenBank database [7].

Amplification included two consecutive rounds of 30 cycles each in a 25 µL reaction mixture containing: 66 mM Tris-HCl (pH 8.4), 16 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.1% Tween 20, 7% glycerol, 100 $\mu L^{-1}$ BSA, 0.2 mM of each dNTP, 1.25 units of Taq DNA polymerase (Thermo Fisher Scientific), 5 pM of each forward and reverse primer, and 10 ng of DNA template.

The first round began with a 3-minute initial denaturation at 95°C, followed by 30 seconds of denaturation at 95°C, 30 seconds of annealing at 60°C, and 30 seconds of elongation at 72°C. The second round was conducted without initial denaturation. Both rounds concluded with a final elongation step of 7 minutes at 72°C. Amplification products were separated by electrophoresis in a 1.5% agarose gel using 1×TBE buffer at 6 V/cm for one hour. Visualization was carried out using a transilluminator at a wavelength of 302 nm.

## RESULTS AND DISCUSSION

Molecular analysis revealed a marked qualitative difference in fungal species associated with aphid populations of diferent species. Visualization of amplification products in samples containing DNA extracted from *Hyalopterus* revealed target sequences of *Penicillium chrysogenum*, *M. fructigena*, and *B. cinerea*. In contrast, no target sequences of the reviewed pathogens were detected in *Dysaphis* samples. The overall results of the PCR analysis using the aforementioned primers are summarized in Table 2.

Table 2. Identification of mycopathogens in the analyzed samples*

| Aphid / Pathogen | *Hyalopterus* | *Dysaphis* |
|---|---|---|
| *P. chrysogenum* | + | - |
| *P. expansum* | - | - |
| *M. fructigena* | + | - |
| *M. laxa* | - | - |
| *B. cinerea* | + | - |

** "+" sign indicates presence of the fungi in the sample, "- "sign - absence*

The emergence of founding female aphids in the main zone of harmful activity is observed from March to early April, coinciding with the bud scale separation of fruit trees. During the growing season, up to 10 generations typically develop. On the plant organs affected by aphids (leaves, annual shoots, flowers, and fruits), sugary secretions appear, which promote the development of saprophytic fungi by serving as an additional nutrient substrate essential for their activity [8].

Thus, in addition to their direct harmful impact on fruit trees, aphid colonies, by acting as vectors for pathogenic fungi, can contribute to the accumulation of damage in the host plant. As a result, a disruption of water balance and loss of nutrients occurs, which may lead to leaf and fruit drop by mid-summer, while the remaining fruits become deformed and begin to rot.

Based on the obtained results, it can be concluded that aphids of the *Hyalopterus* genus are capable of acting as vectors for both polyphagous fungi and specialized species such as *Botrytis cinerea* and *Monilinia fructigena*, potentially serving as sources of infection at the beginning of the growing season and during early fruit development.

Thus, pathological processes in fruit orchards, initially triggered by a single phytopathogen, tend to evolve over time into systemic, multifactorial pathologies involving numerous other organisms. As a result, several models of multi-level interaction systems emerge within agroecosystem pathogenesis, involving insect vectors, phytopathogens, and host plants.

## CONCLUSION

The molecular-genetic analysis demonstrated the ability of early generations of *Hyalopterus* aphids to transmit pathogenic fungi such as *P. chrysogenum*, *Botrytis cinerea*, and *Monilinia fructigena*. A comprehensive assessment of the vector potential of the studied aphid genera requires further investigation and identification of fungal pathogens in fruit trees at different insect developmental stages.

Due to the variability of the mycopathogenic composition in fruit orchards and the pronounced susceptibility of mycopathosystems to abiotic and anthropogenic environmental factors, there is an increasing need for monitoring and timely identification of secondary infection foci to enhance the effectiveness of phytosanitary measures in orchard management.

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# Session II

# HUMAN AND MEDICAL GENETICS

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# HOMEOSTASIS OF CARBOHYDRATE METABOLISM IN EXPERIMENTAL DIABETES UNDER THE INFLUENCE OF THE BIOPREPARATION SEV-3

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***Abstract.*** *Diabetes mellitus is a major public health issue, characterized by chronic hyperglycemia, complex metabolic dysfunctions, and severe systemic complications. This study investigates the impact of the biopreparation SEV-3 on glucose metabolism homeostasis in experimental diabetes induced in laboratory white rats. The conducted research highlights a hypoglycemic effect and stimulation of insulin secretion, suggesting the potential use of natural biopreparations in improving the metabolic imbalances associated with diabetes mellitus.*

***Keywords: Biopreparation, experimental diabetes, homeostasis, glucose metabolism, medicinal plants.***

## INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder caused by insufficient insulin production or peripheral insulin resistance, leading to disruption of glucose homeostasis. This condition affects millions of people worldwide and is associated with a wide range of chronic complications, including retinopathy, nephropathy, and diabetic neuropathy [1; 2]. At the core of the disease pathogenesis lies a profound disturbance in carbohydrate metabolism, which, if left untreated, progressively impacts lipid and protein metabolism and, ultimately, the overall homeostasis of the body.

Maintaining glucose metabolism homeostasis—that is, preserving a dynamic balance between glucose intake, storage, and utilization—is essential for the optimal function of all organs, especially those highly sensitive to glycemic fluctuations, such as the brain, liver, and pancreas.

Glucose homeostasis, ensured by complex hormonal and enzymatic mechanisms, plays a critical role in the prevention and control of diabetes-related complications [2]. Under physiological conditions, this balance is maintained

through a complex interplay between insulin secretion by pancreatic β-cells and the peripheral insulin response in muscle, liver, and adipose tissue. In diabetes, this system is impaired, leading to excessive accumulation of glucose in the bloodstream, with long-term detrimental effects. Moreover, oxidative stress and chronic inflammation, frequently associated with diabetes, further contribute to cellular damage and disruption of hormonal regulatory mechanisms [3].

The imbalance in glucose metabolism leads to systemic complications, necessitating effective interventions for glycemic control. Given the limitations and adverse effects of conventional antidiabetic treatments, interest in alternative and complementary therapies has significantly increased over recent decades. Phytotherapy, especially the use of medicinal plant extracts with hypoglycemic action, has emerged as a promising area in diabetes research [4, 5]. Plant-derived biopreparations can positively influence blood glucose levels by increasing insulin sensitivity, stimulating insulin secretion, or through antioxidant activity, thereby reducing oxidative stress on pancreatic cells [6, 7].

In addition to conventional treatments, the use of natural bioactive compounds has become a growing focus in biomedical research, aiming to reduce hyperglycemia and prevent diabetes-associated complications [8, 9]. In modern experimental research, induced diabetes models, such as the alloxan model, have allowed for the evaluation of potential therapeutic effects on carbohydrate metabolism in a controlled and reproducible manner. Alloxan is commonly used in diabetes research due to its ability to selectively induce cytotoxicity in pancreatic β-cells, thereby mimicking the characteristics of diabetes mellitus [10]. These models are essential for understanding the mechanisms of action of antidiabetic compounds and for validating their efficacy prior to clinical application.

In this context, recent studies have been exploring the effectiveness of natural biopreparations, such as SEV-3, which contains standardized plant extracts with antioxidant, anti-inflammatory, and glycemia-regulating properties. This biopreparation shows promising potential in correcting metabolic disturbances associated with diabetes [7]. Due to its rich content of flavonoids, glycosides, alkaloids, and synergistically acting vitamins, SEV-3 may contribute to restoring glycemic balance and alleviating oxidative stress, thus offering an integrative approach to maintaining glucose homeostasis under diabetic conditions. Some of these bioactive substances may positively interact with insulin receptors on target cell membranes, enhancing insulin signal transduction and facilitating glucose uptake via GLUT4 transporter activation, thereby contributing to the normalization of blood glucose levels. This mechanism can directly improve insulin sensitivity in insulin-dependent tissues, such as skeletal muscle and adipose tissue [6]. As a result, these biological products exert pleiotropic effects, enabling them to act simultaneously on multiple pathological mechanisms while reducing the risk of toxic or allergic complications.

Thus, the aim of this study is to evaluate the effects of the SEV-3 biopreparation on glycemic and insulin parameters in an alloxan-induced diabetes model, highlighting its therapeutic potential in maintaining glucose homeostasis.

## MATERIALS AND RESEARCH METHODS

The investigations were conducted on laboratory white rats of both sexes, with body weights ranging from 180 to 220 g. A total of 60 rats were used in the study, housed under standard vivarium conditions with free access to food and water for 30 days. Experimental diabetes mellitus was induced by intraperitoneal injection of a 5% alloxan solution at a dose of 200 mg/kg. Blood glucose levels were measured using the "On Call Plus" glucometer, and hormone levels were assessed by the enzyme-linked immunosorbent assay (ELISA) method using the Stat Fax 4700 analyzer. Statistical analysis was performed using GraphPad Prism InStat software (version 10.2.3, GraphPad Software Inc., La Jolla, California, USA).

The biopreparation contains the aerial parts of the following medicinal plants: Vitis vinifera, Equisetum arvense, and *Syringa vulgaris*.

A B C

Figure 1. Dried medicinal plants in the composition of the biopreparation SEV-3

*a) Syringa vulgaris b) Equisetum arvense c) Vitis vinifera*

The preparation is administered at a dose of 50 mL per rat per 24 hours, prepared by infusing the mixture of the aforementioned plants (6 g per plant in 600 mL of water).

The biopreparation SEV-3 has a complex phytochemical profile with therapeutic potential in regulating carbohydrate metabolism. The major active compounds of these plants are glycosides, flavonoids, alkaloids, vitamins, and other natural antioxidants that act synergistically on multiple mechanisms involved in

maintaining glucose homeostasis [6, 11]. Thus, the flavonoids and glycosides in Vitis vinifera are well-known for their hypoglycemic effects, acting by increasing the sensitivity of peripheral cells to insulin and regulating intestinal glucose absorption [5, 7]. The antioxidants present in *Syringa vulgaris* and *Equisetum arvense* extracts reduce oxidative stress induced by hyperglycemia, thereby protecting pancreatic β-cells against apoptosis [3, 4]. Vitamin C, phenolic compounds, and saponins contribute to neutralizing free radicals, supporting partial regeneration of pancreatic islets [12]. The alkaloids and bitter compounds in the SEV-3 biopreparation may directly stimulate insulin secretion by activating calcium channels in β-cells [6]. This helps maintain a physiological level of insulin in the blood, essential for glucose metabolism. The presence of essential vitamins and phytonutrients aids in stabilizing lipid and protein metabolism, which are often disrupted in the context of diabetes mellitus [7]. This global action supports the general homeostasis of the organism.

Therefore, the biopreparation SEV-3 may contribute to restoring glycemic balance through multiple actions: reducing blood glucose, protecting β-cells, stimulating insulin secretion, and combating oxidative stress. These effects justify its exploration within complementary therapies for diabetes, especially in the early or moderate stages of the disease.

## RESULTS AND DISCUSSION

Endocrine pancreatic dysfunction, characteristic of diabetes mellitus, has profound pathological implications, contributing to severe disturbances in the homeostasis of carbohydrate metabolism [2, 3]. This dysfunction is primarily associated with damage to the β-cells of the islets of Langerhans, which are responsible for the synthesis and secretion of insulin—the key hormone regulating blood glucose levels. The loss of functionality or destruction of these cells results in a marked decrease in insulin secretion and, consequently, in the body's inability to maintain plasma glucose concentrations within physiological limits [13].

The dysregulation of this hormonal mechanism leads to significant alterations in carbohydrate metabolism, resulting in persistent hyperglycemia. In the absence of effective glycemic control, the excessive accumulation of glucose in the bloodstream serves as a triggering factor for the onset and progression of both microvascular and macrovascular complications associated with diabetes mellitus [2].

In experimental diabetes, insulin deficiency leads to increased protein catabolism in tissues, elevated amino acid levels in the blood, and increased excretion of ammonia and nitrogen in the urine [10]. These changes correlate directly with the degree of insulin deficiency. Reduced circulating insulin impairs the ability of tissues to absorb glucose, leading to elevated blood glucose levels.

In decompensated diabetes mellitus, insulin—the essential hormone for blood glucose regulation—can no longer effectively exert its functions. As a result, glucose metabolism is severely impaired, with direct consequences for overall homeostasis. Normally, insulin facilitates the uptake of glucose into peripheral tissue cells, such as muscle and liver cells, where it is utilized for energy production. In this form of diabetes, whether due to insufficient insulin or increased insulin resistance, this mechanism becomes dysfunctional, and blood glucose remains elevated [7].

Insulin resistance plays a central role in this process. Under certain conditions, cells become less responsive to insulin, a phenomenon often exacerbated by lipid accumulation in tissues. This impairs glucose transport into cells, resulting in glucose build-up in the blood. In compensation, the pancreas increases insulin secretion. However, over time, this compensatory response becomes inadequate, leading to chronic hyperglycemia.

In addition to insulin imbalance, the liver continues to produce glucose through gluconeogenesis—a metabolic pathway by which glucose is synthesized from non-carbohydrate precursors. Normally, this process is suppressed by insulin, but in poorly controlled diabetes, gluconeogenesis persists, contributing to elevated blood glucose levels. Simultaneously, lipolysis (fat breakdown) is activated, releasing free fatty acids into the bloodstream. While these molecules serve as alternative energy sources, their excess conversion into ketone bodies can lead to ketoacidosis—a life-threatening metabolic disturbance that significantly impairs the body's acid-base balance [2, 3].

At the renal level, hyperglycemia can surpass the kidneys' reabsorptive capacity for glucose, leading to glucosuria and placing additional strain on renal function, thereby contributing over time to the development of diabetic nephropathy. Furthermore, hyperglycemia-induced disturbances in electrolyte balance—such as hypokalemia—may adversely affect cardiovascular function and increase the risk of cardiac arrhythmias [10].

Our study confirmed a significant increase in blood glucose levels in experimental diabetes (16.9 ± 0.84 mmol/L), compared to the control group (5.5 ± 0.27 mmol/L). Administration of the SEV-3 biopreparation reduced blood glucose to 10.5 ± 0.52 mmol/L, demonstrating a clear hypoglycemic effect. Furthermore, insulin levels were significantly reduced in the alloxan-induced group (1.51 ± 0.44 pmol/L), while SEV-3 administration stimulated insulin secretion up to 3.03 ± 0.32 pmol/L, compared to the normal value of 4.02 ± 0.39 pmol/L, indicating the biopreparation's potential to ameliorate metabolic dysfunctions associated with diabetes. These findings are consistent with those reported by Zhang et al. (2020) [13], who demonstrated the antidiabetic effects of plant-derived flavonoids. The differences between groups were statistically significant ($p < 0.05$) for both blood glucose and insulin levels between the diabetic group and the group treated with the

SEV-3 biopreparation.

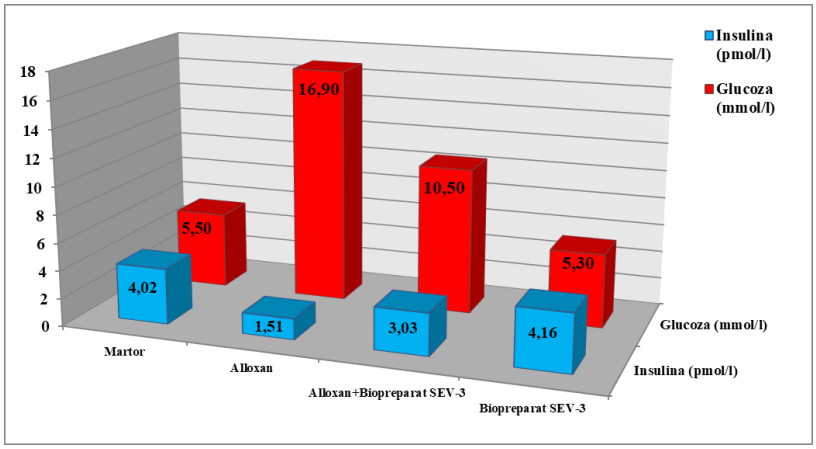


Figure 1. Blood plasma glucose (mmol/l) and insulin (pmol/l) levels following administration of the SEV-3 biopreparation in experimental diabetes.

Insulin plays a central role in the regulation of carbohydrate metabolism. It acts by maintaining blood glucose levels within physiological limits, ensuring the metabolic balance required for optimal organismal function. When blood glucose levels rise, for instance after food intake, the pancreas responds by releasing insulin, which facilitates glucose uptake into cells, where it is utilized as the primary source of energy. Insulin deficiency leads to severe imbalances in glucose metabolism. In the absence of insulin, glucose cannot be efficiently used at the cellular level and accumulates in the bloodstream, resulting in hyperglycemia—a hallmark of diabetes mellitus [2]. In animals, this imbalance can be corrected through the administration of synthetic insulin, a therapeutic approach also widely used in human medicine. Beyond its primary role, insulin also antagonizes the effects of hyperglycemic hormones such as cortisol and adrenaline, which promote glucose release into the bloodstream under stress conditions [3]. Thus, insulin plays a critical role in modulating metabolic responses under both physiological and pathological states.

Preliminary results suggest that administration of SEV-3 may contribute to reducing hyperglycemia and improving metabolic dysfunctions associated with alloxan-induced diabetes. These effects may be attributed to the protection of pancreatic β-cells, attenuation of oxidative stress, and enhancement of glucose utilization in peripheral tissues. This mechanism suggests a beneficial role of SEV-3 in carbohydrate metabolism regulation by promoting glucose uptake and inhibiting excessive endogenous glucose production.

## CONCLUSION

Decompensated diabetes mellitus is characterized by a series of metabolic dysfunctions, including insulin resistance, increased hepatic glucose production, disturbances in lipid metabolism, and the development of ketoacidosis. These processes collectively contribute to sustained hyperglycemia and the onset of severe complications associated with the disease. The data obtained support the use of SEV-3 as a phytotherapeutic adjuvant in strategies aimed at preventing and managing hyperglycemia, particularly in the early stages of diabetes. This effect may be attributed to the bioactive compounds present in SEV-3, which play a role in regulating carbohydrate metabolism and maintaining glucose homeostasis. The SEV-3 biopreparation promoted an increase in insulin secretion in experimental diabetes, suggesting a protective effect on pancreatic β-cells. The observed improvements in glycemic and insulin parameters underscore the potential of this biopreparation to ameliorate metabolic dysfunctions associated with diabetes, thereby justifying further investigations to elucidate its mechanisms of action and therapeutic applicability.

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# FROM VALIDATION TO APPLICATION: IMPLEMENTING A COST-EFFECTIVE QPCR ASSAY FOR PRESYMPTOMATIC AND SYMPTOMATIC SMA DIAGNOSIS

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***Abstract.*** *Spinal muscular atrophy (SMA) is a severe neuromuscular disorder caused mainly by homozygous deletion of exon 7 in the SMN1 gene. Early qualitative and quantitative diagnosis, especially in the presymptomatic stage, is essential for timely access to treatment and improved outcomes. This study focused on developing and implementing a sensitive, rapid, and cost-effective real-time PCR-based method for detecting the exon 7 deletion in SMN1. The test was first validated using 25 clinical samples, then applied in a pilot study on 300 dried blood spot samples to assess its use in presymptomatic newborn screening. The method demonstrated high sensitivity and specificity, a fast turnaround time, and suitability for both symptomatic and presymptomatic diagnosis. Compared to commercial alternatives, the custom assay proved more cost-efficient while maintaining diagnostic reliability. Despite challenges such as lack of national protocols, ethical considerations, and logistical constraints, the findings support integrating this method into routine diagnostics. Its implementation highlights the need for structured policies and infrastructure to enable equitable access to genetic screening for rare diseases like SMA.*

***Keywords: cost-effectiveness, custom genetic test, real-time PCR, SMA, SMN1.***

## INTRODUCTION

Spinal muscular atrophy (SMA) is a group of autosomal recessive inherited neuromuscular disorders characterized by progressive degeneration of motor neurons—nerve cells located in the brainstem and spinal cord that control essential skeletal muscle activity, including speaking, walking, breathing, and swallowing. The loss of motor neurons leads to muscle weakness, atrophy and can be fatal.

Two survival motor neuron (SMN) genes are typically present in the 5q13.2 chromosomal region: *SMN1* and *SMN2*. Homozygous deletion of the telomeric copy, *SMN1* (also known as *SMNtel*), is causally linked to the development of SMA (OMIM: 253300). Although rare, with an estimated incidence of 1–6 cases per

10,000 newborns and a prevalence of 8.43 ± 0.15 per 100,000 in the Republic of Moldova, SMA is one of the most common inherited causes of childhood mortality [1][2]. The *SMN1* gene encodes the survival motor neuron protein, essential for maintaining the structure and function of motor neurons. Reduced levels of this protein lead to neuronal dysfunction and progressive muscular atrophy. The nearly identical centromeric copy, *SMN2*, functions as a pseudogene and modifies disease severity in a copy number-dependent manner. However, *SMN2* alone is insufficient to compensate for *SMN1* loss.

The most frequent causative mutation for SMA is the homozygous deletion of exon 7 in *SMN1*, accounting for approximately 95% of cases. Early identification, especially in the presymptomatic stage, is critical for accessing available therapies, as delayed diagnosis significantly decreases survival and treatment efficacy. Consequently, neonatal presymptomatic screening is vital for improving clinical outcomes in SMA [3].

Spinal muscular atrophy (SMA) is classified into four types based on motor function, with treatment now recommended for newborns lacking SMN1. However, the timing of treatment remains debated in cases with ≥4 SMN2 copies [4]. Although individually rare, such diseases collectively impact many and pose diagnostic challenges due to genetic complexity and limited resources. Since the approval of SMA therapies in 2017, early, accurate, and cost-effective genetic testing—especially presymptomatic—has become essential. This is particularly important for newborn screening programs and maternity wards with access to testing or referral systems [5][6].

The aim of this study was to develop and implement a molecular genetic diagnostic method for detecting exon 7 deletion in the SMN1 gene, associated with spinal muscular atrophy (SMA), and to evaluate the development and customization of genetic testing strategies for rare diseases, with a focus on cost-efficiency and clinical applicability.

## MATERIALS AND METHODS

The molecular genetic test described in this study was developed within Human Molecular Genetics Laboratory (HMGL) with the support of institutional research projects, utilizing authorized access to the HMGL biobank of the Institute of Mother and Child (institutional agreement from 18.09.2020).

The patient cohort consisted of individuals suspected clinically of having spinal muscular atrophy (SMA) who subsequently underwent testing for the deletion associated with SMA using qualitative methods such as PCR-RFLP. A total of 25 DNA samples were included for validation of new test based on real-time PCR, of which 15 were from individuals without SMA and 10 from patients with a confirmed diagnosis of SMA, specifically the deletion of exon 7 in the *SMN1*

gene *(Table 1).*

After validation, the test was used in a pilot study aimed at applying it for presymptomatic molecular genetic screening of this disease. The pilot study included 300 dried blood spot (DBS) samples from newborns as a primary biological material.

The cost-effectiveness of the custom-developed test was evaluated by comparison with a commercially available diagnostic test. Parameters assessed included cost per diagnosis, turnaround time, and diagnostic yield. All procedures were conducted in accordance with national and institutional regulations concerning genetic testing and biobank utilization (approval no. 01-13/591 from 18.09.2020, Institute of Mother and Child).

This custom test was also employed within the framework of a doctoral research project focusing on the implementation of newborn genetic screening, approved by the university's ethics committee (approval no. 3 from 16.02.2021).

***Test Principle and Methodology***

The test is based on the 5' nuclease polymerase chain reaction (PCR) technique with real-time detection. It was specifically designed to identify the SMA-causing mutation in the *SMN1* gene using the amplification control gene *ALB*, from both dried blood spot (DBS) samples collected from newborns and EDTA-stabilized blood samples.

The method allows for the detection of the exon 7 deletion in the SMN1 gene—responsible for SMA—through a sensitive and cost-efficient approach using both commercially available reagents (ThermoFisher Scientific, USA) and custom-developed reagents.

Customization of the assay began with the design of specific primers using Primer-BLAST software (https://www.ncbi.nlm.nih.gov/tools/primer-blast/), followed by synthesis by BioAnalitica SRL. The primer design targeted the c.280TTC>TTT (also referenced as c.840TTC>TTT) transition, which differentiates the *SMN1* gene from its nearly identical paralog *SMN2*. This was achieved using a combined qPCR and High-Resolution Melting (HRM) technique, a molecular assay that merges the real-time quantitative amplification power of qPCR with the high sensitivity and accuracy of HRM analysis. This integrated approach offers a robust tool for detecting and characterizing genetic variations, including point mutations, while enabling qualitative and quantitative analysis of target DNA.

The qPCR+HRM method (in HMG Lab.) provides high sensitivity and specificity, allowing for point mutation detection without the need for post-PCR electrophoretic separation or labeled probes. Amplification and melting curve analysis are performed in real-time, allowing for immediate data interpretation and visualization. Real-Time PCR amplification is initiated using the PowerTrack™ SYBR Green Master Mix (Applied Biosystems™, USA) [7][8], which contains an

intercalating dye—SYBR Green—that fluoresces only in the presence of double-stranded DNA, thereby enabling detection of sequence variations. The custom primers designed for exon 7 detection in *SMN1*, the sequence of nucleotides of forward and reverse primers are included in *Innovation Act nr 562, 07 Mart 2024.* The primer design focused on the exon 7 nucleotide transition (C in *SMN1* vs. T in *SMN2*), which represents a synonymous codon change that critically affects exon splicing. This specificity is crucial, given the high sequence homology between *SMN1* and *SMN2* (only five nucleotide differences, with only one affecting transcription). Therefore, precise primer design is essential to minimize the risk of false-negative results due to nonspecific amplification.

To ensure the accuracy and reproducibility of the results, the method was validated using control samples with known SMA status. Validation was performed on the Applied Biosystems 7500 Real-Time PCR System and demonstrated 100% sensitivity and 100% specificity (*Table 1*). The complete protocol has been documented as part of *Innovation Act nr 562, from 07 Mart 2024.*

Table 1. Method validation through custom test

| | | ***SMA*** | | |
|---|---|---|---|---|
| | | **Not affected** | **SMA positive** | **Total** |
| ***Clinical diagnosis*** | **Not affected** | 15 | 0 | 15 |
| | **SMA** | 0 | 10 | 10 |
| | **Total** | 15 | 10 | 25 |

## RESULTS AND DISCUSSIONS

The proposed method was integrated into a molecular-genetic diagnostic process for the early identification of Spinal Muscular Atrophy (SMA) as part of a pilot study conducted at the Institute of Mother and Child Maternity Hospital during the period 2022–2024. Over this period, more than 300 informed consents and dried blood spot (DBS) samples collected on Guthrie cards were processed.

In the first phase, a pre-analytical analysis was performed, targeting the amplification of exons from *SMN1* and *ALB12* genes using real-time PCR with custom-designed primers. Melting curve interpretation was conducted using high-resolution melting (HRM) analysis. The results from this phase identified a lack of amplification of exon 7 in the *SMN1* gene and altered melting curve profiles in two samples.

***Assessment of the Screening Test Sensitivity for Exon 7 Deletion in SMN1***

The custom screening test employed in this study was designed to detect deletions of exon 7 in the *SMN1* gene, the genetic alteration responsible for

approximately 95% of all SMA cases. Among a cohort of 300 newborns screened, two samples tested positive and were subsequently confirmed using the MLPA (Multiplex Ligation-dependent Probe Amplification) method (*Innovation act no. 483 from 17.03.2022)* as a gold standard for diagnosis. This result indicates a 100% detection rate for this specific mutation. Accordingly: Analytical sensitivity of the test (for exon 7 deletion): 100%. Estimated clinical sensitivity (for detection of all SMA forms, including those caused by rare *SMN1* variants): ~95%. This discrepancy reflects the fact that approximately 5% of clinically diagnosed SMA cases are due to other genetic variants (e.g., point mutations, gene conversions) that are not detectable through this type of molecular screening. The method was further validated through comparison with a commercial real-time PCR kit *NeoNat SCID-SMA Multiplex* targeting SMA-associated genes (*SMN1* and *SMN2*) and an internal control gene (*β-Globin*). (Labsystems Diagnostics, ref no. 8100411) (*Table 2*) [9].

Table 2. Comparative results of applying genetic screening tests for SMA using both customized and commercial tests

| No. | No. of probes | Test Type | Gene / Exon Analyzed | Positive / Negative Results | Interpre-ation | Analytical Sensitivity | Clinical Sensitivity | Specificity | Internal Validation |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 300 | Customized HMGL | SMN1 / exon 7 | 2 / 298 | 2 positives for SMA. 298 negatives | 100% | 95% | 100% | ALB exon 12 – 300/300 ✓ |
| **2** | 75 | Commercial (Neo Nat SCID-SMA) | SMN1 / exon 7 | 1 / 74 | 1 positive for SMA. 74 negatives | 100% | 95% | 100% | β-globin – 75/75 ✓ |

***Cost-Effectiveness Evaluation***. As part of the optimization of the neonatal screening protocol for Spinal Muscular Atrophy (SMA), a comparative cost-effectiveness analysis was performed between a custom laboratory-developed method and a commercial multiplex assay designed for the detection of exon 7 deletions in the *SMN1* gene.

The custom method (HMGL) is based on SYBR Green intercalating dye in Real-Time PCR, comprising two reactions per sample for the target gene (*SMN1*) and one for the internal control gene (*ALB*), enabling analysis of 32 samples per 96-well plate. DNA is extracted from DBS samples or blood on EDTA, and the entire workflow (extraction, amplification, and interpretation) is completed within approximately 4 hours. Both the sensitivity and specificity of this method are 100%.

In contrast, the NeoNat SCID-SMA Multiplex Real-Time PCR kit is a comprehensive commercial assay that includes all necessary reagents (including for DNA extraction) and allows for multiplex detection of mutations (*Table 3*).

Table 3. Comparative table – Cost per round (32 samples)

| Parameter | Custom Test (HMGL) | Commercial Test (NeoNat SCID-SMA) |
|---|---|---|
| **Total reactions/round** | 96 reactions (32 samples in duplicate + controls) | 53 reactions (32 samples + controls) |
| **Total cost/round (MDL)** | ~3052 | ~7485 |
| **Cost/sample (MDL)** | ~95.3 | ~233.9 |
| **Sensitivity/ Specificity** | 100% / 100% | 100% / 100% |
| **Platform used** | 7500 Applied Biosystems | 7500 Applied Biosystems |

However, each kit is optimized for 75 clinical samples, and partial usage for a smaller batch of 32 samples results in significantly higher per-sample costs. A financial analysis of a test round involving 32 clinical samples revealed a substantial cost advantage in favor of the custom method.

Implementing a molecular diagnostic test for *SMN1* exon 7 deletion in neonatal screening revealed critical challenges, including lack of national protocols, ethical concerns, logistical barriers, and limited funding. Despite sharing infrastructure with the national PKU screening program, SMA testing required additional consent procedures, data handling strategies, and specialized communication of results. These limitations highlight the need for cost-effective, ethically sound, and scalable testing strategies for rare genetic diseases.

## CONCLUSIONS

The implementation of a customized qPCR-HRM method for detecting SMN1 exon 7 deletion significantly improved diagnostic accuracy, speed, and applicability for SMA. This approach offers high sensitivity and specificity, rapid turnaround, and utility in both presymptomatic and symptomatic cases, including newborns. Its successful use highlights the need for supportive policies, ethical oversight, and infrastructure to ensure equitable access to genetic screening for rare diseases like SMA. Integration into routine diagnostics is strongly recommended, especially for early detection and carrier confirmation.

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# GENETIC MAPPING OF HUMAN DISEASES: A QUANTITATIVE AND FUNCTIONAL PERSPECTIVE ON GENE–PATHOLOGY RELATIONSHIPS

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**Abstract.** *The classification of human diseases based on genetic contribution has significantly evolved with advancements in genetic research. This study explores the chromosomal distribution of disease-associated genes from a quantitative and functional perspective, aiming to identify genomic regions with a high density of pathogenic associations—so-called genetic hotspots. We integrate data from genome-wide association studies (GWAS) to evaluate genetic overlap among major disease categories, elucidating molecular connections between seemingly unrelated pathologies. The findings highlight the significance of genetic architecture in disease manifestation, offering valuable insights for precision medicine, genetic diagnostics, and targeted therapies.*

***Keywords: genetic mapping, genetic hotspots, gene-pathology relationships, quantitative genetics, genetic overlap.***

## INTRODUCTION

The advancement of genetic research has enabled the classification of human diseases based on the extent and manner in which genetic factors contribute to their onset and progression. Two major perspectives have thus emerged: *qualitative genetics*, which focuses on monogenic diseases, and *quantitative genetics*, which addresses multifactorial or polygenic diseases. Both branches offer critical insights for diagnosis, treatment, and prevention.

*Qualitative genetics* investigates diseases caused by mutations in a single gene or by major chromosomal abnormalities. These are conditions characterized by clear and detectable genetic alterations. Such diseases often follow well-defined Mendelian inheritance patterns—autosomal dominant, autosomal recessive, or X-linked. Classic examples include cystic fibrosis, Duchenne muscular dystrophy, and sickle cell anemia, caused by mutations in the *CFTR* (Cystic Fibrosis Transmembrane Conductance Regulator) gene. This mutation disrupts chloride ion

transport in epithelial tissues, leading to the accumulation of thick mucus in the lungs and digestive tract (Riordan et al., 1989). As an autosomal recessive condition, both parents must be carriers of the mutation for the disease to manifest in their offspring. Similarly, Huntington's disease follows an autosomal dominant inheritance pattern, being caused by a CAG trinucleotide repeat expansion in the *HTT* gene (MacDonald et al., 1993). In such cases, the genotype-phenotype correlation is typically clear and predictable: a specific mutation is strongly associated with disease expression. Monogenic diseases are often severe and have early onset, but their predictable inheritance allows for genetic counseling and preventive testing.

In contrast, most common adult-onset disorders are influenced by numerous genes, each exerting a modest effect. In combination with environmental factors, these genetic contributions shape disease risk. These complex diseases include type 2 diabetes, cardiovascular diseases, obesity, and neuropsychiatric disorders. A typical example is type 2 diabetes, for which over 400 genetic variants have been associated (Mahajan et al., 2018). Genome-wide association studies (GWAS) have identified numerous genetic variants implicated in such diseases, each contributing only marginally to overall risk (Visscher et al., 2017). For these conditions, genetic research employs advanced statistical models, including polygenic risk scores (PRS), to evaluate individual susceptibility (Chatterjee et al., 2016).

Some conditions lie at the intersection of qualitative and quantitative genetics. For instance, hereditary breast cancer associated with BRCA1 and BRCA2 mutations has a clearly defined genetic basis (qualitative), yet the actual cancer risk is modulated by other genetic and environmental factors (quantitative). This overlap illustrates that while the distinction between qualitative and quantitative genetics is pedagogically useful, biological reality is far more nuanced (Mavaddat et al., 2019)

The distinction between these two branches of genetics has direct implications for precision medicine. In monogenic disorders, genetic testing can confirm the diagnosis and guide treatment—as seen in the case of gene therapies for spinal muscular atrophy (Mendell et al., 2017). Conversely, for multifactorial diseases, genetics mainly aids in risk stratification and prevention.

In the era of genomic medicine, understanding the genetic basis of disease is critical for the development of personalized treatments and preventive strategies. Identifying genes associated with various categories of disease offers important insights into the molecular mechanisms underlying these conditions.

This study aims to analyze, from a quantitative and functional perspective, the chromosomal distribution of disease-associated genes, and to identify genomic regions with a high density of pathogenic associations—so-called "genetic hotspots." Furthermore, we assess the degree of genetic overlap among major disease categories, with the goal of identifying molecular connections between

seemingly unrelated pathologies.

## MATERIALS AND METHODS

The current investigations utilized publicly available genomic databases and curated disease-gene association datasets to analyze the chromosomal distribution of disease-associated genes. Statistical and bioinformatic tools were employed to identify genomic hotspots with high densities of pathogenic associations and to assess genetic overlap between major disease categories.

## RESULTS AND DISCUSSION

By combining quantitative data on the number of implicated genes with a comparative analysis across different disease categories, we aimed to obtain an integrative view of the genetic complexity of human diseases. The results obtained can contribute to prioritizing research directions, optimizing genetic testing, and developing targeted therapies based on individual genomic profiles.

The bioinformatic analysis ***of gene distribution associated with various disease categories*** provided a comprehensive overview of the genetic landscape of human pathology. The data summarized in Table 1 reveal not only the extent of current knowledge in medical genetics but also the priority directions for research and intervention in precision medicine. Behind the numbers lie not only real biological differences but also echoes of the evolution of genetic technologies, funding priorities, and the intensity of scientific investigations.

Among all analyzed disease categories, nervous system and sensory organ diseases (NSD) stand out with the highest number of identified genes – 11,862. This impressive figure reflects both the structural and functional complexity of the central nervous system and the diversity of conditions included under this category, ranging from neurodegenerative diseases such as Alzheimer's or Parkinson's to neurological developmental disorders or sensory perception disorders. The pathogenic complexity of these conditions often translates into significant genetic heterogeneity, which justifies the scale of research in the field.

In second place, with 8366 associated genes, are mental and behavioral disorders (MaBD). This finding supports the idea of a consistent genetic contribution to the etiology of these diseases, often considered multifactorial and biologically difficult to define. The presence of an extensive genetic basis offers hope for the development of more effective methods for differential diagnosis and personalized treatment.

Digestive diseases (DSD) and musculoskeletal diseases (MSD) follow, with 7260 and 6688 identified genes respectively. The multiple involvements of these categories in various metabolic and inflammatory processes, as well as the variety

of organs and tissues affected, partially explain the associated genetic volume. At the same time, these figures reflect increased research interest, with direct implications for available treatments.

At the opposite end, infectious and parasitic diseases (IaPd) show the lowest number of associated genes – 3369. This lower value aligns with the nature of these diseases, where an external pathogenic agent plays a dominant role, and the host's genetic predispositions have a relatively secondary impact. However, the number is not negligible and suggests the existence of genetic susceptibility factors, especially in the context of immune response and systemic complications.

Beyond their descriptive value, these results have significant medical implications. Disease categories with a large number of identified genes emerge as promising targets for innovative therapies – including gene therapy, targeted molecular interventions, or predictive genetic testing. For example, treatment strategies in neurological and psychiatric disorders could be shaped based on individual genetic profiles. For diseases characterized by heterogeneous etiology – such as NSD or MaBD – the identification and cataloging of involved genes contribute to the definition of molecular subtypes and, implicitly, to the development of a more precise diagnostic and clinical classification framework. The gene distribution also reflects current scientific investments. Diseases such as Alzheimer's, cancer, or depression have received particular attention in genomic research projects, which has led to better genetic characterization and rapid data accumulation.

Table 1. Identified Genes in the GWAS Catalog Database for Various Disease Categories. *https://www.ebi.ac.uk/gwas/home (21.03.2025)*

| **Disease Category** | **Abbreviation** | **Number of Identified Genes** |
|---|---|---|
| Diseases of the circulatory system | CVD | 5,687 |
| Diseases of the respiratory system | RSD | 3,650 |
| Endocrine, nutritional, and metabolic diseases | ESD | 5,512 |
| Diseases of the digestive system | DSD | 7,260 |
| Diseases of the nervous system and sensory organs | NSD | 11,862 |
| Diseases of the musculoskeletal system and connective tissue | MSD | 6,688 |
| Mental and behavioral disorders | MaBD | 8,366 |
| Infectious and parasitic diseases | IaPd | 3,369 |
| Neoplasms (tumors) | Neo | 6,479 |

This quantitative perspective thus provides a solid starting point for the in-depth exploration of the molecular mechanisms involved in pathology and for

guiding research toward areas with the greatest potential for clinical impact. In addition to the quantitative analysis of genes involved in different disease categories, a further functional perspective can be obtained by evaluating ***their chromosomal distribution***. In this context, understanding chromosomal distribution and genetic overlaps has not only theoretical value but also direct implications in personalized medicine. Knowing the chromosomal location can aid in the development of targeted gene therapies, with minimal risk of side effects by avoiding damage to adjacent regions.

Based on the absolute number of genes per chromosome (Figure 1), the results highlight the intensity of genetic associations and reveal chromosomes 6 (5484 genes), 1 (4934 genes), 2 (4780 genes), 5 (3430 genes), and again 1 (3275 genes) as genomic regions with higher pathological loads — genetic "hotspots" for specific diseases or pathology in general.

Chromosome 6, ranking first, is already known for its region of highest genetic density within the immune system — the Major Histocompatibility Complex (MHC). This region plays a central role in antigen recognition and autoimmunity, justifying its constant presence in the pathogenesis of infectious, autoimmune, and even mental illnesses, where chronic inflammation is an emerging pathogenic mechanism. Conversely, chromosomes 13, 18, 21, and Y have the lowest number of genes associated with diseases. This can be explained by their smaller size and limited genetic content or low density of functional genes. For chromosome Y, its male-specific genetic content has lower relevance for common non-sex-linked diseases.

Although numerically less active, the X chromosome is critical in genetic pathology, especially in sex-linked diseases (e.g., hemophilia, Duchenne muscular dystrophy), due to its unique transmission and random inactivation in females.

The chromosomal distribution of disease-associated genes (Table 2) highlighted a disproportionate concentration on certain chromosomes — particularly those of larger size or containing gene-rich regions involved in nervous system development, metabolism, or immune regulation. For example, chromosomes 1, 6, 11, and X host a significant number of genes associated with neurological diseases, psychiatric disorders, and autoimmune conditions. These findings support the idea of genetic vulnerability “hotspots”.

For each group of conditions, certain chromosomes stand out as "genetic hotspots". For example, in CVD, chromosomes 1 (513 genes), 6 (454 genes), and 2 (445 genes) are predominant. These chromosomes encompass key genes involved in blood pressure regulation, lipid metabolism, and inflammatory response. Respiratory diseases are primarily associated with chromosome 6 (418 genes), reinforcing the hypothesis of an immunogenetic basis in these conditions, followed by chromosomes 1, 2, and 5. Endocrine and metabolic diseases (ESD) are linked to genes located on chromosome 6 (570 genes), followed by 1 (461 genes) and 2 (414

genes), reflecting the genetic importance in diabetes, thyroid disorders, and obesity. Neurological and sensory diseases are determined by genes with closely related values (~1000 genes each), located on chromosomes 1, 2, and 6, supporting the complexity of genetic networks involved in central nervous system function. Neoplasms are determined by oncogenes and tumor suppressor genes with a wide distribution across chromosomes 6 (611 genes), 2 (549 genes) and 1 (510 genes).

Table 2. Chromosomal Breakdown for Each Disease Group

| Chromo -some ID | CVD | RSD | ESD | DSD | NSD | MSD | MaBD | IaPd | Neo | Total of affected genes |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 513 | 305 | 461 | 598 | 1023 | 561 | 680 | 283 | 510 | 4934 |
| 2 | 445 | 294 | 414 | 562 | 1018 | 524 | 743 | 231 | 549 | 4780 |
| 3 | 341 | 213 | 317 | 422 | 734 | 398 | 542 | 224 | 426 | 3617 |
| 4 | 310 | 178 | 310 | 357 | 628 | 379 | 435 | 199 | 302 | 3098 |
| 5 | 328 | 239 | 292 | 421 | 712 | 360 | 506 | 182 | 390 | 3430 |
| 6 | 454 | 418 | 570 | 659 | 1011 | 706 | 737 | 318 | 611 | 5484 |
| 7 | 284 | 182 | 303 | 397 | 615 | 335 | 437 | 179 | 293 | 3025 |
| 8 | 230 | 162 | 226 | 313 | 531 | 297 | 383 | 156 | 336 | 2634 |
| 9 | 189 | 170 | 212 | 279 | 444 | 292 | 304 | 134 | 311 | 2335 |
| 10 | 283 | 164 | 268 | 383 | 521 | 368 | 347 | 145 | 308 | 2787 |
| 11 | 364 | 186 | 317 | 423 | 648 | 380 | 468 | 162 | 327 | 3275 |
| 12 | 314 | 181 | 286 | 369 | 608 | 327 | 433 | 163 | 284 | 2965 |
| 13 | 133 | 83 | 144 | 201 | 336 | 179 | 238 | 96 | 167 | 1577 |
| 14 | 175 | 96 | 142 | 196 | 374 | 174 | 260 | 91 | 190 | 1698 |
| 15 | 181 | 146 | 161 | 217 | 376 | 184 | 269 | 99 | 199 | 1832 |
| 16 | 168 | 97 | 192 | 257 | 400 | 246 | 275 | 101 | 220 | 1956 |
| 17 | 270 | 156 | 225 | 286 | 456 | 240 | 309 | 116 | 238 | 2296 |
| 18 | 106 | 70 | 111 | 151 | 259 | 133 | 168 | 84 | 149 | 1231 |
| 19 | 207 | 105 | 162 | 254 | 404 | 171 | 296 | 114 | 195 | 1908 |
| 20 | 140 | 72 | 147 | 215 | 296 | 155 | 206 | 83 | 217 | 1531 |
| 21 | 64 | 36 | 52 | 78 | 150 | 84 | 104 | 54 | 76 | 698 |
| 22 | 97 | 55 | 100 | 138 | 205 | 119 | 136 | 57 | 125 | 1032 |
| X | 37 | 38 | 77 | 84 | 107 | 74 | 83 | 93 | 56 | 649 |
| Y | 0 | 4 | 0 | 0 | 6 | 1 | 6 | 4 | 0 | 21 |

Thus, understanding the cytogenetic topography of pathologies can be applied in targeted research, personalized medicine and in diagnosis and prevention. Chromosomal distribution can guide the development of sequencing panels focused on the "hot regions" of the genome. Integrating this knowledge into clinical practice will lead to faster diagnoses, targeted therapies, and effective prevention.

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# GENERATION OF PHOTONIC MOLECULAR COMPLEXES UNDER THE ACTION OF ULTRAVIOLET LIGHT IN THE DNA/RNA INACTIVATION PROCESS

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**Abstract**. A semi-classical model (classical by the field and quantum by describing biomolecules) is proposed, which establishes the dependence of the DNA/RNA dimerization rate on ultraviolet C (UVC) radiation intensity. In particular, a nonlinear model based on cooperative processes similar to the Raman-like in quantum optics is developed. The main result of the theory demonstrates that the dimerization process in DNA/RNA strongly depends on the UVC light intensity, thus proving a possible quantum microscopic mechanism of UVC light interactions with DNA. A notion of a photonic molecule consisting of the normal state and the dimer state under the action of the bimodal Raman field is given. This type of molecule exists only in the presence of the excited field, and after it is turned off, the biomolecule remains part of the time in the entangled state after which DNA dimerization is probabilistically established. A nonlinear dependence on the intensity of the applied radiation was established, which was confirmed in a series of experiments on the inactivation of pathogens (or yeast colonies) reproduction under the action of ultraviolet radiation. The main objective of this study was to establish a link between the intensity of UV C radiation and the efficiency of decontamination using metamaterials with an increased contact surface with contaminated fluid by pathogens, that cause nosocomial infections, namely Escherichia coli, Pseudomonas aeruginosa, Candida albicans, and Candida glabrata. These microorganisms were put through different forms of packing, both thick fibers repacked with thin fibers and millimeter balls repacked with sub-millimeter quartz balls. The results demonstrate that bacterial pathogens respond differently to exposure to UV-C radiation, depending on the contact surface created between the elements of the quasi-periodic metamaterials.

***Keywords: raman conversion, cooperative dimerization, critical intensity for dimerization, metamaterials in inactivation, photonic biomolecules.***

## INTRODUCTION

The possibility of creating localized states linked between cellular partners was an idea proposed by Kasha and is actually the basis of the formation of some supracellular structures in which the excited state is relatively stable in time and the electronic subsystem does not recombine in time. Such supracellular patterns could arise upon the

cooperative absorption of photons in the multiple Raman scattering process. This model assumes that between the normal state and the excited state of the bipolar molecules it is possible to form a localized state of the electrons of both molecules so that the system has a relatively long lifetime. If we talk about a system of such bipolar molecules upon excitation, they can enter into a bipolar bond that could lead to the formation of relatively large molecules. A model of multiple excitation of molecular states and the formation of molecular clusters in induced Raman scattering is proposed in this chapter. The model is reduced to the multiple steps of absorption and emission of photons in the scattering process. The model could be used in photosynthesize using natural and artificial photoreceptors, mostly of microbial origin, to be genetically introduced into the cells of interest. For the multiple Raman effect to have an increased efficiency, optical cavities are needed. In biological systems, such optical cavities are observed. For example, in photosynthesis, both the chloroplast membrane and its internal elements are used as a resonator. The chloroplast is a structure in the cells of plants and green algae that is the site of photosynthesis, the process by which light energy is converted into chemical energy, resulting in the production of oxygen and energy-rich organic compounds. With the introduction of photosensitive opsin/rhodopsin it was observed that cells that are naturally insensitive to light can be made photosensitive and addressable by illumination and can be precisely controlled in time and space. If we look at some proteins as radiation accumulation resonators, then possibilities of selective Raman directing when introducing such proteins into the cell can take place at the subcellular level. In other words, the cellular subsystems are activated by applying control elements such as promoters, enhancers and specific targeting sequences to the DNA/proteins encoding the photoreceptor used. This powerful approach enables precise characterization and manipulation of cellular functions and has motivated the development of advanced optical methods for patterned photostimulation.

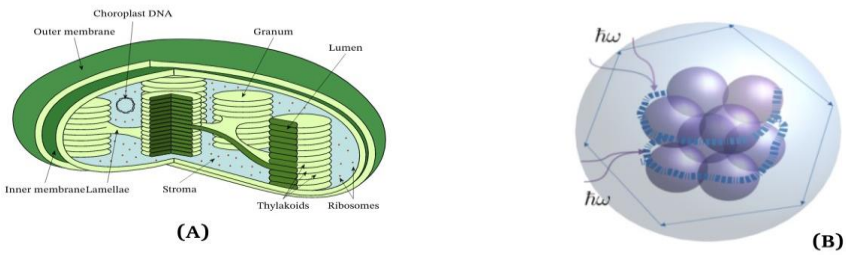


Figure 1. (A) Observation of disc-shaped optical microresonators (photore- ceptors) in chloroplasts in the process of storing and using radiation in photosynthesis. (B) Observation of spherical Hystone elements in the DNA packing [1, 2, 3, 4, 5].

As follows from the literature [1, 2] a nucleosome is the basic structural unit of DNA packaging in eukaryotes and may serves not only support for DNA cain

but the possible electro- magnetic contact through the evanescent zone of the elements of this repached structure in nucleus with dimension about 5- 10 micrometers. The structure of a nucleosome consists of a segment of DNA wound around eight histone proteins [1, 2] and resembles thread wrapped around a spool. The nucleosome is the fundamental subunit of chromatin. Each nucleosome is composed of a little less than two turns of DNA wrapped around a set of eight proteins called histones, which are known as a histone octamer. Each histone octamer is composed of two copies each of the histone proteins H2A, H2B, H3, and H4 (see Ref. [3, 4, 5]). Due to the fact that the refractive index of histone elements is higer than the water one it is posible to brelize the standung or guide waves in such a system like in the traditional optical emements Ref.

In ADN modification, the histones are highly basic proteins abundant in lysine and arginine residues that are found in eukaryotic cell nuclei and play an imortant role in the localization of vibration waves as this take place in microspherical resonators (see Fig. 1) B. They act as spools around which DNA winds to create structural units called nucleosomes [3, 4]. Nucleosomes in turn are wrapped into 30-nanometer fibers that form tightly packed chromatin. Histones prevent DNA from becoming tangled and protect it from DNA damage. In addition, histones play important roles in gene regulation and DNA replication. Without histones, unwound DNA in chromosomes would be very long. For example, each human cell has about 1.8 meters of DNA if completely stretched out; however, when wound about histones, this length is reduced to about 90 micrometers (0.09 mm) of 30 nm diameter chromatin fibers [5]. In other words, the subsystems of the cell nucleus in eukaryotes are activated by applying control elements, such as promoters, enhancers and specific targeting sequences, to the DNA/proteins that encode the photoreceptor used. This powerful approach enables precise characterization and manipulation of cellular functions and has motivated the development of advanced optical methods for patterned photostimulation. A huge catalogue of histone modifications have been described, but a functional understanding of most is still lacking. Collectively, it is thought that histone modifications may underlie a histone code, whereby combinations of histone modifications have specific meanings. However, most functional data concerns individual prominent histone modifications that are biochemically amenable to detailed study.

## Modification of Biomolecular Structural Changes Under UVC Radiation

Experimental results: analyses of dynamical and static regime The core of the equipment proposed in the Refs. [6, 7, 8] was adapted to the new conception of the repacking of studied metamaterial small quartz balls/fibers between the big one. Taking into consideration that numerous pathogens are more sensitive to UVC radiation than eukaryotic cellular structures, we had to substitute these

contaminated dangerous fluids with yeast solution, the DNA of which has larger resistance to UVC radiation in comparison with many viruses or bacteria. In comparison with Ref.[7], where the yeast solution was inactivated after 10 − 15 min, in this investigation the fungal solution was decontaminated after 24 hours of the preparation moment. We suggested inactivating the yeast fungus as a function of exposition time and the applied intensity of UVC radiation. The inactivation ate this yeast fungal solution depends on the tunnelling effect between normal and dimerized DNA states described in the Ref [6]. The measurements were done after 10 and 20 min of inactivation in the dynamic regime (see Figs. 2).

The consecutive action of 6 lamps on the yeast solution during 10 and 20 minutes time duration: Figures (a) represent the preparation of yeast fungal solution before going through the decontamination system for 6 experiments, which contain from one to 6 active UVC lamps; Figures (b) describe the yeast fungus after it has gone through decontamination system about 10 min for 6 experiments in which the lamps were switched off from one to 6 lamps consecutively. Figures (c) correspond to 20 min inactivation time in the same consecutive application of external intensity of UVC sources. This gives us the possibility to find the dependence of the decontamination rate as the function of the intensity of applied UVC radiation in the lamp units. The mediation in the number and dimensions of colonies observed in the 20 experiments, in which of it was observed about 750-800 fungal colonies per figure. Our decontamination core with 0.8 meters length and 2.5 cm in diameter are filled up with a diameter of spheres about 0.5 − 1 mm of the quartz material. The core is covered by 6 mercurial lamps, which a maximum of radiation is 250 − 260 nm. To improve the efficiency of UVCradiation, all the system is placed in an aluminum cylinder with a diameter of about 20 cm so that the intensity of the radiation in the center of the big cylinder increase significantly due to the reflection proprieties of the aluminum. More than this, the researchers and people in the room are well protected from the direct action of UVC radiation obtained from 6 lamps represented in the Figs. 2.

The initial prepared yeast solution. These figures were obtained by multiple testes by the microscope after 24 hours of the prepared yeast solution. We have prepared the yeast solution, dissolving 25 g of fungal yeast into 800 g of water. In order to improve the development of fungi, we add 20 g of agar, C14H24O9. At the initial stage of the development of fungi, we observed the appearance of various numbers of yeast fungus colonies with aleatory dimensions. Such an experiment with the inactivation of fungal colonies in the earlier time development interval of about 15 min (See Refs [6, 7, 8]).

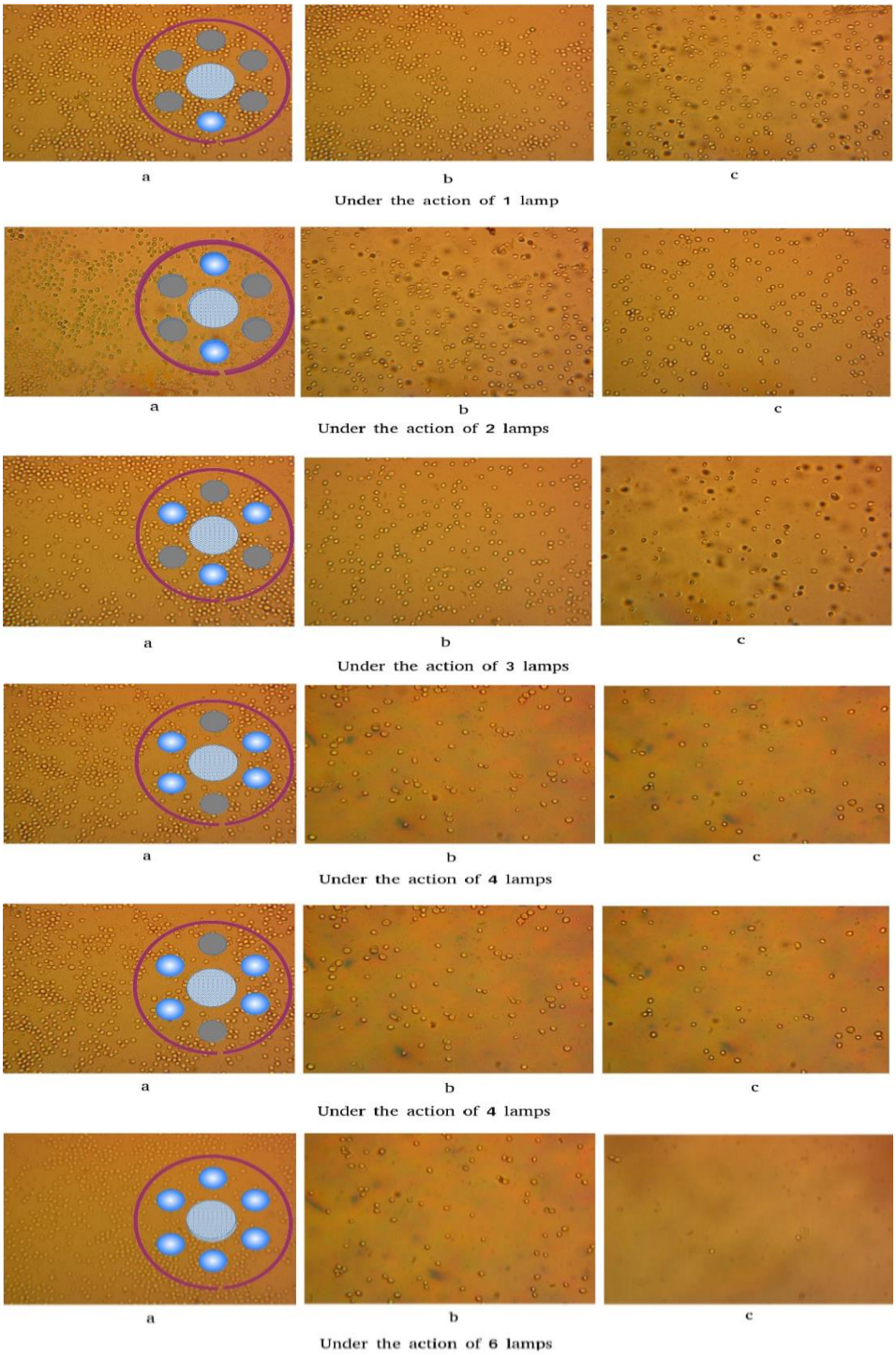


Figure 2. Dynamic regime of decontamination*.

*Figure 2. Dynamic regime of decontamination. *The consecutive action of 6 lamps on the yeast solution during 10 and 20 minutes time duration: Figures (a) represent the preparation of yeast fungal solution before going through the decontamination system for 6 experiments, which contain from one to 6 active UVC lamps; Figures (b) describe the yeast fungus after it has gone through decontamination system about 10 min for 6 experiments in which the lamps were switched off from one to 6 lamps consecutively. Figures (c) correspond to 20 min inactivation time in the same consecutive application of external intensity of UVC sources. This gives us the possibility to find the dependence of the decontamination rate as the function of the intensity of applied UVC radiation in the lamp units. The mediation in the number and dimensions of colonies observed in the 20 experiments, in which of it was observed about 750 - 800 fungal colonies per figure.*

As the development of fungal colonies have some quick developments and some parameters slightly fluctuate in time, we decided to put the inactivation experiments after 24 hours before going through the decontamination equipment. In this experiment, we take into consideration the acceleration of fungal colonies and the intensity dependence of the inactivation rate of yeast fungi by consecutive adding from one to six UVC lamps inside the yeast decontamination core represented in Fig.2.

The first experimental mechanism is connected to improving the contact surface between UVC radiation and contaminated fluid. As we know, the direct radiation on the open surface of non-transparent fluid can not give us the expected result in this area due to the small penetration distance of applied UVC radiation. If we introduce into translucent infected fluid elements of connected quarts metamaterials consisting of optical fibers/spherical elements, we can observe that the total contact surface consists of the surface of each element multiplied by their number. This effect gives us increased decontamination volume, which may be estimated by multiplying the total contact surface of metamaterial elements multiplied by the penetration deep of UVC radiation into the fluid.

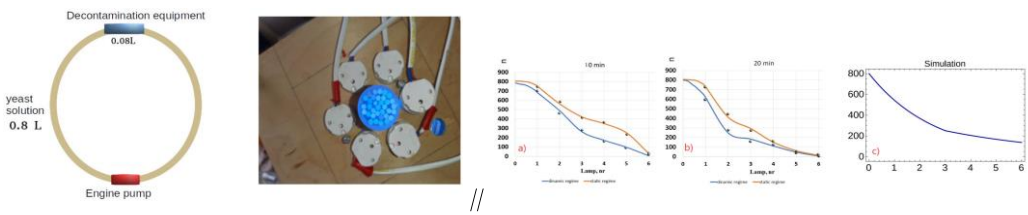


Figure 3. Decontamination speed obtained with left-hand equipment according to the applied intensity of UVC radiation in units of number of lamps:
*a) 10 Min dynamic and static regimes; b) 20 Min dynamic and static regimes; c) The dependence of the inactivation rate according to the intensity applied typically in accordance with the proposed theoretical model.*

The construction and non-uniform arrangement of optical elements of quasi periodic structures create rotation canals for pathogens or infected aerosols from

the fluid. So, during the rotation, as a function of flow velocity, the infected quasi particles or pathogens suffer deposition on the evanescent zone of each element of metamaterial penetrated by UVC radiation, due to the fact that their density is larger than the fluid one. The last acceleration mechanism depends on the fluid velocity stimulated by the pump engine. This pump mechanism generates the rotation of pathogens/droplets around elements of the metamaterial inducing an inertial force to them so that these particles are deposed on the surface of each element of the metamaterial, where the intensity of UVC radiation is significantly larger than between the elements. On the basis of these two effects in our laboratory was elaborate two equipment for the decontamination of infected liquids and gases (see Figs. 3 and ). Experiments have conclusively demonstrated that both quartz spheres and optical fiber metamaterials can effectively annihilate Coliform (including Escherichia coli), or Enterococcus bacteria, as well as yeast and Kombucha cultures.

## Physical Model of Nonlinear Photo-transformation and Dimerization of Biomolecules

The Raman model demonstrates that the dimerization rate of thymine bases (T = T) increases exponentially with the intensity of UVC radiation, suggesting the involvement of a quantum mechanism in dimer formation. This process has been experimentally confirmed through resonant fluorescence techniques, which highlight the disruption of hydrogen bonds and associated structural transitions.

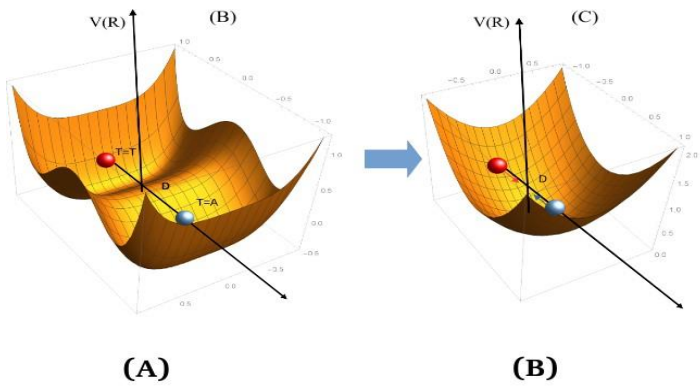


Figure 4. The transition from double well potential
*(A) to single well potential (B) when the UVC field strength increases to a critical value $I_c$.*

For the description of the intensity dependence of the modification of the DNA structure under the action of UVC radiation, we propose the Born-Oppenheimer (BO) frequently used in chemistry and microbiology. In this approach, the nuclei subsystem is characterized by the vectors, R1, R2...Rn , and is

considered a slow subsystem compared to the electronic one, r = {r1,r2...rn}. This allows us to represent the molecular wave function by the product of the wave functions, |Ψe(r,R)⟩|χ(R)⟩, of the subsystems of electrons, |Ψe(r,R)⟩, and the subsystem of nuclei, |χ(R)⟩, of the respective molecules: . The modification of DNA and RNA structures takes place under the action of UVC radiation, as schematically represented in Fig.2 A by modification of covalent bonds, and in figures 2 A,B by changes in interaction potential. Considering that the nuclei positions are fixed for electronic subsystem the Schrodinger equation can be represented in the form He(r,Rs)|Ψe(r,Rs)⟩ = Ee(Rs)|Ψe(r,Rs)⟩. Obviously, the electronic energy eigenvalue Ee depends on the chosen positions R of the nuclei. Varying these positions R in small steps and repeatedly solving the electronic Schrodinger equation, one obtains Ee(Rs) as a function of Rs. We start with DNA dimer modification of the near nucleotide bonds under the action of electromagnetic field. The interaction energy of molecular electrical multi-poles may be approximated with the work of the electrical field during the displacements of electron at distance rj , HI = − ejU(rj), neglecting the magnetic part of the field, where U = rj 0 (d⃗r, ⃗ E(⃗r)) [19].

For the construction of the possible molecular states till and after the action of UVC radiation we propose to represent the covalent bounds of the two steps of nucleobases in DNA [20, 17]. In the formation of covalent bonds, electron orbitals overlap in order to form molecular orbitals, which give us the simplest prototype of molecules with covalent bonds observed in the H2 molecule in the archetypal Lewis pair |Ψ(H2;ζ,R)⟩ = [2(1 + Sab)]−1/2[|φa(1)⟩|φb(2)⟩+|φb(1)⟩|φa(2)⟩],where we involve the overlap integral Sab , defined as, Sab = φa(r)φb(r)dr, is dependent on the orbital exponent, ζ ,and the inter-nuclear separation R . It is considered that in covalent bound take place two electrons a and b.

$$
\begin{aligned}
H^{(n)} = & -\frac{\hbar^2}{2M}\Delta_R \\
& + \frac{M\Omega_u^2(R-Q_0)^2 + M\Omega_d^2(R-D_0-\Theta_0)^2}{2} \\
& - \frac{2(I\alpha)^2 + 2(I\beta)^2}{M\Omega^2} + \Delta V(R) + \mathcal{E}_0(R_0).
\end{aligned}
$$

So, one may conclude that as a function of intensity of the applied field, the potentials can overlap each other. This effect we name the formation of one photon molecula under the action of external coherent field. We point out that in Fig. 4 the first potential corresponds to the normal state of thymine and the second one corresponds to the dimer state and as function of intensity these two states can collapse in one. In the absence of the UVC these states are separated (D0 > |Q|). According to this situation, the thymine nuclei rings are in one of the Fock states, |n⟩u or |n⟩d. The first eigenvector corresponds to the solution of the oscillator

equation, $( - \bar{h}2\Delta Q/(2M) + M\Omega 2Q2/2)|n\rangle u = \bar{h}\Omega u(n + 0.5)|n\rangle u$, and the second corresponds to possible pyrimidine dimerization of DNA/RNA after the action of the electromagnetic field, $(-\bar{h}2\Delta\Theta/(2M)+M\Omega 2\Theta 2/2)|n\rangle d = \bar{h}\Omega d(n+0.5)|n\rangle d$. Now, there could appear a question like: For which intensity of the applied field these two states may become mixed? Looking to the Hamiltonian (1), we observe that in the harmonic approximation two oscillation points may coincide, i.e. $R - Q0 = R - D0 - \Theta 0$. This corresponds to the situation in which $D0 = Q0 - \Theta 0$, see the overlap of two minima in the double-well potential, represented in Fig. 4. In this case the field may act on the carbon rings positions of two adjacent thymine nucleotides (or uracil in RNA strand) in anti-phase, so that the oscillation positions of both protonated bonds between two pyrimidines in the normal and dimer states coincide. In this special case, we also simplify the nuclei Hamiltonian (1), reducing it to the harmonic form $\hat{} H(n) = -\bar{h}\Delta 2 R/(2M) + (M\Omega 2 u + M\Omega 2 d)(R - Q0)2/2$. The stationary solution of this entangled state of the two bonds is found from the Schrodinger equation, $\hat{} H(n)|n\rangle = \bar{h}\,\Omega 2 u + \Omega 2 d(n + 1/2)|n\rangle$. After the decoupling of the external UVC field, this common state is decoupled at distance, D0, and becomes proportional to the sum of the two states, $|n\rangle \sim c1|n\rangle d + c2|n\rangle u$, where c2 1+c2 2 = 1. This process corresponds to the dimerization of DNA/RNA nucleotides. The main problem corresponds to the estimation of the critical intensity of UVC field, $Ic = D0/(ku - kd)$, from which follows that $ku - kd > 0$, and this is possible when the both expressions, $\partial\Pi(0)/\partial Q$ and $\partial\Pi(0)/\partial\Theta$, have opposite signs. With the decreasing of the intensity of the applied field the value of $\Theta 0(I)$ slightly differs from $Q0(I)$ when considered in the definition of $D0 = \Theta 0(Ic) - Q0(Ic)$. According to the perturbation theory, in this case the coordinates of these oscillators may be replaced by the expressions:

$$R - Q0(Ic) + \Delta Q0(I) \text{ and } R - D0 + \Theta 0(Ic) + \Delta\Theta 0(I), \text{where } \Delta Q0(I) = 2(Ic - I)\alpha/(M\Omega 2\, u),\ \Delta\Theta 0(I) = 2(Ic - I)\beta/(M\Omega 2\, d).$$

As result, the perturbed potential shows the attenuation of the tunneling effect with the decreasing of the intensity of the applied UVC field.

## DISCUSSION AND CONCLUSIONS

We conclude that by increasing the intensity of the applied radiation in a fixed time interval, the number of yeast colonies decreases nonlinearly up to a given intensity and after, a slow linear decreasing prevails. This result confirms the theoretical prediction. Particularly, by taking into consideration the conclusions of the perturbation theory, the intensity dependence of the overlapping of the wave functions in the double-well potential can be applied to this experiment. We may consider the efficiency of the tunneling effect between normal and dimerized DNA states proportional to the shifts defined in the theory as $\Delta Q0(I) = 2(Ic - I)\alpha/(M\Omega 2\, u)$ and $\Delta\Theta 0(I) = 2(Ic - I)\beta/(M\Omega 2\, d)$, so the effect correlates the experimental result

obtained from Eq. 1. Therefore, one finds a phenomenological correlation between the experimental observations of yeast inactivation dependent on the intensity of irradiation and the theoretical framework explaining the process of dimerization of yeast's DNA by the UVC irradiation. The decontamination parameter of the system becomes proportional to the compression of the double-well potential and the tunneling of the electrons between two stationary states increases as a function of the intensity of the irradiation.

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# CLINICAL, BIOCHEMICAL, AND GENETIC FINDINGS IN A GROUP OF PATIENTS EVALUATED FOR SUSPECTED MITOCHONDRIAL DISORDERS

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***Abstract.*** Mitochondrial diseases (MDs) are clinically heterogeneous disorders caused by mutations in either mitochondrial DNA (mtDNA) or nuclear genes that impair oxidative phosphorylation. This study aimed to comprehensively characterize the clinical, biochemical, imaging, and molecular features of 37 patients exhibiting mitochondrial involvement, selected from a larger group of 240 individuals with suspected MD based on a Nijmegen Mitochondrial Disease Score ≥3. All patients underwent a standardized evaluation protocol comprising clinical assessment, targeted biochemical and instrumental investigations, qPCR-HRM screening for common mtDNA mutations, and mtDNA sequencing, with additional nuclear gene analysis performed in selected cases. Multisystemic involvement was prevalent, with neuromuscular and central nervous system manifestations being the most frequent. Elevated lactate levels and abnormal neuroimaging findings further supported the clinical diagnosis. Molecular analyses revealed both common and rare mtDNA variants, while nuclear gene defects were identified in a subset of patients, underscoring the genetic complexity of MDs. The integrated approach enabled accurate diagnosis and highlighted the value of combining clinical and molecular data in MD evaluation.

***Keywords: mitochondrial DNA, qPCR-HRM, targeted sequencing, mtDNA mutations, mitochondrial disease.***

## INTRODUCTION

Mitochondrial diseases (MDs) are among the most frequent inherited metabolic disorders, with an estimated prevalence of approximately 1 in 5000 individuals. They stem from genetic alterations that impair oxidative phosphorylation (OXPHOS), disrupting cellular energy homeostasis [1]. The clinical spectrum is remarkably broad, involving neurological, muscular, cardiac, endocrine, gastrointestinal, and ophthalmological manifestations, which adds

considerable complexity to early diagnosis [2]. Given their clinical heterogeneity, the integration of molecular diagnostics has become essential in confirming MD and guiding treatment. Mutations in both mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) can lead to MDs, necessitating a dual-tiered genetic approach [3].

In this study, we describe the clinical, biochemical, instrumental, and molecular characteristics of 37 patients with genetically confirmed mitochondrial disease, selected from a broader group of 240 individuals with suspected MD based on Nijmegen Mitochondrial Disease Score. Our goal was to characterize this subset in detail, exploring potential associations between clinical presentation, NMDS scores, biochemical abnormalities, and the type of underlying genetic variant.

## MATERIALS AND METHODS

This study was conducted between March 2021 and October 2024 at the Institute of Mother and Child, the national reference center for genetic and metabolic disorders in the Republic of Moldova. Patients were included based on a modified Nijmegen Mitochondrial Disease Score [4], ranging from 0 to 8 points, with only individuals scoring ≥3 undergoing further evaluation.

Each selected patient followed a standardized diagnostic protocol, including clinical assessment, targeted biochemical testing, and instrumental investigations. The clinical assessment involved the documentation of neurodevelopmental status, neuromuscular symptoms, growth patterns, and organ-specific dysfunctions. Family history and perinatal data were also recorded when available. Biochemical testing included serum lactate, creatine kinase (CK), lactate dehydrogenase (LDH), electrolytes, ammonia levels, plasma amino acids, acylcarnitine profiles, urinary organic acids, and other parameters tailored to the clinical phenotype. Instrumental investigations comprised electroencephalography (EEG), electromyography (EMG), electrocardiography (ECG), and neuroimaging, which included cranial CT and, when indicated, brain MRI, performed in accordance with clinical necessity.

Molecular testing followed a stepwise approach. All 240 patients underwent qPCR-HRM screening for eight common pathogenic mtDNA point mutations. Based on NMDS >6 and/or positive HRM results, 90 patients were further analyzed by Sanger sequencing of mtDNA. Additional nuclear gene testing was performed in individuals with suggestive clinical features or inconclusive mtDNA results. Variant interpretation followed the American College of Medical Genetics and Genomics (ACMG) guidelines and incorporated in silico tools and public databases (MITOMAP, ClinVar, gnomAD, MitImpact 3D, Ensembl Variant Effect Predictor).

Statistical analyses were performed using IBM SPSS Statistics v27. Categorical variables were expressed as frequencies and percentages, and continuous variables as medians and interquartile ranges. Associations were tested

using Chi-square, and group comparisons with Mann–Whitney U test. A p-value <0.05 was considered statistically significant.

## RESULTS AND DISCUSSIONS

Among the 37 individuals exhibiting mitochondrial involvement, the mean age at clinical evaluation was 48.8 ± 10.55 months. At the time of data analysis, 5 patients (13.5%) were deceased, reflecting the severity and progressive nature of the disease spectrum. Perinatal data were available for 33 individuals (89.2%), among whom 6 (16.2%) presented with intrauterine growth restriction, while pregnancy or birth-related complications were documented in 9 cases (24.3%). These findings highlight the potential early onset and systemic burden of mitochondrial dysfunction from the prenatal period. Building upon these early findings, the clinical spectrum observed in this group was remarkably heterogeneous, encompassing multisystemic involvement with variable age at symptom onset and degrees of severity.

Muscular and neuromotor features were among the most prevalent findings. Hypotonia was the most frequent finding (81.1%), followed by exercise intolerance (64.9%), and episodes of rhabdomyolysis (8.1%). Regression or loss of previously acquired motor skills occurred in 27.0% of individuals, typically in the context of global clinical deterioration.

Neurological involvement spanned a broad spectrum of central and peripheral features. Ataxia was present in 24.3% of cases, including both cerebellar ataxia (10.8%) and broader truncal incoordination. Pyramidal signs were observed in 24.3% and extrapyramidal features in 16.2%, while clinical indicators of brainstem dysfunction—such as dysphagia, dysarthria, or abnormal ocular motility—were noted in 43.2% of patients. Seizures were reported in 70.3%, encompassing generalized, focal, and myoclonic types. Stroke-like episodes occurred in 5.4%, and cortical blindness was noted in 8.1% of cases. Intellectual disability or significant developmental delay was a prominent feature in 62.2%, often accompanied by behavioral or neuropsychiatric disturbances. Peripheral neuropathy, most frequently with a sensorimotor distribution, was present in 24.3%.

The multisystemic nature of mitochondrial dysfunction was further underscored by the frequent association of extra-neurological manifestations. Endocrine and growth abnormalities were among the most prevalent, affecting 59.5% of patients. Gastrointestinal symptoms, such as recurrent vomiting, feeding difficulties, and chronic constipation, were reported in 29.7%. Hepatic involvement, including hepatomegaly and mild transaminase elevation, was observed in 27.0% of cases. Cardiac involvement was observed in 35.1% of patients, including conduction abnormalities (18.9%), dilated cardiomyopathy (5.4%), and, less frequently, hypertrophic cardiomyopathy (2.7%). Ophthalmic

involvement included optic nerve atrophy (40.5%), strabismus (24.3%), nystagmus (29.7%), and ophthalmoplegia (16.2%), while sensorineural hearing loss was present in 8.1% of individuals. Hematologic involvement was documented in 32.4% of patients, most frequently manifesting as coagulation abnormalities (27.0%), followed by thrombocytopenia (16.2%), neutropenia (10.8%), and macrocytic anemia (10.8%). Renal impairment was rare, identified in only 2.7% of patients.

Biochemical testing revealed increased serum lactate levels in 91.9% of patients, with levels exceeding 4 mmol/L in 21.6% of cases. Hyperlactatemia weas significantly associated with developmental impairment ($\chi^2 = 6.11$, $p = 0.013$), highlighting the relevance of metabolic disturbance in early neurodevelopmental impairment. Elevated LDH and ammonia levels were observed in 43.2% and 8.1% of patients, respectively, while increased creatine kinase was detected in 21.6%. Electrolyte disturbances were less frequent and primarily consisted of mild hypocalcemia (21.6%), hyponatremia (18.9%), or hypokalemia (10.8%), without consistent correlation to clinical or imaging findings. Elevated plasma alanine was observed in 32.4% of patients, while altered acylcarnitine profiles and urinary organic acid abnormalities were identified in 13.5% and 10.8%, respectively – findings consistent with metabolic signatures of mitochondrial dysfunction.

Instrumental investigations demonstrated abnormal electroencephalographic activity in 56.8% of individuals, with predominant patterns including hypsarrhythmia (16.2%), polymorphic slow waves (10.8%), frequent epileptiform discharges (5.4%), and atypical complexes potentially indicative of epileptic phenomena (21.6%). Electromyographic studies revealed a myopathic pattern in 21.6% of the assessed patients. Neuroimaging abnormalities were documented in 75.7% of cases, most frequently encompassing cerebral atrophy (40.5%), white matter signal alterations (18.9%), basal ganglia involvement (16.2%), and stroke-like lesions (5.4%). Additionally, bilateral symmetric basal ganglia lesions compatible with Leigh syndrome were observed in 8.1%. Notably, individuals with abnormal neuroimaging exhibited significantly higher rates of psychomotor delay ($\chi^2 = 7.38$, $p = 0.025$) and elevated Nijmegen scores (median 6.82 vs. 6.0; $p = 0.012$, Mann–Whitney U test), supporting an association between structural cerebral involvement and overall disease severity.

Molecular investigations revealed variants in mitochondrial or nuclear genes consistent with the observed phenotypes. Eight patients carried one of the common pathogenic point mutations identified via qPCR-HRM screening: m.3243A>G (n=3), m.8993T>G (n=2), m.11778G>A (n=1), and m.3460G>A (n=2). In the remaining cases, Sanger sequencing of the mitochondrial genome—focused primarily on protein-coding genes—uncovered additional variants, most of which were homoplasmic. Genes most commonly affected were those encoding Complex I subunits (*MT-ND* family) in 25.0% of cases, followed by mitochondrial

RNA genes (21.9%), Complex V subunits (*MT-ATP6/8*) in 15.6%, and Complex III (*MT-CYB*) and Complex IV (*MT-CO2*), each accounting for 3.1%. Mutations spanning multiple respiratory chain complexes were observed in 31.3% of cases, indicating diffuse mitochondrial dysfunction, though not significantly associated with clinical severity ($\chi^2 = 3.44$, $p = 0.064$).

Out of the total, 19 individuals (59.4%) had pathogenic or likely pathogenic variants, while 13 (40.6%) presented VUS with a high likelihood of phenotypic relevance. These VUS were predominantly identified in Complex I-related genes (34.1%), followed by Complex V (29.2%) and Complex IV (17.0%). Patients carrying pathogenic or likely pathogenic variants had significantly higher NMDS scores compared to those with only VUS ($\chi^2 = 6.82$, $p = 0.033$), indicating greater clinical burden in genetically confirmed cases. These distributions are further illustrated in Figure 1, which depicts gene-level frequency, and in Figure 2, which details the classification categories of variants detected exclusively within mtDNA.

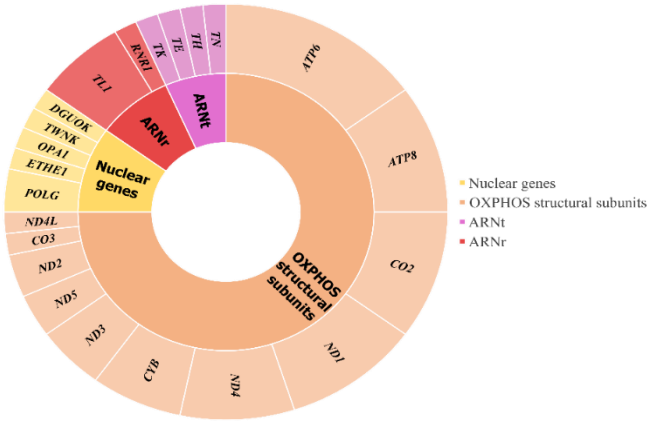


Figure 1. Gene-level distribution of identified variants across mtDNA and nDNA

A subgroup of five individuals was diagnosed with nuclear gene mutations affecting mitochondrial dynamics and replication: *POLG* (n=2), *DGUOK* (n=1), *TWNK* (n=1), and *ETHE1* (n=1), each associated with distinct syndromic forms of mitochondrial dysfunction. Notably, one patient harboring the m.3243A>G variant also carried a pathogenic variant in *OPA1*, a gene implicated in optic atrophy and mitochondrial membrane remodeling.

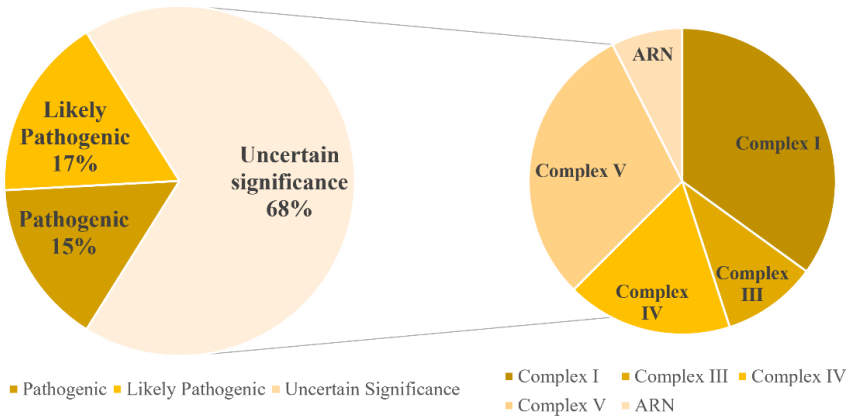


Figure 2. Variant pathogenicity and VUS subdistribution by OXPHOS complexes and RNA genes

Taken together, these findings highlight the heterogeneity and complexity of mitochondrial disorders. The high prevalence of neuromuscular and multisystemic involvement aligns with existing literature [5,6] and reinforces the need for an integrated diagnostic approach combining clinical, biochemical, imaging, and molecular data [7]. The significant associations observed between clinical severity and genetic findings (particularly Nijmegen score and variant classification), as well as with neuroimaging and EEG abnormalities, support the utility of these tools in guiding diagnostic suspicion and stratifying patients for genetic testing [8]. Furthermore, the distribution of mutations across respiratory chain complexes – most notably Complex I – emphasizes its central role in mitochondrial pathology [9]. Despite the presence of VUS in a substantial proportion of patients, their phenotypic relevance and co-segregation with core clinical features suggest their potential pathogenic contribution, warranting future functional validation.

## CONCLUSIONS

This study underscores the clinical and molecular heterogeneity of mitochondrial diseases, revealing consistent patterns of early-onset, multisystemic involvement, with a predominance of neuromuscular and neurodevelopmental features. The Nijmegen score proved valuable not only as a selection tool but also as a proxy for disease severity, correlating significantly with key clinical and imaging parameters. Elevated serum lactate and neuroimaging abnormalities were associated with more severe phenotypes, supporting their relevance as accessible diagnostic markers. Molecular analysis identified diverse mtDNA and nDNA variants, notably in Complex I subunits and mtRNA genes, with pathogenic variants

associating with higher clinical scores. The integration of clinical, biochemical, imaging, and genetic data enabled a refined stratification of patients and highlighted the diagnostic yield of a tiered genetic approach. These findings support the implementation of early and comprehensive evaluation strategies to improve diagnostic accuracy and inform management in suspected MDs.

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# MANIFESTATIONS OF PHYSIOLOGICAL HEMOSTASIS DYSFUNCTIONS

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***Abstract.*** *The study is devoted to the study of hemocoagulation factors in white rats under the action of pyrogenal. It was found that administration of pyrogenal 50 µg/kg increased active recalcification time, active relative thromboplastin time, prothrombin and thrombin time, as well as fibrinogen content. The observed changes indicate an increase in the protective properties of the organism that promote the acceleration of hemocoagulation processes. It is assumed that the administration of this dose of pyrogenal increases the specific and nonspecific resistance of the organism.*

***Keywords: hemostasis, fibrinogen, pyrogenal, trombin, hemocoagulation processes.***

## INTRODUCTION

In the human body, the morpho-functional integrity of blood vessels is maintained under normal conditions due to the action of complex enzymatic systems.

The production of a vascular lesion, no matter how small, triggers a well-determined sequence of reactions that ensure:

- formation of an obstacle that opposes blood loss;
- resumption of normal circulatory flow after the integrity of the damaged vessel has been restored;
- stimulation of tissue repair mechanisms.

The term hemostasis defines precisely this sequence of biological events, in which plasma proteins, cellular components, and humoral components participate in a conjugated manner. Two enzymatic systems with antagonistic action essentially contribute to hemostasis: the coagulation system and the fibrinolytic system. They are normally in a dynamic equilibrium, which is why the term hemostasis is increasingly replaced by the term coagulolytic equilibrium.

Hemostasis is a complex physiological phenomenon, an autocatalytic phenomenon that opposes hemorrhage, ensuring a normal coagulability state through the integrity of vascular walls. Blood coagulation is a process in which blood changes its fluidity due to the transformation of a soluble plasma protein,

fibrinogen, into a three-dimensional, insoluble network. This transformation occurs locally, under the action of specific stimuli, and aims to stop or limit blood loss at the level of vascular lesions, as a result of their sealing.

Blood coagulation involves the interaction of several components: vascular endothelium, platelets, and plasma glycoproteins. It is tightly controlled through positive and negative feedback mechanisms and counterbalanced by the fibrinolytic system.

The endothelium plays a crucial role in maintaining the fluid state of blood and limiting clot formation only at the local level. After producing a vascular lesion, endothelial cells express procoagulant factors such as tissue factor (TF), plasminogen activator inhibitor (PAI), von Willebrand factor (vWF), and protease-activated receptors (PAR), while to inhibit clot formation, they express tissue factor pathway inhibitor (TFPI), heparan sulfate, thrombomodulin, endothelial protein C receptor, tissue plasminogen activator (t-PA), ecto ADP-ase, prostacyclin, nitric oxide, and ADAMTS, a metalloprotease that limits the procoagulant activity of vWF (1,2,3).

Platelets have multiple roles during hemostasis: adhesion, degranulation, aggregation, fusion, and procoagulant (2). When the endothelium is damaged, the subendothelial matrix, rich in vWF and collagen, is exposed, which favors platelet adhesion through glycoprotein receptors (GP) Ib/IX/V and Ia/IIa and initiates activation (3). During adhesion, platelet receptors GP IIb/IIIa are activated, which can bind to fibrinogen and vWF, resulting in platelet aggregation. Activated platelets release the contents of alpha and dense granules into the extracellular space. The degranulation process is dependent on prostaglandin synthesis. Following degranulation, platelets release procoagulant material into the extracellular space such as: vWF, factor V, beta-thromboglobulin, platelet factor 4, fibrinogen, adenosine diphosphate (ADP), serotonin, calcium, etc. The released substances, especially ADP, facilitate a positive feedback loop that enhances ADP and thromboxane A2 release, resulting in secondary aggregation (2). Platelets have a procoagulant role also by providing the phospholipid membrane surface (platelet factor 3-PF3) for the development of coagulation protein reactions.

In the classic model of coagulation, plasma glycoproteins act in a cascade, on two pathways, intrinsic and extrinsic, which meet at the level of factor X activation (common pathway), resulting in the formation of thrombin which cleaves fibrinogen into fibrin monomers that are polymerized and stabilize the platelet clot. This cascade model is useful for understanding laboratory tests but does not reflect the coagulation process in vivo, which is more complex. The current model of coagulation is based on only one common pathway, in which the interaction between TF and factor VII (FVII) has an essential role and unfolds in 3 overlapping phases: initiation, amplification, and propagation (4).

Blood coagulation is a very important mechanism for defending the body

against hemorrhages, involving numerous substances found or formed in plasma and platelets. But sometimes it is influenced by certain factors that trigger major cellular disorders. The interaction between coagulation factors and some stressful states, such as the toxic effect of certain compounds, is extremely complex and still incompletely known. Depending on their origin, toxins can be exogenous or endogenous. Exogenous toxins are: 1. non-biological - exogenous chemical substances that cannot be tolerated at any concentration and chemical substances that become toxic when exceeding the maximum allowed dose 2. biological - toxins produced by living organisms.

Exotoxins cause: ribosylation of intracellular proteins, damage to cell membranes through enzymatic effect, interference with certain neurotransmitters, and endotoxins (lipopolysaccharides) cause the generation of mediators (cytokines, arachidonic acid derivatives, etc.) that act directly on the cell. All of these are pyrogenic substances.

In general, exogenous pyrogens act mainly by inducing the formation of endogenous pyrogens through stimulation of host cells - usually monocytes and macrophages. However, the distinction between exogenous and endogenous pyrogens is sometimes unclear. For example, lipopolysaccharides can act directly on brain endothelial cells to generate fever, while many exogenous products lead to the release of endogenous pyrogens, thus producing fever. Such endogenous substances include antigen-antibody complexes with complement, complement cleavage products, steroid hormone metabolites, bile acids, and some cytokines. [1]

Exogenous pyrogens are represented by: pathogenic microorganisms and their toxins: Gram-negative bacterial endotoxins (LPS), Gram-positive bacterial exotoxins (lipoteichoic acid, peptidoglycans), viruses, fungi, toxic substances - antigen (Ag) - antibody (Ab) complexes that fix Complement, etc. Endogenous pyrogens: are cytokines (IL-1, TNF alpha, IL-6) produced by numerous cells (monocytes, macrophages, tumor cells) and released into circulation, which trigger fever through prostaglandin release. The biological effects of endogenous pyrogens have central action: ↑ threshold of thermoregulatory center regulation with role in triggering the febrile reaction and peripheral actions: - proinflammatory effect, increasing the body's defense capacity: - ↑ leukocyte mobilization at the hematopoietic marrow level ⇒ leukocytosis - induce synthesis of chemotactic factors for monocytes and neutrophils - activation of phagocytosis - ↑ hepatic synthesis of acute phase proteins (IL-6): fibrinogen, C-reactive protein, antiproteases, haptoglobin, complement system - ↓ serum Fe and Zn (trace elements necessary for bacterial multiplication) - activation of B and T lymphocytes - acceleration of muscle catabolism + negative nitrogen balance ⇒ weight loss in prolonged febrile states - synergistic toxic effects ⇒ toxic shock when released in very large quantities. [8,9,10]

The thermoregulatory system is able to maintain the central temperature relatively constant, accepting a variation of approximately 0.2° C. When it rises above the permissible limit, the body's defense mechanisms are also deregulated. [10, 11, 12]

## MATERIALS AND METHODS

The purpose of this work was to study the action of the biogenic preparation (complex of lipopolysaccharides extracted from cell membranes of gram-negative bacteria) pyrogenal in doses of 50µg/kg for 3 days on coagulation factors in laboratory white rats. Before being subjected to the action throughout the experiment, the rats were weighed, body temperature was measured, as well as blood glucose concentration.

After 1, 2, 3 days of experiment, blood samples were collected from laboratory white rats for the determination of 5 coagulation factors: activated recalcification time, activated partial thromboplastin time, prothrombin time, thrombin time, fibrinogen quantity.

To study the action of the preparation on coagulation factors, a set of reagents was used, intended for the investigation of the "plasma chain" of the blood coagulation system, by the method of modeling the blood coagulation process "in vitro", which allows obtaining data regarding the stages of blood coagulation.

## RESULTS AND DISCUSSIONS

Analyzing the obtained data, in the control group of rats we observe that they coordinate with the results obtained by other scientists (Cosmulescu I., 1985; Niguleanu V.N., 1990; Paladi E., Budeanu L., 2004).

In control rats, the activated recalcification time was 12.53 ± 0.65 s., activated partial thromboplastin time - 11.48 ± 0.42 s., prothrombin time - 13.36 ± 0.32s., thrombin time - 11.78 ± 0.18 s., and fibrinogen quantity - 1.71 ±0.08 g/l.

In rats subjected to the action for 1 day, it was found that all the indices investigated by us practically did not change compared to those of the control rats. The activated recalcification time was 13.98 ± 0.33 s., activated partial thromboplastin time - 12.18 ± 0.12 s., prothrombin time - 14.15 ± 0.29s., thrombin time - 11.68 ± 0.8 s., and fibrinogen quantity - 1.75 ±0.09 g/l.

Increasing the duration of administration to 2 days, we found that the changes are still non-essential in the coagulation indices investigated by us compared to the data obtained from control rats and the previous group.

From the obtained results, we found that the activated recalcification time in rats subjected to the biogenic preparation for 2 days remained almost unchanged compared to the previous group (13.98 ± 0.33 s compared to 14.4 ± 0.46 s), but

comparing the results with those from control animals, we detect a small insignificant increase.

Essential changes were obtained in the determination of activated partial thromboplastin time compared to the same index in rats of group 3 (14.98 ± 0.16 s, compared to 12.18 ± 0.12 s).

Analyzing the data obtained in this group for activated partial thromboplastin time in control rats, we observed that this index has changed, constituting an essential difference. In determining prothrombin time in rats of this group, we found almost constant data compared to those of the previous group (13.74 ± 0.26 s, compared to 13.36 ± 0.32 s).

In investigating thrombin time in rats of this group, a small increase in this index was observed compared to that of control animals, which constitutes (12.03 ± 0.17 s compared to 11.68 ± 0.8 s).

The fibrinogen quantity in rats subjected to biogenic preparation administration for 2 days increased compared to that of control rats (1.88±0.03 g/l compared to 1.75±0.09 g/l).

Increasing the duration of action on experimental rats to 3 days, we found that changes in the investigated indices continue to appear (1.97 ±0.13 g/l compared to 1.75±0.09 g/l), but which are not so significant.

## CONCLUSIONS

From the above, we observe a non-significant increase in the 5 investigated indices (activated recalcification time, activated partial thromboplastin time, prothrombin and thrombin time, fibrinogen quantity), which indicates that the rats are subjected to a stressful state, increasing the body's defense capacity when required.

Under the influence of the preparation, essential changes occur in the sympathetic nervous system, which lead to the acceleration of blood coagulability. It is likely that the lipopolysaccharide complex increases the immune response in doses of 50µg/kg as well as the specific and non-specific resistance of the body.

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# Session III

# PLANT GENETICS AND BREEDING

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# EVALUATION OF GENETIC DIVERSITY: A STUDY ON MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS OF LOCAL AND INTRODUCED DURUM WHEAT (*TRITICUM DURUM* DESF.) GENOTYPES

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***Abstract.*** *Wheat is the most widely consumed staple food in the world and provides more nourishment for humans than any other food source. This study evaluated the genetic parameters of selected morphological and physiological traits using 77 durum wheat genotypes (8 local and 69 introduced). The research was conducted from 2022 to 2024 in the Field Laboratory of Grains and Legumes at Azerbaijan State Agrarian University. The wheat was cultivated in Ganja using a completely randomized design under winter wheat growing conditions. Phenological stages were recorded according to the Kuperman method, from emergence to full maturity. Morphological traits such as plant height (PH, cm) excluding awns, spike length (SL, cm), number of spikelets per spike (SNS), number of grains per spike (GNS), grain weight per spike (GWS), thousand kernel weight (TKW, g), and grain yield (GY, kg/m²) were measured. The longest SL was recorded as 12.10 cm in genotype GDP-647, and the shortest was 5.0 cm in genotype Ganja. Twenty-two genotypes showed superior SL performance compared to the check varieties. In the first year, Ganja-8 had 18 SNS, while in the second year it reached 25. The lowest SNS was 13 in genotype GDP-560. Ganja-1 had the highest GNS with 4.30, and GDP-682 had the lowest with 1.2. TKW ranged widely, from 55.30 g (GDP-669) to 31.30 g (GDP-457). The lowest spike density was found in GDP-597 (17.80), while the highest was 35.0 in Ganja-6 and Ganja-7. The study provided a comparative overview of phenological development in local and introduced genotypes, revealing a significant local adaptation effect in introduced genotypes. Among locals, Ganja-5 and Ganja-8 exhibited high phenological performance. Cluster analysis based on estimates grouped genotypes into four main clusters according to traits such as PH, SL, SNS, GNS, GWS, TKW, and spike density. Notably, local genotypes Ganja-5 and Ganja-8 demonstrated strong phenological development and adaptability, indicating their potential for breeding programs.*

***Keywords: durum wheat, genotype, grain quality, productivity, yield components***

## INTRODUCTION

Wheat (*Triticum spp*.) is one of the most vital cereal crops worldwide, contributing significantly to global food security and human caloric intake. Among the wheat species, durum wheat *(Triticum durum* Desf.) plays a prominent role due to its unique grain hardness, high protein content, and superior end-use quality,

especially in pasta and semolina production [4]. As climate change, water scarcity, and emerging diseases increasingly threaten food systems, the improvement of durum wheat through genetic breeding has become a central goal of modern agriculture [6]. The success of any breeding program largely depends on the availability and use of diverse genetic material. Genetic diversity among durum wheat genotypes offers opportunities to select lines with superior traits such as yield potential, drought tolerance, disease resistance, and adaptability to specific agroecological zones [7]. Particularly in dryland regions like Azerbaijan, identifying genotypes that can thrive under fluctuating climatic conditions is vital for stabilizing grain production [5]. Comparing local landraces with introduced germplasm allows breeders to detect useful alleles for adaptive traits and provides insight into genotype × environment (G×E) interactions [3]. Local varieties often exhibit long-term adaptation to regional stress factors, while introduced lines may bring new traits or higher yield potential, albeit requiring careful evaluation in the target environments [1]. The evaluation of morphological and physiological traits such as plant height, spike length, grain weight, number of grains per spike, and thousand kernel weight (TKW) remains a critical step in selecting high-performing and resilient genotypes [6]. The integration of phenotypic evaluation with statistical methods such as cluster analysis enhances the identification of promising materials for durum wheat improvement [2]. This study evaluates the genetic diversity and agronomic traits of durum wheat genotypes under drought conditions in the Northern Great Plains (USA), which is essential for the development of drought-tolerant cultivars [8].

## MATERIALS AND METHODS

The experiment was conducted during the 2022/2023- 2023/2024 growing season at the Field Laboratory of Grains and Legumes, Azerbaijan State Agrarian University, located in Ganja (468 m above sea level; 40°40’N latitude, 46°20’E longitude). The region has a temperate climate suitable for winter wheat cultivation. A total of 77 durum wheat genotypes were used, consisting of 8 local and 69 introduced lines. The experiment followed a completely randomized design (CRD). Each genotype was sown in one row (2 meters in length) with a row spacing of 25 cm. Standard agronomic practices were applied throughout the growing period. Phenological observations were conducted based on the Kuperman methodology, monitoring key development stages from emergence to full maturity. The following traits were recorded: Plant height (PH, cm): Measured (excluding awns) from 10 randomly selected plants per plot before harvest. Spike length (SL, cm): Measured just before harvest. Number of spikelets per spike (SNS). Number of grains per spike (GNS). Grain weight per spike (GWS)Thousand kernel weight (TKW, g):

Weight of 1000 grains. Grain yield (GY, kg/m²): Determined by weighing harvested grains from each plot.

## RESULTS AND DISCUSSIONS

Sowing occurred in early November. Phenological observations showed dynamic development across all genotypes.

Table 1. Average values of agronomic and physiological traits of 77 durum wheat genotypes (2022-2024 growing seasons,Ganja, Azerbaijan).

| № | Genbank number | Origin | PH (cm) | SL (cm) | SNS (no) | SPS (no) | GWS (g) | TGW (g) | SD (no) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | GDP-023 | EGY | 93.00 | 6.00 | 16.50 | 43.00 | 3.00 | 36.00 | 27.40 |
| 2 | GDP-044 | ARG | 105.5 | 7.00 | 18.00 | 40.00 | 1.80 | 44.00 | 26.40 |
| 3 | GDP-049 | ARG | 102.0 | 7.30 | 21.50 | 51.00 | 1.70 | 41.10 | 29.70 |
| 4 | GDP-57 | ARG | 75.00 | 8.00 | 17.50 | 39.00 | 2.00 | 41.30 | 26.90 |
| 5 | GDP-078 | ESP | 82.00 | 6.50 | 18.00 | 53.00 | 2.40 | 46.00 | 28.30 |
| 6 | GDP-115 | CIMMYT | 79.50 | 9.00 | 21.00 | 54.00 | 2.00 | 46.20 | 23.50 |
| 7 | GDP-177 | İCARDA | 63.50 | 7.70 | 20.50 | 46.00 | 2.20 | 42.00 | 26.40 |
| 8 | GDP-182 | İCARDA | 96.00 | 8.80 | 20.50 | 53.00 | 2.00 | 36.40 | 25.50 |
| 9 | GDP-183 | ICARDA | 116.0 | 8.60 | 18.00 | 42.00 | 1.70 | 40.80 | 26.20 |
| 10 | GDP-196 | ICARDA | 93.00 | 6.50 | 16.50 | 37.00 | 1.80 | 39.30 | 27.10 |
| 11 | GDP-204 | AUS | 62.50 | 7.50 | 22.00 | 43.00 | 2.40 | 38.40 | 23.90 |
| 12 | GDP-207 | AUS | 79.00 | 8.80 | 19.00 | 52.00 | 1.40 | 34.30 | 24.00 |
| 13 | GDP-215 | AUT | 83.50 | 7.90 | 18.00 | 39.00 | 1.60 | 42.00 | 27.00 |
| 14 | GDP-215 | AUT | 84.00 | 8.50 | 20.50 | 46.00 | 1.40 | 39.40 | 20.60 |
| 15 | GDP-216 | AUT | 119.5 | 7.00 | 18.50 | 43.00 | 1.40 | 39.70 | 28.70 |
| 16 | GDP-232 | ITA | 109.5 | 7.50 | 21.50 | 33.00 | 1.60 | 39.50 | 26.20 |
| 17 | GDP-235 | ITA | 105.0 | 8.80 | 18.00 | 37.50 | 2.40 | 46.00 | 23.00 |
| 18 | GDP-238 | MAR | 90.0 | 7.70 | 19.50 | 47.50 | 1.80 | 42.60 | 32.50 |
| 19 | GDP-261 | ICARDA | 88.5 | 8.50 | 18.00 | 47.50 | 1.80 | 45.60 | 21.10 |
| 20 | GDP-271 | İCARDA | 91.5 | 8.00 | 20.00 | 42.50 | 1.80 | 43.60 | 24.30 |
| 21 | GDP-310 | ESP | 85.50 | 9.00 | 21.00 | 20.00 | 1.50 | 42.70 | 22.20 |
| 22 | GDP-330 | ESP | 133.0 | 11.00 | 16.00 | 35.50 | 1.80 | 33.50 | 19.00 |
| 23 | GDP-332 | ESP | 88.50 | 7.70 | 16.50 | 51.50 | 2.00 | 38.80 | 20.70 |
| 24 | GDP-341 | FRA | 91.00 | 8.50 | 22.50 | 48.50 | 1.90 | 42.30 | 19.40 |
| 25 | GDP-353 | İTA | 192.0 | 8.70 | 21.50 | 36.00 | 1.50 | 43.50 | 25.50 |
| 26 | GDP-375 | ITA | 69.50 | 10.00 | 19.50 | 42.50 | 2.00 | 45.70 | 21.50 |
| 27 | GDP-376 | ITA | 102.0 | 9.50 | 17.50 | 38.00 | 2.10 | 47.60 | 20.50 |
| 28 | GDP-388 | USA | 68.50 | 8.00 | 20.50 | 41.00 | 1.70 | 41.40 | 22.10 |
| 29 | GDP-395 | ESP | 87.00 | 8.30 | 18.00 | 42.50 | 1.30 | 53.70 | 22.80 |
| 30 | GDP-397 | İND | 69.00 | 7.30 | 18.50 | 54.00 | 1.60 | 34.60 | 22.60 |
| 31 | GDP-403 | ALG | 67.00 | 9.00 | 19.50 | 55.00 | 2.00 | 38.60 | 23.20 |
| 32 | GDP-417 | ICARDA | 72.50 | 7.90 | 19.50 | 41.50 | 1.40 | 37.70 | 24.30 |
| 33 | GDP-419 | İCARDA | 75.00 | 8.50 | 21.00 | 50.00 | 1.60 | 41.50 | 20.50 |

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|---|---|---|---|---|---|---|---|---|---|
| 34 | GDP-420 | ICARDA | 75.00 | 6.90 | 20.00 | 58.50 | 2.10 | 40.00 | 28.10 |
| 35 | GDP-422 | ICARDA | 88.50 | 6.20 | 18.00 | 44.50 | 1.70 | 32.80 | 32.10 |
| 36 | GDP-457 | FRANCE | 94.00 | 7.30 | 21.00 | 46.50 | 1.80 | 31.30 | 27.60 |
| 37 | GDP-466 | FRANCE | 71.50 | 8.50 | 17.00 | 62.00 | 1.60 | 37.30 | 25.60 |
| 38 | GDP-469 | FRANCE | 71.00 | 6.00 | 19.00 | 47.50 | 2.40 | 38.10 | 24.30 |
| 39 | GDP-501 | FRANCE | 82.50 | 8.90 | 20.00 | 59.50 | 1.90 | 37.70 | 29.80 |
| 40 | GDP-503 | FRANCE | 92.50 | 8.50 | 20.50 | 66.50 | 2.20 | 38.30 | 19.40 |
| 41 | GDP-505 | FRANCE | 63.50 | 9.50 | 23.50 | 61.00 | 2.80 | 35.50 | 24.30 |
| 42 | GDP-512 | FRANCE | 102.0 | 8.00 | 22.50 | 49.50 | 2.60 | 35.00 | 25.30 |
| 43 | GDP-534 | FRANCE | 68.00 | 8.00 | 20.50 | 37.00 | 2.30 | 36.60 | 25.00 |
| 44 | GDP-535 | FRANCE | 98.50 | 9.30 | 20.00 | 41.00 | 1.70 | 38.70 | 23.30 |
| 45 | GDP-537 | FRANCE | 59.50 | 7.70 | 18.00 | 51.50 | 1.50 | 42.20 | 24.90 |
| 46 | GDP-551 | ETH | 65.00 | 6.90 | 21.00 | 56.50 | 2.00 | 36.10 | 21.40 |
| 47 | GDP-560 | TUR | 72.00 | 6.80 | 13.00 | 45.00 | 1.50 | 43.10 | 29.10 |
| 48 | GDP-566 | İSRAEL | 78.00 | 6.80 | 20.50 | 38.50 | 1.60 | 46.80 | 25.90 |
| 49 | GDP-574 | KAZ | 113.5 | 7.50 | 18.00 | 48.50 | 1.60 | 44.60 | 28.90 |
| 50 | GDP-590 | OMN | 111.0 | 7.20 | 23.00 | 51.50 | 1.60 | 36.50 | 31.90 |
| 51 | GDP-597 | ESP | 106.5 | 6.30 | 22.50 | 45.50 | 1.30 | 41.50 | 17.80 |
| 52 | GDP-609 | SYR | 103.5 | 8.50 | 16.00 | 46.50 | 1.60 | 47.60 | 30.70 |
| 53 | GDP-611 | IRN | 99.50 | 7.90 | 18.00 | 46.00 | 1.80 | 44.30 | 20.40 |
| 54 | GDP-647 | TUN | 100.0 | 12.10 | 17.50 | 38.50 | 1.90 | 35.60 | 26.30 |
| 55 | GDP-664 | IRQ | 85.50 | 10.00 | 22.00 | 37.20 | 2.30 | 38.90 | 23.80 |
| 56 | GDP-669 | PRT | 109.5 | 7.70 | 18.00 | 53.50 | 1.50 | 55.30 | 18.40 |
| 57 | GDP-671 | TUN | 94.00 | 8.70 | 18.50 | 43.00 | 1.30 | 34.90 | 25.20 |
| 58 | GDP-682 | ETH | 90.00 | 9.00 | 18.50 | 47.00 | 1.20 | 42.60 | 20.00 |
| 59 | GDP-684 | CYP | 102.0 | 9.50 | 18.00 | 47.00 | 2.10 | 45.90 | 22.60 |
| 60 | GDP-694 | TUN | 60.00 | 9.80 | 17.50 | 38.00 | 1.70 | 44.10 | 19.30 |
| 61 | GDP-700 | IRQ | 92.50 | 8.70 | 21.50 | 48.50 | 1.60 | 45.70 | 22.70 |
| 62 | GDP-701 | IRN | 60.50 | 7.70 | 19.00 | 41.00 | 3.30 | 55.30 | 22.10 |
| 63 | GDP-705 | GRC | 69.50 | 10.50 | 20.50 | 45.50 | 2.00 | 50.20 | 21.80 |
| 64 | GDP-737 | UZB | 77.50 | 11.80 | 17.00 | 41.00 | 1.60 | 42.10 | 20.90 |
| 65 | GDP-738 | UZB | 68.00 | 7.90 | 18.50 | 49.50 | 1.50 | 35.50 | 24.10 |
| 66 | GDP-741 | UZB | 86.50 | 8.00 | 21.50 | 49.00 | 1.60 | 39.70 | 30.10 |
| 67 | GDP-748 | ITA | 80.00 | 9.70 | 19.00 | 48.50 | 1.90 | 47.00 | 23.70 |
| 68 | GDP-761 | TUN | 84.50 | 8.00 | 22.50 | 40.00 | 2.00 | 51.00 | 28.30 |
| 69 | GDP-762 | IRN | 88.00 | 6.70 | 22.50 | 47.50 | 1.70 | 51.90 | 32.80 |
| 70 | Ganja-1 | GANJA | 134.0 | 9.20 | 22.50 | 52.00 | 4.30 | 47.60 | 23.90 |
| 71 | Ganja-2 | GANJA | 107.0 | 8.80 | 22.50 | 45.00 | 2.30 | 51.50 | 25.50 |
| 72 | Ganja-3 | GANJA | 95.50 | 9.40 | 21.50 | 48.00 | 2.30 | 43.90 | 19.60 |
| 73 | Ganja-4 | GANJA | 77.00 | 10.40 | 20.50 | 45.50 | 2.00 | 38.30 | 19.60 |
| 74 | Ganja-5 | GANJA | 91.00 | 8.00 | 18.50 | 45.50 | 1.60 | 79.20 | 25.50 |
| 75 | Ganja-6 | GANJA | 118.0 | 5.00 | 21.50 | 42.00 | 3.20 | 47.70 | 35.00 |
| 76 | Ganja-7 | GANJA | 95.00 | 5.90 | 20.00 | 51.50 | 1.80 | 40.00 | 32.50 |
| 77 | Ganja-8 | GANJA | 107.0 | 6.10 | 21.00 | 40.00 | 1.90 | 35.70 | 35.00 |

As observed in Table 1, the genotypes demonstrated wide variability across all examined traits, underlining significant genetic diversity within the panel. The tallest plant height was recorded in genotype GDP-353 (192.00 cm), an introduced Italian line, while the shortest was in GDP-537 (59.50 cm), indicating a near threefold difference in stature. Among local genotypes, Ganja-1 (134.00 cm) and Ganja-6 (118.00 cm) showed strong vertical growth. Spike length (SL) ranged from 5.00 cm (Ganja-6) to 12.10 cm (GDP-647), with 22 genotypes exceeding the check varieties in this trait. Notably, GDP-647 (Tunisia) exhibited exceptional performance, making it a promising candidate for increasing spike size in breeding programs. The number of spikelets per spike (SNS) varied considerably. Ganja-8 demonstrated a significant increase in SNS from 18 in the first season to 25 in the second, suggesting excellent environmental responsiveness. GDP-560 (Turkey) recorded the lowest SNS with 13, indicating potential limitations in reproductive structure development. Grain number per spike (GNS) was highest in Ganja-1 (4.30) and lowest in GDP-682 (1,20), highlighting strong reproductive efficiency in some local genotypes. Ganja-1 also had the highest grain weight per spike (GWS), reinforcing its potential as a high-yielding local cultivar. The thousand-kernel weight (TKW) varied from 31.30 g (GDP-457) to 55.30 g (GDP-669), indicating significant differences in grain filling capacity. GDP-669 (Portugal) and Ganja-2 showed superior kernel weights, which are important traits for grain quality and processing. Lastly, spike density (SD) ranged from 17.80 (GDP-597) to 35.0 (Ganja-6 and Ganja-8), with the local varieties displaying notably higher densities, indicative of their strong adaptability and yield potential in local agro-ecological conditions.

**Principal Component Analysis**

To better understand the interrelationships among the studied morphological and physiological traits, a Principal Component Analysis (PCA) was performed. The analysis identified key components explaining most of the variation in the dataset across 77 durum wheat genotypes. The results are shown in Table2.

The PCA revealed that PC1 was dominated by plant height (PH), accounting for the largest proportion of total variance (78.46%). This suggests that height is a major distinguishing trait among the genotypes. PC2 was influenced by the number of spikelets per spike (SPS), indicating variation in reproductive structures. PC3 was mainly defined by thousand kernel weight (TGW), a critical indicator of grain size and quality. PC4 received high loadings from both spike density (SD) and number of grains per spike (SNS), suggesting an association between spike compactness and reproductive output. PC5 isolated spike length (SL) and PC7 isolated grain weight per spike (GWS) as relatively independent traits. The first three components collectively explained 96.06% of the total variance, underscoring their importance in the characterization of durum wheat genotypes.

Table 2. Principal Component Analysis (PCA)

| | PC 1 | PC 2 | PC 3 | PC 4 | PC 5 | PC 6 | PC 7 |
|---|---|---|---|---|---|---|---|
| PH | 0.9952 | | | | | | |
| SL | | | | | | 0.97765 | |
| SNS | | | | | 0.9981 | | |
| SPS | | 0.83262 | 0.54366 | | | | |
| GWS | | | | | | | 0.99597 |
| TGW | | | 0.83757 | | | | |
| SD | | | | 0.97741 | | | |
| Eigenvalue | 432.61 | 53.81 | 43.23 | 16.09 | 4.00 | 1.21 | 0.44 |
| % variance | 78.46 | 9.76 | 7.84 | 2.92 | 0.73 | 0.22 | 0.08 |

These results can guide breeders in selecting trait-specific genotypes for further improvement and hybridization efforts.

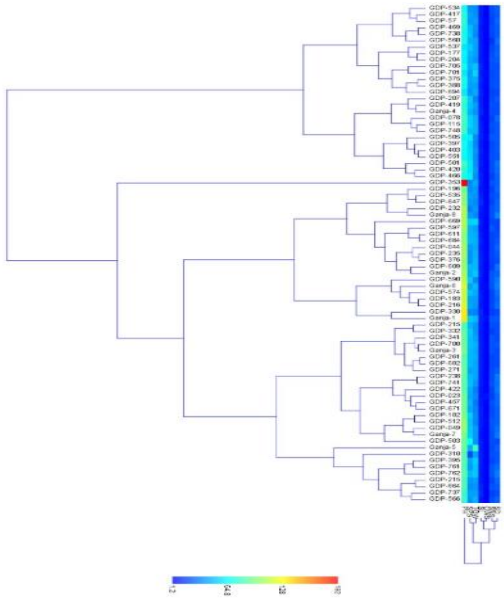

Figure1. Cluster analysis based on estimates grouped the genotypes into four major clusters:

*Cluster I: Subdivided into a and b, included both local and introduced genotypes, indicating shared traits. Cluster II: Contained only GDP-353 (Italy), distinguished primarily by its exceptional plant height. Clusters III and IV: Further divided into two subgroups each, consisting of 14 and 7 genotypes, respectively. These clusters were differentiated by traits such as spike density and grain number.*

## CONCLUSIONS

This study revealed significant genetic variation among 77 durum wheat genotypes, including 8 local and 69 introduced lines, highlighting a rich genetic resource for durum wheat improvement under Azerbaijan's agro-climatic conditions. Among the local genotypes, Ganja-5 and Ganja-8 stood out with superior traits such as spike density reaching up to 35.0, grain number per spike up to 4.3, and plant height up to 134 cm, indicating their strong local adaptation and phenological stability. These genotypes represent valuable candidates for use as parental lines in breeding programs focused on enhancing yield and stress resilience. Introduced genotypes also showed remarkable traits: GDP-647 (Tunisia) exhibited the longest spike length of 12.10 cm, significantly exceeding the local varieties, while GDP-669 (Portugal) achieved the highest thousand-kernel weight of 55.30 g, a key indicator of grain quality and size. The wide range of trait variability observed—such as plant height ranging from 59.5 cm to 192 cm and thousand-kernel weight varying from 31.3 g to 55.3 g—underscores the extensive genetic diversity present in the panel. Cluster analysis effectively grouped genotypes into four main clusters, allowing for the identification of distinct phenotypic profiles and facilitating targeted breeding strategies. This grouping enables breeders to efficiently select genotypes with complementary traits to maximize genetic gains. The combination of locally adapted genotypes with superior yield components and promising introduced lines with desirable traits offers a robust framework for durum wheat improvement in Azerbaijan. Harnessing this genetic diversity will be critical to developing new varieties capable of thriving under local environmental challenges while meeting demands for productivity and grain quality.

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# THE ROLE OF GENETIC RESOURCES IN THE IMPROVEMENT AND DEVELOPMENT OF NEW LEGUME CROP VARIETIES

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***Abstract.*** *A decisive role in generating broad heritable variability in breeding material belongs to germplasm sources. The primary method for developing new ideotypes of pea, soybean, and bean is intraspecific hybridization followed by repeated individual selection. The results obtained represent a genetic advancement in the breeding of these crops, leading to the development of new varieties with high adaptability to natural stress factors, broad ecological plasticity, and minimal fluctuations in yield levels.*

***Keywords: breeding, germplasm, hybridization, variety, pea, soybean, bean***

## INTRODUCTION

The favorable dynamics of the development of the Republic's agricultural sector are closely linked to scientific advancements in crop breeding, particularly the creation and implementation of high-performance varieties and hybrids.

The broad application of innovative results in agricultural practice represents an essential component of the national strategy for ensuring food security. The effectiveness of breeding progress largely depends on the diversification of genetic sources used in hybridization programs. In the development of new legume crop varieties, a decisive role is played by germplasm sources, including forms from global collections, local varieties, and native populations.

This paper presents the practical aspect of utilizing genetic resources for the development of new varieties of pea, soybean, and bean.

## MATERIALS AND METHODS

The germplasm collection maintained in the laboratory represents a valuable genetic resource for legume crop improvement. It currently comprises 120

accessions of pea (*Pisum sativum*), 361 accessions of soybean (*Glycine max*), and 140 accessions of bean (*Phaseolus vulgaris*). This diverse collection provides a broad genetic base that is essential for breeding programs aimed at developing new varieties with enhanced traits such as stress tolerance, yield stability, and disease resistance.

The varieties studied were evaluated in terms of several morpho-productive traits, yield capacity and quality, disease resistance, and earliness.

Following the assessment of the agronomic performance of the studied germplasm, donors of positive traits and characteristics were identified to address the objectives pursued in the breeding programs.

As a result of hybridization work—the fundamental method in the improvement of self-pollinated crops—new ideotypes were developed and tested throughout all stages of the breeding process. Superior homozygous genotypes that became officially registered varieties have been widely adopted in production practice. Throughout the experiments, cultivation technology was strictly followed for all crops included in the study.

## RESULTS AND DISCUSSION

Considering the climate changes observed in the territory of Moldova, research programs have been directed towards developing early-maturing varieties with resistance to hydrothermal stresses.

To reassess the role of pea in crop rotations, research efforts focused on creating new high-yielding technological varieties. From a technological perspective, the varieties *Renata*, *Pitulice*, *Smaragt*, *Flagman*, *Topaz 2*, *Trujenic*, *Zernogradschii 6*, *Orlovcianin* have been identified as possessing high resistance to pod shattering and lodging.

Their use as parental forms has resulted in the development of new varieties such as *Moldavschii Usatîi*, *MZ-7*, and *Valexa*. Valuable parents with high-yield potential include varieties like *Atica*, *Dic From*, *Belcovaia Grozdi*, *Dora*, *Zelenozernîi 1*, *Voroșilovgradschii*, and *Iubileinîi*, originating from diverse geographical backgrounds.

Inclusion of these genotypes in hybridization work led to the creation of varieties such as *Nadia*, *Grațiana*, and *Mazirina*, which exhibit high productive potential (4500–5000 kg/ha) and increased resistance to pod shattering and lodging. This is attributed to the presence of the “afila” and “defa” genes in the plant genome. Possession of genotypes with such valuable traits allows direct harvesting of the crop while minimizing yield losses.

The agronomic value of the new pea varieties will enable the expansion of pea cultivation areas, supporting the revitalization of scientifically justified crop rotations in the agricultural sector of the republic.

Climate changes associated with global warming have had drastic consequences on the yield levels of soybean and bean crops. The occurrence of extreme temperatures combined with acute moisture deficits results in a 50–70% reduction in seed production in these crops.

To urgently address these emerging issues, a new research program was initiated aimed at providing the country's agriculture with highly adaptive varieties and more stable yields. For this purpose, soybean varieties exhibiting stress tolerance and greater ecological plasticity were identified from the germplasm collection. Varieties with notably early maturity and positive traits include *Anușca*, *Livius*, *Simona*, *Iug 3*, *VNIIS-1*, and *Fischebe*, with vegetation periods of 75–85 days. Their use in initial breeding material creation contributed to the development of new early ideotypes with reduced sensitivity to climatic stresses. Early maturing varieties such as *Deia*, *Amedia*, and *Dumitrița* belong to this group.

The higher adaptability of the new varieties is due to architectural modeling of soybean plants, achieved through hybridization with Chinese forms (*Tillin*, Nr. 4), Ukrainian varieties (*Anușca*, *Anastasia*), and Romanian varieties (*Daciana*, *Ileana*).

Phenotypic modeling is expressed by:

- Reduction of leaf surface area;
- Reduction in the number of branches;
- Decrease in leaf insertion angle, etc.

Under current climatic conditions, new varieties such as *Moldovița*, *Flamura*, *Augustina*, and *Viorela* have gained wider agricultural adoption, demonstrating greater yield stability between 3000 and 3500 kg/ha.

A key advantage of soybean varieties is the protein and lipid content of the seeds. Newly registered varieties like *Flamura*, *Augustina*, *Deia*, *Amedia*, and *Albiflora* have shown protein content above 40% and fat content above 23%, resulting from the deliberate use of parental lines with high protein and fat content in breeding programs.

The higher productivity, quality, and adaptability of the new soybean varieties will maintain the brand "soybean—the crop of the future."

Bean cultivation remains one of the most widely used sources of human food. In arid conditions, bean plants show pronounced negative responses during generative phases, leading to severe reductions in seed yield.

To mitigate the negative impact of climatic factors, forms with lower sensitivity were identified from the laboratory's germplasm. Notable varieties include *Haricovscaia 9*, *Dobrudjanscaia 7*, *Motoriscaia Belaia*, *Kentwood*, and *Aurora*, which served as the basis for new varieties such as *Marița*, *Petrela*, *Garofița*, and *Mirabela*, with production potentials of 2500–3500 kg/ha.

In response to domestic and international market demands for larger seed size and varied seed coloration, genotypes from diverse geographical origins such

as *Magna*, *Basarabca 1*, *Muhranula*, *Bistrenschii*, *Borloti*, and local populations like *Iabloana* and *Provadia* have been used as sources to create new varieties.

The new genotypes obtained from these parental lines are currently undergoing breeding evaluation. An important objective in bean breeding has been the improvement of seed quality. Protein donor varieties such as *Nerusa*, *Sanilac*, *Gratiot*, *Aluna*, *Universal*, and *Nebraska* have contributed to the enhancement of protein content in new varieties *Mariţa*, *Garofiţa*, and *Mirabela*, which contain 24–29% protein.

One of the most significant traits for mechanized harvesting suitability is the height of the first pod insertion point. To this end, varieties like *Porumbiţa*, *Timpurie*, *Oca*, *Magna*, and *Tui* have been included in hybridization programs.

The broad adoption of high-performing legume varieties (pea, soybean, bean) will help minimize protein deficits in human nutrition and animal feed.

## CONCLUSIONS

1. The results achieved in the breeding of legume crops (pea, soybean, and bean) in recent years are attributed to the efficient use of available genetic resources.
2. The new pea varieties *Nadia* and *Graţiana* exhibit very good performance in the field in terms of seed yield as well as satisfactory resistance to phytopathogenic agents.
3. The new soybean varieties *Moldoviţa*, *Albiflora*, and *Viorela* belong to the group of technological varieties due to their specific plant architecture, which partially mitigates the negative effects of unfavorable environmental factors.
4. The new bean varieties *Petrela*, *Clarina*, and *Mirabela*, which possess a range of positive traits and high taste qualities, occupy a prominent position among the most appreciated products.
5. The reintegration of pea into the national crop rotation, the cultivation of productive soybean varieties with satisfactory ecological plasticity, and the widespread use of new bean varieties in both domestic and international markets will strengthen the country's agricultural sector, addressing some strategic challenges of the National Research and Innovation Program.

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# NEW TRAINING SYSTEMS FOR HIGH-DENSITY CHERRY PLANTATIONS

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***Abstract.*** *This research evaluated vegetative growth, yield and fruit quality of different cherry varieties using different crown-forming systems and planting densities. The experimental orchards were established in the central (2015) and northern (2018) areas of the Republic of Moldova, using Black Star, Regina, Summit and Sweet Sharetta varieties, grafted on Gisela 6 rootstock. For each variety- rootstock combination and training system, varying densities were established, ranging from 1250 trees/ha (for thin-stem, cup, Kym Green Bush) to 2500 trees/ha (for thin-stem, improved thin-stem, structured vertical axis). Black Star, grown under the thin-stem system, obtained the highest yields between 9.39 and 10.92 t/ha at the age of 7-10 years, significantly exceeding the values obtained for the crowns cup (8.11-9.67 t/ha) and Kym Green Bush (7.65-8.22 t/ha). For varieties Regina, Summit and Sweet Sharetta planted at 4x1 m spacing, the improved thin spindle crown shape had a modest impact on productivity, but provided important benefits on fruit size uniformity along the entire length of the axis.*

***Keywords: C. avium, rootstock, planting distances, crown forms, yield.***

## INTRODUCTION

The crown forms of fruit trees are diverse and depend on the species, variety, pruning and maintenance method, available space, purpose of harvesting and crop management. [In cherry (C. avium), the following forms of crowns are used: round stepped stepped crowns with large volume, well distributed and open branches to allow light penetration and air circulation, pyramidal stepped stepped crowns with shorter and well arranged lateral branches for good light exposure, vase shaped

crowns - Spanish Tufa, Improved vase, Late vase with well spaced lateral branches, fusiform type crowns - Super Fuss Axis, Thin Spindle, Super Slender Ax (SSA), Tall Spindle Axe (TSA), Tall Fusiform Axis, i.e. elongated and narrow with well balanced branching, with main and secondary branches arranged in a balanced manner for uniform light exposure, favoring photosynthesis and fruit development [3,4,5].

In all of the fusiform training systems listed, the key feature is that pruning is mainly carried out on 1-3 year old branches, which is an innovative management approach. However, cherry varieties vary in their adaptability to the plantation structure appropriate to training systems in intensively managed high-density orchards. The objective of this research was to compare training systems suitable for high-density orchards in combination with different varieties, grafted on Gisela 6 rootstock, to assess the control on vegetative growth and productivity.

## MATERIALS AND METHODS

Research on the realization of modern cherry plantations with a high level of productivity is ongoing (years 2018-2024) in the central fruit-growing area, at Ustia SRL "Staragrogroup", Dubasari rayon, and in the northern area, at Sturzeni, Riscani rayon, Republic of Moldova. This research is carried out in two experiments, using the varieties Black Star, Regina, Summit and Sweet Sharetta, grafted on Gisela 6 rootstock.

Experiment 1 was established in the fall of 2015 and includes the cherry variety Black Star, with trees planted at a spacing of 4x2 m. In this experiment, low-volume crown forms were studied, namely Thin Spindle, Cup and Kym Green Bush. Experiment 2 was initiated in the fall of 2018, with trees planted at a distance of 4x1 m, being managed according to the crown forms V1- Thin Spindle, V2-Ax vertical and V3-Improved Thin Spindle [6]. In both experiments the rows of trees are located in the north-south direction.

Physiological studies of the trees were carried out annually at the end of the growing season. On 32 trees, tree height, crown width and crown length were measured, the yield at fruit maturity was determined for each tree, expressed in kg fruit per tree and reported per hectare. During fruit harvest, fruit distribution (kg/pom) was assessed by tree height at 0-160 cm, 160-240 cm and 240-400 cm from the ground on 8 identical trees of each variety. The fruits were divided into 3 fractions according to their diameter (22-26 mm; 26-30 mm; greater than 30 mm). The results of the study were tested at a significant level of 0.05 using Tukey's test. Soil maintenance, irrigation, fertilization and protection of the trees from diseases and pests were carried out as appropriate [7,8].

## RESULTS AND DISCUSSION

Vegetative growth of Black Star cherry variety was studied in dynamics (Table 1). So, the Black Star variety, during the growing and fruiting period, recorded the height of the trees of 375-400 cm, which corresponds to the optimal parameters for cherry orchards with trees grafted on low vigor rootstock, planted at the row spacing of 4 m [9,10]. The crown width of cherry trees shows that with the advancement of the trees in age the crown width increased, reaching optimal sizes in the 6-7 years after planting being 210-240 cm. In the following years the crown width depended on the distance between the rows and the height of the trees and remained at about 250 cm, regardless of the crown shape. As the crown length (200-250 cm) is a basic index in tree formation, it is clear that at the age of 6-7 years after planting the crowns of Black Star trees have joined the rows of trees and formed continuous rows.

Table 1. Phytometric indicators of Black Star cherry trees according to crown shape (planting distance 4x2 m, tree age 6-7 years)

| Crown shape | Tree height, cm | | Crown width, cm | | Crown length, cm | |
|---|---|---|---|---|---|---|
| | Year 2020 | Year 2021 | Year 2020 | Year 2021 | Year 2020 | Year 2021 |
| Thin spindle | 400 | 400 | 220 | 240 | 215 | 245 |
| Cup | 375 | 400 | 210 | 241 | 200 | 250 |
| Kym Green Bush | 380 | 400 | 236 | 241 | 229 | 250 |

Based on morphological indicators of the crown, the level of ground cover with crown projection, lateral area and crown volume were calculated (Table 2). The trees of Black Star variety at the age of 7 years use more than 60% of the area reserved for the tree, form a lateral crown area of 21650-22025 thousand m2/ha, being higher in the case of the crown form Kym Green Bush. The volume of the crown, as a summary indicator of the parameters of the orchard plantation, was 15483-19318 m3/ha. The trees formed after the cup and Kym Green Bush crown system formed the highest values with 14.6-24.7% compared to the thin spindle crown form. In conclusion we mention that the structure of the vegetative assembly of the Black Star cherry plantation, grafted on Gisela 6, planted at a distance of 4x2 m provides a continuous crown volume in the direction of the row, which at the age of 7 years receives more than 60% of solar energy.

Table 2. Structure of the vegetative set in Black Star cherry trees according to crown shape (planting distance 4x2 m, age of trees 7 years)

| Crown shape | Ground cover, % | Lateral crown area, thousand $m^2$/ha | Crown volume, $m^3$ | |
|---|---|---|---|---|
| | | | tree | ha |
| Thin spindle | 60,0 | 21650 | 12,3 | 15483 |
| Cup | 60,2 | 21700 | 14,2 | 17744 |
| Kym Green Bush | 60,2 | 22025 | 15,4 | 19318 |

The technology of cherry cultivation, including the crown management system and orchard management determines the solar energy conversion, earliness, consistent yield and quality of fruit competitive in the market [3,4,7,11,12]. The fruit yield of Black Star cherry trees grafted on Gisela 6 rootstock, planted at 4x2 m spacing, differs by year and crown shape (Table 3). The presented data show that the yield in 2021 was low (2.99-4.40 kg/pom) due to climatic conditions during flowering, and in the following years the yield was 6.12-7.97 kg/pom or 7.96-10.92 t/ha, being significantly higher in trees formed after thin spindle crown. Thus, in the Black Star variety it can be observed that the thin spindle crown during the fruiting period of the trees has the highest distinctly significant values (9.39-10.92 t/ha) compared to the cup (8.11-9.67 t/ha) and Kym Green Bush (7.65-8.22 t/ha) crowns.

Table 3. Fruit yield of Black Star cherry trees by crown shape (planting distance 4x2 m, age of trees 7-10 years)

| Crown shape | Kg/tree | | | | t/ha | | | |
|---|---|---|---|---|---|---|---|---|
| | Year 2021 | Year 2022 | Year 2023 | Year 2024 | Year 2021 | Year 2022 | Year 2023 | Year 2024 |
| Thin spindle | 4,40 | 7,51 | 7,97 | 8,72 | 5,50 | 9,39 | 9,96 | 10,92 |
| Cup | 2,99 | 7,04 | 6,49 | 7,74 | 3,73 | 8,81 | 8,11 | 9,67 |
| Kym Green Bush | 3,12 | 6,37 | 6,12 | 6,58 | 3,90 | 7,96 | 7,65 | 8,22 |
| DL 5% | 0,39 | 0,28 | 1,17 | 1,34 | 0,39 | 0,28 | 1,17 | 1,34 |

The fruit yield (Table 4) in high density plantations was 4.56-4.76 kg/inch for Regina and 6.43-6.97 kg/inch for Sweet Sharetta and Summit with insignificant increase in the crown shape thin spindle compared to vertical axis and improved thin spindle. If we refer to the yield per hectare, compared to the data presented by other authors [13,14], it can be mentioned that the yield of the varieties taken in the study are averages of those considered normal.

Table 4. Fruit yield of cherry trees by variety and crown shape, mm (Planting distance 4x1 m, year 2024)

| Crown shape | Regina variety | | Sweet Sharetta variety | | Summit variety | |
|---|---|---|---|---|---|---|
| | Kg/tree | t/ha | Kg/tree | t/ha | Kg/tree | t/ha |
| V1 | 4,76 | 11,91 | 6,9 | 17,25 | 6,8 | 17 |
| V2 | 4,56 | 11,41 | 6,63 | 16,58 | 6,43 | 16,08 |
| V3 | 4,63 | 11,58 | 6,97 | 17,40 | 6,43 | 16,08 |
| DL 5% | 1,39 | 1,85 | 1,39 | 1,85 | 1,39 | 1,85 |

During the harvest, the distribution of fruits by tree height was evaluated on the sectors of ground distance 0-160 cm, 160-240 cm and 240-400 cm (Table 5). In the case of thin spindle crown shape at ground distance 0-160 cm the diameter of fruits of 22-26 mm constituted 32.8%, 26-30 mm - 48.8% and larger than 30 mm were only 18.4%. In

the central part of the crown (160-240 cm) the number of fruits with the diameter of 26-30 cm (50.03%) and larger than 30 mm (26.43%) increased. In the upper third of the crown the number of fruits with the diameter of more than 30 mm increased significantly and reached 38.03%. The proportion of fruits in the vertical axis system practically changed identically to the thin crown, with 45.52% of fruits with a diameter of 26-30 mm.

Table 5. Distribution of fruits on Regina cherry trees by crown shape, mm (Planting distance 4x1 m, year 2024)

| Crown shape | Distance from ground, cm | Fruit diameter | | |
|---|---|---|---|---|
| | | 22-26 mm | 26-30 mm | Greater than 30 mm |
| | | Fruit weight, % | | |
| Thin spindle | 0-160 | 32,8 | 48,8 | 18,4 |
| | 160-240 | 23,53 | 50,03 | 26,43 |
| | 240-400 | 21,36 | 40,6 | 38,03 |
| | Average | 25,90 | 46,48 | 27,62 |
| Ax vertical | 0-160 | 33,34 | 45,82 | 20,84 |
| | 160-240 | 24,54 | 49,13 | 26,33 |
| | 240-400 | 19,45 | 41,62 | 38,93 |
| | Average | 25,78 | 45,52 | 28,7 |
| Improved thin spindle | 0-160 | 22,53 | 39,06 | 38,4 |
| | 160-240 | 15,03 | 46,8 | 38,16 |
| | 240-400 | 24,43 | 39,96 | 35,6 |
| | Average | 20,66 | 41,94 | 37,39 |

The distribution of fruit on the improved thin-stemmed Fus crown is essentially different compared to the thin-stemmed and vertical axis crowns, with a percentage of fruit larger than 30 mm in size per tree height. Thus, at the distance from the ground 0-160 cm the proportion of fruit larger than 30 mm was 38.4%, at 160-240 cm - 38.16% and 35.6% in the upper part of the crown.

## CONCLUSIONS

The thin spindle crown form used on Black Star cherry, grafted on Gisela 6 rootstock and planted at 4x2 m spacing, proved to be the most effective for ensuring higher yield and continuous crown volume in the direction of the row, which, at 7 years of age, captures more than 60% of the available solar energy.

For the varieties Regina, Summit and Sweet Sharetta, grafted on Gisela 6 and planted at a density of 2500 trees/ha, the improved thin spindle crown shape had a modest impact on productivity. However, it provided important benefits in fruit size uniformity along the entire length of the axis, facilitating more efficient harvesting and marketing.

The thin-spindle and ax vertical crown forms were less effective in

distributing the fruit evenly on the spindle than the improved form. In conclusion, the improved thin-spindle crown shape is recommended for cherry crops grafted on Gisela 6 rootstock, as it provides an optimal balance between productivity and quality and is a suitable choice for high quality and high yielding crops.

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# EVALUATION OF INBRED LINES OF *SALVIA SCLAREA* L.

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***Abstract.*** *In the process of breeding the species Salvia sclarea L., a fundamental objective was to diversify the initial breeding material and select lines with valuable quantitative characters and increased content of essential oils. As a result of research conducted with a large number of inbred lines, it was found that there are lines with full flowering in the first year of vegetation, as well as lines with an increased content of essential oils. Finally, lines with an essential oil content exceeding 2% (dry matter) were identified: M-69 453-82 $S_{16}$ – 2,191%; M-69 428-82 $S_{16}$ – 2,227%; M-69 815-82 $S_{16}$ – 2,140%. Lines with essential oil content exceeding 1%: AP 2-11 $S_6$– 1,642 %; AP 11-11 $S_6$– 1,642 %; AP 28-11 $S_6$– 1,221 %; AP 30-11 $S_6$– 1,912 %; AP 66-11 $S_6$– 1,643 %; AP 67-11 $S_6$– 1,573 %; NC 6 -11 $S_6$– 1,356 %; NC 4 -11 $S_6$– 1,358 %; NC 19 -11 $S_6$– 1,499 %; NC 38 -11 $S_6$– 1,646 %; NC 98 -11 $S_6$– 1,810 % etc. Thus, the selected lines were subjected to inbreeding during the flowering phase, in order to reproduce promising lines, which will later be included in breeding programs. The aim of the investigations was to create, evaluate and select initial breeding material with high-performance genetic potential.*

***Keywords: Salvia sclarea L., inbred lines, inflorescence, vegetation period, essential oil.***

## INTRODUCTION

In the Republic of Moldova, the genus *Salvia* L. is represented by 12 species [5; 6; 7;11; 14]. In cultivation, *Salvia sclarea* L., *S. officinalis* L., as well as the decorative species *S. splendens* Ker. Gawl. are used. As a medicinal plant, *S. sclarea* L. has been known and used in phytotherapy since antiquity [1; 3; 4; 5; 6; 7; 8; 10]. The use of aromatic and medicinal plants in perfumery, cosmetics, pharmacology and the food industry is continuously developing both in our country and in the European Union. The areas cultivated with these species have expanded in recent years. Their importance becomes even more evident in the context of climate change, which requires the creation of varieties resistant to drought and high temperatures, which ensure constant and quality productivity. The research results demonstrate that in dry years the *S. sclarea* L. genotypes included in the

study ensured high productivity [1; 2; 9]. The use of medicinal plants for therapeutic purposes is based on the active principles, the compounds that are synthesized and accumulated. It is known to have antiseptic, antispasmodic, sedative, carminative, diuretic, etc. actions, as well as being used as a flavoring agent and preservative in food products [3; 4; 5; 6; 7;11]. Scientific studies focused on *S. sclarea* L. are focused on diversifying the initial breeding material adapted to climate change. Breeding work is necessary to contribute to the cultivation and expansion of cultivated areas with *S. sclarea* L. genotypes. The process of creating new varieties, with increased plasticity, that meet the requirements of producers depends on the existence of a vast initial breeding material that includes inbred lines with valuable agronomic indices. In this context, the creation, evaluation of inbred lines and the identification of genotypes with valuable breeding indices, which would ensure increased productivity of the raw material, as well as of the essential oil, represent a primary task. The aim of this study is to create and select inbred lines with high-performance breeding qualities, which will later be included in the hybridization process.

## MATERIALS AND METHODS

The biological material used in the present study was represented by 63 inbred lines $S_6$ – $S_{16}$ of different origins: 28 - derived from the Ambra Plus variety; 17 – Natali Clary; 13 – M–69; 2 – Cr. p.; 1–S. - Turkmen/N; 1–S–1122; 1–V–24, second year of vegetation. The field experiment with inbred lines of *S. sclarea* L. began on November 16, 2022. The varieties they came from served as controls for the inbred lines obtained. During the vegetation period, phenological, biometric and visual assessments were carried out. The morphological characters that determine the productivity and amount of essential oil were studied: plant height, inflorescence length, number of branches of I and II degree, as well as the number of whorls on the central spike of the inflorescence. Diversification of the initial breeding material in *S. sclarea* L. in recent years continued by the inbreeding method. The plants selected as parents of future lines were forcibly self-pollinated by closing the inflorescence in an insulator, at the beginning of the flowering phase, forcing pollination with its own pollen. Previously, the inflorescences were subjected to a special toilet [1; 2; 7;10]. The essential oil content was determined in the full flowering phase, by hydro distillation of fresh inflorescences in the Ginsberg apparatus [12; 15; 16;17]. The plant material was sampled in the morning, between 8:00 and 10:00, during the mass flowering period, when the seeds in the vertices of the central spike of the panicle were brown in color. The crushed plant material (100 g of inflorescences) is placed in a flat-bottomed flask, volume 1000 ml, then 200 ml of water is added, the distillation process lasts one hour. At the same time, the moisture content of the raw material is determined for each sample,

using the oven drying method at a temperature of 105°C, for 8 hours. To determine the moisture content, samples of plant material are taken from each sample in aluminum bottles, which are preliminarily weighed. Then, the bottles with plant material are weighed until dry. After the samples are dried, they are weighed. The data are included in the laboratory records, which are later needed to recalculate the essential oil content from the fresh substance to the dry substance, thus avoiding possible errors related to differences in moisture content. The essential oil samples were dehydrated with anhydrous $Na_2SO_4$ and stored in a freezer for further analysis The statistical interpretation of the obtained experimental data was carried out according to the methods in force and using the STATISTICA program. [8;12;13; 15;16].

## RESULTS AND DISCUSSIONS

Multiannual research conducted on the species *S. sclarea* L. has demonstrated that obtaining inbred lines with new, valuable characters through the inbreeding method is effective [1; 7; 10]. Scientific research on the creation and diversification of initial breeding material, its evaluation being carried out within the IGFPP of USM. The investigations focused on the study of inbred lines ($S_6$-$S_{16}$) of *S. sclarea* L. from different origins. Climate change requires the creation of initial breeding material, genotypes resistant to drought and high temperatures. The creation of inbred lines with high essential oil content, which flower in the first year, is an important goal in salvia breeding. The results of the evaluation of the inbred lines demonstrated that they possess certain valuable quantitative characters, such as: plant height, inflorescence length, number of branches of degree I and II, number of whorls on the central spike of the inflorescence, increased essential oil content. Evaluating the inbred lines in 2024, the second year of vegetation, genotypes were selected that stood out for total flowering in the first year of vegetation: AP 2-11 $S_6$; AP 29-11 $S_6$; AP 30-11 $S_6$; AP 30-11 $S_6$; AP 32-11 $S_6$; AP 35-11 $S_6$; AP 41-11 $S_6$; AP 49-11 $S_6$; AP 59-11 $S_6$; AP 62-11 $S_6$; AP 63-11 $S_6$; AP 64-11 $S_6$; AP 66-11 $S_6$; AP 67-11 $S_6$; AP 73-11 $S_6$; AP 77-11 $S_6$; AP 81-11 $S_6$; AP 82-11 $S_6$; AP 97-11 $S_6$; AP 106-11 $S_6$; AP 108-11 $S_6$; AP 113-11 $S_6$; AP 115-11 $S_6$; NC 1-11 $S_6$ ; NC 4-11 $S_6$ ; NC 6-11 $S_6$ ; NC 38-11 $S_6$; NC 45-11 $S_6$; NC 56-11 $S_6$ ; NC 60-11 $S_6$ ; NC 75-11 $S_6$; NC 77-11 $S_6$ ; NC 78 -11 $S_6$; NC 96 -11 $S_6$; M-69 489 $S_{16}$; Cr. p. 11 $S_{16}$; Cr. p. 99 $S_{16}$; V-24-86 844 $S_{14}$; M-69 286 $S_{14}$; M-69 257-82 $S_{16}$. Inbred lines are distinguished by the periods of flowering and technical ripening. In the second year of vegetation, these lines showed satisfactory development: plant height from 95.7 cm in line AP 11-11 $S_6$ to 126.4 cm in line NC 4-11 $S_6$ (Tab. 1). It is important that the proportion of inflorescence length constitutes over 50% of plant height, which promises high productivity. Examining the lines by character - inflorescence length, which varies from 46.8 cm to 68.7 cm, it was found that these

lines have long, large inflorescences with a large number of branches. The length of the inflorescences varied from 50.6 cm in the line (AP 11-11 $S_6$) to 68.7 cm (line NC 6 -11$S_6$). Thus, the length of the inflorescence constitutes over 50% of the plant height. Compact inflorescences, (11.8-19.4) degree I branches and (19.2-49.2) degree II branches. These quantitative characters directly influence the synthesis and accumulation of essential oil in the raw material.

Table 1. Quantitative characters in inbred lines of *Salvia sclarea* L., 2nd year of vegetation, year 2024

| Inbred line code | Plant waistline, cm | Inflorescence length, cm | Number of ramifications | | Essential oil, %, dry matter, |
|---|---|---|---|---|---|
| | | | first degree | second degree | |
| | X ± sX | X ± Sx | X ± sX | X ± sX | |
| AP 2-11 $S_6$ | 87,1±8,9 | 46,8±8,0 | 11,8±1,7 | 21,2±4,7 | 1,642 |
| AP 11-11 $S_6$ | 95,7±6,3 | 50,6±5,4 | 14,2±1,7 | 25,4±6,4 | 1,208 |
| AP 28-11 $S_6$ | 126,0±8,5 | 61,8±4,9 | 16,0±3,2 | 30,8±11,0 | 1,221 |
| AP 29-11 $S_6$ | 121,2±10,5 | 61,2±8,1 | 15,0±1,9 | 29,0±7,0 | 0,940 |
| AP 30-11 $S_6$ | 102,9±4,6 | 59,0±3,0 | 14,0±1,6 | 27,0±9,6 | 1,912 |
| AP 32-11 $S_6$ | 119,1±7,2 | 60,6±5,7 | 14,4±2,0 | 27,0±8,6 | 1,719 |
| AP 33-11 $S_6$ | 110,2±7,3 | 54,7±7,1 | 13,2±1,3 | 26,4±6,5 | 1,586 |
| AP 34-11 $S_6$ | 108,1±6,4 | 56,4±5,1 | 15,8±3,0 | 34,4±12,0 | 0,889 |
| AP 35-11 $S_6$ | 100,0±7,5 | 52,7±4,5 | 13,6±1,5 | 28,4±6,6 | 1,395 |
| AP 41 -11$S_6$ | 114,9±8,9 | 59,7±5,1 | 14,6±2,3 | 28,6±9,6 | 1,476 |
| AP 49-11 $S_6$ | 118,7±3,2 | 62,3±9,5 | 15,2±2,3 | 31,0±8,3 | 0,966 |
| AP 54-11 $S_6$ | 122,2±9,3 | 61,4±6,1 | 15,8±2,3 | 34,0±8,0 | 0,875 |
| AP 59-11 $S_6$ | 125,1±8,0 | 63,7±6,4 | 15,0±2,1 | 28,2±7,7 | 0,742 |
| AP 62-11 $S_6$ | 98,3±9,7 | 52,6±8,6 | 12,8±2,1 | 23,3±10,4 | 1,192 |
| AP 63-11 $S_6$ | 112,3±9,7 | 57,2±5,4 | 14,0±1,8 | 25,2±9,0 | 1,358 |
| AP 64-11 $S_6$ | 119,9±5,5 | 61,9±4,4 | 15,4±2,6 | 30,2±7,8 | 1,279 |
| AP 66-11 $S_6$ | 120,0±5,5 | 65,7±3,7 | 14,8±2,1 | 30,2±8,2 | 1,643 |
| AP 67-11 $S_6$ | 119,7±9,4 | 62,1±4,9 | 16,2±2,3 | 28,6±8,6 | 1,573 |
| AP 68-11 $S_6$ | 99,1±4,7 | 49,7±5,0 | 12,6±0,9 | 22,2±5,0 | 1,008 |
| AP 73-11 $S_6$ | 114,5±6,5 | 59,6±5,8 | 14,2±1,4 | 26,8±4,8 | 1,276 |
| AP 77-11 $S_6$ | 108,3±6,3 | 55,0±5,7 | 13,4±1,3 | 22,4±6,0 | 0,782 |
| AP 81-11 $S_6$ | 108,7±10,8 | 55,0±5,3 | 13,4±1,8 | 27,4±10,0 | 1,165 |
| AP 82-11 $S_6$ | 112,1±6,0 | 53,3±11,8 | 14,4±2,4 | 28,0±9,6 | 0,801 |
| AP 97-11 $S_6$ | 113,5±10,7 | 58,0±6,4 | 14,2±1,1 | 26,0±6,9 | 2,250 |
| AP 106-11 $S_6$ | 102,6±6,3 | 55,9±5,5 | 14,2±1,7 | 25,4±7,0 | 1,485 |
| AP 108-11 $S_6$ | 114,1±5,4 | 60,2±3,4 | 15,0±2,1 | 26,4±7,9 | 1,049 |
| AP 113-11 $S_6$ | 124,4±5,9 | 67,6±3,8 | 17,6±1,8 | 43,4±11,6 | 0,692 |
| AP 115-11 $S_6$ | 120,4±11,7 | 57,9±5,2 | 13,8±1,7 | 24,6±6,9 | 1,232 |
| NC 1 -11 $S_6$ | 108,6±8,5 | 54,4±5,4 | 13,2±1,6 | 26,2±10,4 | 0,949 |
| NC 6-11 $S_6$ | 144,2±11,4 | 68,7±6,9 | 17,6±1,8 | 33,2±10,6 | 1,356 |
| NC 4 -11 $S_6$ | 126,4±4,9 | 61,6±4,7 | 19,4±2,8 | 49,2±14,0 | 1,358 |
| NC 11 -11 $S_6$ | 104,5±6,1 | 56,3±5,8 | 15,6±3,8 | 28,6±9,1 | 1,753 |
| NC 13-11 $S_6$ | 107,0±6,4 | 54,5±6,0 | 14,0±1,8 | 33,8±5,2 | 1,131 |
| NC 19 -11 $S_6$ | 107,9±8,3 | 55,2±4,5 | 13,8±1,9 | 30,0±9,2 | 1,228 |
| NC 38 -11$S_6$ | 131,9±7,2 | 65,3±4,1 | 16,0±2,4 | 35,6±7,4 | 1,646 |
| NC 45 -11$S_6$ | 127,0±9,0 | 60,8±5,3 | 15,0±2,1 | 34,8±10,7 | 1,264 |
| NC 56-11 $S_6$ | 110,7±7,0 | 54,8±4,5 | 14,2±1,9 | 28,2±5,2 | 0,943 |

| | | | | | |
|---|---|---|---|---|---|
| NC 60-11 $S_6$ | 132,9±11,1 | 66,3±8,3 | 16,2±2,3 | 33,4±9,4 | 0,904 |
| NC 61-11 $S_6$ | 112,2±6,7 | 59,8±7,9 | 14,6±2,3 | 32,0±13,3 | 1,703 |
| NC 75-11 $S_6$ | 122,7±7,8 | 60,4±4,1 | 15,4±1,3 | 37,2±12,1 | 0,726 |
| NC 77-11 $S_6$ | 103,5±4,4 | 55,8±2,5 | 13,8±1,7 | 28,0±7,3 | 0,713 |
| NC 78-11 $S_6$ | 102,1±4,1 | 54,3±4,1 | 13,8±1,9 | 25,4±6,0 | 1,202 |
| NC 96-11 $S_6$ | 102,4±7,0 | 55,1±5,2 | 14,6±2,6 | 28,8±8,1 | 1,107 |
| NC 98-11 $S_6$ | 120,8±9,1 | 64,7±6,8 | 14,6±1,8 | 30,0±9,6 | 1,810 |
| NC 100-11$S_6$ | 119,1±5,9 | 58,0±6,6 | 13,6±1,5 | 25,2±5,0 | 1,663 |
| M-69 489 $S_{16}$ | 111,7±4,7 | 57,0±4,8 | 12,6±1,3 | 19,2±4,0 | 0,934 |
| M-69 418 $S_{16}$ | 97,2±2,1 | 50,9±1,6 | 12,3±1,3 | 27,2±6,1 | 1,687 |
| M-69 655 $S_{14}$ | 119,4±5,9 | 61,4±3,9 | 15,4±2,3 | 33,0±7,7 | 1,407 |
| S-1122 60 $S_{14}$ | 132,8±9,7 | 65,9±6,0 | 15,8±2,3 | 28,0±7,3 | 1,208 |
| Cr. p. 11 $S_{16}$ | 117,0±8,1 | 61,2±6,0 | 15,8±3,3 | 35,2±14,0 | 0,845 |
| Cr. p. 99 $S_{16}$ | 119,3±7,9 | 61,6±3,6 | 15,8±2,7 | 37,6±9,4 | 1,554 |
| V-24-86 844 $S_{14}$ | 111,1±3,2 | 60,6±4,1 | 13,8±1,7 | 27,6±4,6 | 1,110 |
| M-69 489 $S_{14}$ | 109,5±8,3 | 61,4±8,1 | 14,6±2,1 | 29,8±7,2 | 1,331 |
| M-69 286 $S_{14}$ | 111,6±4,9 | 57,8±5,2 | 17,8±1,9 | 40,0±12,8 | 1,616 |
| M-69 147 $S_{14}$ | 129,7±9,0 | 63,0±6,3 | 16,6±2,3 | 37,8±12,0 | 0,962 |
| M-69 453-82$S_{14}$ | 116,3±8,9 | 58,1±5,4 | 13,6±1,2 | 27,6±5,7 | 2,192 |
| M-69 257-82 $S_{16}$ | 96,4±5,6 | 53,3±5,3 | 12,8±1,3 | 22,0±5,0 | 1,203 |
| M-69 485-82 $S_{16}$ | 100,6±6,6 | 55,1±5,7 | 13,8±1,9 | 24,2±5,6 | 1,531 |
| M-69 403-82 $S_{16}$ | 87,4±7,5 | 47,4±3,9 | 13,2±1,0 | 21,8±3,9 | 1,709 |
| M-69 756-82 $S_{16}$ | 112,8±4,0 | 61,1±6,1 | 14,0±2,3 | 20,4±4,5 | 1,692 |
| M-69 428-82 $S_{16}$ | 98,8±5,4 | 51,8±2,7 | 25,8±5,8 | 25,8±5,8 | 2,227 |
| M-69 815-82 $S_{16}$ | 117,0±4,3 | 62,4±6,9 | 13,6±1,5 | 29,0±9,2 | 2,140 |

In inbred lines: AP 29-11 $S_6$; AP 34-11 $S_6$; AP 49-11 $S_6$; AP 54-11 $S_6$; AP 64-11 $S_6$; AP 66-11 $S_6$; AP 113-11 $S_6$; NC 6-11 $S_6$; NC 4-11 $S_6$; NC 11-11 $S_6$; NC 13-11 $S_6$; NC 38-11 $S_6$; NC 45-11 $S_6$; NC 60-11 $S_6$; NC 61-11 $S_6$; NC 75-11 $S_6$; NC 98-11 $S_6$, compact inflorescences were observed, with a large number of branches (30.8-49.2) of the second degree (Tab.1).

The high and very high oil content in *S. sclarea* L. is ensured by quantitative characters, which directly influence productivity. Also, inbred lines $S_6$ – $S_{16}$, not subject to inbreeding depression, with very high essential oil content in the proportion of 2.192 – 2.250%, were highlighted. The lines synthesized and accumulated essential oil in inflorescences that exceeded the quota of 1.5% (dry matter): AP 30-11 $S_6$ - 1,912 %; AP 32-11 $S_6$ - 1,719 %; AP 66-11 $S_6$ - 1,643 %; AP 97-11 $S_6$ - 2,250%; NC 11 -11 $S_6$ - 1,753 %; NC 38 -11 $S_6$ - 1,646 %; NC 98 -11 $S_6$ - 1,810 %; NC 100 -11 $S_6$ - 1,663 % etc. (Tab.1).

## CONCLUSIONS

63 inbred lines $S_6$ – $S_{16}$ of different origins were created and studied: 28 - derived from the Ambra Plus variety; 17 – s. Natali Clary; 13 – s. M–69; 2–Cr. p.; 1–S. s. Turkmen/N; 1–S–1122; 1–V–24. The evaluated inbred lines have different flowering and ripening periods: early, mid-early and late.

The inbred lines $S_6$ – $S_{16}$ from the second year of vegetation developed

plants with a height of (87.4 – 126.2 cm), very long inflorescences (46.8 – 68.7 cm), compact, first-degree branches (11.8-19.4) and second-degree branches (19.2-49.2).

Early, medium and late lines with high content (1.005 – 1.694%) of essential oil were selected for use in hybridizations as parental forms.

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# USE OF BIOFORTIFICATION PRINCIPLES IN DEVELOPING A MAIZE GERMPLASM COLLECTION

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**Abstract**: *This study explores the application of biofortification principles in developing a maize genetic collection with specific germplasm aimed at improving nutritional traits. The main objective was to evaluate and classify 174 maize genotypes based on their genetic potential for enhanced nutrient content using traditional and modern breeding tools. The research was conducted at the Laboratory of Applied Genetics and Biotechnology, Technical University of Moldova. A comprehensive methodological framework was employed, including classical selection, induced mutagenesis, polyploidization, haploidization, and molecular diagnostics. The results revealed a significant proportion of genotypes carrying key endosperm mutations such as o2, su1, wx1, and fl2, with 31% of the collection exhibiting double recessive combinations. Additionally, 9 lines and 15 hybrids were successfully converted into tetraploids. The study confirms the potential of these genotypes as valuable breeding material for biofortification. The genetic collection developed represents a strategic resource for enhancing the nutritional quality of maize and contributes to advancing sustainable agriculture and food security.*

***Keywords: biofortification, endosperm mutations, genetic diversity, maize, polyploidy***.

## INTRODUCTION

In the last decade, a new research direction has emerged in modern agricultural biology — biofortification. Biofortification is considered a set of scientific methods and technologies aimed at improving the nutritional qualities of cultivated plants using breeding methods and a range of biotechnologies [1,2]. Today, biofortification is recognized as part of the sustainable development strategy (contributing to the UN Sustainable Development Goals – SDGs) [3]. The leading research strategy in biofortification is characterized by an interdisciplinary approach, combining modern breeding-genetic and physiological-biochemical methods used to intentionally modify and/or enhance the nutritional qualities of key agricultural crops at various levels [4,5]. In this context, there is a strong link,

primarily, between biofortification and genetics, which can be clearly substantiated by the following points:

1. The foundation of biofortification lies in identifying and utilizing plant genotypes that naturally contain higher levels of desirable nutrients and critical micronutrients. This requires extensive research into genetic diversity within and between related species [4,6]. Advanced genetic methodologies, in combination with principles of metabolic engineering and bioinformatics, play a crucial role in identifying such "wild" or traditional genotypes [7].

2. Traditional breeding methods are fully applicable to solving many biofortification problems. For example, by crossing and selecting plants with desirable traits (e.g., in maize — high iron or provitamin A content), breeders can develop new varieties with improved nutritional value [1,7]. This process is based on the genetic laws of inheritance. However, it can be lengthy and may not always guarantee the desired result, as selection for one trait can affect other genes (pleiotropy and genetic correlations).

3. At the modern level, solutions to biofortification challenges involve targeted genome modification (introducing genes from other organisms responsible for synthesizing or accumulating specific nutrients into plant genomes) or genome editing (introducing precise DNA changes through insertion, deletion, or relocation of fragments using specifically engineered endonucleases) [2].

4. The acceleration of the breeding process aimed at achieving the main goals of biofortification can also be successfully implemented through marker-assisted selection (MAS). Thanks to advances in gene and QTL mapping, molecular markers can be used for the rapid and efficient selection of plants with desired biofortification-related genes [7].

In recent years, maize biofortification research has been actively developed at the Laboratory of Applied Genetics and Biotechnology, Technical University of Moldova — the successor of the former Department of Plant Breeding and Genetics led by Academician Andrei Palii. Building on national and international achievements in maize biochemical breeding, this work forms a foundation for advancing biofortification in Moldova [8–11]. While biofortification requires a comprehensive approach integrating agronomic and breeding considerations, this study focuses specifically on applying its principles to develop a maize germplasm collection with targeted genetic traits.

## MATERIALS AND METHODS

The subject of this study was an active maize germplasm collection comprising 174 genotypes, developed at the Laboratory of Applied Genetics and Biotechnology, Faculty of Agricultural, Forestry, and Environmental Sciences, Technical University of Moldova. The selection of optimal biofortification methods

followed a systematic approach, taking into account the nature of the targeted traits, technological feasibility, as well as temporal and economic constraints.

Priority has been given to plant breeding methods closely aligned with biofortification objectives, including traditional Mendelian selection, spontaneous and induced mutagenesis, polyploidization, haploid techniques, heterosis breeding, molecular diagnostics based on storage protein profiles, and biochemical characterization of the maize genotypes.

## RESULTS AND DISCUSSIONS

Maize genotypes in the specific germplasm collection were classified according to the following criteria:

*A. Donor-originator centers*

*B. Taxonomic characteristics (subspecies)*

*C. Endosperm structure genes*

*D. Ploidy level.*

*A.* The maize genotype collection was developed using materials from the following breeding centers: Republic of Moldova (National Seed Research and Production Center; successor of the State Agrarian University of Moldova — Faculty of Agricultural, Forestry, and Environmental Sciences, Technical University of Moldova); Ukraine (Yuriev Institute of Plant Production, Kharkiv); Russia (Krasnodar Agricultural Research Institute named after P. P. Lukyanenko, now part of the National Grain Center); Croatia (EU Seeds Gallery — International online seed store [12]). Selection and systematization principles of the materials from these originators are presented in sections B, C, and D.

*B.* In addition to the maize types traditionally used in Moldovan breeding and adapted to local agroclimatic conditions — including flint, dent, sweet, starch, waxy, popping, and pod corn — the collection also includes several subtropical forms. These forms, visually characterized by Figure 1, carry genetic traits associated with enhanced carbohydrate metabolism, photoperiod sensitivity typical of subtropical maize, lodging resistance, and anthocyanin pigmentation. The latter is particularly relevant for classical genetic studies using morphological markers.

*C.* A clear indicator of the targeted development of foundational breeding material with specific maize germplasm—serving as a basis for experimental biofortification efforts in Moldova—is that 118 out of the 174 genotypes in the collection are mutant lines with known endosperm structure genes. Figure 2 illustrates a pie chart summarizing the distribution of these mutations.

The chart is divided into two major categories: monogenic mutants (69%) and double recessive mutants (31%). Among the monogenic forms, the genotypic distribution is as follows: *o2* — 48%, *su1* — 22%, *wx1* — 18.5%, *fl2* — 9%, *su2* — 2.5%.

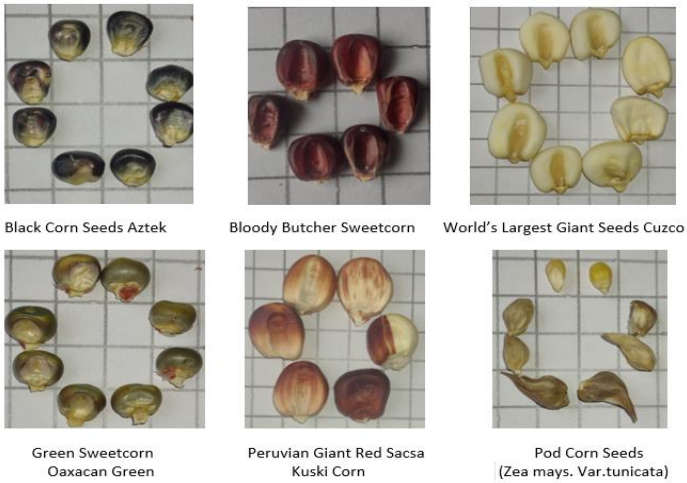


Figure 1. Visual phenotype of subtropical maize forms included in the working genetic collection with specific germplasm (on a 10mm grid).

Figure 2. Pie chart illustrating the percentage of maize mutants by endosperm structure genes in the working genetic collection.

Within the double recessive category, the combinations are distributed as: *wx1y1* — 68%, *o2wx1* — 11%, *o2su2* — 8%, *o2sh2* — 5%.

Additionally, 8% of the genotypes possess the *R1-nj* anthocyanin pigmentation marker, used for the identification of in vivo induced haploids in maize. This supports the dual focus on nutritional enhancement and doubled haploid technology in the breeding program.

*D.* The development of the maize germplasm collection also provided the opportunity to expand into polyploid breeding. As a result, nine genotypes—comprising both normal lines and mutant forms—were successfully converted into tetraploids. As illustrated in Figure 3A, 56% of these tetraploid lines carry the *o2* gene, 11% carry *fl2*, while the remaining 33% represent tetraploid analogs of standard diploid lines. In addition to these, eighteen Moldovan maize hybrids from the FAO 100–400 maturity groups were also converted into tetraploid forms. These

hybrids served as source material for the development of fifteen new tetraploid populations. According to Figure 3B, 87% of these populations carry the *o2* gene, 7% carry *fl2*, and 6% combine the *su1o2* double recessive mutations.

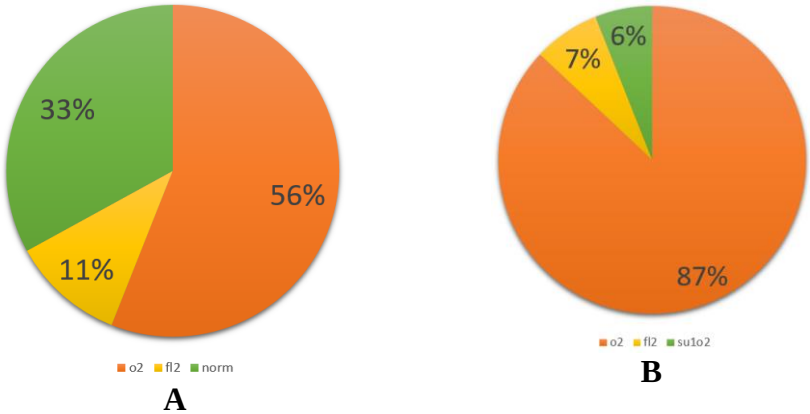


Figure 3. Sector charts showing:
*A – percentages of normal and mutant lines converted to tetraploids; B – tetraploid populations developed from hybrid combinations*

*E.* The maize germplasm collection has been approached innovatively through several integrated research directions. Over 170 genotypes derived from various hybrids have undergone dihaploid induction to accelerate trait fixation and genetic uniformity. Comparative analyses have been conducted between classical heterotic groups—such as BSSS, Iodent, Lacaune, and Lancaster—and new heterotic patterns emerging from specific germplasm tailored to biofortification goals. The overarching focus remains on enhancing biochemical trait expression, supported by advanced diagnostic methods — biochemical, physiological, and molecular — employed within the Laboratory of Applied Genetics and Biotechnology at the Technical University of Moldova.

## CONCLUSIONS

It has been confirmed that the active maize germplasm collection is strategically oriented toward the effective application of biofortification principles, contributing to sustainable agriculture and food security. A detailed analysis reveals that most genotypes—developed through spontaneous mutagenesis, polyploidization, and haploidization—contain specific germplasm and constitute a valuable genetic resource. This collection supports both the development of theoretical frameworks for advanced biofortification diagnostics and the practical implementation of complex breeding strategies, offering a solid foundation for addressing current challenges in maize biofortification.

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# INFLUENCE OF PLANTING DISTANCE AND CROWN SHAPE ON THE GROWTH, PRODUCTIVITY AND QUALITY OF CHERRY FRUITS, GRAFTED ON GISEALA 6

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***Abstract.*** *The study on the influence of planting distance and crown shape on cherry fruits in the pedoclimatic conditions of the central and northern areas of the Republic of Moldova in the period 2018-2024 aimed at assessing the impact of these factors on the growth, productivity and quality of the fruits. In the research, trees of the Early Star and Samba varieties were grafted onto the Gisela 6 rootstock and managed under the Thin Spindle, Cupă and Kym Green Bush crown forms, planted at a density of 1250 trees/ha. The Kordia and Sweet Stefany varieties were grafted onto the same rootstock, but managed according to the Thin Spindle, Structured Vertical Axis and Improved Thin Spindle crown systems, planted at a distance of 4x1 m, with a density of 2500 trees/ha. For all variants, parameters such as total yield, diameter and mass of cherries, as well as soluble dry matter content and titratable acidity of the fruits were determined. The Early Star variety achieved the highest yield in 2022 when the trees were managed according to the Thin Spindle crown shape, with an average of 8.8 kg/tree. The Kordia variety recorded the highest yield (9.64 kg/tree) when the trees were managed according to the Structured Thin Spindle crown shape. Although the Sweet Stefany variety has a lower yield overall, the highest production was obtained in the variant with management according to the Structured Vertical Axis system, with a yield of 7.11 kg/tree.*

***Keywords: cherry variety, crown shape, harvest, fruit quality.***

## INTRODUCTION

According to data presented by FAO, in 2020 cherry was cultivated in 64 countries of the world. The estimated area for the last 10 years is 400-450 thousand ha,

with a small increase in the last two years. And the evolution of world cherry production, in the last 30 years, increased by 1.8 times. The average global harvest, in the last 10 years, was 5.3-6.2 t/ha [1, 2, 3].

The climatic conditions of the Republic of Moldova are favorable for cherry cultivation, which occupies 3.6% of the area of orchards. Modern technologies for cherry production offer new opportunities on the international market and, as a result, there has been a greater interest in cherry cultivation.

Currently, in the Republic, the wild cherry (Cerasus avium, L.) and Mahaleb (Cerasus mahaleb, L.) rootstocks are still used in cherry cultivation. Rootstocks of low (Gisela 5), medium-low (Gisela 6, Krymsk 6) and medium vigor (Maxima 14, Krymsk 5, Piku 1, Piku 4) are not fully used and there are still few studies on this subject in our country. Taking this situation into account, the Early Star, Samba, Kordia, Sweet Stefanny varieties grafted onto Gisela 6, at different planting distances and crown shapes, were studied [4].

In modern orchards, cherry tree pruning is applied every year, in order to maintain the balance between growth and fruiting, as well as to produce high yields of quality fruits [5, 6, 7]. In order to make cherry cultivation more efficient, it is necessary to reduce the volume and height of the trees when pruning trees and harvesting fruits. This goal can be achieved by using rootstocks of low and medium vigor, but also by applying pruning appropriate to the variety-rootstock association [ 8, 9, 10].

Cherry cultivation in modern orchards requires simplicity in the process of crown formation, pruning and maintenance, relatively free forms with reduced volume with a central axis and cup-shaped, early and stable quality harvests. [11, 12, 113, 14]. Small-volume, narrow fusiform crowns with a thickness of no more than 3 m, associated with small planting distances ensure the efficient use of solar energy in the formation of large harvests of qualitative fruits, labor productivity in manual work on pruning and harvesting fruits and a high degree of mechanization of technological work [15, 7,16]. Formation and pruning are important procedures for cherry trees, as they ensure the formation of an airy and balanced crown in space, early harvests, maintaining the final parameters of trees according to the reserved space [17, 18].

The issues addressed are aimed at promoting sustainable cropping systems that ensure early entry of cherry trees into economic fruiting, produce quality and healthy fruits, demanded and competitive on the market by identifying planting distances, the system of tree formation and pruning, as well as maintaining a balance between growth and fruiting. Currently, all sustainable, integrated cropping systems are being successfully introduced, based on geographical conditions, the degree of natural soil fertility, the relative vigor of the variety-rootstock association, planting density, simple crown shapes, early, high-quality and efficient harvests.

## MATERIAL AND METHODS

The experiments were organized in 4 repetitions of 8 representative trees in each. The interaction of planting distance and crown shape was monitored, as basic factors that determine the entry of trees into fruit, the harvest and the quality of the fruits. Agrotechnical measures in orchards are carried out in accordance with the recommendations in force. In the orchards of SRL "StarAgro Group" in Dubăsari district and in the northern area, in Sturzeni, Rîșcani district, drip irrigation is used, and to monitor soil moisture, Watermark transducers are used, installed at 20, 40 and 60 cm depth in each plot. Water is distributed through mains with drippers fixed 40 cm from the ground in the direction of the row.

In the experiments, in the first two years after planting the trees, the soil was maintained as a cultivated field, in the following years the distance between the rows remained grassed naturally or artificially. The strips between the rows, 2–2.5 m wide, with weeds that grow naturally and artificially, are mowed as necessary and remain as mulch. Herbicides or 2–3 mechanical plows are applied along the row of trees, with a rotary tiller with a feeler.

Experiment 1 was established in the central orchard area in the fall of 2015 with the cherry varieties Early Star, and Samba, grafted on the Gisela 6 rootstock, with trees planted at a distance of 4x2 m, 1250 trees/ha. The rows of trees are located in the north-south direction. The following crown shapes with reduced volume were studied: C1 - Thin spindle; C2 - Cup; C3 - Kym Green Bush.

Experiment 2 is carried out in the northern fruit growing area with the varieties Kordia and Sweet Stefanny, grafted on Gisela 6, planted in 2018 at a distance of 4 x 1 m, 2500 trees/ha. Crown shapes are used: V1- Thin spindle; V2 - Structured vertical axis; V3 - Improved thin spindle.

## RESULTS AND DISCUSSION

Cherry trees grafted onto Gisela 6 rootstock at the age of 6-7 years reached the optimum height, width and crown length for such orchards with trees planted at a distance of 4x2 m (tab. 1). In 6-year-old trees, higher values of tree height were recorded for the Early Star variety (400 cm) where the trees were trained according to the Cup crown shape. In 7-year-old trees, this index recorded higher values for trees trained according to the Thin Fus and Cup crown shapes in the Samba variety (400 cm). Crown width differs depending on the variety, the age of the trees and the crown shape. Therefore, in 6-year-old trees this index varies from 230 cm in the Samba variety with the Cup crown shape and Kym Green Bush to 250 cm in the Early Star variety with the Thin Fusiform crown shape. In the following year, an increase in crown width is observed in both varieties, but the Early Star variety records higher values compared to the Samba variety and this index varies from 243

cm in trees formed according to the Cup crown shape to 255 cm in trees formed according to the Kim Greem Bush crown shape.

Table 1. Phytometric indicators of cherry trees depending on the tree management system

*(Gisela 6 rootstock, planting distance 4x2 m, tree age 6-7 years, SRL "StarAgroGroop")*

| Crown shape | Tree height, cm | | Tree height, cm | | Tree height, cm | |
|---|---|---|---|---|---|---|
| | The year 2020 | The year 2021 | The year 2020 | The year 2021 | The year 2020 | The year 2021 |
| Variety Early Star | | | | | | |
| Slim Waist | 369 | 385 | 250 | 244 | 211 | 240 |
| Cup | 400 | 375 | 236 | 243 | 220 | 251 |
| Kym Green Bush | 392 | 397 | 262 | 255 | 232 | 240 |
| Variety Soiul Samba | | | | | | |
| Slim Waist | 397 | 400 | 236 | 253 | 200 | 250 |
| Cup | 385 | 400 | 230 | 246 | 234 | 245 |
| Kym Green Bush | 380 | 397 | 230 | 251 | 230 | 250 |

The crown length also varies from one variety to another and depending on the crown shape. In 6-year-old trees managed according to the Thin Fus crown shape, a crown length of 200 cm is recorded for the Samba variety to 211 cm for the Early Star variety. A greater crown length is observed in varieties formed according to the Cup crown shape 220-234 cm. Trees with the Kym Green Bush crown shape have a greater crown length compared to trees managed according to the other crown shapes 230-232 cm.

Research on the length and width of the crown of cherry trees in intensive cultivation systems demonstrates that the crown parameters depend on the variety and crown shape.

The growth of branches is determined by the variety and age of the trees. Thus, in the orchard of SRL "StarAgroGroop", in the 6th year after planting, the average length of annual branches (tab. 2) in the studied trees was 35.8-54.2 cm. In 7-year-old trees, the average length of annual branches varies from one variety to another and depending on the crown shape studied. The Early Star variety is characterized by a greater length of annual branches compared to the Samba variety.

Therefore, the average length of annual branches in the Samba variety is from 32.5 cm in trees managed according to the Cupă crown shape, up to 34.8 cm in trees managed according to the Thin Shaft and Kym Green Bush crown shapes. The average length of annual branches in the Early Star variety varies from 45.2-49.5 cm.

Analyzing the total length of annual branches, it is observed that the Early Star variety registers higher values compared to the Samba variety.

Table 2. Average and total length of annual branches of cherry trees depending on variety and crown shape, cm

*(Gisela 6 rootstock, planting distance 4x2 m, tree age 6-7 years, SRL "StarAgroGroop")*

| Crown shape | Average length, cm | | Total length, m | |
|---|---|---|---|---|
| | *2018* | *2019* | *2020* | *2021* |
| *Variety Early Star* | | | | |
| Slim Waist | 53,6 | 48,9 | 50,2 | 48,2 |
| Cup | 49,6 | 45,2 | 45,2 | 42,2 |
| Kym Green Bush | 54,2 | 49,5 | 47,4 | 45,4 |
| *DL 5%* | *8,9* | *16,4* | *4,12* | *7,24* |
| *Variety Samba* | | | | |
| Slim Waist | 42,7 | 34,8 | 37,5 | 35,5 |
| Cup | 35,8 | 32,5 | 42,3 | 32,3 |
| Kym Green Bush | 40,5 | 34,8 | 35,1 | 33,1 |
| *DL 5%* | *8,9* | *16,4* | *4,12* | *7,24* |

In 6-year-old trees, in the Early Star variety, the total length of annual branches varies depending on the crown shape and varies from 45.2 m in trees trained according to the Cup crown shape to 50.2 m in trees trained according to the Thin Spine crown shape. In 7-year-old trees, this index decreased slightly, in all studied crown shapes and the largest total length of annual branches is recorded in trees trained according to the Thin Spine – 48.2 m. The Samba variety is characterized by shorter growth compared to the Early Star variety. In 7-year-old trees, the total length of annual branches varies from 32.3 m in the variant where the trees were trained according to the Cup crown shape, to 35.5 m in trees trained according to the Thin Spine crown shape. Regarding the harvest of the Early Star variety (Fig. 1) depending on the crown formation system, the highest average yield was obtained in 2022 in the case of the thin spindle crown form – 8.8 kg/tree. In the case of the Cup and Kym Green Bush crown forms in all the years of study, there are no large differences between the harvests.

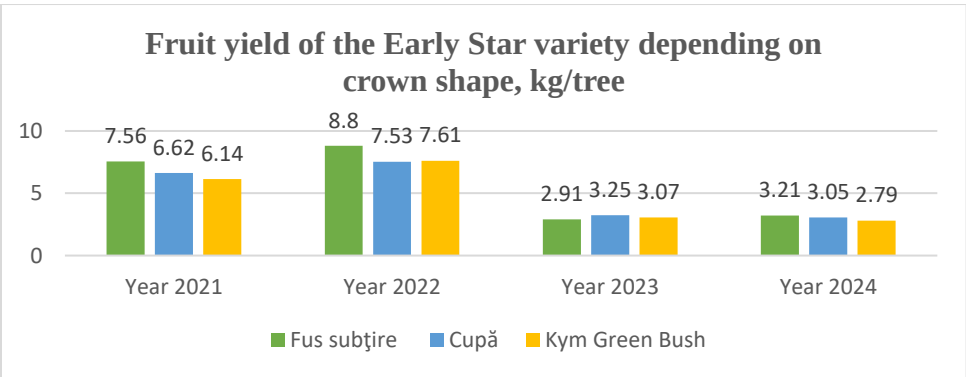


Figure 1. Fruit yield of the Early Star cherry variety depending on the crown shape *(Gisela 6 rootstock, planting distance 4x2 m, tree age 4-10 years, SRL "StarAgroGroop")*

In 2022, the Early Star variety recorded maximum yields in all crown shapes studied, but trees managed according to the Thin Spine crown shape are highlighted by a higher yield of 8.8 kg/tree compared to the Cup crown shape (7.53 kg/tree) and Kym Green Bush (7.61 kg/tree). In 7-year-old trees, the yield varies depending on the variety and crown shape (tab. 3).

The Kordia variety recorded a higher yield in the variant where the trees were managed according to the Thin Spine Structured crown shape (9.64 kg/tree) followed by the variant where the trees were managed according to the Thin Spine crown shape (9.32 kg/tree), and a lower yield in variant 3, the trees being managed according to the Improved Thin Spine shape (9.25 kg/tree).

The Sweet Stefanny variety is characterized by a lower yield compared to the Kordia variety, but we can mention that a higher yield is recorded in the variant where the trees were trained according to the Structured Vertical Axis (7.11 kg/tree). We note that the biological particularities of the variety, the crown shape and the planting distance greatly influence the fruit harvest.

Table 3. Fruit yield of cherry trees depending on variety and crown shape
*(Gisela 6 rootstock, planting distance 4x1 m, year 2024)*

| Crown shape | Variety Kordia | | Variety Sweet Stefanny | |
|---|---|---|---|---|
| | Kg/tree | t/ha | Kg/tree | t/ha |
| Submachine gun | 9,32 | 23,30 | 6,55 | 16,37 |
| Same vertical structuret | 9,64 | 24,10 | 7,11 | 17,77 |
| Upgraded sub-spindle. | 9,25 | 23,12 | 6,66 | 16,65 |
| *DL 5%* | 1,39 | 1,85 | 1,39 | 1,85 |

All registered sunflowers in the study were harvested in a variant where apples had a fusiform crown shape, with a minimum planting distance (4x1 m) of crown shape. și Kym Green Bush (4x2 m).

Fruit yield shows that, similar to the majority of soybeans, the surplus unit remains an essential factor in assessing the technological efficiencies of cultivating its shareholder value. adaptability to modern cultivation technology, in varied natural conditions.

Fruit quality is genetic in nature, give this pubertal influence of action factor of technological means, is interested in obtaining higher fruit with ridiculous commercial value representing a greater representative pomology and soiului (tab.4). The variability of the maximum diameter of the masses around this sea depends on its shape, crown shape, period of harvest and the climatic conditions of the annuli. The threshold studied registered a diameter at the fruit of 28.41 mm the Kordia threshold to 30.82 mm the Sweet Stefanny threshold. Luate sun in studio produces high quality fruit.

Table 4. Influence of soil on cherry fruit quality
*(Gisela 6, plant distance 4x1 m, submerged spindle crown, apple green 7 years, year 2024)*

| Vsriety | Screw diameter, mm | The target mass, g | Soluble extracted substance, Brix% | The firmness of cherries, kg/cm$^2$ | Acidity of cherries, g malic acid/100 g fresh fruit |
|---|---|---|---|---|---|
| Kordia | 28,41 | 10,21 | 18,65 | 3,04 | 0,67 |
| Sweet Stefanny | 30,82 | 12,11 | 19,05 | 3,26 | 0,65 |
| *DL 5%* | 1,25 | 0,45 | 1,13 | 0,12 | 0,12 |

Thus, the firmness of the fruits was 3.04-3.26 kg/cm2, the soluble dry matter was 18.65-19.05 °Brix, and the titratable acidity in the fruits was 0.65-0.67 g malic acid/100 g fresh fruit. We note that these results for the Kordia and Sweet Stefanny varieties, grafted on the Gisela 6 rootstock, are constant values and differ little from climatic conditions.

## CONCLUSIONS

The study confirms that the crown shape and planting distance significantly influence the production and quality of cherry fruits, being essential for optimizing cultivation technologies in the specific conditions of the studied area.

The crown width differs depending on the variety, the age of the trees and the crown shape. Thus, in 6-year-old trees this index varies from 230 cm in the Samba variety with the Cup crown shape and Kym Green Bush to 250 cm in the Early Star variety with the Thin Spindle crown shape.

The crown length also varies from one variety to another and depending on the crown shape. In 6-year-old trees managed according to the Thin Spindle crown shape, a crown length of 200 cm is recorded in the Samba variety to 211 cm in the Early Star variety. A greater crown length is observed in varieties formed according to the Cup crown shape 220-234 cm.

In 2022, the Early Star variety recorded maximum yields in all crown shapes studied, but trees managed according to the Thin Spindle crown shape are highlighted by a higher yield of 8.8 kg/tree compared to the Cup crown shape (7.53 kg/tree) and Kym Green Bush (7.61 kg/tree).

The Kordia variety recorded a higher yield in the variant where the trees were managed according to the Thin Spindle structured crown shape (9.64 kg/tree) followed by the variant where the trees were managed according to the Thin Spindle crown shape (9.32 kg/tree), and a lower yield in variant 3, the trees being managed according to the Improved Thin Spindle shape (9.25 kg/tree).

The Sweet Stefanny variety is manifested by a lower yield compared to the Kordia variety, but we can mention that a higher yield is recorded in the variant where the trees were managed according to the Vertical Structured Axis (7.11

kg/tree). The variability in the size, diameter and mass of cherries is large and depends on the variety, crown shape, fruit harvest period and climatic conditions of the year. The studied varieties recorded a fruit diameter from 28.41 mm in the Kordia variety to 30.82 mm in the Sweet Stefanny variety.

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# RESULTS OF EARLY CORN BREEDING IN THE REPUBLIC OF MOLDOVA

## REZULTATELE AMELIORĂRII PORUMBULUI TIMPURIU ÎN REPUBLICA MOLDOVA

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***Abstract.*** *The main results of a breeding program for early maize, realized in R. Moldova, are presented in this article. More useful germplasm for inbred lines development at present are alternative heterotic groups Euroflint, Iodent, BSSS-B37 and Lancaster. The analysis of hybrid performance in advanced testing established a better adaptation of genotypes from Iodent x BSSS-B37 heterotic pattern in Moldova and Iodent x Euroflint, BSSS-B37 x Euroflint in Belarus. Commercial seed production include 10 hybrids FAO 180-240 registered in Belarus and 4 single cross hybrids FAO 300-320 registered in Moldova.*

***Keywords: breeding, early maize, germplasm groups, heterotic patterns, hybrids, inbred lines.***

## INTRODUCERE

Ameliorarea porumbului timpuriu a fost inițiată în anul 1974 din momentul creării Institutului de Cercetări Științifice pentru Porumb și Sorg. Primul hibrid dublu Moldavskii 330MRf, cu maturitate FAO 330, inițial a fost omologat în 1984 în Moldova și ulterior a fost înregistrat pentru export în Rusia, Belarus, Țările Baltice și Kazahstan. În 1982 se formează actualul laborator de ameliorare a porumbului pentru zonele nordice, având ca parteneri colaboratori ai Institutului de Cercetări Științifice în Agricultură din Belarus. Hibrizii de tip dublu și triliniar Porumbeni 176MRf, Moldavskii 257CRf, Moldavskii 215MRf, Bemo 181CRf, Bemo 182CRf, Bemo 172CRf și Nemo 216CRf cu maturitate FAO 190-210 și Moldavskii 330MRf au constituit baza exportului de semințe cu un volum anual de 43,3 mii tone în perioada 1988-1993 și 10,5 mii tone în anii 1994-2013 [1]. În ultimii 15 ani cantitatea de semințe exportate s-a redus până la 3,2-4,5 mii tone anual, cu o cotă mai semnificativă în R. Belarus. Analiza retrospectivă atestă

anumite schimbări esențiale a germoplasmei de porumb utilizate la crearea liniilor consangvinizate, tipurilor de încrucișări a formelor parentale și a metodologiei de sintetizare a combinațiilor hibride. Scopul prezentei publicații constă în sinteza principalelor rezultate ale ameliorării porumbului timpuriu în ultimii ani de activitate.

## MATERIAL ȘI METODE

În procesul de dezvoltare a liniilor consangvinizate se folosește metoda pedigreului, selecția fenotipică între descendențele din diverse generații de consangvinizare și testarea capacității de combinare în încrucișări sistemice de tip topcross. Materialul inițial sub formă de hibrizi simpli, încrucișări între linii înrudite și încrucișări regresive (backcross) include germoplasma grupelor heterotice Euroflint, Iodent, BSSS-B37 și Lancaster, evidențiate cu anumite performanțe ameliorative. În colecția operațională sunt transferate descendențele fenotipic omogenizate cu valori superioare ale capacității generale de combinare. Liniile noi sunt apreciate după caracterele agronomice *per se* și după capacitatea de producție realizată în hibrizi experimentali. Anual la compartimentul formelor parentale sunt evaluate 39-78 linii și circa 80 forme materne modificate în baza liniilor înrudite. Toleranța la temperaturi suboptimale în faza de germinare a semințelor este apreciată prin semănatul în câmp la final de martie - epoca extratimpurie și peste 15 zile - epoca timpurie. La sintetizarea hibrizilor se folosesc încrucișările liniilor de tip simplu – A x B, simplu modificat – (A x $A_1$) x B și triliniar - (A x $B_)$ x C. Evaluarea hibrizilor în culturi comparative de orientare (2 repetiții), preconcurs (3 repetiții), concurs (6 repetiții) și ecologice se finalizează cu selectarea mostrelor performante pentru promovare la testări oficiale de stat

## REZULTATE ȘI DISCUȚII

La prima etapă de ameliorare (1981-1990) materialul inițial pentru crearea liniilor consangvinizate includea hibrizi din Europa de Vest și SUA cu pedigreul necunoscut cu o cotă de 32,2 %, hibrizi cu 3-4 linii -30,5 %, hibrizi simpli -25,6 %, soiuri indurata -5,2 %, populații sintetice cu bază genetică largă -3,6 % și populații cu 50 % în genom a unei linii performante -2,9 %. După anul 2011 în calitate de material inițial preponderent s-au utilizat încrucișările între linii înrudite, hibrizi simpli și încrucișări regresive $BC_1$, sintetizate cu germoplasma grupelor heterotice alternative Euroflint, Iodent, BSSS-B37 și Lancaster [2]. În ultimele trei grupe de germoplasmă cu bob dentat materialul inițial a fost creat în încrucișări a liniilor elită semitardive cu surse mai timpurii. La etapa actuală majoritatea materialului inițial prezintă recombinări a liniilor timpurii autohtone folosite ca forme parentale în hibrizi comerciali și de perspectivă. În ultimii 5 ani colecția

operațională a inclus 71 linii consangvinizate originale, dintre care 49,3 % aparțin grupei heterotice Iodent cu subgrupele MKP61, MKP601, MKP63, MKP65, 22,5 % - Euroflint, 15,5 % - BSSS-B37, 9,9 % - Lancaster și 2,8 % - Dent mixt [3]. În grupa de maturitate extratimpurie sunt încadrate 13 linii, 45 linii aparțin grupei timpurii și 13 linii sunt incluse în grupa semitimpurie. Din momentul includerii liniilor noi în colecție se inițiază transferarea în citoplasmă sterilizantă iar restauratorii fertilității polenului se creează la formele paterne ale hibrizilor cu performanțe în testări avansate.

Evaluarea liniilor din colecția operațională după toleranța la temperaturi joase în faza de germinare a semințelor a constatat o diferențiere a indicilor germinației de câmp și a masei unei plantule în faza de 5-6 frunze embrionale (tabelul 1). În medie pe anii 2022-2024 germinația boabelor a constituit 54,7 % la liniile cu bob sticlos, semănate în epoca extratimpurie, comparativ cu 48,6 % la grupa de germoplasmă BSSS-B37 și circa 51 % la liniile Iodent și Lancaster. Temperaturile mai înalte ale solului pentru epoca timpurie, semănată cu un interval de 15 zile, au favorizat germinarea semințelor, înregistrându-se valori maxime de 79,1 % la grupa Lancaster și minime de 72,2 % la grupa Iodent.

Tabelul 1. Toleranța la temperaturi joase ale solului a liniilor consangvinizate.

| Grupele de germoplasmă | Germinația semințelor, % | | | Masa unei plantule, g | | |
|---|---|---|---|---|---|---|
| | epoca extratimpurie | epoca timpurie | media | epoca extratimpurie | epoca timpurie | Media |
| Euroflint | 54,7 | 76,1 | 65,4 | 2,43 | 3,16 | 2,80 |
| Iodent | 50,7 | 72,2 | 61,5 | 2,65 | 2,93 | 2,79 |
| Lancaster | 51,1 | 79,1 | 65,1 | 2,45 | 2,88 | 2,67 |
| BSSS-B37 | 48,6 | 76,6 | 62,6 | 2,06 | 2,80 | 2,43 |

Ritmul de creștere a plantulelor în faza de 5-6 frunze embrionale, exprimat prin masa unei plantule în stare proaspătă, a fost mai intens la mostrele Iodent în prima epocă de semănat - 2,65 g și la liniile Euroflint - 3,16 g în următoarea epocă de semănat. Datele experimentale pe 3 ani au permis evidențierea a 5 linii cu o medie pe ambele epoci de 73,4 – 75,4 % semințe viabile, considerate ca tolerante și 8 linii cu toleranță medie cu valori de 70,2 – 72,4 % a germinației de câmp la diferența limită $DL_{05}$ = 3,8 %.

Aprecierile liniilor în anii 2021- 2023 (tabelul 2) au furnizat informație utilă la sintetizarea combinațiilor hibride, inclusiv excluderea decalajului la înfloritul organelor reproductive la formele parentale și pretabilitatea de folosire ca forme materne sau paterne.

Tabelul 2. Principalii indicii agronomici al liniilor consangvinizate timpurii

| Grupele de germoplasmă | Nr. de linii | Zile de la răsărit până la | | Producția de boabe, t/ha | | Umiditatea boabelor, % | |
|---|---|---|---|---|---|---|---|
| | | mătăsit | maturitate | Media | variația | media | Variația |
| Euroflint | 5-14 | 57,6 | 99,5 | 2,37 | 1,19-3,18 | 13,6 | 14,2-22,1 |
| Iodent | 17-24 | 62,4 | 111,0 | 3,34 | 2,11-4,49 | 14,8 | 12,0-18,0 |
| BSSS-B37 | 6-8 | 61,7 | 110,2 | 2,68 | 2,07-3,68 | 14,7 | 12,1-17,4 |
| Lancaster | 2-6 | 64,4 | 113,5 | 2,40 | 1,80-3,12 | 15,9 | 14,2-17,6 |

Liniile grupelor de germopasmă Euroflint și Lancaster, respectiv cu 57,6 zile și 64,4 zile a duratei fenofazei răsărit – înflorit, au manifestat o adaptabilitate ecologică mai redusă, realizând producții de boabe inferioare. Prin urmare liniile respective pot fi mai eficient folosite în calitate de forme paterne, iar liniile grupelor Iodent și BSSS-B37, cu potențial înalt de producție, corespund cerințelor față de formele materne. Cercetările au constatat o expresie stabilă a androsterilității citoplasmatice de tip M la majoritatea liniilor și încrucișărilor înrudite din grupa Iodent. Analiza liniilor din colecția operațională comparativ cu formele parentale ale hibrizilor cultivați în secolul trecut atestă un progres genetic semnificativ după rezistența plantei la frângerea tulpinii, corelată cu caracterul *stay green,* productivitate, umiditate scăzută în boabe, toleranță la maladiile plantei și a știuleților. Germoplasma grupei Iodent, dezvoltată în baza liniilor tardive P101, P343 și a donatorului semitimpuriu D29, a înregistrat performanțe după precocitate, capacitate generală de combinare și pierderea rapidă a umidității boabelor. La liniile originale MKP60 și MKP61 din primul ciclu de ameliorare perioada până la înfloritul organelor reproductive a fost redusă cu circa 10 zile comparativ cu progenitorul P101. Includerea în material inițial a liniilor elită semitardive MK01, AS587/02, DK205/710 ca donatori de gene favorabile a rezultat cu evidențierea unui șir de linii timpurii cu capacitate înaltă de producție în combinații hibride. Grupa de germoplasmă BSSS-B37 se caracterizează cu capacitate generală de combinare înaltă, însușire moștenită de la donatorii semitimpurii D27, 016/12 și linia semitardivă Oh43. Liniile MKP70 și MKP71 din primul ciclu de ameliorare, folosite intens în material inițial, transmit descendențelor toleranța la pătarea cenușie a frunzelor, sensibilitatea la tăciunele comun. Grupa de germoplasmă Lancaster, în arealele cu secete atmosferice și de sol frecvente, manifestă o adaptare ecologică mai redusă. Grupa Euroflint în prezent include germoplasma liniilor extrase din soiurile Lacaune, Lizargarate, Ottava Flint, Morano, Gelber Landmais. Liniile originale sunt menținătoare a androsterilității citoplasmatice de tip C și datorită calității boabelor, toleranței la frig ar putea fi folosite în calitate de forme materne. Un neajuns esențial al germoplasmei respective îl constituie capacitatea generală de combinare relativ inferioară, care impune identificarea liniilor cu

capacitate specifică de combinare.

În procesul de sintetizare a hibrizilor experimentali la etapa actuală predomină încrucișările de tip simplu și simplu modificat, iar hibrizii triliniari ocupă o pondere nesemnificativă. Cercetările anterioare au constatat eficiența utilizării hibrizilor simpli modificați, care manifestă producții de boabe și uniformitate a plantelor apropiată hibrizilor simpli. Avantajele tipului respectiv se manifestă în producerea de semințe, datorită formei materne cu vigoare și potențial de producție mai înalt comparativ cu liniile consangvinizate sensibile la condițiile climaterice stresante [4]. Clasificarea liniilor consangvinizate în grupe de germoplasmă și identificarea perechilor de grupe cu performanțe ameliorative în încrucișări, desemnate în literatura de specialitate ca modele heterotice – *heterotic patterns*, înlesnește procesul de sintetizare a combinațiilor hibride. Analiza a 758 hibrizi din testări avansate (preconcurs și concurs) a anilor 2021-2024 în condițiile Moldovei (tabelul 3) constată că din cadrul celor 3 modele heterotice cu perioada de la răsărit până la mătăsit de 55,1-55,9 zile și 102,1-103,6 zile până la maturitatea fiziologică se evidențiază varianta Iodent x Euroflint cu o medie de 5,26 t/ha boabe și 6,12 t/ha în combinații specifice. Formula de încrucișări Iodent x BSSS-B37 cu producție în medie pe 300 hibrizi de 5,88 t/ha boabe și 6,66 t/ha în combinații individuale poate fi considerată ca cel mai performant model heterotic, luând în calcul și eficiența producerii de semințe. Rezultatele testării ecologice în R. Belarus în anii 2021 -2024 (localitatea Jodino, regiunea Minsk), a 117 hibrizi selectați în cultură comparativă de concurs sunt redate în tabelul 4.

Tabelul 3. Performanțele ameliorative ale hibrizilor timpurii testați în Moldova

| Modelul heterotic | Nr. de hibrizi | Zile de la răsărit până la | | Umiditatea boabelor, % | | Producția de boabe, t/ha | |
|---|---|---|---|---|---|---|---|
| | | mătăsit | maturitate | media | minimă | media | Maximă |
| IodentxEuroflint | 250 | 55,1 | 102,8 | 16,9 | 15,5 | 5,26 | 6,12 |
| EuroflintxIodent | 42 | 55,3 | 102,1 | 16,5 | 15,8 | 5,18 | 5,98 |
| BSSS-B37xEuroflint | 76 | 55,9 | 103,6 | 16,9 | 15,6 | 5,04 | 5,61 |
| IodentxBSSS-B37 | 300 | 58,1 | 106,9 | 16,4 | 14,7 | 5,88 | 6,66 |
| BSSS-B37xIodent | 90 | 56,8 | 104,8 | 16,2 | 15,3 | 5,63 | 6,17 |

Cu performanțe la producția de substanță uscată a fost evidențiat modelul heterotic BSSS-B37 x Euroflint, care a înregistrat 15,02 t/ha în medie pe 19 hibrizi și 16,0 t/ha în combinații specifice. După valorile medii ale perioadei până la înflorit, producției și umidității boabelor între hibrizii realizați în formulele de încrucișări Iodent x Euroflint, BSSS-B37 x Euroflint și Euroflint x Iodent nu s-au înregistrat diferențe semnificative, cu excepția producției de 8,18 t/ha boabe în combinații specifice la Euroflint x Iodent. În cadrul celor 3 modele heterotice cu bob semisticlos s-au semnalat reacții specifice la condițiile climaterice ale anilor de

testare. Rezultatele testării hibrizilor din grupa de maturitate FAO 200 -220, sintetizați în încrucișări reciproce ale grupelor de germoplasmă dentiformis Iodent și BSSS-B37, au confirmat adaptabilitatea acestora pentru cultivare la boabe și siloz în zonele cu regim termic limitat. Menționăm că varianta BSSS-B37 x Iodent cu un eșantion de 11 hibrizi evaluați în anii 2023-2024 a înregistrat valori medii apropiate de varianta Iodent x BSSS-B37 după precocitate, producție de substanță uscată și boabe.

Tabelul 4. Caracteristica hibrizilor testați în R. Belarus

| Modelul heterotic | Nr. de hibrizi | Perioada răsărit – înflorit, zile | Producția de substanță uscată, t/ha | | Producția de boabe, t/ha | | Umidi-tatea boabe-lor, % |
|---|---|---|---|---|---|---|---|
| | | | media | maximă | media | maximă | |
| IodentxEuroflint | 46 | 68,6 | 14,04 | 15,62 | 7,63 | 8,63 | 36,4 |
| BSSS-B37xEuroflint | 19 | 69,7 | 15,02 | 16,00 | 7,76 | 8,68 | 36,4 |
| EuroflintxIodent | 11 | 69,2 | 14,17 | 15,06 | 7,66 | 8,18 | 36,2 |
| IodentxBSSS-B37 | 30 | 71,2 | 14,55 | 15,77 | 7,90 | 8,66 | 38,1 |
| BSSS-B37xIodent | 11 | 69,8 | 15,11 | 15,85 | 9,11 | 9,44 | 31,2 |

În prezent producerea și comercializarea semințelor de porumb pentru export este delegată firmei Forever în baza licenței exclusive, care include 10 hibrizi omologați în R. Belarus. Hibrizii Porumbeni 176 MRf, Porumbeni 180 și Bemo 203 cu indice de maturitate FAO 180-200 reprezintă modelul heterotic Iodent x Euroflint, iar Porumbeni 220, Porumbeni 221, Porumbeni 230, Porumbeni 231, Bemo 235, Farmec și Porumbeni 243 cu maturitate FAO 220-240 au fost creați în formulele de încrucișări heterotice Iodent x BSSS-B37. Din cadrul acestora 2 hibrizi sunt de tip triliniar, 4 - simpli modificați și 4 de tip simplu. În componența hibrizilor FAO 180 -240 sunt folosite 13 linii consangvinizate ca forme parentale, inclusiv 7 analogi androsterili cms M și 5 restauratori ai fertilității polenului MRf. Hibrizii simpli Porumbeni 305, Porumbeni 310 și Porumbeni 315 cu maturitate semitimpurie FAO 300 -310 au fost înregistrați în Moldova, iar Porumbeni 310 se exportă în România și Ucraina. Pentru utilizare în scopuri alimentare a fost creat și omologat hibridul simplu Alimentar 325 cu bob sticlos. Multiplicarea formelor parentale ale hibrizilor nominalizați se efectuează în laboratorul de producere a semințelor. Menționăm că secetele atmosferice și de sol frecvente în ultimii ani au semnalat vulnerabilitatea producerii de semințe a hibrizilor simpli. Din aceste considerente eforturile amelioratorilor sunt orientate spre majorarea productivității formelor materne prin modificarea acesteia în baza încrucișărilor înrudite cu un anumit grad de heterozis reproductiv și adaptiv.

## CONCLUZII

1. În procesul de creare a liniilor consangvinizate prioritate se acordă încrucișărilor între linii înrudite, încrucișările regresive $BC_1$ și hibrizilor simpli, care asigură omogenizarea mai rapidă a descendențelor și păstrarea genomului genitorilor performanți.

2. Baza genetică a liniilor originale o constituie germoplasma grupelor heterotice alternative Euroflint, Iodent, BSSS-B37 și Lancaster. Liniile și încrucișările înrudite din grupa heterotică Iodent manifestă indici ameliorativi superiori, fiind folosite în calitate de forme materne cu impact economic în producerea de semințe hibride.

3. Combinațiile hibride în modelele heterotice Iodent × Euroflint și BSSS-B37 × Euroflint asigură producții de boabe și substanță uscată superioare în zonele cu regim termic redus, iar hibrizii obținuți în modelul heterotic Iodent × BSSS-B37 prezintă interes pentru regiunile cu temperaturi moderate.

4. Lucrările de ameliorare a porumbului timpuriu s-au finalizat cu crearea a 10 hibrizi cu indice de maturitate FAO 180-240 omologați în R. Belarus și 4 hibrizi semitimpurii FAO 300-320 înregistrați pentru cultivare în Moldova.

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# BREEDING THE DOMESTIC APPLE TREE (*MALUS DOMESTICA* BORKH.) AS A FAMILY CREATIVE ACTIVITY

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***Abstract.*** *The aim of the work is to make the domestic apple tree breeding process more efficient with the acquisition of new original varieties in the interaction between human and nature also in the form of creative activity of the whole family. In this process, it is possible to use many wild seedlings (WGS) grown from the seeds of domestic apple tree fruits along roadsides, in fields, old gardens and orchards. In the experiment, 39 WGS were selected from different locations in Slovakia, with average fruit weights ranging from 57.45 g to 197.59 g. The fruits were determined to be of different shapes, most often heart-shaped, and varying in colour from light to dark. The colour of the flesh was white, light yellow and slightly red. Many of the seedlings excelled in original aroma and flavour. Process is very simple. In late August, look for suitable seedlings with attractive fruit, take a sample of the fruit and mark the seedling's location. Then take budded cuttings from the seedlings in January for grafting onto prepared rootstocks. In the third year, the first harvests can be evaluated on the WGS. Through this process, each member of the family can get their own original variety in the garden, not only for enjoyment but also for eating the original fruit.*

***Keywords: ampelographic evaluation of fruits, domestic apple tree, Malus domestica Borkh., rootstocks wild seedlings, selection of suitable genotypes.***

## INTRODUCTION

Apples are still one of the most consumed fruits in many countries and cultures, with an annual per capita consumption of around 20-30 kg. By 2050, average daily fruit consumption worldwide is expected to increase from 204 to 242 g per person [1]. When properly grown, stored and used, they are considered a healthy food with significant sources of nutrients and biologically active components [1, 2].

Domestic apple breeding programs primarily focus on improving adaptability to the growing environment, improving tolerance to biotic and abiotic

factors, increasing yield, long-term fruit storage, marketable appearance, fruit size, market pressure, and ever-increasing consumer and producer expectations [3]. Plant breeding is still a powerful and indispensable tool to achieve "harmony" between agriculture and the environment. For this to become a reality, partnerships between plant breeders, ecologists, policy makers and, most importantly, consumers of apple fruit and food products are needed [4].

Although plant breeding has had a very long evolution, at least throughout recorded history, it has rarely lacked new ideas and concepts that have made breeding practices increasingly innovative and efficient. The development of new technologies in recent years has contributed to the strength and versatility of the science and art of plant breeding. Some of these changes have been spectacular, others rather modest. Science and plant breeding are very much intertwined, even if in many cases compromised. This is especially true of so-called basic research. Secretary of Defense Charles Wilson under President Eisenhower defined basic research as follows: "Basic research is when you don't know what you are doing", which basically means that the researcher does not know in advance the answer to the problem being addressed. In doing so, there is a general perception that plant breeders do not conduct any basic research, which is a big misconception [5].

As with other species, hybridisation is the most widely used in apple breeding programmes. This method makes it possible to broaden the genetic base of the selected populations to enhance the heterozygosity of the offspring. In general, intraspecific hybridisation is mainly used, based on the principle of 'crossing the best with the best parent'. It takes 20-25 years to breed a new variety in this process. As the best varieties were also the best known and most widely distributed in the world, relatively few parental components were used in the new breeding programmes. As a result, a small number of parental varieties were repeatedly used during apple breeding [6], and the best-known varieties thus became the parental forms of most of the new varieties. Therefore, many modern varieties found in the world range are related to each other and have common parents or close origins or ancestry, i.e. they share a common genetic basis [7, 8].

At the same time, it is common knowledge that in the early days of plant breeding, seeds from local varieties were used for many plant species and also for apple trees. An example of this was the situation in Finland, where apple trees are grown at the limits of their northern growing possibilities and conditions. Because of these constraints, it was found in practice that growing and thus breeding new varieties by selecting promising individuals from the population of seedlings grown from seed was the most appropriate method for obtaining new varieties that provided a fruit crop for growers in the north. This created a unique culturally and genetically rich indigenous gene pool of local varieties that met the diverse needs of apple growers and consumers from the late 18th century to the mid-19th century [9].

The research and breeding collective at the Julius Kuehn Institute in Dresden-Pillnitz has a long-standing focus on breeding resistant apple varieties. Between 1972 and 1998 they used 52 000 seeds from apple trees to obtain new varieties. From this number of seedlings, at the end of the experiment, they selected and registered in 2006 3 resistant varieties under the name (Re-cultivars, Recolor, Rekarda). The production of the three cultivars from approximately 52 000 seeds means that 17 000 seeds were needed to obtain one cultivar. Only one of the approximately 9 000 seedlings was resistant to scab and was of sufficient quality to become a variety. This proportion documents the enormous effort required to develop a new cultivar [10].

At present, research and breeding teams are focusing mainly on improving the nutritional and therapeutic value of apples, with a priority on increasing the content of flavonoids and other nutrients. An important example is the Department of Primary Industries and Regional Development (DPIRD) and the University of Western Australia (UWA) who are conducting research into breeding flavonoid-rich apples – healthier apples for consumers. The national project focuses on aspects of the breeding process and the market knowledge needed to deliver a healthier apple to the consumer. Apples are bred to maximise certain characteristics such as initial eating quality, appearance, shelf life and resistance to pests and diseases. Maximising the nutrient content of the fruit is increasingly in demand and project staff are investigating how to breed future high flavonoid varieties with additional positive attributes [11].

## MATERIALS AND METHODS

The aim of the present work is to document how the complex and long-term breeding process of the domestic apple tree can be made more efficient with the acquisition of "new varieties" in the interaction between man and nature in the form of creative activity of the whole family. The essence of this process is the search for suitable wild seedlings grown in nature at the time of fruit ripening. The seedlings with the best fruit are propagated on prepared rootstocks and planted in gardens and orchards for use.

***Specific objectives***

(a) Exploration and search for wild seedlings in selected areas of Slovakia as genetic resources for breeding new varieties,

(b) Determination of the economic value of the fruits of wild seedlings in morphological traits.

***Biological material***

Wild seedlings in number 39 obtained by selection of fruiting wild seedlings grown from seeds of domestic apple tree fruits next to roads, in fields, old gardens

and orchards, pastures and other places. In our experiments we referred to them with the abbreviation "WGS - wild seedlings".

***Implemented working procedures***

(a) The search and selection of genotypes from wild seedlings was carried out in selected regions during the period of fruit ripening,

(b) Criteria for selection of wild seedlings - good health without disease symptoms, fruit size and shape and bush habit,

(c) Location of the selected genotype was recorded under registration number with simultaneous determination of its geographical location by GPS device,

(d)The image records of the genotype were made with Fuji FinePix S 7000, Panasonic DMC FZ50 and Zeiss Discovery V12 stereomacroscope with 3 Mpx digital camera ICc3.

***Morphometric analysis***

The following agrophysical parameters were evaluated for the fruits - fruit height (mm), fruit width (mm), fruit weight (g), pedicel pit depth (mm), calyx pit depth (mm), carpel width (mm), carpel length (mm). In this study we present the weight of the fruits, their shape and the colour of fruits the pulp.

## RESULTS AND DISCUSSIONS

It is well known that plant breeding, including domestic apple breeding, is an intellectually, time-consuming, technically, technologically and financially very expensive process [12]. According to the Law of the Slovak Republic, "plant variety breeding is the process by which new varieties of plants are discovered, created and developed, and by which their traits and characteristics are maintained during reproduction" [13]. It also follows from the Act that a variety can be created and developed using classical intra- or interspecific hybridisation or also using modern genetic technologies. This process requires knowledge of new scientific knowledge in the fields of genetics, breeding, physiology, biochemistry, plant nutrition, trends in human nutrition, plant protection against diseases and animal pests, current requirements for cultivation technologies, storage and marketing technologies, consumer and processor requirements for fruit quality and other scientific areas that are complexly related to the breeding of a new variety for large-scale cultivation. Indeed, even today, this process requires 20-25 years to breed a single variety from a domesticated apple tree [12]. But there is also the possibility of obtaining a new variety from domestic apple trees in the form of its discovery from bud mutations [14] or with the selection of suitable wild seedlings grown from seed in nature [10]. This process was also implemented in our experiment. In the conditions of Slovakia, we searched for and selected 39 wild seedlings for evaluation based on a survey. The search is very easy because WGS grow in the form of a shrub. They do not have a separate stem from the crown (Figure 1). Seedlings can form a variety

of even exotic shrub shapes.

Figure 1. Habitus of a wild seedling of a domestic apple tree in flowering season
*(Photo Š. Hajdu, 2010)*

We searched for WGS during the ripening period according to the above methodology. In this publication, due to the limited number of pages, we only present results from fruit weight and some external fruit quality traits. Comparisons of fruit weights from the first WGS group are presented in Figure 2. The comparison shows that the fruit weight was determined in the range from 57.45 g (WGS021) to 197.59 g (WGS003). This means that even with WGS it is possible to find seedlings with lower or even higher fruit weight. This is a criterion of the purposefulness of the use of the fruits. However, the optimum fruit weight of apples for different purposes varies. For marketing purposes, a minimum weight of 90 grams is generally accepted for apples, but smaller sizes may be acceptable if they meet certain quality standards. For storage, the average weight of apples for the Golden Delicious variety is 160 ± 60 grams. In general, an average weight of around 140 grams is required for a medium-sized apple [15].

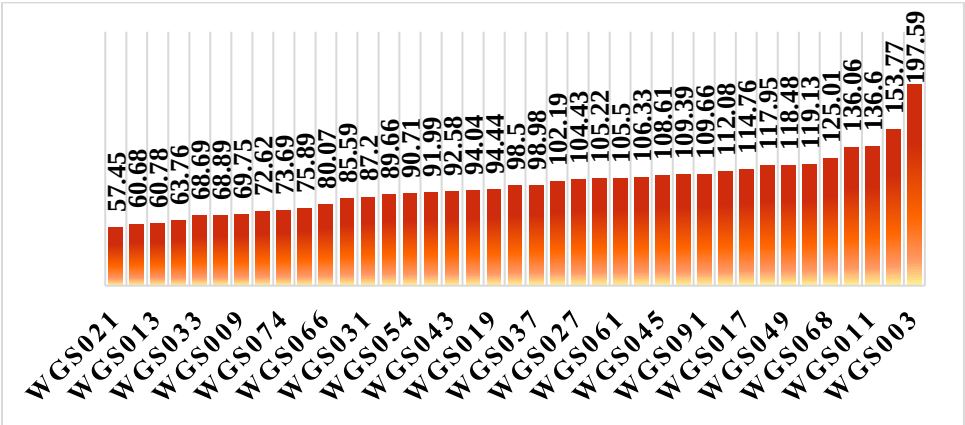


Figure 2 Comparison of evaluated wild apple seedlings in fruit weight (g)

In addition to fruit weight, many consumers prefer the colour of apple fruit, which is the most influential factor affecting market acceptance and price. Consumers judge fruit by sight; specifically, consumers "eat with their eyes" [16]. Red-skinned varieties are generally more desirable [17]. In nature, WGS can be found with a variety of fruit shape and colour. This is documented by the presentation of the shape and colour of some of the WGS evaluated in Figure 3.

Figure 3 Comparison of the selected WGS group in fruit shape, size and colour
*(Photo A. Oravec, 2011)*

The presented results, even in short form, clearly document that WGS are very interesting genotypes of domestic apple from which it is possible to search and, after their comprehensive evaluation, to select a potentially new variety.

## CONCLUSIONS

Although many breeders and researchers do not recognise the breeding of native apple trees from seed and consider the process to be 'unscientific', this system has its merits. This is evidenced by the dozens of varieties of native apple trees, typified by Golden Delicius and others, which have been obtained from WGS. For breeders, this is a great creative activity, a chance and an opportunity to select WGS from the wild from domestic apple trees with interesting fruit shape and colour and to propagate them on prepared rootstocks in the garden. Each member of the family can have their own original variety "even with their own registration". This system is also suitable for so-called "open science". Many so called "naturalists" can provide breeders with very interesting information about unique WGS from their regions, which stand out for some morphological traits on fruits but also on other parts of the plants and can be potentially and practically useful as new genetic

resources. This system allows for a more active expansion, maintenance and use of biodiversity in fruit production, thus eliminating the priority cultivation of only a few dozen modern apple varieties in home gardens and orchards.

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# PRODUCTIVITY INDICES IN *LAVANDULA ANGUSTIFOLIA* MILL. VARIETIES, SIXTH YEAR OF VEGETATION

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***Abstract.*** *The evaluation of quantitative character indices – lant height and diameter, number of flowering stems per plant, inflorescence and floral spike length, number of whorls on the floral spike, raw material yield, and essential oil content – allowed for the characterization and assessment of each lavender variety-clone. The varieties under study synthesized and accumulated an essential oil content at standard humidity (60%) exceeding 2% (Svetlana – 2.199%; Alba 7 – 2.188%), and on dry matter basis exceeding 5% (Svetlana – 5.498%; Alba 7 – 5.472%). Raw material yield varied from 3.6 t/ha to 7.7 t/ha depending on the variety, with the most productive being Svetlana (7.7 t/ha), Alba 7 (7.3 t/ha), and Favoare (6.7 t/ha). The essential oil yield in the evaluated varieties-clones ranged from 11.6 kg/t to 22.0 kg/t. The highest yields were recorded in the varieties: Svetlana (22.0), Alba 7 (21.8), and Favoare (17.1) kg of essential oil per ton of fresh inflorescences.*

***Keywords: breeding, productivity, varieties, yield, essential oil.***

## INTRODUCTION

The cultivation of the species *Lavandula angustifolia* Mill. in the Republic of Moldova has increased in recent years, currently covering over 2.000 hectares. In order to accelerate the expansion of cultivated areas, breeding research has been carried out to develop varieties adapted to local pedoclimatic conditions, such as critical low winter temperatures, drought, summer heat, and so on, as well as different vegetation periods, high productivity, and elevated essential oil content [1; 5; 6; 7; 8].

The production and quality of essential oil are strongly influenced by pedoclimatic conditions, soil, and the cultivated varieties. For therapeutic purposes, the volatile oil is used in aromatherapy as a relaxant, antispasmodic, carminative, analgesic, sedative, hypotensive, antimicrobial, antitussive, antioxidant, and antibacterial agent [5; 6; 7; 8; 9; 11].

One of the main tasks of the research group was to create lavender varieties-clones with resistance potential and adaptive plasticity to unfavorable environmental factors, as well as the ability to accumulate valuable chemical compounds. Over the course of several years, the breeding team from the Aromatic and Medicinal Plants Laboratory of the IGFPP, USM, developed six lavender variety-clones with varying vegetation periods (early, medium, and late), distinctive quantitative traits of productivity, high essential oil content in raw material, and superior quality. These have been registered, patented, and four of them have been implemented in the agriculture and economy of the Republic of Moldova [1; 2; 3; 4; 5; 6; 10]. The objective of the present research is to evaluate *L. angustifolia* clones according to high productivity indices in their sixth year of vegetation.

## MATERIALS AND METHODS

This study included six local lavender varieties and three promising hybrids. The research was conducted in the Comparative Contest Crops nursery, in the sixth year of vegetation. The planting scheme used was 1.4 x 0.6 meters. In early spring, the winter hardiness and frost resistance of the studied varieties were assessed by evaluating the number of frozen shoots per plant, scored on a scale from 1 to 5 [13].

The evaluation of the variety-clones included in the research was carried out in accordance with current methodologies, based on a series of quantitative traits that directly influence productivity: plant height and diameter, inflorescence length, floral stem and floral spike length, number of whorls per inflorescence, vegetation period, and essential oil content. The methods used for setting up the experiments, evaluating the material, and statistical data interpretation followed validated lavender breeding protocols [13].

Essential oil was extracted from fresh inflorescences at full bloom using Ginsberg-type distillation apparatus [12]. The essential oil content was determined and recalculated on a dry mass basis.

## RESULTS AND DISCUSSION

In the sixth year of vegetation, the lavender varieties from different maturity groups were evaluated in the CCC (Comparative Contest Crops): early (Favoare and Moldoveanca 4), medium (Cr.13S-6-7; Cr.13S-6-35; VM-18V; Vis Magic 10; Aroma Unica), and late (Svetlana and Alba 7). The species' resistance to abiotic factors (frost, wintering) was assessed. It was found that the varieties Svetlana, Favoare, Moldoveanca 4, and Aroma Unica exhibited high resistance, scoring 5 points, while Vis Magic 10, Alba 7, Cr.13S-6-35, Cr.13S-6-7, and VM-18V were rated above average, with 4 points. The quantitative traits influencing productivity

-plant height and diameter, and number of flowering stems per plant were evaluated at the full flowering stage of the lavender plants.

Under severe drought conditions that persisted throughout the vegetation period, the varieties in their sixth year of growth developed plants with heights ranging from 68.5 cm to 94.8 cm. Plant diameters ranged from 95.6 cm to 132.1 cm. These data are shown in Figure 1. These two traits indicate plant vigor. Noteworthy performances for these traits were observed in the following varieties: Svetlana with a height exceeding 130.0 cm and a diameter of 94.8 cm; Cr.13S-6-7 (122.1 cm and 80.2 cm); Cr.13S-6-35 (121.9 cm and 85.9 cm). The hybrid genotype VM-18 had the shortest height of 95.6 cm and the smallest diameter of 68.5 cm (Figure 1).

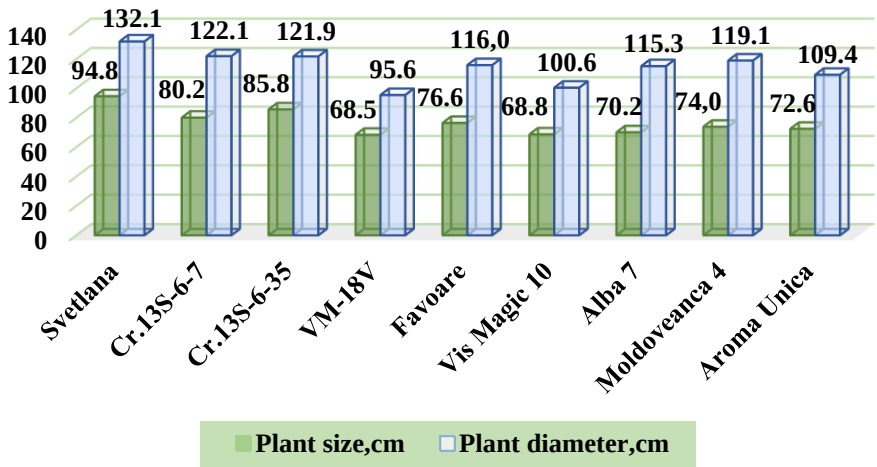


Figure 1. Height and diameter of *L. angustifolia* Mill. varieties in CCC (sixth year of vegetation), 2024

The number of flowering stems per plant is a key trait determining the production of fresh raw material. This index varied from 940 to 3023 units per plant. The highest number of flowering stems was recorded in the early variety Favoare (2503 units), followed by the medium hybrid Cr.13S-6-7 (2842 units.) and the late variety Svetlana (3023 units).

In the lavender clones evaluated in the Comparative Contest Crops, traits related to the inflorescence were also studied – namely, the length of the inflorescence and floral spike, and the number of whorls on the floral spike (Figure 2).

Based on the evaluation, the varieties with the longest inflorescences - greater than 30.0 cm - were: Favoare (32.4 cm), Cr.13S-6-7V (32.4 cm), and

Svetlana (38.7 cm). Six varieties showed inflorescence lengths above 20.0 cm: Moldoveanca 4 – 26.7 cm; Alba 7 – 28.2 cm; VM-18V – 28.5 cm; Aroma Unica – 29.4 cm; Cr.13S-6-35 – 29.4 cm; and Vis Magic 10 – 29.8 cm. For the floral spike length, lavender varieties in the sixth year of vegetation showed values ranging from 7.5 cm to 10.9 cm. The highest values were recorded for the clones: Aroma Unica (8.8 cm), Favoare (9.1 cm), and Svetlana (10.9 cm). The number of whorls on the floral spike in the tested varieties is a key index that determines the essential oil content in the raw material (inflorescences). The more whorls a variety forms - and the more flowers per inflorescence - the higher the essential oil content, as most oil glands are located on the flower calyx [8]. In the sixth year of vegetation, the number of whorls per spike ranged from 5.5 to 7.5 units.

The varieties that formed more than 6 whorls were: Alba 7 (6.2 units), Aroma Unica and Vis Magic 10 (6.3 units.), and Favoare (6.8 units.). The highest number of whorls (7.5 units.) was recorded in the late-maturing variety Svetlana, while the lowest (5.7 units.) was found in the early-maturing Moldoveanca 4 (Figure 2).

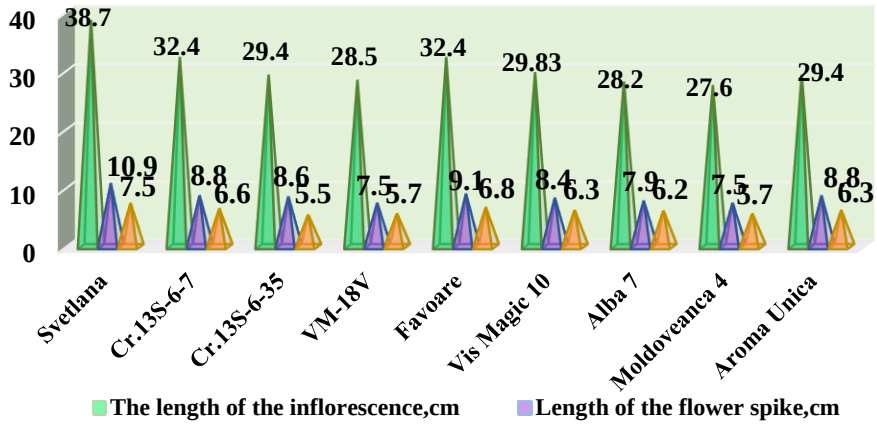


Figure 2. Characteristics of *L. angustifolia* Mill. varieties in CCC based on quantitative inflorescence traits, 2024.

In all lavender clones evaluated in the sixth year of vegetation, productivity indices were determined: raw material yield, essential oil content, essential oil production, and yield per variety (Table 1). The raw material yield ranged from 3.6 t/ha to 7.7 t/ha, depending on the variety. The highest inflorescence yields were recorded for the following varieties: Favoare (6.7 t/ha), Alba 7 (7.3 t/ha), and Svetlana (7.7 t/ha).

The research results show that the dry year of 2024 was favorable for lavender cultivation, encouraging the accumulation and synthesis of essential oil in

the raw material. Some evaluated lavender varieties accumulated an essential oil content at standard humidity (60%) of more than 2% (Alba 7 – 2.188% and Svetlana – 2.199%), recalculated to dry matter exceeding 5% (Alba 7 – 5.472%, Svetlana – 5.498%). The lowest essential oil content was found in the hybrid Cr.13S-6-7, with only 2.897% (dry matter).

Essential oil production in the evaluated clones varied from 45.5 kg/ha to 169.3 kg/ha. The highest production levels were recorded for the following varieties: Favoare (114.5 kg/ha), Alba 7 (159.7 kg/ha), and Svetlana (169.3 kg/ha).

Based on the obtained data, it can be stated that the lavender clones included in the Comparative Contest Crops also achieved a high essential oil yield, ranging from 11.6 kg/t to 22.0 kg/t. The highest yields were recorded for the varieties: Svetlana (22.0), Alba 7 (21.8), and Favoare (17.1) kg of essential oil per ton of fresh inflorescences (Table 1).

Table 1. Productivity of lavender variety-clones in CCC (2024)

| Varieties - clones | Raw material production, t/ha | Essential oil content, % | | Essential oil production, kg/ha | Essential oil yield, kg/t |
|---|---|---|---|---|---|
| | | stand. humid, (60%) | dry matter | | |
| Svetlana | 7.7 | 2.199 | 5.498 | 169.3 | 22.0 |
| Cr.13S-6-7 | 6.5 | 1.159 | 2.897 | 75.3 | 11.6 |
| Cr.13S-6-3 | 5.5 | 1.210 | 3.026 | 66.5 | 12.1 |
| VM-18V | 3.6 | 1.263 | 3.196 | 45.5 | 12.6 |
| Favoare | 6.7 | 1.709 | 4.380 | 114.5 | 17.1 |
| VisMagic10 | 4.9 | 1.424 | 3.632 | 69.7 | 14.3 |
| Alba 7 | 7.3 | 2.188 | 5.472 | 159.7 | 21.8 |
| Moldoveanca4 | 6.5 | 1.434 | 3.596 | 93.2 | 14.4 |
| AromaUnica | 5.4 | 1.277 | 3.192 | 68.9 | 12.7 |

The evaluation of the varieties in the sixth year of vegetation (2024) in the Comparative Contest Crops demonstrated that the most productive variety was the late-maturing Svetlana, which stood out for its high raw material yield, high essential oil content and production, and high oil yield.

## CONCLUSIONS

The research conducted in the Comparative Contest Crops (CCC) on lavender variety-clones revealed well-developed plants that produce a large number of flowering stems, as well as inflorescences with long floral spikes and a high number of whorls per spike.

The essential oil content, a key breeding index in lavender, varied by variety from 2.897% in Cr.13S-6-7 to 5.498% (dry matter) in Svetlana.

The evaluation of the varieties in the sixth year of vegetation (2024) demonstrated that the most productive was the late-maturing variety Svetlana, which showed high raw material yield, high essential oil content, and high essential oil production.

The lavender variety-clones included in the comparative trials also displayed high essential oil yields, ranging from 11.6 kg/t to 22.0 kg/t. The highest yields were recorded for the varieties Svetlana (22.0), Alba 7 (21.8), and Favoare (17.1) kg/t.

The *L. angustifolia* variety-clones developed and patented are well adapted to the pedoclimatic conditions of the Republic of Moldova, maintaining their distinctiveness, productivity, and high values of quantitative traits, including an elevated essential oil content in high-quality raw material—meeting the requirements of agricultural producers and companies specialized.

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# SCREENING OF CORN GENOTYPES FOR SALINITY RESISTANCE

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***Abstract***. *The aim of the study was to evaluate salt resistance of corn lines and hybrids at early stages of plant growth and development, as well as at the level of haploid male gametophyte. Under stress conditions of chloride salinity, the variability of such traits as "seed germination", "seedling length", "rootlet length", "number of rootlets" was studied. Correlations and regressions of traits, effects of the factors "genotype", "salinity stress", as well as their interaction were assessed. As a result, reliable positive correlations of the studied traits of early growth and development under salt stress were established, and reliable effects of the "genotype" factor in the variability of traits at the diploid and haploid levels were determined. The hybrid combination with the maternal component L276xP165, as well as the lines P343, L1866, Mo17, Co125 turned out to be genotypes with increased salt resistance.*

***Keywords: corn, salinity, resistance, pollen.***

## INTRODUCTION

At present, it is relevant and important to study the mechanisms of salt tolerance in corn and to develop technologies for growing this crop on saline soils [1]. It should be noted that significant progress has been made in understanding the genetic variability that determines salt tolerance in corn [2]. In further selection for salt tolerance, the study of morphological and physiological indicators of corn seedlings in NaCl solutions is particularly emphasized [3], and it is recommended to use them as selection criteria in corn breeding programs for salt resistance [4]. Researchers have also shown [5] that corn genotypes resistant to salinity are also more resistant to plumbum stress.The aim of our study was to screen corn lines and hybrids under chloride salinity conditions both at the stages of early growth and development of plant and at the stage of mature pollen grains.

## MATERIALS AND METHODS

In the experiment 8 inbred lines (P343, A285, XL12, B73, Co125, L1866, Mo17, P346) were used as the initial material, as well as the following hybrid combinations: (459xMK390)xA285 (trilinear hybrid), (L1866x092) x (459xM11) x ((P343xP346)xF2)

(synthetic population), (459xMK390)x(A239xP502) (double hybrid), 5 hybrid combinations in which the paternal genotype was (459xMK390)x(P165xB73) double hybrid and as the maternal genotypes were used simple hybrids (Mo17xW23), (A285xW23), (A285xRf7), (XL12xN6), (L276xP165), and hybrid combination (A285xN6) x ((459 xP502) x P101) - simple hybrid as maternal genotype and trilinear hybrid as paternal genotype. Sodium chloride solutions (in concentrations 1,5-2,0% (seeds) and 10% (pollen)) were used to determine the resistance to salinity. At the initial stages of plant development under NaCl stress were estimated: the frequency of seed germination, the length of seedlings, rootlets and the number of rootlets. At the haploid level the variability of "pollen grain diameter" character was studied. The values of the this trait (in eyepiece micrometer units) were determined using the ocular micrometer MOB-1-15x). The experiments were carried out according to the schemes of the ANOVA, correlation and regression analysis. Statistical processing of data was performed using STATISTICA 7 software.

## RESULTS AND DISCUSSION

The values of the trait «seed germination under saline stress» in the genotypes used in the experiment varied significantly. The seed germination in NaCl solutions for the best lines (Co125, L1866, Mo17) by this indicator was 77.3%, 66.7% and 99%, respectively. The seed germination indicators for hybrids varied from 72.7% to 95.8%. The lines P343, A285, XL12, B73, P343 were characterized by seed germination frequency from 13.5% to 57.6%. Correlation analysis of the variability of traits characterizing the early growth and development of plants under salt stress revealed the presence of significant positive correlations (table 1). In hybrids, the highest values of the correlation coefficient were noted for the traits "rootlet length" and "number of rootlets".

Table 1. Correlation and regression coefficients of early growth and development traits under NaCl stress.

| Trait | «seedling length» | "rootlet length" | "number of rootlets" |
|---|---|---|---|
| «seedling length» | - | Lines r=0.67*** $b_{yx}$=0.95* | Lines r=0.74*** $b_{yx}$=0.58*** |
| "rootlet length" | Hybrids r=0.37* $b_{yx}$=0.57* | - | Lines r=0.72*** $b_{yx}$=1.3*** |
| "number of rootlets" | Hybrids r=0.47* $b_{yx}$=0.4*** | Hybrids r=0.6*** $b_{yx}$=0.53*** | - |

**-p<0.05, ***-p<0.001*

The correlation of the trait "seedling length" with the trait "number of

rootlets" was weaker. The lowest value of the correlation coefficient had the relationship between the traits "rootlet length" and "seedling length". It should be noted that the inbred lines had higher values of the correlation coefficients for these traits. The traits "rootlet length" and "seedling length" showed the highest values of the correlation coefficient with the trait "number of rootlets". The highest difference in the correlation coefficient values for the lines and hybrids was found between the traits "seedling length" and "rootlet length". Regression analysis revealed significant values of the regression coefficients, and their values were higher in inbred. The dispersion analysis of the variability of the traits of the seedlings and rootlets under salt stress conditions showed a significant effect of the "genotype" factor (Table 2). The highest values of the effect of this factor were determined for such traits as "rootlet length" (hybrids) and "number of rootlets" (lines and hybrids).

Table 2. ANOVA results of traits variability under NaCl stress.

| Traits variability | MS | F | $\eta_p^2$ | $R^2$ |
|---|---|---|---|---|
| Lines «seedling length» | 8.1 | 3.3 | 0.59* | 0.77* |
| Lines "rootlet length" | 36.4 | 5.8 | 0.72*** | 0.85*** |
| Lines "number of rootlets" | 3.8 | 2.8 | 0.83* | 0.74* |
| Hybrids «seedling length» | 0.27 | 6.17 | 0.73*** | 0.82*** |
| Hybrids "rootlet length" | 0.83 | 10.4 | 0.82*** | 0.91*** |
| Hybrids "number of rootlets" | 5.5 | 15 | 0.87*** | 0.93*** |

**-p<0.05 , ***-p<0.001*

Almost equal values of the effect size of the "genotype" factor were found out for the traits "rootlet length" (lines) and "seedling length" (hybrids). The effect size of the "genotype" factor was the smallest for the trait "seedling length" (lines). The coefficients of determination of the experimental model for each trait in hybrids had very high values, and in inbred lines similar indicators for the rootlet traits were significantly lower. The hybrids also had a higher determination coefficient for the trait "seedling length". The lines had the highest determination coefficient for the trait "rootlet length", and the values of the determination coefficients for the other two traits were approximately at the same level.

High average values of the trait "rootlet length" under salinity stress were noted in the lines P343, L1866, Mo17, while the other lines were characterized by very low average values of this trait (Table 3). For hybrid combinations, the average values of the "rootlet length" trait under salt stress conditions did not exceed 1.6 cm and were significantly inferior to similar values for the lines P343, L1866 and Mo17. It should be noted that only three lines used in this study were characterized by the lowest values of the "number of rootlets" trait (A285, XL12, P346).

Table 3. Average values of early growth and development traits under salinity stress.

| Geno Type | Lines | | | Geno type | Hybrids | | |
|---|---|---|---|---|---|---|---|
| | seedling length,cm | rootlet length,cm | number of rootlets | | seedling length, cm | rootlet length, cm | number of rootlets |
| P343 | 4.65 | 9.45 | 4.0 | 1 | 0.83 | 0.73 | 1.0 |
| A285 | 0.27 | 0.43 | 1.0 | 2 | 0.17 | 0.97 | 1.0 |
| XL12 | 0.2 | 0.5 | 1.0 | 3 | 0.6 | 0.34 | 0.83 |
| B73 | 0.85 | 0.9 | 2.0 | 4 | 0.3 | 0.4 | 1.0 |
| P346 | 1.0 | 0.95 | 1.0 | 5 | 0.95 | 1.15 | 2.0 |
| Co125 | 3.83 | 0.53 | 3.0 | 6 | 0.3 | 0.3 | 1.0 |
| L1866 | 2.75 | 6.0 | 3.0 | 7 | 0.47 | 1.43 | 1.0 |
| Mo17 | 2.77 | 5.61 | 2.0 | 8 | 0.7 | 1.57 | 2.5 |
| $LSD_{0.05}$ | 2.79 | 4.35 | 2.03 | 9 | 0.93 | 1.6 | 5.0 |
| Mean | 2.04 | 3.05 | 2.12 | $LSD_{0.05}$ | 0.36 | 0.54 | 1.04 |

*1-(459xMK390)xA285, 2-(L1866x092)x(459xM11)x((P343xP346)xF2), 3-(459xMK390)x(A239xP502), 4-(Mo17xW23)x((459xMK390)x(P165xB73)), 5(A285xW23)x((459xMK390)x(P165xB73)), 6(A285xRf7)x((459xMK390)x(P165xB73)), 7-(XL12xN6)x((459xMK390)x(P165xB73)), 8-(A285xN6)x((459 xP502) x P101), 9-(L276xP165)x((459xMK390)x(P165xB73))*

The best values of this trait were in the lines P343, Co125, L1866, while these indicators exceeded the average values of the "number of rootlets" trait in all hybrids, except for the combination in which the maternal genotype was L276 x P165 simple hybrid, which was characterized by the best indicator of this trait in the experiment. Under salt stress conditions, the average values of the "seedling length" trait did not exceed 1.0 cm in hybrids and in the A285, XL12, B73 lines. The highest values of this trait were found in the P343 line, as well as in the Co125, L1866, Mo17 lines.

The assessment of genotype resistance to chloride salinity at the haploid level of the male gametophyte showed that the "salt stress" factor and its interaction with the "genotype" factor had the greatest values of effect size ($\eta_p^2$=0. 34*** and $\eta_p^2$=0.3***, respectively) on the variability of the "pollen grain diameter" trait. The effect size of the "genotype" factor was lower ($\eta_p^2$=0.26***). The determination coefficient in this experimental model was high and significant ($R^2$=0.75***). The average values of the "pollen grain diameter" trait were significantly higher in

hybrid combinations where simple hybrids A285xN6 and L276xP165 were used as maternal genotypes (Table 4). The remaining hybrids differed from each other with an insignificant difference.

Table 4. Mean values of the pollen grain diameter (PGD) under chloride salinity.

| genotype | PGD mic.units | genotype | variant | PGD mic.units |
|---|---|---|---|---|
| 1 | 147.4 | 1-(Mo17xW23)x ((459xMK390)x(P165xB73)) | control | 157.6 |
| 2 | 156.4 | | NaCl | 137.2 |
| 3 | 142.7 | 2-(L276xP165) x ((459xMK390)x(P165xB73)) | control | 168.1 |
| 4 | 154.2 | | NaCl | 144.7 |
| 5 | 144.4 | 3-(A285xW23) x ((459xMK390)x(P165xB73)) | control | 137.6 |
| 6 | 142.2 | | NaCl | 147.8 |
| $LSD_{0.05}$ | 7.63 | 4-(A285xN6) x ((459 xP502) x P101) | control | 167.5 |
| Mean value (control) | 154.7 | | NaCl | 141.0 |
| Mean value (NaCl) | 141.07 | 5-(XL12xN6) x ((459xMK390)x(P165xB73)) | control | 151.7 |
| $LSD_{0.05}$ | 4.4 | | NaCl | 137.0 |
| Mean value of the experiment | 147.9 | 6-(A285xRf7) x ((459xMK390)x(P165xB73)) | control | 145.7 |
| $LSD_{0.05}$ | 4.41 | | NaCl | 138.7 |

The average values of the control and stress variants were significantly different, and the effect of salinity significantly reduced the value of the studied trait. The smallest values of the pollen grain diameter under chloride salinity were demonstrated by hybrids with the maternal genotypes Mo17xW23, XL12xN6, A285xRf7. The hybrid combination with the maternal genotype A285xW23 turned out to be significantly better in terms of the male gametophyte trait value under stress conditions. The smallest difference between the control and stress variants was noted in the hybrid with the maternal genotype A285xRf7.

## CONCLUSIONS

At the early stages of growth and development of corn plants, the traits "seedling length", "rootlet length", "number of rootlets" under chloride salinity conditions realized significant positive correlations, and inbred lines had higher values of correlation and regression coefficients than hybrid combinations.

A significant effect size of the "genotype" factor on the variability of early growth and development traits under salt stress was revealed.

The best lines by average values of the studied traits at the early stages of growth and development under stress conditions were P343, L1866, Mo17, Co125. Among the hybrid combinations, the genotype with the maternal component L275xP165 proved to be more salt-resistant.

At the haploid level of the male gametophyte, the effect size of the “stress” factor was the greatest; hybrid combinations with maternal genotypes A285xN6 and L276xP165 stood out for the highest average value of the “pollen grain diameter” trait.

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# EVALUATION OF MID-PARENT AND BEST-PARENT HETEROSIS FOR GRAIN YIELD IN SELECTED MAIZE INBRED LINES

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***Abstract.*** *Maize (Zea mays L.) is a major crop with global importance for food, feed, and industry. Improving yield through hybrid breeding remains a key objective, and heterosis plays a central role in achieving superior performance. This study evaluated mid-parent heterosis (MPH) and best-parent heterosis (BPH) for grain yield in 19 maize hybrids derived from genetically diverse inbred lines. Field experiments were conducted in 2024 near Cucuruzeni, Orhei district, Republic of Moldova, under chernozem soil conditions and moderate drought stress. A partial diallel mating design was used, and trials were arranged in a randomized incomplete block design with two replications. Yield was measured at harvest, and heterosis was calculated using standard formulas. Results revealed significant variability, with MPH ranging from 33.7% to 76.3% and BPH from 13.3% to 71.6%. The hybrid 2110 × 2126 showed the highest yield and BPH, while 2132 × 2127 had the highest MPH. The findings confirm the role of non-additive gene effects and highlight promising parental combinations for future hybrid development.*

***Keywords: best-parent heterosis, grain yield, inbred lines, maize, mid-parent heterosis.***

## INTRODUCTION

Maize (*Zea mays* L.) holds a position of major importance in global agriculture, serving as a staple food crop, a key component in animal feed, and a valuable raw material for numerous industries [1]. As one of the most widely cultivated cereals worldwide, maize plays a fundamental role in agricultural systems, rural livelihoods, and agricultural economies in both developed and developing countries. Its versatility and adaptability across diverse agroecological zones make it a cornerstone of agricultural production, with critical implications for food security and sustainable development [2,3].

Maize breeding programs have made significant progress in recent decades, evolving from traditional selection methods to advanced molecular and genomic techniques [4,5]. The integration of marker-assisted selection, genomic selection,

and gene editing technologies has substantially increased the precision and efficiency of breeding processes [6,7]. These innovations have enabled the development of maize varieties with enhanced productivity, improved tolerance to stress, greater nutrient use efficiency, and increased resistance to diseases and pests [8,9].

Currently, yield improvement in maize is primarily achieved by exploiting the phenomenon of heterosis, which arises from crosses between genetically distinct inbred lines, typically from divergent heterotic groups [4]. Heterosis, or hybrid vigor, refers to the superior performance of hybrids compared to their parental lines. This superiority can manifest in traits such as vigor, disease resistance, and yield. The expression of heterosis in $F_1$ hybrids is commonly assessed relative to the mid-parent value (mid-parent heterosis, MPH) or the best parent (best-parent heterosis, BPH) [3,4]. These evaluations are labor-intensive and are conducted continuously at all stages of breeding material assessment.

The genetic basis of heterosis is complex, involving additive, dominance, and epistatic gene interactions. While the dominance hypothesis attributes heterosis to the masking of deleterious recessive alleles, the overdominance and epistasis models suggest that specific allele combinations at individual or interacting loci contribute to superior hybrid performance [10]. Identifying parental lines that exhibit strong combining ability is thus critical for maximizing heterotic gains in breeding pipelines [11].

In this context, evaluating MPH and BPH provides valuable insights into the potential of new inbred lines to contribute to superior hybrid performance. Such assessments support the selection of elite parental combinations for further testing and commercialization. The objective of this study was to evaluate mid-parent and best-parent heterosis for grain yield in a set of maize hybrids derived from diverse inbred lines, with the aim of identifying promising crosses for inclusion in future hybrid development programs.

## MATERIALS AND METHODS

The experiments were conducted in 2024 on an experimental field near the village of Cucuruzeni, Orhei District, at the farm SRL Pleurotis. The area is characterized by a hilly landscape typical of the central region of the Republic of Moldova, with soil conditions favorable for agricultural experimentation. The predominant soil type is chernozem. The research was carried out under climatic conditions marked by above-average temperatures and a water deficit during the critical growth stages of the maize crop.

The biological material consisted of 28 maize inbred lines, which were crossed in a partial diallel mating scheme. Sowing was carried out in two replications, using an incomplete randomized block design. In this article, we

present results only for 19 hybrid combinations that exhibited a heterosis effect exceeding 30%, a threshold commonly used by breeders for assigning lines to heterotic groups [12].

In the experimental field, standard agronomic practices for maize cultivation were applied to ensure optimal plant development under controlled conditions. The evaluation of reproductive heterosis was based on yield analysis at harvest.

MPH and BPH were calculated using standard formulas:

$MPH_{(\%)} = \frac{F1-MP}{MP} \times 100,$

$BPH_{(\%)} = \frac{F1-BP}{BP} \times 100$

where

$F_1$ = hybrid yield, $MP$ = mid-parent mean, and $BP$ = best-parent yield.

All collected data were processed and statistically analyzed using the R environment [13]

## RESULTS AND DISCUSSIONS

The grain yield analysis of the 19 maize hybrids revealed a wide range of performance across combinations, reflecting both the diversity of the parental lines and the varying degrees of hybrid vigor. Hybrid yields ranged from 3407.7 kg/ha to 6134.4 kg/ha, with substantial differences in mid-parent and best-parent heterosis values (

Tabel 1). This variation highlights the influence of genetic distance and complementarity between specific parental lines.

The cross between inbred lines 2110 × 2126 recorded the highest hybrid yield (6134.4 kg/ha) and one of the most significant heterotic responses, with a mid-parent heterosis (MPH) of 71.8% and best-parent heterosis (BPH) of 71.6%. This near-equal expression of MPH and BPH suggests not only high hybrid performance but also strong dominance and potential overdominance effects between these two lines.

Similarly, the combination 2132×2127 exhibited the highest MPH (76.3%), though the best-parent heterosis was more moderate at 38.6%. The strong MPH in this case indicates that the hybrid significantly exceeded the average of its parents, despite one of them (2127) having a relatively low yield potential. Such hybrids can be considered promising for exploiting heterosis through complementary gene action, even when one parent is agronomically weaker.

Several other hybrids displayed consistently high heterotic effects:

2133 × 2126 showed an MPH of 65.0% and a BPH of 55.7%.

2108 × 2112 produced 6055.2 kg/ha with a BPH of 62.2%.

2108 × 2133 achieved a respectable 5344.3 kg/ha and BPH of 46.1%.

These combinations suggest that certain inbred lines — particularly 2108,

2110, and 2133 — possess good general combining ability (GCA) and may serve as recurrent parents in future hybrid development.

Conversely, hybrids such as 2135 × 2134 and 2127 × 2128 performed below expectations, with both relatively low yield and heterosis (BPH of 36.5% and 14.0%, respectively). These cases may reflect poor genetic complementation or limited dominance interactions, reinforcing the necessity of pre-screening inbred lines not only for per se performance, but also for combining ability.

Table 1. Grain yield and heterosis values for 19 maize hybrids derived from selected inbred lines.

| Female line ID | Male line ID | Hybrid Yield, kg/ha | Female Mean, kg/ha | Male Mean, kg/ha | Mid-Parent Mean, kg/ha | MPH (%) | BPH (%) |
|---|---|---|---|---|---|---|---|
| 2126 | 2111 | 4309.0 | 3567.4 | 2880.0 | 3223.7 | 33.7 | 20.8 |
| 2133 | 2112 | 4646.7 | 3165.1 | 3732.6 | 3448.8 | 34.7 | 24.5 |
| 2127 | 2128 | 3718.4 | 2249.6 | 3261.1 | 2755.3 | 35.0 | 14.0 |
| 2110 | 2112 | 4956.4 | 3574.3 | 3732.6 | 3653.5 | 35.7 | 32.8 |
| 2128 | 2136 | 4427.1 | 3261.1 | 3259.5 | 3260.3 | 35.8 | 35.8 |
| 2112 | 2130 | 4229.7 | 3732.6 | 2490.6 | 3111.6 | 35.9 | 13.3 |
| 2107 | 2126 | 4600.3 | 3174.9 | 3567.4 | 3371.2 | 36.5 | 29.0 |
| 2135 | 2134 | 3407.7 | 2337.0 | 2496.5 | 2416.8 | 41.0 | 36.5 |
| 2112 | 2128 | 4944.1 | 3732.6 | 3261.1 | 3496.9 | 41.4 | 32.5 |
| 2107 | 2111 | 4284.2 | 3174.9 | 2880.0 | 3027.5 | 41.5 | 34.9 |
| 2135 | 2112 | 4393.2 | 2337.0 | 3732.6 | 3034.8 | 44.8 | 17.7 |
| 2130 | 2134 | 3611.7 | 2490.6 | 2496.5 | 2493.6 | 44.8 | 44.7 |
| 2110 | 2132 | 5524.1 | 3574.3 | 3929.1 | 3751.7 | 47.2 | 40.6 |
| 2131 | 2128 | 4229.8 | 2335.3 | 3261.1 | 2798.2 | 51.2 | 29.7 |
| 2108 | 2133 | 5344.3 | 3657.7 | 3165.1 | 3411.4 | 56.7 | 46.1 |
| 2108 | 2112 | 6055.2 | 3657.7 | 3732.6 | 3695.2 | 63.9 | 62.2 |
| 2133 | 2126 | 5555.3 | 3165.1 | 3567.4 | 3366.2 | 65.0 | 55.7 |
| 2110 | 2126 | 6134.4 | 3574.3 | 3567.4 | 3570.9 | 71.8 | 71.6 |
| 2132 | 2127 | 5447.2 | 3929.1 | 2249.6 | 3089.3 | 76.3 | 38.6 |

Interestingly, crosses involving line 2112 revealed varying outcomes. While it contributed to high-yielding hybrids (e.g., 2110 × 2112, 4956.4 kg/ha), its performance also varied depending on the female partner, suggesting a complex interaction between parental backgrounds and direction of the cross.

The substantial variation observed in hybrid yield and heterotic response among the 19 maize crosses confirms the complex genetic interactions that underpin hybrid performance. The results are consistent with previous findings that heterosis, particularly for yield, is strongly influenced by the genetic divergence and combining ability of parental lines [4,8].

The top-performing hybrids, such as 2110 × 2126 and 2132 × 2127, which exhibited over 70% mid-parent heterosis, align with the theory that greater genetic

diversity between parents can enhance heterotic expression [14]. These crosses likely benefited from complementary gene action and dominance effects across multiple loci, as proposed in the dominance and overdominance hypotheses [10].

Overall, the results confirm that high heterosis is not uniformly distributed across combinations, but rather is contingent on the specific pairing of lines. The most promising hybrids identified in this study should be considered for advanced multilocation trials to validate yield stability and adaptability. These findings also reinforce the role of heterosis as a strategic tool in hybrid maize breeding, especially when guided by careful parental selection and combining ability testing.

## CONCLUSIONS

This study assessed grain yield performance and heterosis expression in 19 maize hybrids derived from genetically diverse inbred lines. The results demonstrated considerable variability in both mid-parent and best-parent heterosis, with several hybrid combinations exhibiting strong heterotic responses and superior yield performance.

The hybrid 2110 × 2126 was the top performer in terms of yield (6134.4 kg/ha) and best-parent heterosis (71.6%), indicating its potential for commercial cultivation. Similarly, hybrids such as 2132 × 2127, 2133 × 2126, and 2108 × 2112 showed high mid-parent heterosis exceeding 60%, making them promising candidates for advanced yield trials.

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# STORAGE POTENTIAL OF THE EGGPLANT (*SOLANUM MELONGENA* L.) COLLECTION ACCESSIONS FOR *EX SITU* CONSERVATION IN A PLANT GENE BANK

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***Abstract.*** *To determine the storage potential (SP) of samples from the eggplant collection in the gene bank's active collections, two tests were used: the accelerated seed aging test ( AA ) and the electrical conductivity test (EC). After conducting the AA test to the seeds, which was conducted at an elevated air temperature (45-47°C) and elevated humidity (90-100%), the morphophysiological and biochemical parameters of the seeds and seedlings were determined. Based on the set of parameters studied, the eggplant samples were divided into two groups: Group 1 - with high seed viability (genotypes Sucleischii, Varatic, Rada, Almaz, Acvamarin, Laura), Group 2 - with average seed viability (Magda, Belaia Lilia, Flower Goddess, Black Beauty genotypes). Determining the storage potential of seeds enables us to predict the storage life of eggplant collection samples in a plant gene bank during ex situ conservation, as well as to make recommendations on seed reproduction times.*

***Keywords: gene bank, eggplant, germination, germination, root length, root biomass, peroxidase accelerated aging, active collections, longevity, ex situ conservation, storage potential.***

## INTRODUCTION

One of the main tasks of plant genetic banks in the *ex situ* conservation of germplasm is to preserve the viability of seed material. This is because complex aging processes occur in seeds, leading to a gradual loss of viability and genetic uniformity [1, 2, 3]. The deterioration of the physiological quality of seeds contributes to impaired plant growth and development, as well as reduced resistance to abiotic and biotic stresses. Therefore, it is necessary to assess the SP of collection samples before they are placed in long-term storage. This comprehensive indicator is widely used in various gene banks around the world, as it enables conclusions to be drawn about the longevity of samples under *ex situ* conservation conditions, as well as enabling them to be graded according to storage potential [1, 4, 5, 6]. To determine seed viability, an accelerated seed aging test is performed under

conditions of elevated temperature and humidity for a short period of time [7]. Following the test, seed viability can be characterized by various morphophysiological and biochemical parameters, enabling differences between collection samples to be assessed [8, 9]. This test enables the identification of samples that are not suitable for long-term storage in a plant gene bank, the provision of recommendations on reproduction times for seeds, and the indication of the need to restore the viability of valuable collection samples.

The aim of this study was to determine the viability of eggplant collection samples prior to their placement in long-term storage in a plant gene bank.

## MATERIALS AND METHODS

The objects of the study were collection samples of eggplant from active collections of the plant genetic bank. Ten samples were tested: *Sucleischii*, *Varatic*, *Rada*, *Almaz*, *Acvamarin*, *Laura*, *Magda*, *Belaia Lilia*, *Flower Goddess*, and *Black Beauty*. To determine the germination capacity of eggplant seeds, we used the accelerated seed aging test according to the Hampton, Tekrony method [7] and the seed electrical conductivity (EC) test [4], which are included in the International Seed Testing Association [10]. According to international standards, the seed aging test for eggplant seeds should be conducted at an air temperature of 42°C, air humidity of 90-100%, and an aging period of 72 hours. However, when testing these samples, it was found that the above temperature does not allow the seeds to age. Therefore, we proposed new temperature conditions (45-47°C), which helped to achieve the desired effect. Following the test, the morphophysiological parameters of seeds and seedlings were determined: germination energy (GE) and germination (G) of seeds, root length (RL), wet (WB) and dry (DB) biomass of roots [10], electrical conductivity of solutions [4], and peroxidase enzyme activity [11]. Normal seeds were used as controls. The data were processed using the *Statistica 7* software package.

## REZULTS AND DISCUSSIONS

After conducting the AA test on eggplant seeds, a decrease in GE and G of seeds was observed. The decrease in these parameters in different genotypes

compared to the control was 13.0-32.5% and 11.0-33.0%, respectively, which indicates their genetic specificity. Based on changes in EP, the genotypes were

divided into two groups: Group 1: *Almaz*, *Acvamarin*, *Belaia Lilia*, *Black Beauty*, *Magda*, *Flower Goddess* (EP decrease of 9.5-18.0%); Group 2: *Sucleischii*, *Varatic*, *Rada*, *Laura* (EP decrease of 19.0-32.5%). Based on seed germination, the genotypes were also divided into two groups: Group 1: *Sucleischii*, *Varatic*, *Rada*,

*Almaz*, *Acvamarin*, *Laura* (decrease in the parameter by 11.0-18.0%) (Fig. 1). Group 2: *Magda*, *Belaia Lilia*, *Flower Goddess*, *Black Beauty* (decrease by 20.0-33.0 %) (Fig. 2). After the treatment of eggplant seeds, a decrease in other morphophysiological parameters was also observed.

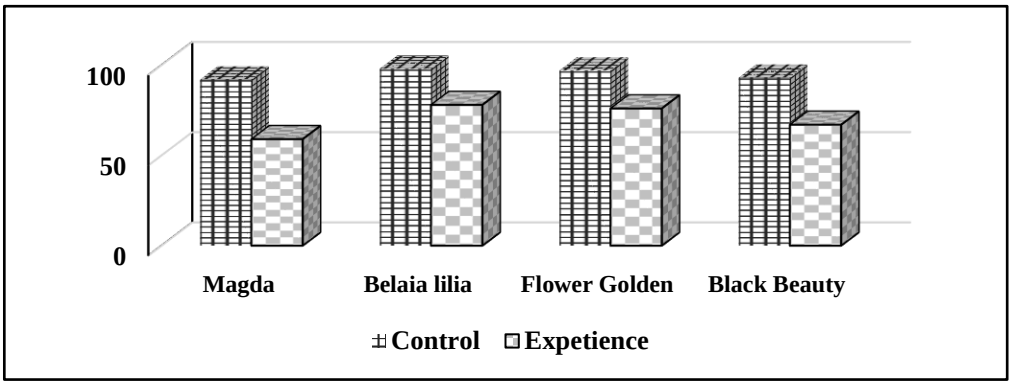


Figure 1. Germination of eggplant seeds following the action of the accelerated seed aging test, % (1st group)

The diameter of the seeds after treatment decreased by an average of 1.5-5.0 mm compared to the control, depending on the genotype (Fig. 3). The strongest inhibition of seedling root growth was observed in the genotypes *Varatic*, *Almaz*, *Belaia Lilia*, *Black Beauty*, *Flower Goddess*, and the weakest in the genotypes *Acvamarin* and *Laura*.

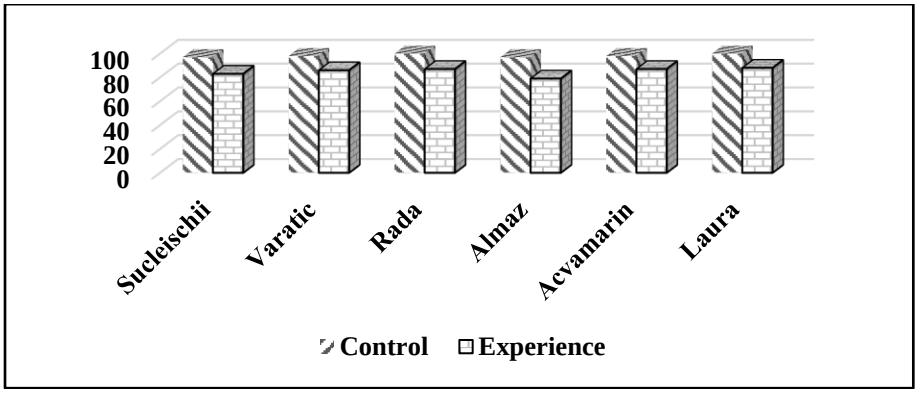


Figure 2. Germination of eggplant seeds following the action of the accelerated seed aging test, % (2nd group)

Determination of the wet and dry biomass of seedlings after seed treatment revealed

a similar pattern. The raw biomass of the roots decreased compared to the control by an average of 17.5-61.0 mg depending on the genotype, with the maximum decrease in this parameter observed in the genotypes *Varatic*, *Almaz*, and *Black Beauty*. A sharp drop in morphophysiological parameters in certain genotypes after seed ST testing indicates their lower resistance to high temperature and humidity. Almost all eggplant collection samples showed a significant positive correlation between seed germination and germination energy (r=+0.59), root length (r=+0.62), wet (=+0.64) and dry (r=+0.53) biomass.

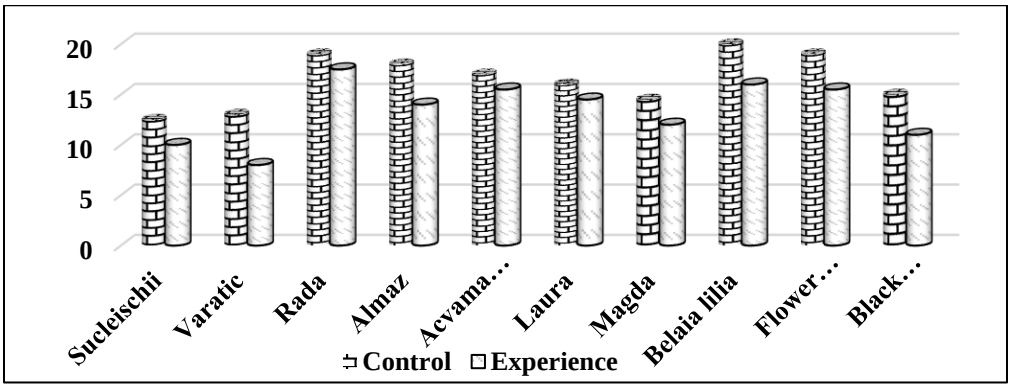


Figure 3. Variability of root length of eggplant seedlings after accelerated seed aging, mm

The electrolyte yield from seeds was also determined by the electrical conductivity of solutions with normal and aged seeds at two swelling exposures: 24 and 48 hours. At 48 hours of exposure, the difference between genotypes became more pronounced. After accelerated seed aging test the electrical conductivity of the solutions increased on average by 1.1-1.5 times compared to the control, depending on the genotype (Table 1). The greatest increase in electrical conductivity was observed in the genotypes *Varatic*, *Belaia Lilia*, and *Black Beauty*, and the smallest in the genotypes Sucleischii, *Almaz*, and *Laura*. A slight increase in electrolyte yield indicates a small increase in protoplasm permeability after accelerated seed aging.

This fact is favorable for the successful *ex situ* conservation of samples. The content of the peroxidase enzyme in the roots of seedlings increased after accelerated seed aging compared to the control by 1.3-2.5 times, depending on the genotype, which is characteristic of the effect of thermal stress on seeds (Table 2).

Table 1. Electrical conductivity of eggplant seed solutions following the accelerated seed aging test, mS/m$^2$

| Order No. | Genotype | Control | Experience |
|---|---|---|---|
| 1 | *Sucleischii* | 1,50 ± 0,15 | 1,72 ± 0,13 |
| 2 | *Varatic* | 0,85 ± 0,11 | 1,29 ± 0,17 |
| 3 | *Rada* | 0,69 ± 0,12 | 0,90 ± 0,09 |
| 4 | *Almaz* | 3,35 ± 0,11 | 3,55 ± 0,14 |
| 5 | *Acvamarin* | 2,85 ± 0,12 | 3,45 ±0,11* |
| 6 | *Laura* | 3,52 ± 0,07 | 3,85 ± 0,10 |
| 7 | *Magda* | 3,30 ± 0,14 | 3,75 ± 0,15 |
| 8 | *Belaia Lilia* | 0,75 ± 0,09 | 1,10 ± 0,07* |
| 9 | *Flower Golden* | 3,65 ± 0,15 | 4,10 ± 0,12* |
| 10 | *Black Beauty* | 2,75 ± 0,11 | 3,65 ± 0,13* |

**- the difference is significant at p<0.05*

The greatest increase in enzyme activity in the radicles of seedlings after accelerated seed aging was observed in the genotypes *Sucleischii*, *Almaz*, and *Magda* (2.2; 2.2 and 2.5 times, respectively), and the smallest increase was observed in the genotypes *Acvamarin*, *Belaia Lilia*, *Flower Goddess* (1.3; 1.5 and 1.5 times, respectively).

Table 2. Peroxidase activity in the roots of eggplant sprouts following the accelerated seed aging test, conventional units

| Order No. | Genotype | Control | Experience |
|---|---|---|---|
| 1 | *Sucleischii* | 0,19 ± 0,02 | 0,42 ± 0,03* |
| 2 | *Varatic* | 0,27 ± 0,02 | 0,44 ± 0,02* |
| 3 | *Rada* | 0,19 ± 0,01 | 0,37 ± 0,02* |
| 4 | *Almaz* | 0,20 ± 0,02 | 0,44 ± 0,03* |
| 5 | *Acvamarin* | 0,27 ± 0,02 | 0,34 ±0,03 |
| 6 | *Laura* | 0,30 ± 0,03 | 0,60 ± 0,04* |
| 7 | *Magda* | 0,20 ± 0,03 | 0,49 ± 0,03* |
| 8 | *Belaia Lilia* | 0,28 ± 0,03 | 0,42 ± 0,04* |
| 9 | *Flower Golden* | 0,31 ± 0,03 | 0,45 ± 0,04* |
| 10 | *Black Beauty* | 0,29 ± 0,03 | 0,55 ± 0,04* |

**- the difference is significant at p<0.05*

Based on the combination of morphophysiological and biochemical parameters of eggplant seeds and seedlings, the following genotypes had the highest storage potential: *Sucleischii*, *Varatic*, *Rada*, *Almaz*, *Acvamarin*, and *Laura*.

## CONCLUSIONS

1. Another air temperature was proposed and tested for the accelerated aging of eggplant seeds: 45-47°C.

2. Using the accelerated seed aging test on eggplant seeds and determining their morphophysiological and biochemical parameters made it possible to assess the storage potential of samples from active gene bank collections and grade them accordingly.

3. All of the samples from the eggplant collection studied are suitable for *ex situ* conservation in a plant gene bank.

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# EVALUATION OF NEW HYBRIDS OF *SALVIA SCLAREA* L., WITH HIGH ESSENTIAL OIL CONTENT

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**Abstract.** The importance of the species *Salvia sclarea* L., its multiple uses, have stimulated genetic and breeding work to create new hybrids with high essential oil content, resistant to drought, frost and wintering. The biological material used is 33 $F_2$ hybrids, simple, triple, double, stepped, complex, in the first, and second year of vegetation. The evaluated hybrids are distinguished by high height, long, compact inflorescences, due to the large number of branches (40 – 52) of the first (12.6 – 17.8) and second (22.4 – 35.6) order of the inflorescence. Determination of the essential oil content has shown that 72.7% of the evaluated hybrids have accumulated an essential oil content greater than 1,000%. In the first year of vegetation, the oil content recorded values ranging from 0.924 – 1.992%, and in the second year, 0.745 – 1.761%. 8 $F_2$ hybrids with very high essential oil content of 1.409–1.761% were selected. The simple hybrid [AP115-11 $S_4$ x AP 112-11 $S_4$]$F_2$ was highlighted for this character, with 1.587% in the first year and 1.761% essential oil recalculated to dry matter, in the second year of vegetation.

***Keywords*: *essential oil, hybrid, the quantitative characters, Salvia sclarea* L.**

## INTRODUCTION

Cultivation of the species *Salvia sclarea* L., (Sage) for the production of essential oil is of indisputable importance. Sage oil is highly appreciated in the perfumery industry [2], being highly appreciated for its excellent aroma and quality as a fixative of aromas [5]. Like other essential oils, clary sage oil can be used in medicinal practice as an antiseptic [5; 8]. The oil of this species has been studied in particular for its antioxidant, antibacterial, analgesic, anti-inflammatory action and qualities [8; 9]. The beneficial action of sage oil in the treatment of diabetes mellitus [10] is attested. Good results have been recorded in Moldova in the treatment of osteoarthritis and arterial hypertension [11]. Sage oil is also used in the manufacture of beer instead of hops, as well as in the production of soft drinks. In the production of liqueurs, scherlai oil is part of the "vanilla cream", and in winemaking it is used

to make vermouth and muscat-type wines [14].

The importance of the *S. sclarea* L. species, the multiple uses of the essential oil, has stimulated a series of genetic and breeding research, which aim to create new, more productive genotypes, resistant to drought, frost and wintering, adapted to the pedoclimatic conditions of the Republic of Moldova. Breeding work on the *S. sclarea* L. species has resulted in the creation and evaluation of over 550 genotypes.

## MATERIALS AND METHODS

The biological material used in the research is 33 $F_2$ hybrids, including 13 – simple, 4 – triple, 2 – double, 5 – step and 9 – complex, in the first and second year of vegetation (2023-2024). Sowing was carried out in the second decade of November, 2022. Cultivation technology – usual for sherlai [6, 14].

During the vegetation period, phenological estimates were made and the phases of plant development were determined. In the first year, the vegetation period begins with the emergence of seedlings, in the second year, from full regeneration, followed by the formation of a leaf rosette, the appearance of internodes, budding, the beginning of flowering, mass flowering, technical ripening of seeds. The evaluation of the hybrids was carried out by studying the morphological characters: plant height, inflorescence length, number of first and second degree branches of the inflorescence [6, 15].

The essential oil content was determined by hydrodistillation in Ginsberg apparatus and recalculated to dry matter to avoid errors caused by the difference in humidity (development phase) of the analyzed samples from each genotype [12].

The statistical interpretation of the obtained experimental data was carried out according to the methods in force [13].

## RESULTS AND DISCUSSIONS

Genetic and breeding work on *S. sclarea* L. has resulted in the creation of hybrids of various types with valuable quantitative traits and high essential oil content. The appreciation of the species, by evaluating the initial breeding material used to create hybrids and performance varieties, aims to obtain new genotypes with high essential oil content, supported by valuable quantitative traits [3]. The second generation ($F_2$) hybrid genotypes evaluated and described in this work are distinguished by quantitative traits that directly influence productivity: plant height, inflorescence length, number of inflorescence branches and essential oil content. Simple, triple, double, stepped and complex hybrids of *S. sclarea* L., bloom in the first and second year of vegetation, respectively, are distinguished by high height (103.2 – 136.2 cm), very long inflorescences (65.0 – 70.0 cm), compact, due to the

large number of branches (40.0 – 52.0) of the first order (12.6 – 17.8), and, respectively, of the second order (22.4 – 35.6). As a result of the evaluation of quantitative characters in $F_2$ hybrids, it was demonstrated that they possess excessive variability. The height of the plants ranged from 103.2 cm to 136.2 cm, depending on the genotype. Plants taller than 124 cm formed the triple hybrid [AP 26-11 $S_4$ x (M-69 487-82 $S_3$ x Rubin 115 b 77)$F_{11}$]$F_2$ (124.2 cm) and the stepped hybrid [(0-32 $S_3$ x 0-41 $S_5$)$F_{11}$ x (0-42 x Rubin)$F_1$ x S-786)$F_9$]$F_2$ (124.5 cm) (Figure 1, n. 16, 24). In the genotype [M-69 489 $S_{12}$ x [(S-1122 60 $S_{10}$ x (M-69 10 $S_4$ x L-15)$F_9$)]$F_5$]$F_2$ this index constituted 128.9 cm, two other step hybrids [(0-57 $S_5$ x 0-20 $S_5$)$F_{11}$ x (0-42 x Rubin)$F_1$ x S-786)$F_9$]$F_2$ and [(S-1122 528 $S_3$ x H-453)$F_1$ x S-1122 390 $S_4$)$F_{11}$ x AP 11-11 $S_4$]$F_2$ were practically at the same level and the plant height was 130.4 (Figure 1, n. 20, 25, 21). The highest index for the "plant height" trait was attested to the simple hybrid [AP 115-11 $S_4$ x AP 112-11 $S_4$]$F_2$ – 136.2 cm (Figure 1, n. 10).

Another quantitative character, which influences productivity, "inflorescence length", varied from 52.0 to 69.9cm in different hybrids (Figure 1). With vigorous inflorescences, two simple hybrids [NC 60-11 $S_4$ x AP 115-11 $S_4$]$F_2$ (65.9 cm), [AP 115-11 $S_4$ x AP 112-11 $S_4$]$F_2$ (68.9 cm), a trilinear hybrid [AP 26-11 $S_4$ x (M-69 487-82 $S_3$ x Rubin 115 b 77)$F_{11}$]$F_2$ (66.6 cm), as well as four stepwise hybrid combinations – [AP 70-11 $S_4$ x (0-42 x Rubin)$F_1$ x S-786)$B_7$]$F_2$ (65.0 cm), [M-69 489 $S_{12}$ x [(S-1122 60 $S_{10}$ x (M-69 10 $S_4$ x L-15)$F_9$)]$F_5$]$F_2$ (65.2 cm), [(0-57 $S_5$ x 0-20 $S_5$)$F_{11}$ x (0-42 x Rubin)$F_1$ x S-786)$F_9$]$F_2$ (68.3 cm) and [(S-1122 528 $S_3$ x H-453)$F_1$ x S-1122 390 $S_4$)$F_{11}$ x AP 11-11 $S_4$]$F_2$, (69.9 cm) (Figure 1, n. 9, 10, 16, 26, 20, 25, 21).

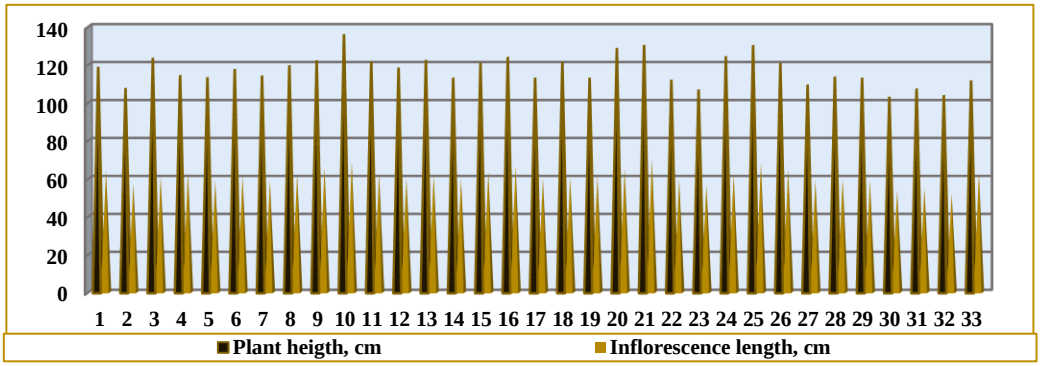


Figure 1. Quantitative characteristics of the plant in the $F_2$ hybrids of *S sclarea* L.

Vigorous inflorescences with a large number of branches are important characters for sage. Both the first and second degree branching of the inflorescence

influence the production of raw material and the essential oil content, because the oil glands in *S. sclarea* L. are exogenous, located on the epidermis of the inflorescence branches and the conger eel sepals of the flowers [3]. Thus, the number of first-degree branches of the inflorescence in the evaluated hybrids varied from 12.6 in the complex hybrid [M-69 655 $S_9$ x (M-69 429-82 $S_3$ x 0-40 $S_5$)$F_7$]$F_6$] x [(S-3 x $H_2S_3$)$F_2$ x 0-32 $S_3$)$F_8$ x NC 11-11 $S_2$]$F_2$]$F_2$, to 17.8 branches in the simple hybrid [NC 60-11 $S_4$ x AP 115-11 $S_4$]$F_2$ (Figure 2, n. 32, 9). The inflorescences of the evaluated hybrids formed a number of second-degree branches, which ranged from 22.4 to 35.6, indices recorded by the simple hybrids [AP 32-11 $S_4$ x AP 26-11 $S_4$]$F_2$ and [NC 77-11 $S_4$ x V-24-86 844 $S_{12}$]$F_2$ (Figure 2, n. 12, 4). The simple hybrid [NC 60-11 $S_4$ x AP 115-11 $S_4$]$F_2$ and the complex hybrid [M-69 655 $S_9$ x (M-69 429-82 $S_3$ x 0-40 $S_5$)$F_7$]$F_6$ x [NC 34 -11$S_2$ x (S-1122 528 $S_3$ x S.s.Tien-Shan/sud))$F_6$]$F_2$]$F_2$ stood out with a very high number in the character "number of branches" of degree I and II of the inflorescence, which in sum constituted 49.0 and 49.2 branches respectively (Figure 2, n. 9, 31). Exceptional in this character were the stepped hybrid combination [(0-32 $S_3$ x 0-41 $S_5$)$F_{11}$ x (0-42 x Rubin)$F_1$ x S-786)$F_9$]$F_2$ and the simple hybrid [NC 77-11 $S_4$ x V-24-86 844 $S_{12}$]$F_2$, with (16.4-34.6) 51 and (16.6-35.6) 52.2 branches, respectively (Figure 2, n. 24, 4).

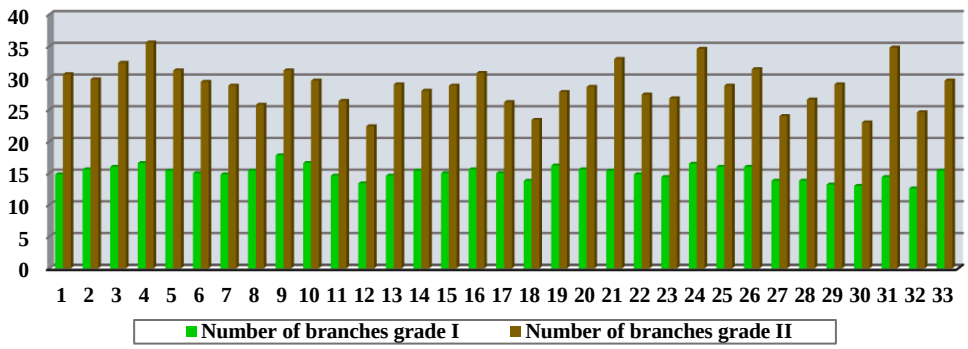


Figure 2. Number of first and second degree branches of the inflorescence in the $F_2$ hybrids of *Salvia Sclarea*

One of the priority objectives of *S. sclarea* L. is the creation of varieties, hybrids of different types, with high content of essential oil of superior quality [6]. The characteristics of the inflorescence, as well as the large number of branches of the first and second order, always ensure the accumulation of a high content of essential oil. The determination of the essential oil content, recalculated to the dry matter, demonstrated that some hybrids synthesized and accumulated a more modest essential oil content, of 0.745 – 0.997%, but the vast majority of them accumulated an essential oil content higher than 1,000%. Thus, in the first year of

vegetation, the essential oil content in the evaluated hybrids recorded values ranging between 0.924 – 1.992% (dry matter), and in the second year, 0.745 – 1.761% (dry matter) in different hybrids. As a result of the evaluation of $F_2$ hybrids of *S. sclarea* L., it was demonstrated that 8 genotypes with very high essential oil content were identified in the second year of vegetation. Thus, the simple hybrid [NC 77-11 $S_4$ x AP 89-11 $S_4$]$F_2$, the trilinear hybrid (0-57 $S_5$ x 0-20 $S_5$)$F_{11}$ x AP 115-11 $S_4$]$F_2$ and the complex hybrid [(M-69 655 $S_9$ x (S-1122 528 $S_3$ x (Rubin x S-786) $F_1$ x (0-33 $S_3$ x L-15)$F_7$)]$F_6$ x [M-69 655 $S_9$ x (K-36 x0-41)$F_2$ x 0-19)$B_5$]$F_5$]$F_2$, accumulated 1,409; 1,440 and 1,443% essential oil recalculated to dry matter, respectively (Figure 3. n. 5, 17, 29). The simple hybrid [AP 113-11 $S_4$ x M-69 489 $S_{14}$]$F_2$ as well as the triple hybrid combination were at a level and the essential oil content constituted 1.483% (dry matter) (Figure 3. n. 3, 14). We note that very high oil content was attested to 2 simple hybrids [NC 60-11 $S_4$ x AP 115-11 $S_4$]$F_2$ and [AP 26-11 $S_4$ x AP 115-11 $S_4$]$F_2$ and this important index constituted 1.548 and 1.665% (dry matter) respectively (Figure 3. n. 9, 2). Exceptional in this character was the hybrid [AP 115-11 $S_4$ x AP 112-11 $S_4$]$F_2$, with a content of 1,587% in the first year and 1,761% essential oil recalculated to dry matter, in the second year of vegetation (Figure 3. n. 10).

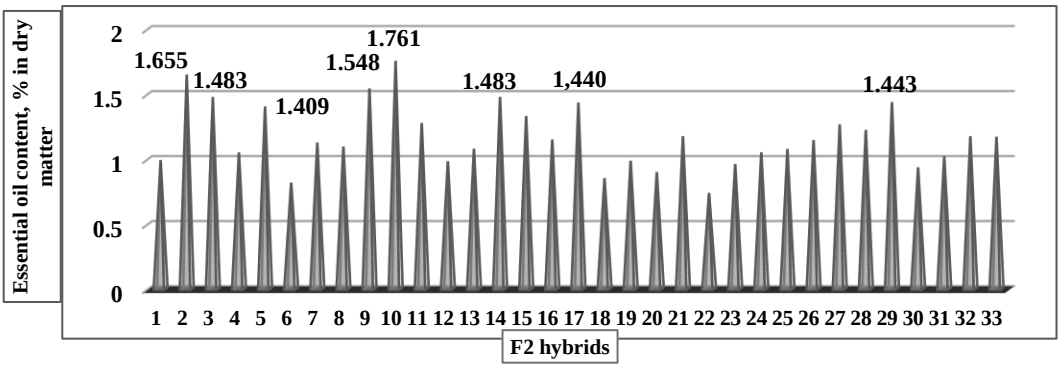


Figure 3. Essential oil content in $F_2$ hybrids of *Salvia Sclarea* in the 2nd year of vegetation

As a result of the research, it was found that 72.7% of the $F_2$ hybrids accumulated essential oil greater than 1.0% (dry matter), and in 24.2% of the genotypes evaluated, the essential oil content was very high and amounted to 1.4 – 1.7% (dry matter).

## CONCLUSIONS

33 simple, triple, double, stepped and complex $F_2$ hybrids of *S. sclarea* L. were evaluated. The evaluated hybrids possess valuable quantitative characters:

high height (103.2 – 136.2 cm), very long inflorescences (65 – 69 cm), compact, due to the large number of branches (40 – 52) of the first order (12.6 – 17.8), and, respectively, of the second order (22.4 – 35.6).

For the character "inflorescence length", the step hybrids [(0-57 $S_5$ x 0-20 $S_5$)$F_{11}$ x (0-42 x Rubin)$F_1$ x S-786)$F_9$]$F_2$ (68.3 cm) and [(S-1122 528 $S_3$ x H-453)$F_1$ x S-1122 390 $S_4$)$F_{11}$ x AP 11-11 $S_4$]$F_2$, (69.9 cm) were highlighted. The highest number of I and II degree branches of the inflorescence was demonstrated by the step hybrid combination [(0-32 $S_3$ x 0-41 $S_5$) F11 x (0-42 x Rubin) $F_1$ x S-786)$F_9$]$F_2$ (51) and the simple hybrid [NC 77-11 $S_4$ x V-24-86 844 $S_{12}$]$F_2$ (52.2).

It was found that 72.7% of the evaluated hybrids accumulated essential oil greater than 1.0% (dry matter). As a result of the evaluation, 8 hybrids with very high essential oil content were selected: 1,409 – 1,761% (dry matter). The simple hybrid [AP 115-11 $S_4$ x AP 112-11 $S_4$]$F_2$ was highlighted, with 1,587% in the first year of vegetation and 1,761% essential oil recalculated to dry matter, in the second year of vegetation.

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# RESEARCH ON THE INTERACTION PECULIARITIES OF COMMON WHEAT WITH *FUSARIUM OXYSPORUM* ISOLATES

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***Abstract.*** The purpose of the research was to establish the cluster organization of *Fusarium oxysporum* isolates, collected in different localities of the Republic of Moldova, the variability and heritability of wheat plant growth and development traits in interaction with the pathogen. Methods for isolating and identifying fungi, preparing culture filtrates, infecting plants, morphometric, and statistical data processing were applied. Treatment of common wheat grains with culture filtrates of 9 *F. oxysporum* isolates, demonstrated their different virulence, which allowed their successful classification into clusters with different pathogenic potential. Factorial analysis of the weight of plant genotype, *F. oxysporum* isolate and their interaction in the source of variation of growth characters demonstrated that the genotype recorded: 48 ... 63%, the isolate factor: 17 ... 23%, the *genotype x isolate* interaction – 11 ... 23%. Relatively high values were recorded for the heritability coefficient in the broad sense (0.53 ... 0.69) and genetic advantage (13.39 ... 24.74%) for growth and development traits in the interaction of common wheat with *F. oxysporum* isolates, which reveals the high opportunities for creating resistant wheat genotypes in limited terms.

***Keywords: Fusarium oxysporum, grown traits, virulence, wheat.***

## INTRODUCTION

Plant-fungal interactions can have beneficial or detrimental effects on host plants and can give rise to various changes at both the plant and fungal levels. The reduction or prevention of fungal diseases of plants depends on resistant crop varieties or fungicide treatment. Plant-fungal interactions need to be known to understand the mechanisms of fungal plant diseases, their prevention, and to improve plant productivity [1].

Incorporating genetic resistance into crop plants is considered the most effective and sustainable solution to combat root rot. However, resistance is often quantitative in nature, i.e. variable and dependent on environmental conditions. The genetic method of combating the disease by creating resistant genotypes is considered a safe and ecologically harmless way to reduce the consequences of root rot. However, it should be noted that it is quite difficult to solve this problem due

to the specificity of the *plant x pathogen* interaction, a specificity that is most directly reflected in the variability and heritability of growth traits.

The heritability pattern of a trait is important for plant breeding because it provides information on the extent to which a particular trait can be transmitted from parent to offspring [2], and heritability estimation provides information on the level of genetic control of the trait [3]. High broad-sense heritability, associated with high genetic progress, reveals the significant contribution of additive genetic variance in trait expression and the fact that selection based on these traits plays a vital role in improving quantitative traits [4].

The genotypic coefficient of variation is the best index for assessing genetic variance, and high heritability coefficients for different traits indicate a high contribution of the genotypic component [5]. To quantify variability and compare traits, the genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability and genetic advantage are assessed according to [6].

In connection with the complexity of the factor systems on which the manifestations of root rot in wheat depend, the purpose of the research was to establish the cluster organization of *F. oxysporum* isolates, collected in different localities of the Republic of Moldova, the variability and heritability of plant growth and development traits in interaction with the pathogen.

## MATERIALS AND METHODS

As research material, 3 genotypes of common winter wheat served – L S/BT/S, L Sel./Accent, Moldova 16 and 9 isolates of *F. oxysporum* from 3 different localities – Br F.o.1, Br F.o.2, Br F.o.3 (Braviceni commune), V F.o.1, V F.o.3, V F.o. 5 (Chișinău city), L F.o.2, L F.o.3, L F.o.5 (Leova city). *F. oxysporum* isolates were selected according to the principle of the difference in geographical areas from which they were sampled and the ability to grow at high temperature (35°C) *in vitro* conditions. Thus, the isolates from Braviceni commune Br1, Br2, Br3 were moderately resistant (MR), susceptible (S), resistant (R); Chișinău town – V1, V3, V5 – moderately resistant (MR), resistant (R), susceptible (S); Leova town – L2, L3, L5 – resistant (R), moderately resistant (MR), resistant (R), respectively.

Culture filtrates (CF) were prepared on the basis of Cszapek liquid nutrient medium. After inoculation with fungal mycelium, Cszapek medium was maintained for 21 days at 24°C [7].

Wheat grains were kept for 18 hours in CF after which they were rinsed 2 times with distilled water and placed on filter paper moistened with distilled water in Petri dishes, 30 grains in each. After 6 days of cultivation at 18-19°C, the seedlings were subjected to morphometric measurements (germination, %; radicle length, mm; stem length, mm; seedling length, cm). The vigor index was calculated based on the formula: germination (%) x seedling length (cm).

To analyze the genetic variability of wheat seedling growth traits, the methods proposed by [6] were used: where $\sigma^2_g$ – genotypic variance; $\sigma^2_{ph}$ – phenotypic variance; $\sigma^2_e$ (error variance, or VE) = MSE; $h^2$ – broad heritability coefficient; PCV – phenotypic coefficient of variation; GCV – genotypic coefficient of variation; X – general mean of the trait; GA – genetic advantage; K – selection differential = 2.06 at 5% selection pressure; $\sigma_{ph}$ – general standard deviation of the trait.

The experiment was carried out in 3 repetitions. The data were statistically processed according to variance analysis, clusterian (dendrograms – agglomerative-iterative method, *k*-means – centroid method), factorial analysis in the STATISTICA 7 software package.

## RESULTS AND DISCUSSIONS

Biometric analysis data demonstrated that the characters of the genotypes under study reacted differently to the treatment of grains with culture filtrates (CF) of *F. oxysporum* isolates, their sensitivity depending on both the genotype of the plant and the isolate (Fig. 1).

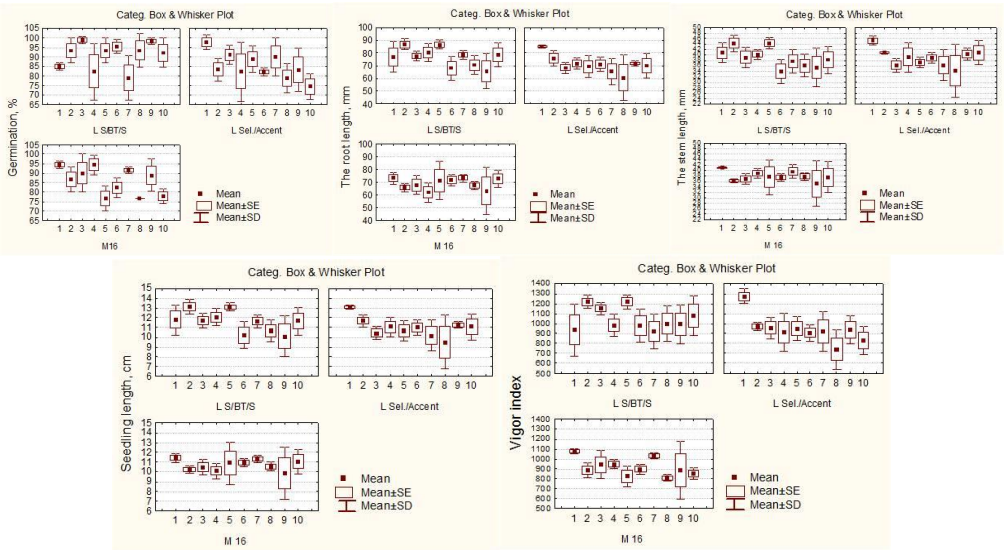


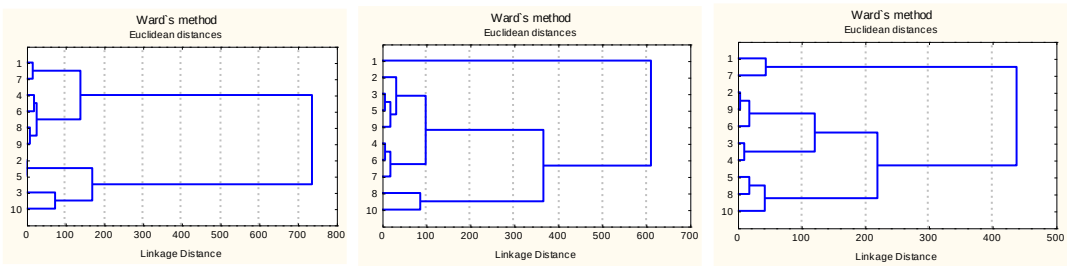

Figure 1. Influence of the *F. oxysporum* culture filtrates on growth characteristics of common winter wheat plants

*Horizontally: 1 – $H_2O$, 2 – Br1, 3 – Br2, 4 – Br3, 5 – V1, 6 – V3, 7 – V5, 8 – L2, 9 – L3, 10 – L5*

The difference in the average of the 9 *F. oxysporum* isolates compared to the control was in the case of *germination*: +6.8%, -14.4%, -9.4%; *radicle length:* -0.1%, -18.6%, -11.4%; *stem length*: -4.9%, -15.3%, -8.3%; *seedling length*: +0.2%,

-17.6%, -7.0%; *vigor index*: +13.5%, -29.3%, -16.7%, respectively, for the genotypes L S/BT/S, L Sel./Accent, Moldova 16. It is worth mentioning that the genotype L S/BT/S differed from the other two in that the values of growth and development characters in the 9 variants with FC were practically at the level of the control variant ($H_2O$), and in the case of germination, FC that stimulate the character were also recorded. This denotes the resistance of the genotype to the action of all *F. oxysporum* isolates.

Cluster analysis using the dendrogram construction method demonstrated that the classification of isolates based on the effect on germination (%), radicle length (mm), stem length (mm), seedling length (cm), and vigor index is different for the 3 genotypes, which denotes that the similarity of isolates according to virulence capacity depends on the genotype of the wheat plant (Fig. 2).




**L S/BT/S** **L Sel/Accent** **Moldova 16**

Figure 2. Dendrogram of distribution of *F. oxysporum* isolates based on their action on the growth and development organs of common wheat plants

*Horizontally: 1 – $H_2O$, 2 – Br1, 3 – Br2, 4 – Br3, 5 – V1, 6 – V3, 7 – V5, 8 – L2, 9 – L3, 10 – L5*

Through the centroid classification analysis (*k*-means method) of the isolates, it was found that the intercluster variance was greater than the intracluster variance for the 3 wheat genotypes and, at the same time, for all the analyzed characters, which reveals a successful classification of the isolates based on virulence capacity (Tab. 1-3).

It is worth noting that in the case of the resistant genotype L S/BT/S, the ratio of intercluster variance to intracluster variance is much lower than in the case of L Sel./Accent and M16. This indicates that although the differentiation of isolates into virulence clusters was successful for L S/BT/S, the differences between clusters are less pronounced than in the case of the other 2 genotypes.

Table 1. Intra- and intercluster variance analysis in the reaction of wheat genotype L S/BT/S with *F. oxysporum* isolates

| Variant | Intercluster variance (1) | df | Intracluster variance (2) | df | F | p | Ratio 1/2 |
|---|---|---|---|---|---|---|---|
| Germination, % | 226.1 | 2 | 189.24 | 7 | 4.18 | 0.06 | 1.20 |
| The length of the radicle, mm | 227.2 | 2 | 224.03 | 7 | 3.55 | 0.09 | 1.01 |
| The length of the stem, mm | 62.2 | 2 | 46.30 | 7 | 4.70 | 0.05 | 1.34 |
| The seedling length, mm | 5.3 | 2 | 4.45 | 7 | 4.21 | 0.06 | 1.19 |
| Vigor index | 105442.3 | 2 | 10092.21 | 7 | 36.57 | 0.00 | 10.45 |

Table 2. Intra- and intercluster variance analysis in the reaction of wheat genotype L Sel./Accent with *F. oxysporum* isolates

| Variant | Intercluster variance (1) | df | Intracluster variance (2) | df | F | p | Ratio 1/2 |
|---|---|---|---|---|---|---|---|
| Germination, % | 306.8 | 2 | 103.01 | 7 | 10.42 | 0.01 | 2.98 |
| The length of the radicle, mm | 272.7 | 2 | 105.64 | 7 | 9.04 | 0.01 | 2.58 |
| The length of the stem, mm | 45.1 | 2 | 43.11 | 7 | 3.66 | 0.08 | 1.05 |
| The seedling length, mm | 5.4 | 2 | 2.68 | 7 | 7.04 | 0.02 | 2.02 |
| Vigor index | 162437.3 | 2 | 7142.64 | 7 | 79.60 | 0.00 | 22.74 |

Table 3. Intra- and intercluster variance analysis in the reaction of wheat genotype M16 with *F. oxysporum* isolates

| Variant | Intercluster variance (1) | df | Intracluster variance (2) | df | F | p | Ratio 1/2 |
|---|---|---|---|---|---|---|---|
| Germination, % | 369.9 | 2 | 84.84 | 7 | 15.26 | 0.00 | 4.36 |
| The length of the radicle, mm | 83.6 | 2 | 75.10 | 7 | 3.90 | 0.07 | 1.11 |
| The length of the stem, mm | 16.1 | 2 | 8.88 | 7 | 6.33 | 0.03 | 1.81 |
| The seedling length, mm | 1.7 | 2 | 0.80 | 7 | 7.26 | 0.02 | 2.13 |
| Vigor index | 61916.1 | 2 | 5979.12 | 7 | 36.24 | 0.00 | 10.36 |

Through descriptive statistical analysis (Tab. 4-6), the clusters of isolates that produced the most significant inhibitions of growth traits were found, but the presence of isolates L2 (R), L5 (R) (or. Leova) in the cluster with the strongest negative effect for all 3 genotypes is observed.

Table 4. Descriptive statistics of clusters of *F. oxysporum* isolates in interaction with the wheat line L S/BT/S

| Variant | Mean, % | σ | Member of the cluster |
|---|---|---|---|
| ***Cluster 1, n = 3*** | | | |
| Germination, % | 95,23 | 3.18 | 2 – Br1 (MR),<br>3 – Br2 (S),<br>5 – V1 (MR). |
| The length of the radicle, mm | 83,77 | 5.26 | |
| The length of the stem, mm | 42,53 | 2.97 | |
| Seedling length, mm | 12,63 | 0.81 | |
| Vigor index | 1199,25 | 38.15 | |
| ***Cluster 2, n = 5*** | | | |
| Germination, % | 92.32 | 6.13 | 4 – Br3 (R),<br>6 – V3 (R),<br>8 – L2 (MR),<br>9 – L3 (R),<br>10 – L5 (S). |
| The length of the radicle, mm | 72.70 | 6.48 | |
| The length of the stem, mm | 36.78 | 2.44 | |
| The seedling length, mm | 10.94 | 0.88 | |
| Vigor index | 1007.78 | 42.06 | |
| ***Cluster 3, n =2*** | | | |
| Germination, % | 81.95 | 4.31 | 1 (M – $H_2O$),<br>7 – V5 (S). |
| The length of the radicle, mm | 77.85 | 1.06 | |
| The length of the stem, mm | 39.25 | 2.19 | |
| The seedling length, mm | 11.71 | 0.11 | |
| Vigor index | 928.12 | 10.30 | |

Factorial analysis of the weight of plant genotype, *F. oxysporum* isolate, genotype x isolate interactions in the source of variation of the characters under study returned the genotype that recorded: 48, 63, 51, 60%, respectively, of germination, radicle length, seedling length, vigor index. The weight of the isolate factor constituted: 20, 17, 23, 17%, and of genotype x isolate interaction – 23, 11, 14, 15%, respectively, of germination, radicle length, seedling length, vigor index (Fig. 3).

By calculating the genetic parameters of genotypic ($\sigma^2_G$) and phenotypic ($\sigma^2_{Ph}$) variance, it was found that the broad sense heritability coefficient ($h^2$) varied within the limits of 0.53 ... 0.69, which denotes a relatively high capacity for hereditary transmission of growth characters in the interaction of wheat plants with *F. oxysporum* isolates.

Table 5. Descriptive statistics of clusters of *F. oxysporum* isolates in interaction with the wheat line L Sel./Accent

| Variant | Mean, % | σ | Member of the cluster |
|---|---|---|---|
| ***Cluster 1, n = 1*** | | | |
| Germination, % | 97.77 | 0.00 | 1 – ($H_2O$). |
| The length of the radicle, mm | 85.32 | 0.00 | |
| The length of the stem, mm | 45.24 | 0.00 | |
| The seedling length, mm | 13.05 | 0.00 | |
| Vigor index | 1276.73 | 0.00 | |
| ***Cluster 2, n = 7*** | | | |
| Germination, % | 85.88 | 3.94 | 2 – Br1 (MR), 3 – Br2 (S), 4 – Br3 (R), 5 – V1 (MR), 6 – V3 (R), 7 – V5 (S), 9 – L3 (R). |
| The length of the radicle, mm | 70.55 | 3.24 | |
| The length of the stem, mm | 38.45 | 1.87 | |
| The seedling length, mm | 10.90 | 0.50 | |
| Vigor index | 935.89 | 23.93 | |
| ***Cluster 3, n =2*** | | | |
| Germination, % | 76.67 | 3.11 | 8 – L2 (R), 10 – L5 (R). |
| The length of the radicle, mm | 65.24 | 6.51 | |
| The length of the stem, mm | 37.58 | 4.70 | |
| The seedling length, mm | 10.26 | 1.08 | |
| Vigor index | 783.58 | 60.88 | |

Table 6. Descriptive statistics of clusters of *F. oxysporum* isolates in interaction with the wheat line M16

| Varian | Mean, % | σ | Member of the cluster |
|---|---|---|---|
| ***Cluster 1, n = 2*** | | | |
| Germination, % | 93.06 | 1.96 | 1 – ($H_2O$), 7 – V5 (S). |
| The length of the radicle, mm | 73.26 | 0.05 | |
| The length of the stem, mm | 40.33 | 1.08 | |
| The seedling length, mm | 11.36 | 0.10 | |
| Vigor index | 1056.65 | 31.37 | |
| ***Cluster 2, n = 5*** | | | |
| Germination, % | 88.45 | 4.48 | 2 – Br1 (MR), 3 – Br2 (S), 4 – Br3 (R), 6 – V3 (R), 9 – L3 (R). |
| The length of the radicle, mm | 66.17 | 3.90 | |
| The length of the stem, mm | 37,0 | 1.39 | |
| The seedling length, mm | 10.31 | 0.41 | |
| Vigor index | 912.95 | 31.35 | |
| ***Cluster 3, n =3*** | | | |
| Germination, % | 77.05 | 0.63 | 5 – V1 (MR), 8 – L2 (R), 10 – L5 (R). |
| The length of the radicle, mm | 70.57 | 2.68 | |
| The length of the stem, mm | 37.66 | 0.15 | |
| The seedling length, mm | 10.82 | 0.25 | |
| Vigor index | 829.76 | 23.06 | |

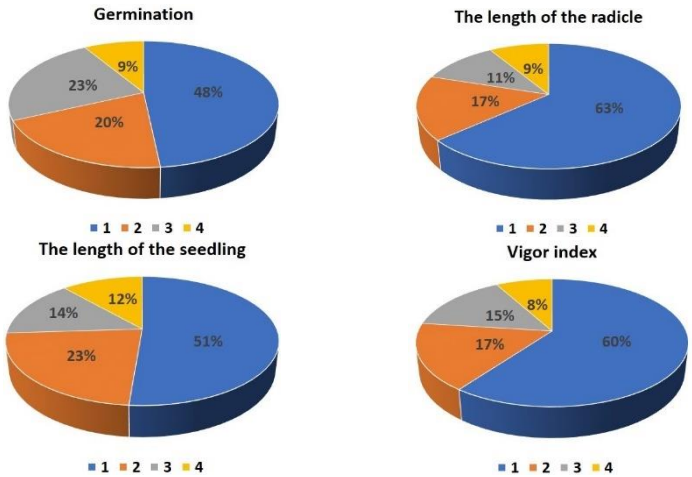



Figure 3. Share of plant genotype factors (1), isolate (2), genotype x isolate interactions (3), random factors (4) in the source of variation in growth and development traits of common wheat

Table 7. Variability and heritability of common wheat growth characters in interaction with *F. oxysporum* isolates

| Parameter | Germination | The length of the radicle | The length of the seedling | Vigor index |
|---|---|---|---|---|
| $\sigma^2_G$ | 85.03 | 153.3 | 1.73 | 43572 |
| $\sigma^2_{Ph}$ | 140.3 | 226.6 | 3.24 | 63548 |
| $h^2$ | **0.61** | **0.68** | **0.53** | **0.69** |
| GCV, % | 10.55 | 17.13 | 11.83 | 21.55 |
| PCV, % | 13.55 | 20.82 | 16.23 | 26.03 |
| GAM, % | 13.44 | 18.77 | 13.39 | 24.74 |

The genotypic coefficient of variation (GCV, %) and genetic advantage (GAM, %) recorded medium-high values: 10.55 ... 21.55%, 13.39 ... 24.74%, respectively. The association of relatively high values of the $h^2$ and GAM (%) parameters reveals real opportunities for obtaining common wheat genotypes resistant to the fungus *F. oxysporum*.

## CONCLUSIONS

Treatment of common wheat grains with culture filtrates of 9 F. oxysporum isolates, collected in different areas of the Republic of Moldova (Brăviceni commune, Chișinău city, Leova city), and distinguished according to their

resistance to high temperature (35°C), demonstrated their different virulence, which allowed their successful classification into clusters with different pathogenic potential.

Factorial analysis of the weight of plant genotype, *F. oxysporum* isolate and their interaction in the source of variation of growth characters demonstrated that the genotype recorded: 48, 63, 51, 60% in the source of variation of germination, radicle length, seedling length, vigor index, respectively. The weight of the isolate factor constituted: 20, 17, 23, 17%, and of *genotype x isolate* interaction – 23, 11, 14, 15%, respectively, of germination, radicle length, seedling length, vigor index.

Relatively high values were recorded for the heritability coefficient in the broad sense (0.53 ... 0.69) and genetic advantage (13.39 ... 24.74%) for growth and development traits in the interaction of common wheat with *F. oxysporum* isolates, which reveals the high opportunities for creating resistant wheat genotypes in limited terms.

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# AGRONOMICAL CHARACTERISTICS THE BREEDING GERMPLASM OF CHICKPEA

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***Abstract.*** *Investigation material comprised twenty(F5) and eight ten (F3) breeding germplasm of chickpea derived from 2 crosses ♀Alexandrite×♂Ichel and ♀Botna×♂Ichel respectively, as check was v. Botna. The objective of the study was evaluation and selection germplasm by yield and analysis 5 yield attributing characters: number seeds and seeds yield per plant, hundred seeds weight, plant height and full maturity. Method of breeding is "pedigree". By productivity were selected 10(F4) and 12(F5) progenies, of them B121, B144, B126, B130, B137, B143, B147 and A170, A181, A190 were evaluated as high yielded (116.1 – 124.2g/m$^2$), which superior the check v. Botna (106.5g/m$^2$). Analysis of traits revealed that no. of seeds and seeds yield per plant are principal in yield enhancing. Larger seeds germplasm coupled with taller plant for most progenies increases yield per plant. Most high yielded germplasm is middle-maturity. Strong relationship exhibited trait seeds yield per plant with plant height (r=0.855), hundred seeds weight (r=0.881) and with no. seeds per plant (r=0.891) in F4 and F6 generation, respectively.*

***Keywords: chickpea germplasm, F4, F6-generation, yield attributing traits, yield.***

## INTRODUCTION

Despite recent growth in chickpea production, challenges like climate change, emerging diseases and market issues remain significant. To overcome these issues through crop improvement, genetic variability and diversity studies are the fundamental requirement [1]. India is the leading country of chickpea gross production. Shreyasee Seth *et al*. (2020) reported about yield varieties in India: Uday- 1058 kg/ha, JG 14- 908 kg/ha, Anuradha - 904 kg/ha, Vaibhav -787 kg/ha) [2]. Romanian researchers Pânzaru Radu Lucian *et al.* (2022) in their work reported: the culture presents a productive potential for Romania, between 1000 and 1500 kg/ha, under the conditions of use, in production, of local populations and varieties [3]. About yield attributing traits Moulya G. et al. (2024) related: correlation studies revealed that, seed yield was in positive association with number of pods per plant, plant height and hundred seed weight [1]. In Ethiopia Doda Gemeda A. and Nepir Gurmu G. (2024) in his research concluded that seed yield in chickpea can be improved by selecting the plant that have greater number of pods per plant and number of seeds per plant [4]. In aspect yield per area Sweta Soni *et*

*al*. (2024) communicate: that plot yield in grams had significant and positive correlation with hundred seed weight, harvest index and seed yield per plant [5]. In R. Moldova released for cultivation 4 varieties: Botna, Ichel, Cogâlnic and Ovidel [6]. Alexandrite is Ukrainian variety. In this work presented results of evaluation breeding germplasm F4 and F6 for yield and main yield attributing characters.

## MATERIALS AND METHODS

Method of breeding the germplasm is "pedigree"[7]. Eight ten (F3) and twenty (F5) breeding germplasm of chickpea derived from 2 crosses Botna ×Ichel and Alexandrite× Ichel, respectively were evaluated on yield and 5 yield attributing characters: seeds and seeds yield per plant, hundred seeds weight, plant height and full maturity of plants. The field experiment was carried out in 2024. Material laid out random in 2 replications, rows 3.0 m length, inter row distance 50 cm and plant to plant -10cm. As check was used cv. Botna. The sowing was 3-4 April. Seven plant from each entry were randomly collected for data seeds & seeds yield per plant. Plant height were measured before plant maturing. Selection criterion based on productivity at the check level and higher. Observation of maturity: first date 50%, second one 100% plants were full maturity. Seeds data (type, color and HSW) described according to the descriptors for chickpea.

## RESULTS AND DISCUSSIONS

By productivity in the F4 generation were selected 10 progenies, namely: B121, B123, B126, B130, B137, B143, B144, B147, B150 and B152. Type of seeds these genotypes are: *desi* only genotype B152 as well as check v. Botna. Genotypes B121, B126 and B137 had segregation into *desi* and *gulabi*, others - *kabuli* (genotypes B143 and B144 had a little amount *gulabi/desi* and intermediate d*esi-gulabi* type). By seed color genotypes B126 and B 152 as well as v. Botna are brown. Genotypes B121, B137, B143 and B144 had segregation into brown and green, B123, B130, B147 and B150 – beige (Table1).

In F6 generation by yield were selected 12 germplasm: A157, A170, A171, A173, A181, A184, A186, A192, A201and A202 by type seeds are kabuli, A204 – gulabi and A191- intermediate gulabi-kabuli. Color of seeds: A157 and A190 – light-brown, A201 and A202 – beige, A192 – Brown-green, A204 – orange-yellow others are brown (Table2).

Table 1. Type and seeds' color of germplasm, F4 generation (Botna ×Ichel)

| Name of progenies | Type of seeds | Color of seeds | Name progenies | Type of seeds | Color of seeds |
|---|---|---|---|---|---|
| v. Botna | Desi | Brown | B143 | Kabuli* | Brown/green |
| B121 | Desi/Gulabi | Brown/green | B144 | Kabuli** | Brown/green |
| B123 | Kabuli | Beige | B147 | Kabuli | Beige |
| B126 | Desi/Gulabi | Brown | B150 | Kabuli | Beige |
| B130 | Kabuli | Beige | B152 | Desi | Brown |
| B137 | Desi/Gulabi | Brown/green | - | - | - |

**g/d and **d-g / little amount of seeds*

Five yield attributing characters were measured /observed in selected germplasm: seeds and seeds yield per plant, hundred seed weight, plant height, time of full maturity. As resulting character - seed yield/m$^2$. The range four of them in descending order for F4/F6 constituted following values: plant height (11.5/13.3), hundred seed weight (6.1/2.8), seeds per plant (4.64/4.36) and seed yield per plant (2.0/1.7).

Table 2. Type and seeds' color germplasm, F6-generation (Alexandrite ×Ichel)

| Name of progenies | Type of seeds | Color of seeds | Name progenies | Type of seeds | Color of seeds |
|---|---|---|---|---|---|
| A157 | Kabuli | Light-brown | A186 | Kabuli | Brown |
| A170 | Kabuli | Brown | A190 | G-k | Light-brown |
| A171 | Kabuli | Brown | A192 | Kabuli | Brown-green |
| A173 | Kabuli | Brown | A201 | Kabuli | Beige |
| A181 | Kabuli | Brown | A202 | Kabuli | Beige |
| A184 | Kabuli | Brown | A204 | Gulabi | Orange-yellow |

Thus, PH had maximum range for both generation, in regard HSW in F4 generation was greater than in F6 one, range of seeds and seed yield per plant are commensurate between generation. Difference between mean values at F4 and F6 also in descending order are: plant height (6.43cm, 50.44/ 56.87), HSW (2.59g, 26.43/ 29.02), mean seeds per plant at F4 (30.71) a little greater than at F6(28.24) and seeds yield per plant (8.12/8.2) without significant difference. Range of yield per m$^2$ in F4 generation was 104.3g – 122.5g, in F5 one 105.4g – 124.2g(commensurate) but mean values in F4 -114.54g a little greater than F6-110.86g. (Table3,4).

Full maturity of v. Botna was observed 18 July, at F4 generation closely by this character were by genotypes B126, B150 and B152 – 18-19 July. Genotypes B123, B130, B137, B143 and B144 full maturity observed on19-20 July, little later genotypes B121 and B147 (20-21 July). Thus, full maturity was at 1-2 days later (except B121, B147) than v. Botna (Table3).

Table 3. Yield and yield attributing characters, F4-generation (v. Botna × v. Ichel)

| Progenies | Seeds/Pl | SY/Pl | 100-SW | Plant Height | Maturity | SY/m$^2$ |
|---|---|---|---|---|---|---|
| v. Botna | 27.86±1.13 | 7.56 | 27.1 ±0.22 | 45.7±0.37 | 18/07 | 105.7 |
| B121 | 31.76±1.14 | 8.67 | 27.3±0.45 | 51.3±0.51 | 20-21.07 | 122.5 |
| B123 | 30.5 ±1.22 | 7.15 | 23.4±0.35 | 49.8±0.46 | 19-20.07 | 104.3 |
| B126 | 31.1±1.15 | 9.05 | 29.1±0.43 | 55.4±0.49 | 18-19.07 | 119.4 |
| B130 | 32.0±1.25 | 9.15 | 28.6±0.36 | 57.2±0.53 | 19-20.07 | 120.8 |
| B137 | 30.5±1.17 | 9.0 | 29.5±0.42 | 52.5±0.48 | 19-20.07 | 120.1 |
| B143 | 31.6 ±0.98 | 7.7 | 24.5±0.47 | 48.1±0.52 | 19-20.07 | 117.5 |
| B144 | 32.5±1.27 | 8.22 | 25.3±0.45 | 50.7±0.51 | 19-20.07 | 121.3 |
| B147 | 30.71±1.18 | 7.96 | 25.9±0.29 | 48.3±0.47 | 20-21.07 | 116.1 |
| B150 | 29.88±1.16 | 7.44 | 24.9±0.27 | 47.8±0.46 | 18-19.07 | 106.4 |
| B152 | 29.44±1.12 | 7.42 | 25.2±0.26 | 48.1±0.48 | 18-19.07 | 105.9 |
| Min | 27.86 | 7.15 | 23.4 | 45.7 | 18-19.07 | 104.3 |
| Max/range | 32.5/ 4.64 | 9.15/2 | 29.5/ 6.1 | 57.2/ 11.5 | 20-21.07 | 122.5 |
| Mean | 30.71 | 8.12 | 26.43 | 50.44 | - | 114.54 |
| CV,% | 4.3 | 9.0 | 7.57 | 6.88 | - | 6.4 |

Maturity at F6 generation: germplasm as A190, A202 and A204 full maturity observed 19 July, 6 germplasm, namely: A170, A171, A181, A186, A192 and A201 had the latest maturation 22-23 July, intermediate were A157, A173 and A184 – 20-21 July. Range of maturity at F6 generation was 5 days (19-23.07) 2 days greater compared to F4 one, 3 days (19-21.07). So, ripening germplasm of F6 generation had somewhat later than F4 one (Table 4).

Table 4. Yield and yield attributing traits F6-generation (v. Alexandrite ×v. Ichel)

| Progenies | Seeds/Pl | SY/Pl | 100-SW | Height Plant | Maturity | SY/m$^2$ |
|---|---|---|---|---|---|---|
| v. Botna | 27.9±1.15 | 7.62 | 27.3 ±0.22 | 46.9±0.37 | 18.07 | 107.3 |
| A157 | 27.6±1.18 | 7.81 | 28.3±0.29 | 56.7±0.47 | 19-20.07 | 106.5 |
| A170 | 30.93±1.22 | 9.28 | 30.0±0.32 | 59.6±0.49 | 22-23.07 | 124.2 |
| A171 | 27.74±1.21 | 8.1 | 29.2±0.31 | 59.1±0.48 | 22-23.07 | 108.4 |
| A173 | 26.87±0.98 | 7.95 | 29.6±0.28 | 57.3±0.42 | 20-21.07 | 107.1 |
| A181 | 30.53±1.24 | 9.19 | 30.1±0.32 | 60.2±0.50 | 22-23.07 | 121.8 |
| A184 | 27.86±1.19 | 8.22 | 29.5±0.27 | 55.9±0.45 | 19-20.07 | 109.2 |
| A186 | 27.5±1.13 | 8.17 | 29.7±0.29 | 57.6±0.48 | 22-23.07 | 108.6 |
| A190 | 29.76±1.23 | 8.78 | 29.5±0.30 | 56.7±0.44 | 19.07 | 120.3 |
| A192 | 27.54±1.17 | 7.85 | 28.5±0.33 | 58.4±0.47 | 22-23.07 | 106.7 |
| A201 | 26.57±1.09 | 7.89 | 29.7±0.29 | 59.5±0.52 | 22-23.07 | 105.4 |
| A202 | 28.26±1.14 | 7.8 | 27.6±0.26 | 55.3±0.46 | 19.07 | 107.5 |
| A204 | 28.06±1.09 | 7.94 | 28.3±0.25 | 56.2±0.47 | 19.07 | 108.2 |
| Min | 26.57 | 7.62 | 27.3 | 46.9 | 18.07 | 105.4 |
| Max/range | 30.93/ 4.36 | 9.28/1.7 | 30.1/ 2.8 | 60.2/ 13.3 | 22-23.07 | 124.2 |
| Mean | 28.24 | 8.2 | 29.02 | 56.87 | - | 110.86 |
| CV, % | 4.71 | 6.58 | 3.17 | 5.92 | - | 5.9 |

Coefficient of correlation: Plant height exhibited significant positive relationship with HSW in both generation F4(r=0.656), F6(r=0.746). PH with no. seeds per plant showed significant value only in F4(r=0.614), with seeds yield per plant were observed strong association in F4(r=0.855) and moderate one in F6(r=0.506). Hundred seeds weight with seeds per plant had negligible value in F4(r=0.069) and weak one in F6(r=0.314), with seeds yield per plant highly significant in F4(r=0.881) and significant in F6(r=0.711).

Table5 Phenotypic correlation at F4 and F6 progenies

| Characters | F4 | F6 | Characters | F4 | F6 |
|---|---|---|---|---|---|
| Plant height – HSW | 0.656* | 0.746* | HSW – no. S/Pl | 0.069 | 0.314 |
| Plant height – no. S/Pl | 0.614* | 0.202 | HSW - SY/Pl | 0.881** | 0.711* |
| Plant height – SY/Pl | 0.855** | 0.506* | no. S/Pl - SY/Pl | 0.533* | 0.891** |

*, **- *significant at level P≤0.05 and P≤0.01*

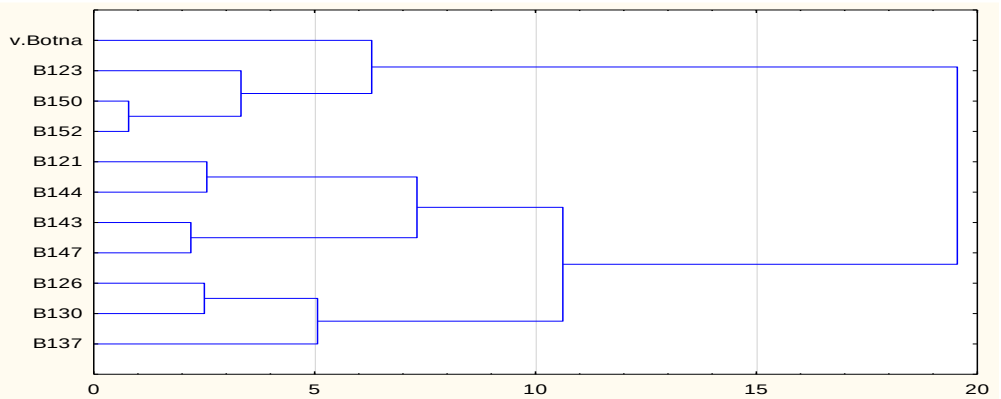


Figure1. Dendrogram of germplasm (F4 generation) based on 5 traits

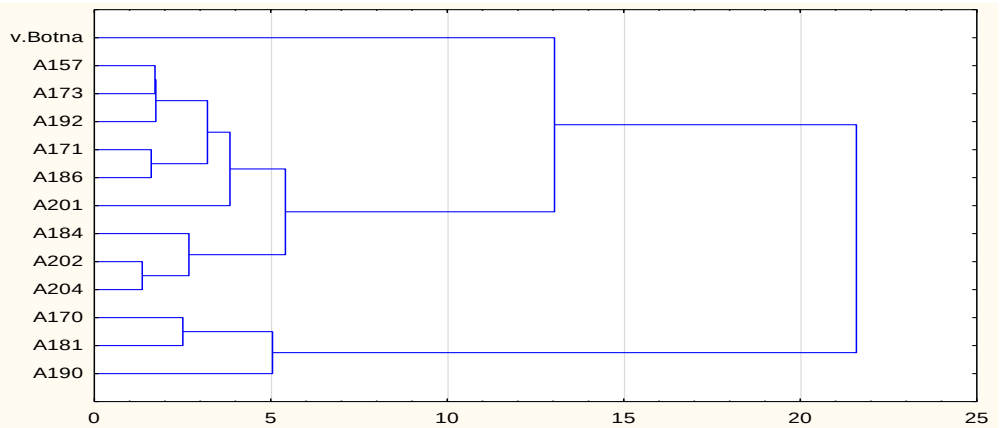


Figure 2. Dendrogram of germplasm (F6 generation) based on 5 traits

Between variables seeds per plant and seeds yield per plant were determined moderate association in F4(0.533) and highly significant in F6(r=0.891), (Table5).

Both dendrogram have been done by complete linkage method (Figure 1,2). In F4 generation germplasm were partitioned into 4 clusters: CL1 – v. Botna, B123, B150, B152; CL2 – B121, B144; CL3 – B143, B147; CL4 – B126, B130 and B137(Figure1). In F6 generation were formed 3 clusters: CL(I) – A157, A173, A192, A171, A186, A201; CL(II) – A184, A202, A204; CL(III) - A170, A181 and A190. Variety Botna did not enter any of the clusters but by yield/m$^2$ combined with CL(I) and CL(II), Figure 1,2.

Table 6. Mean performance of agronomic traits partitioning into clusters

| CLs | Germplasm | Seeds /Pl | SY/Pl (g) | 100-SW | Height Plant | SY/ M$^2$(g) | Maturity |
|---|---|---|---|---|---|---|---|
| 2 | B121,B144 | 32.13 | 8.44 | 26.3 | 51.0 | 121.9 | 19-21/7 |
| 4 | B126, B130, B137 | 31.2 | 9.06 | 29.06 | 55.03 | 120.1 | 19-20/7 |
| 3 | B143, B147 | 31.15 | 7.83 | 25.2 | 48.2 | 116.8 | 19-21/7 |
| 1 | Botna, B123, B150, B152 | 29.42 | 7.39 | 25.12 | 47.85 | 105.6 | 18-20/7 |
| III | A170, A181, A190 | 30.40 | 9.08 | 29.86 | 58.83 | 122.1 | 19, 22-23/7 |
| II | A184, A202, A204 | 28.06 | 7.98 | 28.46 | 55.8 | 108.3 | 19-20/7 |
| I | A157, A173, A192, A171, A186, A201 | 27.39 | 7.91 | 28.9 | 56.5 | 107.14 | 19-21, 22-23/7 |

Mean performance of agronomic traits by clusters presented in Table 6 in descending order of yield/m$^2$. In F4 generation germplasm pertaining to CL2 and CL4 were the most productive with mean yield 121.9g and 120.1g/m$^2$, respectively. Distinction germplasm of GL4 from CL2 are: the plants are a little taller, with larger seeds, and little difference into traits seeds and seeds yield per plant. Germplasm at CL3 intermediate by yield (116.8g/m$^2$) by seeds and SY/Pl, HSW and PH little inferior compared to germplasm of CL2. In cluster 1, entering germplasm B123, B150, B152 and v. Botna with mean productivity105.6g/m$^2$, them differ from CL3 a little reduce seeds and SY per plant. Maturity: germplasm of CL1 a little earlier than others one. In F6 generation 3 germplasm A170, A181 and A190 pertaining to Cl(III) with mean yield 122.1g/m$^2$ superior by yield other germplasm and v. Botna (108.3, 107.14g/m$^2$). Main advantage germplasm of Cl(III) compared to CL(II) and CL(I) are higher values of seeds and SY per plant. In F6 generation germplasm of CL(II) as well as A190, A157 and A173 by ripening were a little earlier than remaining. In generally late maturing is undesirable traits, of them only 2 out of 6 progenies had increased yield.

Number of fruit-bearing plants(FBP) traits that should also be taken into consideration. Analysis of FBP the F4 generation in clusters showed following mean values per m$^2$: 14.2, 14.52, 15.0 and 13.3 in clusters 1,2,3 and 4, respectively;

in F6 one – without significant difference (13.47 – 13.54). Thus, all germplasm of F6 and belonging to CL4(F4) had some reduced values.

## CONCLUSIONS

As a result of evaluation breeding germplasm on yield/agronomic traits, were selected 22 progenies of them that superior the check was 7(B121, B144, B126, B130, B137, B143, B147) and 3(A170, A181, A190) germplasm in F4 and F6 generation, respectively. Analysis yield traits revealed that number of seeds and seeds yield per plant is the most significant. Plant height is significant coupled with hundred seeds weight, for most part of progenies increasing these traits enhanced yield per plant and per plot. Most (8 out of 10) of high yielded germplasm were middle matured, thus later maturing is undesirable trait.

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# INDUCTION OF ARTIFICIAL MUTATIONS IN *LINUM USITATISSIMUM* L. BY EXPOSING SEEDS TO DIFFERENT DOSES OF *X*-RAYS

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***Abstract:*** *The artificial induction of mutations represents an effective method for generating new sources of germplasm, which are essential in plant breeding. In the present study, induced mutagenesis was applied to flax by exposure to X-rays. As biological material, three flax (Linum usitatissimum L.) genotypes were selected: Kaufmann and Blaringhem (oilseed flax), and Belinka (fiber flax). Seeds were irradiated with X-rays at three doses: 500, 700, and 900 Gy, using the RS-2400-Q installation. The treatment led to a significant reduction in plant viability, especially for the Blaringhem and Belinka genotypes, which were completely eliminated within the first 24–33 days. In the case of the Kaufmann genotype, the survival rate at the 500 Gy dose was only 0.7%, also indicating high sensitivity to irradiation. However, plant mortality during various developmental stages was also influenced by environmental stress factors (hydrological drought, high temperatures).*

*Analysis of the $M_0$ generation descendants revealed significant morphological changes compared to the control: increased number of branches, flowers, and fruits, but a pronounced reduction in seed mass and quality. The $M_0$ descendants exhibited an imbalance between vegetative and reproductive growth.*

***Keywords: flax, linseed, Linum usitatissimum L., physical mutagenesis, X rays.***

## INTRODUCTION

Flax or linseed (*Linum usitatissimum* L., 2n = 2x = 30) - technical, oilseed, medicinal, melliferous, nutritional and ornamental plant. It is an annual, dicotyledonous, self-pollinated plant species, with a relatively small genome size, estimated at ~370-455 Mb, depending on the genotypes [1, 2].

Currently, approximately 61,000 germplasm accessions of linseed including 1,127 wild accessions are conserved in genebanks (1,750 - GB) and/or institutes worldwide [3, 4]. However, estimates show that only 10,000-15,000 of them are unique [5, 6]. Including 12 mutant flax varieties developed through induced mutagenesis, all of

which have been officially approved and registered in the MVD of FAO/IAEA.

According to the FAO/IAEA Mutant Varieties Database (MVD), the total number of officially recorded mutant varieties currently stands at 3,460, derived from over 200 to 240 plant species [7, 8, 9, 10, 11, 12, 13]. This figure reflects a significant increase since the 1990s, with the number of registered varieties rising from 1,936 in 1990 to 2,724 in 2000, 3,222 in 2010, and 3,397 by 2020 [7].

The MVD database indicates that the species with the highest number of mutant varieties include rice (over 876 entries), barley (309), chrysanthemum (288), wheat (276), soybean (184) and maize (89) etc. In the case of flax, 12 mutant varieties have been registered, corresponding to two different species: *Linum usitatissimum* L. (6 – textile flax, 5 – oilseed flax) and *Linum grandiflorum* Desf. (1 – red flax, decorative). The countries with the most mutant crops: China (825), Japan (501), India (346), Russian Federation (216), Netherlands (176) etc. [13, 14].

The earliest flax mutant variety recorded in the Mutant Variety Database (MVD) is *"Redwood 65"*, an oilseed flax cultivar officially registered in Canada in 1965. This variety was developed through *X*-ray-induced mutagenesis, a method involving physical mutagens. The most recent mutant oilseed flax variety, *"DLV 20"*, was registered in 2019 in India and was obtained via ethyl methanesulfonate (EMS)-induced mutagenesis, a chemical mutagenesis technique. In the case of textile flax, the most recently recorded mutant varieties are *"Baltyuchai"* and *"M-5"*, both registered in the Russian Federation in 1991. These were developed using chemical mutagens, specifically nitrosoethylurea (NEU) and dimethyl sulfate (DMS). Additionally, the ornamental flax variety *"Zorepad"*, belonging to the species *Linum grandiflorum* Desf., was developed in Ukraine through EMS-induced chemical mutagenesis and was officially registered in 2003 [7].

Physical mutagens, mostly ionizing radiation, can increase the natural mutation rate by 1,000 to 1 million-fold, and have been widely used to induce heritable genetic changes. More than 70% of induced and released mutant crop varieties have been developed using physical mutagens. Since the 1960s, gamma rays have become the most commonly used mutagenic agent in plant mutation breeding [15].

## MATERIALS AND METHODS

The scientific research was conducted at the Institute of Genetics, Physiology, and Plant Protection of Moldova State University, as well as at the Slovak University of Agriculture in Nitra, Institute of Plant and Environmental Sciences.

For the research, three flax genotypes from the *ex situ* collection of the Gene Bank were used: *Kaufmann* and *Blaringhem* (oil flax), and *Belinka* (fiber flax).

The flax seeds was subjected to *X*-ray irradiation treatment using the RS-2400-Q installation, equipped with an ionizing *X*-ray emitter (Rad Source

Technologies). The irradiation was performed in a single session, using 1000 seeds for each experimental variant. Three radiation doses were applied: 500 Gy (with an exposure time of 44 minutes), 700 Gy (62 minutes) and 900 Gy (80 minutes). During the treatment, the seeds were placed in cylindrical carbon fiber canister. The control samples consisted of untreated flax seeds, used for comparison with the irradiated variants.

The assessment of biological, morphological, and agrotechnical parameters was conducted in accordance with the criteria established in the *Descriptors List for Flax (Linum usitatissimum L.),* Nitra, 2016 [16]. The evaluations were carried out following the technical standards and methodological guidelines officially approved for plant breeding activities [17, 18].

## RESULTS AND DISCUSSIONS

For obtaining induced mutations in plants, physical mutagens are used (gamma rays, *X*-rays and ultraviolet (*UV*) radiation). These methods allow the induction of heritable changes in the plant genome, which can then be selected based on desired traits (disease resistance, productivity, fruit size etc.).

The effects of ionizing radiation on plants vary significantly. The ultimate impact of the mutagenic agent, including *X*-rays, on plants is contingent upon the administered dose, the nature of the mutagen, the botanical species involved and the susceptibility of the genotype under investigation.

During the growing season, a significant elimination of the irradiated material was recorded. Under the influence of radiation and limiting environmental factors, the *Blaringhem* (after 24 days) and *Belinka* (after 33 days) varieties proved to be the least resistant, with the irradiated plants being completely eliminated. Also, the percentage of remaining plants was very low for the *Kaufmann* variety as well, reaching only 0.7% (500 Gy) (Table 1).

The analysis of the data presented in Table 2 highlights significant differences between the descendants from the $M_0$ variant (irradiated plants) and *Kaufmann* (control). In the case of $M_0$ descendants, the technical stem length could not be determined, as these plants developed basal branches, unlike the control plants, in which branching predominantly occurred in the upper part of the stem. A specific characteristic of the irradiated forms is the higher number of branches, flowers, and fruits produced. However, these plants show a considerable reduction in the number of viable seeds, seed mass, and seed quality. Thus, in the $M_0$ variant, although a double number of main branches was recorded (14.14) and flowering was abundant (~98.14 flowers/plant), only 45.0 of these developed into fruits, with a total seed mass of only 0.93 g. In comparison, the control plants showed an average of 7.14 branches, 32.0 flowers, and 26.9 capsules (fruits), associated with a correspondingly higher seed mass.

Table 1. Determination of the degree of elimination of plants under the influence of *X*-rays

| Day | Dose of ionising radiation, Gy | Genotype | | | Day | Genotype | | |
|---|---|---|---|---|---|---|---|---|
| | | Kaufmann | Belinka | Blaringham | | Kaufmann | Belinka | Blaringham |
| **d. 3** | 500 | 65.0% | 77.5% | 54.6% | **d. 27** | 4.7% | 3.8% | 0 |
| | 700 | 51.0% | 55.1% | 52.5% | | 0.3% | 0.1% | 0 |
| | 900 | 72.7% | 50.0% | 24.4% | | 0.4% | 0 | 0 |
| | Martor | 100% | 100% | 100% | | 50% | 50% | 50% |
| **d. 6** | 500 | 60.0% | 57.5% | 43.4% | **d. 29** | 2.5% | 1.0% | 0 |
| | 700 | 31.7% | 27.1% | 39.2% | | 0.1% | 0.1% | 0 |
| | 900 | 30.3% | 20.0% | 11.1% | | 0.1% | 0 | 0 |
| | Martor | 100% | 100% | 100% | | 50% | 50% | 50% |
| **d. 9** | 500 | 48.6% | 44.3% | 24.1% | **d. 31** | 1.9% | 0.4% | 0 |
| | **700**$^{+}$ | 28.8% | 25.9% | 27.1% | | 0.1% | **0**$^{+}$ | 0 |
| | **900**$^{*}$ | 25.7% | 24.4% | 66.0% | | **0**$^{*}$ | 0 | 0 |
| | Martor | 100% | 100% | 100% | | 50% | 50% | 50% |
| **d. 12** | **500**$^{+}$ | 41.4% | 40.5% | 17.5% | **d. 33** | 1.3% | **0**$^{+}$ | 0 |
| | **700**$^{*}$ | 21.6% | 19.2% | 13.3% | | **0**$^{*}$ | 0 | 0 |
| | 900 | 19.2% | 66.0% | 0.5% | | 0 | 0 | 0 |
| | Martor | 100% | 100% | 100% | | 50% | 50% | 50% |
| **d. 16** | 500 | 34.3% | 29.7% | 3.0% | **d. 37** | 1.1% | 0 | 0 |
| | 700 | 15.0% | 12.0% | 2.0% | | 0 | 0 | 0 |
| | **900**$^{-}$ | 10.0% | 15.0% | **0**$^{-}$ | | 0 | 0 | 0 |
| | Martor | 90% | 90% | 90% | | 50% | 50% | 50% |
| **d. 19** | **500**$^{-}$ | 21.7% | 21.3% | **0**$^{-}$ | **d. 38** | 10 | 0 | 0 |
| | 700 | 6.7% | 1.6% | 0.3% | | 0 | 0 | 0 |
| | 900 | 3.5% | 0.5% | 0 | | 0 | 0 | 0 |
| | Martor | 75%% | 75%% | 75% | | 50% | 50% | 50% |
| **d. 24** | 500 | 9.0% | 7.0% | 0 | **d. 40** | 0.9% | 0 | 0 |
| | **700**$^{-}$ | 0.7% | 0.3% | **0**$^{-}$ | | 0 | 0 | 0 |
| | 900 | 0.6% | 0.1% | 0 | | 0 | 0 | 0 |
| | Martor | 75% | 75% | 75% | | 50% | 50% | 50% |
| **d. 26** | 500 | 5.4% | 5.0% | 0 | **d. 125** | 0.7% | 0 | 0 |
| | 700 | 0.4% | 0.1% | 0 | | 0 | 0 | 0 |
| | **900**$^{+}$ | 0.5% | **0**$^{+}$ | 0 | | 0 | 0 | 0 |
| | Martor | 50% | 50% | 50% | | 49% | 49% | 49% |

The ratio between the number of flowers and the number of fruits formed is 53.14 in the $M_0$ variant, compared to only 5.1 in the control variant, indicating low reproductive efficiency in the irradiated plants, despite their seemingly greater morphological potential.

Table 2. Comparative analysis of linear and quantitative parameters of the *Kaufmann* control with the descendants from generation $M_0$ (500 Gy)

| **Parameters per plant** | ***Kaufmann*** | |
|---|---|---|
| | **Control** | **$M_0$ (500 Gy)** |
| Plant height, cm | 45±1,20 | 41±1,63 |
| Technical stem length, cm | 26±1,36 | 0 |
| No. of branches, un. | 7,14±0,4 | 14,14±1,47 |
| No. of capsules, un. | 26,9±3,62 | 45,0±10,44 |
| No. of flowers, un. | 32,0±3,72 | 98,14±10,19 |
| No. of seeds per plant, un | 7,14±0,74 | 5,71±0,57 |
| Mass of seeds per plant, g | 1,28±0,12 | 0,93±0,34 |

## CONCLUSIONS

The $M_0$ generation plants, subjected to *X*-ray radiation treatment, exhibited heightened sensitivity under simultaneous exposure to abiotic stress factors such as hydrological drought and high temperatures, which prevailed during the vegetation period. The cumulative interaction of these factors led to a significant increase in the physiological vulnerability of the plants, as they were unable to develop effective tolerance mechanisms to ionizing radiation in the context of combined environmental stress.

The study aimed to evaluate the response of several genotypes (*Kaufmann*, *Blaringhem*, *Belinka*) to the combined action of physical mutagenic factors (*X*-ray irradiation) and environmental conditions. The results highlighted that the *Kaufmann* genotype exhibited the highest resistance under the applied experimental conditions, demonstrating superior tolerance to the induced stress. Regarding the effectiveness of the irradiation doses, the 500 Gy treatment proved to be the most effective in stimulating mutagenic processes. These findings suggest that the *Kaufmann* genotype and the 500 Gy dose may represent an optimal combination for generating genetic variability in breeding programs.

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# COMPARATIVE DROUGHT RESISTANCE IN SUNFLOWER UNDER CONTROLLED CONDITIONS

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***Abstract.*** *Under changing climatic conditions, sunflower (Helianthus annuus L.) cultivation is increasingly challenged by abiotic stresses such as drought. This study evaluates the response of three sunflower genotypes to osmotic stress induced by polyethylene glycol (PEG) at concentrations of 10%, 20%, and 30% during germination and early seedling development under controlled conditions. The germination rate was evaluated daily over a 10-day period. Seeds were exposed to PEG to assess germination rate, seedling growth, and stress tolerance indices. Genotype H3 demonstrated moderate tolerance, maintaining satisfactory germination at 10% PEG but showing a marked decline at higher concentrations. H1 was highly sensitive, with poor germination and severely restricted growth of 20% and 30% PEG. In contrast, H2 displayed strong resilience, maintaining consistent germination, root development even under severe stress. These highlight the genotypic variability in sunflowers drought tolerance and suggest that H2 is a promising candidate for cultivation in water-limited environments. H1 may be suitable under moderate stress, while H1 may require additional agronomic interventions. Overall, the selection of stress-resilient sunflower genotypes emerges as a key strategy for enhancing productivity in drought-prone regions.*

***Keywords*: *sunflower, drought stress, PEG, germination, seedling***

## INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a crop of high economic value due to its oil-rich seeds and its contributions to both global food and biofuel production. However, sunflower cultivation is often constrained by abiotic stresses, with

drought being the most critical. Water deficit particularly affects key developmental stages such as germination and early vegetative growth [1-3], ultimately reducing yield potential. These vulnerabilities underscore the importance of targeted breeding strategies to improve drought tolerance.

Enhancing osmotic stress resilience is a central goal of sunflower breeding programs, especially in actual conditions of climate changes. The identification and selection of genotypes with improved adaptive responses to drought are crucial for ensuring stable yields under water-limited conditions. Additionally, other abiotic factors, such as soil salinity and extreme temperatures, further compromise plant performance, reinforcing the demand for robust, multi-stress-tolerant cultivars [3-5].

Osmotic stress, especially that induced by drought, represents a major environmental challenge that significantly affects plant growth and development. The capacity of plants to tolerate such stress varies considerably across species and genotypes, highlighting the need for the understanding of the genetic and physiological mechanisms of resistance. This knowledge is essential for the development of crops with enhanced resilience to the adverse effects of climate change [5].

While field evaluations remain a valuable approach for identifying drought- and heat-tolerant genotypes, they are often constrained by seasonal dependency, high labor demands, and extended timelines. Alternatively, early-stage laboratory assessments under controlled conditions provide a more practical and time-efficient method for evaluating the stress response of genotypes. Polyethylene glycol (PEG), a hydrophilic polymer, is widely used to simulate drought conditions in laboratory settings. This method enables consistent and reproducible evaluation of plant responses to water stress, particularly during early growth stages, and provides critical insight into tolerance mechanisms. Systematic evaluations under PEG-induced osmotic stress are therefore essential for identifying genotypes with superior drought resilience [6-8].

The main objectives of this study are to evaluate the germination, growth, and physiological responses of three sunflower genotypes subjected to osmotic stress induced by different concentrations of PEG-6000.

## MATERIALS AND METHODS

The experimental design involved three local sunflower genotypes (H1, H2, and H3), each characterized by a distinct response to drought stress under field conditions, with H2 identified as the most tolerant to hydric stress, followed by H1, and H3 being the most sensitive. The plants were subjected to controlled laboratory conditions (25±3°C) during the germination and early seedling stages. Before the experiment, the seeds of each sunflower genotype were surface-sterilized by

immersing them in 70% ethanol for 2 minutes, followed by treatment with a 10% sodium hypochlorite solution for 10 minutes, and finally rinsed thoroughly with distilled water. Each genotype was subjected to osmotic stress using three concentrations of polyethylene glycol (PEG-6000): 10%, 20%, and 30%, alongside a control group with distilled water. Twenty seeds per genotype were placed in Petri dishes lined with Whatman No. 1 filter paper and moistened with the respective PEG solutions or water. All treatments were performed in duplicate. After 10 days, germination percentage, plant height, root length, fresh weight, and dry biomass were recorded. The germination rate (GR) was calculated based on the formula by George (1967): *GR=(Germinated seeds/Total seeds) × 100* [9].

The stress tolerance indices for plant height (PHSI), root length (RLSI), and dry matter (DMSI) were calculated as follows:

- *PHSI=(Height of stressed plant / Height of control plant) × 100*
- *RLSI=(Root length of stressed plant / Root length of control plant) × 100*
- *DMSI=(Dry matter of stressed plant / Dry matter of control plant) × 100*

Statistical analyses were conducted using the methods outlined by Dospekhov (1985) to assess significant differences among treatments [10].

## RESULTS AND DISCUSSIONS

Drought stress affects *Helianthus annuus* by disrupting essential physiological processes, particularly seed germination, a critical phase for successful crop establishment. In this context, the primary objective of the study was to evaluate the germination response and drought tolerance of three sunflower genotypes under osmotic stress conditions induced by polyethylene glycol (PEG).

Numerous studies have investigated the germination rate (GR) of sunflower under water deficit conditions, highlighting genotype-specific responses to osmotic stress. The germination behavior of sunflower genotypes under PEG-induced osmotic stress observed in the present study aligns with previous findings by Praveen et al. (2021), Razzaq et al. (2017), Toscano et al. (2017) [11-13], and others, who reported a gradual reduction in germination rate (GR) with increasing osmotic stress, accompanied by significant variability in drought tolerance and germination dynamics among different genotypes. Similar trends were also noted by Akhter et al. (2025) [14], who emphasized that higher PEG concentrations reduce seed moisture uptake, ultimately resulting in decreased seed germination percentages.

According to present study under control conditions, all genotypes exhibited high and stable GR, ranging from 93,3 to 96,7%. At a 10% PEG6000 concentration, the hybrids maintained successful germination, with GRs remaining stable around 90%. This suggests that a low concentration of PEG6000 imposes only a mild

osmotic stress that does not significantly affect the germination process in the tested genotypes (Figure 1).

At moderate stress levels (20% PEG6000), GR declined to approximately 80% across genotypes. Under severe osmotic stress (30% PEG6000), pronounced genotype-dependent differences were observed. Thus, GR decreased to 60% in genotype H2, while H3 declined further to 46.7%. In contrast, genotype H1 failed to germinate entirely. These findings underscore the varying degrees of drought tolerance among genotypes, with H2 demonstrating the highest resilience under severe osmotic stress during the germination stage. Similar results were reported by Meng et al. (2023) [15], who observed near-total germination failure at high PEG concentrations, including complete inhibition of germination in *Chamaecytisus palmensis* at 30% PEG.

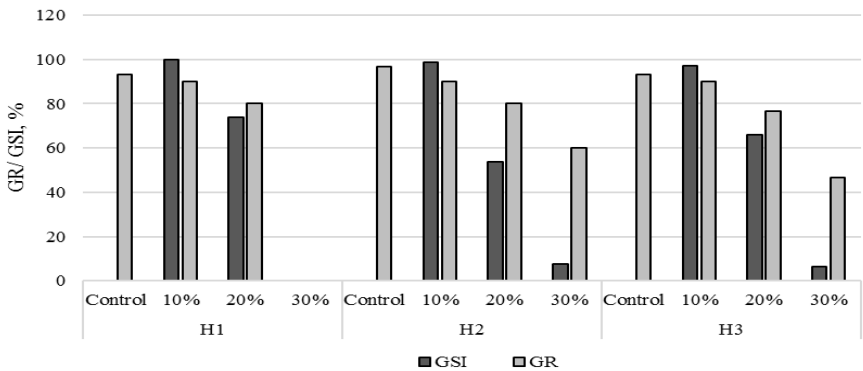


Figure 1. Effect of different PEG concentrations on final germination rate (GR) and germination stress tolerance index (GSI) in some sunflower hybrids

Compared to the control, the mean germination rate of the hybrids was notably delayed under 30% PEG-induced stress. Under control conditions and at low stress levels, nearly all seeds had germinated by the second day of incubation. In contrast, at the highest stress level, the first signs of germination were observed only on the seventh day. A delay in germination was also evident at the moderate stress level (20% PEG6000); by the second day, the germination rates were 40% for H1, 37% for H2, and 20% for H3, respectively. These results are in agreement with previous observations reported for sunflower by El Midaoui et al. (2001) and for *Brassica napus* L. by Willenborg et al. (2004) [16, 17].

The germination stress tolerance index (GSI) values for the tested sunflower hybrids varied notably across osmotic stress levels. Under mild stress conditions, the germination stress index (GSI) remained very high across all genotypes, averaging around 98%. At 20% PEG, GSI values decreased moderately, ranging from 53.9% for H2 to 73.9% for H1, indicating that H1 maintains relatively strong

germination performance even under moderate osmotic stress. In contrast, under severe stress (30% PEG6000), H1 exhibited a GSI of 0%, while the other hybrids showed a dramatic decline, with GSI values of 6.52% and 7.41%, respectively, highlighting a high degree of sensitivity to elevated osmotic stress.

Osmotic stress is known to affect sunflower shoot height, thereby influencing genotype performance during early developmental stages. This trend has been observed by several authors. For example, *Ahmad et al.* (2009) reported that plant height significantly decreased under osmotic stress conditions [8].

The physiological indices such as the Plant Height Stress Index (PHSI), Root Length Stress Index (RLSI), and Dry Matter Stress Index (DMSI) were used to evaluate the response of sunflower hybrids to PEG-induced water stress. A significant reduction in shoot length was observed in all tested genotypes. Shoot growth declined across all genotypes as PEG6000 concentration increased, and hypocotyl elongation was completely inhibited at 30% PEG in most of the analyzed hybrids, consistent with the findings of Toosi et al. (2014) [18].

At 10% PEG6000, hybrid H2 demonstrated the highest shoot height tolerance, with a Plant Height Stress Index (PHSI) of 96.6%, whereas H1 and H3 exhibited substantially lower values of 43.1% and 36.0%, respectively. At 20% PEG, shoot growth was further suppressed: H2 declined to 14.8%, and H3 to 10.9%, while no shoot elongation was recorded for H1. Under severe stress conditions (30% PEG6000), only H2 maintained measurable shoot growth (PHSI of 6.0%), suggesting greater relative tolerance compared to the other genotypes (Figure 2).

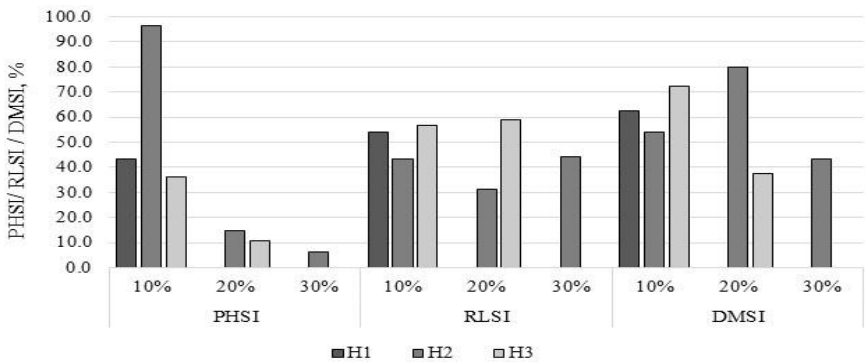


Figure 2. Effect of different PEG concentrations on:
*plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI) and dry matter stress tolerance index (DMSI) of some sunflower hybrids*

Root growth responded differently, under 10% PEG, RLSI ranged from 43.4% in H2 to 56.7% in H3, with H1 showing 53.9%. At 20% PEG, H3 retained relatively stable root growth (58.9%), while H2 declined to 31.4%, and H1 data was not reported. Under severe stress (30% PEG), H2 recovered slightly (44.0%), while

data was unavailable for the others, indicating a relatively better root development capacity in H2 under high osmotic pressure.

DMSI values followed a decreasing trend with increased stress. At 10% PEG, dry matter accumulation was highest in H3 (72.4%), followed by H1 (62.3%) and H2 (53.8%). Under 20% PEG, H2 demonstrated a superior response with a DMSI of 79.7%, while H3 dropped to 37.6%. At 30% PEG, H2 still maintained a moderate DMSI (43.3%), suggesting greater biomass retention capacity under drought stress. The results are similar with those reported by Ahmad et al. (2009) and Saensee K. et al. (2012) [8, 19].

Overall, H2 demonstrated the highest level of tolerance to PEG-induced osmotic stress based on shoot height, root length, and dry matter accumulation indices. These results indicate that H2 could be a promising candidate for cultivation under moderate to severe drought conditions during the early phases of development.

## CONCLUSIONS

The results demonstrate clear genotypic variation in drought tolerance among the three sunflower genotypes tested under PEG-induced osmotic stress. Genotype H2 emerged as the most drought-tolerant, maintaining high germination rates, vigorous seedling growth, enhanced root development, and relatively stable biomass accumulation across all stress levels. H3 exhibited moderate tolerance, with noticeable reductions under severe drought conditions, while H1 proved highly sensitive, showing poor germination and growth performance even under moderate stress.

These laboratory-based findings align closely with field observations, where H2 also demonstrated the highest drought resilience, confirming its adaptability to water-limited conditions. Similarly, H3 showed an intermediate response in both environments, while H1 consistently performed poorly under drought stress. This correlation between controlled laboratory screening and field performance highlights the reliability of PEG-based assays for early-stage genotype evaluation. It supports the use of H2 for cultivation in regions characterized by water deficiency, as well as its inclusion in breeding programs aimed at enhancing drought tolerance in sunflower.

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# ADAPTATION OF BEECH PLANTS FROM DIFFERENT PROVENANCES TO NATURAL GROWTH CONDITIONS

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***Abstract.*** *This study assessed the adaptive potential of Fagus sylvatica L. seedlings from various provenances under natural growth conditions in the Republic of Moldova. Between 2022 and 2024, seedlings of different ages and origins (Slovakia, Romania, Moldova, and Ukraine) were transplanted and monitored. Key indicators included adaptation rate, survival rate and annual growth. Seedlings from Slovak provenances (Nitra-19 and Rožňava-22) showed the highest initial transplant resistance, with survival rates of 96.15% and 97.00%, respectively. Plants from Cioreşti-21 (Republic of Moldova) followed closely, demonstrating the highest survival rate (87.00%) one year after transplantation. Growth assessments showed that although Romanian seedlings (Humosu-20) were initially taller, those from Slovakia and Republic of Moldova surpassed them in growth after one year. Adaptation was influenced by genetic and epigenetic factors, seedling morphology, precipitation levels, and climatic similarity between the origin site and the planting location. These results highlight the importance of selecting suitable provenances sites for beech seed collection and conditions for successful conservation and restoration of beech forests.*

***Keywords: Fagus sylvatica, beech seedlings, adaptation, survival***.

## INTRODUCTION

*Fagus sylvatica* L., commonly known as European beech, is the most important native forest tree species in Central Europe, with a lifespan of up to 300 years and late reproductive maturity [1]. In Republic of Moldova, beech forests cover about 400 hectares, representing the easternmost range of the species in Europe. These forests play a vital ecological role, supporting biodiversity, soil and water protection, and climate regulation [2, 3]. Due to illegal logging, land use changes, and climate extremes, beech forests in Republic of Moldova have been significantly reduced [4]. Restoration is essential for preserving rare species,

enhancing ecological resilience, and ensuring long-term sustainability [5, 6]. Successful regeneration depends on local adaptation, especially under increasing heat and drought stress [3, 6]. High genetic heterogeneity in seedlings is crucial for future resilience. Therefore, the aim of this study was to assess the adaptive potential of European beech (*Fagus sylvatica* L.) plants from different provenances to natural growth conditions in the Republic of Moldova

## MATERIALS AND METODS

Study objects: beech plants obtained under greenhouse conditions from seeds of different provenances and collected in different years, namely: Nitra-19 and Rožňava-22 (Slovakia); Humosu-20 (Romania); Plaiul Fagului-20, Codrii-20, Hîrjauca-20, Hîrjauca-21, Cioreşti-21 and Plaiul Fagului-23 (Republic of Moldova); Chernivtsi-20 and Ivano-Frankivsk-20 (Ukraine). Beech seedlings from various provenances and ages were planted under natural growth conditions over three consecutive years. On March 24, 2022, 135 three-year-old seedlings from Slovakia (Nitra-19) and 115 two-year-old seedlings from Romania (Humosu-20) were planted. In 2023 and 2024, additional seedlings from Republic of Moldova, Ukraine, Romania, Slovakia, and Austria were introduced to enrich the genetic diversity of the collection.

The monitoring of the adaptive capacities of beech plants was carried out 2–3 times per year. The following indicators were studied: the adaptation rate of the plants, the survival rate over one growing season, and the annual growth of the transplanted beech plants.

## RESULTS ANS DISCUSSIONS

The monitoring revealed that beech plants obtained from seeds of both Slovak populations – Nitra-19 and Roznava-22 demonstrated the highest initial resistance to transplantation, with survival rates of 96.15% and 97.00%, respectively, three months after being transferred (Fig. 1). The second-highest three-month survival rate was recorded for plants from Cioreşti-21, Republic of Moldova, at 90.00%. The lowest survival rate was observed in plants of Romanian provenance, Humosu-20 (Fig. 1). One year after transplantation, plants from Cioreşti-21 (Republic of Moldova) became the leaders in survival, with a survival rate of 87.0%. Plants from Nitra-19 (Slovakia) had a survival rate of 68.79%, while those from Humosu-20 (Romania) had only 43.01%. After two years of observation, the Slovak population Nitra-19 led in terms of surviving plants, while the remaining populations continue to be monitored (Fig. 1). The overall condition of all plants in 2024 is more than satisfactory, due to a high level of precipitation during spring and the first two decades of June 2024.

The height of seedlings at the time of transplantation varied significantly,

ranging on average from 17.41 cm to 26.40 cm across populations (Fig. 2). The Nitra-19 seedlings from Slovakia were the shortest (19.73 cm), although they were in their third year of growth when transplanted, compared to seedlings from other provenances, which were in their second year. Humosu-20 plants from Romania were the tallest at the time of transplantation, measuring an average of 26.4 cm. Three months after transplantation, the growth of seedlings from Cioreşti-21 (Republic of Moldova) and Roznava-22 (Slovakia) was nearly equal to that of Nitra-19 (Slovakia). Humosu-20 seedlings remained 4.9–7.4 cm taller than other populations on average.

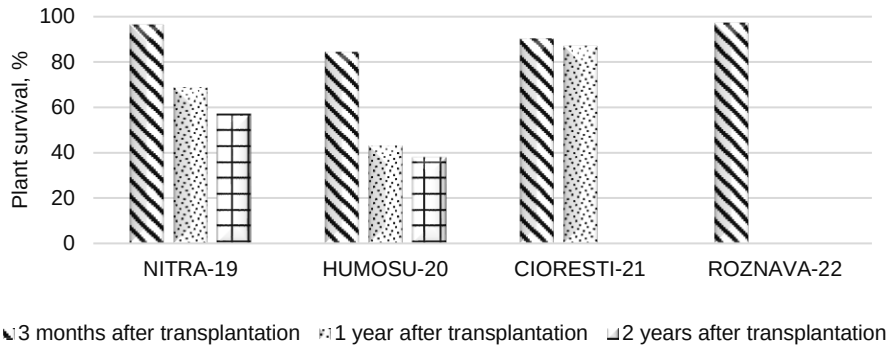


Figure 1. Survival dynamics of *F. sylvatica* seedlings from different provenances (Republic of Moldova, Romania and Slovakia) under natural growth conditions for the period 2022-2024, "Plaiul Fagului" Nature Reserve.

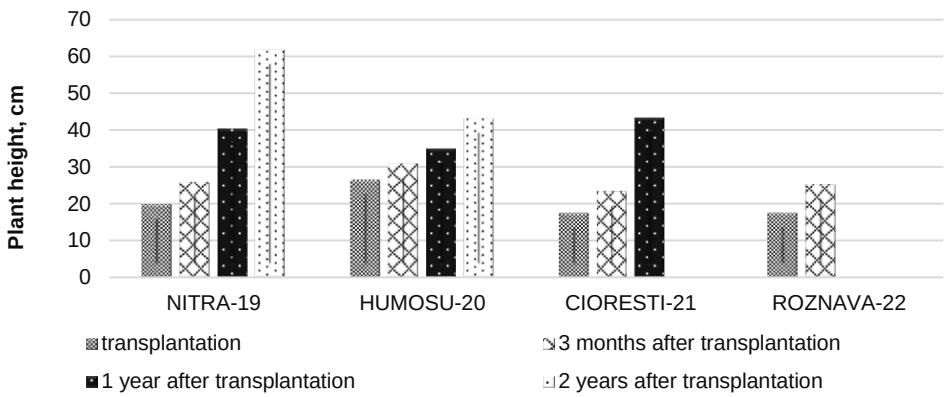


Figure 2. Height of *Fagus sylvatica* beech plants from different provenances (Republic of Moldova, Romania, and Slovakia) under natural growth conditions during the period 2022–2024, "Plaiul Fagului" Nature Reserve.

However, one year after transplantation, seedlings from Slovakia (Nitra-19 and Roznava-22) and Moldova (Cioreşti-21) outpaced the growth rate of Romanian seedlings (Humosu-20) by an average of 5.4–8.3 cm.

## CONCLUSIONS

The monitoring revealed that the Slovak population Nitra-19, transplanted in its third year of growth, demonstrated a higher adaptation rate compared to the Romanian population Humosu-20, while the Cioreşti-21 (Moldova) and Roznava-22 (Slovakia) populations, transplanted in their second year, showed similarly high survival rates, confirming that adaptation in natural conditions depends on a complex interplay of genetic, epigenetic, morphological, climatic, and topographic factors.

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# INFLUENCE OF PHOTOPERIOD ON PRODUCTIVITY AND SPECTRAL INDICES OF LEAF BLADES OF GARDEN CRESS SAMPLES GROWN UNDER LIGHT CULTURE CONDITIONS

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***Abstract.*** *Identification of optimal growing conditions and promising genotypes for specific environmental parameters using modern non-invasive methods is an urgent task. In this work, spectral indices of leaf blades of 17 accessions of garden cress (Lepidium sativum L.) from the VIR collection were studied for the first time under strictly controlled conditions of an agrobiopolygon under different photoperiods (12, 14, 16 h), as well as the productivity of these accessions. As a result of the work, photoperiods of 12 and 16 h were determined as the most favorable for growing garden cress accessions under light culture conditions. Accessions of garden cress, k-112 (Local, Azerbaijan) and k-165 (Gartenkresse, Belgium), were selected as the most productive and promising for cultivation under light culture conditions. Based on the data obtained, it was established that optical indicators can be an effective non-invasive criterion for identifying the most optimal duration of photoperiod for realizing the production potential of garden cress samples, and also serve as predictors for determining the most productive genotypes of interest for subsequent breeding work.*

***Keywords: garden cress, Lepidium sativum L., non-invasive methods, light culture, spectral indices***

## INTRODUCTION

Garden cress (*Lepidium sativum* L.) is an early maturing, responsive crop with valuable biochemical composition, which makes it a promising crop for cultivation under light culture conditions [5, 6]. Light culture implements an approach based on satisfying all the basic needs of the plant and eliminating stress factors affecting their growth and development, and also allows for the selection of optimal environmental conditions for specific crops or genotypes. One of the important objects of selection and regulation are the parameters of the light environment, in particular, the photoperiod [3]. Identifying optimal growing conditions and promising genotypes for specific environmental parameters using modern non-invasive methods is an urgent task.

The purpose of the study is to study the effect of photoperiod on the productivity and optical parameters of leaf blades of garden cress samples grown under light culture conditions.

## MATERIALS AND METHODS

The objects of the study were 17 accessions of garden cress (*Lepidium sativum* L.) of different origin, obtained from the collection of Vavilov all-Russian Institute of Plant Genetic Resources (VIR): k-1 (Garten kress, Germany), k-27 (Fine curled, Canada), k-41 (Broad leaf, Canada), k-42 (Narrow-leaved 3, Russia), k-48 (no name, Azerbaijan), k-63 (no name, Georgia), k-66 (no name, Afghanistan), k-78 (Local, Russia), k-79 (Local, Russia), k-85 (Cresson aienois, France), k-91 (no name, Iran), k-97 (Local, Armenia), k-112 (Local, Azerbaijan), k-118 (no name, Pakistan), k-127 (Local, Azerbaijan), k-165 (Gartenkresse, Belgium), k-185 (Almindelig, Denmark). The vegetation experiments were conducted under strictly controlled conditions of the agrobiological testing ground of the Agrophysical Research Institute (AFI). The plants were grown using flow hydroponics under three light conditions: 12, 14 and 16 hour photoperiod. The light source was gas-discharge mirrored sodium lamps DNaZ-400 (manufactured by OOO Reflax, Russia), and the substrate was peat-based soil Agrobalt-S (OOO Pindstrup, Russia). A modified Knop solution was used for flow hydroponics. The experiment was repeated three times, the sample size was 16 plants. The morphological description of the cress plants was carried out in the phase of technical maturity on the 26-29th day after sowing, in accordance with the methods of the Federal State Budgetary Institution State Commission for Variety Testing - RTG/02485/1; RTG/01/3 (OOS test methods). Reflectance spectra of leaves were recorded before harvesting using a miniature fiber-optic spectrometric system from Ocean Optics (USA). The following indices obtained as a result of contact spectroscopy were evaluated: chlorophyll index ChlRI, carotenoid to chlorophyll ratio index SIPI, photochemical index PRI [4, 5]. The PRI index in this work was used with the modification ((C1-PRI)*C2; C1=0.1; C2=10), hereinafter referred to as PRImod, and characterizes non-photochemical quenching. Statistical data processing was performed using the STATISTICA v.10.0 program (StatSoft Inc., USA).

## RESULTS AND DISCUSSION

Plant mass is one of the features characterizing productivity for green crops. Under photoperiods of 12 and 16 hours, the production potential of garden cress samples was realized most fully – significantly higher mass values were noted than under a photoperiod of 14 hours (by 1.01 g and 1.34 g, $LSD_{05}$=1.01). Most of the optical parameters we used also changed significantly in plants grown under different photoperiods (Table 1).

The chlorophyll index value increased significantly with increasing photoperiod, indicating the accumulation of green pigments under longer illumination. Similar dynamics of an increase in the content of green pigments was previously obtained for other representatives of the *Brassicáceae* family under light

culture conditions [6]. Under the least favorable photoperiod of 14 hours for the accumulation of green mass, the SIPI index, representing the ratio of carotenoids to chlorophyll, increased. The growth of this ratio was caused by a significant increase in the amount of carotenoids, which is part of the adaptation to unfavorable conditions. No reliable differences in the $PRI_{mod}$ index were noted, which is associated with high variability within the set of studied genotypes for this indicator.

Table 1. Productivity of garden cress (*L. sativum*) samples and some optical parameters, depending on the photoperiod

| Indicator | Photoperiod, h | | | $LSD_{05}$ |
|---|---|---|---|---|
| | 12 | 14 | 16 | |
| Mass of 1 plant, g | 3,55±0,44 | 2,54±0,18 | 3,88±0,28 | 1,01 |
| ChlRI | 0,37±0,01 | 0,40±0,01 | 0,41±0,01 | 0,05 |
| SIPI | 1,00±0,00 | 1,01±0,00 | 1,00±0,00 | 0,01 |
| $PRI_{mod}$ | 0,34±0,16 | 0,32±0,14 | 0,33±0,15 | 0,46 |

*Note: n=16, mean ± SEM. LSD - Least Significant Difference.*

As a result of the study, the most promising genotypes for growing in photoculture were also noted - samples k-112 (Local, Azerbaijan) and k-165 (Gartenkresse, Belgium). They demonstrated high mass values relative to the average for the studied samples under favorable photoperiods of 12 and 16 hours (Table 2).

Table 2. Average weight of 1 plant of isolated samples of watercress (*L. sativum*), compared with the average for the studied varieties, under different photoperiods

| Samples | Photoperiod, h | | |
|---|---|---|---|
| | 12 | 14 | 16 |
| k-112 | 6,04±0,35 | 3,19±0,13 | 5,64±0,58 |
| k-165 | 5,91±0,32 | 3,00±0,16 | 5,45±0,33 |
| Average across samples | 3,60±0,46 | 2,54±0,18 | 3,88±0,28 |
| $LSD_{05}$ | 1,17 | 0,66 | 1,29 |

*Note: mean ± SEM. LSD - Least Significant Difference.*

Both samples were resistant to early stem formation under light culture conditions under all studied photoperiods – the beginning of stem formation was not recorded by the end of the growing season (26-29 days).

The non-photochemical quenching index $PRI_{mod}$ had an inverse dynamic with the mass of 1 plant trait in the selected samples. Thus, the values of the $PRI_{mod}$ index in the genotypes selected by mass - k-112 and k-165 were below the average for the samples (the difference was from 0.45 to 0.79, with $LSD_{05}$ from 0.09 to 0.12).

## CONCLUSION

In this work, the productivity and optical indices of 17 garden cress (*Lepidium sativum* L.) accessions from the VIR collection grown under strictly controlled conditions were investigated for the first time. Photoperiods of 12 and 16 h were identified as the most favorable for growing garden cress accessions under light culture conditions. Garden cress accessions k-112 (Local, Azerbaijan) and k-165 (Gartenkresse, Belgium) were identified as having the highest productivity and resistance to early stemming under light culture conditions under all studied photoperiods. Based on the data obtained, it was established that some optical indices can be an effective non-invasive criterion for identifying the most optimal photoperiod duration for realizing the production potential of garden cress accessions, as well as predictors for determining the most productive accessions with traits of interest for further breeding work.

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# BREEDING OF COMON WHEAT (*TRITICUM AESTIVUM*) IN THE CENTRAL PART OF RM

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***Abstract.*** *During 2023-24 year we have studied 920 lines of wheat in selection field and the best of them were sowed in control and competition field. The best of them Bijuteria Zîmbrenilor as productivity and quality have been transferred to the State Commission for Crops Variety Testing. This sort of wheat has a productivity potential of 8,4- 9,0 t/ha and is zoned in all 3 agricultural parts of the Republic Moldova since 2025. It had gotten the volume of bread 646 cm$^3$ with porose crumb and smooth upper crust on the bread, better than M 5.*

***Keywords: high productivity, resistance to abiotic and biotic factors, protein, gluten, baking qualities.***

## INTRODUCTION

Common wheat (*Triticum aestivum*) is the main bread-making cereal crop cultivated in our country and in many other regions of the globe (approximately 100 countries). Metaphorically speaking, it is the backbone of life because it is found daily on the table of every person in our country as a bakery, pastry or confectionery product. According to statistical data from our country, wheat is the second cereal crop after corn in terms of sown area and harvested production volume. According to several authors, the contribution of breeding to increasing production capacity per hectare is from one percent to 50-60%. The continuous increase in the number of common wheat varieties created (diversification of the assortment) is one of the most effective measures that can contribute to the growth and stabilization of yields of this crop (Starodub, V. 2015). In the Applied Genetics Laboratory of IGFPP, several new varieties of common winter wheat have been created from the beginning to the present: Basarabianca, Moldova 77, 79, 16, 5, 11, 66, Moldova 614, etc. Of which the last 4 varieties have been approved in our country.

That is why the problem of creating new varieties of common winter wheat

that are more productive, of better quality, and more resistant to abiotic and biotic factors of the environment than the existing ones approved at the present time is always relevant.

## MATERIALS AND METHODS

To create new lines of common wheat, we used intraspecific hybridization. We used as initial material the varieties of common winter wheat created in our laboratory, both domestic and from other countries (varieties, lines, forms).

In the selection field during the year 2023-2024, we studied 920 lines of common wheat from which we selected the best forms, which were sown in the control and competition fields. The length of the plots in the selection field was 1.5 m. and the distance between the rows was 40 cm. As a control variety, the Kuialnic variety from Odessa, Ukraine was sown. The control field was 5 m2, the competition field – 10m2, and the demonstration and multiplication field was 100 m2. The selection field was sown manually and the control, competition, demonstration and multiplication field of the variety seed was sown mechanized. Sowing was done from the end of September to October 20. Phenological observations were made during the ontogenesis period. The harvesting of the lines in the selection field was done manually with subsequent sorting of the sheaves harvested at the batose. The yield from the control, competition, demonstration and multiplication fields was collected using the Sampo combine. The yield was recorded for all genotypes and varieties harvested. The protein percentage was determined according to Childall's method. The gluten content by washing the dough. We also determined the volume of bread from 100 g of flour.

## REZULTS AND DISCUSIONS

As the initial material for the creation of new varieties, the best geographically distant varieties in terms of productivity, quality, and resistance to abiotic and biotic factors were used - Blogodarca Odesskaia, Polevic, Pisanca, Epoha Odesskaia Kuialnic Zolotocolosa, Bezostaia 100, Trublion, Căpriana, Lăutar, Meleag, Moldova 5, 11, 79, 66, 614 and others. Starting with the second generation, due to individual selection, a whole set of lines belonging to the semi-intensive ecotype were identified and studied at different stages of the breeding process. Our varieties Moldova 5, 11, 66, 614, approved in our Republic, meet the requirements of semi-intensive varieties. We selected elite spikelets with a number of spikelets in the spikelet not less than 20 and with fertile spikelets one hundred per spikelet. Elite spikes were selected from plants with a height of 80 to 105 cm, resistant to falling, diseases, drought, with well-filled grains and a weight of 1000 grains from 36 g to 46 g. During the 2023-2024 growing season, 920 lines and forms

selected from different hybrid populations were studied in the selection field. In 2024, the best lines were lines selected from hybrid combinations, SI from M55-1, SI from M55-2, SI pop 57/17, SI pop Arctic and others. Compared to the control variety Kuialnic (200 g 0.6 sq m), 17 lines had a yield from 200 to 350 g 0.6 sq m. The highest yield in the selection field was in the line selected from the spontaneous hybrid combination SI M55-1 = 350 g 0.6 sq m, which constitutes an increase of 150 g compared to the control variety. According to the control field data from 2024, out of 40 genotypes sown in a single repetition, 10 lines had a yield at the level of the control variety Kuialnic (4.2 t/ha) or higher. The best 2(two genotypes in this field were the lines KK 23/24 and KK 39/24 which had a yield equal to 4.96 t/ha, higher by 0.76 t/ha than the control variety Kuialnic. The lines named above were resistant to rust, powdery mildew, drought and had a height of 100 and 97 cm respectively. In the field with comparative competition crops, 6 lines were sown in 3 repetitions, of which the most fruitful was the line KK11/23 – 4.7t/ha, and in the control variety Kuialnic- 4.0 t/ha, which is 0.7 t/ha more than in the control variety. This line is tolerant to rust, powdery mildew, drought, falling and has a height of 90 cm. The best lines in the control and comparative competition fields in autumn 2024 were sown in the following links of the breeding process (competition and multiplication).

Table 1. Productivity of the best lines and varieties of common winter wheat from the multiplication field and demonstration

| Name | Plant height | Resistance, points | | Average | |
|---|---|---|---|---|---|
| | | lodging | drought | t/ha | +control |
| Kuialnic, martor | 0,80 | 9 | 9 | 3,6 | - |
| Pisanca | 0,82 | 9 | 9 | 3,7 | +0,1 |
| SI pop 47/20 | 0,73 | 9 | 9 | 3,9 | +0,3 |
| SI pop 42/20 | 0,71 | 9 | 9 | 3,6 | - |
| Moldova 614 | 1,07 | 8 | 9 | 3,8 | +0,2 |
| Bijuteria Zîmbrenilor | 0,70 | 9 | 9 | 3,9 | +0,3 |

In 2023-24, 12 varieties, lines and forms of wheat selected from the comparative competition field were sown in this field. According to the data in Table 1, only 3 (three) varieties and one line surpassed the Kuialnic control variety. Our varieties approved in the RM, Moldova 5,11 and 66 are more resistant to lodging than the Kuialnic variety and had a yield at the level of the control variety. The Moldova 614 variety, which was approved in the northern area of the Republic of Moldova in 2024, is weaker to lodging than the control variety in years with a lot of moisture. This variety had a high resistance to wintering, drought, grain shaking, brown rust, powdery mildew, septoria, fusarium. The Kuialnic and M 614 varieties doubled and tripled their yield in years favorable for humidity. This

variety, Moldova 614, was submitted to the State Commission in the fall of 2019. It had a yield of 9.2 t/ha at the Pelenia CSTS state sector in 2023. The highest yield during multiplication was the Bijuteria Zimbrenilor variety of 3.9 t/ha, which is 0.3 t/ha more than the control variety (table 1). According to the State Commission data, it is resistant to lodging, shaking, drought, wintering, brown rust, powdery mildew, fusarium and grain bug.

Table 2. Regeneration capacity and green mass and grain yield of different varieties of cereal crops in 2024, IGFPP

| Name of the cereal variety | Plant height, m | Green mass yield at earing, kg/m2 | Full maturity grain yield, g/m2 | Grain yield after first harvest, g/m2 | Ratio between first harvest and second harvest, % | Level of adaptability and drought resistance |
|---|---|---|---|---|---|---|
| Zîmbreni 70 (secară) | 1,70 | 5,0 | 784 | 162 | 20,7 | high |
| Kuialnic | 0,80 | 3,4 | 836 | 92 | 11,0 | good |
| Bijuteria Zîmbren | 0,70 | 3,6 | 873 | 97 | 11,1 | good |
| Moldova 5 | 0,72 | 3,5 | 791 | 81 | 10,3 | average |
| Mold. 614 | 1,10 | 5,6 | 859 | 94 | 10,9 | good |

In order to detect common wheat varieties with a high degree of adaptability, and with a vigorous root system, resistant to drought, the studied varieties were harvested for multiplication on plots of 1 m2 in 3 repetitions in the earing phase when 50-60% of ears appeared and were cut at a height of 5-6 cm from the soil surface. As a control, the same varieties harvested at full grain maturity (1st mowing) - 1 m2 were taken. Also, the plots that gave ears after the 1st mowing were harvested. As can be seen from Table 2, the Bijuteria Zimbrenilor variety (3.6 kg/m2) had a higher green mass yield than the control variety Kuialnic (3.4 kg/m2). The Bijuteria Zîmbrenilor variety has an adaptability level approximately the same as the control variety Kuialnic. This variety has a yield ratio between the first and second harvest of 11.1% and the control of 11% (tab 2)

Table 3. Biological and economic properties of the common wheat variety Bijuteria Zîmbrenilor (3-year average)

| Indices | Unit of measurement | Kuialnic | Bijuteria Zîmbrenilor | + - relative to the control variety |
|---|---|---|---|---|
| Producţia de boabe | t/ha | 5,0 | 5,2 | 0,2 |
| Masa hectolitrică | 1 litru | 76,0 | 76,0 | - |
| Masa la 1000 boabe | Grame | 41,0 | 38,0 | -3,0 |
| Sticlozitatea | % | 68,0 | 69,0 | 1,0 |
| Conţinutul glutenului brut | % | 26,8 | 27,0 | 0,2 |
| Conţinutul proteinei brute | % | 13,8 | 14,1 | 0,3 |
| Calitatea de panificaţie | puncte | 5 | 5 | - |

On average over 3 years of study, the Bijuteria Zîmbrenilor variety in the competition field had an average yield of 5.2 t/ha compared to the Kuialnic variety - 5.0 t/ha. It has a better protein, gluten and glassiness content than the control variety Kuialnic (tab. 3). The volume of bread in the Bijuteria Zîmbrenilor variety is 646 cm3 from one hundred grams of flour, which is higher than in Moldova 11 and Moldova 5 but lower than in Moldova 614 and Kuialnic. It has a Romanian bread crust with good porosity and superior taste qualities than the other 2 varieties M 5 and Kuialnic (tab. 4).

Table 4. Analysis of the baking qualities of common winter wheat varieties

| № | Variety name | Bread volume, cm3 |
|---|---|---|
| 1 | Kuialnic | 663 |
| 2 | Moldova 11 | 530 |
| 3 | Bijuteria Zîmbrenilor | 646 |
| 4 | Moldova 614 | 665 |
| 5 | Moldova 5 | 544 |
| 6 | Secară, Tetram | 220 |

The Bijuteria Zîmbrenilor (M55) variety was tested at the State Commission for 2 (two) years -2023,2024 and had positive results on average per republic of 6.73 t/ha, and the control 6.62t/ha which is 1.7% more and was approved for the republic, all 3 agricultural zones. It is a variety of semi-intensive and universal ecotype. (tab 5)

Table 5. Results of testing the common winter wheat variety Bijuteria Zîmbrenilor in Zones (RM) in 2023-24 (t/ha)

| Soiul | Northern area | | | Central area | | | Southern area | Average per country | |
|---|---|---|---|---|---|---|---|---|---|
| | 2023 | 2024 | Media pe 2 ani | 2023 | 2024 | Media pe 2 ani | Media pe 2 ani | Pe 2 ani, țară | % față de martor |
| Media mr.pe an | 7,03 | 7,57 | 7,3 | 8,75 | 7,58 | 8,17 | 4,39 | 6,62 | 100,0 |
| Bijuteria Zîmbrenilor | 6,7 | 8,1 | 7,4 | 8,68 | 8,13 | 8,41 | 4,37 | 6,73 | 101,7 |

Fig.1 Bread volume.

According to CSTS data, the Bijuteria Zîmbrenilor (BZ) variety had an average of 2 years in the North of 7.4 t/ha, and the control - 7.3 t/ha. On average for 2 years, in the northern zone, the Bijuteria Zîmbrenilor (BZ) variety exceeded the control variety by 1.4%. Central zone, control - 8.17t/ha, BZ -8.41t/ha. South Zone, the control-4.39t/ha, and BZ-4.37t/ha almost equal. Year of Registration in the CSTS catalog- 2025 This variety is part of the Lutescens variety. The Bijuteria Zimbrenilor variety was obtained by the method of individual selection from the selected hybrid population of the Moldova 5 variety that blooms openly, more than 50%. White spike, cylindrical, without awns at the tip of the spike, short awns of 1-2 cm., length (9.0-10.5cm) and density (20-24 spikelets per 10 cm) average. Red grain, the mass of 1000 grains varies from 36-40gr, in a spikelet 2-3 grains. The length of the vegetation period is 270-274 days and it is part of the group of plants with medium precocity. The height of the plants is 60-80cm, the number of siblings per plant is 2-3. It is resistant to drought, wintering and diseases (powdery mildew, brown rust, fusarium, septoria, etc.). The productivity potential is 8.4-9.0t/ha. It has good biochemical and baking qualities. It has vigorous roots and is well adapted to changing climate conditions. Therefore, since 2025, the Bijuteria Zîmbrenilor variety has been approved in the republic, in all 3 agricultural zones.

## CONCLUSIONS

In the selection field in 2023-24, we studied 920 lines of common wheat. As a result of phenological observations and biometric measurements, several lines resistant to wintering, drought, diseases and others and with higher productivity than the control variety (200g/0.6mp) were selected. They were sown in the control field and then the best ones in the competition field. As a result of the tests, one of the best varieties Bijuteria Zîmbrenilor (in terms of productivity, resistance to various abiotic and biotic factors) was submitted to the State Commission in the fall of 2022, having a productivity potential of 8.4-9.0 t/ha. It is approved in all 3 agricultural zones of the Republic of Moldova since 2025. The varieties Bijuteria Zîmbrenilor (BZ), Moldova 5, and Kuialnic had a bread volume of 646, 544, and 663 cm3. The bread of the (BZ) variety had a smooth crust and uniform porosity of the core.

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# IMPACT OF NON-OPTIMAL TEMPERATURE AND BIOREGULATOR ON ROOT AND SEEDLING VIGOUR OF CORN

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***Abstract.*** *Take into account that the corn vigour has the strong correlated field emergence and yield, the purpose of this work was to evaluate the combined influence of non-optimal temperatures and a natural bioregulator on the vigour of corn root and seedling. The seeds of Porumbeni 455 corn hybrid pre-treated with natural bioregulator (JS) extracted from Juniperus sabina were subjected to treatment at 50°C (heat test) and at 10°C (cold test). The vigour of root and seedling was assessed after seed germination. Temperature above the optimum significantly affected both the vigour of root and the vigour of seedling, but temperature below the optimum only reduced the root vigour of the tested corn hybrid. The JS bioregulator contributed to an increase in root system vigour. Corn seeds pre-treated with JS had root vigour greater than untreated ones by 13.08 and 18.46%, respectively in the cold and heat test. The evaluation of factor impact showed that the temperature influenced both the root and seedling vigour more than the treatment with the bioregulator, and their combined effect significantly modified only the root vigour.*

***Keywords: bioregulator, cold test, corn, heat test, germination, vigour.***

## INTRODUCTION

The corn vigour determined in laboratory condition strong correlated with both the laboratory overall germination (0.93-0.99) of corn seed and their field emergence (0.82) [1, 2]. Thus, the vigour of corn seeds predicts a higher rate and uniformity of emergence in the field, as well as crop yield. It is well known, that seedling emergence can be affected by many abiotic factors such as high or low temperatures, water deficit, etc. [3]. However, the pre-treatment with plant growth regulators can induce a rapid adaptive response to stress conditions [4, 5], including the non-optimal temperature. Therefore, the aim of this work was to evaluate the

combined influence of non-optimal temperatures and a natural bioregulator on the vigour of corn root and seedling.

## MATERIALS AND METODS

***Corn seeds*** of hybrid Porumbeni 455 (P455) that belongs to the FAO 450 corn group and has dent grain were tested. Weight of 1000-grain is 330-340 g. ***Laboratory testing.*** Seeds were pre-soaked for 24 hours in water or in solution with concentration 0.0001% of natural bioregulator (JS) obtained from aerial part of plant *Juniperus sabina* L. [6]. Then seeds were subjected to heat treatment at 50°C for 30 minutes (heat test) or to cold test. Germination of intact (control) and heated seeds was carried out in accordance with the international rules [7]. To conduct the cold test, seeds were germinated for seven days at temperature of 10°C, and then 4 days at 25°C [8]. The overall seed germination, root/seedling length, root/seedling vigour was assessed [9]. ***Statistical analysis*** was performed by Statgraphics Plus 5.0 software.

## RESULTS ANS DISCUSSIONS

Treatment of control seeds with the bioregulator JS did not contribute to an increase in roots vigour. The average values of root vigour of intact and JS-treated seeds differed insignificantly (Fig. 1). However, the root vigour of corn seeds subjected to both heat and cold tests decreased significantly ($p \leq 0.001$) compared to the control seeds. The positive effect of JS treatment on the increase of root vigour was evaluated after heat and cold test with the difference values of 76.55 (cold-test) and 100.92 (heat-test) (Fig. 1). Thus, the corn seeds pre-treated with JS had roots vigour greater than the untreated ones by 13.08 and 18.46%, respectively in the cold and heat test.

In growth conditions with non-optimal temperatures, the use of pre-treatment of corn seeds with JS natural bioregulator leads to an increase in the vigour of the root system, which is very important for the early phase of corn growth and its adaptation to stressful conditions. Treatment of control seeds with JS also resulted in an increase in seedlings vigour by 20.78% (Fig. 2). It should to be noted, that the cold test itself had a positive effect ($p \leq 0.001$) on the seedling vigour of germinated seeds without JS treatment. However, no significant impact of JS treatment was found on the seedlings vigour of the P455 corn seeds subjected to both the heat and cold tests (Fig. 2).

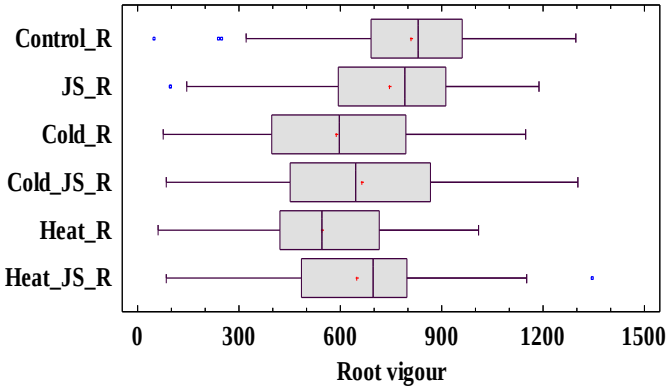


Figure.1. Root vigour of corn germinated from seeds exposed different treatments:
*control – untreated; JS – treated with bioregulator; Cold – exposed to cold test; Cold_JS - exposed to cold test and treated with bioregulator; Heat - exposed to heating; Heat_JS - exposed to heating and treated with bioregulator*.

Previously [10], different physiological responses of super sweet, semi-flint and popcorn seeds to heat and cold test were shown, specifying their different ability to adapt to stress conditions.

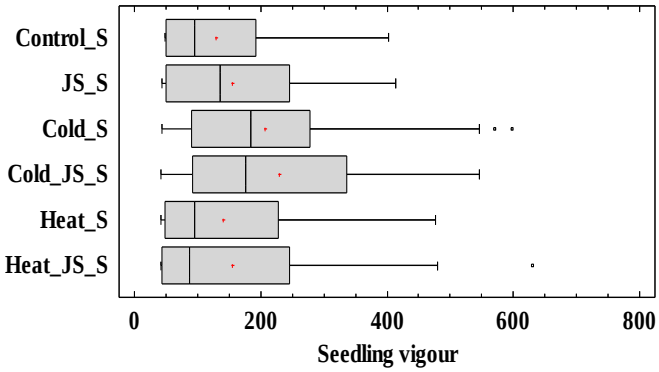


Figure.2. Seedlings vigour of corn germinated from seeds exposed different treatments:
*control – untreated; JS – treated with bioregulator; Cold – exposed to cold test; Cold_JS - exposed to cold test and treated with bioregulator; Heat - exposed to heating; Heat_JS - exposed to heating and treated with bioregulator*.

The evaluation of factor impact such as temperature and JS treatment of corn seed on root and seedling vigour of hybrid P455 was carried out by ANOVA analysis (Table). The temperature significantly ($p \leq 0.05$) influenced both root and seedling vigour more than the treatment with bioregulator. The interaction between temperature and bioregulator factors contributed to changes significantly ($p \leq 0.05$)

only in root vigour (Table 1). The contribution of temperature from total impact was 9.19% and 7.44%, respectively in root and seedling vigour changes.

Table 1. Factor impact on root and seedling vigour

| Factor | Sum of squares | Df | Mean square | F-ratio | P-value |
|---|---|---|---|---|---|
| **Root vigour** | | | | | |
| Temperature (T) | 3.339E6 | 2 | 1.669E6 | 23.58 | 0.0000* |
| Bioregulator (JS) | 180517.0 | 1 | 180517.0 | 2.87 | 0.0906 |
| Interactions T-JS | 719026.0 | 2 | 359513.0 | 5.72 | 0.0035* |
| Total | 3.6332E7 | 518 | | | |
| **Seedling vigour** | | | | | |
| Temperature (T) | 618890.0 | 2 | 309445.0 | 20.80 | 0.0000* |
| Bioregulator (JS) | 56450.3 | 1 | 56450.3 | 3.79 | 0.0520 |
| Interactions T-JS | 3710.65 | 2 | 1855.32 | 0.12 | 0.8828 |
| Total | 8.3190E6 | 513 | | | |

Note: * - denotes the statistically significant difference at P≤0.05

## CONCLUSIONS

Under the influence of non-optimal temperatures, the contribution of the JS natural bioregulator to increasing the vigour of the root system and seedling of the P455corn hybrid is significantly reduced.

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# STUDY OF VARIABILITY IN PRODUCTIVITY ELEMENTS OF WINTER TRITICALE COLLECTION

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***Abstract.*** *Arid conditions, which are increasingly common in the world, have a negative impact on the development of agricultural crops and pose a great threat to the implementation of potential plant productivity. In this regard, the purpose of this study is to evaluate collection variety samples for economically valuable traits and to select promising genotypes for use in the breeding process. This article presents the results of a study of 36 triticale variety samples of different geographical origins for the main economically valuable traits (plant height, main spike length, number of spikelets per spike, number of grains, grain weight and 1000-grain weight). Analysis of the productivity elements of the studied samples revealed high variability in the number of grains and grain weight per spike, which makes it possible to select valuable genotypes. Promising samples have been identified based on individual productivity traits and their complex for targeted use in selection of parental pairs included in hybridization.*

***Keywords: collection, triticale, variability, vegetation period, yield structure.***

## INTRODUCTION

Triticale (x *Triticosecale Wittmack*) is a hybrid of wheat (*Triticum aestivum*) and rye (Secale cereale), combining the positive properties of both cereals. Triticale as a new cereal plant has become increasingly well-known in the world due to its adaptability and valuable agronomic properties, and is grown in more than 30 countries. According to FAO, the geography of triticale distribution is quite wide and is cultivated on an area of more than 4 million hectares worldwide, giving an increase to world grain production of more than 15 million tons per year. Poland, France, Germany, Belarus, Russia and Ukraine play the most important role as the largest European breeding centers and producers of triticale grain [1].

The growing interest in the triticale crop is caused by its high productivity, winter hardiness, grain and green mass quality, low susceptibility to a number of diseases, the possibility of cultivation on poor soils - these valuable properties and

qualities acquired by this crop from wheat and rye are important for its use in agricultural production. Triticale is used as a food and forage crop. The crop is of undoubted interest for the bakery, confectionery, starch, brewing, alcohol and other industries [2].

The consequences of climate change in the world (frequent droughts) cause great damage to agriculture, as a result of which the harvest shortfall is many tons of grain of cereal crops and negatively affects the quality of the grain. In this regard, it is important to expand the gene pool, create new primary and secondary triticale with the involvement of new highly productive forms and varieties. In the Republic of Moldova, triticale can be grown in all regions, so the area of distribution should increase every year. [3].

In breeding for resistance to stress factors of the external environment, an important task is to find sources for a particular stress. One of the methods is the evaluation of collection samples and promising varieties in specific soil and climatic conditions capable of realizing their genetic potential [4].

The main goal of triticale breeding at the present stage is the creation of new high-yielding varieties with increased adaptability, resistance to biotic and abiotic stresses, with high feed, technological and baking qualities [5].

The purpose of this study is to evaluate collection samples for economically valuable traits and identify promising genotypes with a complex of valuable traits for their use in the breeding process.

## MATERIALS AND METHODS

The experimental part was carried out on the experimental field of the Institute of Genetics, Physiology and Plant Protection of the Moldova State University. The experiment was preceded by black fallow. The object of the research was 36 collection samples of triticale of different genetic and geographical origin (Romania, Bulgaria, Ukraine, Russia, Belarus, Poland, Germany). The winter triticale variety Ingen 93 of the Institute of Genetics, Physiology and Plant Protection was used as a standard. The collection material was assessed by morphobiological indicators (plant height, spikelet length, number of spikelets per spike, number of grains, grain weight and 1000-grain weight). The productivity elements were studied by the method of structural analysis of a sample consisting of 20 plants. The laying of experiments, phenological observations and field records were carried out in accordance with the methods of state variety testing of agricultural crops by the Center for Testing Plant Varieties of the Republic of Moldova. The experimental data were processed mathematically using the Microsoft Office Excel software package.

## RESULTS AND DISCUSSIONS

Numerous selection and genetic studies have proven the need to create an identified gene pool of cultivated plants, as well as the importance of choosing the source material, which is the basis for obtaining new forms and varieties of agricultural crops. Based on the results of the studies, the main economically valuable traits of breeding interest were determined. A triticale collection consisting of 36 variety samples was studied for the following biological properties and economic traits: length of the vegetation period, winter hardiness, drought resistance, resistance to diseases, lodging and productivity.

In terms of the length of the growing season for our conditions, medium early and medium genotypes (255-260 days) are of interest. These groups include samples from Romania, Bulgaria and Poland, Germany (Amol, Haiduc, Stil, L.Ir.Bulgaria, Presto, LT 76872, Agendus, etc.). Late groups include forms from Ukraine and Russia (Salanda, Cernobrivet, Buchet, L.16441-5, AD 1128, etc.). In terms of the length of the growing season, the collection of winter triticale was divided into 3 groups: medium early (length of the growing season up to 255 days), medium (256-260 days) and late (261-267 days) (Table 1).

Table 1. Distribution of triticale collection by maturity groups

| Maturity groups | Length of vegetation period, days | Number of samples | % | Name of samples |
|---|---|---|---|---|
| Medium early | 255 and Less | 10 | 27,8 | Amol, Haiduc, Stil, Mezin, Negoiu, Oda, L.Ir.Bulgaria, Presto, LT 76872, и др |
| Medium | 256-260 | 18 | 50,0 | Marchian, Salanda, Cernobrivet, Eltei, Canar,Atlet,Tulus и др |
| Late | 261 –267 | 8 | 22,2 | Buchet, AD1128, L.16441-5, Duet, B-7, и др |
| St. | 259 | | | Ingen 93 |

The obtained data show that out of 36 genotypes of the collection, 10 samples belong to the mid-early group, which is 27.8%. This group of triticale ripened 2-4 days earlier than the standard variety Ingen 93. It mainly included forms and varieties created in Bulgaria, Romania and Poland. The mid-season group includes 18 samples, which is 50%. They ripen at the same time as the standard variety or 2-3 days later than the previous group and included samples from Ukraine, Romania, Germany, etc. The late-ripening group included 8 samples or 22.2%. They ripened at least 3-8 days later than the standard and these are mainly samples from Ukraine and Russia. Samples and varieties of triticale of the early and mid-season groups were widely used in hybridization.

In genetic and selection studies of triticale, great importance is attached to the variability of traits, determined not only by the genotype, but also by environmental conditions. The degree of variability of traits reveals the norm of the genotype's reaction in different environmental conditions [6]. Table 1 presents experimental data on the productivity traits of collection samples of winter triticale (plant height, length of the main spike, number of spikelets per spike, number of grains per spike, weight of grains per spike, weight of 1000 grains).

Lodging of plants significantly reduces the yield and complicates mechanical harvesting, therefore the plant height trait is of great importance. Analysis of the plant height of the studied variety samples shows that the values varied widely - from 96 (Agendus) to 139 cm (95W73-2-1), the variation coefficient fluctuated from 1.5 to 3.8%, with an average value of 2.3%. The following samples were the shortest (96-100 cm): Agendus, Tadeus, Oda. The main components of the yield structure are the length of the spike the number of grains in the spike, the productivity of one spike and the weight of 1000 grains. Our studies have shown that the values for the main spike length trait in the variety samples ranged from 9.6 to 15.3 cm. The variability of this trait varied from 2.4 to 13.4%, and the average value was 6.94%.

Important components of the yield are productivity elements, such as the number of spikelets and grains in the spike and their weight. The number of spikelets in the main spike ranged from 24.8 pcs (Negoiu) to 37.6 pcs (Salanda). The variation in this feature is from 3.1 to 11.8% with an average value of 7.0%. The number of grains from the main spike ranged from 41.3 (Roudkrider) to 86.9 pcs (Tulus), the variation coefficient was 6.8 - 27.3%, and the average value was 15.0%. Spike productivity varied from 2.0 g (Roudkrider) to 3.8 g (Duet), the variation coefficient was 8.0 - 26.1% (average 18.1%). The weight of 1000 grains is the most important indicator of the yield structure and the usefulness of seeds, its value depends on the weather conditions during the period of earing and wax ripeness. The weight of 1000 grains was within the range of 33.1 g (Agendus) to 52.8 g (L.16441-5). The coefficient of variation for this feature varied from 4.6 to 15.6%, with an average value of 10.0%. In terms of productivity, the best forms were from Germany (Duet), Ukraine (Salanda) and Russia (L.16441-5, 95W73-2-1, L.12).

The variability of the main breeding-valuable traits of triticale collection variety samples was within 1.5-29.5%. Among the productivity elements, the most stable traits were: spike length 3.8-13.4% (Vcp=6.9%) and number of spikelets 3.1-11.8% (Vcp=7.0%). The lowest variability was observed for plant height 1.4-3.8% (Vcp=2.3%). High variability was observed for the number of grains per spike 6.8-27.3% (Vcp=15.0%) and grain weight per spike 8.0-29.5% (Vcp=18.1%). Variability of economically valuable traits is important for breeding work, as it allows identifying and using the most valuable genotypes to create new varieties of triticale.

Table 2. Productivity elements of collection samples of winter triticale

| Sample | Height of plants | | Length of main spike | | Number of spikelets in main spike | | Number of grains in main spike | | Weight of grains from main spike | | Weight of 1000 grains | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X, cm | V,% | X, cm | V,% | X, pcs | V,% | X, pcs | V,% | X, g | V,% | X, g | V,% |
| Obrii Mironov. | 115 | 1,5 | 14,9 | 7,7 | 33,6 | 11,6 | 54,0 | 20,1 | 3,0 | 22,1 | 47,3 | 8,7 |
| Marchian | 122 | 3,8 | 13,4 | 5,6 | 31,4 | 7,4 | 64,6 | 16,4 | 2,9 | 20,1 | 44,4 | 14,6 |
| Salanda | 120 | 2,3 | 14,9 | 4,4 | 37,6 | 3,1 | 75,8 | 10,9 | 3,7 | 17,9 | 48,4 | 10,8 |
| Buchet | 122 | 2,4 | 14,0 | 8,9 | 32,5 | 5,5 | 72,1 | 17,8 | 3,0 | 19,9 | 41,5 | 4,6 |
| Cernobriv. | 120 | 2,0 | 12,2 | 4,4 | 29,8 | 6,5 | 51,0 | 15,1 | 2,2 | 14,9 | 43,9 | 8,9 |
| Eltei | 109 | 2,9 | 14,1 | 8,3 | 32,1 | 7,7 | 59,1 | 17,3 | 2,6 | 22,0 | 44,4 | 14,6 |
| Amol | 111 | 2,3 | 12,1 | 3,8 | 29,3 | 6,5 | 68,2 | 6,8 | 2,5 | 8,0 | 37,3 | 5,1 |
| Haiduc | 102 | 2,1 | 11,5 | 7,7 | 30,8 | 10,1 | 64,2 | 9,6 | 3,0 | 14,2 | 46,2 | 10,3 |
| Stil | 108 | 3,2 | 12,0 | 7,5 | 29,7 | 11,0 | 84,1 | 11,7 | 3,5 | 19,7 | 41,9 | 11,3 |
| Mezin | 106 | 1,5 | 9,8 | 8,8 | 24,8 | 11,8 | 54,9 | 14,4 | 2,4 | 18,5 | 42,8 | 11,2 |
| Negoiu | 105 | 2,0 | 10,1 | 5,9 | 24,8 | 6,8 | 65,4 | 23,3 | 2,8 | 23,6 | 43,5 | 14,7 |
| Oda | 97 | 3,1 | 10,7 | 7,4 | 28,6 | 9,1 | 59,0 | 16,3 | 2,4 | 19,7 | 40,1 | 11,1 |
| Canar | 118 | 2,7 | 15,3 | 6,6 | 33,0 | 7,7 | 78,9 | 10,7 | 3,0 | 13,3 | 37,9 | 9,5 |
| L. Ir. Bulg | 130 | 2,5 | 12,1 | 8,9 | 27,7 | 5,1 | 57,4 | 27,3 | 2,3 | 29,5 | 40,0 | 8,7 |
| 161/88-233 | 121 | 2,0 | 12,2 | 4,4 | 31,7 | 3,3 | 67,2 | 9,1 | 2,8 | 14,9 | 41,0 | 9,6 |
| LT 76872 | 123 | 2,1 | 13,6 | 5,4 | 32,7 | 5,6 | 71,5 | 12,5 | 2,5 | 15,8 | 34,7 | 8,2 |
| 188TR 5021 | 125 | 2,6 | 13,3 | 6,9 | 31,4 | 9,4 | 68,0 | 17,1 | 2,3 | 26,1 | 34,1 | 10,4 |
| Castor | 123 | 2,3 | 11,3 | 7,3 | 30,8 | 7,0 | 55,5 | 15,9 | 1,9 | 17,5 | 34,4 | 11,4 |
| Atlet | 124 | 2,3 | 14,0 | 8,6 | 32,8 | 9,3 | 64,4 | 13,4 | 2,8 | 18,1 | 42,9 | 11,4 |
| Tril | 113 | 2,0 | 11,7 | 9,7 | 29,7 | 7,1 | 57,8 | 7,6 | 2,6 | 10,9 | 45,3 | 6,9 |
| Titan | 119 | 3,0 | 15,3 | 6,6 | 31,9 | 7,5 | 76,9 | 11,4 | 3,1 | 9,5 | 40,6 | 6,0 |
| Rozovscoe | 125 | 2,3 | 13,9 | 6,3 | 30,5 | 5,2 | 70,3 | 16,7 | 2,7 | 19,2 | 37,9 | 8,2 |
| Roudkrider | 137 | 2,1 | 9,6 | 4,7 | 26,5 | 7,2 | 41,3 | 18,5 | 2,0 | 12,6 | 43,7 | 12,4 |
| Linia 12 | 125 | 3,5 | 12,3 | 13,4 | 31,2 | 9,6 | 64,3 | 13,7 | 3,1 | 18,6 | 48,5 | 14,6 |
| Linia 16441-5 | 133 | 3,2 | 12,6 | 8,7 | 29,6 | 5,8 | 47,9 | 22,6 | 2,5 | 25,4 | 52,8 | 8,6 |
| 95W73-2-I | 139 | 1,4 | 12,0 | 7,4 | 30,9 | 6,2 | 70,9 | 16,9 | 3,5 | 18,6 | 49,7 | 4,9 |
| AD 1467 | 135 | 2,2 | 13,9 | 7,9 | 31,1 | 5,1 | 77,2 | 15,3 | 3,5 | 18,9 | 44,9 | 11,9 |
| Presto | 138 | 2,3 | 10,2 | 4,1 | 31,2 | 4,5 | 61,8 | 12,5 | 2,8 | 22,7 | 45,1 | 10,3 |
| AD 1128 | 139 | 2,1 | 10,4 | 6,3 | 31,3 | 8,1 | 61,9 | 13,9 | 2,4 | 19,2 | 39,2 | 8,8 |
| Agendus | 96 | 3,1 | 10,7 | 2,4 | 27,4 | 3,5 | 61,5 | 16,1 | 2,0 | 17,7 | 33,1 | 6,6 |
| Tulus | 102 | 1,7 | 14,6 | 4,7 | 34,8 | 6,9 | 86,9 | 9,5 | 3,4 | 14,5 | 39,1 | 10,8 |
| Tadeus | 97 | 2,7 | 13,7 | 4,3 | 33,4 | 3,2 | 62,1 | 13,3 | 2,3 | 16,1 | 37,9 | 14,6 |
| Duet | 123 | 1,7 | 14,0 | 7,5 | 35,3 | 5,7 | 76,3 | 10,7 | 3,8 | 13,9 | 49,9 | 15,6 |
| B - 7 | 122 | 1,5 | 12,5 | 9,1 | 34,2 | 6,0 | 79,7 | 23,9 | 3,5 | 22,5 | 43,9 | 13,7 |
| Linia 2405 | 106 | 1,5 | 14,7 | 8,5 | 31,7 | 7,6 | 66,8 | 13,3 | 2,6 | 14,3 | 38,5 | 4,6 |
| Ing. 93 (st) | 99 | 1,7 | 12,1 | 10,0 | 30,2 | 8,2 | 52,5 | 18,5 | 2,4 | 16,1 | 39,3 | 7,6 |
| Cv (%) cp. | | 2,3 | | 6,9 | | 7,0 | | 15,0 | | 18,1 | | 10,0 |

## CONCLUSIONS

A comprehensive assessment of collection samples of triticale of different geographical origins was carried out based on the main structural elements of productivity. As a result of studying the main economically valuable traits, forms with a whole complex of economically valuable traits were identified, which will be used in

further selection as genetic sources for creating new varieties with specified traits. In terms of productivity, the best samples were from Germany (Duet), Ukraine (Salanda) and Russia (L.16441-5, 95W73-2-1, L.12). Highly productive forms were identified from Romania (Haiduc, Oda), Poland (Presto), Ukraine (Obrii Mironovskii, Buchet) and Russia (95W73-2-1, AD1467). Analysis of the variation coefficients showed that the greatest variability in triticale variety samples is inherent in the number of grains and the weight of grains per spike. Low variability is characteristic of plant height.

Samples and varieties of early-ripening and mid-ripening triticale with high productivity potential were included in hybridization and the resulting hybrid forms are at various stages of the selection process.

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DOI: https://doi.org/10.53040/cga12.36 UDC: 633.111.1:632.4

# INFLUENCE OF THE *FUSARIUM OXYSPORUM* FUNGUS ON THE TRANSGRESSIVE POTENTIAL OF GROWTH TRAITS OF COMMON WHEAT

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***Abstract.*** the purpose of the research was to establish the reaction of 7 parents, 4 pairs of $F_2$ hybrids of common winter wheat, obtained based on 4 pairs of reciprocal $F_1$ hybrids, and the transgressive potential of growth and development organs to the treatment of grains with *Fusarium oxysporum* culture filtrate. The pathogen was isolated from wheat plants with root rot symptoms and identified by macro- and microscopic methods. The culture filtrate (CF) was prepared on the basis of Cszapek liquid medium. After treating the grains for 18 hours with CF, the seedlings were cultivated for 6 days, after which morphometric analyses of growth characters were performed. Plant distribution histograms were analyzed according to variant and character. The degree (Tg), the frequency (Tf) of transgressions were calculated and the important role of the plant-pathogen interaction mode, the parental factor in the spectrum of phenotypic classes and transgressive manifestations in segregating populations were found.

***Keywords: Fusarium oxysporum, traits, transgressions, wheat.***

## INTRODUCTION

Interactions between plants and fungi can have beneficial or detrimental effects on host plants and can give rise to various changes at both the plant and the fungus levels. Plant fungal pathogens are economically important due to the threat they pose to the production and yield of agricultural crops.

The reduction or prevention of fungal diseases of plants depends on resistant crop varieties or fungicide treatment. Plant-fungi interactions need to be known to understand the mechanisms of fungal plant diseases, their prevention, and to improve plant productivity [1, 2].

The fungus *Fusarium oxysporum* Snyd. et Hans. has a wide distribution both geographically and in terms of expansion on different host plants, which demonstrates the possession of a strong fermentative apparatus that gives it the possibility to utilize as many biochemical substrates as possible. It is a fungus with vascular activity, being better known for its ability to produce seed/grain rot at the

germination stage, root system, stem base and plant wilting [3].

Plant populations, like all living organisms, are the level of organization through which the species exists and realizes its biological potential under specific conditions. In this regard, there is little research on the influence of fungi on the population level of plants.

Due to the phenomenon of crossing-over in meiotic prophase I, which involves the exchange of fragments between homologous chromosomes, recombinant genetic material is obtained. It is this phenomenon that underlies the generation of a wide spectrum of genotypes with recombinant characters in the $F_2$ population, which offers broad opportunities for theoretical research and practical applications in biology and agriculture.

The success of genetic improvement is based on the knowledge and correct use of available germplasm, in order to identify the varieties to be used as parents in such a way that when crossed they generate an $F_1$ offspring with a high heterotic effect, allowing the emergence of transgressive variants that can be selected in segregating generations. According to the author [4], transgressive segregation consists of the production of $F_2$ offspring or subsequent generations with phenotypes that exceed the range of the parental populations from which they are derived.

According to the author [5], transgressive phenotypes are the result of positive or negative complementation of additive alleles, epistatic interactions of unique parental attributes, unmasking of recessive alleles from a heterozygous parent, or various combinations of these mechanisms.

The author [6] proposed that transgression occurs by crossing parents with similar values for the variable under study, but with different genes controlling the trait, which allows some $F_2$ individuals to contain new gene combinations with a greater or lesser number of genes that present a more favorable effect than that of the parents.

The aim of the present research was to elucidate the influence of the phytopathogen F. oxysporum on the spectrum of phenotypic classes in $F_2$ populations of common wheat, descendants of reciprocal $F_1$ hybrids, and the degree and frequency of transgressions of some growth and development characters.

## MATERIALS AND METHODS

The study material consisted of 7 common winter wheat parents – Moldova 11 (M11), Moldova 16 (M16), Moldova 66 (M66), Cuialnic, Amor, Miranda, Centurion and 4 pairs of $F_2$ hybrids – descendants of reciprocal $F_1$ hybrids. The culture filtrate of the *F. oxysporum* isolate from wheat plant with signs of stem base rot was obtained on the basis of Cszapek liquid nutrient medium (figure 1).

**A** **B** **C**

Figure 1. Isolation of the fungi from the stem base of:
*wheat (A); colony (B) and microconidia (C, 300x) of F. oxysporum*

Wheat grains were treated for 18 hours with *F. oxysporum* CF. Morphometric analysis of the length of the embryonic radicle and the stem was performed on day 6 of seedling growth. The experiment was performed in replicates, 30 grains each for the parents and 120 – for the $F_2$ hybrids.

**The degree of transgression** (Tg) was calculated based on the formula: Tg = [(Ch • 100%) : Cp] – 100, where Tg – degree of transgression of the trait in percentage; $C_h$ – maximum value of the trait in the $F_2$ generation hybrid (average of the 3 best plants); $C_p$ – maximum value of the trait of the best parent (average of the 3 best plants).

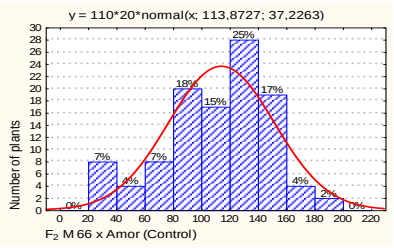
***The transgression frequency*** ($T_f$) was determined according to the formula: $T_f$ = (H • 100%) : $N_h$, where $T_f$ – transgression frequency in percentage; H – number of $F_2$ hybrid plants that exceed the best parent (average of 3 best plants) by the analyzed character; $N_h$ – total number of $F_2$ hybrid plants analyzed [7].

When describing histograms, the following 3 basic characteristics were taken into account: ***modality*** – the number of "peaks" of the distribution (***unimodal*** – with a single peak, ***bi-, multimodal*** – two or more "peaks"); ***skewness*** – the tendency of increasing or decreasing values: *skewed to the right* – a tendency of increasing the investigated characteristic and is called a positive distribution, *skewed to the left* – a tendency of decreasing the investigated characteristic – a negative distribution, the lack of skewness signifies a symmetric distribution; ***flattening*** – if the distribution is very flattened it means that the scores/values vary a lot, and if it is sharp – the scores/values vary little [8]. The data were statistically processed in the STATISTICA 7 software package.

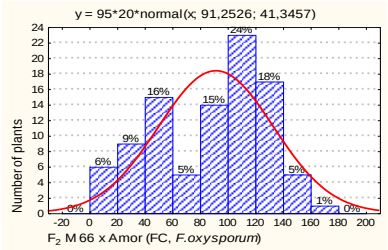
## RESULTS AND DISCUSSIONS

***Radicle length***. It was found that *F. oxysporum* CF produced both inhibition and stimulation of radicle growth in the $F_2$ M66 x Amor population. In the control variant it recorded 113.9 mm, and in the F. ox. CF variant – 91.3 mm, which means a decrease of 19.9%. At the same time, in the $F_2$ Amor x M66 population in the control variant, the radicle length was 56.4 mm, and under the influence of CF – 70

mm, thus showing growth stimulation by 24.1% (figure 2).

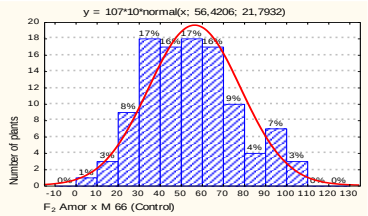



**A**

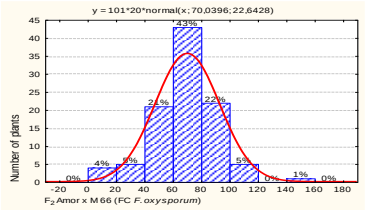



**B**

**$F_2$ Moldova 66 x Amor**

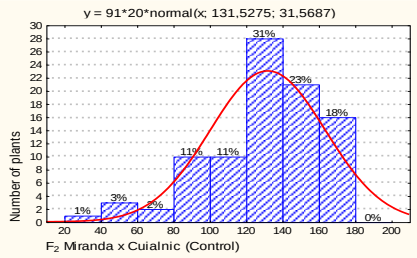



**A**

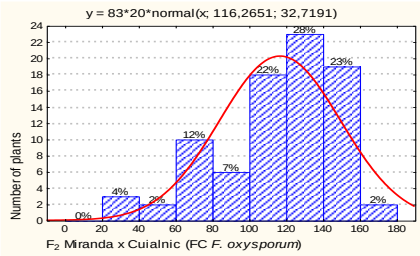



**B**

**$F_2$ Amor x Moldova 66**

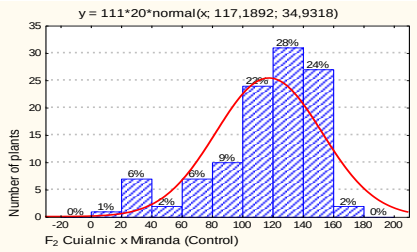



**A**

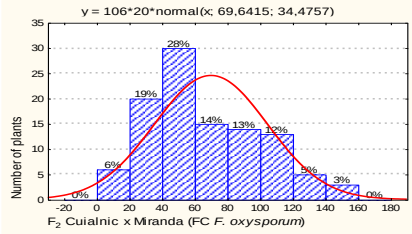



**B**

**$F_2$ Miranda x Cuialnic**




**A**




**B**

**$F_2$ Cuialnic x Miranda**

Figure 2. Spectrum of phenotypic classes (raicle length, mm) in $F_2$ populations of common wheat:

*A – control, B – F. oxysporum FC*

In the case of the $F_2$ Miranda Cuialnic and $F_2$ Cuialnic x Miranda hybrids, a decrease in radicle length under the influence of *F. oxysporum* CF was recorded by 11.6% and 40.6%, respectively. The data indicate that the reaction of wheat to F. oxysporum is specific, and the pattern of plant-pathogen interaction has the parental factor as a determinant in the creation of new genotypes.

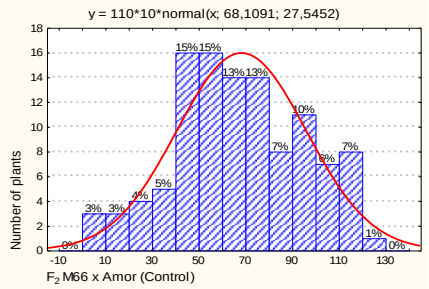
Disregarding the average values of the populations for the characters under study, which often camouflage different particularities and subcomponents within the system, we find that the pathogen influenced the profile of the distribution histograms of the phenotypic classes differently.

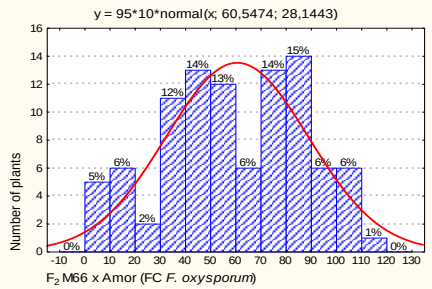
For example, under the pressure of the fungus *F. oxysporum*, in the $F_2$ M66 X Amor hybrid, a tendency for the population to decompose into 2 subpopulations with their own characteristics was observed - amplitude, maximum of value distribution, number of phenotypic classes, flattening. In the $F_2$ Amor x Moldova 66 population in the variant with *F. oxysporum*, no phenomena of decomposition of the initial population were observed, on the other hand, the maximum of value distribution with adjacent classes was narrower, which means lower variability.

Regarding the combinations $F_2$ Miranda x Cuialnic and $F_2$ Cuialnic x Miranda, it was observed that in the variant with *F. oxysporum* CF the rate of plants with high values of radicle length decreased compared to the control. For example, regarding the phenotypic classes 140-160 and 160-180 mm, in the combination $F_2$ Miranda x Cuialnic the summary rate of plants constituted 41 and 25%, respectively, of the control and *F. oxysporum* CF and in the reciprocal combination $F_2$ Cuialnic x Miranda – 26 and 3%, respectively, of the control and *F. oxysporum* CF variants.

***Stem length***. A decrease in the character was found – by 11.1% in the $F_2$ M66 x Amor combination and a stimulation by 8.6% – in the $F_2$ Amor x M66 combination under the influence of the pathogen. Unlike the radicle, in the $F_2$ Amor x M66 combination, at the same time, in the variant with fungus, the spectrum of phenotypic classes was expanded (figure 3).

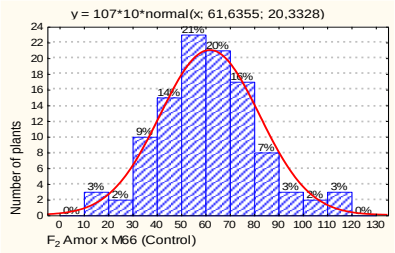
The character average of the combinations $F_2$ Miranda x Cuialnic, $F_2$ Cuialnic x Miranda in the variant with FC recorded +1.2% and -24.9%, respectively, in relation to the control. The $F_2$ Miranda x Cuialnic population was uneven in the control variant, but under the influence of the fungus, it slightly became uniform. In the $F_2$ Cuialnic x Miranda population, under the action of FC, the seedlings suffered a distribution tilted to the left, in the direction of low values.

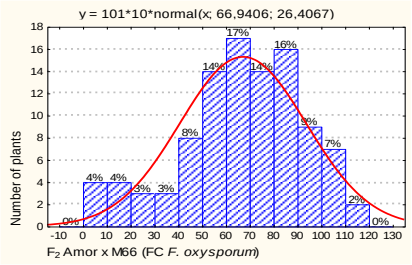



**A** **B**

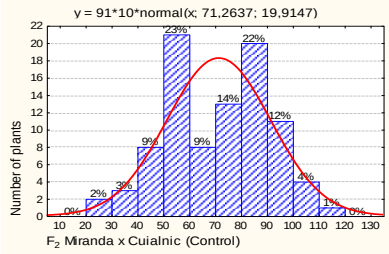
**$F_2$ Moldova 66 x Amor**

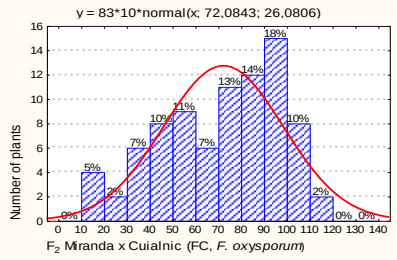



**A** **B**

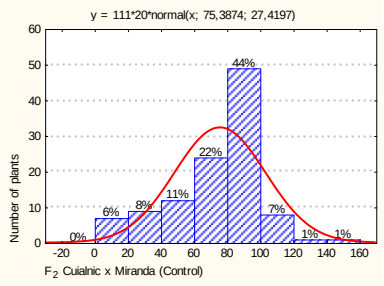
**$F_2$ Amor x Moldova 66**

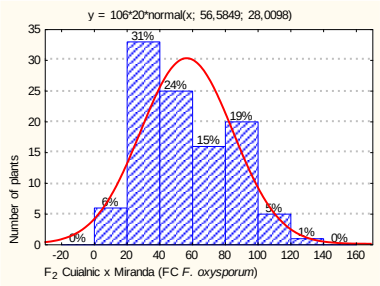



**A** **B**

**$F_2$ Miranda x Cuialnic**




**A** **B**

**$F_2$ Cuialnic x Miranda**

Figure 3. Spectrum of phenotypic classes (stem length, mm) in $F_2$ populations of common wheat:

*A – control, B – F. oxysporum CF*

The calculation of the degree (Tg) and frequency of transgressions (Tf) for the characters under study demonstrated the prevalence of negative transgressions in the case of the radicle and positive ones – in the stem (table).

Taking as a basis the postulate that transgressive phenotypes are determined mainly by the complementary action of allelic genes, we can admit that this mechanism can provide several positive "extreme" phenotypes for the less sensitive character (the stem) to the action of the phytopathogen, and at the same time - several negative "extreme" phenotypes for the sensitive character - the embryonic radicle, as demonstrated in previous research [9].

Starting from the practical interest in genotypes that manifest positive transgressions in the interaction with pathogens, in particular of sensitive characters but important for the vitality of the plant, we consider that the combinations $F_2$ Amor x Moldova 16, Cuialnic x Miranda, Centurion x Moldova 11 are promising, whose rate of positive segregants for radicle length on a background of biotic pressure varied within the limits of 3.77 ... 5.66%, the degree of positive transgression varying within the limits of 4.44 ... 11.09%.

Table 1. Degree (Tg) and frequency of transgressions (Tf) of some growth characters in the interaction of common wheat with *F. oxysporum*

| $F_2$ combination | Variant | The length of radicle | | The length of stem | |
|---|---|---|---|---|---|
| | | $T_g$ | $T_f$ | $T_g$ | $T_f$ |
| Amor x Moldova 66 | Martor | -1,96 | 0,91 | 10,49 | 4,67 |
| | FC *F. ox.* | -27,99 | 0,01 | 2,4 | 1,98 |
| Moldova 66 x Amor | Martor | -43,14 | 0,01 | 17,05 | 12,73 |
| | FC *F. ox.* | -4,83 | 1,05 | 0,90 | 1,05 |
| Amor x Moldova 16 | Martor | -3,70 | 1,80 | 12,73 | 4,72 |
| | FC *F. ox.* | **9,86** | **4,04** | 0,29 | 1,01 |
| Moldova 16 x Amor | Martor | -3,70 | 1,80 | 15,76 | 5,41 |
| | FC *F. ox.* | -5,13 | 0,02 | 5,80 | 2,83 |
| Miranda x Cuialnic | Martor | -23,17 | 0,01 | -16,3 | 0,02 |
| | FC *F. ox.* | -2,35 | 0,96 | 9,03 | 9,62 |
| Cuialnic x Miranda | Martor | **1,78** | **3,67** | 10,14 | 7,34 |
| | FC *F. ox.* | **11,09** | **5,66** | -10,65 | 0,01 |
| Moldova 11 x Centurion | Martor | **7,29** | **4,42** | 8,26 | 24,78 |
| | FC *F. ox.* | -6,45 | 0,01 | 26,18 | 19,54 |
| Centurion x Moldova 11 | Martor | -24,70 | 0,02 | -15,34 | 0,98 |
| | FC *F. ox.* | **4,44** | **3,77** | 25,09 | 27,12 |

## CONCLUSIONS

1. It was found that treating common wheat grains with F. oxysporum culture filtrate led to inhibition or stimulation of embryonic radicle and stem growth.

2. Under the influence of the fungus, changes occurred in the profile of plant distribution spectra in $F_2$ segregating populations – the appearance of bi- or trimodal histograms, the orientation of the slope, flattening (expanded, contracted).

3. The $F_2$ combinations Amor x Moldova 16, Cuialnic x Miranda, Centurion x Moldova 11 are considered promising, whose degree and rate of positive segregants for radicle length on a background of biotic pressure varied within the limits of 3.77 ... 5.66%, 4.44 ... 11.09%, respectively.

**Acknowledgments**. The research was carried out within the framework of project "23.70105.5107.04. Identifying and capitalizing on the valuable parents of agricultural crops in the creation of a native genetic base of socio-economic interest", financed by the National Agency for Research and Development of the Republic of Moldova (2024-2025).

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DOI: https://doi.org/10.53040/cga12.37 UDC: 635.64:631.528

# THE BREEDING OF HIGHLY RESISTANT TOMATO $F_1$ HYBRIDS TO STRESS ABIOTIC FACTORS

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***Abstract.*** *The paper presents the results of studying the adaptive potential of $F_1$ tomato hybrids obtained from crossing genotypes with different combinations of mutant marker genes. The assessment of resistance to high and low temperatures is evaluated at the stage of mature male gametophyte under in vitro conditions and fruiting under in vivo conditions Genotype-specific differences in the character of manifestation of traits between $F_1$ hybrids, both depending on the features of the initial forms and the type of temperature stress factor, were established. A stable character of manifestation of the studied traits of male gametophyte is observed in hybrids from combinations with genotypes of cultural type and mutant forms - L8/234 × Mo835, L33/241 × Mo835, L111 × Mo 341, Fakel × Mo 341, MaKrista × Mo 341. Strong variability was characteristic of $F_1$ intermutant hybrids (L33/16 × Mo 835, L11069 × L28, L45/16 × Mo 341) under both stress backgrounds (45°C and +6°C) including fruiting in three different years of research (2021-2023).*

***Keywords: $F_1$ tomato hybrids, fruit setting, male gametophyte traits, temperature stress factors, resistance.***

## INTRODUCTION

Creation of heterotic hybrids of tomato $F_1$ is one of the most priority directions of breeding programs. Heterotic power of plants, their higher productivity, early maturity, resistance to diseases in comparison with the original forms and released varieties, shows high efficiency of hybrid cultivation, both in the conditions of open and protected soil. It has been established [1] that the greatest effect of heterosis on yield and resistance to stress abiotic factors have hybrids obtained from crossing forms that differ sharply in biological and morphological traits. The crossing of such forms provides for their genetic diversity, which determines higher heterozygosity of hybrids, being the basis of heterosis. Therefore, mutant forms obtained by different methods are of particular interest in this regard [2, 3]. They are carriers of a wide range of genes, the involvement of which in the breeding process can meet the requirements for modern varieties and hybrids regarding their resistance to diseases, increased content of biologically valuable components in fruits, marketable appearance – shape, size, coloring, high transportability, etc., as well as their ability to be used in the breeding process.

Genes controlling plant growth and suppressing the growth of lateral shoots are also preferred. In addition, modern breeding technologies should be aimed at the creation and introduction into production of $F_1$ tomato hybrids that have not only a wide range of economically valuable traits, but also have high adaptive capabilities for cultivation under conditions with limited control of environmental parameters. The temperature factor is of special importance in this respect. Exceeding the boundary of optimum temperature leads to a sharp decrease in fruit formation due to the resulting inconsistency with the genotypic norm of the response of cultivated hybrids. This necessitates the selection of $F_1$ hybrids with the ability to set fruit stably in a wider range of environmental temperature regimes. The solution to such a complex problem is the involvement of a wide variety of tomato *Solanum lycopersicum* L. gene pool, including mutant forms in the breeding process, with the simultaneous use of a combination of traditional selection methods with modern techniques for working with the sporophytic and gametophytic [4] phases of plants: pollen selection and provocative backgrounds of different degrees of severity.

The aim of the research is to study and select tomato forms with higher gametophyte resistance rates *in vitro* and fruit set *in vivo*, obtained using different mutant genes in comparison with existing $F_1$ hybrids.

## MATERIALS AND METHODS

The initial material for the research was 12 $F_1$ tomato hybrid combinations obtained using mutant forms of Mo 835 (*Ln*), Mo 341 ($Wo^m$), Mo 443 (*ls*), semi-mutant lines L 111 ($sp^{\pm}$, *nor, u, hp-1, j-2*), L11069 ($sp^+$, *u, nor, Tm-2a, ls, br, j-2*), L33/16 (*sp, ps-2*), L45/16 (*sp, ps*) and lines homozygous for *rin* and *nor* genes – L 28, L186, L8/234, 33|341, L201 and high-tech tomato varieties of own selection Stefani, Fakel, MaKrista. The work was carried out in several stages: identification of donors and genetic sources of the most important traits; crosses; study and evaluation of the general and specific combinative ability of parental forms; evaluation of the adaptive potential of $F_1$ hybrids. Plants were grown in greenhouses of the Institute of Genetics, Physiology and Plant Protection, in three different climatic years (2021-2023), on plots of 10 $m^2$, 35-40 accounting plants from each hybrid combination [5]. Morphological description of plants of $F_1$ hybrids and the degree of phenotypic expression of traits, including mutant marker traits in $F_1$, were carried out using well-known methods [6]. At the same time, indices of such traits as: type of plant growth, inflorescence type, number of flowers on plants and number of fruit set from them in three different years were taken into account. In laboratory conditions, $F_1$ hybrids were tested for tolerance to high and low temperatures according to male gametophyte traits using artificially created stress backgrounds [4]. For this purpose, freshly collected pollen from fully opened flowers of each hybrid was divided into 3 parts. One part was immediately sown on

artificial nutrient medium consisting of 15% sucrose and 0.006% boric acid and germinated under *in vitro* conditions for 3 hours at 25°C. Viability (*p, %*) was determined and pollen tube length (*l, µm*) was measured in ocular micrometer divisions, this served as control (C). The second part of pollen was used to evaluate heat resistance by treating it at 45°C for 8 h, followed by germination and determination of viability (*p, %*) and pollen tube length (*l, µm*). Cold tolerance of $F_1$ hybrids was determined by sowing the third part of pollen on nutrient medium and germinating it in the thermostat at +6°C for 24 hours. For each $F_1$ hybrid and in each research variant, at least 500 pollen grains were analyzed. Preparations with pollen were studied under a Zeiss light microscope. Resistance was judged on the basis of the ratio of parameters in the experiment (E) to the control (C). Statistical analysis allowed us to evaluate the variability (V, %) and genetic stability of the studied traits in $F_1$ tomato hybrids depending on the characteristics of the initial forms and growing conditions.

## RESULTS AND DISCUSSION

When assessing the resistance of tomato genotypes to environmental stresses, it is necessary to take into account that the level of favorable temperature intervals for the development of reproductive organs is narrower than for vegetative organs. In this regard, the male gametophyte is of special interest. It is the most sensitive to the influence and change of environmental conditions, unlike the female gametophyte, which is covered with thick layers of somatic tissues. Therefore, pollen quality can serve as a criterion of genotypes' reaction to changes in environmental conditions, which is reflected in the reproductive ability of plants [7] and the formation of high-quality seeds [8]. Taking this into account, we evaluated the resistance of new $F_1$ tomato hybrids to high- and low-temperature stress factors, considering into account the nature of manifestation according to male gametophyte traits – pollen viability, pollen tube length, resistance according to pollen germination under the influence of stress temperatures and the ability of germinated grains to form pollen tubes long enough for fertilization (three pollen grain diameters).

The results obtained showed a complex character of the manifestation of the studied traits in $F_1$ hybrids and strongly pronounced differences between hybrids, both with respect to the reaction of their pollen to high (45°C) and low (+6°C) temperatures, and depending on the initial forms used in crosses (Tables 1 and 2). The degree of variability (V, %) of both pollen traits in individual hybrids was very high and, on the contrary, very low with respect to two different stress factors. For example, pollen from hybrids from the combinations L8/234 × Mo835, L33/241 × Mo835, L111 × Mo 341, Fakel × Mo 341, MaKrista × Mo 341 and the standard sample $F_1$ Ingstar responded less to high-temperature exposure. Consistently high

indicators in three different years of research (2021-2023) are confirmed by low variation coefficients for the "viability-germination" trait – 5.7-9.4% (Table 1) and the "pollen tube length" trait – 6.7-9.6% (Table 2). Similar high results for these $F_1$ hybrids were obtained when their pollen was germinated and at low temperature (Table 1). The average coefficient of variability for the trait "cold tolerance in pollen germination" was consistently low in three different years of the study, ranging from 3.2 to 9.6% (Table 1). The same low variability was observed for "pollen tube length", 4.4-8.2% (Table 2). This indicates the high adaptive potential of these $F_1$ tomato hybrids to both high and low temperatures. A high degree of variability (V, %) of the trait "pollen germination" under both stress backgrounds was observed in hybrid combinations – L33/16 × Mo 835, L11069 × L28, L45/16 × Mo 341 and Mo 443 × Fakel (Table 1).

Table 1. The influence of high and low temperatures on pollen viability-germination (p, %), of different $F_1$ tomato hybrids

| **Hybrids $F_1$** | **Pollen germination at temperature** | | | | | |
|---|---|---|---|---|---|---|
| | **25°C - 3 hour (control)** | | **45°C - 8 hours** | | **6°C – 24 hours** | |
| | **Average for three years (2021-2023),%** | | | | | |
| | **x** | **v,%** | **x** | **v,%** | **x** | **v,%** |
| L8/234 × Mo 835 | 88.9 | 18.9 | 76.3 | 7.9 | 80.1 | 11.4 |
| L33/16 × Mo 835 | 19.4 | 20.7 | 7.9 | 36.3 | 27.6 | 33.2 |
| Stefani × Mo 835 | 42.3 | 7.8 | 20.4 | 10.6 | 64.7 | 9.6 |
| L33/241 × Stefani | 58.2 | 5.7 | 55.5 | 9.4 | 71.4 | 7.3 |
| L186 × Mo 835 | 70.0 | 13.1 | 68.3 | 14.7 | 69.1 | 9.2 |
| L11069 × L28 | 24.5 | 41.4 | 11.1 | 56.6 | 47.3 | 28.7 |
| L45/16 × Mo 341 | 10.7 | 31.3 | 4.1 | 42.8 | 5.6 | 37.5 |
| L111 × Mo 341 | 63.9 | 6.4 | 51.2 | 8.7 | 60.4 | 4.8 |
| L 201 × Mo341 | 66.4 | 8.7 | 53.6 | 10.1 | 15.5 | 24.4 |
| Mo 443 × Fakel | 33.1 | 28.2 | 12.7 | 24.6 | 13.3 | 30.1 |
| Fakel × Mo341 | 31.2 | 11.2 | 45.2 | 8.4 | 18.1 | 10.6 |
| MaKrista × Mo 341 | 83.9 | 3.4 | 72.0 | 5.7 | 88.9 | 3.2 |
| Standard - $F_1$ Ingstar | 77.4 | 10.3 | 64.1 | 7.2 | 71.7 | 4.8 |

Similar response of pollen of these $F_1$ hybrids to temperature stressors was observed for the trait "tube length" (Table 2). The coefficient of variability for both traits (average for three years) is very high for pollen from 36.3% to 56.6% (45°C) and from 28.7 to 33.2% (+6°C), while for pollen tube length from 21.7 to 51.6% – 45°C and from 20.3 to 24.4% – + 6°C (Tables 1 and 2). It can be assumed that the high degree of variability of both pollen traits is determined by the peculiarities of parental lines 33/16, 11069, 45/16 and Mo 443, which are carriers of *ls, br, ps ps-2*

genes. The dominant character of inheritance of these genes by $F_1$ hybrids is a consequence of high morpho-biological heterogeneity of pollen grains in their populations. Morphological abnormalities in the structure of flowers and their individual elements, as well as the presence in the populations of these $F_1$ hybrids of a large number of small, puny pollen grains with disturbed integrity, and reflected in the character of the manifestation of traits both in freshly collected pollen (control) and under the influence of temperature stress factors.

In addition to those noted, $F_1$ hybrids with ambiguous reaction of their pollen to temperature stressors were also identified. $F_1$ Stefani × Mo835 pollen was susceptible to high temperature, but it germinated perfectly at low temperature, forming very long tubes at the same time. Conversely, pollen from hybrids L201 × Mo341 and Fakel × Mo341 showed high tolerance to high temperature (45°C/8 hours) and very poor germination at low temperature (6°C/24 hours) (Tabl.1 and 2).

Table 2. Effect of temperature on pollen tube length (*l, μm*) of different $F_1$ tomato hybrids

| **Hybrids $F_1$** | **Length of pollen tubes at temperature** | | | | | |
|---|---|---|---|---|---|---|
| | **25°C - 3 hour (control)** | | **45°C - 8 hours** | | **6°C – 24 hours** | |
| | **average for three years (2021-2023) in ocular-micrometer divisions (*l, μm*)** | | | | | |
| | **x** | **v,%** | **x** | **v,%** | **x** | **v,%** |
| L8/234 × Mo 835 | 90.1 | 8.5 | 69.6 | 6.8 | 75.0 | 8.2 |
| L33/16× Mo 835 | 42.0 | 26.4 | 31.2 | 21.7 | 39.6 | 20.3 |
| Stefani × Mo 835 | 58.6 | 10.2 | 30.0 | 9.6 | 56.8 | 10.1 |
| L33/241 × Stefani | 71.2 | 6.3 | 64.7 | 8.7 | 77.4 | 4.3 |
| L186 × Mo 835 | 60.3 | 8.1 | 61.5 | 7.4 | 57.4 | 11.2 |
| L11069 × L28 | 33.4 | 32.7 | 24.2 | 44.9 | 41.3 | 23.7 |
| L45/16 × Mo 341 | 36.2 | 40.3 | 24.8 | 51.6 | 10.0 | 24.4 |
| L111 × Mo 341 | 67.7 | 7.7 | 47.4 | 9.2 | 52.2 | 5.6 |
| L 201 × Mo341 | 88.7 | 9.3 | 67.3 | 10.7 | 20.0 | 31.2 |
| Mo 443 × Fakel | 44.1 | 18.4 | 40.2 | 21.2 | 43.6 | 22.3 |
| Fakel × Mo341 | 40.6 | 14.8 | 48.3 | 6.7 | 25.1 | 19.6 |
| MaKrista × Mo 341 | 64.4 | 4.7 | 61.7 | 8.6 | 71.2 | 7.4 |
| Standard - $F_1$ Ingstar | 96.2 | 3.9 | 74.1 | 9.7 | 82.4 | 10.2 |

This approach to research allowed us to identify the response of pollen of each $F_1$ hybrid to the action of high and low temperatures on the manifestation of two traits of male gametophyte (pollen germination and pollen tube length), which suggests its use as an express method for evaluation and selection of genotypes resistant to temperature stresses.

The study and analysis of fruit set in $F_1$ hybrids from different crossing combinations also showed significant differences between them. At the same time, a pronounced relationship between pollen traits and fruit formation level in hybrids from different crossing combinations should and be noted. For example, in hybrids (Mo443 × Fakel, L33/16 × Mo 835, L45/16 × Mo 341 and Fakel × Mo341) with high variability of pollen traits (Tables 1 and 2) in three different years of study (2021-2023), the number of fruit set from the number of flowers on the plant was below 50% and respectively amounted to 40.0%, 40.4%, 42.6% and 48.5%. Whereas the percentage of fruit set was higher than the standard sample ($F_1$ Ingstar - 84.2%) in 4 $F_1$ hybrids from combinations: L111 × Mo341, 85.4%; L8/234 × Mo835, 86.3%; L33/241 × Mo835, 89.5%; and L186 × Mo835, 89.9%. These $F_1$ tomato hybrids also showed high tolerance to high and low temperature stressors according to male gametophyte traits (Tables 1 and 2). The ability of pollen grains germinated under the influence of stress temperatures to form long pollen tubes confirms their high reproductive potential. Such a pollen tube in vivo provides fertilization, contributing to high fruit set and the formation of a large number of full-fledged high-quality seeds. Lower than the standard, but quite high fruit set (from 68.9% to 81.2%) was also observed in other studied $F_1$ hybrid combinations - Stefani × Mo835, L11069 × L28, L201 × Mo341, MaKrista × Mo341.

These two groups of hybrids are characterized by a wide range and original combination of economically valuable traits, including resistance to one or both high and low temperatures simultaneously. Consequently, with minimal improvement, they can be recommended for cultivation in production conditions in the Republic of Moldova

## CONCLUSIONS

Genotype-specific differences between $F_1$ tomato hybrids have been shown, both with respect to the response of their pollen to high and low temperature stress factors and the manifestation of these traits depending on the characteristics of their original parental forms and the conditions of plant cultivation in three different climatic years (2021-2023). These results may become the basis for a deeper study and identification of the influence of various combinations of mutant genes in heterozygous offspring on the character of manifestation of traits of key importance for practical tomato breeding and, especially, in the creation of heterozygous hybrids with high indices of economically valuable traits and increased adaptability to temperature stresses.

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# EVALUATION OF OSMOTIC STRESS TOLERANCE DURING THE GERMINATION STAGE IN SOYBEAN GENOTYPES

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***Abstract:*** *To evaluate the tolerance of soybean genotypes to osmotic stress, a controlled germination experiment was performed on six soybean lines: three obtained by gamma-ray-induced mutagenesis (C15M$_7$200, B2M$_8$200, Z21M$_{12}$200) and three by hybridization (L.4, L.8, L.86). Seeds were treated with three concentrations of PEG 6000 (0%, 10%, 15%) to simulate a water stress gradient. The analyzed parameters – germination rate and root length – were recorded 5 and 7 days after sowing. A significant reduction of both parameters was found with increasing PEG concentration, highlighting a different sensitivity between genotypes. Lines C15M$_7$200 and B2M$_8$200 showed better tolerance, while L.8 and Z21M$_{12}$200 were the most affected. The results highlight genetic variability and support the utility of PEG testing in the early stages of breeding programs for the selection of drought-tolerant genotypes.*

***Keywords: drought tolerance, germination, PEG, soybean.***

## INTRODUCTION

Under current climate change conditions, drought and water stress represent one of the greatest threats to global food security, severely affecting agricultural production, especially in arid and semi-arid regions. Osmotic stress, caused by soil water deficit, profoundly disrupts plant physiological processes, reducing germination rate, root system development and, ultimately, crop productivity. These effects are accentuated by increasing average temperatures and unpredictable rainfall, factors that also contribute to soil salinization and reduced water availability for plants [6].

To better understand and evaluate plant responses to water stress, agricultural research often uses polyethylene glycol (PEG), a compound that mimics water deficit conditions by creating a negative osmotic potential [3]. The use of PEG in germination experiments allows for the simulation of a controlled environment of osmotic stress and facilitates the comparison of drought tolerance between different genotypes. These studies provide relevant data on drought tolerance in the early stages of development, contributing to the selection of varieties with high agronomic potential under stress conditions [4].

In this context, soybean (*Glycine max*), a legume of strategic importance for both human and animal nutrition, is becoming a species of major interest. Due to its high protein content and its ability to fix atmospheric nitrogen, soybean plays a key role in sustainable agricultural systems [1]. However, this crop is particularly sensitive to water stress, especially in the early stages of germination and emergence, which can compromise the stability of production under adverse climatic conditions [2].

The identification of soybean genotypes with increased tolerance to osmotic stress holds significant practical relevance in the context of modern agriculture, which is increasingly marked by the frequency and intensity of drought episodes. Germination tests based on polyethylene glycol (PEG) enable rapid and efficient selection of promising genetic material, contributing to the shortening of the breeding cycle and the development of cultivars adapted to water stress conditions. Moreover, the strong correlation between germination parameters (such as germination rate, radicle emergence, and root elongation) and root system development provides reliable criteria for the indirect selection of tolerant genotypes [5].

Genotypes that demonstrate resilience during the germination phase generally show superior field establishment, greater biomass accumulation, and better yield performance under limited water availability [4]. The implementation of these findings in breeding programs can accelerate the development of high-performing varieties, contributing both to enhanced food security and to the long-term sustainability of agricultural production in increasingly unpredictable climatic conditions.

In this context, the present study aims to evaluate the response of six soybean genotypes to osmotic stress induced by polyethylene glycol (PEG 6000) under controlled laboratory conditions. The goal is to identify genetically valuable and tolerant material that can be further used in breeding programs focused on adapting soybean cultivation to increasingly unpredictable climatic conditions.

## MATERIALS AND METHODS

To evaluate osmotic stress tolerance, a germination experiment was performed under controlled conditions, using six soybean genotypes from the "Applied Genetics" laboratory of the Institute of Genetics, Physiology and Plant Protection (IGFPP), USM. Three of these ($C15M_7200$, $B2M_8200$, $Z21M_{12}200$) were obtained by experimental gamma-ray mutagenesis, and the other three (L.4, L.8, L.86) by conventional hybridization. For each genotype, 10 seeds per experimental variant were used, in three repetitions, covering the following treatments:

0% PRG (control) – distilled water, no stress

10% PEG – moderate stress
15% PEG – severe stress

The concentrations of polyethylene glycol (PEG 6000) were established according to the values proposed by Michel and Kaufmann [3], and the solutions were prepared in distilled water. Germination was performed in sterile Petri dishes, lined with filter paper, onto which 5 ml of each treatment solution was distributed. The seeds were previously disinfected with a 1% sodium hypochlorite solution for 5 minutes, followed by three successive rinses with sterile distilled water. The Petri dishes were placed in a climate chamber, under controlled conditions of temperature (25 ± 1°C) and relative humidity (70%). Germination assessments were performed 5 and 7 days after the initiation of the experiment. A seed was considered germinated if the radicle exceeded 2 mm in length. The experimental data were statistically analyzed using STATISTICA 7 software, and the graphs and presentation tables were processed in Microsoft Excel.

## RESULTS AND DISCUSSION

The results obtained from germination tests performed at 5 and 7 days highlight a significant influence of osmotic stress on the germination capacity of the soybean genotypes analyzed. The data presented in Table 1 show that at the control level (0% PEG), most genotypes showed a high germination rate, over 80%, with the exception of genotypes L.8 and L.86, which recorded 70% and 60%, respectively. This indicates a generally good viability of the seeds, but also genetic differences in terms of initial vigor.

With the application of osmotic stress, all genotypes underwent significant reductions in germination. Lines C15$M_7$200 and B2$M_8$200 were noted for maintaining a relatively high germination percentage at 10% PEG (80–90%), but suffered a drastic decrease at 15% PEG, indicating moderate tolerance to moderate stress and increased sensitivity to severe stress. Line Z21$M_{12}$200 showed reduced tolerance, with only 5% germination at 5 days under 15% PEG conditions, but increased to 15% at 7 days, suggesting a delay in the germination process, possibly due to a protective strategy under unfavorable conditions. This delayed response may be associated with metabolic adjustments or activation of specific stress-related pathways, allowing certain seeds to overcome the initial inhibitory effects of PEG. The observation highlights the importance of evaluating germination dynamics over multiple time points, rather than relying solely on early measurements, which may underestimate the potential of some genotypes to adapt or recover under stress conditions. Among the hybrid lines, L.4 performed best under moderate stress (70–80%), but performed very poorly at 15% PEG.

Table 1. Effect of PEG on germination percentage at 5 and 7 days

| Genotype | Treatment | Number of seeds | Germination in 5 days | Germination in 5 days, % | Germination in 7 days | Germination in 5 days, % |
|---|---|---|---|---|---|---|
| C15M$_7$200 | Control | 30 | 27 | 90% | 30 | 100% |
| C15M$_7$200 | 10% PEG | 30 | 24 | 80% | 27 | 90% |
| C15M7200 | 15% PEG | 30 | 3 | 10% | 4 | 15% |
| B2M$_8$ 200 | Control | 30 | 30 | 100% | 30 | 100% |
| B2M$_8$ 200 | 10% PEG | 30 | 24 | 80% | 24 | 80% |
| B2M$_8$ 200 | 15% PEG | 30 | 9 | 30% | 9 | 30% |
| Z21M$_{12}$ 200 | Control | 30 | 25 | 85% | 30 | 100% |
| Z21M$_{12}$ 200 | 10% PEG | 30 | 19 | 65% | 22 | 75% |
| Z21M$_{12}$ 200 | 15% PEG | 30 | 1 | 5% | 4 | 15% |
| L.4 | Control | 30 | 30 | 100% | 30 | 100% |
| L.4 | 10% PEG | 30 | 21 | 70% | 24 | 80% |
| L.4 | 15% PEG | 30 | 1 | 5% | 1 | 5% |
| L.8 | Control | 30 | 21 | 70% | 24 | 80% |
| L.8 | 10% PEG | 30 | 15 | 50% | 15 | 50% |
| L.8 | 15% PEG | 30 | 0 | 0% | 1 | 5% |
| L.86 | Control | 30 | 18 | 60% | 21 | 70% |
| L.86 | 10% PEG | 30 | 13 | 45% | 16 | 55% |
| L.86 | 15% PEG | 30 | 4 | 15% | 6 | 20% |

Figure 1 highlights the clear negative effect of PEG-induced water stress on germination percentage at 7 days. A progressive decrease in germination capacity is observed with increasing PEG concentration from 0% (control) to 15%, confirming the general sensitivity of soybean seeds to osmotic stress.

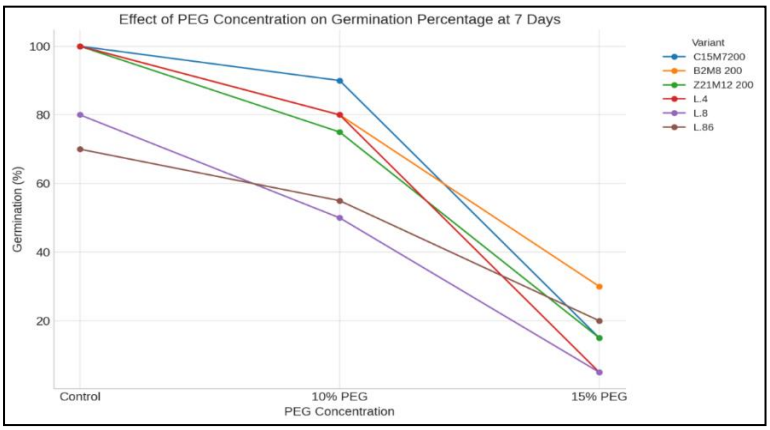


Figure 1. Germination percentage at 7 days as a function of PEG concentration for six soybean genotypes

Analyzing the variation of root length in the soybean genotypes studied, we

can mention that osmotic stress had a significant negative impact on the development of the root system. As the PEG concentration increased, the root length decreased considerably in all genotypes (fig.2).

The C15M$_7$200 and B2M$_8$200 genotypes were distinguished by the largest root lengths under control conditions, frequently exceeding 8–10 cm, but also by a better capacity to maintain root growth at 10% PEG. This behavior indicates a superior tolerance to moderate stress and a possible adaptation strategy by more efficient exploration of the substrate in search of water.

In contrast, the L.8 and Z21M$_{12}$200 genotypes showed a drastic reduction in root length, with very low average values at 10% and 15% PEG concentrations, suggesting a severe inhibition of root growth under osmotic stress.

Also, genotypes L.4 and L.86 showed intermediate resilience, maintaining some root growth at 10% PEG, but with a sharp decrease at 15%.

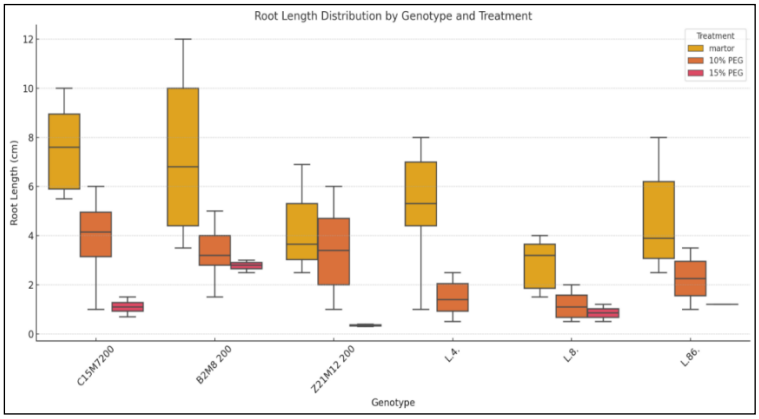


Figure 2. Effect of PEG-induced osmotic stress (0%, 10%, 15%) on root length in different soybean genotypes, evaluated 7 days after germination

Correlating these data with the germination percentage (figure 1), it is observed that root length is a good indicator of tolerance to water stress in the early development phase, and genotypes that maintained better root growth under stress generally also recorded higher germination rates.

## CONCLUSIONS

The results obtained in this study highlight the significant impact of osmotic stress, induced by the use of polyethylene glycol (PEG 6000), on germination and root development of six soybean genotypes. Both the germination percentage and root length decreased progressively with increasing PEG concentration, confirming the sensitivity of soybean to water deficit in the early stages of development.

At the same time, the differences between genotypes demonstrated the existence of significant genetic variability in the response to water stress, an essential aspect for breeding programs. The genotypes C15$M_7$200 and B2$M_8$200 stood out for their superior performances in both germination and root system development, especially under moderate stress (10% PEG), suggesting a high adaptive capacity and valuable potential for selection.

On the other hand, genotypes L.8, L.86 and Z21$M_{12}$200 showed increased sensitivity, with drastic decreases in germination and root growth, especially under severe stress conditions (15% PEG), indicating reduced tolerance to water deficit.

Testing for osmotic stress tolerance during the germination phase, using PEG as a simulant, proved to be an effective method for differentiating soybean genotypes and identifying promising genetic sources. The tolerant genotypes identified in this study represent valuable material for use in breeding programs aimed at improving drought resistance. Their inclusion can support the development of more stable and resilient soybean varieties, better adapted to the challenges posed by climate change.

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# VARIATION IN TOMATO GENOTYPES RESPONSE TO X-RAY IRRADIATION AND ESTIMATION OF THE EFFECTIVE DOSES

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***Abstract:*** *The study investigates the response of 15 tomato genotypes (cultivated and wild forms) to X-ray irradiation, aiming to determine effective mutagenic doses that result in a 50% reduction in key quantitative parameters: germination rate, radicle length, hypocotyl height, and seedling height. Seeds were exposed to 10 irradiation doses (ranging from 100 to 530 Gy), and the effects were assessed in comparison to a non-irradiated control. Linear, nonlinear, and polynomial regression models were applied, along with dose–response curve analysis, to estimate the effective inhibitory doses ($ID_{50}$/$ID_{30}$) for the efficient use of induced mutagenesis in the $M_0$ and subsequent generations. The results reveal considerable variability among genotypes, including stimulatory (hormetic) effects at lower doses. These findings are essential for optimizing mutagenesis protocols in tomato breeding programs.*

***Keywords: mutagenesis, tomato, regression, variability, X-rays.***

## INTRODUCTION

The induction of mutations using physical agents, such as ionizing radiation (gamma rays, X-rays), is a well-established approach for increasing genetic variability beneficial to plant breeding programs. This method enables the development of novel genotypes exhibiting superior agronomic traits, including stress tolerance, disease resistance, and improved nutritional or technological qualities [1]. However, the success of induced mutagenesis is strongly influenced by a combination of biological, technical, and experimental factors that affect the plant's response to radiation treatments. A key determinant is the proper preparation of seed material. Seed moisture content at the time of irradiation directly affects energy transfer efficiency and consequently influences the frequency and type of induced mutations. Seeds with higher water content are more sensitive to radiation due to the generation of free radicals through water radiolysis,

which can interact with DNA and other cellular structures. Therefore, strict control of seed moisture (typically reduced to 12-14%), according to standardized protocols is essential to ensure the repeatability and comparability of experimental results.

Other critical variables include the radiation dose and the type of radiation source, both of which influence the intensity and specificity of the mutagenic effects. In addition, the physiological stage of the biological material (seeds, seedlings, callus, etc.) and the genetic background of the treated genotypes contribute significantly to variations in radiosensitivity and the plant's capacity to repair radiation-induced damage [2; 3]. Genotypes vary in their response to mutagenic stress, necessitating individualized calibration of radiation doses for each variant under study. This is typically achieved through the determination of parameters such as the median inhibitory dose ($ID_{50}$). Establishing an effective dose is a critical step to ensure an optimal mutation rate while avoiding lethal damage to plant tissues.

In tomato, the literature reports a wide range of effective doses depending on genotype and the type of radiation or facility used [4; 5; 6]. Moreover, assessment methods such as germination rate, hypocotyl/plant length reduction, and seedling vigor are commonly used to identify promising genotypes for further breeding cycles.

In this context, the primary objective of the present study is to evaluate the differential radiosensitivity of 15 tomato genotypes and to estimate their $LD_{50}$ values based on relevant morphological parameters. By applying mathematical modeling approaches, including both linear and nonlinear regression, we aim to accurately estimate optimal radiation doses and to characterize the dose–response relationships. This contributes to the rational planning of mutagenesis experiments. Therefore, the rigorous integration of these factors into the experimental design is essential for generating reliable, reproducible, and practically applicable results in modern plant improvement programs.

## MATERIALS AND METHODS

An essential prerequisite for X-ray treatment is the determination of initial seed moisture content, which in our case was assessed using the gravimetric method, according to [7]. Representative seed samples (three replicates of 500 mg each) were analyzed for three genotypes. The optimal moisture content was standardized to 12–13% [8] through controlled humidification. Seeds were hermetically sealed prior to irradiation to maintain consistent moisture levels.

The evaluation of genotype radiosensitivity and the identification of effective doses were carried out following IAEA protocols [9] and methodologies described by Bado et al. [6]. The irradiation response was tested in vivo across 15 tomato genotypes (including varieties, intra- and interspecific recombinant forms,

and spontaneous variants). A total of 10 X-ray doses ranging from 100 to 530 Gy were applied, with six selected doses (100, 150, 200, 250, 300, and 350 Gy) retained for further experiments based on their preliminary depressive effects. Following irradiation, seeds were placed either in Petri dishes (for germination rate assessment) or directly into soil substrate for seedling development. X-ray treatments were performed using a calibrated Rad Source RS-2400 unit.

The dose–response relationship was analyzed using the mean values of measured traits, as well as by the estimation of the $DI_{50}$ (inhibitory dose causing a 50% reduction in the parameter relative to the control). Quantitative indices used to assess the effects of irradiation included germination rate over time, radicle length, hypocotyl length, and total plant height. Measurements were carried out dynamically, starting 72 hours after seed soaking. Germination was assessed *in vivo* using three replicates of 30 seeds per genotype per dose. Seedling measurements were performed 21 days after germination on 10 plants per replicate (3 replicates). Germination was conducted in darkness at approximately 20 °C. Seedlings were grown under controlled laboratory conditions in soil substrate.

Data were analyzed using Excel and Statgraphics Plus software. Linear, nonlinear, and polynomial regression models were applied, along with dose–response curve analysis to estimate effective irradiation doses.

## RESULTS AND DISCUSSION

According to the literature data, the optimal doses of ionizing radiation (X-rays and γ-rays) for tomatoes typically range between 200 and 400 Gy. However, these values must be adjusted based on the specific genotype tested. To determine the optimal impact of X-rays, we initially assessed a wide range of doses (100–530 Gy) on key indicators in five genotypes using a limited sample of seedlings and young plants. Based on preliminary lethality observations, we selected six doses between 100 and 350 Gy, where mortality at the seedling stage did not exceed 30%.

In subsequent experiments, the 15 genotypes included in the study exhibited differential responses depending on both the radiation dose and the evaluated trait. In this study, we applied targeted indicators and statistical methods to evaluate their relevance in determining the radiation impact on tomatoes—particularly in assessing the effects of various doses on quantitative traits through the calculation of $ID_{50}$ values.

One of the first measurable indicators was the seed germination rate over time. Starting from 120 hours post-imbibition, we observed a gradual decrease in germination speed with increasing radiation dose, compared to the control. Suppression reached up to 90% at doses of 300–350 Gy. However, beginning at 178–216 hours, the differences in germination rate between irradiated and control groups diminished significantly, with the initial suppressive effect disappearing

entirely. This suggests that irradiation primarily causes a delay in germination rather than a reduction in final germination rate.

Additional indicators, such as radicle length, hypocotyl length, and total seedling length, also showed significant reductions with increasing irradiation dose, varying by genotype (Table 1, 2). A 30–50% decrease in radicle length compared to the control ($ID_{50}$) was observed across a wide range of doses (150–250 Gy), while a similar decrease in total seedling length (ID $ID_{50}$) ranged between 150–350 Gy, depending strongly on the genotype. Notably, at the dose 350 Gy, although the final germination rate was not significantly reduced, radicle length decreased by 75–90% and seedling length by 47–100% compared to the control. These findings align with international literature, which emphasizes genotype-dependent variation and radiation type [4, 6].

Further analysis focused primarily on plant height as a function of radiation dose across genotypes, although other indicators could also serve as references for assessing radiation impact. According to our observations, plant height is a more informative and reliable trait for establishing standard impact indices such as $ID_{50}$. As radiation dose increased, so did the proportion of individuals that remained at the cotyledon stage (BBCH 10), whereas control and some irradiated individuals progressed to BBCH 11–12. Thus, we used two comparative evaluation models: (1) "Plant" model, height was recorded only for individuals that had developed at least one true leaf (BBCH 11 or higher); otherwise, height was recorded as zero, (2)"Cotyledon" model, height was measured for all individuals, regardless of whether they had only cotyledons or true leaves.

Results indicate that data from individuals with true leaves (BBCH 11–13) are more relevant. Higher radiation doses often inhibit further development in seedlings that form only cotyledons, resulting in relatively tall hypocotyls but no progression to later stages. This is confirmed by diagrams showing that including cotyledon-only individuals in the $ID_{50}$ or $ID_{30}$ estimation artificially inflates the doses required to suppress seedling height by 50% or 30%, sometimes beyond 350 Gy – a threshold that does not reflect biological reality, as severe damage to radicle or seedling development occurs at these levels (Figure 1A, Table 3). Moreover, when calculating mean plant height based solely on individuals that had progressed to at least BBCH 11 (i.e., with true leaves), we can more confidently assume a higher likelihood of survival – though this is not guaranteed. From the dose–response diagrams for mean plant height (Figure 1B), we observe that the $ID_{50}$ doses fall within the 250–300 Gy range for over 50% of the genotypes studied.

In the present study, we aimed to apply a variety of statistical techniques and methods to highlight the relevance of each factor – genotype, radiation dose, evaluation index, assessment matrix, and both statistical and empirical approaches – in determining the impact of radiation on plants. Specifically, our objective was to identify effective mutagenic doses that induce minimal adverse effects on plant

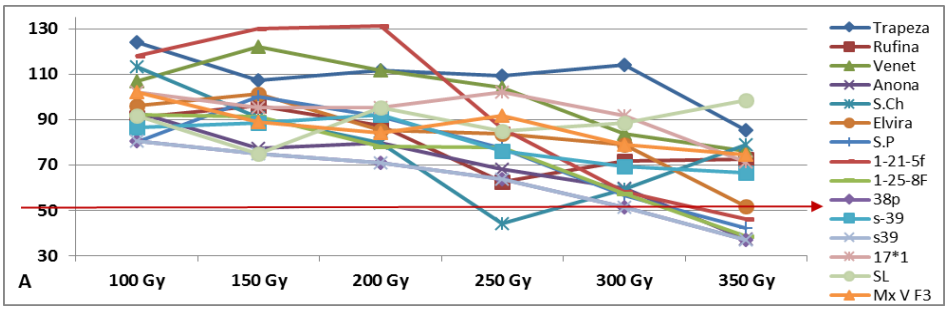
vitality, as well as to determine the $ID_{50}$ (inhibitory dose for 50% effect).

By employing dedicated statistical methods, we were able to assess the radiation impact either at the level of individual genotypes, genotype groups, or specific irradiation doses. When analyzing the mean radiation impact expressed as ID across 15 genotypes, a clear decrease in ID values was observed with increasing doses (Table 1). For instance, a dose of 100 Gy led to a 3.8% reduction in average plant height ($ID_{50}$) compared to the control, while a 300 Gy dose resulted in a 47.1% reduction. Furthermore, analysis using Multiple Range Tests (a component of dispersion analysis) confirmed that different radiation doses produced significantly distinct effects on the suppression index (ID) compared to the control. Statistically significant differences were recorded for each of the doses ranging from 250 to 350 Gy (Table 1). A similar trend is also observed in the average plant height (expressed in cm) across 15 genotypes exposed to six radiation dose levels ranging from 0 to 350 Gy.

Table 1. Multiple range tests for plant height and percent values relative to the control (ID), according to radiation dose (100–350 Gy), summarized across 15 genotypes. Method: 95.0% LSD

| X ray dose, Gy | Genotypes number | LS Mean | Homogeneous Groups | X ray dose, Gy | Total evaluation | LS Mean | Homogeneous Groups |
|---|---|---|---|---|---|---|---|
| ID – trait value, % of control | | | | Plant height, cm, LS Sigma - 0.09 | | | |
| 350 | 15 | 42.9 | C | 350 | 311 | 3.64 | F |
| 300 | 15 | 56.7 | CB | 300 | 311 | 4.72 | E |
| 250 | 15 | 72.0 | B | 250 | 311 | 5.23 | D |
| 200 | 15 | 90.0 | A | 200 | 311 | 6.05 | C |
| 100 | 15 | 96.2 | A | 150 | 311 | 6.50 | B |
| 150 | 15 | 100.1 | A | 100 | 311 | 6.58 | AB |
| | | *LS Sigma 5.9* | | 0 | 311 | 6.75 | A |

It is also worth noting that repeated experiments revealed a hormesis effect in some genotypes, typically at doses between 100 and 150 Gy, with certain exceptions. This phenomenon is illustrated in Figure 1.



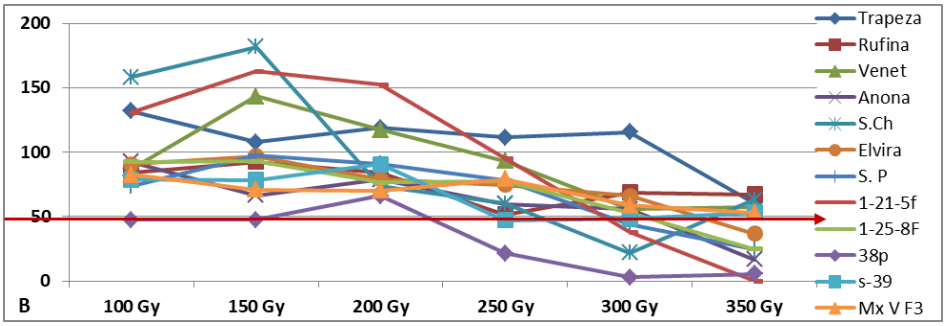



Figure 1. Variation in the trait *Plant height* as predicted by Model 1 (B – 15 genotypes) and Model 2 (A – 12 genotypes), in response to increasing X-ray radiation dose, expressed as a percentage of the control value.

The divergence in response becomes apparent starting at 200 Gy, with the height of plants irradiated at 350 Gy showing a reduction of approximately 46% compared to the control (Table 1). However, the response of plants to ionizing radiation varied significantly depending on the genotype. The application of Multiple Range Tests revealed the classification of seven statistically homogeneous groups with respect to the overall effect of radiation doses (0–350 Gy) on plant height. The differences between group means were consistent and statistically significant (Table 2).

Table 2. Multiple Range Tests for plant height affected by X-Ray exposure, (*LS Sigma* - 0,13)

| *Genotype* | 8 | 5 | 10 | 9 | 12 | 7 | 1 | 2 | 4 | 11 | 3 | 6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Count* | 168 | 168 | 168 | 168 | 168 | 168 | 168 | 168 | 168 | 168 | 168 | 168 |
| *LS Mean, cm* | 2,64 | 2,87 | 3,11 | 3,92 | 4,42 | 4,48 | 4,84 | 4,96 | 5,13 | 5,34 | 5,93 | 6,65 |
| *Groups* | H | GH | G | F | E | E | D | D | CD | C | B | A |

Note: Genotypes code: 1 - Trapeza, 2 - Rufina, 3 - Veneț, 4 - Anona, 5 - *Solanum cheesmaniae* (S.Ch), 6 - Elvira, 7 - *S. pimpinellifolium* (S.P), 8 - 1-215f, 9 - 1-218f, 10 - 38p, 11 - S-39, 12 – F3 Mary Gratefully x Venet

The relationship between variables is effectively illustrated through regression analysis, which allows us to investigate how a dependent variable (e.g., growth suppression) changes in response to one or more independent variables (e.g., radiation dose). Moreover, regression models can support the prediction of trends and the estimation of effect magnitude.

Using linear, nonlinear, or polynomial regression equations generated in

Excel or other data analysis software, it is possible to estimate the dose required to achieve a 50% or 30% suppression of a trait's value ($ID_{50}$, $ID_{30}$), either for individual genotypes or across multiple genotypes within a given experiment. In this study, 12 genotypes were subjected to six irradiation doses ranging from 100 to 350 Gy, based on 90 observations (mean values of trait suppression). The results revealed a strong negative correlation between radiation dose and the evaluated index (R=-0,6) (Table 3).

Table 3. Summary of $ID_{50}$ index for plant height based on different regression types and trait evaluation modes (Mode 1/2)

| Traits | | Type of regression | | | | |
|---|---|---|---|---|---|---|
| | | Linear simple Y=a+b*X | | Polynomial Y=a+b*X-c*X$^2$ | | Nonlinear Y=d / (1+(X/e) ^b) |
| | | Model 1 | Model 2 | Model 1 | Model 2 | Model 1 |
| Plante height, | ID50 | 328 Gy | 422 Gy | 321 Gy | 388 Gy | 316 Gy |
| | | $R^2$=37 | $R^2$ =36 | $R^2$=39 | $R^2$=38 | $R^2$=40 |
| | | R=-0,6 | R=-0,6 | | | |
| Hypocotyl height, | ID50 | 418 Gy | | 392 Gy | | |
| | | $R^2$=47 | | $R^2$=48 | | |
| | | R=-0,7 | | | | |

| Individual genotype evaluation | | | |
|---|---|---|---|
| Genotype | Equation | $R^2$ | ID50, Gy |
| Trapeza | y = -0.2041x + 124.49 | $R^2$ = 0.87 | 364 |
| Venet | y = -0.2238x + 121.19 | $R^2$ = 0.72 | 318 |
| S.P | y = -0.2476x + 117.94 | $R^2$ = 0.93 | 274 |

Returning to earlier discussions on how plant height should be measured, we compared results using two models: Model 1, which included only plants that reached at least BBCH stage 11, and Model 2, which included also seedlings measured at the hypocotyl stage. The $ID_{50}$ values derived from these two models – both under linear and polynomial regression – differed significantly. However, when comparing the modes' predictive accuracy to actual observed responses, Model 1 yielded more consistent results, with estimated $ID_{50}$ values of 321 Gy (linear regression) and 328 Gy (polynomial regression), compared to Model 2 with $ID_{50}$ values of 422 and 388 Gy, respectively. In parallel, a third nonlinear regression mode, commonly applied in mutagenesis and pharmacological studies, was also used. The $ID_{50}$ value derived from this method was likewise 316 Gy, closely aligning with the other estimates. This suggests that applying any of these doses would likely result in a 50% reduction in trait value compared to the control. An alternative and recommended approach would be also to estimate ID values for each

genotype individually, based on irradiation dose. These ID values showed wide variation across genotypes, but these were not always consistent with the biological manifestations, as reflected also in the distribution graphs presented in Figure 1.

A key metric for assessing the quality of model fit in regression analysis is the coefficient of determination ($R^2$). The closer $R^2$ is to 1, the better the model explains the variance in the dependent variable. In our analyses, $R^2$ values ranged between 0.36 and 0.40, indicating that, depending on the model used, approximately 40% of the total variability in the observed data is explained (Table 3). Although the level of correlation observed in our case is moderate, it can be considered acceptable under complex experimental conditions or in the presence of high biological variability. However, when evaluating individual genotypes, the coefficient of determination ($R^2$) shows relatively high values, suggesting a greater accuracy of the resulting equation in capturing the variability of the trait.

## CONCLUSIONS

The study highlights the importance of comprehensive assessment in determining radiosensitivity, taking into account both individual genotype responses and aggregated data across the entire genotype set. The application of regression models – linear, polynomial, and nonlinear and the analysis of dose–response curves facilitates the identification of optimal doses for mutation induction without compromising seedling viability. These findings are essential for the effective design of mutagenesis strategies.

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# RANKS AND RANK STABILITY OF WHEAT CULTIVARS TESTED IN NATIONAL YIELD TRIALS IN ROMANIA

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***Abstract.*** *Cultivars can play a key role in ensuring high wheat grain yields and reducing its variation under the increasing environmental variability. We used data on grain yield and coefficients of variation from sixty yield trials with Romanian winter wheat cultivars, conducted in four regions of the country, for four years (2021-2024) to analyze the ranks and rank variances of the tested cultivars. Despite the large variation of environments, we identified cultivars with better average ranks and with lower rank variances, which could contribute to higher and more stable yields in farms. Results are also useful for establishing priorities in breeding future cultivars.*

***Keywords: ranks, variation, wheat, yield.***

## INTRODUCTION

Most farmers recognize cultivars as one of the most dynamic and economically efficient factors in wheat production, with a decisive influence on yield level and response to environmental conditions. Public breeding programs in Romania have continuously contributed to providing better cultivars to farms. As a result, from 1971 to present, Romanian wheat cultivars have occupied on about 40 to 69 % of the total certified seed produced in Romania [1], achieving a significant genetic progress in yield and other important traits [2]. To promote the best new cultivars, NARDI Fundulea has coordinated, since its foundation, a National uniform yield trial with winter wheat cultivar, at sites all over the country. The present paper analyzes average ranks and rank variance recorded in these trials conducted during 2021-2024.

## MATERIAL AND METHODS

We analyzed yield trials results obtained in Romanian winter wheat cultivars released from the National Agricultural Research & Development Institute Fundulea, and from the Agricultural Research & Development Stations Turda, Lovrin – Oradea, Șimnic and Caracal, along with the historical check Bezostaya 1.

Yield trials with balanced lattice design, on 5 or 10 $m^2$ plots with twenty-five entries and three replications, were conducted countrywide to test winter wheat yielding ability and adaptation to various regions of Romania.

We analyzed yield results from yield trials conducted with crop management recommended for each environment, including the recommended Nitrogen fertilization, which varied from 82 to 143 kg. N/ha, at sites grouped on regions:

**Southern region** including: Agricultural Research & Development Station (ARDS) Valu lui Traian - Lat. 44º16' N, Long. 28º51'E; NARDI Fundulea - Lat 44°30`N, Long 26°51`E; ARDS Teleorman (Drăgănești-Vlașca)– Lat. 44°07'N, Long. 25°45'E; ARDS Mărculești – Lat. 44°25′N, Long. 27°29′E; ARDS Brăila – Lat. 45°16′N, Long. 27°57′E; ARDS Pitești – Lat 44º81'N, Long.24 º86'E; Craiova University - ARDS Caracal – Lat. 44º11'N. Long. 24º37'E; ARDS Șimnic -, 44°20′N 23°49′E.

**Western region** including: ARDS Lovrin - Lat 45°57'N, Long. 20°46'; ARDS Oradea – Lat. 47°02'N Long. - 21°54'E; ARDS Livada Lat. 47°52'N, Long. 23°08'E;

**Central region** including: ARDS Turda – Lat. 46º58' N, Long. 23º78'E; RDSCB Târgu-Mureș – Lat. 46°32'N, Long. 24°33'E.

**Eastern region** including: ARDS Secuieni – Lat. 46ºN, Long. 26º 86'E; ARDS Perieni Lat. 46°18′N, Lat. 27°37′E.

The testing sites covered a large diversity of soils, from chernozems to luvisoils with pH from 5.02 to 7.6, and humus content from 1.71 to 3.6%. Weather conditions during the period 2021-2024 at all sites reflected present climate changes and were diverse, as illustrated by rainfall, which varied from 211.2 to 613.8 mm/year.

We analyzed ranks of the cultivars tested all testing years in a two-way table with K rows (genotypes) and N columns (environments), ranking the K phenotypic values within each column (environment) separately (best value = rank of 1 and worse value = rank of K). We calculated the average rank and the variance of all ranks for each genotype. The average rank is a convenient measure of cultivar competitiveness, while the variance of ranks is a nonparametric measure of stability, a genotype being considered stable over environments if its ranks are similar over environments, i.e., maximum stability = equal ranks over environments{3].

Graphical representations of average ranks and rank variances for each

group of yield trials facilitated identification of genotypes that combine above average performance and stability, placed in the down-right quarter of the graph.

## RESULTS

In thirty-two trials from the **Southern region**, cultivars *FDL Columna*, *Ursita*, *FDL Consecvent* and *FDL Abund* had the best average ranks, while lowest average rank was found in the historical check *Bezostaya 1*. Rank variance varied from 7.56 in *Bezostaya 1*, to more than 27 in cultivar *A4-10*. We can explain the high rank stability of *Bezostaya 1* by the fact that it was inferior to the newer, semidwarf cultivar in most trials, while the large variation observed in A4-10 was due to the fact that the region included ARDS Pitesti, where the line is very adapted, but also sites where lodging affected its yields.

We observed higher rank stability in lowest yielding cultivar *Bezostaya1*, but also in two of the cultivars with highest average ranks (*FDL Columna* and *Ursita*) (Figure 1).

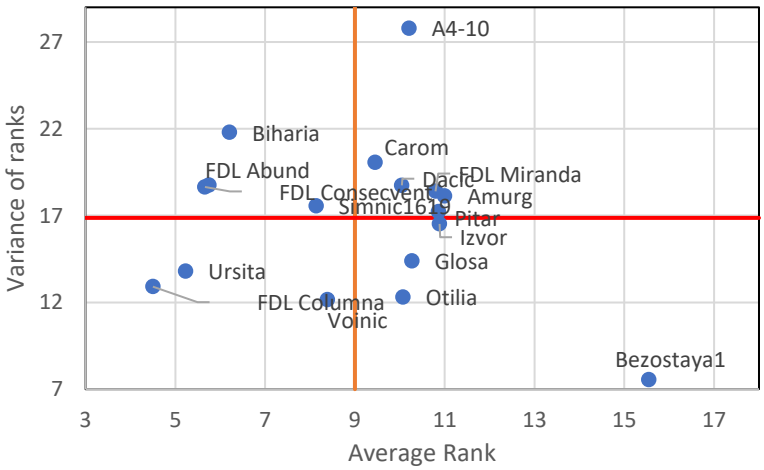


Figure 1. The relationship between cultivar average ranks and their stability in yield trials conducted in the Southern region.

In twelve trials from the **Western region** cultivars *FDL Columna*, *FDL Consecvent* and *Biharia* had best average ranks while *Bezostaya 1* had the lowest average rank. Highest variation in ranks was observed in cultivars *Otilia* and *Dacic*, while the lowest variation was observed again in *Bezostaya1*, but also in cultivars *Biharia* and *FDL Columna*. It is worth mentioning that cultivars *Biharia*, *FDL Columna* and *FDL Consecvent* proved to have at the same time the best average ranks and best rank stability caused by environmental conditions in this region (Figure 2).

Rank stability of cultivar *Biharia* was much better in the Western region than

in the Southern region. This shows better adaptation of this cultivar in West Romania and suggests that *Biharia* might be more affected by harsher weather conditions, more frequent in the Southern region.

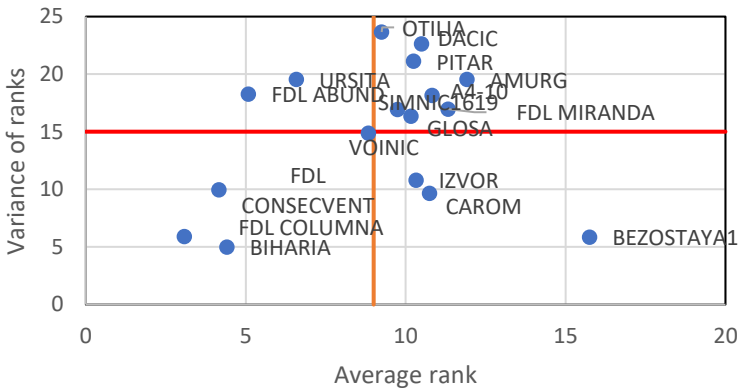


Figure 2. The relationship between cultivar average ranks and their stability in yield trials conducted in the Western region.

In the eight trials conducted in the **Central region** (Transylvania), where yields were highest of all regions, we found the best average ranks in cultivars *T75-16, FDL Consecvent, T42-17* and *Luminiţa.* Cultivars *FDL Consecvent, T42-17* and *Luminiţa*, along with *FDL Abund* and *T109-12* showed better than average performance both for average ranks and variances (Figure 3).

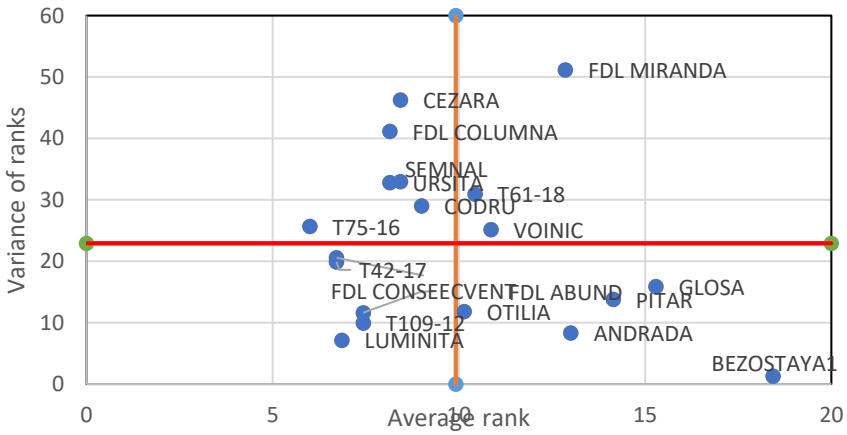


Figure 3. The relationship between cultivar average ranks and their stability in yield trials conducted in the Central region.

Cultivars *Bezostaya 1, Andrada, Glosa* and *Pitar*, showed lower rank

variances, but lower yields in this region, related to their lower yielding potential or to their earliness, which did not allow full use of the available weather resources.

Data from eight trials conducted in the **Eastern region**, showed the best average ranking in cultivars *FDL Columna* and *FDL Consecvent*, followed at considerable distance by *Voinic* and *FDL Abund*. The old non semidwarf cultivar *Bezostaya 1* had the worse ranking, as in all other regions. Cultivars *FDL Columna, Bezostaya 1* and *Andrada* had the smallest variance of ranks.

Cultivars *FDL Columna, FDL Consecvent, FDL Abund* and *Ursita* combined average ranks and variance of ranks better than the average of all studied cultivars (Figure 4).

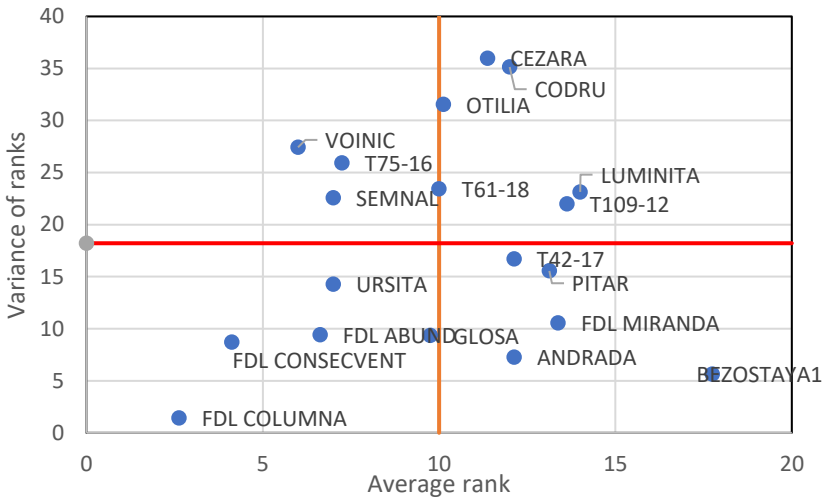


Figure 4. The relationship between cultivar average ranks and their stability in yield trials conducted in the Eastern region.

## DISCUSSION

As underlined by Huehn [3] "Nonparametric measures for phenotypic stability based on ranks provide a useful alternative to parametric measures currently used, which are based on absolute data". Among the advantages of nonparametric stability statistics, he mentioned the fact that "no assumptions are needed about the distribution of the phenotypic values" and "for many applications, for example selections in breeding and testing programs, the rank orders of the genotypes are the most essential information."

Using this approach, we analyzed the results of sixty yield trials with winter wheat cultivars, conducted during four years at fifteen sites from four regions of

Romania. Both average ranks and rank variances varied much among cultivars, allowing identification of cultivars with better relative performance and with better rank stability. We did not find any correlation between ranks and their stability, the lowest rank variances being found in the historical check *Bezostaya 1,* but also in cultivars with highest performance.

The most recently released cultivars, and especially *FDL Columna*, showed the best average ranks for yield in most regions, combined with good rank stability, superior to older cultivars.

## CONCLUSIONS

Testing wheat cultivars in sixty yield trials conducted for four years in diverse conditions allowed identification of cultivars able to provide higher yields, with smaller variation induced by environmental conditions, Data included in this paper can facilitate better choice of cultivars for each of the analyzed regions and this could contribute to higher and more stable wheat production in farms. Presented results can also be useful for establishing priorities in breeding future cultivars.

**Acknowledgements:** We are grateful to the collaborators for the trials carried out within the national research network.

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# COMPARATIVE ANALYSIS OF RESISTANCE TO HIGH TEMPERATURE STRESS IN PROSPECTIVE TOMATO LINES

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***Abstract.*** *The paper presents the results of the evaluation of tomato lines obtained as a result of intraspecific hybridization based on resistance to high temperatures (40°, 42°C) in early ontogenesis. Seedlings grown at 25°C served as a control variant. The analysis of the variability of the resistance trait was carried out based on the length of the embryonic radicle, stem, and whole plantlet length. It was found that at a temperature of 40°C all lines showed increased resistance to growth traits, and at a temperature of 42°C a significant inhibition of the radicle, stem, and whole plantlet length was observed. Through cluster analysis (k-means) the variety Prestij and lines 203, 204, 205 were identified with complex resistance of growth characters to high temperatures (40 and 43°C), which can be implemented in breeding programs to create varieties with complex resistance to thermal stress.*

***Keywords: tomato, resistance, temperature, variability.***

## INTRODUCTION

Temperature is one of the main environmental factors that influences the entire range of vital processes of vegetable plants: seed germination, plant growth, flowering, fruiting, as well as assimilation, respiration, transpiration and other physiological processes that normally occur only in the presence of an optimal temperature. Climate change represents one of the main challenges of humanity which will thus require the implementation of new strategies for adapting newly created varieties in a timely manner to local conditions in order to reduce the effect of stress temperatures [1, 2].

High temperatures have a negative impact on several physiological processes in the plant (disruption of the functioning of the photosynthetic apparatus

components, a number of enzymes in mitochondria and other cellular components [3, 4, 5], which leads to a decrease in plant productivity [6, 7].

In many species, including tomatoes, productivity decreases due to a decrease in fruit set during high-temperature seasons. Previous research has shown that resistant tomato genotypes have the ability to form a much larger number of fruits than susceptible ones under stress conditions [8,9].

The purpose of our research was to evaluate the influence of high temperatures on the growth and development traits of promising tomato lines in the selection field and to identify those with potential for use in breeding programs.

## MATERIALS AND METHODS

The Prestij variety and 8 lines selected from the intraspecific combinations $F_4$ Mary Gratefully x Florina (L 202), $F_4$ Deşteptarea x Florina (L 203, L 204, L 205), F**4** Prestij x Pontina (L 206), $F_6$ Zolotaia oseni x L 310 (L 207), $F_5$ Santa Maria x Rozovîi velican (L 208), $F_4$ Deşteptarea x Pontina (L 209) served as the research material.

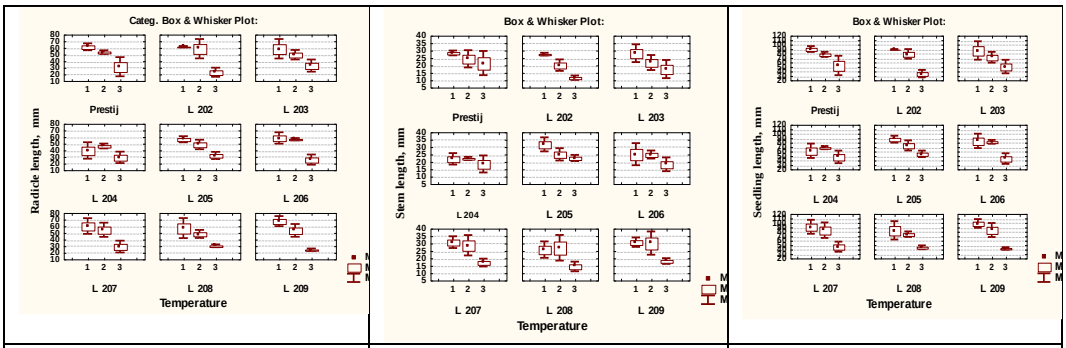
The genotypes were tested at 3 temperature levels: optimum – 25°C and heat stress: 40 and 42°C. The assessment of the resistance of tomato genotypes to high temperatures was carried out according to the growth capacity of the embryonic radicle, stem and intact seedling for 7 days after maintaining them on day 4 at temperatures of 40° and 42°C for 6 hours [10]. Seedlings kept constantly at 25°C served as a control. The resistance of the plants (%) was calculated according to the ratio of the values of the investigated characters from the stress variant to the control variant, and the tomato samples were differentiated by resistance groups: 81-100% – highly resistant, 61-80% – resistant, 41-60% – medium resistant, weakly resistant 21-40% and 0-20% – non-resistant.

The cluster analysis of the degree of similarity / difference of tomato genotypes based on growth and development characters at different temperatures was carried out according to the centroid method of *k*-means, successfully used in the genetics and improvement research [11]. The obtained data were statistically processed in the STATISTICA 7 software package.

## RESULTS AND DISCUSSION

The evaluation of the perspective lines based on the reaction of tomato plants to high temperatures demonstrated that under the action of temperature stress, in most of the genotypes studied there was a strong inhibition of growth characters. As can be seen from the data presented (Fig. 1) the influence of stressful temperatures on the growth of the embryonic radicle, stem and whole plantlet in the tomato genotypes included in the study is different. The length of the radicle in the

lines studied under optimal conditions was 39,2...67,3 mm, at 40°C – 46,3...58,3 mm, and 42°C – 22,7...32,8 mm, respectively (Fig. 1A). The decrease in the character in relation to the control varied within the limits of 3,3...21,0% at 40°C, and the resistance was 84,5 – 117,3%. Stimulation by 17,3% at $40^0$C was recorded in line L 204 selected from the $F_4$ combination Deşteptarea x Florina. The data obtained show that all lines analyzed are characterized by increased resistance, even at $40^0$C.




| A | B | C |
|---|---|---|

Fig. 1. Influence of temperature on the length of the radicle (A), stem (B) and seedling (C) of tomato

Horizontally: 1 – Martor (25˚C), 2 – 40˚C, 3 – 42˚C.

Under the action of the $42^0$C temperature, the growth of the embryonic radicle was repressed in all the analyzed forms, its values representing -49,1%, -62,6%, -43,6%, -26,3%, -43,6%, -56,4%, -52,2%, -47,1%, -64,3% in relation to the control, and their resistance was 50,9%, 37,1%, 56,4%, 73,7%, 56,4%, 43,6%, 47,3%, 52,9%, 35,7%, respectively, for the Prestij variety and the L 202-L 209 lines. So, with the exception of the L 204 line which showed a lower depression reaction, in the other genotypes a repression of more than 43,0% of the radicle length occurred, which denotes stronger sensitivity to temperature $42^0$C. Complex resistance was manifested by the L 204.

A wide amplitude of stem length variability was also recorded in response to stress temperatures. Stem inhibition relative to the control at 40°C ranged from 2.0 to 27,3%, and at $42^0$C, from 16,1 to 57,9% (Fig. 1B). The most strongly influenced by 420C were the lines L 202, L 207, L 208 and L 209. Stimulation was observed in two cases at $40^0$C, L 204 (1,4%) and L 208 (5,1%). It is worth mentioning that the stem was less affected by thermal stress, and if in the case of the radicle, complex resistance was manifested by only one line, then in the case of the stem, complex resistance was manifested by the variety Prestij and the lines L 204, L 205 and L 206, in which the resistance at a temperature of 40°C was 85,8,

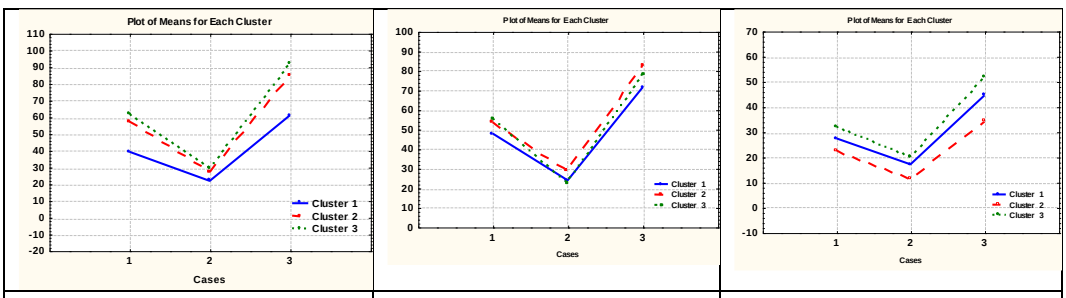
101,4, 78,5, 99,2%, and at 42°C – 75,5, 83,9, 70,9, 72,7%, respectively.

The reduction in seedling length compared to the control ranged from 2,5 ... 17,7% at $40^0$C and 22,6 ... 61,6% - $42^0$C. At the same time, the temperature of $42^0$C most strongly influenced the lines L 202 (61,6%), L 209 (57,2%). (Fig.1C). Complex resistance to stress temperatures based on this character was manifested by the lines L 204 and L 205, showing interest in breeding.

By the cluster analysis which recorded that the intercluster variance was much higher than the intracluster variance in the case of radicle, stem and seedling length both under optimal and heat stress conditions, we can conclude that the tomato genotypes were successfully classified into clusters based on growth characters (Tab. 1, Fig. 2).

Table 1. Analysis of variance of growth traits in the interaction of tomato genotypes with different temperature levels

| Variant | Intercluster variance | df | Intracluster variance | df | F | p |
|---|---|---|---|---|---|---|
| **25°C** | | | | | | |
| Length of the radicle | 430,0947 | 2 | 36,68750 | 6 | 35,16959 | 0,000486 |
| Length of the stem | 43,5806 | 2 | 35,95500 | 6 | 3,63626 | 0,092383 |
| The seedling length | 750,7125 | 2 | 69,22748 | 6 | 32,53242 | 0,000602 |
| **40°C** | | | | | | |
| Length of the radicle | 120,4983 | 2 | 20,10166 | 6 | 17,98334 | 0,002922 |
| Length of the stem | 54,3658 | 2 | 32,79417 | 6 | 4,97337 | 0,053265 |
| The seedling length | 202,3058 | 2 | 27,43417 | 6 | 22,12269 | 0,001703 |
| **42°C** | | | | | | |
| Length of the radicle | 73,3409 | 2 | 28,01467 | 6 | 7,85384 | 0,021116 |
| Length of the stem | 60,8556 | 2 | 25,36666 | 6 | 7,19711 | 0,025464 |
| The seedling length | 267,8275 | 2 | 24,58801 | 6 | 32,67782 | 0,000595 |




A B C

Figure 2. Differentiation of genotype clusters under optimal conditions (A) – 25°C and heat stress B (40°C), C (42°C)

*Growth characters: 1 – radicle length, 2 – stem length, 3 – seedling length*

By classifying the genotypes based on the evaluated characters, it was found that in the variant with the temperature regime of 40°C, the Prestij variety and the lines L 202, L 206, and at the temperature of 42°C the Prestij variety and the lines L 203 and L 205, formed clusters with the highest values of growth characters, which denotes that they are promising in creating new varieties resistant to high temperatures.

Table 2. Descriptive cluster analysis

| Cluster | Caracter | x | Genotype | x | Genotype | x | Genotype |
|---|---|---|---|---|---|---|---|
| | | 25°C | | 40°C | | 42°C | |
| 1 | Length of the radicle, mm | 39,20 | L 204 | 47,85 | L 203, L 204, L 205, L 208 | 27,42 | L 204, L 206, L 207, L 208, L 209 |
| | Length of the stem, mm | 21,80 | | 23,87 | | 17,10 | |
| | Length of the seedlings, mm | 61,00 | | 71,47 | | 44,52 | |
| 2 | Length of the radicle, mm | 57,30 | L 203, L 205, L 206, L 208 | 53,65 | L 207, L 209 | 22,70 | L 202 |
| | Length of the stem, mm | 27,52 | | 29,30 | | 11,50 | |
| | Length of the seedlings, mm | 84,62 | | 83,00 | | 34,10 | |
| 3 | Length of the radicle, mm | 62,37 | Prestij, L 202, L 207, L 209 | 55,93 | Prestij, L 202, L 206 | 31,87 | Prestij, L 203, L 205 |
| | Length of the stem, mm | 29,17 | | 22,93 | | 20,337 | |
| | Length of the seedlings, mm | 91,60 | | 78,87 | | 52,207 | |

## CONCLUSIONS

It was found that the temperature of 42ºC compared to the temperature of 40ºC significantly influences the early ontogenesis of tomato genotypes by strongly repressing the growth of the radicle, stem and seedling.

Cluster analysis using the k-means method showed that both the optimal and stressful temperature exhibited a high discriminant capacity of tomato clusters for all characters, which reveals that the tomato genotypes were successfully classified into clusters based on growth characters.

The variety Prestij and lines 203, 204, 205 with complex resistance of growth characters to high temperatures (40 and 43ºC) were identified, which can be successfully implemented in breeding programs for the creation of varieties with complex resistance to thermal stress.

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# VARIABILITY OF THE PHYSICOCHEMICAL PROPERTIES OF SELECTED MAIZE GENOTYPES AND PROSPECTS OF THEIR APPLICATION FOR VARIOUS PURPOSES

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***Abstract.*** *This study aimed to analyze the grain's physical properties and chemical composition from fifteen maize hybrids produced and cultivated at the Maize Research Institute Zemun Polje, Serbia. The investigated genotypes were selected based on their diverse genetic backgrounds and different FAO maturity groups (100-700). The results showed notable differences in the physical and chemical characteristics of the investigated maize genotypes, indicating they could be beneficial for various uses in food and animal feed production, as well as in different processing industries. The 1000 kernel weight, a desirable attribute for wet milling as it typically results in increased starch and protein yields, varied between 255.70 g and 411.10 g, while starch content ranged from 65.45 to 70.18 % dry matter (d.m.) basis. Protein content (10.31-12.12%) further qualifies studied grains as valuable sources of nutrients. These findings suggest that selecting appropriate hybrids based on their genetic traits can optimize yield and quality for specific applications. Further research is needed to explore the potential of these hybrids in enhancing food security and sustainability within agricultural practices.*

***Keywords: chemical composition, genetic variability, maize genotypes, physical properties, potential application.***

## INTRODUCTION

Maize (*Zea mays* L.) is regarded as one of the most important cereal crops utilized as a raw material for food, feed, energy, and industrial applications. Approximately 1.22 billion metric tons of maize were produced globally during the 2024–2025 timeframe, according to data from USDA (2025) [1]. Despite maize being a crucial food crop, its demand as livestock feed has markedly risen over the past twenty years. This was mostly attributable to the swift economic growth in the densely populated regions of Asia, the Middle East, and South America, which heightened the demand for food products from chicken and cattle among affluent consumers.

The chemical makeup of maize grains largely determines their usable value; however, the intrinsic value of maize depends on its application; each new use increases its worth [2]. The extensive variety of maize grain characteristics enables modifications in grain composition concerning the quantity and quality of specific components. This is accomplished through the breeding process [3]. Maize grain comprises roughly 70% starch on average [2]. Additionally, certain experts assert that maize serves as an important framework for investigating genetics, evolution, and domestication. The Organisation for Economic Co-operation and Development (OECD), in conjunction with the Food and Agriculture Organization of the United Nations (FAO), states that maize will persist in its expansion and diversification as a research model, industrial resource, and crop for feed and fuel in the foreseeable future (OECD/FAO, 2013). Serbia is a significant global producer of maize, and the surplus of this cereal grain, not utilized for food or animal feed, should be judiciously channelled towards the production of sustainable and eco-friendly products such as biodegradable packaging, bio plastics, biofuels, etc.

## MATERIALS AND METHODS

Fifteen maize hybrids (FAO 100-700) were cultivated in a two-replicate trial following the randomized complete-block design at the experimental field of the Maize Research Institute, Zemun Polje, Serbia (44°52'N, 20°19'E, 81 m asl). Maize ears from each replication were collected at full physiological maturity from two inner rows. The experiment was conducted under rain-fed conditions in 2023. Ten representative maize ears per replication were chosen for subsequent laboratory tests. The methodologies employed to ascertain kernel physical properties, including 1000-kernel weight (TKW), test weight (TW), flotation index (FI), milling response (MR), percentage of hard endosperm (HE) in kernel weight, percentage of soft endosperm (SE) in kernel weight, and water absorption index (WAI), were thoroughly elucidated in a previously published paper (Radosavljević et al., 2000). The water absorption index was determined by quantifying the volume of water absorbed by a specific quantity of maize kernels under rigorously controlled conditions. The milling response (MR) assessed by the Stenvert-Pomeranz test (Pomeranz et al., 1985) quantifies kernel hardness, indicating the duration (s) required to grind 20 g of maize kernels in a micro hammer mill operating at 3600 rpm until the material passes through a 2-mm sieve and accumulates to a volume of 17 ml in a glass cylinder (125×25 mm). The cornmeal samples collected post-grinding of maize kernels during the experiment are combined and filtered using a 0.5 mm diameter nylon mesh. The portion that passes through the sieve is referred to as the soft endosperm, while the portion that remains is termed the hard endosperm. The fractions were subsequently measured, and the hard and soft endosperm portions were quantified. The whole grain maize flour was produced by a dry grind milling method (Perten Instruments, Hägersten, Sweden) for fine sample preparation (mesh 0.5 mm). The moisture content of the kernels

was assessed by drying the ground samples at 105˚C until a consistent weight was achieved. The starch concentration was assessed using the Ewers polarimetric method (ISO 10520, 1997). The total protein content was ascertained using the Kjeldahl method, calculated as total nitrogen multiplied by 6.25 (AOAC, 1990). The oil content was assessed using the Soxhlet standard method (AOAC, 2000). The crude fiber content was assessed using the Weende method modified for FibretecTM Systems, Foss, Denmark (Agricultural food products, 1993). The ash concentration was ascertained through the gradual burning of the sample at 650°C (AOAC, 1990). The results are shown as percentages of dry matter (d.m.). All analyses were conducted in duplicate.

## RESULTS AND DISCUSSION

The results obtained after physical and chemical characterisation of the selected maize genotypes' grain are shown in Tables 1 and 2.

Table 1. Physical properties of maize hybrid grain

| Hybrid | TKW | TW | FI | WAI | MR | HF | SF |
|---|---|---|---|---|---|---|---|
| ZP 161 | 255.70 | 827.06 | 17.45 | 0.208 | 13.80 | 62.82 | 37.18 |
| ZP 200 | 285.36 | 791.91 | 82.13 | 0.247 | 12.07 | 57.30 | 42.70 |
| ZP 341 | 374.83 | 822.65 | 32.45 | 0.254 | 11.67 | 58.56 | 41.44 |
| ZP 4242 | 350.58 | 791.63 | 75.86 | 0.242 | 12.70 | 35.15 | 44.85 |
| ZP 5090 | 339.20 | 778.16 | 75.70 | 0.271 | 11.17 | 57.02 | 42.98 |
| ZP 555 | 368.71 | 827.81 | 26.53 | 0.253 | 13.20 | 63.28 | 36.72 |
| ZP 5550 | 369.92 | 819.43 | 31.43 | 0.224 | 12.47 | 58.68 | 41.32 |
| ZP 5011 | 377.10 | 821.68 | 25.24 | 0.219 | 14.43 | 62.24 | 37.76 |
| ZP 606 | 381.88 | 810.32 | 60.87 | 0.204 | 12.50 | 57.79 | 42.21 |
| ZP 6364 | 410.82 | 830.51 | 25.68 | 0.212 | 12.30 | 61.34 | 38.66 |
| ZP 6715 | 353.68 | 835.90 | 21.92 | 0.224 | 12.37 | 60.81 | 39.19 |
| ZP 707 | 411.10 | 824.86 | 29.61 | 0.196 | 13.20 | 60.72 | 39.28 |
| ZP 7357 | 409.47 | 828.43 | 15.35 | 0.192 | 13.37 | 62.41 | 37.59 |
| ZP 7578 | 379.72 | 819.82 | 61.31 | 0.212 | 11.50 | 55.36 | 44.64 |
| ZP 7900 | 407.64 | 787.11 | 89.74 | 0.231 | 11.07 | 56.17 | 43.83 |
| Min | 255.70 | 778.16 | 15.35 | 0.19 | 11.07 | 35.15 | 36.72 |
| Max | 411.10 | 835.90 | 89.74 | 0.27 | 14.43 | 63.28 | 44.85 |
| Average | 365.05 | 814.49 | 44.75 | 0.23 | 12.52 | 57.98 | 40.69 |
| SD | 44.80 | 18.21 | 26.25 | 0.02 | 0.96 | 6.81 | 2.80 |

**TKW – 1000 kernel weight (g); TW – test weight (kg·m$^{-3}$);FI – flotation index; WAI — water absorption index; MR – milling response (s); HE – percentage of hard endosperm portion in kernel weight; SE – percentage of soft endosperm portion in kernel weight.*

The maize genotype ZP 161 exhibited a TKW of 255.70 g, indicating a relatively high value of this parameter, which is often associated with higher grain quality and yield potential. The TW of 827.06 kg/m³ suggests good density and indicates that the grain is likely to have favorable storage and handling

characteristics. The Flotation Index (FI) of 17.45 indicates a moderate level of grain integrity, with higher values typically correlating to better quality. The WAI of 0.208 suggests a reasonable capacity for water uptake, which can influence cooking and processing characteristics. The MR of 13.80 s indicates a relatively quick milling process, which is advantageous for efficiency in grain processing. The HF of 62.82% and SF of 37.18% reveal a predominance of hard over soft endosperm, which is favourable for wholegrain flour production.

Table 2. Chemical composition of maize hybrid grain

| Hybrid | Starch (%) | Protein (%) | Oil (%) | Crude fiber (%) | Ash (%) |
|---|---|---|---|---|---|
| ZP 161 | 66.92 | 11.30 | 4.15 | 2.46 | 1.55 |
| ZP 200 | 65.45 | 11.45 | 4.12 | 2.41 | 1.53 |
| ZP 341 | 66.96 | 10.38 | 4.54 | 2.70 | 1.55 |
| ZP 4242 | 67.87 | 10.31 | 3.67 | 2.10 | 1.49 |
| ZP 5090 | 66.53 | 11.87 | 4.33 | 2.63 | 1.55 |
| ZP 555 | 67.72 | 11.45 | 4.36 | 2.77 | 1.47 |
| ZP 5550 | 67.89 | 10.91 | 4.67 | 2.49 | 1.36 |
| ZP 5011 | 67.43 | 12.12 | 3.82 | 2.46 | 1.54 |
| ZP 606 | 69.17 | 10.54 | 2.70 | 2.24 | 1.34 |
| ZP 6364 | 68.80 | 11.12 | 3.11 | 2.03 | 1.49 |
| ZP 6715 | 67.10 | 11.46 | 4.29 | 2.61 | 1.41 |
| ZP 707 | 67.80 | 11.62 | 4.15 | 2.37 | 1.51 |
| ZP 7357 | 70.18 | 11.18 | 4.01 | 2.35 | 1.50 |
| ZP 7578 | 66.80 | 10.63 | 4.30 | 2.30 | 1.43 |
| ZP 7900 | 67.79 | 11.00 | 4.25 | 2.05 | 1.46 |
| Min | 65.45 | 10.31 | 2.70 | 2.03 | 1.34 |
| Max | 70.18 | 12.12 | 4.67 | 2.77 | 1.55 |
| Average | 67.63 | 11.16 | 4.03 | 2.40 | 1.48 |
| SD | 1.15 | 0.53 | 0.53 | 0.23 | 0.07 |

Starch (%) had the highest mean value (67.65%) among all genotypes, with a moderate standard deviation (0.60), indicating some variability across hybrids. The starch content ranged from 65.45% to 70.18%. Protein (%) averages 11.16% with a standard deviation of 0.58, suggesting relatively consistent protein levels among the hybrids (range: 10.31% to 12.12%). Oil (%) had a mean of 3.99% and a standard deviation of 0.58, with values ranging from 2.70% to 4.67%. Crude fiber (%) shows a mean of 2.40% and a low standard deviation (0.24), indicating little variation (range: 2.03% to 2.77%). - Ash (%) has the lowest mean (1.48%) and the lowest variability (std dev 0.07), with values between 1.34% and 1.55%. Overall, starch was the predominant component, while ash and crude fiber were present in

smaller and more consistent amounts. The relatively low standard deviations for most components suggest that the chemical composition is fairly stable across the maize hybrids in this investigation. The results are in accordance with previously published studies conducted in Maize Research Institute, Zemun Polje [2, 3, 9, 10].

## CONCLUSIONS

The grain of the investigated maize genotypes showed significant differences regarding physical and chemical properties, offering a diverse spectrum of applications in the production of food, animal feed and various industries.

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# SOURCES OF ECONOMICALLY VALUABLE TRAITS FOR *BRASSICA RAPA* L. VEGETABLES CROPS BREEDING

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***Abstract.*** *Vegetable crops of Brassica rapa L. compose an integral part of the world economy, providing fresh vitamin products for humanity. They are actively cultivated in East Asian countries for various purposes: food, medical, cosmetic and decorative. In Russia, most of them belong to the local non-traditional crops, but the interest of the population to these crops is increasing due to their valuable biochemical composition, high yield and simplicity of cultivation.*

*The aim of the study: to identify the source material in terms of economically valuable traits in the VIR Brassica rapa L. crops world-wide collection for use in the breeding process.*

*The material of the study included 23 accessions of the species belonging to different botanical and ecological-geographical groups: Chinese cabbage - 7 accessions, pak-choi - 2, tacai - 1, mizuna - 1, leaf turnip - 1 and root turnip - 11. The field study was carried out on the basis of NPB «Pushkin and Pavlovsk laboratories of VIR» (St. Petersburg, North-West region of the Russian Federation) in 2024 with the use of VIR Methodic guidelines for the study and maintenance of the world cabbage collection (1988), Methodic of the State cultivar testing of agricultural crops, assessment of the degree of Alternaria ssp. - according to the Methodic of Ph.B. Gannibal (2011), biochemical analysis - according to the Methodics of the VIR Department of Biochemistry (Ermakov et al., 1987).*

*As a result of the study, valuable accessions with a complex of economic, phytopathologic and biochemical traits were identified, representing promising source material for breeding programs aimed at expanding the range of vegetable crops.*

***Keywords: Alternaria spp., Brassica rapa L., breeding value, genetic diversity, sources of resistance for breeding.***

## INTRODUCTION

*Brassica rapa* L. is a representative of the widely distributed and multifarious Brassicaceae Burnett family. For thousands of years, this species has accompanied the development of agriculture, shaped the culinary traditions of entire nations, and continued to evolve alongside humanity's needs. The leafy,

rooted, and oilseed crops of *Brassica rapa* are actively used in various spheres including food processing, cosmetic industries, and medicine due to their valuable biochemical composition. The content of vitamin C in the leaves reaches 40-80 mg/100g of fresh weight, β-carotene - 2-10 mg/100g, chlorophylls - 50-200 mg/100g, a large amount of organic acids and essential amino acids (valine, isoleucine, tryptophan), phenolic compounds, fiber, red-colored forms contain anthocyanins, that exhibit antioxidant and anti-inflammatory effects, stimulate the immune system, and prevent the development of cardiovascular diseases. The roots contain 21-35 mg/100 g of vitamin C, 0,09-0,12 mg/100 g of vitamin B6, 0,9-1,1 g of protein, 0,1-0,2 g of fats, and 10-30 mg of glucosinolates. The combination of low fat content with high water and protein levels results in low caloric value, making these plants particularly valuable for dietary nutrition. Furthermore, they serve as an excellent source of essential mineral elements including potassium, calcium, sulfur, phosphorus, zinc, iron, and manganese [1,2,3,4].

The population of Southeast Asian countries has been actively cultivating and using vegetable plants of this species in cooking since ancient times. In Russia, B. rapa vegetables are cultivated on a more limited scale and primarily used as salad greens. The most common is Chinese cabbage, which is grown in open and protected ground. The rest of the crops are grown in the field of private vegetable growing. In the modern conditions interest in these crops continues to grow annually due to their early maturity, ease of cultivation, and high nutritional value [5]. There is a need to expand the range of cultivated vegetable crops and create cultivars and hybrids with a complex of valuable characteristics.

Black spot is one of the limiting factors in Russia and around the world that inhibits the growth and development of plants of the species and leads to the loss of marketable qualities of fresh vitamin products. The disease is highly harmful. Under favorable conditions yield losses reach 50-80%. The pathogens *Alternaria brassicae* (Berk.) Sacc. and *A. brassicicola* (Schwein) Wiltshire produce mycotoxins that contaminate agricultural products. Monitoring of the pathogen becomes an important process, as part of the observed range expansion and increased aggressiveness. In the conditions of the North-Western region of Russia, black spot is a serious problem and requires the search for sources of resistance among natural varietal populations [6,7,8,9,10].

The species *Brassica rapa* L. serves as a valuable genetic resource for breeding programs for early maturity, productivity, disease resistance, and a high content of biologically active compounds.

The VIR Brassicaceae Burnett collection is unique, contains 10,997 accessions originating from 98 countries and including representatives of 11 genera and 32 species. The *Brassica rapa* L. vegetable crop collection contains 1,700 accessions of leafy and rooted vegetable crops.

## MATERIALS AND METHODS

The study was conducted in 2024 at the experimental site of NPB «Pushkin and Pavlovsk laboratories of VIR» (St. Petersburg, North-West region of Russian Federation). The research material included 23 accessions of various *Brassica rapa* L. subspecies: 7 accessions of Chinese cabbage, 2 of pak-choi, 1 of tacai, 1 of mizuna, 1 of leaf turnip, and 11 of root turnip. Field sowing was performed in early July using a ridge planting system with 70×30 cm spacing, in three replications with 15 plants per replication. The experiment and description of plants were carried out according to the VIR Methodic guidelines for the study and maintenance of the world wide brassicas collection (1988) [11], Methodic of the State cultivar testing of agricultural crops [12] and the «Methodology of field experience in vegetable growing» (2011) [13]. The degree of development of *Alternaria* ssp. was assessed according to the method of Gannibal Ph.B. (2011) [14] according to a 4-point system and to 3 tiers of the leaf rosette of plants. Biochemical analysis was conducted at the VIR Biochemistry Department laboratory - according to the Methodics of Ermakov et al. (1987). The preparation protocol included analysis of fresh material derived from three individual plants representing each accession. Statistical data processing was performed using STATISTICA 12 software (StatSoft, USA).

## RESULTS AND DISCUSSIONS

During the study of various cultivars and hybrids representing different crops of *Brassica rapa* L., Table 1 was formed, containing the main agrobiological characteristics. Vegetation Period is one of the key features in the breeding of vegetable crops, it determines the yield potential and adaptation to stress factors. The accessions were classified into the following maturity groups: early-maturing (33-40 days), mid-maturing (45-60 days), late-maturing (70-75 days). Accessions of pak-choi demonstrated particularly early maturity, making them suitable for cultivation in risk farming zones with limited growing seasons. The earliest-maturing accession was pak-choi k-647 from Tajikistan (33-35 days), representing valuable genetic material for breeding ultra-early forms. Tacai, mizuna and leaf turnip showed intermediate growing periods (40-48 days). Chinese cabbage and root turnip exhibited the longest vegetation period (50-75 days).

The variability of morphological features (CV: 12,86-27,37%) reflects the collection's wide phenotypic diversity. According to the parameters of the leaf rosette, the leaders are root turnip (diameter 34,0-46,4 cm; height 25,0-40,3 cm) and Chinese cabbage (diameter 35,75-41,7 cm; height 26,7-39,24 cm). Tacai turned out to be the most compact (diameter 25,1 cm; height 19,1 cm). Chinese cabbage had the highest average leaf size (length 24,1-37,85 cm; width 17,2-24,7 cm). The

leaves of the accession of mizuna Shirojuki Sensuji Kyo Mizuna (k-434, Japan) are strongly dissected, forming a dense spherical rosette. The accession can be used for decorative purposes. Chinese cabbage demonstrated the highest average product organ mass at 1,33 kg. The biggest head weight was shown by the Japanese accessions: Raioh 90 F1 (vr.k-1558) - 1,584 kg, Udzi M80 (k-399) - 1,551 kg, Udzi M70 (k-398) - 1387,3 kg. Pak-choi followed with an average mass of 0,85 kg. The Russian accession Lastochka (k-623) demonstrated leaf rosette mass with 1,004 kg.

Table 1. Phenological, morphological, and phytopathological features of the studied *Brassica rapa* L. accessions

| VIR Catalog No. | Name and Origin | Vegetation Period, days | Rosette Diameter, cm | Rosette Height, cm | Leaf Blade Length, cm | Leaf Blade Width, cm | Product Organ Mass, kg | Yield, kg/m² | Degree of Alternaria Infection, avg. score | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | by tiers | average |
| ssp. *pekinensis* (Lour.) Hanelt | | | | | | | | | | |
| 36 | Khibinskay, Russia | 42-45 | 40,3±1,0 | 35,71±0,8 | 32,8±1,85 | 17,47±0,8 | 0,971±0,01 | 4,62±1,13 | 3<br>2,13<br>1,2 | 2,11±0,2 |
| 1556 | Kapella, Russia | 48-50 | 35,75±0,71 | 39,24±0,59 | 37,85±0,8 | 18,7±0,9 | 1,298±0,04 | 6,17±0,76 | 3,78<br>3,11<br>1,98 | 2,95±0,15 |
| 405 | Spring sun 60 F1, Japan | 50-55 | 37,45±0,98 | 31,8±1,37 | 30,9±3,1 | 24,7±2,0 | 1,545±0,19 | 7,35±2,11 | 3,1<br>1,77<br>1,03 | 1,96±0,22 |
| 1557 | Mini raioh 50 F1, Japan | 50-55 | 37±0,65 | 26,7±1,9 | 24,1±0,96 | 17,2±0,87 | 1,382±0,05 | 6,57±2,0 | 3,82<br>3,07<br>1,43 | 2,77±0,13 |
| 398 | Udzi M70, Japan | 60-65 | 41,7±0,79 | 36,48±0,9 | 34,4±1,89 | 21,2±1,3 | 1387,3±0,11 | 6,6±1,54 | 2,0<br>1,58<br>0,26 | 1,28±0,35 |
| 399 | Udzi M80, Japan | 60-65 | 40,17±1,23 | 35±1,14 | 33,9±1,7 | 21,8±1,27 | 1551,1±0,07 | 7,38±0,89 | 2,27<br>1,52<br>0,2 | 1,31±0,31 |
| 1558 | Raioh 90 F1, Japan | 70-72 | 41,13±1,1 | 35±2,2 | 33,1±2,0 | 20,11±0,9 | 1,584±0,04 | 7,53±0,44 | 2,0<br>0,61<br>0,3 | 0,97±0,19 |
| ssp. *chinensis* (L.) Hanelt | | | | | | | | | | |
| 647 | Unnamed, к-647, Tajikistan | 33-35 | 30,8±1,4 | 31,4±1,76 | 17,2±0,99 | 12,8±0,76 | 0,694±0,05 | 3,3±0,8 | 3,42<br>2,16<br>1,13 | 2,24±0,1 |
| 623 | Lastochka, Russia | 38-42 | 35,81±0,76 | 32,1±1,0 | 23±2,2 | 14,4±1,4 | 1,004±0,03 | 4,78±0,68 | 3,27<br>1,89<br>0,74 | 1,95±0,18 |
| ssp. *chinensis* var. *rosularis* (Tsen et Lee) Hanelt | | | | | | | | | | |
| 695 | Xiao Ba Je Ta Cai, China | 35 - 40 | | | | | 0,435±0,01 | 2,07±1,47 | 1,77<br>0,93 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 25,1±1,28 | 19,1±0,64 | 8,4±1,4 | 8±0,71 | | | 0,1 | 0,93±0,24 |
| ssp. *nipposinica* (Bailey) Hanelt) | | | | | | | | | | |
| 434 | Shirojuki Sensuji Kyo Mizuna, Japan | 45-48 | 34,8±0,7 | 23±1,8 | 11,7±1,63 | 7,35±0,92 | 0,497±0,06 | 2,36±1,89 | 2,73<br>2,33<br>1,6 | 2,57±0,1 |
| ssp. *rapa* L. f. *komatsuna* Makino | | | | | | | | | | |
| 330 | Toraziroh F1, Japan | 50-55 | 39±0,52 | 40,3±0,73 | 26,17±1,0 | 17,2±1,2 | 0,871±0,03 | 4,14±0,58 | 3,47<br>1,99<br>0,89 | 2,12±0,11 |
| ssp. *rapa* L. | | | | | | | | | | |
| 1398 | Palitra, Russia | 60-62 | 44,2±0,88 | 28,3±0,91 | 28,8±1,7 | 14,5±1,0 | 0,722±0,02 | 3,43±2,11 | 2,37<br>1,0<br>0,24 | 1,20±0,20 |
| 1422 | Hinona, USA | 60-62 | 34±0,55 | 35,4±1,27 | 32,8±1,17 | 9,1±1,58 | 0,307±0,01 | 1,46±1,51 | 1,25<br>1,05<br>0,31 | 0,87±0,14 |
| 1424 | Golden Ball, Finland | 58-62 | 42,1±0,49 | 30,2±0,93 | 26,4±1,04 | 13,8±0,76 | 0,389±0,07 | 1,85±0,14 | 2,37<br>1,64<br>0,81 | 1,6±0,15 |
| 1121 | Repa listovaya. na silos, Finland | 60-65 | 42±1,23 | 32,9±2,5 | 33,1±0,95 | 17±1,5 | 0,746±0,12 | 3,55±1,84 | 3,23<br>1,85<br>1,15 | 2,08±0,17 |
| 830 | Petrovskaya 1, Russia | 65-68 | 41,1±0,75 | 25±1,18 | 23,2±1,23 | 10,3±0,79 | 0,678±0,01 | 3,22±1,72 | 2,37<br>1,65<br>0,95 | 1,66±0,2 |
| 803 | Solovetskaya, Russia | 65-70 | 41,4±0,69 | 26,2±0,89 | 26,3±2,14 | 16,8±1,99 | 0,615±0,04 | 2,92±0,94 | 2,93<br>1,52<br>1 | 1,81±0,12 |
| 1421 | BRA 2842-96, Iraq | 65-70 | 43±0,84 | 38,4±1,1 | 37,7±0,81 | 16,1±0,93 | 0,977±0,08 | 4,65±0,22 | 3,0<br>2,25<br>1,41 | 2,22±0,11 |
| 1423 | Salusia 4x, Germany | 68-70 | 45±0,9 | 38,3±0,4 | 35,3±0,68 | 17,4±0,48 | 0,701±0,07 | 3,33±0,73 | 1,09<br>0,77<br>0,19 | 0,68±0,11 |
| 1415 | Peterburgskaya noch', Russia | 68-72 | 45,7±1,04 | 33,3±0,55 | 29,4±0,58 | 18,6±1,83 | 0,748±0,05 | 3,56±2,01 | 2,7<br>2,04<br>0,78 | 1,84±0,13 |
| 1405 | Ova daehnfeldt, Denmark | 70-75 | 43,3±0,93 | 39±0,84 | 35±2,3 | 18,5±0,79 | 0,899±0,02 | 4,2±1,0 | 2,0<br>1,25<br>0,3 | 1,18±0,21 |
| 1403 | Purpurnaya Primor'ya, Russia | 70-75 | 46,4±1,0 | 38,9±2,0 | 32,6±1,82 | 18,1±1,54 | 0,584±0,15 | 2,77±0,29 | 1,94<br>1,33<br>0,62 | 1,29±0,16 |
| Min | | | 25,1 | 19,1 | 8,4 | 7,35 | 0,307 | 1,46 | 0,68 | |
| Max | | | 46,4 | 40,3 | 37,85 | 24,7 | 1,584 | 7,53 | 2,95 | |
| Std. Dev | | | 5,07 | 5,75 | 7,78 | 4,39 | 0,38 | 1,85 | 0,62 | |
| Std. Err | | | 1,05 | 1,2 | 1,62 | 0,91 | 0,08 | 0,386 | 0,12 | |
| CV, % | | | 12,86 | 17,61 | 27,37 | 27,23 | 43,53 | 43,59 | 36,18 | |

Source: own research

Chinese cabbage (6,61 kg/m$^2$) and pak-choi (4,04 kg/m$^2$) are the leaders in yield. The following accessions stood out: Chinese cabbage - Raioh 90 F1 (7,53 kg/m$^2$), Udzi M80 (7,38 kg/m$^2$), Spring sun 60 F1 (7,35 kg/m$^2$), Udzi M70 (6,6 kg/m$^2$), pak-choi - Lastochka (4,78 kg/m$^2$), root turnip - BRA 2842-96 (4,65 kg/m$^2$) and Ova daehnfeldt (4,2 kg/m$^2$).

Nine accessions were identified as relatively resistant to *Alternaria* ssp. (infection severity up to 1,35 points), twelve as moderately resistant (1,36-2,7 points), and two as susceptible (2,71-4 points).The lowest degree of infection was noted of root turnip accessions - Salusia 4x (k-1423, Germany) - 0,68 points, Hinona (k-1422, USA) - 0,87 points, Ova daehnfeldt (k-1405, Denmark) – 1,18 points; Tacai - Xiao Ba Je Ta Cai (k-695, China) - 0,93 points; Chinese cabbage - Raioh 90 F1 (vr.k-1558, Japan) - 0,97 points, Udzi M70, (k-398, Japan) - 1,28 points. Figure 1 shows a difference in symptom severity. Outer leaves were commonly affected in all accessions with varying degrees of infection. The middle leaves are affected less often. The internal structures forming the head of the cabbage are affected by a minimum.

*Figure 4. Chinese cabbage: Raioh 90 F1* (vr. 1558, Japan)

*Source: field trial data, 16.09.2024*

The coefficients of variation (36,18-43,58%) confirm sufficient genetic diversity in the studied set for effective breeding work, particularly for yield and resistance to *Alternaria* ssp.

Consequently, the Japanese breeding of Chinese cabbage is distinguished by a combination of key features: balanced rosette structure, high yield, and moderate resistance to Alternaria infection. Accessions of pak-choi exhibit moderate yield and early maturity, but intermediate resistance to Alternaria. Accessions of root turnip and tacai can be used as sources of resistance. According to the complex of economically valuable characteristics, samples of Chinese cabbage can be distinguished: Raioh 90 F1 (vr. 1558, Japan), Udzi M70 (k-398, Japan), Udzi M80 (k-399, Japan) and root turnip: Ova daehnfeldt (k-1405, Denmark).

Table 2 shows the main components of the biochemical analysis of the accessions.

Table 2. Biochemical characteristics of the studied *Brassica rapa* L. accessions

| Accession | Ascorbic acid mg/100g | Monosaccharides% | Cchlorophyll a, mg/100g | Cchlorophyll b, mg/100g | Carotenoids, mg/100g | β-carotene mg/100g | Pphenolic compounds mg *GAE/g | Anthocyanins mg/100g |
|---|---|---|---|---|---|---|---|---|
| ssp. *pekinensis* (Lour.) Hanelt | | | | | | | | |
| Khibinskaya | 18,56 | 2,00 | 5,88 | 1,35 | 5,84 | 0,366 | 0,17 | - |
| Kapella | 24,32 | 1,90 | 1,29 | 0,05 | 2,07 | 0,143 | 0,26 | - |
| Spring sun 60 F1 | 24,00 | 2,92 | 39,67 | 18,05 | 4,61 | 2,461 | 0,19 | - |
| Mini raioh 50 F1 | 27,20 | 3,03 | 7,86 | 2,00 | 0,53 | 0,518 | 0,25 | - |
| Udzi M70 | 18,40 | 2,88 | 1,18 | 0,23 | 1,31 | 0,085 | 0,16 | - |
| Udzi M80 | 16,00 | 2,82 | 15,17 | 5,08 | 12,55 | 0,852 | 0,18 | - |
| Raioh 90 F1 | 24,00 | 3,23 | 9,31 | 2,44 | 7,97 | 0,533 | 0,19 | - |
| ssp. *chinensis* (L.) Hanelt | | | | | | | | |
| Unnamed, к-647 (leaves) | 85,44 | 2,33 | 80,55 | 25,39 | 14,27 | 4,751 | 1,38 | - |
| Unnamed, к-647 (petioles) | 19,52 | 4,58 | 11,32 | 2,83 | 1,50 | 0,608 | 0,15 | - |
| Lastochka (leaves) | 94,40 | 3,41 | 73,94 | 18,64 | 16,58 | 4,264 | 1,08 | - |
| Lastochka (petioles) | 25,60 | 4,03 | 17,77 | 5,45 | 4,22 | 0,997 | 0,18 | - |
| ssp. *chinensis* var. *rosularis* (Tsen et Lee) Hanelt | | | | | | | | |
| Xiao Ba Je Ta Cai | 72,00 | 2,45 | 134,17 | 47,70 | 38,88 | 8,557 | 1,15 | - |
| ssp. *nipposinica* (Bailey) Hanelt | | | | | | | | |
| Shirojuki Sensuji Kyo Mizuna | 46,40 | 1,94 | 20,06 | 4,72 | 11,31 | 1,145 | 0,60 | - |
| ssp. *rapa* L. f. *komatsuna* Makino | | | | | | | | |
| Toraziroh F1 | 62,40 | 4,54 | 122,46 | 46,79 | 29,99 | 7,678 | 0,86 | - |
| ssp. *rapa* L. | | | | | | | | |
| Palitra (leaves) | 106,65 | 2,21 | 40,73 | 14,86 | 17,22 | 2,615 | 0,96 | 143,01 |
| Palitra (root) | 35,55 | 7,22 | 0,87 | 0,25 | 1,02 | 0,104 | 0,38 | 369,56 |
| Hinona (leaves) | 110,21 | 2,35 | 50,85 | 22,17 | 20,30 | 3,249 | 0,82 | 1046,40 |
| Hinona (root) | 32,00 | 5,98 | 0,40 | 0,13 | 0,52 | 0,046 | 0,35 | 1906,77 |
| Golden Ball (root) | 40,53 | 5,87 | 0,19 | 0,18 | 0,82 | 0,108 | 0,20 | - |
| Repa listovaya na silos (root) | 43,37 | 5,32 | 1,15 | 0,33 | 0,80 | 0,102 | 0,17 | - |
| Petrovskaya 1(root) | 49,77 | 6,16 | 0,54 | 0,25 | 1,09 | 0,132 | 0,27 | - |
| Solovetskaya (leaves) | 88,88 | 3,58 | 49,96 | 14,88 | 19,36 | 3,085 | 0,89 | - |
| Solovetskaya (root) | 35,55 | 5,30 | 1,06 | 0,27 | 1,12 | 0,131 | 0,27 | - |
| BRA 2842-96 (root) | 44,08 | 5,13 | 0,40 | 0,15 | 0,34 | 0,050 | 0,19 | 362,61 |
| Salusia 4x (leaves) | 127,98 | 4,93 | 58,70 | 28,57 | 22,28 | 3,811 | 0,92 | 266,00 |
| Salusia 4x (root) | 32,00 | 7,32 | 4,19 | 1,02 | 2,25 | 0,278 | 0,28 | 247,49 |
| Peterburgskaya noch' (root) | 35,55 | 4,34 | 0,14 | 0,13 | 0,24 | 0,040 | 0,22 | - |
| Ova daehnfeldt (root) | 49,77 | 5,97 | 0,45 | 0,20 | 0,52 | 0,084 | 0,22 | - |
| Purpurnaya Primor'ya (root) | 37,68 | 6,01 | 0,11 | 0,07 | 0,21 | 0,030 | 0,23 | 418,82 |

*Source: own research*

The leaves of root turnip (108,43 mg/100 g) and tacai (72,0 mg/100 g) are characterized by the highest content of ascorbic acid. They also have a minimal degree of Alternaria infection. The maximum vitamin C content was found in the leaves of the Salusia 4x (k-1423, Germany - 127,98 mg/100 g). The largest amount

of sugars was found in root forms – 6,04%. The maximum content was noted in Palitra (k-1398, Russia) - 7,22%. The accumulation of monosaccharides in the leaves of root crops is significantly lower – 3,27%, as well as Chinese cabbage – 3%, which positively affects the degree of Alternaria infection (3 resistant accessions of Chinese cabbage and 5 of root turnip). Tacai (181,87mg/100 g) and leaf turnip (169,25mg/100 g) had the highest chlorophyll content. These accessions are also leaders in the content of carotenoids (38,88 mg /100 g and 29,99 mg/100 g) and β-carotene (8,55 mg/100 g and 7,67 mg/100 g). They can be used in breeding process for an increased content of biologically active components and for resistance to stress. The 22-25% β-carotene share of total carotenoids across *Brassica rapa* L. accessions confirms the high bioavailability of provitamin A.

The highest content of phenolic compounds was found in pak-choi (1,23 mg*GAE/g), tacai (1,15 mg*GAE/g), root turnip (0,89 mg*GAE/g) and leaf turnip (0,86 mg*GAE/g). The presence of anthocyanins was noted in 5 accessions. The highest content was noted in the root turnip Hinona (k-1422, USA) - it accumulates a large amount of anthocyanins both in the root crop (1906,77 mg/100g) and in the leaves (1046,40 mg/100g), which exceeds the content in other root crops by 355-426% and leaves by 293-632%. In combination with a low degree of Alternaria infection, it is a valuable breeding material.

The biochemical composition in comparison with the degree of Alternaria infection revealed correlations (according to Pearson): A strong negative correlation is observed between resistance to the disease and the content of anthocyanins (r = -0,72) and phenolic compounds (r = -0.61), moderate - between the content of ascorbic acid (r = -0,45), carotenoids (r = -0,39), beta-carotene (r = -0,41) and the content of chlorophyll a (r = -0,32), low - between the content of chlorophyll b (r = -0,28). A low positive correlation is observed between stability and the content of monosaccharides (r = 0,12). Thus, anthocyanins have the greatest protective function, which may be related to their antimicrobial activity. Phenolic compounds have a strong protective function, which may be related to their fungitoxic properties, which inhibit pathogen development, participate in lignification by strengthening the physical barrier to pathogen penetration and act as signalling molecules, activating plant defence genes. Ascorbic acid also influences defence mechanisms, which may be related to its antioxidant function and the synthesis of specific proteins and lignin that prevent pathogen penetration and participate in the activation of signalling pathways associated with systemic acquired resistance (SAR). Carotenoids and chlorophyll have a supporting role in plant defence mechanisms and are involved in photosynthetic activity, providing energy for defence reactions. The stronger effect of chlorophyll a may be due to its higher amount and closer association with carotenoids (including β-carotene), which protect plants against oxidative stress. Thus, selection in breeding for

resistance should be primarily based on the content of anthocyanins and phenolic compounds.

## CONCLUSION

The study of a set of accessions of different crops of *Brassica rapa* L. allowed to reveal a diverse manifestation of morphological and economically valuable plant traits under conditions of the North-West region of the Russian Federation. According to the level of resistance to *Alternaria* ssp and the content of protective bioactive compounds the most valuable for breeding are accessions of root turnip - Hinona (k- 1422, USA), Salusia 4x (k-1423, Germany), tacai - Xiao Ba Je Ta Cai (k-695, China). Sources of yield and resistance to *Alternaria* ssp. - Chinese cabbage accessions: Raioh 90 F1 (vr.k-1558, Japan), Udzi M70 (k-398, Japan), early maturity and high content of biologically active components - k-647 (Tajikistan).

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# RESERCHES REGARDING ADAPTABILITY OF NEW WALNUT VARIETIES IN THE CONDITIONS OF REPUBLIC OF MOLDOVA

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***Abstract***. *On the basis of comparative researches of local Moldovan walnut varieties with some new introduced ones (Chandler, Fernor, Fernette, Franquette, Lara etc.) was established a high ecological plasticity and adaptability considering pedological and changeable climatic conditions of Central and partially North pomological zone of Republic of Moldova. Preliminary obtained data regarding compatibility of introduced and local varieties during pollination, as well as fruit setting demonstrate good perspective for industrial orchards establishment according general agrobiological recommendations for walnut culture.*

***Keywords: adaptability, new varieties, walnut, Republic of Moldova.***

## INTRODUCTION

Through the fruit growing cultures, the walnut deserves an essential attention [1, 7-9]. Walnut (Juglans regia L.) are one of the oldest fruit trees species traditionally planted within republic of Moldova. For Moldovan people nuts always were and will remain a valuable product for food, trade, etc. 1,[4,7, 8]. Long time seed multiplication of local selected biotypes actually represents a sustainable possibility for long-term biodiversity conservation within the species Juglans regia L. [5]. Actually Moldovan registered for industrial culture walnut assortment include local varieties with high level of resistance to winter and spring temperature stresses and low sensibility to blight, others diseases. Usually trees productivity increases rapidly and reaches 2-2.5 tonnes of kernel per hectare in 8-9 years after plantation. For temporary testation in orchards there are included 15 foreign varieties [5]. Main trials of its promotion are: sustainable productivity and nuts qualities. But special attention it should be done to resistance to late spring frost, usually being in the period of flowering of walnut pistillate flowers.

## MATERIALS AND METHODS

It was studied the characteristics of pistillate flower flowering processes, receptivity to pollination with determination terms of more effective pollination of

mainly protandrous introduced varieties (Chandler, Fernor, Fernette, Franquette, Lara etc.) and the condition of central and partially north pomological zone of the Republic of Moldova. Researchers are based on methodologically approved experimental agrobiological and embryological studies [3,7-9] of walnut intraspecific hybridization of some locally created varieties with introduced varieties, also the degree of compatibility and the resistance of pistillate flowers to low temperatures within walnut flowering period. Pollination compatibility of introduced protandrous varieties with Moldovan ones were studied.

## RESULTS AND DISCUSSIONS

In comparative collaborative scientific researchers there where established good ecological plasticity and adaptability of the main Moldavian varieties also in the neighbor countries Ukraine, Romania, Hungary, Serbia etc. with manifestation of good kernel quality (tab. 1). As a result of different agrobiological researches of walnut production [3, 7-9] there were established basic cultural considerations. Between its important biological features o Moldavian

Table 1. General characteristics of nut and kernel of main walnut varieties registered for production in rep. of Moldova (source: multiannual biochemical data obtained by dr. Radusinskaia I.)

| Variety | Medium weight of nuts, gr. | Kernel content % | Chemical composition of kernel | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Total sugars, % | Lipids, % | Proteins, % | Carbon % | Carotene (A), mg/% | Tiamin ($B_1$) mg/% | Riboflavin ($B_2$) mg/% |
| Kalarasski | 14,3 | 51,3 | 5,31 | 66,42 | 18,00 | 5,31 | 0,135 | 0,208 | 0,345 |
| Kişineovski | 10,1 | 49,3 | 6,42 | 66,96 | 16,44 | 6,42 | 0,212 | 0,199 | 0,119 |
| Kostiujenski | 14,1 | 50,4 | 5,14 | 66,37 | 19,00 | 5,14 | 0,492 | 0,192 | 0,234 |
| Korjeutki | 10,2 | 49,7 | 7,76 | 66,52 | 18,81 | 7,76 | 0,308 | 0,114 | 0,153 |
| Skinosski | 13,2 | 50,0 | 8,59 | 67,17 | 17,44 | 8,79 | 0,254 | 0,466 | 0,153 |

varieties. High exigency given the light, which is well seen due to its rare crown. It means the light should get in its crown so high, as well as from the lateral parts. It should be noticed also that in luminosity conditions, the fit of the most dangerous disease which provokes the harvest loss is fainter. Even if the walnut trees in the rep. of Moldova during the winter supports normally the temperatures of minus 20-25 degrees, the tardy spring frosts represent a permanent danger [3,7]. That is why, while choosing the varieties for the orchards setting up, it is necessary to choose the varieties with a tardy disburgeoning and development of pistillate flowers. This will assure a more tardive flourishing, first of all for protandrous

genotypes (tab. 2,3; fig. 1-3) and the damages of pistilatte juvenile flowers avoidance from the respective late spring frosts.

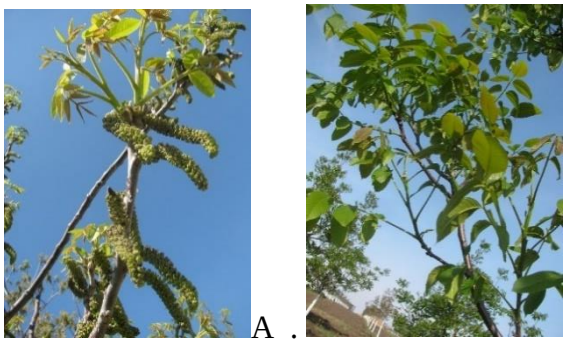
Table 2. Average walnut flowering data in the Central Pomological zone of the rep. of Moldova. Local varieties.

| Variety | Male flowers | | | Female flowers | | |
|---|---|---|---|---|---|---|
| | Beginning | End | Period (days) | Beginning | End | Period (days) |
| Cazacu | 11.05 | 15.05 | 5 | 05.05 | 15.05 | 11 |
| Calaraşski | 07.05 | 13.05 | 7 | 16.05 | 21.05 | 6 |
| Costiujenski | 05.05 | 11.05 | 7 | 15.05 | 23.05 | 9 |
| Schinoski | 12.05 | 18.05 | 7 | 05.05 | 12.05 | 8 |
| Chişinevski | 16.05 | 24.05 | 9 | 15.05 | 22.05 | 8 |
| Cogâlniceanu | 13.05 | 18.05 | 6 | 10.05 | 15.05 | 6 |
| Pescianskii | 14.05 | 20.05 | 7 | 07.05 | 13.05 | 7 |

Table 3. Average introduced walnut varieties flowering data in the Central Pomological zone of the rep. of Moldova.

| Variety | Male flowers | | | Female flowers | | |
|---|---|---|---|---|---|---|
| | Beginning | End | Period (days) | Beginning | End | Period (days) |
| Milotai | 11.05 | 15.05 | 5 | 05.05 | 15.05 | 11 |
| Aloszentivanvi | 07.05 | 13.05 | 7 | 16.05 | 20.05 | 5 |
| Chandler | 10.05 | 14.05 | 5 | 17.05 | 22.05 | 6 |
| Fernor | 05.05 | 11.05 | 7 | 15.05 | 23.05 | 9 |
| Fernette | 12.05 | 18.05 | 7 | 05.05 | 12.05 | 8 |
| Franquette | 13.05 | 20.05 | 8 | 16.05 | 25.05 | 10 |
| Lara | 10.05 | 18.05 | 9 | 11.05 | 17.05 | 7 |

Difference between terms of flowering of female and male flowers (days) of varieties with coincidence of effective receptivity of female flowers to pollination period (tab. 2, 3) represent possibility of interpollination of local with introduced varieties. As a result of studies of morfohystochemical pecularities of pistillate flower receptivity to pollination depending on flowering stage were established terms of more effective pollination of dichogamous local and introduced varieties within condition of Republic of Moldova. Based on cytoembryological studies of walnut intraspecific hybridization studied introduced and local varieties the optimal degree and the peculirities of compatibility of all dichogamous types was also established [3].



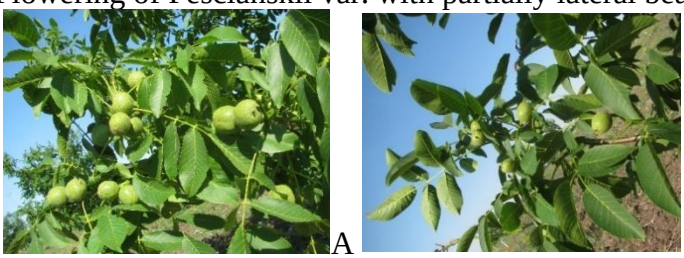
A . B

Figure 1. Different stage of flowering of introduced varieties: A-Franquette var. B-Fernor var.

A B

Figure 2. Homogamous Flowering-Chisinevskii.-A, Pistillate full flowering of Chandler var.-B.

Figure 3. Flowering of Pescianskii var. with partially lateral bearing.



A B

Figure 4. Development of fruits (nuts). A -Lara. B-Fernor. Lateral bearing.

Figure 5. Development of fruits (nuts). A-Chandler. B- Fernette. Lateral bearing.

Preliminary result of cultivation (within the frame oftemporal testation) within central pomological zone of Republic of Moldova of varieties Franquette, Chandler, Fernor, Fernette, etc. shows good results of adaptability regarding initiation and development of vegetative and flower buds, resistance to extremal temperatures, compatibility to some local varieties, as well as optimal development of fruits (tab. 2, 3, fig. 1-5), optimal harvests in the areas with annual medium daily temperature of 8-9 degrees. Our observations shows that even if the walnut trees in the rep. of Moldova during the winter supports normally the temperatures of minus 20-25 degrees, the tardy spring frosts represent a permanent danger. That is why, while choosing the varieties for the orchards setting up, it is necessary to choose the varieties with a tardy disburgeoning and tardy flowering of pistillate flowers. So, introduced protandrous and late homogamous varieties like Chandler, Fernette, Fernor, Franquette, as well as Moldovan ones: Costiujenskii, Cazacu, Chisiniovskii, could will assure a more tardive flourishing of pistillate flower and avoid damage from the respective late spring frosts.

## CONCLUSIONS

Protandrous varieties Chandler, Fergean, Fernor, Franquette, as well as Moldovan dichogamous ones: Costiujenskii, Cazacu, Pescianskii, Chisiniovskii with a high potential of the ecological adaptability and plasticity with special refference to the resistance at the biotic factors (diseases and pests) and abiotics ( the pedo climatic conditions, technological approaches). This assortment there are important for the implementation within conventional production, as well as for development of new resilient agricultural practices (organic production, etc.).

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# TEMPORAL GENE EXPRESSION PATTERNS DURING MICROSPOROGENESIS IN SUNFLOWER

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***Abstract:*** *The objective of this study was to characterize the transcriptional variation of a selected set of genes in fertile sunflower (Helianthus annuus) anthers by quantifying their expression levels using Ral-time PCR across four stages of microsporogenesis. The results reveal that over 90% of the analyzed genes exhibit dynamic or oscillatory expression patterns, reflecting stage-specific regulatory responses. A moderate increase in gene expression variability during the tetrad and microspore stages, compared to the earlier phases of microsporogenesis, such as pachytene and meiotic division, was observed. The prevalence of dynamic expression highlights complex transcriptional regulation required for maintaining physiological homeostasis during microsporogenesis. These results contribute to the functional validation of candidate genes and offer insights into the genetic and physiological mechanisms underlying male fertility in sunflower.*

***Keywords: candidate genes, gene expression dynamics, microsporogenesis, qPCR, sunflower.***

## INTRODUCTION

Microsporogenesis represents a critical phase in the formation of the male gametophyte, requiring extensive transcriptomic reprogramming to coordinate cellular differentiation and structural transitions. Although co-regulation of gene expression is a common feature of stress and developmental responses [1], it remains highly gene-specific and dependent on the physiological context.

Over the past years, numerous transcriptomic studies have been conducted in various crop species, including sunflower (*Helianthus annuus*), focusing on tissue-specific gene expression under diverse experimental conditions. These efforts have primarily explored plant responses to abiotic [2, 3] and biotic stressors [4, 5], as well as strategies aimed at improving fertility and yield performance [6, 7]. However, despite the growing interest in sunflower genomics, investigations of differential gene expression specifically focused on male reproductive development remain relatively limited compared to those conducted in other major crop species.

The objective of this study was to characterize the transcriptional variation of a selected set of genes across different stages of microsporogenesis in fertile sunflower anthers, in order to reveal stage-specific patterns in gene expression dynamics.

The analysis of candidate gene expression represents is an essential step in the experimental validation and functional annotation of genes, particularly in the case of species whose genome is partially functionally characterized, as is the case of Helianthus annuus. Also, expression data can significantly contribute to refining existing gene annotations and deepening our understanding of their biological roles.

## MATERIALS AND METHODS

**Plant materials.** Anthers of sunflower plants from the inbred line SW501 (Magroselect ASP, Soroca, Republic of Moldova) were used as the biological material. For accurate selection of anthers corresponding to specific stages of microsporogenesis, florets and anthers were subjected to cytological and histological analysis, as described in the previous study [8].

**RNA isolation and RT-qPCR analysis.** Total RNA was isolated from anthers at four microsporogenesis stages: pachytene (P), meiotic divisions (D), tetrads (T), and young microspores (M), using TRIzol reagent (Invitrogen, Thermo Fisher Scientific), according to the manufacturer's protocol. cDNA synthesis was performed using RevertAid Reverse Transcriptase (Thermo Fisher Scientific) with arbitrary hexamer primers and oligo(dT)18 primers. Gene expression analysis was carried out with the Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific).

Relative gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ method, with *actin* (GenBank: AF282624.1) as the reference gene. Expression differences (fold change, FC) between microsporogenesis stages were considered biologically relevant at $FC \geq 1.5$ and statistically significant at $p \leq 0.05$, according to ANOVA, and Bonferroni correction.

Gene expression analysis covered 108 genes, mostly candidates with putative functions, grouped into five functional categories based on Gene Ontology terms.

## RESULTS AND DISCUSSIONS

The variation in gene expression levels in the anthers of fertile sunflower plants under normal physiological conditions reflects the internal dynamics of male reproductive development. Thus, heatmap analysis of relative expression values across different phases of microsporogenesis (pachytene, meiotic divisions, tetrads, and microspores) revealed genes with similar expression patterns, indicating the existence of co-regulated gene groups, which suggests their involvement in common biological processes. Additionally, the chromatic contrast distribution on the expression map reveals a certain degree of heterogeneity both among genes and between the phases of microsporogenesis. It is also observed that gene expression levels are higher for a larger number of genes in the microspore phase compared to the other three analyzed phases.

For a detailed characterization of transcript content variation in anthers

depending on the phases of microsporogenesis, boxplot representations of relative expression data and agglomerative hierarchical clustering analysis were used (Figure 1).

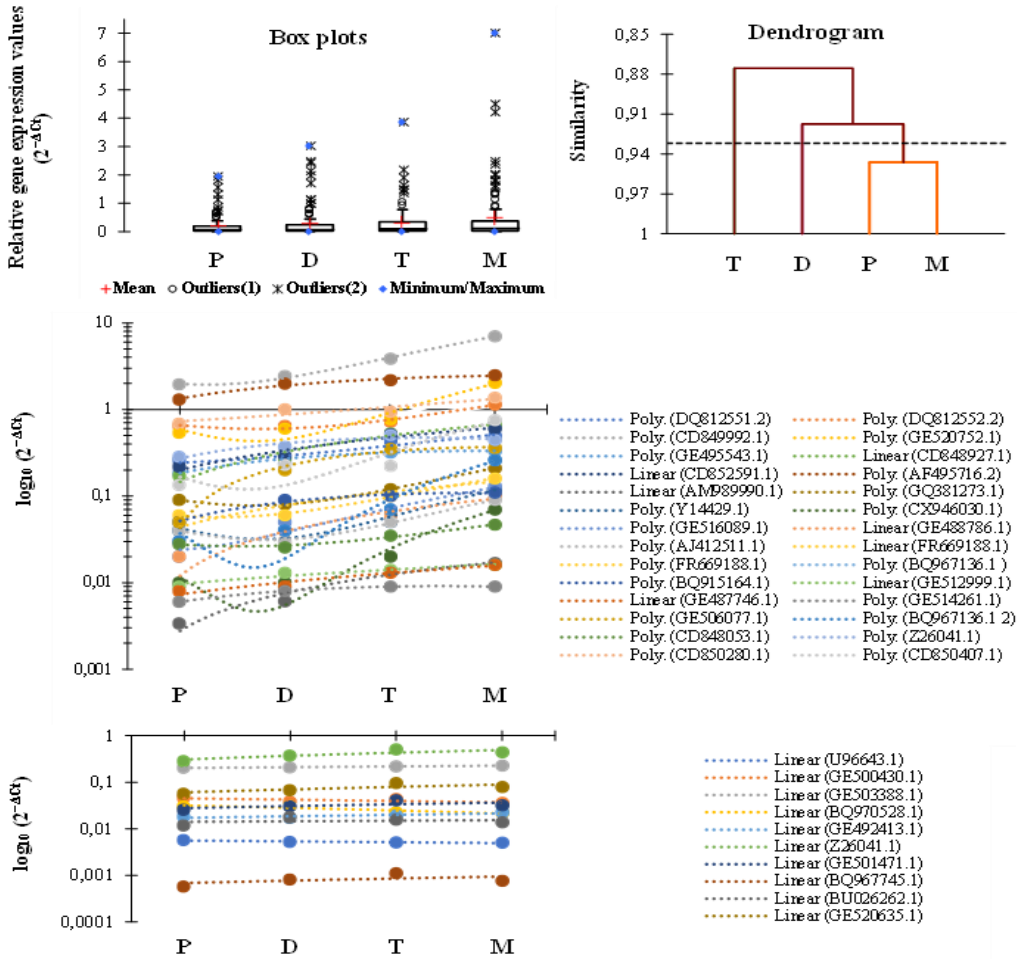


Figure 1. Variation in gene expression across microsporogenesis stages in sunflower anthers of the SW501 homozygous fertile line (boxplots, UPGMA-based hierarchical clustering and regression trend lines of relative expression values)

The relatively close medians observed in the boxplots suggest moderately variable gene expression between phases. At the same time, the extreme values (minimum and maximum) indicate a more pronounced dispersion of transcript levels, particularly in the tetrad phase, compared to the pachytene and division phases. The tetrad formation phase

is characterized by a higher degree of value dispersion, but with a symmetric distribution around the median, suggesting heterogeneous, but balanced gene expression. In contrast, the microspore phase shows the greatest variability, reflected by a high number of extreme values, indicating divergent expression levels both in comparison to other genes and to the expression of the same genes in earlier stages.

The hierarchical clustering analysis confirms the differentiation of gene expression data in the microspore phase, which is separated first, followed by the tetrad phase, which in turn shows a low degree of similarity with the pachytene and meiotic division phases. However, the overall high degree of similarity between phases (above 0.90) indicates a well-defined pattern of gene interaction and suggests the presence of fine regulatory mechanisms of gene expression during microsporogenesis in a physiologically normal (homeostatic) conditions.

In conclusion, the comparative analysis of gene expression values distributions across different phases of microsporogenesis in fertile anthers of the homozygous plants SW501 line, using heatmap, boxplot representations, and hierarchical clustering analysis, revealed:

- a moderate increase in gene expression variability during the tetrad and microspore stages compared to the earlier phases of microsporogenesis, such as pachytene and meiotic division,
- groups of co-expressed genes, which may be attributed to their involvement in the same metabolic pathways or physiological processes, supporting the validity of the functional category association results. Moreover, this gene expression correlation (co-expression) may reflect common regulatory factors or synchronized responses to internal signals associated with specific physiological states.

To gain a deeper understanding of the temporal dynamics of the studied gene expression during microsporogenesis and to identify genes with functional roles in the key developmental stages of microsporocytes and microspores, genes were classified based on natural patterns of expression variation under normal physiological conditions. Such an approach allows for the distinction between genes with relatively constant expression and those with variable expression (fluctuating, increasing, or decreasing), providing insights into the metabolic or regulatory processes in which these genes are involved.

Thus, in the next stage of the analysis, expression trends across the four studied phases (P, D, T and M) were examined, and the genes were clustered into three groups: those with progressively increasing expression levels, relatively constant expression, and oscillatory expression, potentially involved in the regulation of transient or cyclical processes and, implicitly, in physiological responses to various intra- or intercellular signals.

**Group 1: Genes with a progressively increasing expression profile.** Out of the 108 genes analyzed, 27 exhibited a progressively increasing expression pattern. These

genes belong to all five functional categories examined in this study, as follows:

- **category I – Redox homeostasis and cellular stress response:** *MSD1, MSD2, APX1, TRX2, BADH, GPX1, GSH, DHAR2, MATE.*
- **category II – Signal perception and transduction, hormonal interactions:** *NDPK1, NDK2, NDPK3, GA20-OX1, IAA27, PP2A.*
- **category III – Growth and development of reproductive structures:** *PEL18, TMT2, PME2.*
- **category IV – Mitochondrial activity and biogenesis:** *UCP1, ACP2, GAPB, VDAC, ATAD1B.*
- **category V – Genome integrity maintenance, DNA replication and repair, cell division:** *RAN3, NTH, MUTYH, MSH1.*

These genes exhibit a clear upward trend in transcript levels over time (P–M) (Figure 1). Additionally, based on their expression level values, the genes were conventionally divided into two subgroups:

- *Subgroup 1.1* includes genes that showed relatively high minimum and maximum expression values (relative expression range: 0.1 – 7.0). The genes in this subgroup are: *MSD1, MSD2, APX1, TRX2, NDPK1, NDPK3, TMT2, UCP1, ACP2, VDAC, RAN3, PP2A.*
- *Subgroup 1.2* includes genes with significantly lower relative expression levels—approximately 10- to 20-fold lower compared to the subgroup 1.1 (the relative expression range: 0.003–0.40). The genes in this subgroup are: *BADH, GPX1, GSH, DHAR2, MATE, GA20-OX1, IAA27, PEL18, PME2, GAPB, ATAD1B, NTH, MUTYH, MSH1, NDK2.*

**Group 2: Genes with relatively constant expression.** This group includes 9 genes that exhibit a relatively stable expression profile across the analyzed phases (fold change < 1.5), with relative expression values ranging from 0.0006 to 0.25. Their distribution across functional categories is as follows: five genes from category II (*RLPK, MRK3, GID1C, PERK1, CBL2*), two genes from category III (*MC1, GCL1*), one from category IV (*SDH1*) and one from category V (*MTSSB*).

**Group 3: Genes with oscillatory expression.** This group includes the largest number of genes (72), showing significant variation in expression across phases:

- **category I – Redox homeostasis and cellular stress response:** *SOD1CU, SOD2CU, PRXIIF, CATA2, CATA3, GST, CATA4, APX4, CATA, NTRB.*
- **category II – Signal perception and transduction, hormonal interactions:** *CAM, NTF4, RAC1, TIR1, GGPS. PI3K, SERK1, SERK2, MAPKKK7, MKP1, ACA9, GA2OX1, GAI1, RGL2, ABP1, GID2, TMK1, GDI1*
- **category III – Growth and development of reproductive structures:** *AG, BGAL5, EXPA8, EXPA1, GPAT1, DAD1, BAXI1, MC4, HXK1, HOG1, LSD1, KN1, KN2, HB-8, CDF3, MYB1, MYB-PHL7, MYB4, HEN1, GSL12, XTH6, BAG3, MYC2, XTH23,*

*DRH.*

- **category IV – Mitochondrial activity and biogenesis:** *NDB1, NDC1, DL1C, CYTC-1, NDA2.*
- **category V – Genome integrity maintenance, DNA replication and repair, cell division:** *HIS1, WHY2, Histone H3.2, DYAD, MMH-1, OGG1, MSH5, OSB2, RECA3, BFN1, SKP1-1B, CDC2A, H2B, PCNA.*

Based on expression level values, these genes were conventionally classified into three subgroups.

*Subgroup 3.1* includes genes with expression levels ranging from 0.1 to 5,0: *SOD1CU, SOD2CU, CAM, HOG1, DRH, PRXIIF, SKP1-1B, CYTC-1, CDC2A, NTRB, BGAL5, BAXI1, GPAT1, HIS1, RAC1, DAD1, CATA, NDA2, NTF4, GID2, TMK1, H2B, MC4, WHY2, GGPS.*

*Subgroup 3.2* comprises genes with moderate expression levels, ranging from 0.01 to 1. The expression values of these genes are approximately 5 to 10 times lower than those in subgroup 3.1: *EXPA8, EXPA1, HXK1, LSD1, PCNA, NDB1, PI3K, HEN1, SERK1, AG, TIR1, CATA3, GST, SERK2, MAPKKK7, MKP1, GA2OX1, ABP1, KN1, HB-8, CDF3, GAI1, MYB1, NDC1, DL1C, DYAD, MMH-1, OGG1, RGL2, OSB2, APX4, MYB-PHL7, XTH6, XTH23, RECA3, KN2, BFN1, GDI1, CATA4.*

*Subgroup 3.3* includes genes with predominantly low expression values (< 0.01), specifically within the range of 0.0003 to 0.015: *CATA2, MYC2, MYB4, GSL12, BAG3, histone H3.2, ACA9, MSH5.* Compared to subgroup 3.2, the expression levels of genes in subgroup 3.3 are approximately 30 to 70 times lower.

The synthesis of the obtained data reveals that the majority of the analyzed genes (over 90%) exhibit oscillatory or dynamic expression patterns. The predominant group of oscillatory genes is mainly represented by those classified in Categories I, II and V, whose molecular functions are associated with signal transduction and hormonal interactions, growth and development of reproductive structures, as well as in cell division, genome integrity maintenance, DNA replication and repair. These findings suggest the presence of an active and adaptive temporal regulation in response to the physiological transitions occurring between successive stages of anther development.

A comparatively large number of genes with progressively increasing expression levels belong to Categories I and IV, indicating a gradual activation of processes involved in redox homeostasis, cellular stress responses, mitochondrial biogenesis and function. This may reflect the high metabolic activity within the microsporangium, particularly in tapetal cells, which play a key role in nourishing developing microspores.

Within this study, only a small number of genes exhibited either gradually decreasing expression levels across microsporogenesis stages (functional repression) or relatively stable expression throughout all four stages.

## CONCLUSIONS

The comparative analysis of gene expression value distributions across different phases of microsporogenesis in fertile anthers of the homozygous plants SW501 line revealed a moderate increase in gene expression variability during the tetrad and microspore stages, compared to the earlier phases of microsporogenesis, such as pachytene and meiotic division.

The prevalence of dynamically expressed genes, whether progressively upregulated or fluctuating, within the transcriptomic profile of anthers during microsporogenesis highlights the involvement of complex regulatory mechanisms that maintain physiological homeostasis. Disruption of this biochemical and physiological equilibrium during male gametophyte development may result in partial or complete male sterility.

This study adds valuable scientific evidence concerning the experimental validation and functional characterization of candidate genes, thereby facilitating the functional the functional annotation process and the integration of these genes into molecular networks.

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# INITIAL GENETIC DIVERSITY ASSESSMENT OF ENDEMIC HYRCANIAN WILD FIG POPULATIONS IN AZERBAIJAN

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***Abstract.*** *This study presents the first genetic assessment of wild Ficus carica populations from the Hyrcanian forests of Azerbaijan using Inter Simple Sequence Repeat (ISSR) markers. Fifteen genotypes collected from Hirkan National Park were analyzed using five ISSR primers, which revealed a moderate level of genetic diversity (GDI = 0.62). All accessions were successfully differentiated, highlighting the high discriminatory power of the selected primers. Jaccard similarity indices ranged from 0.14 to 0.89, with an overall mean of 0.53. Cluster and Principal Coordinates Analyses revealed three major genetic clusters and significant intra-population diversity, with no correlation to geographic proximity. Unique bands were observed in several genotypes, and variation in genetic diversity indices across primers emphasized the importance of marker selection. These findings underscore the substantial genetic variation among wild fig populations in Azerbaijan and confirm the utility of ISSR markers for conservation, breeding, and taxonomic studies of underutilized fig germplasm.*

***Keywords: Ficus hyrcana, genetic diversity, ISSR, wild fig.***

## INTRODUCTION

The genus *Ficus* L. (Moraceae) is one of the largest among flowering plants, comprising over 800 species that are predominantly distributed across tropical and subtropical regions. *Ficus carica* L., commonly known as the common fig, holds significant economic and cultural importance and is widely cultivated throughout the Mediterranean basin and West Asia, owing to its broad ecological adaptability [1]. Cytogenetically, it is diploid, with a somatic chromosome number of 2n = 2x = 26 [2].

Despite its long-standing cultivation and domestication, the exact origin of *F. carica* remains debated. Some researchers propose western Asia or southern Arabia as the center of origin, where both wild and caprifig populations may be found [3]. Others suggest that the species may have originated along the Caspian coastlines or in northwestern Turkey, where dense natural populations are still present [2]. Over time, fig trees became naturalized across a broad area, encompassing the Mediterranean, Iran, Central Asia, and the Transcaucasus [4]. This broad distribution, coupled with long-term cultivation, has led to the emergence of a rich diversity of local cultivars, many of which are believed to have arisen from centuries of unconscious selection among wild seedlings [5]. Wild fig populations continue to serve as vital reservoirs of genetic diversity, especially in regions such as Turkey and Transcaucasia.

In this context, Azerbaijan, a Transcaucasian country, represents an important but underexplored center of fig diversity. The wild fig populations native to the Hyrcanian forests in southeastern Azerbaijan are currently recognized as rare and underutilized genetic resources within the *F. carica* complex. First described by the botanist Alexander Grossheim in 1939 as *Ficus hyrcana*, these trees are now commonly regarded as a local ecotype or synonym of *F. carica* subsp. *carica* [6]. Notably resilient to both abiotic and biotic stressors, Hyrcanian wild figs may possess valuable alleles with potential applications in fig breeding. Their comprehensive study could improve conservation efforts and support their use as rootstocks or grafting material for cultivated *F. carica* varieties.

To effectively evaluate and conserve such genetic resources, molecular markers are increasingly favored over conventional morphological approaches. Among these, Inter Simple Sequence Repeat (ISSR) markers are particularly useful due to their reproducibility, cost-effectiveness, and ability to detect polymorphisms without requiring prior sequence information. ISSR markers amplify regions between microsatellites, providing high levels of resolution for genetic diversity studies in various plant species, including figs [7].

Despite their ecological importance and potential genetic value, Hyrcanian wild figs remain largely neglected and are classified as a vulnerable plant group in need of conservation. Previously, we conducted a comprehensive morphological assessment of these trees [8], which provided the groundwork for subsequent molecular investigations. The objective of the present study is to provide the first and an initial assessment of the genetic diversity and relationships among rare and underutilized wild fig populations from the Hyrcanian forests of Azerbaijan using ISSR markers.

## MATERIALS AND METHODS

During a field expedition to Hirkan National Park in Azerbaijan, in May 2025, young leaf samples were collected from wild *Ficus carica* L. (syn. *F. hyrcana* Grossh.) trees and placed into sterile tubes for preservation. Genomic DNA was extracted at the Department of Molecular Genetics and Genomics, Genetic Resources Institute, Ministry of Science and Education of Azerbaijan, using a modified cetyltrimethylammonium bromide (CTAB) method as described by Doyle and Doyle [9]. The purity and quantity of the extracted DNA were evaluated with a NanoDrop spectrophotometer (Thermo Scientific, 2000). Five ISSR primers were selected based on their ability to produce clear, reproducible, and polymorphic amplification profiles (Table 1). PCR amplification and subsequent separation of amplified fragments were carried out using 1.5% agarose gel electrophoresis, following the protocol described by Qurbanova et al. [10].

Only clearly distinguishable and reproducible bands were manually scored as present (1) or absent (0), forming a binary matrix for analysis. To evaluate the informativeness of each ISSR primer, various genetic diversity metrics were calculated, including the genetic diversity index (GDI) [11], polymorphism information content (PIC), effective multiplex ratio (EMR), marker index (MI), resolving power (RP), and mean resolving power (MRP). Genetic relationships among the fig accessions were inferred through unweighted neighbor-joining (NJ) clustering and principal coordinate analysis (PCoA), both performed using the Jaccard similarity coefficient in DARwin 6.0 software [12].

## RESULTS AND DISCUSSIONS

A total of five ISSR primers were used to evaluate genetic diversity in 15 Hyrcanian wild fig genotypes collected from the Hirkan National Park in Azerbaijan. The primers generated 41 bands in total, of which 17 (41.2%) were polymorphic, indicating a moderate level of genetic variability within the studied accessions (Table 1). The number of bands per primer ranged from 7 (UBC 818) to 9 (UBC 834 and UBC 835), with the number of polymorphic bands (Np) ranging from 2 to 5. These results are consistent with the findings of Khadari et al. [13], who reported a total of 37 markers generated by 8 ISSR primers, with the number of markers per primer ranging from 2 to 8, and an average of 4.6 markers per primer. In contrast, a study on 24 cultivated and wild fig accessions from Palestine using 15 RAPD markers revealed an average of 6.1 alleles per primer [14].

Among the primers, UBC 835 exhibited the highest polymorphism percentage (56%), followed closely by UBC 823 (50%), suggesting these primers were more effective in detecting variation among the accessions. In contrast, UBC 818 showed the lowest polymorphism (29%), indicating relatively lower variability

captured by this primer.

Table 1. Genetic diversity parameters in wild fig accessions from Azerbaijan based on ISSR markers

| Primer | $N_t$ | $N_p$ | P, % | MI | EMR | RP | MRP | PIC | GDI |
|---|---|---|---|---|---|---|---|---|---|
| UBC 818 | 7 | 2 | 29 | 0.21 | 0.57 | 0.94 | 0.47 | 0.36 | 0.42 |
| UBC 823 | 8 | 4 | 50 | 0.76 | 2 | 2.8 | 0.70 | 0.38 | 0.76 |
| UBC 834 | 9 | 3 | 33 | 0.45 | 1 | 2.14 | 0.71 | 0.45 | 0.72 |
| UBC 835 | 9 | 5 | 56 | 0.50 | 2.8 | 1.1 | 0.22 | 0.18 | 0.66 |
| UBC 840 | 8 | 3 | 38 | 0.36 | 1.1 | 1.32 | 0.44 | 0.33 | 0.52 |
| Total | 41 | 17 | - | - | - | - | - | - | - |
| Mean | 8.2 | 3.4 | 41.2 | 0.46 | 1.5 | 1.53 | 0.34 | 0.34 | 0.62 |

Despite the limited number of studied genotypes, unique bands were observed in some accessions with certain primers. For example, using the UBC 835 primer (Figure 1), a unique band of 1210 bp was amplified in the Hyrcan 2 accession, while a distinct 860 bp fragment was observed in Hyrcan 12. Additionally, a 950 bp fragment was present in all accessions except Hyrcan 3, making this absence a unique profile feature for that tree.

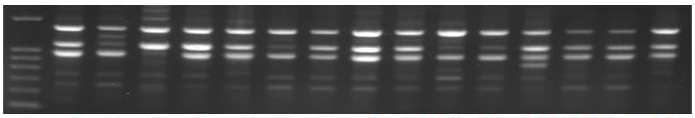


Figure 1. Genetic profile of wild Hyrcanian fig accessions generated using ISSR primer UBC 835

Several genetic parameters were calculated for the ISSR primers used. Marker index values, reflecting both informativeness and multiplexing capacity, ranged from 0.21 (UBC 818) to 0.76 (UBC 823), supporting the high utility of UBC 823 in genetic studies of Hyrcanian wild figs. The effective multiplex ratio ranged from 0.57 to 2.8, with an average of 1.5. In the study by Khadari et al. [7], ISSR markers demonstrated higher efficiency compared to RAPD and SSR markers when evaluated based on the EMR across 30 fig cultivars. The primers UBC 823 and UBC 818 exhibited the highest and lowest resolving power values (2.8 and 0.94), respectively. Similarly, UBC 834 and UBC 823 showed the greatest MRP, whereas UBC 835 displayed the lowest.

One of the main parameters used to evaluate the efficiency of molecular markers, the GDI, showed considerable variation among the primers, ranging from 0.42 for UBC 818 to 0.76 for UBC 823. On average, the genetic diversity within the collection was 0.62, indicating a moderate level of variation among the samples. PIC values ranged from 0.18 (UBC 835) to 0.45 (UBC 834), with an average of

0.34. These values fall within the typical range for dominant markers and reflect moderate informativeness of the primers. UBC 823, with the highest PIC and resolving power (RP = 2.8), was particularly efficient in differentiating among genotypes. Overall, the ISSR marker system proved to be a valuable tool in assessing genetic diversity among Hyrcanian wild fig accessions.

Jaccard similarity indices ranged from 0.14 (between Hyrcan 5 and Hyrcan 13) to 0.89 (between Hyrcan 10 and Hyrcan 12), with an overall mean of 0.53. This moderate average similarity indicates a substantial degree of genetic variability within the studied population, consistent with the expectation for wild fig populations that have evolved under varying ecological conditions. Cluster analysis using UPGMA revealed the formation of three major clusters, each comprising several subclusters (Figure 2). Notably, no clear clustering based on geographic proximity was observed. Genotypes from different villages and distant areas of the national park often grouped together, while others collected from adjacent locations were placed in separate clusters. This pattern suggests that geographic distance is not the primary driver of genetic similarity in these populations.

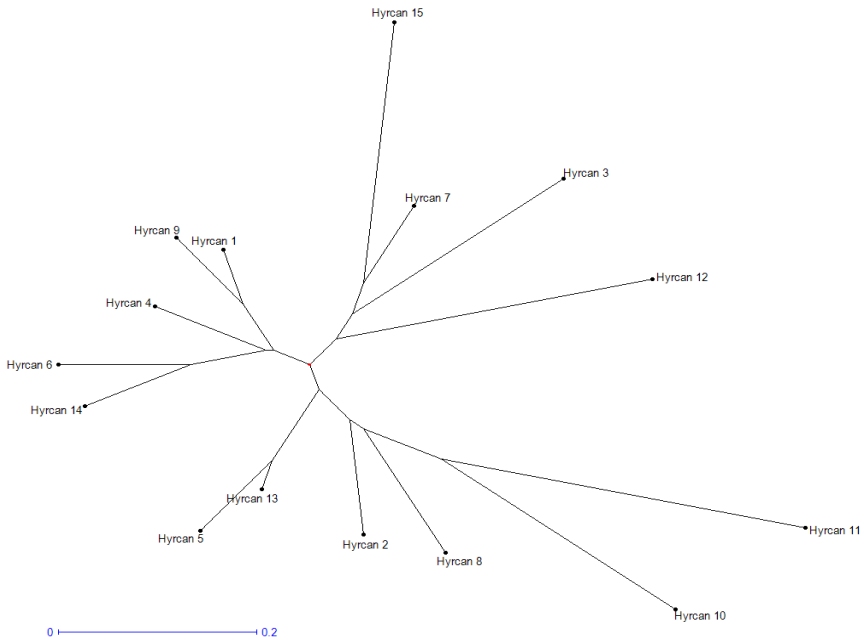


Figure 2. Genetic relationships among wild fig accessions revealed by ISSR markers

Cluster I included four genotypes, among which Hyrcan 3 and Hyrcan 15 were noteworthy. Despite being sampled from different regions, both were located near rivers. Cluster II was composed of five genotypes, with Hyrcan 1 and Hyrcan 9 showing the greatest genetic similarity within this group. The remaining

genotypes were assigned to Cluster III, within which, Hyrcan 10 and Hyrcan 11 exhibited the greatest divergence, with pairwise genetic distances ranging from 0.50 to 0.71 when compared to other members of the cluster.

The PCoA further supported these findings (Figure 3). The first two axes explained 49.4% of the total genetic variation, while the first five axes accounted for 85.2%, indicating a high resolution of the genetic structure within the dataset. The scattered distribution of genotypes in the PCoA plot, without the formation of tight clusters, reflects a high level of genetic differentiation among individuals, supporting the hypothesis of significant intra-population diversity. Interestingly, only Hyrcan 10 and Hyrcan 11 were placed distinctly apart from the main group of genotypes on the PCoA plot, underscoring their unique genetic profile. Additionally, Hyrcan 15, collected from the highest elevation site (287 m a.s.l.), was positioned at the extreme edge along the negative Y-axis, suggesting possible altitudinal effects on genetic differentiation.

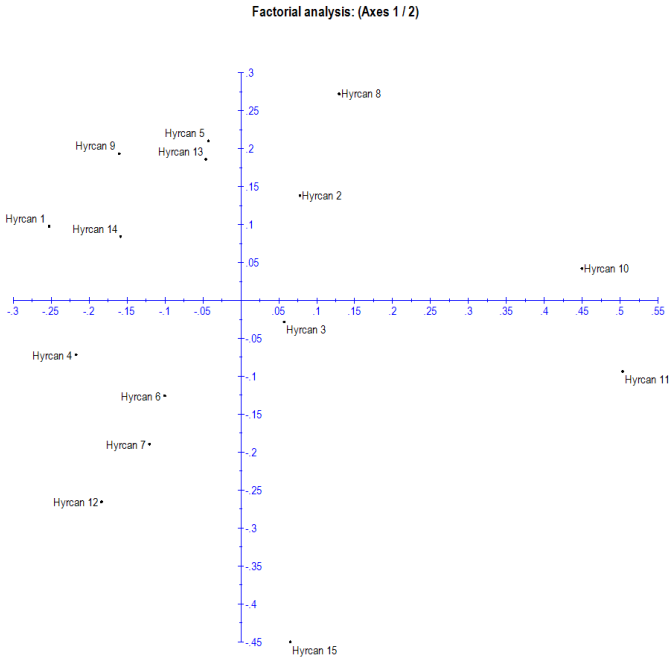


Figure 3. PCoA plot showing genetic variation among wild Hyrcanian figs of Accessions

Previous studies have focused predominantly on cultivated figs, with limited attention given to wild populations. For example, Khadari et al. [13] analyzed 75 fig accessions using eight ISSR and six SSR markers but were unable to fully discriminate between all cultivars due to genetic heterogeneity and naming

inconsistencies. In contrast, the current study successfully achieved complete discrimination of all 15 wild fig accessions using only five ISSR primers, demonstrating the high discriminatory power and efficiency of the selected markers in assessing genetic diversity among wild individuals. Our findings align with recent work by Haffar et al. [15], who reported clear genetic differentiation between cultivated and wild fig gene pools using RGA markers. Their results suggested that distinct RGA loci, potentially associated with adaptive traits such as resistance to biotic and abiotic stresses, have accumulated in wild populations. This further highlights the evolutionary and ecological significance of conserving wild fig germplasm as a reservoir of genetic diversity and resilience traits.

## CONCLUSIONS

This first genetic study of Hyrcanian figs demonstrated that ISSR markers are highly effective for assessing genetic diversity in wild *F. carica* populations. Using just five primers, all 15 genotypes were successfully differentiated, revealing moderate overall diversity and distinct genetic profiles. Cluster and PCoA analyses indicated substantial intra-population variation, unstructured by geographic proximity, suggesting the influence of gene flow and environmental heterogeneity. These results highlight the genetic variation among wild figs in Azerbaijan and emphasize the value of ISSR markers for their conservation, breeding, and taxonomic study.

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# CLIMATYPE BREEDING FEATURES OF *Pinus sylvestris* L. cv. Negorelskaya

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***Abstract***. *How can a new cultivar of Pinus sylvestris* L. cv. Negorelskaya*, ecologically sustainable and with increased productivity, be obtained by breeding 65 climatypes of Scots pine? The breeding of cultivars of higher plants has always been of interest and often occurred during the succession of several generations of scientists. The first generation of scientists founded the primary in 1959, and the secondary provenance trial plantation in 1965. The second generation continued the breeding process and received a breeder's patent in 2014. The first seed production of clones of Pinus sylvestris* L. cv. Negorelskaya *on a grafted forest-seed plantation begins at the age of 5; at the age of 15-17, the actual yield of varietal seeds on a hybrid-seed plantation is 11-15 kg per 1 ha.*

***Keywords: climate change, Pinus sylvestris L. cv. Negorelskaya, plant climatype, population breeding, provenance trial plantation.***

## INTRODUCTION

The Scots pine (*Pinus sylvestris* L.) has the world's widest range of vegetation (intrazonality) and economic use of plantings both in its natural form and in forest crops.

*P. sylvestris* grows on quite different types of soils, including sandy and rocky ones, due to the natural aerial seeding [1], which is carried out in its natural form due to the peculiarities of the physiological structure of the seed, which has a filmy wing. Physiologically, *P. sylvestris* seeds are similar in light [2], that is, there is no need for seeds to be embedded in the soil to initiate the germination mechanism by genes. Moreover, in the absence of lighting, the germination process

is inhibited. Therefore, the seed, having reached the crevice between the stones, and catching a portion of photons of solar radiation during the day, germinates, becomes fixed and continues to develop as a plant.

Due to the gradual warming of the climate [3], many international grant projects for the restoration of agroforestry landscapes are planning a roadmap for future experiments with coniferous plants, based on the principle of "moving seeds along the climatic gradient [4,5]", and, accordingly, the latitude–longitude gradient, from the place of growth to the place of experiment, most often from areas with a higher average annual temperature in a zone with a lower average annual temperature [5].

The conservation of genetic resources [6], as well as the breeding process of new Scots pine cultivars, are always based on the desire to ensure the best productivity and stress resistance of the future plant.

An interesting method of breeding *P. sylvestris* for obtaining new cultivars is the selection of seed sources according to provenance and the creation of a primary provenance trial plantation from selected individuals [7].

Firstly, when observing the ontogenesis of different *P. sylvestris* climatypes, it is possible to select the most promising climatypes based on the high level of genetic variability, which consists of the levels of variability in the stands of the donor regions of reproductive material. Secondly, it is possible to select according to the degree of resistance of climatypes to the influence of limiting environmental factors of the acceptor region. Thirdly, it is possible to select according to productivity and basic biometric indicators. The application of breeding methods [8,9] in the practice of forestry will make it possible to increase the productivity and environmental sustainability of the created stands.

We will demonstrate the peculiarities of breeding the cultivar of the common pine "Negorelskaya" based on climatypes.

## MATERIALS AND METHODS

In 1958, enterprising young scientists, Vasily Grigorievich Mishnev, a geobotanist, and Evgeny Dmitrievich Mantsevich, a specialist in the field of forest breeding, who worked at the Belarusian State Technological University (Minsk, Belarus), agreed on the issue of obtaining 200 different seed lots of *P. sylvestris* with the Central Control Station of Forest Seeds and the Belarusian Control Station of Forest seeds. After conducting a thorough forest typological analysis of the obtained seed batches, they selected 65 experimental batches of seeds collected in the most characteristic locations, from 48° to 62° north latitude and from 22° to 111° east longitude.

In 1959, in the Dzerzhinsky district of the Minsk region, on the territory of the Negorelsky Educational and experimental forestry of the Belarusian State

Technological University, a rectangular primary provenance trial plantation was established, elongated from north to south.

Topologically, geographical crops, including separate plots with an area of 0.1 hectares (40×25 m) of each of the 65 climatypes presented, are arranged as follows: from north to south – plots with seeds from large climatic regions, from west to east – according to the greatest geographical difference.

Geomorphologically, the site of geographical crops was a site after logging, where cultural and technical land reclamation was carried out with the uprooting of stumps and harvesting of felling residues, leveling the surface and plowing the cohesive sandy soil to a depth of 25 cm.

Annually, agrotechnical maintenance was carried out at the primary provenance trial plantation to remove weeds and improve gas exchange by mechanically loosening the soil with a special unit.

In 1965, on test-sites for *P. sylvestris* crop climatypes: Belgorod's (the sum of effective accumulated temperatures above 5.0 °C GDD5 = 2600; hydrothermal coefficient GTK = 1.3), Voronezh's (GDD5 = 2800; GTK = 1.2), Saratov;s (GDD5 = 3200; GTK = 0.8), Kirov's (GDD5 = 1800; GTK = 1.4) and Minsk's (GDD5 = 2550; GTK = 1.7) provenances [10] only female flowering was observed, therefore, a secondary provenance trial plantation was laid from the harvest of hybrid seeds (from pollination between each group and background pollen from the local *P. sylvestris* stand).

A detailed description of the breeding process from the climatypes of the Negorelskaya cultivar is presented by S.V. Rebko and co-authors [10], published in the journal "Botany. Research" at the V. F. Kuprevich Institute of Experimental Botany of the National Academy of Sciences of Belarus (Minsk). The variety has been included in the State Register of Tree and Shrub Varieties of the Republic of Belarus since 01.01.2014, and co-authors S. Rebko and L. Poplavskaya received a breeder's certificate.

## RESULTS AND DISCUSSION

The appearance of any cultivar is always associated with lengthy field experiments and causes extensive discussion, especially in matters of productivity.

From observations of plant growth and development, it has been established that the Negorelskaya cultivar is characterized by early and abundant seed production (the first seed production of clones on a grafted forest-seed plantation begins at the age of 5; at the age of 15-17 years, the actual yield of varietal seeds on a hybrid seed plantation is 11-15 kg of seeds per 1 ha), the presence of unusual cluster-like seed production for the common pine as one of the signs of reproductive heterosis (there may be from 3 to 32 cones in one bunch), more intensive growth of varietal offspring in test crops (an increase in height It can reach up to 50-80 cm in

one growing season).

For comprehensive research of the Negorelskaya cultivar, various experimental plantations have been established in the Minsk region over the years, the most extreme of which is in the Educational and Experimental Forestry of the Voronezh State Forestry University [11] (Voronezh Region, Russia) in the fall of 2023, in the spring and autumn of 2024, an experimental plantation of seedlings with closed roots produced from 1200 seeds of the Negorelskaya cultivar with known gravimetric and optometric characteristics within the framework of the Russian Science Foundation, grant 23-26-00228, included in the technological passport "seed – culture" [11].

An interesting question may be the question of the degree of reproduction of full-fledged seeds (sound seed) by plants of the Negorelskaya cultivar, as well as the effect of the seed coat color [14].

As shown by M. Sourso [12], the inbreeding coefficient of higher coniferous plants varies greatly when moving along the gradients of latitude and longitude, therefore, the number of germ-free seeds [13], unable to germinate, could have strong variability in experimental batches of geographical crops.

It would be interesting in future research by data [15] to consider the geographical seed plantation used for the breeding of the Negorelskaya cultivar according to the indicator (coefficient F) of inbred depression: how much and in what direction it has changed in geographical crops since the establishment of the forest-seed plot over 65 growing seasons.

## CONCLUSIONS

Breeding according to the climatypes of the Scots pine species provides sufficiently hardy and productive offspring, characterized by cluster-like fruiting, as well as increased activity of growth processes, which is not characteristic of *P. sylvestris* plants in natural habitats.

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# THE POSSIBILITY OF INTEGRATING THE REPUBLIC OF MOLDOVA INTO THE MULTILATERAL SYSTEM OF THE INTERNATIONAL TREATY ON PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE

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***Abstract.*** *Due to interdependence among nations regarding access to genetic materials, international cooperation through legal frameworks such as the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) is essential. The Treaty establishes the Multilateral System (MLS) to facilitate access to PGRFA and ensure fair and equitable benefit-sharing among stakeholders. This paper explores the global significance of the ITPGRFA and evaluates the Republic of Moldova's progress in implementing the Treaty and establishing the MLS. It outlines key challenges Moldova faces, including limited financial resources, lack of integrated agrobiodiversity policies, and the absence of a approved National Programme for PGRFA. Despite acceding to the Treaty in 2015 and participating in international sessions, Moldova has yet to fully integrate its national genetic resource collections into the MLS, but intend to do this. The study emphasizes the need for strengthened national legislation, stakeholder capacity building, and the inclusion of national data in MLS. It concludes that the ITPGRFA represents a vital tool for international collaboration and that Moldova must enhance its institutional and legislative frameworks to fully benefit from this global initiative.*

***Keywords: conservation, plant genetic resources, international cooperation, International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), Multilateral System (MLS).***

## INTRODUCTION

Effective use of plant genetic resources is vital for increasing food security and reducing poverty. While the vast majority of countries depend on plant genetic resources originating beyond their borders, international cooperation and agreements are essential for their access and conservation. There are three main reason for international cooperation in plant genetic resource conservation [1]

In first of all plant genetic resources (PGR) are essential for agricultural development, especially for increasing food production in order to promoting economic growth. The FAO estimates that global food production must rise by 70% by 2050 to meet increasing demand, despite the strain on natural resources, that are

limited [2]. PGR holds two key types of value: immediate value, where specific genes and traits—like pest resistance, drought tolerance, or flavor - are directly useful to plant breeders, and farmers and; long-term value, where overall genetic diversity acts as a safeguard against future challenges, helping ensure the resilience of farming systems locally and globally. Continued progress in plant breeding, using diverse genetic resources, is crucial to meet the rising global demand for food. Plant breeding is essential for developing high-yield, resilient crops that can withstand pests, diseases, and extreme weather. To achieve this, conserving crop diversity and ensuring access to it for breeders and farmers is critical. Secondly, collaboration is vital because countries heavily rely on plant genetic resources from other regions, especially for major crops. Even with local conservation efforts, continued access to diversity from the centers of origin of the crop species, is necessary to maintain traits. Thirdly, cooperation is economically necessary because genetic erosion, driven by the replacement of diverse traditional crop varieties with uniform modern ones, is accelerating. As plant genetic resources are a global public good, their conservation requires international agreement and action.

Many nations around the world are facing serious food security challenges, which are further intensified by global issues such as climate change, overpopulation, poverty, and the decline of agricultural biodiversity. Despite these obstacles, there is still hope—if each country acts individually and the global community collaborates, progress can be made. Working together to conserve, sustainably use, and share plant genetic resources is essential to securing food supplies now and in the future. This collaborative approach forms the foundation of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), which aims to provide countries with a legal structure to support these efforts and to promote fair and equitable sharing of the benefits derived from these vital resources.

The International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) represents a major step in global cooperation to fight hunger and poverty. It benefits various groups: consumers gain food variety and security; farmers' rights are protected; researchers will have access to vital plant genetic materials to aid in plant breeding and scientific study; international agricultural research centers (IARCs) will be acknowledged for their contributions and benefit from a legal framework supporting plant breeding, conservation, and access to their genetic collections; and both public and private sectors can use a broader genetic base for agricultural development [3]

The Treaty, adopted during the 31$^{st}$ session of FAO in November 2001 at Rome and came in force since June 2004 and ratified by 154 parties (data updated on April 1, 2025), aims to ensure sustainable agriculture and global food security. Its three core goals are the conservation of plant genetic resources (PGRFA), their

sustainable use, and the fair sharing of benefits arising from their use [4]. To support these objectives, the Multilateral System (MLS) was established under Article 10 of the ITPGRFA to facilitate access to PGRFA for plant breeders, scientists, and various other users and stakeholders including intermediaries as well as for fair and equitable sharing of monetary and non-monetary benefits arising from the utilization of PGRFA. This system enables the pooling and exchange of genetic materials from 64 essential food and forage crops (listed in Annex I) These crops were chosen due to their significance to global food security and the interdependence of countries, determined by historical factors like migration, trade, and climate change [5].

The MLS includes resources that are:

- Managed and publicly controlled by Contracting Parties;
- Voluntarily contributed by individuals or organizations;
- Held by international institutions with agreements under the Treaty.

Parties are required to adopt legal or suitable measures at the national level to implement the MLS and facilitate access to this global genetic pool.

The main concept behind a multilateral system for accessing and sharing the benefits of PGRFA is based on the principle, also affirmed by the Convention on Biological Diversity (CBD), in particular Article 15, that States have sovereign rights over their resources [6]. However, ITPGRFA recognizes that applying the CBD's model of negotiating access and benefits on a case-by-case and country-by-country basis is not suitable for agricultural biodiversity. This is because food crops have been cultivated, shared, and adapted across different regions of the world for thousands of years, often far from their place of origin. Modern collection and distribution practices have further spread genetic material globally, making it difficult to identify where a particular crop originated. As a result, determining benefits only based on the current location of the resource could lead to unfair outcomes. Furthermore, agricultural systems of all countries, depend on receiving genetic material from other countries and regions. Given this high level of interdependence, a multilateral approach—one that provides access to a wide pool of genetic resources—is more practical and fairer than trying to negotiate numerous individual agreements with each resource holder.

The multilateral system described in Part IV of the ITPGRFA serves two main goals: first, to provide easier access to PGRFA, thereby supporting the preservation and advancement of agricultural biodiversity; and second, to ensure that the benefits derived from using these resources are shared fairly and equitably, supporting broader sustainable development objectives [7]

The Plant Genetic Resources for Food and Agriculture (PGRFA) in the Multilateral System come from three main sources [8]:

- PGRFA listed in Annex 1 of the ITPGRFA that are publicly managed and controlled by Contracting Parties, that have ratified the treaty are automatically

included in the system (Article 11.2 of the ITGRFA).

- Natural and legal persons may voluntarily include PGRFA in the multilateral system, according to Annex 1. Contracting Parties are encouraged to promote such voluntary inclusion (Article 11.3)
- PGRFA maintained in ex situ collections held in trust by international institutions with Article 15 agreements are included. These institutions use the Standard Material Transfer Agreement (SMTA) to distribute these materials and may also use the SMTA for non-Annex 1 materials.

All materials shared through the multilateral system are transferred under the Standard Material Transfer Agreement (SMTA), adopted by the Governing Body of ITPGRFA in 2006. This agreement requires providers to offer the materials either free of charge or at minimal cost and to report all transfers to the Governing Body. It is important to follow certain conditions, including [9]:

1. Using the materials only for research, breeding, and training related to food and agriculture, not for industrial uses unrelated to food or feed (Article 12.3(a) of the ITPGRFA).
2. Not to seek intellectual property rights that would limit others' access to the materials 'in the form received' (Article 13.2(b) iii) of the ITPGRFA).
3. Contributing to the ITPGRFA's Benefit-sharing Fund if they commercialize new products using materials from the multilateral system and prevent others from using those products for further research or breeding.
4. Accepting that a third-party beneficiary, acting on behalf of the multilateral system, may request information to ensure compliance and initiate dispute resolution procedures if the terms of the SMTA are violated.

The Treaty established detailed rules and contract templates of SMTA, that can be used by individuals and organizations [10]

MLS is the largest pool of genetic resources available for exchange worldwide. According to Data Store of the International Treaty about germplasm flow within the Multilateral System through the Global Information System on Plant Genetic Resources for Food and Agriculture (GLIS), dated on 04.06.2025, 7.058.670 accessions are available. Of the total amount of material transferred, 87,7% was distributed by Contracting Parties, while 12,3% was distributed by non-Contracting Parties. Totally, 116.279 SMTA were signed, including 68 Provider Countries and 189 Recipient Countries [11]

The paper emphasizes that, alongside national conservation initiatives, globally managed collections, remain essential for safeguarding plant genetic resources on behalf of all humanity. It also highlights the need for a unified legal framework that includes international agreements, such as International Treaty on Plant Genetic Resources for Food and Agriculture, in order to enhance the collaboration at the international level for sustainable use of agrobiodiversity. These elements are important to securing both the conservation and accessibility of plant

genetic resources for food and agriculture, while also promoting fair benefit-sharing through the multilateral system of ITPGRFA. At the same time, this article analyzes the progress that the Republic of Moldova has achieved and is planning to make toward implementing the ITPGRFA and establishing the Multilateral System (MLS).

## MATERIALS AND METHODS

Primary reference materials used in present work included international legal instruments such as the International Treaty on Plant Genetic Resources for Food and Agriculture and the Convention on Biological Diversity. At the national level Law No. 94 of May 14, 2015, concerning the accession of the Republic of Moldova to the International Treaty on Plant Genetic Resources for Food and Agriculture, was analyzed. Additionally, was analyzed reports on the implementation of the MLS and on the Availability of Material in the Multilateral System. issued by the Secretariat of the International Treaty for the years 2023 (IT/GB-10/23/9.1) and 2022 (IT/GB-9/22/9.1), available at https://www.fao.org/plant-treaty/areas-of-work/the-multilateral-system/implementation-mls/en/. A comprehensive review and analysis of various official documents and statistical data published by the Food and Agriculture Organization (FAO) were also conducted. To assess the current status and operational effectiveness of the Treaty, international databases such as GLIS (Global Information System), WIEWS (World Information and Early Warning System on Plant Genetic Resources), as well as the ReGen national information system, were utilized.

## RESULTS AND DISCUSSIONS

The International Treaty on Plant Genetic Resources for Food and Agriculture was established in light of increasing concerns over the erosion of plant genetic diversity and the urgent need to safeguard these resources and promote their sustainable use for the benefit of future generations.

Moldova confronts with multiple obstacles in preserving its agrobiodiversity. The main problem is the absence of robust strategies to prevent agrobiodiversity loss. Financial incentives aimed at meeting biodiversity goals are limited, and both scientific and traditional conservation practices remain underutilized. Moreover, agrobiodiversity protection is not sufficiently incorporated into the country’s economic development plans or sectoral policies. During the 2016-2017 in the Republic of Moldova the FAO Project TCP / MOL / 3504 "Support to the development of a National Program for Plant Genetic Resources for Food and Agriculture (PGRFA) in Moldova" was implemented. The

main objective of the Project was to develop the National Program of the Republic of Moldova in the field of evaluation, conservation and sustainable use of plant genetic resources for food and agriculture. The aim of the program was to make the protection of agricultural biodiversity more efficient and to ensure the country's food security. Within the framework of this project, FAO provided technical support for strengthening and modernizing the capacity to conserve plant genetic resources. A large amount of analytical work has been done. As a result, the draft National Programme has been proposed. This document presents the deep analysis of existing problems and specific action plan developed for the nearest years. Unfortunately, due to some unclear reasons, the National Programme for PGRFA has not yet been approved by the decision-making authorities

In 2015, the Republic of Moldova acceded to the International Treaty on Plant Genetic Resources for Food and Agriculture (Law No. 94 of 14-05-2015). The Republic of Moldova participated in several meetings of the Governing Body of ITPGRFA. Between September 15–25, 2022, the Republic of Moldova participated in the 9th Session of the Governing Body of the International Treaty on Plant Genetic Resources for Food and Agriculture, organized by the Food and Agriculture Organization (FAO) of the United Nations. The event took place in New Delhi, India, in a hybrid format, and brought together approximately 600 participants from 150 countries that are signatories to the Treaty. During the regional consultations organized by the ITPGRFA Secretariat, the Republic of Moldova was designated as the coordinator of the European Regional Group. Among the main issues discussed at GB 9, which are critical to the future of plant genetic resources for food and agriculture, were: the enhancement of the Multilateral System (MLS) for Access and Benefit-Sharing (ABS), which had already been a central topic at GB 8; progress concerning farmers' rights, a key pillar in fulfilling the Treaty’s mandate; the connection with the ongoing negotiations for a post-2020 Global Biodiversity Framework (GBF) under the Convention on Biological Diversity (CBD), which aims to put biodiversity on a fast track to recovery.

The tenth session of its Governing Body (GB 10), in which the Republic of Moldova also participated, was held in Rome, Italy, from 20 to 24 November, under the theme “From seeds to innovative solutions, safeguarding our future: contributing to the implementation of the Global Biodiversity Framework for sustainable food systems.” At its 10th session, the Governing Body focused on advancing the implementation of the Treaty and its Multilateral System (MLS) for access and benefit-sharing (ABS). Key discussions included the progress and future steps in this direction. Topics addressed included digital sequence information, expanding the Annex I crop list, and revising the payment structure. The session also covered issues related to conservation, sustainable use, farmers’ rights, and collaboration with international frameworks, particularly the Convention on

Biological Diversity (CBD) and the Global Biodiversity Framework.

On ratifying the Treaty, countries agree to make their genetic diversity and related information about the crops stored in their genebanks and public domains available to all through the MLS. This obligation is stipulated in Articles 10–13 of the aforementioned Treaty. A report on the compliance with the provisions of the ITPGRFA was developed in the Republic of Moldova, highlighting both the strengths and weaknesses of the Treaty's implementation in our country. One identified weakness is the non-compliance with Article 13 of the Treaty – that is, data on plant germplasm collections conserved by institutions holding such collections at the national level are not included in the Multilateral System, as outlined in Annex I of the Treaty. In this context, it is necessary to initiate the preparation and submission of available data, stored in ReGen, regarding the conservation of agricultural diversity at the national level, to the Multilateral System of the ITPGRFA, through the Standard Material Transfer Agreement (SMTA).

## CONCLUSIONS

The ITPGRFA initiates a new direction in the history of international cooperation in field of sustainable conservation of plant genetic resources for food and agriculture and is an important instrument against hunger and poverty. While the Treaty is legally binding for all ratifying countries, this alone does not guarantee its effective implementation. Each country must establish appropriate regulatory frameworks to operationalize the Treaty's provisions and, where necessary, adopt or amend national laws to align with its requirements. The success in implementation of the ITPGRFA, especially of MLS depends on more than just legal measures. Expanded initiatives are required to strengthen the abilities of key stakeholders—such as plant breeders, gene banks, academic institutions, and farmers' organizations. To achieve this goal, the Republic of Moldova needs to adopt specific legislation on access to genetic resources and benefit-sharing at the national level."

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# EFFECT OF DROUGHT ON THE TOTAL PROTEIN COMPOSITION IN SUNFLOWER SEEDLINGS

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**Abstract.** *The aim of the work was to study the protein composition of seedlings of several sunflower genotypes under the influence of simulated drought. The protein composition was studied using SDS electrophoresis. The drought effect was created using PEG (6000). Drought-induced changes in protein composition were detected in only two of the six studied genotypes. In the Rf 2 genotype, under the influence of PEG (30%), 2 zones with low molecular weights accumulated over 6 days. In the ASC3 genotype, 3 zones of proteins with low molecular weights began to appear already at the first stage of drought, and they accumulated as the drought intensified. The amount of these zones increased under severe drought by almost 5-10 times compared to the control. The molecular weights of the proteins that accumulate during drought (D1, D2 and D3) range from 22 to 20 kDa, which is consistent with the molecular weights of dehydrins, which found in Helianthus annuus. The enhanced accumulation of these proteins under drought conditions may suggest that the ASC3 genotype exhibited greater drought tolerance and could be a promising parental line for breeding purposes.*

***Keywords: dehydrins, drought, genotype, protein composition, sunflower.***

## INTRODUCTION

Drought is one of the main climatic stresses, significantly reducing the yield of many agricultural plants [1]. Like other types of stress, water stress causes oxidative stress associated with an increase in the amount of reactive oxygen species (ROS) in plant cells [2]. ROS are highly reactive and can cause irreversible changes in the structures of proteins, nucleic acids, and membrane lipids [3]. Thus, water stress causes significant morphological, physiological, and biochemical changes in plants, which can lead to delayed plant growth and sometimes to the loss of part of the crop [4]. In plants resistant to various types of stress, the processes of ROS accumulation are usually inhibited. [5]. Plants capable of biosynthesis of various protective compounds have a high antioxidant status, allowing them to withstand unfavorable climatic conditions [6]. Therefore, analyzing breeding material for this trait is a crucial step in for effective selection.

An important role in maintaining plant viability under drought conditions

may be played by hydrophilic proteins, which help protect cells from dehydration [7]. These proteins belong to the group of dehydrins, which are classified as LEA proteins of class II [8]. The structural features of dehydrins include the presence of conserved sequences, designated K-, S- and Y-segments. The highly conserved K-segment is mandatory for all dehydrins [8]. The classification of dehydrins developed by Close T. is based on the number and combination of conserved segments in their structure. There are six classes of dehydrins: YnSKn, Kn, KnS, SKn, YnKn and FnSKn. Among them, the most common class of dehydrins is the YnSK2 class.

Dehydrins usually accumulate in seeds at late stages of plant development, but they are also found in vegetative tissues of plants under the influence of various stress factors [9]. Dehydrins are believed to prevent protein denaturation during dehydration and can also bind significant amounts of water, preventing tissue dehydration [10]. For example, barley dehydrins DHN1-DHN4, DHN6 and DHN9, which have the YSK2 structure, accumulate in seedlings in response to dehydration. [11]. Also, high levels of dehydrins have been found to correlate with plant resistance to drought and other types of stress. For sorghum plants (Sorghum bicolor (L) Moench), a correlation was also found between the drought resistance of varieties and the accumulation of dehydrins in their tissues [12]. Also was discovered that water stress increased the transcriptional activity of the dehydrin gene HaDhn2 of sunflower (*Heliantus annuus* L.) much more strongly in plants of the drought-tolerant variety compared to the drought-sensitive variety [13].

Dehydrins play an important role in alleviating dehydration stress in various rice species [14]. Among the dehydrins studied, the DHN8 gene of O. sativa ssp. japonica showed up to 448-fold increase in expression on the first day of stress treatment. In maize (*Z. mays*) seedlings, the level of transcripts for various dehydrins was significantly elevated under water deficiency [15]. However, the accumulation of dehydrin transcripts does not directly correlate with the content of the corresponding proteins. Therefore, the aim of our study was to investigate changes in the protein composition of seedlings of different sunflower genotypes under the influence of water stress.

## MATERIALS AND METHODS

The material for the study was seedlings of 6 sunflower genotypes: three lines with cytoplasmic androsterility (ASC1-ASC3) and three androfertile lines with restored fertility (Rf1-Rf3). To induce drought stress, a solution of PEG (6000) was used in a concentration of 20% and 30%. Plants watered with distilled water served as a control. Experimental samples at the stage of the first true leaf pair were treated with PEG for 3 or 6 days. Some of the plants were rehydrated after 3 days of PEG treatment, while in other samples the process of creating drought was

continued. Thus, 4 variants of experimental samples were obtained, differing in the degree of drought: 20% PEG (3 days), 30% PEG (3 days), 20% PEG (6 days), and 30% PEG (6 days).

For protein extraction from plant material was used 0.1 M Tris-HCl buffer pH 8.0 (100 mM EDTA, 0.2% ascorbic acid). The sample was ground in a mortar and extracted by stirring for 1 hour in a sample/buffer ratio of 1:4. After centrifugation at 12000 for 10 minutes, a sample was precipitated using TCA. The precipitated protein was washed from impurities three times: with 70% ethanol, 96% ethanol and acetone. Then the protein was dissolved in protein buffer for SDS-electrophoresis. Proteins were separated by denaturing SDS PAGE [16]. A Mini-PROTEAN Tetra Cell chamber (Bio-Rad Laboratories, Inc., USA) was used for electrophoresis. The concentration of the separating gel was 12.5% AA, and the stacking gel was 4%. All tracks were aligned by the amount of protein added. Protein concentration was measured using Bradford protein assay [17]. Protein Marker IV peqGOLD (Life Science) were used as markers. Stained and washed electrophoregrams were scanned on an Epson Expression 10000XL scanner (GE Healthcare, USA). The evaluation of each sample (Rf value of all bands in the lane, calculation of molecular weights based on the markers, and determination of bands intensity) was carried out using the Phoretix 1D Advansed software (TotalLab, Ltd., Great Britain).

## RESULTS AND DISCUSSIONS

The study of the total protein composition in sunflower seedlings leaves under drought stress of varying severity showed significant polymorphism of the response in different genotypes. Electrophoretic analysis of the studied sunflower genotypes under drought conditions showed that only two genotypes revealed noticeable changes in the total protein composition (Fig. 1). In the Rf 2 genotype, under the influence of severe drought, 2 protein zones (provisionally designated D1 and D3) with molecular masses of 21.7 kD and 19.6 kD accumulate. The abundance of zone D1 increases by 36%, and zone D3 - by 21% (Fig. 2).

In another sunflower line (ASC3), more significant changes in the protein composition were observed (Fig. 1). In this genotype, as water deficiency increased, three protein zones accumulated: D1 (Mr 21.7 kD), D2 (Mr 20.8 kD), and D3 (Mr 19.6 kD). The dynamics of accumulation of these zones, reflected in the diagram, indicates that in this genotype, already at the first stage of water stress (20% PEG/3 days), sharply increased synthesis of proteins with low molecular weight (Fig.2). Further intensification of drought stress resulted in even greater accumulation of these zones. The level of D1 protein level increased threefold at the first stage and further rose 4.5-fold as the drought intensified. Similarly, the amount of D2 protein also was accumulated 3.5-4.9 times. Zone D3 accumulates more than all others, it

increases 6.5 times at the first stage and 10 times at the last stage. The massive accumulation of low molecular weight proteins under drought stress in these two genotypes, as well as molecular weights of proteins, fluctuating from 22 to 20 kDa, may indicate that these proteins are dehydrins and play an important role in plant resistance to water stress.

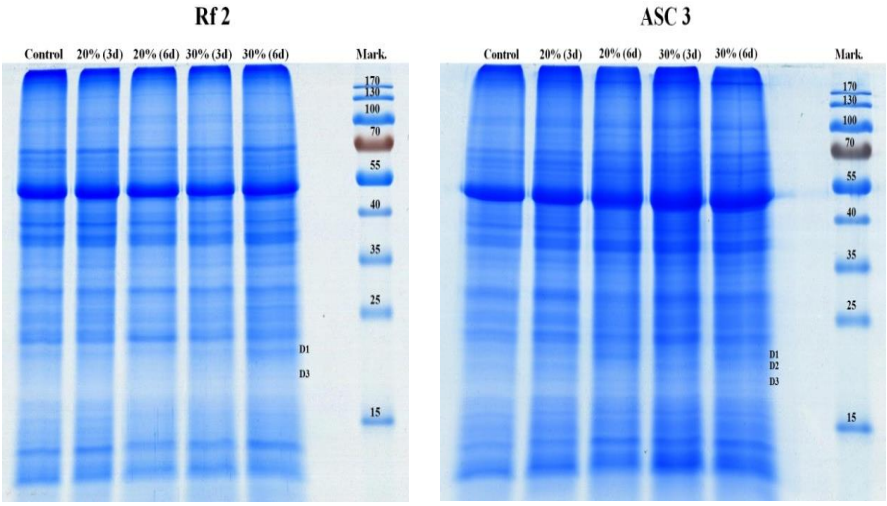


Fig. 1. Electrophoretic profiles of total protein in samples obtained during drought stress in sunflower genotype Rf 2 and ASC 3. The drought stress conditions are indicated above the tracks, the rightmost lane is the molecular mass markers (170-15 kDa).

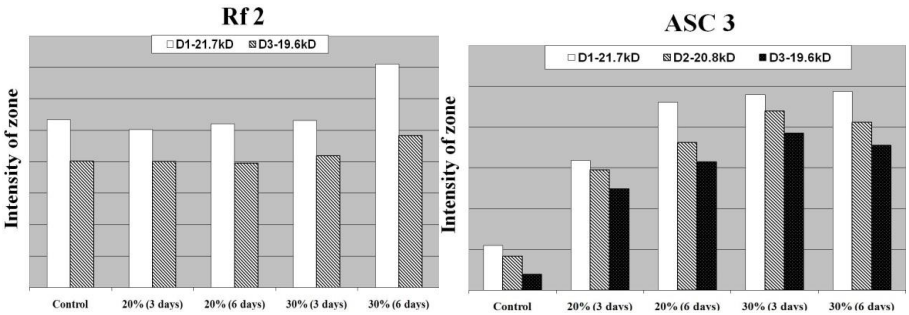


Fig. 2. Diagram of changes in the intensity of zones D1-D3 during increasing drought stress in the sunflower genotypes Rf 2 and ASC3.

In the drought-resistant sunflower genotype, the genes *HaDhn1* and *HaDhn2*, which accumulate under drought conditions, were identified [18]. The proteins encoded by these genes belong to the dehydrin family. The accumulation of *HaDhn1*, *HaDhn2* transcripts was compared in the resistant R1 genotype and in

the sensitive S1 genotype subjected to progressive drought. The results of the study confirmed that under drought conditions in the leaves of the resistant genotype R1, the accumulation of transcripts of dehydrins *HaDhn1, HaDhn2* was much higher than in the drought-sensitive genotype S1 plants [13]. According to the UniProtKB database, the molecular weights of dehydrin proteins found in sunflower range from 26 to 16 kDa [19].

## CONCLUSIONS

Accurate assessment of resistance of plant genotypes is crucial for the successful development of drought-tolerant varieties. Coordinated approaches involving traditional plant breeding as well as molecular approaches should be used to identify and develop drought-tolerant varieties. To speed up the selection process, laboratory methods for early diagnosis of seeds and seedlings are used, which makes it possible to carry out assessment all year round and study large volumes of selection material.

This study identified two sunflower genotypes exhibiting noticeable alterations in seedling protein composition under drought conditions. These two lines also displayed significant differences in both the intensity and the timing of this response. In the Rf 2 genotype, changes in protein composition were observed only under very severe drought conditions (30% PEG/6 days), with the increase in the quantity of two protein zones (D1 and D3) being insignificant, at 36% and 21%, respectively. In contrast, the ASC 3 line showed an increase in the amount of D1, D2, and D3 proteins even under mild drought conditions (20% PEG/3 days). The accumulation of these three protein zones continued to increase with escalating water stress, reaching an almost fivefold increase at the severe stress stage (30% PEG/6 days).

The molecular weights of the proteins that accumulated during water stress (D1, D2, and D3) ranged from 22 to 18 kDa, consistent with the molecular weights of dehydrins reported in *Helianthus annuus* [18, 19]. The enhanced accumulation of proteins under drought conditions suggests that the ASC3 genotype possesses greater drought tolerance and may serve a promising parental line for breeding purposes. To further substantiate these findings, subsequent studies should focus on evaluating the antioxidant activity in seedlings of the investigated genotypes under drought stress conditions. A concurrent analysis of antioxidant responses alongside other components of the plant defense system will provide a more robust and comprehensive assessment of genotype-specific drought tolerance.

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# Session IV

# BIOTECHNOLOGIES

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# INTEGRATION OF BIOINFORMATICS AND CHEMINFORMATICS FOR THE ANALYSIS AND CLASSIFICATION OF BIOACTIVE COMPOUNDS USED IN CARDIOVASCULAR DISEASES

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***Abstract***.*This paper presents a literature-based analysis on the integration of bioinformatics and chemoinformatics methods for identifying, classifying, and evaluating bioactive compounds with therapeutic potential in cardiovascular diseases. A detailed review of computational tools and molecular databases was conducted, emphasizing methods like QSAR modeling, virtual screening, and multi-omics integration. The research outlines a conceptual framework combining cheminformatics, bioinformatics, and machine learning techniques to enable efficient drug candidate classification and predictive modeling. The study concludes with directions for future research, particularly in enhancing data harmonization and model generalizability.*

***Keywords: artificial intelligence, bioinformatics, cardiovascular diseases, chemoinformatics, natural compounds.***

## INTRODUCTION

Cardiovascular diseases (CVDs) are the foremost cause of mortality globally, accounting for nearly 18 million deaths each year according to the World Health Organization. The increasing prevalence of heart-related disorders, coupled with the growing resistance to conventional drug therapies, underscores the urgent need for innovative approaches in the discovery and development of cardiovascular therapeutics. Traditional drug discovery processes are often time-consuming, expensive, and limited in scope when it comes to handling the complexity of cardiovascular pathophysiology. In this context, computational biology, particularly the integration of bioinformatics and chemoinformatics, provides a powerful alternative to traditional experimental workflows.

Bioinformatics facilitates the interpretation of complex biological data such as genomics, transcriptomics, and proteomics, offering insights into disease mechanisms, biomarker identification, and gene expression patterns [3]. Chemoinformatics, on the other hand, enables the analysis of chemical properties, structure–activity relationships, and virtual screening of large molecular libraries. The synergy between these two domains allows for a more comprehensive

understanding of how compounds interact with biological systems. The integration of these approaches is especially promising in the field of cardiovascular pharmacology, where understanding multi-target interactions and network effects is crucial.

Advances in artificial intelligence and machine learning have further accelerated this trend, enabling the construction of predictive models that can classify compounds based on toxicity, efficacy, and pharmacokinetic properties [1, 2]. These models are now being used to identify novel drug candidates from both synthetic and natural compound libraries with enhanced precision [4].

This paper aims to review recent developments in the use of integrated bioinformatics and chemoinformatics frameworks for the analysis and classification of bioactive compounds targeting cardiovascular diseases. It presents an overview of tools, databases, and algorithms that support this integration, highlights successful case studies, and proposes a conceptual model for streamlining candidate selection in early-stage drug discovery.

## MATERIALS AND METHODS

This paper is based on a comprehensive literature review combined with methodological analysis of current computational tools used in the integration of bioinformatics and chemoinformatics for cardiovascular drug discovery. The selection criteria for literature sources included peer-reviewed articles published between 2013 and 2024 that focused on cardiovascular pharmacology, compound screening, and the application of machine learning or multi-omics approaches. Databases such as PubMed, Scopus, and Web of Science were used to identify relevant publications using keywords including “cardiovascular diseases,” “bioinformatics,” “chemoinformatics,” “QSAR,” “molecular docking,” “multi-omics,” and “machine learning” [1].

To simulate a standardized methodological pipeline, several open-access and academic-grade software tools were analysed:

- **KNIME Analytics Platform** was used for data integration, feature engineering, and machine learning model construction. Its modular design allowed integration of cheminformatics nodes (RDKit) and bioinformatics workflows (e.g., sequence analysis, BLAST, GO term mapping).
- **AutoDock Vina** served as the primary tool for molecular docking simulations. Selected ligands were docked against known cardiovascular protein targets including ACE (angiotensin-converting enzyme), HMG-CoA reductase, and β1-adrenergic receptor. Protein structures were retrieved from the Protein Data Bank (PDB).
- **SwissADME** and **pkCSM** were employed to predict pharmacokinetic and toxicity profiles of selected compounds. These tools assess ADMET properties such

as gastrointestinal absorption, blood–brain barrier penetration, CYP450 inhibition, and mutagenicity.

- **STRING** and **Cytoscape** were used for protein–protein interaction (PPI) network construction and visualization. These tools allowed identification of key hub proteins and signaling pathways associated with hypertension, atherosclerosis, and arrhythmias.
- **Multi-omics data** (transcriptomics, proteomics, metabolomics) were extracted from public repositories such as the Gene Expression Omnibus (GEO) and EMBL-EBI ArrayExpress. Data normalization was carried out using standard z-score and quantile transformation methods.

The compound datasets analyzed included natural product libraries (e.g., NPASS, COCONUT) and DrugBank cardiovascular drug subsets. Structural descriptors such as topological polar surface area (TPSA), molecular weight, rotatable bonds, and logP were computed using PaDEL and RDKit. Biological descriptors included gene expression fold change, pathway activation scores, and disease association indices.

Machine learning classifiers such as support vector machines (SVM), random forest (RF), and convolutional neural networks (CNN) were trained using compound–target interaction matrices as input. Model performance was evaluated using 10-fold cross-validation, with metrics including accuracy, precision, recall, and area under the ROC curve (AUC).

All analytical steps adhered to FAIR data principles and reproducibility best practices. The proposed pipeline serves as the foundation for the conceptual model presented in the following sections.

## RESULTS AND DISCUSSIONS

The literature review confirmed the increasing importance of combining bioinformatics and chemoinformatics in the discovery and analysis of compounds used in cardiovascular therapy. This section presents key thematic findings derived from recent scientific publications.

### 1. Computational Tools in Literature

Numerous reviewed studies report successful use of silico tools to accelerate compound screening for cardiovascular diseases. Cheminformatics techniques such as QSAR modeling and molecular docking are frequently applied to evaluate ligand–target interactions for enzymes like HMG-CoA reductase, ACE, and β-adrenergic receptors. Studies like Osipova et al. [1] show how machine learning models can be trained on drug–target interaction data to support early-stage candidate prioritization.

**2. Integration with Bioinformatics**

An important trend in recent publications is the integration of chemical structure-based predictions with bioinformatics data. This includes pathway analysis, protein–protein interaction networks, and transcriptomic profiles relevant to cardiovascular conditions such as atherosclerosis, hypertension, and myocardial infarction. Zhang et al. [3] describes how multi-omics data improves the biological relevance of compound selection, linking predicted molecular effects to gene expression and disease pathways.

**3. Machine Learning and Predictive Modeling**

Several articles reviewed demonstrate the use of supervised learning techniques such as SVM, random forests, and deep learning for compound classification. These models are built using descriptors like molecular fingerprints and structural data, combined with biological effect data from experimental studies. Ivanov et al. [2] reported high accuracy in toxicity classification of cardiovascular compounds using convolutional neural networks on molecular images, suggesting promise for automated compound triage.

**4. Relevance of Natural Compounds**

The review also highlighted the significant role of natural products in cardiovascular pharmacology. Natural compounds such as flavonoids, alkaloids, and terpenoids were frequently reported as effective against oxidative stress, endothelial dysfunction, and inflammation. The work of Newman and Cragg [4] reinforces the notion that natural scaffolds remain a major source of novel cardiovascular agents.

**5. Challenges Reported in Literature**

Despite encouraging progress, researchers repeatedly point out limitations such as data inconsistency across databases, lack of standardization in omics formats, and limited availability of high-quality labeled datasets for training machine learning models. A recurring theme is the need for interoperable frameworks and annotated compound–disease interaction databases.

## CONCLUSIONS

This literature review highlights the growing convergence of bioinformatics and chemoinformatics in cardiovascular drug research. The analysis of recent studies suggests that this integration offers significant potential for improving the prediction, classification, and understanding of bioactive compounds relevant to cardiovascular diseases.

Publications reviewed in this paper consistently emphasize that cheminformatics tools — particularly QSAR modeling, molecular docking, and chemical fingerprinting — are widely used to predict compound–target interactions. Meanwhile, bioinformatics provides the biological context, offering

insights into gene expression patterns, molecular pathways, and protein–protein interaction networks involved in cardiovascular pathology.

Together, these fields form the basis for data-driven frameworks capable of supporting early-stage drug discovery. Studies using machine learning models on integrated datasets demonstrate promising accuracy in toxicity prediction, target affinity estimation, and therapeutic classification. This approach reduces experimental costs and supports high-throughput compound triage.

A particularly strong theme in the reviewed literature is the relevance of natural compounds in cardiovascular pharmacology. Numerous studies report the successful identification of polyphenolic, alkaloid, and terpenoid compounds with multi-target effects, often mediated through anti-inflammatory, antioxidant, or vasodilatory mechanisms.

However, the integration of bioinformatics and chemoinformatics is not without its limitations. Many studies cite challenges such as data heterogeneity, lack of standardized formats for biological data, and limited publicly available, well-annotated datasets. These issues limit reproducibility and the scalability of predictive models.

In conclusion, while the reviewed evidence supports the effectiveness of integrated computational approaches in cardiovascular drug research, further efforts are needed to develop unified platforms, standardized descriptors, and high-quality open-access datasets. These advancements will be essential for translating computational predictions into clinically relevant drug candidates.

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# ANTIFUNGAL ACTIVITY OF LIPID FRACTIONS OF MEDICINAL PLANTS AGAINST FUSARIUM ISOLATES FROM INFECTED GARLIC CLOVES

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**Abstract.** Garlic (*Allium sativum* L.) is widely cultivated for its culinary and medicinal value but is highly susceptible to fungal diseases, particularly those caused by Fusarium species. This study evaluated the antifungal activity of lipid fractions from seven medicinal plants against Fusarium isolates obtained from infected garlic cloves. The lipid fractions were added to PDA medium and fungal growth was monitored over nine days. The lipid fractions of Eucalyptus mellidora and Salvia officinalis significantly reduced mycelial growth of both isolates compared to the control, suggesting strong antifungal potential. Other oils exhibited moderate or low activity. These findings support the potential use of selected plant-derived lipid fractions as natural fungicides in garlic protection. Further research is needed to validate efficacy under field conditions.

***Keywords: antifungal activity, essential oils, Fusarium spp., garlic, medicinal plants.***

## INTRODUCTION

Garlic (*Allium sativum* L.) is a globally cultivated vegetable crop valued not only for its culinary uses but also for its medicinal properties, including antimicrobial, anti-inflammatory, and antioxidant activities [1]. Despite its bioactive potential, garlic is highly susceptible to a range of soil-borne fungal pathogens, among which species of the genus *Fusarium* are among the most damaging [2]. *Fusarium* spp. cause serious diseases such as basal rot and bulb rot,

leading to significant yield losses during both cultivation and post-harvest storage [3]. Infected garlic cloves typically show symptoms like softening, discoloration, and decay of the basal plate, which compromise both market quality and seed viability.

Control of *Fusarium*-related diseases remains challenging due to the lack of effective and environmentally safe fungicides registered for use on garlic. Furthermore, the development of fungicide resistance among fungal populations and increasing regulatory restrictions on chemical pesticides have underscored the need for alternative plant protection strategies [4].

Despite growing interest in phytochemicals for disease control, relatively few studies have focused on their use against *Fusarium* isolates from garlic, and even fewer have examined the comparative activity of multiple plant oils under uniform *in vitro* conditions. Therefore, a systematic evaluation of essential oils from selected medicinal plants is warranted to identify potential biofungicides that can be incorporated into pre- and post-harvest management strategies for garlic crops.

In this context, essential oils derived from aromatic and medicinal plants have emerged as promising candidates for integrated disease management [5-7]. These lipid-based fractions are complex mixtures of volatile organic compounds, including monoterpenes, sesquiterpenes, and phenolic compounds, known for their broad-spectrum antimicrobial activities [8]. Numerous studies have demonstrated the antifungal effects of essential oils against plant pathogens, including various *Fusarium* species. However, their efficacy is highly variable and depends on the botanical source, chemical composition, mode of application, and target organism.

The present study was designed to evaluate the *in vitro* antifungal activity of lipid fractions extracted from the aerial parts of seven medicinal plant species against *Fusarium* spp. By comparing the inhibition of radial mycelial growth across treatments, this research aims to identify plant-derived substances with strong antifungal potential and practical relevance for sustainable agriculture.

## MATERIALS AND METHODS

### Fungal Isolates

Two *Fusarium* spp. isolates were obtained from the basal plates of garlic cloves collected in 2023. Isolates were cultured on Potato Dextrose Agar (PDA) for 10 days at room temperature.

### Essential Oil Samples

Lipid fractions (essential oils) were obtained from the aerial parts of seven medicinal plants: *Laurus nobilis* (bay leaf), *Cetraria islandica* (Iceland moss), *Eucalyptus mellidora*, *Salvia officinalis* (sage), *Amaranthus* sp., *Origanum vulgare* (oregano), and *Calendula officinalis* (calendula). The lipid fractions were prepared via low-temperature liquefied gas extraction using chlorodifluoromethane [9, 10]. The

extraction was carried out at temperatures ranging from 12 °C to 32 °C and pressures from 0.6 to 1.2 MPa over a 10-hour period. The resulting extract had a yield of about 5% from the dry mass.

**Preparation of Media and Treatments**

To evaluate antifungal activity, 1% (v/v) of each essential oil was mixed into melted PDA medium supplemented with 0.05% (v/v) Tween 80 (Polysorbate 80) as a non-ionic emulsifier to facilitate oil dispersion. The PDA-oil mixtures were immediately poured into sterile 90 mm diameter Petri dishes (10 mL per dish). After solidification, a 5 mm agar plug taken from the periphery of a 7-day-old *Fusarium* colony was placed mycelium-side down in the center of each dish. The control group consisted of PDA plates containing Tween 80 but no essential oils.

Each treatment (essential oil × isolate combination) was replicated ten times. All plates were incubated at room temperature, and radial mycelial growth was measured every 24 hours using a millimeter ruler until the control colonies reached the edges of the Petri dish.

**Growth Rate and Inhibition Analysis**

The antifungal effect was quantified by calculating the daily radial growth rate (V) using the formula: $V = (Rx - R0)/T$,

where $R_x$ is colony radius on day x, $R_0$ is initial radius, and T is the number of incubation days.

Statistical analysis of mean growth rates and standard deviations was performed using STATISTICA, version 14.0.0.15 from TIBCO Software Inc. (Palo Alto, California, USA). Significance of inhibition was evaluated visually through growth kinetics over 9 days, with particular attention to variance between isolates.

## RESULTS AND DISCUSSION

The antifungal activity of lipid fractions derived from various medicinal plants was assessed by measuring the radial growth of two *Fusarium* isolates over a 9-day period. The results clearly demonstrated significant inhibition of mycelial growth for both isolates compared to the control, with the level of suppression varying depending on the plant species from which the lipid fraction was derived.

Figure 1 illustrates the progressive growth of both *Fusarium* isolates in untreated control conditions, showing rapid radial expansion and complete colonization of the Petri dish by day 9. In contrast, essential oils from several plant species reduced colony expansion substantially, as seen in the daily measurements (Table 1).

Among the tested essential oils, those from *Eucalyptus mellidora* and *Salvia officinalis* demonstrated the most pronounced antifungal effects. On day 9, the average colony diameter of F1 in the presence of *Eucalyptus* oil was $1.39 \pm 0.12$ mm/day, and for F2, it was only $0.95 \pm 0.05$ mm/day—both markedly lower than the respective control values (2.5 mm/day and 2.64 mm/day). Similarly, *Salvia officinalis* inhibited F1 to

1.09 ± 0.02 mm/day and F2 to 0.96 ± 0.04 mm/day by day 9.

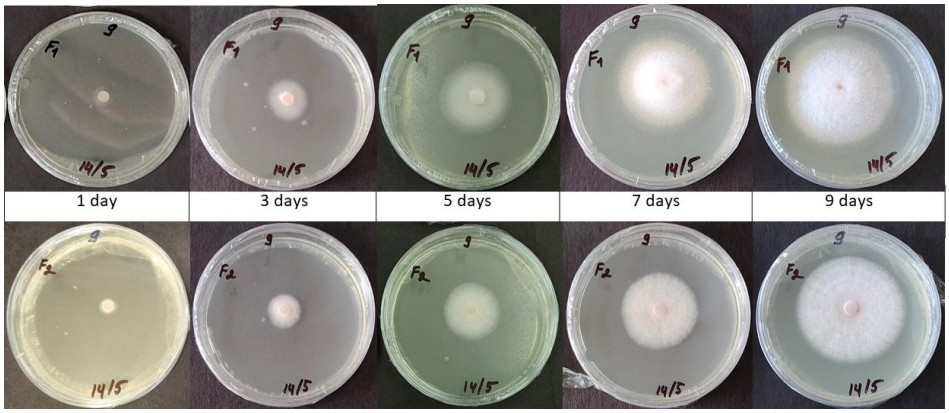


Figure 1. Progressive radial growth of two Fusarium isolates on control PDA medium over 1, 3, 5, 7, and 9 days of incubation at room temperature.
*Colonies expand rapidly in the absence of essential oil treatment, reaching full Petri dish coverage by day 9.*

Table 1. Daily radial growth (mm/day) of Fusarium isolates cultured on PDA medium supplemented with 1% essential oils from selected medicinal plants.
*Values are expressed as mean ± standard deviation (n = 10)*

| Fusarium isolates | F 1 | | | F 2 | | |
|---|---|---|---|---|---|---|
| Days of incubation | 1 | 5 | 9 | 1 | 5 | 9 |
| Control | 2,0±0,281 | 3,36±0,079 | 2,5±0,000 | 1,79±0,100 | 2,53±0,090 | 2,64±0,061 |
| *Laurus nobilis* | 1,75±0,144 | 2,93±0,011 | 2,89±0,016 | 1,04±0,042 | 2,28±0,040 | 2,46±0,017 |
| *Cetraria islandica* | 1,29±0,164 | 2,28±0,060 | 2,37±0,054 | 1,33±0,083 | 2,28±0,028 | 2,01±0,021 |
| *Eukaliptus sp.* | 0,88±0,056 | 1,37±0,011 | 1,39±0,120 | 0,71±0,100 | 0,97±0,054 | 0,95±0,049 |
| *Salvia officinalic* | 0,88±0,191 | 1,19±0,035 | 1,09±0,019 | 0,83±0,105 | 0,94±0,035 | 0,96±0,035 |
| *Amaranthus sp.* | 1,37±0,155 | 2,23±0,060 | 1,88±0,067 | 1,08±0,083 | 1,54±0,057 | 1,49±0,049 |
| *Oregano vulgaris* | 1,38±0,107 | 2,64±0,045 | 2,07±0,035 | 1,42±0,083 | 1,69±0,039 | 1,54±0,017 |
| *Kalendula officinalis* | 1,38±0,085 | 1,69±0,051 | 1,56±0,023 | 0,96±0,042 | 1,04±0,052 | 1,26±0,086 |

This consistent and substantial inhibition suggests a strong fungistatic or even fungicidal effect, likely attributable to the high concentrations of bioactive monoterpenes such as eucalyptol and thujone. These compounds have well-documented antimicrobial properties, including disruption of fungal membrane

integrity and interference with spore germination [11].

Other essential oils such as those from *Cetraria islandica*, *Amaranthus* sp., and *Origanum vulgare* also demonstrated moderate inhibitory effects, with reductions in growth ranging between 20–40% compared to controls. Lipid fractions from *Calendula officinalis* and *Laurus nobilis* showed the least antifungal activity but still provided measurable suppression relative to untreated conditions.

While both *Fusarium* isolates responded similarly to most treatments, slight differences in sensitivity were observed. For example, F2 was marginally more sensitive to *Salvia* lipid fractions at all time points, suggesting a possible variation in membrane composition or metabolic adaptability between species.

These findings support the hypothesis that essential oils from selected medicinal plants can serve as natural antifungal agents against soil-borne pathogens. The strong inhibitory effect of *Eucalyptus* and *Salvia* oils underscores their potential for integration into eco-friendly disease management programs in garlic cultivation. However, further *in vivo* testing and formulation development are required to optimize delivery methods, dosage, and stability under field conditions.

## CONCLUSIONS

The results of this study demonstrate that essential oils derived from medicinal plants possess notable antifungal properties against two pathogenic *Fusarium* isolates affecting garlic. In particular, lipid fractions from *Eucalyptus mellidora* and *Salvia officinalis* exhibited the most significant inhibitory effects on radial mycelial growth of both *Fusarium* isolates under *in vitro* conditions. These findings are consistent with the known bioactivity of monoterpenes such as eucalyptol and thujone, which likely contribute to the observed antifungal effects.

The broad-spectrum activity and reproducibility of these oils suggest that they hold strong potential as environmentally friendly alternatives to synthetic fungicides, which are increasingly limited by resistance development and regulatory restrictions. Importantly, the ability to suppress pathogenic fungi at relatively low concentrations (1% v/v) highlights their practical value for integrated disease management strategies.

Nevertheless, the transition from laboratory efficacy to field application requires further investigation. Future research should focus on evaluating the performance of these essential oils in greenhouse and open-field trials, their phytotoxicity to garlic plants, their formulation stability, and their long-term impact on soil microbiota and crop yield. Additionally, synergistic interactions among multiple plant oils and their components warrant exploration to optimize antifungal formulations.

In summary, the study supports the feasibility of incorporating plant-derived

lipid fractions, especially from *Eucalyptus* and *Salvia*, into sustainable garlic protection practices and contributes to the growing body of evidence advocating for biopesticide development from botanical sources.

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# BIOTECHNOLOGICAL VALORIZATION OF *ARTHROSPIRA PLATENSIS* FOR HEAVY METAL AND RARE EARTH ELEMENT RECOVERY FROM MULTIMETALLIC EFFLUENTS

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Abstract

*This study aimed to evaluate the bioaccumulation capacity of the cyanobacterium Arthrospira platensis for heavy metals and rare earth elements (REEs) from mono- and polymetallic effluents. Experimental systems were enriched with metals such as Ni, Cu, Zn, Cr, Fe, Sr, Mo, and REEs (Dy, La, Tb, Nd, Sm, Yb) at various concentrations. Metal accumulation in biomass was quantified using neutron activation analysis analysis. Biomass productivity was assessed spectrophotometrically based on a calibration curve, while oxidative stress was evaluated via malondialdehyde (MDA) content using the TBARS assay. Results demonstrated high accumulation capacities, with notable values for Cu (2870 mg/kg), Zn (up to 22500 mg/kg), Ni (1310 mg/kg), and La (>90% recovery). Biomass growth and MDA levels varied depending on the metal combination and concentration, indicating stress responses. A. platensis showed consistent resilience and was effective as a biosorbent, especially under moderate oxidative stress. The findings highlight its applicability in the bioremediation of wastewater containing diverse metal contaminants and its potential use as a matrix for biofortified supplements.*

***Keywords***: ***Arthrospira platensis, bioaccumulation, heavy metals, oxidative stress, rare earth elements.***

## INTRODUCTION

Environmental pollution by toxic metals and rare earth elements (REEs) is an escalating global challenge, largely driven by urbanization, industrial growth, and unsustainable resource use. As a result, hazardous substances, including heavy metals and REEs, are increasingly released into natural ecosystems, with water resources being especially vulnerable. Less than 3% of the world's water is

drinkable, and millions of tons of waste, including hundreds of tons of heavy metals, enter aquatic systems annually [1].

Research priorities have shifted from municipal and agricultural wastes to more complex contaminant mixtures, including microcontaminants, plastics, persistent organics, and REEs, all of which threaten environmental stability [2]. Effective management now requires innovative, sustainable solutions that move beyond traditional, energy-intensive physico-chemical treatments [3-6]. Biotechnological approaches, particularly bioremediation, offer eco-friendly and cost-effective alternatives by utilizing the metabolic potential of microorganisms to remove or transform pollutants.

Phototrophic cyanobacteria such as *Arthrospira platensis (Spirulina)* are promising bioremediators due to their minimal environmental requirements, mainly $CO_2$ and light [7-9], and high metabolic versatility. These microorganisms can efficiently accumulate and transform a broad spectrum of metal ions, including REEs, and their resulting biomass can be valorized for use as fertilizers or sources of bioactive compounds.

The application of *A. platensis* for metal and REE recovery aligns with recent advances in metabolic engineering and systems biology, enabling improved bioremediation efficiency and product valorization [10]. Its ability to thrive in diverse environments and its GRAS status further support its potential as a sustainable resource for wastewater treatment and resource recovery.

This study investigates the biotechnological valorization of *Arthrospira platensis* for recovering heavy metals and rare earth elements from multimetallic effluents, with the goal of developing integrated processes for both environmental remediation and biomass utilization.

## MATERIALS AND METHODS

*Object of Study.*The object of study was the cyanobacterium *Arthrospira platensis*, a well-characterized strain (CNMN–CB-02) obtained from the National Collection of Nonpathogenic Microorganisms (Chisinau, Republic of Moldova).

*Experimental Designs.* To comprehensively evaluate the bioaccumulation capacity and physiological responses of *A. platensis*, four experimental models were established, each simulating different types of multimetallic contamination or supplementation scenarios relevant to environmental and biotechnological applications:

1. Nickel-Based Multimetallic Effluent Model: *A. platensis* was cultivated in mineral medium supplemented with nickel as a single metal or in combination with chromium, iron, copper, zinc, strontium, and molybdenum. Cultures underwent four consecutive cultivation cycles to simulate long-term exposure and adaptation to multimetallic synthetic effluents.

2. Zinc-Based Multimetallic Systems: Cultivation was performed in four systems: (i) zinc alone, (ii) zinc with copper and strontium, (iii) zinc with copper and nickel, and (iv) zinc with copper, strontium, and barium. In each case, three increasing concentrations of metal ions were added on the fifth day of growth, at the onset of the stationary phase. The exposure lasted for 24 hours before biomass harvesting.

3. Rare Earth Element (REE) Model: *A. platensis* was exposed separately to dysprosium (Dy), samarium (Sm), terbium (Tb), lanthanum (La), neodymium (Nd), and ytterbium (Yb), each at three concentrations (10, 20, and 30 mg/L). Cultures were maintained for five days, after which the biomass was harvested for analysis.

4. Lithium Bioaccumulation Model: Lithium carbonate ($Li_2CO_3$) was introduced to the growth medium at concentrations ranging from 5 to 500 mg/L, either at inoculation or during the exponential growth phase, to investigate lithium enrichment and its physiological impact.

*Analytical Methods*

Following cultivation, the biomass was collected, washed, and dried for subsequent analyses. Metal accumulation in the biomass was quantified using neutron activation analysis (NAA) for transition metals and rare earth elements, and Proton Induced Gamma Emission (PIGE) analysis for lithium. Total protein content was measured spectrophotometrically by the Lowry method. The malondialdehyde (MDA) content was measured using the thiobarbituric acid reactive substances (TBARS) assay. All experiments were conducted in at least three replicates, and results were evaluated for statistical significance.

## RESULTS AND DISCUSSION

This study comprehensively evaluated the bioaccumulation capacity and biochemical responses of Arthrospira platensis under exposure to a wide spectrum of metals, including transition metals, rare earth elements, and lithium, using four complementary experimental models.

Heavy Metal and Multimetallic Effluent Exposure. The first two experimental models focused on the ability of A. platensis to accumulate nickel, zinc, and other metals from mono- and multimetallic synthetic effluents. The results, summarized in Table 1, show that both nickel and zinc were efficiently accumulated from all tested systems, with the highest nickel content (1310 mg/kg) observed in the Ni/Cr/Fe variant and maximum zinc accumulation (up to 23-fold over control) in certain zinc-based mixtures. Alongside nickel and zinc, substantial uptake of additional metals - chromium, iron, copper, strontium, barium, and molybdenum, was observed depending on system composition. Notably, in most systems, accumulation peaked in the first or second cultivation cycle, then

stabilized or declined, reflecting both high initial uptake and subsequent cellular adaptation or efflux.

Exposure to metal mixtures led to a transient reduction in biomass productivity (up to 12% after the first cycle), but yields typically recovered in subsequent cycles, indicating physiological adaptation. Total protein content showed a similar dynamic: initial declines (up to 35% in some multi-metallic systems) were followed by partial or full recovery with prolonged cultivation. MDA levels, measured by the TBARS assay, increased during the initial exposure cycles (up to 2.5-fold), especially under multi-metallic stress, but subsequently decreased, indicating an enhanced antioxidative response in adapted cells.

Table 1. Metal bioaccumulation, protein content, and MDA Levels in *Arthrospira platensis* exposed to mono- and multimetallic systems

| **Element /elements** | **Metal concentration, mg/l** | **Total proteins % of biomass** | **MDA, nM/g of dry biomass** | **Bioaccumulation capacity, mg/kg of biomass** |
|---|---|---|---|---|
| Zn | 2,5-10,0 | 64.1 – 71.4 | 12.0 – 14.8 | 90 - 16000 |
| Zn/Cu/Sr | Zn 2,5-10,0<br>Cu 1,0-5,0<br>Sr 0.5-2,0 | 42.2 – 65.3 | 15.2 – 20.7 | Zn 250.0-20000.0<br>Cu 125.0-500.0<br>Sr 5.0-50.0- |
| Zn/Cu/Ni | Zn 2,5-10,0<br>Cu 0,5-2,0<br>Ni 0.5-2,0 | 43.2 – 66.0 | 15.2 – 18.9 | Zn 750.0-20500.0<br>Cu 60.0-250.0<br>Ni 40.0-240.0 |
| Zn/Cu/Sr/Ba | Zn 2,5-10,0<br>Cu 0,5-2,0<br>Ni 0.5-2,0<br>Ba 0,5-1,0 | 43.1 – 66.0 | 15.0 – 15.9 | Zn 120.0-22500.0<br>Cu 125.0-500.0<br>Sr 5.0-50.0<br>Ba 60.0-25.00 |
| Ni | 2,5-10 | 52.5 - 71.4 | 10.4 – 16.5 | 50-250 |
| Ni/Cr/Fe | Ni 2,5-10<br>Cr 1,0-5,0<br>Fe 1,0-5,0 | 51.0 – 65.4 | 15.8 – 20.8 | Ni 50.0-1310.0<br>Cr 1.05-110.0<br>Fe 2500.0-17000.0 |
| Ni/Cu/Sr/Zn | Ni 2,5-10<br>Cu 0,5-1,0<br>Sr 1,0-5,0<br>Zn 0,5-2,0 | 51.8 – 72.1 | 17.2 – 28.4 | Ni 60.0-51.00<br>Cu 70.0-820.0<br>Sr105.0-1370.0<br>Zn 20.0-1860.0 |
| Ni/Cu/Zn/Mo | Ni 2,5-10<br>Cu 1,0-5,0<br>Zn 0,5-2,0<br>Mo 0,5 | 40.2 – 64.3 | 15.8 – 44.6 | Ni 40.0-480.0<br>Cu 50.0-2870.0<br>Zn 20.0-1750.0<br>Mo 3,5-4,4 |

*Rare Earth Element (REE) Bioaccumulation*. In the third experimental model, A. platensis demonstrated selective and efficient bioaccumulation of rare earth elements, particularly La and Dy (up to 39.8 and 25.5 mg/g, respectively),

with removal efficiencies exceeding 85% even at elevated concentrations. Other elements (Sm, Nb) were accumulated at moderate levels, while Tb and Yb showed limited uptake. REE exposure had minimal impact on biomass productivity and total protein content, except for high ytterbium concentrations, which reduced growth and increased oxidative stress (MDA up to 60% above control). For most REEs, MDA and protein content remained near control values, suggesting low toxicity under tested conditions (Table 2).

Table 2. RREs bioaccumulation, protein content, and MDA levels

| **Element** | **Element concentration, mg/l** | **Total proteins % of biomass** | **MDA µM/L** | **Bioaccumulation capacity, %** |
|---|---|---|---|---|
| La | 10-30 | 64 - 66 | 1.10 – 1.30 | 88-95% |
| Dy | 10-30 | 66 - 70 | 1.20 – 1.60 | 85-90% |
| Tb | 10-30 | 62 - 65 | 0.70 – 0.95 | <20% |
| Yb | 10-30 | 62 - 66 | 2.00 – 2.10 | <20% |
| Sm | 10-30 | 59 - 62 | 1.10 – 1.35 | 42-97% |
| Nd | 10-30 | 60 - 66 | 1.75 – 1.90 | 46-86% |

*Lithium Enrichment.* The final model assessed lithium enrichment of *A. platensis* via bioaccumulation. The strain displayed high tolerance to lithium, with stable biomass production across the tested concentration range (5–500 mg/L). Lithium content in the biomass increased proportionally to medium concentration, reaching 2.1–2.5 mg/g dry weight. Importantly, total protein content remained stable (60–66% of biomass), and MDA increases (11–141% over control) were moderate and within safe dietary limits.

Collectively, these results highlight the remarkable adaptability of *A. platensis* to a range of metallic stressors. The organism combines efficient metal bioaccumulation with the ability to maintain, or quickly restore, key physiological parameters such as protein content and oxidative balance. These properties, together with the specific selectivity for different metal ions demonstrated in each experimental scenario, underline its potential for biotechnological applications in the remediation of multimetallic wastewaters and the production of functionalized biomass with added nutritional or technological value.

## CONCLUSIONS

This study demonstrates that *Arthrospira platensis* is highly adaptable and effective in accumulating a broad range of metals, including heavy metals, rare earth elements, and lithium, from both mono- and multimetallic systems. Despite initial reductions in biomass productivity and protein content under metal stress, the strain exhibited strong physiological adaptation, with growth and protein levels recovering over time.

The results highlight the organism's high metal uptake capacity and resilience, enabling not only efficient bioremediation of complex, metal-rich effluents but also the production of protein-rich biomass with retained nutritional value. Notably, lithium enrichment did not compromise biomass yield or protein synthesis, further supporting its application in functional food development.

Overall, *A. platensis* stands out as a robust candidate for sustainable bioremediation and biotechnological valorization, combining metal recovery with the generation of valuable biomass for diverse applications.

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# EVALUATION OF VARIABILITY OF QUANTITATIVE CHARACTERS IN $SC_4$ TRITICLE SOMACLONES WITH VALUABLE AGRONOMIC PROPERTIES

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***Abstract.*** *As a result of research on the influence of in vitro culture and gamma radiation on quantitative traits, significant variations in the coefficient of variation values were observed in $SC_4$ triticale somaclones. Both in vitro culture and radiation caused changes in the coefficient of variation of quantitative traits in the studied genotypes, compared to the control. The variability assessment revealed low to medium variation, indicating individual similarity across the studied traits. Radiation showed a major action on the following traits: length of the last internode, plant height and number of fertile siblings. The factors studied represent a significant source of variation, with their influence intensified by the specific characteristics of the genotype.*

***Keywords: in vitro, quantitative traits, radiation, somaclones, triticale.***

## INTRODUCTION

Improving many agronomic traits, including cereal quality, requires the separation of their genetic variability from non-genetic influences. For many years, the main goal of breeding programs was to replace traditional species with high-yielding, disease resistant modern species, which led to a reduction in genetic diversity, especially in terms of quality traits and nutritional properties [2].

Genetic diversity can be estimated using morphological traits. Continuous development and availability of new genetic resources are essential for improvement of species, while phenotypic characterization can be used to differentiate genotypes [5].

Somaclones, obtained through *in vitro* culture, exhibit a wide range of morphological and biochemical trait variation, confirming the efficiency of callus culture in inducing variability and producing somaclones of agronomic interest [4]. The advantages of somaclonal variation and mutagenesis are well documented. In most cases, somaclonal variation in different plant species has been determined based on morphological and physiological traits [3]. The effectiveness of gamma radiation as an inducer of genetic variation in barley has been confirmed through the creation of mutant varieties with superior traits related to yield, quality, plant architecture, and disease resistance [1].

Therefore, it is of interest to evaluate the effect of *in vitro* culture alone and in combination with gamma radiation as genetic modifiers on agro-morphological traits in $SC_4$ triticale somaclones to identify valuable genotypes for integration into a breeding program.

## MATERIALS AND METHODS

*Plant material*: The study material included triticale somaclones with valuable agronomic traits selected from $SC_3$ descendants of three triticale species (x Triticosecale): Ingen 35, Ingen 93 (standard), and 188TR 5021.

*Determining somaclonal variation:* $SC_3$ somaclone seeds were sown in randomized blocks with three replicates of 60 seeds per genotype. Each variant included three triticale genotypes: the control, gamma irradiated forms, and forms obtained via *in vitro* culture. Phenological observations were made. Plants were collected at the vegetation phase (according to the Zadocks scale): full maturity (99), and under laboratory conditions, they were analyzed based on several agro-morphological traits: plant height, number of fertile tillers, main spike length, number of internodes, number of grains per spike, grain weight, and 1000 kernel weight.

*Statistical analysis:* The impact of *in vitro* culture and radiation on quantitative trait variation was assessed using analysis of variance (ANOVA), influence power (IP, %), and coefficient of variation (CV, %). Mathematical data analysis was conducted using STATGRAPHICS Plus 5.0 software.

## RESULTS AND DISCUSSION

Biometric analysis of quantitative traits in $SC_4$ triticale somaclones induced by gamma rays and *in vitro* culture revealed distinct traits compared to initial forms. Phenological observations, trait evaluation, and assessment of $SC_4$ somaclones were conducted. The vegetation period, including phases and stages, was determined using the Zadocks scale. Observations indicated that all triticale plants were at the same vegetation phase but at different developmental stages. This developmental lag persisted in the *flowering* and *grain formation* phases, as well as at the *milk ripeness* stage. The evaluation revealed variation in the developmental stages within this phase, advancing by 1-2 stages. Thus, the *in vitro* derived $SC_4$ somaclones and the control variant 188TR 5021 (Figure 1) were at stage 71 on the Zadocks scale, corresponding to the *watery ripe stage (first formed grains are half of their final size*), while the irradiated $SC_4$ 188TR 5021 and its control were at stage 69 (full flowering). The Ingen 35 somaclones, both irradiated and *in vitro*-derived, were at stages 70-71 (early grain formation - watery ripe), while the $SC_4$ control was at stage 69, *full flowering*. For the Ingen 93 somaclones control, irradiated, and *in vitro* derived the same vegetation phase, *early grain formation*, was observed.

Plants were collected at full maturity (stage 99). Under laboratory conditions, morphometric parameters of agronomic interest were evaluated.

**A** **B** **C**

Figure 1. $SC_4$ somaclones at different vegetation phases:
*A - 188TR 5021; B - Ingen 35; C - Ingen 93*

Table 1. Bio-morphological trait variation in $SC_4$ somaclones summarized

| **Character/ Genotype** | **Number of fertile tillers** | **Plant height (cm)** | **Length of the main spike (cm)** | **Length of the last internode** | **Grains per spike** | **Grain weight (g)** | **1000 kernel weight (g)** |
|---|---|---|---|---|---|---|---|
| | **$\bar{x}$ ± ES CV,%** | **$\bar{x}$ ± ES CV,%** | **$\bar{x}$ ± ES CV,%** | **$\bar{x}$ ± ES CV,%** | **$\bar{x}$ ± ES CV,%** | **$\bar{x}$ ± ES CV,%** | **$\bar{x}$ ± ES CV,%** |
| Control Ingen 35 | 1,86±0,11 33,68 | 114,6±0,98 4,70 | 13,58±0,18 7,35 | 35,41±0,40 6,29 | 75,03±1,98 14,46 | 3,54±0,09 15,34 | 47,26±0,67 7,86 |
| RAD Ingen 35 | 2,5±0,14 ***32,80 | 136,6±1,99 *** 8,01 | 13,89±0,17 6,81 | 49,32±1,02 *** 11,42 | **90,36±2,18 *** 13,22** | 3,71±0,12 18,53 | 41,02±0,83 ***11,11 |
| *In vitro* Ingen 35 | 2,43±0,17 ***38,4 | 120,4±0,99 *** 4,51 | 14,26±0,19 * 7,40 | 36,36±0,43 6,52 | 74,53±1,56 11,52 | 3,09±0,09 **17,46 | 41,39±0,90 ***11,94 |
| Control Ingen 93 | 2,16±0,12 32,25 | 110,9±1,11 5,50 | 14,37±0,22 8,44 | 32,49±0,60 10,25 | 68,83±1,42 11,34 | 2,88±0,06 12,49 | 42,09±0,88 11,49 |
| RAD Ingen 93 | 2,66±0,14 *30,08 | 121,7±1,42 ***6,46 | 13,68±0,20 *8,00 | 44,88±0,68 ***8,35 | 69,66±2,35 18,54 | 2,95±0,10 18,81 | 42,52±0,53 6,86 |
| *In vitro* Ingen 93 | 2,23±0,14 34,65 | **116,07±1,05 ** 4,99** | **15,58±0,19 *** 6,87** | 32,88±0,77 12,86 | **74,06±1,28 **9,46** | 3,44±0,07 11,82 | **46,52±0,57 ***6,75** |
| Conrol 188TR5021 | 2,13±0,12 31,94 | 114,37±1,45 6,97 | 14,23±0,23 9,02 | 39,73±0,69 9,55 | 88,06±2,33 14,49 | 3,17±0,11 19,00 | 36,04±0,80 12,28 |
| RAD 188TR5021 | 2,37±0,09 20,75 | 114,39±0,97 4,59 | 14,35±0,17 6,48 | 39,24±0,50 6,93 | 86,68±2,18 13,57 | 3,01±0,09 17,78 | 34,72±0,59 9,19 |
| *In vitro* 188TR5021 | 2,06±0,15 40,04 | 142,97±1,56 ***6,01 | 14,89±0,19 * 7,26 | 47,97±0,77 ***8,83 | 79,5±2,47 * 17,05 | **3,85±0,14 ***20,80** | **48,40±0,81 *** 9,16** |

*Note: *, **, *** - significant differences for P ≤ 0,05; 0,01; 0,001.*

To assess somaclonal variability, morphological parameters were evaluated: plant height, length of the main spike and last internode, number of fertile tillers and internodes per plant, grains per spike, grain weight, and 1000 kernel weight. Bio-morphological evaluations showed significant variation (95-99% confidence level) in agro-morphological traits of SC4 triticale somaclones depending on genotype and analyzed factor (Table 1).

Analysis of the data on the *number of fertile tillers per plant* highlighted that genotypes Ingen 93(RAD) and Ingen 35(RAD) exhibited 23,14–34,40% higher values compared to the control, with differences significant at the 99,9% level. The coefficient of variation for this trait was medium (20,75–40,04%).

Mean *plant height* varied among *in vitro* derived somaclones and was genotype dependent. Ingen 93(*in vitro*), Ingen 35(*in vitro*), and 188TR 5021(*in vitro*) displayed higher mean values than their respective controls (by 5,06–25%), significant at the 95–99,9% level. The highest *plant height* (142,97 cm) was recorded in genotype 188TR 5021(*in vitro*). Gamma irradiated variants of Ingen 93 and Ingen 35 also showed increased values compared to the control (by 9,78–19,19%), while the lowest values were noted in 188TR 5021(RAD). The coefficient of variation was low (4,51–6,97%), indicating trait stability in $SC_4$ generation.

For the *main spike length*, genotype specific responses were observed. Ingen 93(*in vitro*), Ingen 35(*in vitro*), and 188TR 5021(*in vitro*) had 4,68–8,42% higher values compared to the control, significant at the 95–99.9% level, while Ingen 93(RAD) had a 5% lower mean, significant at the 95% level. The longest spike (15,58 cm) was recorded in Ingen 93(*in vitro*). The coefficient of variation ranged from 6,48–9,02%, indicating low variability.

The mean value for *last internode length* increased significantly in gamma irradiated variants (38,13–39,28%) compared to the control, with significance at the 99,9% level for Ingen 93(RAD) and Ingen 35(RAD). *In vitro* derived 188TR 5021 somaclones also had a 20,73% increase. The variability for this trait ranged from low to medium (6,29–12,86%).

For the *number of grains per spike*, Ingen 35(RAD) showed a 20,43% increase over the control, significant at the 99,9% level. The effect of *in vitro* culture showed a 7,59% increase for Ingen 93(*in vitro*) but a 1,10 fold decrease for 188TR 5021(*in vitro*) compared to their control. The variability for this trait ranged from low to medium (9,46–18,54%).

The mean *grain weight* differed significantly only for Ingen 35(*in vitro*), which had a 13% lower value compared to its control. The highest value, 21,06% above control, was recorded for 188TR 5021(*in vitro*), significant at the 99,9% level. The coefficient of variation for this trait ranged from medium (11,82–20,80%).

For the *1000 kernel weight*, *in vitro* culture led to an increase of 10,52–34,29% in Ingen 93 and 188TR 5021 genotypes, with differences significant at the 99,9% level. Gamma radiation decreased this trait by 14% in Ingen 35(RAD). The coefficient of variation ranged from 6,86–11,94%, indicating low variability.

Based on the evaluations, $SC_4$ somaclones of Ingen 35(RAD), Ingen 93(*in vitro*), and 188TR 5021(*in vitro*) were noteworthy for superior average values in traits such as *the number of fertile tillers, plant height, main spike length, last internode length, grain number per spike,* and *1000 kernel weight.*

Table 2. Analysis of bio-morphological indices in $SC_4$ (ANOVA test)

| Source of variation | Sum of squares | Degrees of Freedom (DF) | Mean Square ($S^2$) | Fisher Test (F) | IP, % |
|---|---|---|---|---|---|
| **Plant height** | | | | | |
| Genotype | 3356,89 | 2 | 1678,45 | 29,23*** | 18,50 |
| RAD | 3709,11 | 1 | 3709,11 | 64,60*** | 20,44 |
| Genotype-RAD | 2808,41 | 2 | 1404,20 | 24,46*** | 15,47 |
| Residual | 8267,93 | 144 | 57,41 | | |
| Total | 18142,30 | 149 | | | |
| Genotype | 6325,34 | 2 | 3162,67 | 69,75*** | 25,76 |
| *In vitro* culture | 6552,49 | 1 | 6552,49 | 144,50*** | 2,66 |
| Genotype-*in vitro* | 5143,68 | 2 | 2571,84 | 56,72*** | 20,95 |
| Residual | 6529,62 | 144 | 45,34 | | |
| Total | 24551,1 | 149 | | | |
| **Length of the main spike** | | | | | |
| Genotype | 8,56 | 2 | 4,28 | 3,66 | 4,54 |
| RAD | 0,61 | 1 | 0,61 | 0,53 | 0,32 |
| Genotype-RAD | 10,81 | 2 | 5,40 | 4,62* | 5,74 |
| Residual | 168,40 | 144 | 1,17 | | |
| Total | 188,40 | 149 | | | |
| Genotype | 29,47 | 2 | 14,73 | 11,99*** | 12,50 |
| *In vitro* culture | 26,80 | 1 | 26,80 | 21,82*** | 11,37 |
| Genotype-*in vitro* | 2,46 | 2 | 1,23 | 1,00 | 1,04 |
| Residual | 176,89 | 144 | 1,23 | | |
| Total | 235,62 | 149 | | | |
| **Grains per spike** | | | | | |
| Genotype | 10151,60 | 2 | 5075,81 | 38,52*** | **31,74** |
| RAD | 798,11 | 1 | 798,11 | 6,06* | 2,49 |
| Genotype-RAD | 2054,41 | 2 | 1027,21 | 7,79*** | 6,42 |
| Residual | 18977,20 | 144 | 131,786 | | |
| Total | 31981,30 | 149 | | | |
| Genotype | 4847,61 | 2 | 2423,81 | 22,18*** | 22,25 |
| *In vitro* culture | 40,56 | 1 | 40,56 | 0,37 | 0,18 |
| Genotype-*in vitro* | 1157,08 | 2 | 578,54 | 5,29* | 5,31 |
| Residual | 15734,20 | 144 | 109,27 | | |
| Total | 21779,50 | 149 | | | |
| **Grain Weight** | | | | | |
| Genotype | 16,25 | 2 | 8,12 | 26,49*** | 26,64 |
| RAD | 0,0024 | 1 | 0,0024 | 0,01 | - |
| Genotype-RAD | 0,57 | 2 | 0,28 | 0,92 | 0,92 |
| Residual | 41,17 | 144 | 0,31 | | |
| Total | 60,98 | 149 | | | |
| Genotype | 4,10 | 2 | 2,05 | 6,50** | 6,53 |
| *In vitro* culture | 1,97 | 1 | 1,97 | 6,25* | 3,13 |
| Genotype-*in vitro* | 11,32 | 2 | 5,66 | 17,94*** | 18,01 |
| Residual | 45,42 | 144 | 0,32 | | |
| Total | 62,81 | 149 | | | |

| Source of variation | Sum of squares | Degrees of Freedom (DF) | Mean Square ($S^2$) | Fisher Test (F) | IP, % |
|---|---|---|---|---|---|
| **1000 kernel weight** | | | | | |
| Genotype | 2137,08 | 2 | 1068,54 | 65,32*** | **42,11** |
| RAD | 273,08 | 1 | 273,08 | 16,69*** | 5,38 |
| Genotype - RAD | 308,73 | 2 | 154,36 | 9,44*** | 6,08 |
| Residual | 2355,77 | 144 | 16,36 | | |
| Total | 5074,65 | 149 | | | |
| Genotype | 174,57 | 2 | 87,28 | 4,74* | 3,30 |
| *In vitro* culture | 335,55 | 1 | 335,55 | 18,24*** | 6,34 |
| Genotype-*in vitro* | 2129,71 | 2 | 1064,86 | 57,88*** | **40,26** |
| Residual | 2649,04 | 144 | 18,40 | | |
| Total | 5388,87 | 149 | | | |

*Note*: * ,**, *** - significant differences for P $\leq$0,05; 0,01; 0,001.

Variability assessment confirmed low to medium values, suggesting uniformity among individuals for the studied traits.

The ANOVA test for quantitative traits in $SC_4$ triticale somaclones showed that genotype, radiation, *in vitro* culture, and their interactions had a significant impact (95–99,9% confidence) (Table 2). Radiation had a major effect on *last internode length* (40,81%), *plant height* (20,44%), and *number of fertile tillers* (7,47%), all significant at the 99,9% level. The *in vitro* culture influence ranged from 0,18–11,37%, with the highest on spike length.

Radiation exhibited a major effect on the following traits: *length of the last internode* (40,81%), *plant height* (20,44%), and the *number of fertile tillers* (7,47%), all significant at the 99,9% level. The influence power of *in vitro* culture ranged between 0,18% and 11,37%, with the highest value observed for the trait *main spike length*. The interaction between genotype and radiation, depending on the trait, showed influence values ranging from 0,92% to 23,97%. The strongest influence was found for the traits: *length of the last internode* (23,97%), *plant height* (15,47%), and the *number of grains per spike* (6,42%). The interaction between genotype and *in vitro* culture had influence values ranging from 1,04% to 40,26%, with the highest values recorded for *1000 kernel weight, plant height* and *grain weight*. Genotype influence in $SC_4$ somaclones ranged from 0,59% to 53,43%. The highest influence was recorded for the traits: *number of grains per spike* (31,74%) and *1000 kernel weight* (42,11%), both highly significant (99,9%). The most substantial genotype influence was observed for the trait *length of the last internode* (53,43%).

These results highlight the potential of the studied factors to expand genetic diversity and variability in triticale. This data supports the necessity of using somaclones in subsequent generations for selecting genotypes with superior agronomic traits.

## CONCLUSION

1. Following the evaluations, the $SC_4$ somaclones of genotypes Ingen 35(RAD)**,** Ingen 93(*in vitro*), and 188TR 5021(*in vitro*) stood out compared to the control. The most determinative traits were: *number of fertile tillers per plant, plant height, main spike length, last internode length, number of grains per spike*, and *1000 kernel weight*.

2.The variability assessment revealed low to medium variation, indicating individual similarity across the studied traits.

3. Radiation had a major effect on the traits: *last internode length* (40,81%), *plant height* (20,44%), and *number of fertile tillers* (7,47%), all statistically significant at 99,9%. The genotype × radiation interaction had a maximal influence for the traits: *last internode length* (23,97%), *plant height* (15,47%), and *number of grains per spike* (6,42%). The genotype × *in vitro* culture interaction reached values between 1,04% and 40,26%, with the highest for *1000 kernel weight, plant height,* and *grain weight*. Genotypic influence was greatest for the traits: *number of grains per spike* (31,74%) and *1000 kernel weight* (42,11%), both significant at 99,9%. The maximum influence from genotype was on *last internode length* (53,43%).

4. Productivity related indices *plant height, main spike length, last internode length, number of grains per spike,* and *1000 kernel weight* can be used as key indicators for evaluating somaclonal diversity induced by gamma radiation and *in vitro* culture. Therefore, they should be given top priority in the selection process for triticale breeding.

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# TESTING OF THE RIHTER WDG PREPARATION IN THE CONTROL OF APPLE SCAB (*VENTURIA INAEQUALIS* WINT)

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***Abstract.*** *The results of the testing of the chemical fungicide Rihter WDG in the control of apple scab variety "Renet Simirenko" in the conditions of the Republic of Moldova. The research was carried out on the experimental apple orchard of the IGFPP of MSU. Three preparation consumption norms were tested: 1,8 kg/ha, 2,2 kg/ha and 2,5 kg/ha. Used once by the spraying method. As a result, the biological efficiency against apple SCAB was 94,3%,94,0%,91,3%*

***Keywords: Apple scab, biological efficacy, rihter, Venturia inaequalis wint.***

## INTRODUCTION

The favourable climatic conditions of the Republic of Moldova contribute to the intensive development of agriculture. In horticulture among perennial crops the leading place is occupied by apple trees.

About 350-400 thousand tonnes of apples are exported annually from the Republic of Moldova. The total area of apple orchards is more than 70 thousand ha out of 3 384 ha covering the entire territory of the Republic of Moldova. Thanks to the rich diversity of the soil and the possibilities for storage, apples can be eaten fresh all year round. The flavour of our apples is easily recognised on the foreign market. All foreign citizens who have tasted Moldovan fruit have been impressed by its quality and flavour - the "apple taste", as Moldovan apples are still called [1].

At first glance, the apple tree may seem to be quite a strong tree that bears not only fragrant, but also beautiful fruit. At the same time, the plant is quite often faced with a variety of diseases. Therefore, a huge role is played by timely and quality prevention, as well as treatment of the apple tree, if the infection is not avoided.

The tree is susceptible to many diseases and often suffers from pests. The greatest threat to apple trees is powdery mildew, Cytospora, fruit rot, black cancer, rust and others. They can be attributed to those problems that are caused by fungi. Much more rarely, the tree is faced with a viral or bactericidal infection [2].

The purpose of these tests was to establish the biological efficacy of Rihter

fungicide, WDG formulation (Captan, 800 g/kg) against apple scab (*Venturia inaequalis*). According to the plan proposed by the company, it was necessary to study and establish the biological efficacy of the product against the above diseases.

## MATERIALS AND METHODS

Studies were conducted in 2022 at the experimental orchard of the Institute of Genetics, Physiology and Plant Protection of the MSU, variety ”Renet Simirenko”. The object of research was a chimical fungicide based on the captan, 800 g/kg.

Field experiments on testing fungicide against apple scab (*Venturia inaequalis)* on apple trees were conducted in apple orchard. The experiments were laid on the variety ”Renet Simirenko”, late maturity. During the growing season, the necessary agrotechnical measures were carried out in the orchard. It should be noted that in order to eliminate the influence of various factors on the results of the tested fungicide, as well as for the application of statistical methods of processing the obtained results of research, the method of randomization was used when placing the variants of the experiment. Treatments were carried out by knapsack sprayer at the rate of 1000 liters per hectare. Determination of harmfulness and biological efficacy of this preparation was carried out according to the Methodological instructions on testing of chemical and biological means of plant protection, issued by the State Centre for certification and approbation of phytosanitary means [3].

Mathematical processing of the obtained results was carried out according to the method of one-factor variance analysis with the use of computer methods of data processing on ABC Pascal platform.

## RESULTS AND DISCUSSIONS

The most dangerous periods for the primary infection of leaf parsch are the phases of ”pink bud”, ”flowering” and immediately after flowering. In this regard, the main treatments were timed to these phenophases.

Under the conditions of the current year, the indicators of development and spread of parsch were at an average level in the spring period. The manifestation of the disease on leaves was noted in the second decade of May.

The degree of parsha spread on the leaf apparatus at the time of the first count in the control variant was 42.0% with the intensity of disease development of 7.8%. In the variant with application of fungicide Rihter, WDG at the rate of consumption of 1.8 kg/ha the degree of spread was 15 % with intensity of development of 2.4 %, at the rate of consumption of 2.2 kg/ha the degree of spread was 17.3 % with intensity of development of 1.9 %. With increasing the fungicide

rate to 2.5 kg/ha, the spread and development of the disease were 19.7% and 1.8%, respectively. The biological efficiency at the time of the first record for the rate of 1.8 kg/ha was 68.8 %, for the rate of 2.2 kg/ha was 75.9, and for the rate of 2.5 kg/ha was 77.2 % (Table 2).

Table 2. The biological efficacy of Rihter fungicide, WDG in reducing the development of apple scab (*Venturia inaequalis* Wint.) on leaves

| № | Variant | Dosa, litres, kg/ha | Disease progress, % | Disease intensity, % | Biological efficiency, % |
|---|---|---|---|---|---|
| 1 | Control | - | 42.0 | 7.8 | - |
| 2 | Etalon Merpan 80 WG | 2 | 22.0 | 2.0 | 74.5 |
| 3 | Rihter, WDG | 1.8 | 15.0 | 2.4 | 68.8 |
| 4 | Rihter, WDG | 2.2 | 17.3 | 1.9 | 75.9 |
| 5 | Rihter, WDG | 2.5 | 19.7 | 1.8 | 77.2 |
| HCP $_{0,05}$ | | | 7.5 | 2.2 | |

Subsequent surveys during the growing season allowed to establish rather high biological efficiency of Rihter, WDG fungicide application in controlling parsley both on leaves and fruits.

During the second control recording, progression of the disease was noted. The percentage of leaf lesions was 52.3%, and the intensity of disease development was 13.5%. In the experimental variant with the application of the fungicide *Rihter WDG* at a consumption rate of 1.8 kg/ha, the degree of disease spread on the leaf apparatus was 14.3%, with an intensity of development of 1.2%. At a consumption rate of 2.2 kg/ha, the degree of disease spread on the leaf apparatus was 11.7%, with an intensity of development of 0.8%. At a consumption rate of 2.5 kg/ha, these indicators were: degree of spread, 12.7%; intensity of development, 0.8%.

The biological efficiency of Rihter WDG at a dosage of 1.8 kg/ha was 91.3%, at a dosage of 2.2 kg/ha it was 94.0%, and at a dosage of 2.5 kg/ha it was 94.3%. The biological efficiency of the chemical reference Merpan 80 WG was 93.2% at an intensity of 0.8% (Table 3).

Surveys during the harvesting period made it possible to determine the degree of development and spread of parsch on apple fruits. In the control, the level of damaged fruits was 10 % with pathogen development intensity of 0.6 %. In the variant with application of fungicide *Rihter, WDG* at a dosage of 1.8 kg/ha the degree of disease spread on fruits was 2.3 %, the intensity of disease development on fruits was 0.058 %. With the increase of fungicide dosage up to 2.5 kg/ha, the spread of disease was 1 %, with the intensity of disease development - 0.025 %.

Table 3. The biological efficacy of Rihter fungicide, WDG in reducing the development of apple scab (*Venturia inaequalis* Wint.) on leaves.

| № | Variant | Dosa, litres, kg/ha | Disease progress, % | Disease intensity, % | Biological efficiency, % |
|---|---|---|---|---|---|
| 1 | Control | - | 52.3 | 13.5 | - |
| 2 | Etalon Merpan 80 WG | 2 | 12.7 | 0.9 | 93.2 |
| 3 | Rihter, WDG | 1.8 | 14.3 | 1.2 | 91.3 |
| 4 | Rihter, WDG | 2.2 | 11.7 | 0.8 | 94.0 |
| 5 | Rihter, WDG | 2.5 | 12.7 | 0.8 | 94.3 |
| HCP $_{0,05}$ | | | 6.6 | 3.2 | |

The biological efficiency of fungicide in the variant with the dose of 1.8 kg/ha was 90.7%, in the variant with the rate of consumption of 2.8 kg/ha - 96%. (Table 4)

Table 4. The biological efficacy of fungicide Rihter, WDG in reducing the development of apple scab on apple fruits

| № | Variant | Dosa, litres, kg/ha | Disease progress, % | Disease intensity, % | Biological efficiency, % |
|---|---|---|---|---|---|
| 1 | Control | - | 10.0 | 0.63 | - |
| 2 | Etalon Merpan 80 WG | 2 | 1.7 | 0.042 | 93.3 |
| 3 | Rihter, WDG | 1.8 | 2.3 | 0.058 | 90.7 |
| 4 | Rihter, WDG | 2.2 | 1.3 | 0.033 | 94.7 |
| | Rihter, WDG | 2.5 | 1.0 | 0.025 | 96.0 |
| HCP $_{0,05}$ | | | 1.7 | 0.17 | |

Mathematical processing of the data proves that the obtained results of testing the tested doses of the drug in the control of apple parsha and moniliosis are significant in relation to the control and are not inferior in efficiency to the chemical reference Rihter, WDG.

The obtained results of testing the fungicide *Rihter, WDG* in the Republic of Moldova under the existing agroclimatic conditions indicate that the drug is quite effective against apple parsha and can be recommended for inclusion in the system of integrated crop protection.

## CONCLUSIONS

As a result of the ongoing tests, the following conclusion can be drawn:

1. In the current year's climatic conditions with rainfall at 52.6% of the norm, the fungicide *Rihter WDG*, applied at rates of 1.8, 2.2, and 2.5 kg/ha, effectively inhibited the development and spread of parsha on apple trees.

2. The biological efficiency of the preparation in controlling leaf parsha

during the growing season was 80% at a rate of 1.8 kg/ha, 85% at a rate of 2.2 kg/ha, and 85.7% at a rate of 2.5 kg/ha.

3. The biological efficiency of the fungicide *Rihter, WDG*, in controlling fruit parsha was 90.7% at a rate of 1.8 kg/ha, 94.7% at a rate of 2.2 kg/ha, and 96% at a rate of 2.5 kg/ha.

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# EVALUATION OF ZEUZERA PYRINA INFESTATION IN WALNUT ORCHARDS AND PROPOSAL OF AN ALTERNATIVE ECOLOGICAL CONTROL METHOD

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***Abstract****: The study assessed the impact of the xylophagous pest Zeuzera pyrina on walnut orchards in the central region of the Republic of Moldova, providing a detailed phenological and ecological profile. Monitoring of adult populations revealed an active flight period between May and August, correlated with temperatures above 25°C. Field data indicated an average infestation rate of 24.4% among the analyzed trees, with 58.1% of their branches being affected. The larvae produce galleries up to 50 cm in length, causing significant structural damage. An unusually high trophic plasticity was observed, with larvae also feeding on foliage under limiting conditions. Given the difficulty of applying contact insecticides, the study proposes an innovative ecological control method: detection of larvae using acoustic sensors and injection of a biologically active substance. This approach offers ecological, selective advantages and is compatible with modern technologies, representing a viable solution for sustainable pest management and the preservation of walnut orchard health.*

***Keywords***: ***ecological control, Juglans regia, phenological monitoring, larval stage, Zeuzera pyrina.***

## INTRODUCTION

*Zeuzera pyrina* L. (Lepidoptera: Cossidae), also known as the "leopard moth" or "wood borer," is a polyphagous pest of major economic importance for orchards of apple, pear, plum, cherry, and especially walnut. Its negative impact on tree health and productivity is associated exclusively with the larval stage. Adult females, with a lifespan of 8–10 days, lay eggs in bark crevices or wounded areas of the trees, in groups of 50–200, totaling up to 800–2000 eggs over their lifetime, depending on climatic conditions and region. After an incubation period of 10–15 days, larvae hatch and penetrate the woody tissues of the host trees [1].

Newly hatched larvae initially settle in soft tissues (leaves, shoots), then bore into branches and trunks, creating ascending or labyrinthine galleries with diameters up to 7 mm and lengths exceeding 30–50 cm. These galleries severely compromise sap circulation, weaken the wood structure, and may lead to wilting or drying of branches, or even the death of young trees. The larvae also exhibit migratory behavior within the tree under favorable conditions of nighttime humidity, contributing to a high invasive potential and increased difficulty of control [2].

In studies conducted in walnut orchards in the central region of the Republic of Moldova between 2022 and 2024, a significant infestation rate was observed: on one hectare, 30% of trees were affected, with 58% of the branches on infested trees showing active galleries.

The biological cycle of *Z. pyrina* varies by region: in temperate areas it is predominantly biennial, while in warmer regions it can be completed within a single year. The pest overwinters in the larval stage inside the galleries. In the central region of Moldova, adult flight occurs from the second decade of June to the first decade of August, with a peak in July. In 2022, this schedule was significantly delayed (starting only on July 27), a phenomenon correlated with local climatic anomalies such as low temperatures and lack of precipitation.

Population monitoring of *Z. pyrina* was carried out using pheromone traps in walnut plantations in Sadova (Călărași district) and Bubuieci (Chișinău municipality), where attraction levels were three times higher in walnut orchards than in apple orchards. These phenological data are essential for planning phytosanitary measures, as the flight period coincides with oviposition — a critical moment for preventing infestations. Monitoring adult populations serves both informative and preventive purposes, providing valuable data for determining optimal intervention times.

The main problem lies in the damage caused by Z. pyrina in its larval stage. Control measures must focus primarily on this stage, as it is the most destructive and hardest to manage — the larvae are protected inside woody tissues, making them practically inaccessible to most conventional treatments.

Unfortunately, control measures targeting the larval stage are still underdeveloped and insufficiently researched. The existing literature offers few effective solutions, and current methods — such as systemic chemical treatments or the introduction of natural parasitoids — have limited efficacy, are difficult to implement, or carry uncertain ecological impacts. Moreover, the lack of technologies well-adapted to walnut crops further limits intervention possibilities. Therefore, there is an urgent need for in-depth research into larval-stage control methods, as well as for the development of integrated, sustainable strategies that are practically applicable in commercial plantations.

## MATERIALS AND METHODS

For monitoring the species Z. pyrina, Δ type pheromone traps equipped with species-specific pheromones were used to attract males. Three traps were placed in a walnut orchard of the Fernor variety (1.5 ha, Sadova, Călărași district, Republic of Moldova), at a height of 1.5–2 meters within the tree canopy, spaced evenly to ensure uniform coverage. The traps were checked weekly, and the captures were recorded and analyzed to identify peak flight periods.

The data were correlated with climatic conditions (temperature, humidity) to understand their influence on flight dynamics. Visual inspections of the trees were also

conducted to detect larval galleries and specific symptoms of infestation (bored branches, dried shoots). The collected information allowed for the determination of the optimal timing for implementing control measures.

## RESULTS AND DISCUSSIONS

The study dedicated to assessing the impact of *Zeuzera pyrina* on walnut (*Juglans regia* L.) orchards in the central region of the Republic of Moldova enabled the development of a detailed phenological and ecological profile of this xylophagous species, with significant implications for tree health and plantation productivity. Monitoring the dynamics of adult populations revealed that the flight of male moths consistently begins in the third decade of May and remains active until the second decade of August, with a peak recorded in July. Adult activity is closely correlated with the thermal regime of the period, being favored by stable temperatures above 25°C and calm, windless nights—conditions that facilitate mating and oviposition behaviors.

Analysis of pheromone traps indicated an average capture rate of three males per trap per week, which, although seemingly low, must be interpreted in the context of the species' cryptic and endophagous nature—where the larval stage is the destructive component of the biological cycle.

Field data regarding the infestation level showed that, on average, 24.4% of the analyzed trees were affected by Z. pyrina larvae, indicating that nearly a quarter of the tree population is under significant biotic stress. A detailed analysis of the main branches of affected trees revealed that approximately 58.1% of branches showed clear signs of infestation, indicating not only the presence of larvae but also active damage, directly impacting the conductive vessels and woody tissues of the tree. Proportionally, this translates into about 14.2% of the total number of branches at the plantation level being damaged by this insect, a value relevant for estimating the decline in photosynthetic capacity and overall productivity of the orchards.

Each infested tree showed, on average, between 3 and 5 larval galleries, each ranging from 15 to 30 cm in length, with severe cases reaching up to 50 cm. Assuming a conservative average of 4 galleries per tree and a gallery length of 25 cm, one infested tree can suffer approximately 1 linear meter of dislocated woody tissue—equivalent to significant internal damage compromising the tree's structural integrity and sap flow (both raw and elaborated). These injuries are often accompanied by visible external perforations (1–5 per tree), appearing as circular holes 5–20 mm in diameter, serving as both entry points and exits for frass and wood debris.

An interesting and ecologically important observation is the larvae's ability to shift their food source depending on availability. During periods when woody resources are limited or inaccessible — either due to high larval density or severe tree weakening— they may switch to feeding on foliage. This behavior can significantly damage leaves, indirectly impairing photosynthesis. Such trophic plasticity is remarkable and requires a

reevaluation of control strategies, as not only the woody structure but also the tree's overall vitality is at risk. Moreover, foliar feeding in the later part of the season may compromise carbohydrate reserve accumulation necessary for overwintering, reducing the tree's resilience during the cold season.

Furthermore, analysis of the ratio of galleries to the number of main branches revealed that, on average, 50.8% of the primary branches of an infested tree were affected, indicating that the damage is diffuse and not localized—making it harder to isolate and treat. In a typical tree with five main branches, where three are infested and each has an average of two galleries, this results in 6–10 infestation channels per tree — a density sufficient to compromise long-term tree development.

Another revealing aspect is that late-stage larvae are largely inaccessible to contact treatments, as they are sheltered deep within the wood. This biological adaptation makes Z. pyrina a highly resilient pest, requiring systemic or innovative biological control solutions, such as the introduction of entomopathogenic nematodes or specific predators. Preliminary pheromone trap data also suggest the presence of predator species that could be utilized in integrated biological control.

An effective strategy for controlling xylophagous pests like Z. pyrina involves using modern detection technologies and precisely applying biological agents. The proposed method involves locating larvae within branches using a device equipped with acoustic vibration sensors, capable of accurately identifying larval galleries [3]. Such systems have already been developed and tested for detecting other wood-boring insects.

Subsequently, in infested zones, a controlled injection of a biologically active substance can be carried out, with a dual purpose:

- To attract the larva to the injection site through chemical signals that mimic the composition of preferred plant tissues;
- To release, upon contact, a biocidal compound or pathogenic biological agent, such as a granulovirus specific to lepidopteran larvae [4], or an entomopathogenic fungus (e.g., *Beauveria bassiana*) [5], enzymes, or volatile proteins mimicking natural phloem/xylem signals [6, 7].

Consequently, the larva is lured to the injection point, comes into contact with the active agent, and is neutralized inside the gallery. Alternatively, if drawn to the surface, it can be mechanically removed or locally destroyed [8].

This method offers several advantages:

- Minimally invasive – does not require bark removal or branch cutting, preserving tree integrity;
- Ecological – eliminates the need for synthetic insecticides;
- Selective – attractant enzymes and biological agents can be tailored to target *Z. pyrina* specifically;
- Compatible with precision technology – well-suited for modern, sustainability-focused orchards.

Implementing this method could represent a major step forward in developing

smart, sustainable, and environmentally efficient agriculture.

## CONCLUSIONS

Zeuzera pyrina is an especially dangerous wood-boring pest for walnut orchards in central Moldova, affecting tree health and plantation productivity. Its activity, with a flight peak in July, is closely linked to favorable climatic conditions, allowing the synchronization of monitoring and control interventions. The high infestation level observed in the field and the severity of the damage caused confirm the significant impact of this pest.

The endophagous larvae cause severe damage by boring into branches, impairing the trees' physiological functions and leading to losses in vitality and yield. Furthermore, the larvae's trophic adaptability and the inefficiency of conventional contact treatments highlight the need for innovative solutions.

In this context, modern methods such as acoustic detection and targeted injection of biological agents, combined with technology based on attracting and eliminating larvae using specific organisms, offer ecological, effective, and sustainable alternatives for protecting walnut orchards.

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# OIL CONTENT IN SEEDS BY NMR RELAXATION METHOD

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**Abstract.** *The aim of our work was to evaluate the capability of NMR-relaxation method of the determination of oil content in walnut kernels and soybean seeds. NMR-relaxation spin-echoes for protons of the studied seeds were studied on a modernized NMR-relaxometer, using a sequence of 90°- and 180°- high-frequency pulses, with an interval 3.5 ms. As standards in NMR calibration for walnut kernels and for soybean seeds were used different oils. The oil content of commercial walnut kernels SRL "Monicol" is 69±1%, which corresponds to the typical range for the crop, 64-72%.*

*After harvest 2021 the minimal oil content was in the seeds of soybean variety Pentata (14,6±0,2% for the seeds with dry weight 100-150 mg and 14,9±0,4% for the seeds with dry weight 200-250 mg) and the maximal oil content was in seeds of soybean variety Ladutsa (19,3±0,3% for the seeds with dry weight 100-150 mg and 19,7±0,5% for the seeds with dry weight 150-200 mg).*

***Keywords: NMR relaxation, oil, soybean, walnut.***

## INTRODUCTION

Seed oil content is important in the study of the seed biodiversity. Cultivated soybean seeds have an oil content of approximately 18–22%, whereas wild soybean seeds contain about 8–10% oil [1]. Fat is the main component of the nut kernel and generally accounts for 64%-72% [2]. As a biochemical utilization of a physical method, nuclear magnetic resonance and its improvements allow for numerous rapid determinations of seed oil content within a short period of time and greatly

improve the throughput of analysis [3- 5]. Spin echo method is one of the elegant and most useful features in pulsed nuclear magnetic resonance (NMR). Determination of oil content in seeds by NMR relaxation method is a clear, nondestructive, fast and accurate alternative to traditional liquid chemistry methods. The oil content determined by NMR relaxation method strictly corresponds to the amount of oil extracted from the walnut kernels by organic solvents [6]: oil was extracted with Petroleum Ether using a Soxhlet apparatus, dehusked seeds also were analyzed for oil content by NMR method. Comparison between NMR and Soxhlet method certified that for low oil content walnut seeds the data of NMR method were 62.58 ± 0.03% compared to 64.35 ± 0.28% for Soxhlet method; for medium oil content walnut seeds the data of NMR method were 67.99 ± 0.11% compared to 68.09 ± 0.66% for Soxhlet method; for high oil content walnut seeds the data of NMR method were 72.60 ± 0.01% compared to 73.17 ± 0.22% for Soxhlet method.

The aim of our work was to evaluate the capability of NMR-relaxation method of the determination of oil content in walnut kernels and soybean seeds.

## MATERIALS AND METHODS

NMR-relaxation spin-echoes for protons of the studied seeds were studied on a modernized NMR-relaxometer [7], using a sequence of 90°- and 180°- high-frequency pulses [8], with an interval 3.5 *ms*. As standards in NMR calibration for walnut kernels and for soybean seeds were used different oils. For walnut kernels was used Pure Walnut Carrier Oil manufactured by RV Essential (Republic of India): Juglans Regia (100% Pure and Natural Cold Pressed). For soybean calibration was used soybean oil, obtained from soybean seeds variety Bucuria (the oil from the seeds was obtained from soybean meal after two extractions by toluene). Walnut kernels were obtained from walnut orchard of SRL "Monicol". Soybean seeds of different maturity groups (MG) were used: Ladutsa (MG II) and Pentata (MG III).

## RESULTS AND DISCUSSIONS

Fig. 1 shows the samples and ampoule for determination of oil content in seeds by NMR relaxation method. The determination of oil content by NMR-relaxation requires significantly less material compared to biochemical extraction methods: while biochemical extraction uses 10 g of seeds [6], samples for measurement by NMR-relaxation, as a rule, do not exceed 0.2 g (and the sensitivity of the device, not less than 1 mg of oil, allows the use of small samples, weighing from 10 mg). For soybean the NMR measurements were performed on individual

seeds. The oil content of seeds was determined by comparing echo-signals from seeds with echo-signal of calibration standards.

Figure 1. General view of the samples and ampoule for determination of oil content in seeds by NMR relaxation method
*(208 mg soybean seed and 182 mg sample from native walnut kernel)*

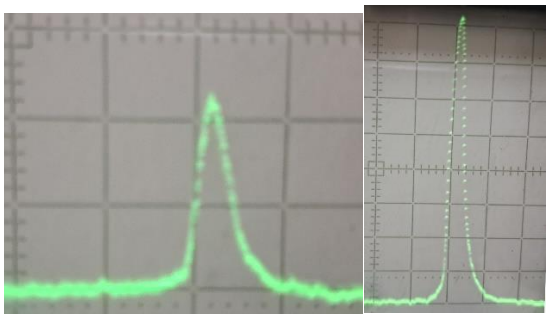

Figure 2. NMR spin echo signals recorded according to [8], at a time distance of 3.5 ms between 90°- and 180°-pulses for seed samples from different crops *(208 mg soybean seed and 182 mg sample from native walnut kernel)*

Figure 2 shows the differences of spin echo signals from seeds of different crops in intensity and shape: the echo signal from walnut kernel protons is significantly more intense and narrower. The echo signal from the protons of pure walnut oil used to construct the calibration curve is also characterized by a narrow shape, similar to the NMR spin echo signal from samples from native walnut kernels.

To avoid the influence of differences in the quality of the oils studied (for different crops or one crop of different origin), it is recommended to adjust the

nuclear magnetic resonance relaxometer on a pure chemical standard (glycerol).

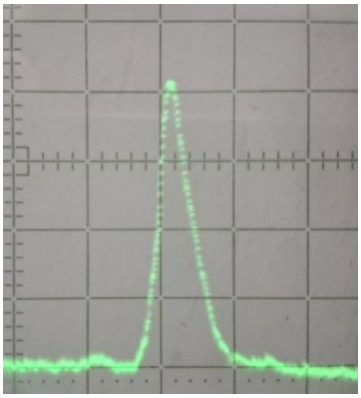

Figure 3. NMR spin-echo from standard of glycerol.

According to the NMR relaxation method, the oil content of commercial walnut kernels SRL "Monicol" is 69±1%, which corresponds to the typical range for the crop, 64-72%.

Oil content data for individual soybean seeds of Ladutsa and Pentata varieties (2021 harvest) are presented in Figure 4.

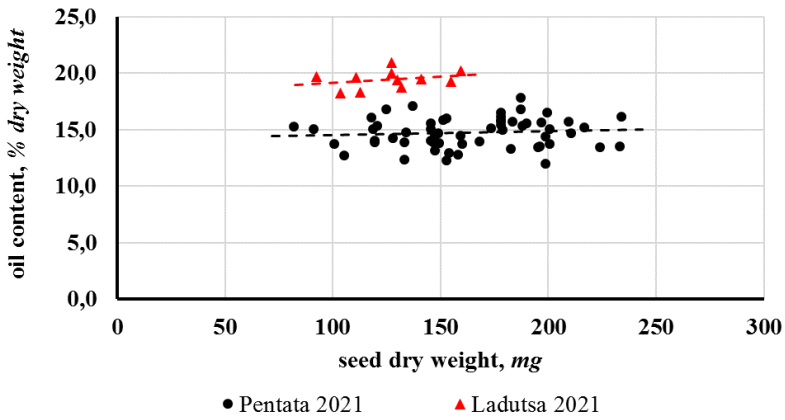


Figure 4. The oil content in soybean seeds of Pentata and Ladutsa varieties (harvest 2021)

*Each dot corresponds to a separate individual seed*

Table 1 shows the data on oil content determination by NMR relaxation method for different soybean varieties according the seed weight.

At harvest 2021 the minimal oil content in seeds was determined for soybean variety Pentata – 14,6-15,0% dry weight (14,6±0,2% for the seeds with

dry weight 100-150 mg and 14,9±0,4% for the seeds with dry weight 200-250 mg).

Table 1. Oil content in seeds of different weight for soybean varieties Pentata and Ladutsa (harvest 2021)

| Variety | Individual seed weight, *mg* | | Oil content, *% dry weight* |
|---|---|---|---|
| | range | average | |
| Pentata | 100-150 | 128±4 | **14,6**±0,2 |
| | 150-200 | 177±2 | **14,8**±0,2 |
| | 200-250 | 214±5 | **14,9**±0,4 |
| | 250-300 | 269±16 | **15,0**±0,3 |
| Ladutsa | 100-150 | 123±4 | **19,3**±0,3 |
| | 150-200 | 157±2 | **19,7**±0,5 |

The maximal oil content in seeds was determined for soybean variety Ladutsa – 19,3-19,7% dry weight (19,3±0,3% for the seeds with dry weight 100-150 mg and 19,7±0,5% for the seeds with dry weight 150-200 mg).

## CONCLUSIONS

The echo signal from walnut kernel protons is significantly more intense and narrower comparing to soybean seeds. The echo signal from the protons of pure walnut oil used to construct the calibration curve is also characterized by a narrow shape, similar to the NMR spin echo signal from samples from native walnut kernels. The oil content of commercial walnut kernels SRL “Monicol” is 69±1%, which corresponds to the typical range for the crop, 64-72%.

At harvest 2021 the minimal oil content in soybean seeds was determined for variety Pentata – 14,6-15,0% dry weight (14,6±0,2% for the seeds with dry weight 100-150 mg and 14,9±0,4% for the seeds with dry weight 200-250 mg). The maximal oil content in seeds was determined for soybean variety Ladutsa – 19,3-19,7% dry weight (19,3±0,3% for the seeds with dry weight 100-150 mg and 19,7±0,5% for the seeds with dry weight 150-200 mg).

To avoid the influence of differences in the quality of the oils studied (for different crops or one crop of different origin), it is recommended to adjust the nuclear magnetic resonance relaxometer on a pure chemical standard (glycerol).

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# HIBERNATING ARTHROPODS' COMPLEX IN THE PLUM TREE ORCHARD UNDER THE INFLUENCE OF GREEN MANURES AND NECTARIFEROUS PLANTS

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***Abstract.*** *The work represents the highlighting of the arthropod complex, which populates and hibernates in the cardboard trap belts of the plum orchard influenced by green manures and nectariferous crops. This complex is composed of phytophagous, saprophagous, predators and parasites. In the trapping belts, polyphagous species of five families of spiders dominated, which constituted an average of 33.5% of the total individuals highlighted, and coccinellids (seven species) - an average of 6.5%.*

*The parasitoid Scambus inanis achieved a degree of parasitism of moth larvae in average 29.4%. The highest percentage was recorded in the standard and experimental variants (46.0% and 35.3%) respectively.*

***Keywords: nectariferous plants, green manures, predators, parasites, plum.***

## INTRODUCTION

Currently, in plant protection systems, the attention is no longer paid to the reserve of the pest complex and their natural regulators that have gone into hibernation. The hibernation in natural environments exposes arthropods to drastic eradications. The hibernation in artificial environments such as cardboard trap belts is more successful. The percentage of parasitism of pests by parasites from the order Hymenoptera and other orders in the autumn-spring period depends on the degree of additional feeding that nectariferous and sidereal plants, which have long flowering periods such as *Lobullaria maritima* or regeneration periods such as grasses from spontaneous flora, can provide.

Chemical treatments against pests in perennial crops are ending with the

period of fruit ripening, which also coincides with the dry period, where the natural flowering grass carpet is poor and beneficial insects lack additional food, considering that they have a vital activity until the second decade of November.

Investigations aimed at determining the complex of arthropods that hibernate in artificial environments began decades ago. However, trap belts were mostly used as a method of controlling moths and other pests of fruit crops, without taking into account the other beneficial taxa present, thus sometimes destroying them as well. Later, the belts were modified, being used as a more ecological method of protecting beneficial fauna [1]. Some difficulties during the eight years period of investigations in plum cultivation were observed regarding the integrity of the trap belts used, often being destroyed by birds and mammals. The modification of their protection in which the arthropod complex hibernates is obvious. Based on this, the purpose of the investigation was to highlight the complex of arthropods hibernating in plum tree traps belts against the background of green manure and nectariferous crops.

## MATERIALS AND METHODS

The research materials were individuals of arthropod species from various taxa groups of different ontogenetic stages from solitary eggs, eggs in the nest inside the cocoon, larvae without and in the cocoon, free and in the cocoon pupae, free and in the cocoon adults. This biological material was obtained from the trap belts, placed around the trunk of model plum trees, in the second decade of July, when the migration to the summer diapause of plum borer larvae took place. The new type of trap belts were mounted at a height of five centimeters from the ground level, which corresponds to the requirements for plum borer. The extraction of the belts was carried out in the third decade of January, their analysis in the third decade of February, which lasted until the first decade of April. The collected belts, placed in bags and labeled, were kept at temperatures below +5°C to stop the reactivation of the biological material. To fix the belts to the model tree trunks, double corrugated cardboard was used, using different types of fixing supports: polyester fiber mesh with Velcro, adhesive tape and palisade thread. And, to obtain individuals for diagnosis up to taxa, the eggs found were activated at temperatures above +10 °C. Later, when the predator larvae appeared, they were fed with individuals of the aphids *Aphis gossypii*, *Schizaphis graminum*, the mite *Tetranychus urticae* and bean weevil eggs in their diet. To determine the percentage of parasitism of the hibernating moth larvae of the Tortricidae family, the cutting was carried out along with portions of cardboard for larvae in the cocoon. The selected larvae were introduced into the Florinschii test tube. Females of *Pseudoccocus comstoki* parasitized by *Stemmatosteres bohemicus* were introduced into the Ulinguta test tubes for parasite flight for further analysis.

The exemplars of the Cicadellidae family, found dead in the belts, were used for the preparation of wings and genitals. The material was introduced into a 40% lactic acid solution for decolorization and was subsequently used for the fabrication of temporary preparations and identified under Optika and Euromex digital microscopes. Exemplars of the order Aranea, after extraction from the belts, were placed into 70% ethyl alcohol. Spiders from the cocoons were hatched under uncontrolled conditions and subsequently fed with aphids till their development to the adults. The species keying was carried out according to special classical guides [2, 3, 4, 5] and current ones [6, 7, 8].

## RESULTS AND DISCUSSIONS

The biological material accumulated in the trap belts was quite varied and consisted of groups of phytophagous, predatory and parasitic arthropods.

In the plum crop, under the climatic conditions of 2024, phytophagous arthropods were represented by a number of species, which varied from 12 in the experimental variant and 17 in the standard and control, respectively. The dominant species were the tortricid moths, larvae and adults of unidentified coleopterans. Therefore, the larvae of the moths detected belong to the plum fruit moth, the oriental fruit moth, the coddling moth and an unknown species of moth. The number of larvae on average of 5.7 specimens per belt was noted in the experimental variant, 1 in the standard and 1.5 in the control, respectively.

The pest species, such as the previously found *Pseudococcus comstoki* in the 2024 was represented by eggs, ovipositing females, males and first instar larvae with a low average number per belt of 3 specimens in the experimental variant, 1.4 in the standard and 2.3 in the control. *Zignia flamigera* was also noted in the belts. The larval exuviae of the *Metcalfa pruniosa*, which was present in the plantation during three years were not found. The invasive species, the oak leaf beetle *Corythucha arcuata*, was also found in the belts, being present during six years there. In 2024 it was observed in small numbers, thus being located more in the experimental and standard variant. The average number per belt was 1.5 in experimental variant and 2.2 individuals in standard and 1 in the control. Therefore, phytophagous and saprophagous were the larvae of the *Partenolecanium corni*, individuals of the Ptiliidae family, *Phalacrus fimetarius* (Phalacridae), which is often found in composite inflorescences, the mycophagous *Anthelephila pedestris* (Anthicidae), *Roglius alboacuminatus* (Rhyparochromidae) and three species of unidentified Coleoptera.

The group of predators in the belts was represented by thrips from the family Phlaeothripidae, larvae in and without the cocoons of neuropters from the families Coneopterigidae and Chrysopidae (*Chrysopa carnea* and *Chrysopa ciliata*, species from the family Coccinellidae such as: *Chilocorus bipustulatus*, *Scimnus suturalis*,

*Stethorus punctillum*, *Coccinella septembunctata*, *Cryptolaemus monthozerei*, *Oenopia* sp., *Tytthaspis sedecempunctata*, the carabid *Microlestes minutulus* and the staphylinid *Platystethus nodifrons*. The anthocorid predatory bug *Amphyareus obscuriceps* was also found in single specimens, primarily females.

Of the total taxa analyzed, the largest number were predators from the order Araneae represented by more than five families. However, the dominant species were the species from the families Thomisidae and Phylodromidae, which constituted 45.7% of the total number of spiders. The families that followed were: Salticidae – 22.0%, Dictynidae and other species of 11.0-11.7% respectively; Clubionidae and Gnaphosidae of 7.3 and 2% respectively. As can be seen from Figure 1, the highest number of them was noted in plot two (standard) influenced by the green manures culture (*Medicago sativa* + *Eruca sativa*) and in the one influenced by the conventional plant protection system plot one (control). Totally, the araneids were 5.0% less in plot three (experimental variant). In both the control and standard, the litter was planted with grasses from the spontaneous flora.

Of the spider species previously noted on plum trees, in 2024 in the belts *Cheiracanthium* sp. (Cheiracanthiidae), *Aphantaulax trifasciata* (Gnaphosidae), *Erigone atra* (Linyphiidae), *Leptorchestes berolinensis*, *Sinageles dalmaticus* (Salticidae), *Pistius truncatus* (Thomisidae) were noted. Cocoons with spider eggs of an unidentified species were also noted, which require time to obtain adults. In a cocoon there were from 20 to 40 eggs found, which can later change the dominance position of the aforementioned ones. As a result, the phytophagous: predator ratio was 1:1 in the control and standard and 1.3:1 in the experimental variant.

It is known that, in each family of parasitoids from the suborders Ichneumonoidea, Chalcidoidea, there are families within which there are also genera of phytophagous insects such as the family Pteromalidae can be found. In order to detect the presence of these useful species, it is important to use the method of individual hatching of parasitoid species from the larvae of phytophagous moths. As a result, the flight of adults of *Scambus inanis* (= *annulatus*) adults out of them was observed. Exposure of moth larvae to individual hatching brought to their parasitism at a level of 46.0% in the standard, 35.3% in the experimental variant and 7% in the control. The difference of 10.7% between the experimental variant and the standard can be explained by the vegetative activity of the green manures and a resistance to the drought conditions of the year. The percentage of parasitism obtained cannot be attributed to a specific moth species, given that in addition to the plum fruit moth, the oriental fruit moth, the coddling moth and an unkeyed species of moth were also present. In such diagnostic investigations it is vitally important to correctly attribute the percentage of parasitism by *S. inanis* to the certain species. This can only be achieved by diagnosing the residues left after parasitation, such as the cephalic capsules of the larvae left after the activities of both internal and external parasites.

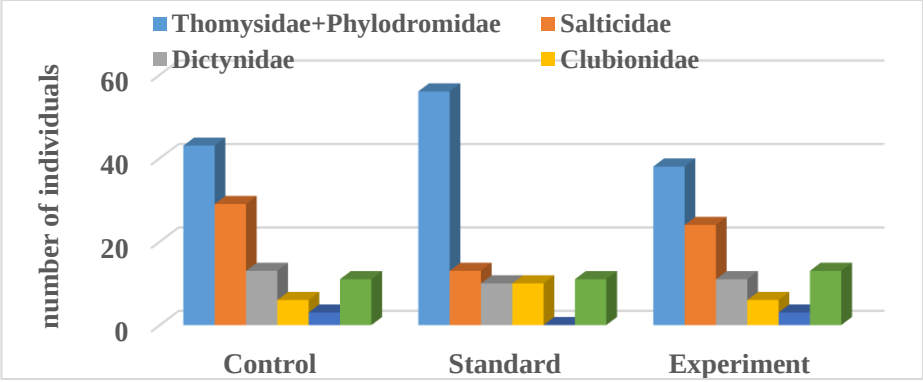


Figure 1. Distribution of araneids by plum tree variants in trap belts, year 2024.

Another group of parasitoids were those from the suborders Chalcidoidea and Bethyloidea, which were represented by individuals from the families Scelionidae, Platigastridae and Bethylidae.

Figure 2 (in original). Predators and parasites in the winter reserve of plum:
*1- Scimnus suturalis; 2 - larva of Malachius bipustulatus; 3 - adult of the Scelionidae family; 4 - spider Aphantaulax trifasciata; 5 - tachina fly hatched from the puparium; 6 - spider eggs nest; 7 - larva of Coniopterygidae Conwetzia sp.*

As a result, the phytophagous:parasitoid ratio was 5:1 in the control, 4.5:1 in the experimental variant and 1:1.5 in the standard. The most numerous were the scelionid individuals with an average number per belt of two in the experimental variant, four in the standard and one in the control. However, we note the fact that they are parasitic species of eggs of pests and also of some predators. These are parasitoids of eggs of praying mantises, crickets, harvestmen, locusts, the harmful beetle *Zabrus tenebrioides*, eggs of predatory carabids from the *Amara* and *Harpalus* genera. Several specimens of the genus *Telenomus* are parasitoids of true bugs eggs. There are also, the eggs of species from the orders Lepidoptera and Neuroptera (Chrysopidae). Therefore, it is important to key these specimens to species level.

## CONCLUSIONS

It was observed that the most effective fixation for the trap belts preservation for maintaining the arthropod complex in field conditions is the one with double corrugation and holes of different diameters equipped with polyester thread mesh with Velcro.

The arthropod complex hibernating in the modified trap belts had quite significant quantities, where polyphagous predators (arachnids) from the families Thomysidae and Phylodromidae dominated to a large extent, which constituted 45.7%.

The predators of aphids, mites and mealybug *Psedoccocus comstoki* were represented by 15 species from three orders Neuroptera, Coleoptera and Thysanoptera.

The percentage of parasitism of *Scambus inanis* on moth larvae hibernating in the belts was determined on variants where it was established that the difference of 10.7% between the standard and the experimental variant was influenced by the vegetative period of the green manure.

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# EXPLORING THE PHYTOCHEMICAL PROFILE AND SENSORY PROPERTIES OF GREEN AND ROASTED *COFFEA ARABICA* L. BEANS FROM DIVERSE GEOGRAPHIC REGIONS

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***Abstract.*** *The aim of study was to evaluate the phytochemical quality indicators (dry matter, ash, crude protein, antioxidant activity, total flavonoids, polyphenols and phenolic acids) of 100% Arabica (Coffea arabica L.) from Brazil, Colombia, Bolivia, Tanzania, El Salvador and Cuba. The samples were first analyzed as raw green coffee and then after the roasting process (medium roast 240 °C, 14 minutes). The same measurements were then performed on the roasted coffee as on the green coffee, to compare the samples and draw attention to the changes that occur during the roasting process. Finally, a sensory analysis (color, taste, aroma) was performed (the coffee was served using the French press method). The total dry matter content in green coffee was on average 93.05%, the crude protein content was 13.07% and the ash content was 2.94%. Using the DPPH method was determined the antioxidant activity, which was in the range of 7.32 – 21.55 mg TEAC/g (TEAC – Trolox equivalent antioxidant capacity) and the total content of polyphenols was on average 45.65 mg GAE/g (GAE – gallic acid equivalent). Subsequently, we determined the total content of flavonoids on average 31.91 mg QE/g (QE – quercetin equivalent) and the content of phenolic acids on average 13.14 mg CAE/g (CAE – caffeic acid equivalent). This was followed by roasting and repeating all measurements, with which was found an increase in dry matter by 5.93% and ash by 0.19%. When determining the total crude protein, only imperceptible changes occurred. The most significant decrease was observed in polyphenols and flavonoids, the total contents of which decreased during roasting, since these are relatively thermolabile substances. The content of total polyphenols after roasting in the tested samples ranged from 26.58 to 34.36 mg GAE/g, which is a decrease of more than 25%. As for flavonoids, the value ranged between 10.56 and 17.79 mg QE/g. The antioxidant activity did not change significantly, since various other bioactive substances participating in the antioxidant activity formed during roasting. The crude protein content also did not change significantly. The sensory evaluation took place without problems and all samples were evaluated relatively positively, the respondents evaluated the color, aroma, taste.*

***Keywords: Coffea spp., polyphenols, antioxidant activity, sensory evaluation, roasting, DPPH.***

## INTRODUCTION

*Coffea arabica* L., also known as Arabica coffee, is one of the most popular coffee species worldwide. It is believed to be the first coffee species to be commercially cultivated and is the dominant cultivar, accounting for about 60% of global production (Lashermes et al., 1999*). Coffea arabica* was first mentioned in Yemen and documented as early as the 12th century (Söndahl, 2005). Wild plants are 9 to 12 m tall and have an open branching system; the leaves are opposite, simple elliptic-ovate to oblong, 6–12 cm long and 4–8 cm broad, glossy dark green. The flowers are white, 10–15 mm in diameter, and grow in axillary clusters. The seeds are contained in a drupe 10-15 mm in diameter, maturing bright red to purple and usually contain two seeds. Dried coffee berries, stripped of pulp and skins, contain similar acids, proteins and caffeine as roasted coffee. However, roasting coffee acquires sensory properties that green beans lack, primarily the characteristic smell, aroma, taste and color. Dry green beans intended for roasting contain: 12% water, 13% protein, 12% fat, 9% carbohydrates, 1-1.5% caffeine, 9% acid, 35% cellulose and 4% ash (Wintgens, 2009). The coffee beans after medium roast are already a bit darker and larger than in the first stage. Their surface is dry. The aroma of the coffee is slightly spicy and distinctly fruity. The fruit can also be felt in taste. Despite the still dominant acidity, a slightly sweet taste also appears in the coffee. The body develops and the aftertaste lasts a bit longer. Espresso has a thin, light creama. Coffee roasted in this way is still little used commercially, due to its distinctly exotic and sour taste. The non-volatile fraction of green coffee is primarily composed of water, carbohydrates, proteins, free amino acids, lipids, minerals, organic acids, chlorogenic acids, trigonelline, and caffeine. Of these compounds found in green coffee, chlorogenic acids are the most abundant, caffeine, trigonelline, and diterpenes from the lipid fraction are most likely bioactive and may also be important contributors to the beverage after roasting. In addition, traces of theophylline and theobromine have been identified in the seeds and reported to be metabolites of caffeine (Mazzafera et. al,. 2009). Many studies have shown that coffee consumption may help prevent several chronic diseases. Long-term coffee consumption is associated with a significant dose-dependent reduction in the risk of developing type 2 diabetes (Nawrot et. al., 2003). Coffee intake also reduces the risk of liver damage in people at high risk of liver disease, including liver damage, cirrhosis, and hepatocellular carcinoma (Homan and Mobarhan, 2006). The risk of Alzheimer's disease is lower in individuals who regularly consume caffeinated coffee than in those who do not (Mendonca and Cunha, 2010). Coffee has also been shown to improve endurance performance in long-term physical activities. Interestingly, the relative risk of suicide was reduced by 13% for each cup of coffee consumed per day (Kawachi et. al., 1996). Coffee consumption is generally inversely associated with cancer risk at various sites, including the liver and colon

and rectum, there is no universal.

The aim of this study was to evaluate the phytochemical quality indicators of 100% Arabica coffee *(Coffea arabica* L.) sourced from Brazil, Colombia, Bolivia, Tanzania, El Salvador, and Cuba. It was assessed various parameters, including dry matter, ash content, crude protein, antioxidant activity, total flavonoids, polyphenols, and phenolic acids. The coffee beans were analyzed in their green form and subsequently after medium roasting to determine how the roasting process influences these phytochemical properties.

## MATERIALS AND METHODS

### Material

The five coffee samples (100 Arabica) – Brazil, Bolivia, Tanzania, El Salvador and Cuba were provided by the private supplier from Nitra (Slovakia). Green coffee (100 g) was fed to a micro-roaster coffee machine (Gene Café, CBR-101, Korea). The beans were roasted by using medium roasting style –240 °C, 14 minutes. Roasted coffee bean was ground in a grinder machine (Krups, GVX 2, Bur Grinder, Germany). The samples were ground to an average particle size (20 mesh), suitable for preparation in a paper filter. To prepare the extract, 0.1 g of sample was extracted with 20 ml 95 °C distilled water, after 5-minute extraction filtered through filtrate paper (Whatman no 1) and used for analysis (antioxidant activity, total polyphenol, flavonoid and phenolic acid content).

### Chemical

All the chemicals used were of analytical grade and were purchased from Sigma- Adrich (St. Louis, MO, USA) and CentralChem (Slovakia).

### Dry matter, ash, and crude protein content

AACC method 08-01 used to determine the dry matter and ash content (AACC, 1996). The semi-micro-Kjeldahl method was used to calculate the nitrogen content. The usual factor of 6.25 was used to convert nitrogen to protein.

### DPPH – Free radical scavenging activity

According to the steps outlined by Sanchéz-Moreno et al. (1998), the 2,2-difenyl-1-picrylhydrazyl (DPPH) assay used to measure the samples' capacity to scavenge free radicals using a spectrophotometer (Jenway 6320D, UV VIS, UK). Trolox (10 – 100 mg/L; $R^2 = 0.989$) was used as a standard.

### Total polyphenol content

The total polyphenol content of samples determined spectrophotometrically (Jenway 6320D, UV VIS, UK) using modified Folin-Ciocalteu method by

Singleton et al. (1965). Gallic acid (25 – 300 mg/L; $R^2$ = 0.999) was used as a standard.

**Total Flavonoid Content**

Total flavonoids will be determined spectrophotometrically (Jenway 6320D, UV VIS, UK) using a modified method of Willett (2002). Quercetin (10 – 500 mg/L; $R^2$ = 0.999) was used as a standard.

**Total Phenolic Acid Content**

Total phenolic acids content will be determined (Jenway 6320D, UV VIS, UK) using the method of Jain et al. (2017). Caffeic acid (10 – 550 mg/L; $R^2$ = 0.988) was used as a standard.

**Sensory analysis**

A sensory panel of 30 panelists – 15 women and 15 men, aged 22 to 65 – determined the organoleptic properties of roasted coffee prepared in style French Press. The panelists were asked to assess the color, taste and aroma. A 9-point hedonic scale was used to rate the samples, with values ranging from 9 (like very much) to 1 (strongly dislike).

**Statistical analysis**

All experiments were carried out in triplicate and the results reported are the results of those replicate determinations with standard deviations.

## RESULTS AND DISCUSSION

**Dry matter, ash, and crude protein content**

When determining the dry matter, it was found that the average value for green coffee was 93.05% (Tab. 1). The highest value was measured in the sample from Colombia, which had a dry matter content of 93.822%. In contrast, the lowest value was found in the sample from El Salvador, with a dry matter content of 92.24%. Thus, it can be concluded that coffee is a sustainable crop, primarily due to its low moisture content and high dry matter content. However, we must adhere to the correct storage and packaging parameters to maintain this moisture level, as increased humidity poses a risk for the proliferation of bacteria and microscopic filamentous fungi. After roasting, the dry matter content increased in all tested samples (Tab. 1). The composition of the seed changes dramatically during roasting due to pyrolysis, caramelization and Maillard reactions. The moisture content of roasted coffee (1.5% - 5%) is much lower than that of green coffee and varies depending on the degree of roasting. The beans become more brittle and can be chewed relatively easily, unlike green beans. However, even though roasted coffee seems to be a durable product in terms of dry matter and moisture content,

care should be taken to store it properly, in a closed container and in a cool room.

When determining the total crude protein, it was measured at an average value of 13% (Tab. 1). The highest measured value of crude protein was determined for the Cuba sample at 13.78% and the lowest for the Salvador sample at 12.06%. All samples had a similar protein content without large deviations. After roasting, the samples measured average values of 13.93%. The highest crude protein content was found in the Cuba sample (14.91%), which was an increase of 1.13% compared to the same green coffee sample. The lowest crude protein content was found in the Tanzania sample (13.16%). Overall, it can be concluded that the crude protein content did not change much during the roasting process (Tab. 1). Part of the coffee protein is degraded, and free amino acids and peptides are consumed by Strecker reactions. Some of these amino acids react with reducing sugars to form (via the Maillard reaction) low molecular weight compounds and melanoidins, which incorporate other components such as chlorogenic acids, galactomannans and arabinogalactan proteins into their structures. Globulin 11S is the main protein of coffee beans – 45% of the total protein, but there is no conclusion yet about its participation in coffee quality. It is now generally known that free amino acids and peptides are required for the formation of coffee aroma. However, the mechanisms leading to the defined mixtures of free amino acids and peptides remain unknown (Bhattarai et al., 2022).

The ash content determined in green coffee samples was on average 2.94%, with the highest content being reached in the Bolivia sample with a value of 2.96%. The lowest ash content was reached in samples from Brazil, Cuba, and El Salvador with a content of 2.93% (Tab.1). After roasting, a slight increase was recorded compared to green coffee beans, which is due to the difference in the water/dry matter ratio in green coffee and coffee after roasting. The highest difference was recorded in the El Salvador sample, where the ash content increased from 2.93% to 3.14%. The lowest difference was in the Bolivia sample, where the ash content increased from 2.96% to 3.1%. The average value of all samples was 3.13%, which is an increase of 0.19% compared to green coffee beans (Tab. 1). Regarding the mineral content of roasted coffee, data from the literature are sparse and show a small increase related to weight loss during roasting (Ferreira et.al., 1971; Clarke, 1985). There is evidence that both mineral content and profiles in coffee can be influenced by growing conditions (e.g. fertilizers, maturity), prior processing operations (dry vs. wet processing) and fermentation (Clarke, 1985). Since the mineral content of coffee is not affected by roasting and is known to vary only according to the maturity of the coffee beans, elemental analysis could be a potential candidate for differentiating between defective and non-functional beans in roasted coffee. Oliveira et. al., (2006) and Vasconcelos et. al., (2007) evaluated the total mineral content of defective coffee beans compared to non-defective ones. Both studies reported higher mineral contents of defective beans (~6%) compared to healthy ones (~5%) before and after roasting.

Table 1. Results of dry matter, ash and protein content in green and roast coffee samples

| Sample | Dry matter (%) | Ash content (%) | Crude protein (%) |
|---|---|---|---|
| **Brazil green** | 93.38 ±0.11 | 2.93 ±0.02 | 13.39 ±0.14 |
| **Brazil roast** | 99.06 ±0.13 | 3.16 ±0.01 | 13.94 ±0.02 |
| **Tanzania green** | 92.73 ±0.09 | 2.94 ±0.04 | 12.06 ±0.11 |
| **Tanzania roast** | 98.75 ±0.09 | 3.12 ±0.02 | 13.16 ±0.09 |
| **El Salvator green** | 92.24 ±0.14 | 2.93 ±0.01 | 12.07 ±0.01 |
| **El Salvator roast** | 99.91 ±0.14 | 3.14 ±0.05 | 13.28 ±0.01 |
| **Colombia green** | 93.82 ±0.05 | 2.95 ±0.05 | 13.21 ±0.04 |
| **Colombia roast** | 99.12 ±0.14 | 3.17 ±0.02 | 14.14 ±0.03 |
| **Bolivia green** | 92.75 ±0.12 | 2.96 ±0.05 | 13.54 ±0.02 |
| **Bolivia roast** | 99.16 ±0.08 | 3.13 ±0.02 | 14.18 ±0.01 |
| **Cuba green** | 93.44 ±0.04 | 2.93 ±0.01 | 13.78 ±0.05 |
| **Cuba roast** | 98.91 ±0.01 | 3.11 ±0.02 | 14.91 ±0.05 |

*mean± standard deviation*

The average antioxidant activity value of green coffee was 14.131 mg TEAC/g. As we can see in Tab. 2, the highest value was measured in a coffee sample from Tanzania with a value of 21.55 mg TEAC/g, which is a big difference compared to the sample with the lowest value of El Salvador 7.32 mg TEAC/g. With relatively high antioxidant activity are the samples from Bolivia 17.54 mg TEAC/g and Colombia 14.01 mg TEAC/g. The differences between the samples can be explained by the differences in the countries and localities where they came from different agro-ecological conditions, which have a significant impact on how many antioxidant compounds are synthesized in coffee.

The average antioxidant activity value of roasted coffee was 8.38 mg TEAC/g, which is a significant decrease compared to the average value of green coffee of 14.13 mg TEAC/g. The highest antioxidant activity value was recorded in the Tanzania sample of 10.27 mg TEAC/g, which is a difference compared to the green sample of this coffee of 11.28 mg TEAC/g. The lowest value was determined in the Salvador sample of 4.28 mg TEAC/g, which overall showed the lowest antioxidant activity of all samples, whether before or after roasting (Tab. 2). Czakóová, (2019) measured the antioxidant activity of roasted coffee beans using the same DPHH method. She followed the same method, but used a roasting level called cinnamon roast, which uses slightly lower roasting temperatures than in roasting regime used for sample of this study (medium roast). Czakóová, (2019) used different mixtures of samples to determine antioxidant activity, but for the Tanzania Iyela PB sample she measured a value of 53.47 mg TEAC/L, so for our Tanzania sample we determined a value of 10.27 mg TEAC/L. If we compared the results in the same units, our antioxidant activity value in the Tanzania sample would be lower, which could result in the influence of agroecological factors such as vintage, soil, processing method, type of roasting.

By determining the total polyphenols in green coffee samples, it was measured

an average value of 45.65 mg GAE/g (Tab. 2). The highest polyphenol value was achieved in the sample from Brazil at 52.8 mg GAE/g. The lowest value was found in the sample from Colombia at 39.69 mg GAE/g. The highest limit was also reached in samples from Tanzania at 51.91 mg GAE/g and Cuba at 44.58 mg GAE/g. When determining the total polyphenols of roasted coffee, an average value of 31.708 mg GAE/g was measured, which is a decrease of 13.943 mg GAE/g compared to the total polyphenol content of green coffee. The highest measured value was reached by the Brazil sample at 34.36 mg GAE/g and the lowest by Cuba at 26.58 mg GAE/g. The lowest decrease compared to green coffee was in the Colombia sample, where the total amount of polyphenols decreased from the original 39.69 mg GAE/g to 33.13 mg GAE/g (Tab. 2). There is growing evidence that some polyphenols, such as chlorogenic acid, have biological effects in the small intestine that alter the form of glucose taken up. Chlorogenic acid has been shown to inhibit the action of glucose-6-phosphate, which plays a key role in regulating glucose metabolism. It increases alertness and motivation, helps reduce the risk of depression, and increases the need for exercise. Long before epidemiological studies suggested a link between coffee consumption and health, the antimutagenic properties of chlorogenic acids and their metabolites were demonstrated in a series of animal and in vitro studies. Recent studies have confirmed these findings and elucidated several mechanisms of action, including scavenging free radicals, metal chelation, inactivation of reactive compounds, and alterations in metabolic pathways.

The total flavonoid content in the green coffee samples was on average 31.91 mg QE/g. The highest measured value was found in the sample from Tanzania at 37.98 mg QE/g and the lowest measured value was in the sample from Cuba at 28.74 mg QE/g (Tab. 2). After roasting, a decrease of almost 50% was observed in each sample compared to the green coffee samples before roasting, which can be explained by the fact that flavonoids are the most sensitive to high temperatures in terms of stability among polyphenolic compounds (Tab. 2). Flavonoids are effective free radical scavengers as shown *in vitro* studies. However, even with very high flavonoid intakes, plasma and intracellular flavonoid concentrations in humans are probably 100 to 1,000 times lower than the concentrations of other antioxidants, e.g. ascorbate (vitamin C), uric acid and glutathione. Flavonoids are considered the most common, important and widely distributed group of phenols present in plants with highly effective antioxidants. Flavonoids inhibit metal-initiated lipid oxidation by forming complexes with metal ions (Mestanza et al., 2023).

The measured value of phenolic acids in green coffee samples was on average 13,136 mg CAE/g. The highest content of phenolic acids was in the sample from Tanzania, 16.01 mg CAE/g, and the lowest in the sample from El Salvador, 10.79 mg CAE/g (Tab.2). When determining the total phenolic acid content of roasted coffee, a decrease was recorded compared to green coffee. The average phenolic acid content of roasted coffee was 6.808 mg CAE/g, which is 6.328 mg CAE/g less than in green coffee samples. The highest value was achieved by the Tanzania sample, 8.02 mg CAE/g, while

the lowest value of the total phenolic acid content was achieved by the Cuba sample, only 4.4 mg CAE/g. The highest decrease compared to the same green coffee sample was observed in the Tanzania sample, a decrease of 7.99 mg CAE/g (Tab. 2).

Phenolic acids are substances that act as primary antioxidants, as they react with free radicals that are formed in the body. The activity depends on the number of hydroxyl groups in the molecule (Hírešová, 2015). Active antioxidants are cinnamic acids and their esters, so-called depsides, primarily quinic acid and chlorogenic acid (but more than 30 cinnamic acid esters are found in coffee). Chlorogenic acid refers to all natural quinic acid esters. These are the main acids of green coffee, where they occur in an amount of 7-10% of dry matter. During roasting, approximately 30-70% of it degrades to form phenols (benzoic acid, pyrogallol, pyrocatechol, resorcinol) and other compounds.

Table 2 Results of antioxidant activity (DPPH), total polyphenols (TPC), flavonoids (TFC) and phenolic acids (TPAC) in green and roast coffee samples

| Sample | DPPH (mg TEAC/g) | TPC (mg GAE/g) | TFC (mg QE/g) | TPAC (mg CAE/g) |
|---|---|---|---|---|
| **Brazil green** | 11.20 ±1.11 | 52.81 ±0.12 | 30.17 ±0.04 | 11.77 ±0.12 |
| **Brazil roast** | 8.14 ±0.03 | 34.36 ±0.12 | 14.84 ±0.01 | 7.11 ±0.12 |
| **Tanzania green** | 21.55 ±0.13 | 51.91 ±0.14 | 37.98 ±0.08 | 16.01 ±0.14 |
| **Tanzania roast** | 10.27 ±0.03 | 33.02 ±0.11 | 17.79 ±0.09 | 8.02 ±0.11 |
| **El Salvator green** | 7.32 ±0.01 | 42.80 ±0.01 | 31.79 ±0.04 | 10.79 ±0.01 |
| **El Salvator roast** | 4.28 ±0.02 | 30.36 ±0.04 | 17.51 ±0.02 | 8.01 ±0.04 |
| **Colombia green** | 14.10 ±0.15 | 39.69 ±0.05 | 28.84 ±0.03 | 12.36 ±0.05 |
| **Colombia roast** | 9.25 ±0.04 | 33.13 ±0.02 | 15.79 ±0.03 | 6.62 ±0.02 |
| **Bolivia green** | 17.54 ±0.02 | 42.13 ±0.03 | 33.98 ±0.07 | 14.67 ±0.03 |
| **Bolivia roast** | 9.94 ±0.11 | 32.80 ±0.02 | 13.50 ±0.01 | 6.71 ±0.02 |
| **Cuba green** | 13.17 ±0.11 | 44.58 ±0.01 | 28.74 ±0.04 | 13.22 ±0.01 |
| **Cuba roast** | 8.43 ±0.07 | 26.58 ±0.02 | 10.56 ±0.05 | 4.41 ±0.02 |

*mean± standard deviation; DPPH – 2,2-difenyl-1-picrylhydrazyl; TEAC – Trolox equivalent antioxidant capacity; GAE – gallic acid equivalent; QE – quercetin equivalent; CAE – caffeic acid equivalent*

**Sensory analysis**

When evaluating coffee color, distinct characteristics emerge based on origin and scoring: Brazil (4 points) exhibits a muted color, suggesting a darker, less vibrant roast, Colombia (9 points) bright and lively appearance, indicating a well-developed roast with golden to deep brown hues, often linked to higher acidity and fruitiness. Tanzania (9 points) similar to Colombia, it shows a vibrant color profile, reflecting fruity and floral characteristics, El Salvador (7 points) presents a balanced color, suggesting a moderate roast with sweetness and complexity, Bolivia (8 points) rich and inviting color, indicating a well-crafted roast with nuanced flavors, Cuba (4 points) similar to Brazil, it has a less vibrant color, possibly reflecting a darker, traditional roasting style (Figure 1).

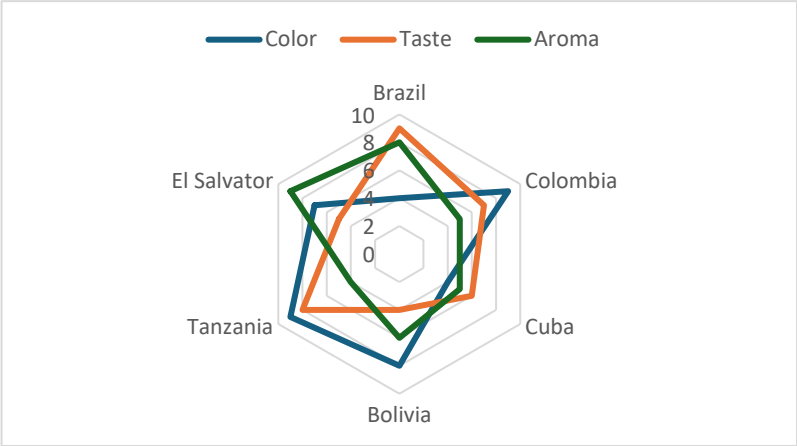


Figure 1. Results of sensory analysis of roasted coffee samples (sum of all evaluators)

The sensory evaluation of coffee reveals distinct taste profiles among various origins. Brazilian coffee stands out with a score of 9, showcasing a rich, well-rounded flavor often characterized by chocolate and nutty notes, along with a pleasant sweetness. Colombian coffee, rated at 7, offers a balanced taste with bright acidity and fruity undertones, though slightly less complex than its Brazilian counterpart. Tanzanian coffee, scoring 8, is notable for its vibrant acidity and berry-like flavors, providing an intriguing contrast to the smoother Brazilian profile. El Salvador's coffee, with a score of 5, tends to be milder and less memorable, lacking the depth found in higher-rated origins. Bolivia, at 4, presents a more subdued flavor profile that may come across as flat or lacking in character. Finally, Cuban coffee, rated 6, offers a unique taste experience, often marked by earthy and herbal notes, but it does not quite reach the level of complexity found in the top-rated coffees. Overall, the sensory evaluation highlights a spectrum of flavors, with Brazilian and Tanzanian coffees leading the pack in taste quality (Fig. 1). The sensory evaluation of coffee aromas reveals a diverse range of characteristics across different origins. Brazilian coffee stands out with a score of 8 points, indicating a rich and well-rounded aromatic profile that likely includes nutty and chocolatey notes. In contrast, Colombian coffee, with a score of 5, presents a more subdued aroma, typically featuring fruity and floral nuances but lacking the complexity found in Brazilian varieties. Tanzanian coffee, scoring 4 points, offers a less pronounced aroma, often marked by mild citrus hints but lacking depth. El Salvador's coffee excels with a top score of 9, showcasing a vibrant and captivating aroma, possibly rich in caramel and spicy undertones. Bolivian coffee scores 6 points, suggesting a balanced aroma with a moderate level of sweetness and earthiness. Finally, Cuban coffee, also scoring 5 points, presents a familiar yet less distinctive aromatic profile, often

characterized by a blend of earthy and mild floral notes. Overall, the evaluation highlights the distinct sensory properties of each coffee origin, with El Salvador and Brazil leading in aromatic complexity (Fig. 1).

## CONCLUSIONS

The total dry matter content in green coffee was on average 93.05%, the crude protein content was 13.07%, and the ash content was 2.94%. Using the DPPH method, the antioxidant activity was determined in the range of 7.32 - 21.55 mg TEAC/g and the total polyphenol content was on average 45.65 mg GAE/g. The total flavonoid content was on average 31.91 mg QE/g and the phenolic acid content was on average 13.13 mg CAE/g. This was followed by roasting and repeating all measurements, during which an increase in dry matter by 5.93% and ash by 0.19% was found. Only imperceptible changes occurred in the determination of the total crude protein. The most significant decrease was recorded in polyphenols and flavonoids, which can be explained by the fact that these are relatively thermolabile substances. The content of total polyphenols after roasting ranged from 26.58 to 34.36 mg GAE/g, which is a decrease of more than 25%. As for flavonoids, the value ranged between 10.56 and 17.79 mg QE/g. Antioxidant activity varied, but not significantly, since various other bioactive substances are formed during roasting, which support antioxidant activity. The content of crude protein also did not change significantly. Sensory analysis showed that all samples were evaluated relatively positively, respondents evaluated the color, aroma, and taste. Overall, it can be concluded that coffee in our diet contributes to a very important source of bioactive substances, but consumption is necessary only in adequate quantities, since excessive drinking coffee can have an adverse effect on the body due to the content of alkaloids. However, it is important, especially to store ground coffee correctly. Therefore, we recommend that everyone drinks coffee mainly from freshly ground beans.

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# INFLUENCE OF ROOT ENVIRONMENT HUMIDITY ON THE GENERATION OF POTENTIAL DIFFERENCE IN A PLANT BIOELECTROCHEMICAL SYSTEM

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***Abstract:*** *The dynamics of potential difference formation in bioelectrochemical systems with different root environment humidity levels from 13% to 60% was studied when growing bok choi cabbage. The highest voltage values reached 320 mV, specific power 0.01 mW/m$^2$ and were typical for samples with higher humidity. A weak correlation (r=0.3) between the root environment humidity indicators and the generated potential difference was obtained. It was suggested that, along with the properties of the root environment, the state of plants and the intensity of metabolic processes also play a significant role in voltage formation.*

***Keywords: bioelectrogenesis, bok choi, green energy, monitoring, root system.***

## INTRODUCTION

Bioelectrogenic processes occurring in the root environment of plants, associated with the generation of potential differences during oxidation-reduction reactions and ion diffusion accompanying the development of the root system, are the basis for the creation of alternative devices for obtaining renewable, environmentally friendly, resource-saving energy – bioelectrochemical systems (BES) [1-3]. Electricity generation using biotechnological devices can be combined with production processes, one of which is the production of plant products. The properties of the root environment play a significant role in the formation of the electrogenic parameters of BES. Thus, it has been shown that when growing plants using peat, the average potential difference formed in the root environment of lettuce plants was about 300 mV [4], and when cultivating using the panoponics method, about 200 mV [5]. A change in the potential difference depending on the soil depth has been determined [6]. It has been suggested that the flow of water introduced into the system daily triggers a number of physical and chemical reactions on the electrodes – diffusion, oxidation-reduction processes, the intensity of which decreases as the liquid moves deeper into the soil.

Thus, the humidity of the root environment is one of the key parameters affecting the energy characteristics of the BES. The method of non-invasive measurement of bioelectric potentials in the soil-plant system, based on a new

method of providing surface contact between the root system and the electrodes, was used to study the responses of the BES to water deficiency stress and watering [7]. It has been shown that watering the plant from above causes a jump in biopotential at a level of 20-150 mV depending on the duration of the plant's exposure to water, while the influx of water from below leads to a smooth change in the bioelectric potential with an output at a level corresponding to the new physiological state of the plant. The aim of this work was to study the influence of humidity parameters of the root environment on the formation of electrical properties of bioelectrochemical systems when growing plants in them.

## MATERIALS AND METHODS

The phyto-test object was pak choi (*Brassica rapa* L.), an early maturing plant rich in fiber and vitamin C.

The bioelectrochemical system was a 440 $cm^3$ plant growing container. Electrical characteristics were measured by placing 8*8 cm biocompatible electrode systems in the root environment, which ensured surface electrical contact with the roots and root zone. The lower electrode was located at a distance of 3 cm from the bottom of the container, the upper electrode was placed at a distance of 3 cm from the lower electrode (Fig. 1a). In this experiment, a BES configuration with an upper electrode made of stainless steel and a lower electrode made of graphite felt was used.

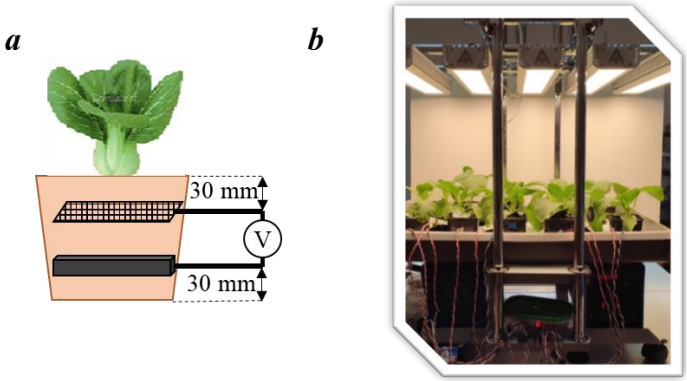


Figure 1. a – schematic representation of BES based on pak choi cabbage, b – vegetation-irradiation installation Ecofil.

The experiments were conducted in the controlled conditions of the agrobiological testing ground of the FGBNU AFI using the Ecofil vegetative-irradiation installation (Fig. 1b). The light source was AFI-3000 – LED lamps with a color temperature of 3000 K with a spectral composition in the visible region close to natural light, with a photosynthetic photon flux density PPFD of 320±20

μmol $m^{-2}$ $s^{-1}$. The duration of the light period was 14 hours per day. The root environment was Agrobalt S peat soil (Pindstrup LLC, Russia) based on low-decomposition high-moor peat containing at least: N - 150 mg/l, P2O5 - 150 mg/l, K2O - 250 mg/l, Mg - 30 mg/l, Ca - 120 mg/l. Watering was carried out with Knop solution.

Monitoring of changes in the potential difference in the BES was carried out every 15 minutes using the Arduino Mega hardware platform built on the ATmega2560 microcontroller [10].

Ten BES variants were studied, differing in the level of humidity of the root environment from 13% to 60%, which was provided by daily automatic watering with Knop's solution in different volumes. The humidity indicators of the root environment were obtained by measuring in five places of the BES with subsequent averaging using a capacitive sensor.

## RESULTS AND DISCUSSIONS

The average humidity value in the root environment was: BES-1 – 10.9 relative units, BES-2 – 18.3 relative units, BES-3 – 10.8 relative units, BES-4 – 10.9 relative units, BES-5 – 22.1 relative units, BES-6 – 13.8 relative units, BES-7 – 16.8 relative units, BES-8 – 16.7 relative units, BES-9 – 8.7 relative units, BES-10 – 21.5 relative units.

Fig. 2 shows the dynamics of changes in the potential difference when growing pak choi plants in a BES with variable humidity values of the root environment. The average voltage value in plant systems was: BES-1 – 312±36 mV, BES-2 – 314±48, BES-3 – 310±34 mV, BES-4 – 298±27 mV, BES-5 – 282±32 mV, BES-6 – 286±27 mV, BES-7 – 310±37 mV, BES-8 – 327±30 mV, BES-9 – 264±27 mV, BES-10 – 317±52 mV.

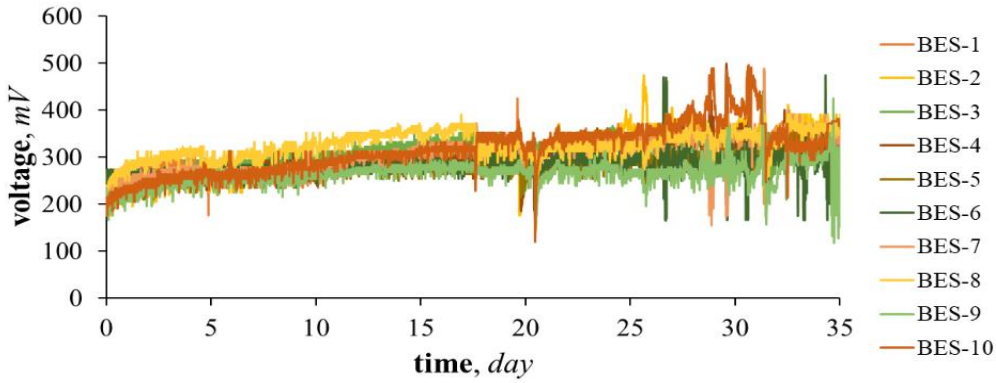


Figure 2. Changes in the potential difference in the BES during the growing season of pak choi at different humidity levels of the root environment.

BES-8 and BES-10 with the highest humidity were characterized by the

highest voltage generation. However, for the BES-5 sample with comparable humidity indicators, the potential difference was smaller. A weak correlation (r=0.3) was obtained between the humidity indicators of the root-inhabited environment and the generated potential difference (Fig. 3). It is likely that, along with the properties of the root environment, the state of the plants and the intensity of metabolic processes also play a significant role in the formation of voltage.

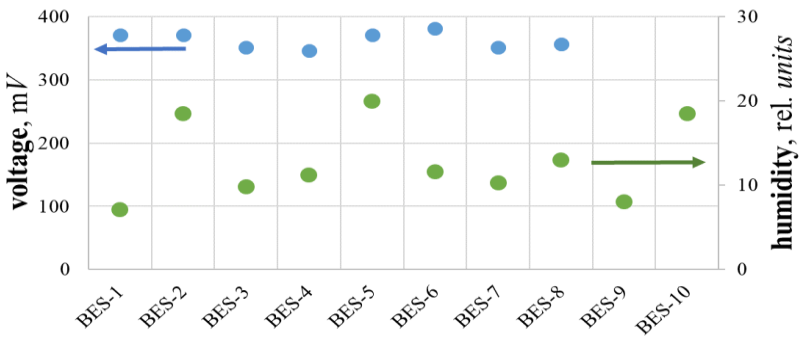


Figure 3. Comparison of the humidity indicators of the root environment and the generated potential difference when growing pak choi in a BES on the 36th day.

Polarization curves were measured for the studied BES with different humidity levels, taken on the 36th day of pak choi vegetation. For BES-10 with the highest humidity, the maximum power generation of 0.01 mW was observed, the lowest indicator – 0.005 mW was characteristic of BES-6 with one of the lowest values of root environment humidity. The obtained morphometric characteristics of pak choi cabbage samples grown with different root environment humidity are presented in Table 1. It is evident that the plants have a strong spread in physiological indicators. The largest mass of the above-ground part and leaf area were characteristic of the BES-1 and BES-4 variants, and the lowest indicators for BES-10, which was inversely proportional to the formed average root environment humidity for these variants.

Table 3. Morphometric characteristics of BES with different humidity levels.

| BES options | BES 1 | BES 2 | BES 3 | BES 4 | BES 5 | BES 6 | BES 7 | BES 8 | BES 9 | BES 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Height, cm** | 14±1 | 15±2 | 15±1 | 19±2 | 10±1 | 9±2 | 10±1 | 15±1 | 10±1 | 11±1 |
| **Diameter, cm** | 27±2 | 24±3 | 23±1 | 30±3 | 20±3 | 8±2 | 21±2 | 12±1 | 13±1 | 12±1 |
| **Weight, g** | 185±14 | 69±8 | 95±4 | 200±20 | 64±9 | 61±11 | 118±13 | 114±7 | 90±6 | 46±55 |
| **Leaf area, $cm^2$** | 156±12 | 130±15 | 110±5 | 195±20 | 64±9 | 56±11 | 100±11 | 99±6 | 63±4 | 60±6 |

Thus, the role of the humidity of the root environment in the formation of the potential difference in the BES during the development of pak choi plants was established - the highest voltage values were characteristic of samples with higher

humidity, the specific power reached 0.01 mW/m$^2$.

## CONCLUSIONS

The possibility of using bioelectrogenesis in the root environment-plants system as a new green source of electric power has great potential for use, first of all, in agriculture to provide power to low-power devices for monitoring the state of the environment and plants, as well as partial power supply of devices for supporting the life of plants in artificial agroecosystems, which is especially important in areas remote from power grids.

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# CATALYTIC OXIDATION OF GALLIC: MECHANISMS, KINETICS, AND BYPRODUCT ANALYSIS

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***Abstract.*** *This paper investigates the catalytic oxidation of gallic acid utilizing Fenton and Fenton/UV reactions, focusing on elucidating the mechanisms, kinetics, and byproducts of the oxidation processes. Gallic acid, a naturally occurring phenolic compound, presents both health benefits and environmental challenges due to its persistence and potential toxicity. The Fenton and Fenton/UV reactions generate highly reactive hydroxyl radicals, initiating the oxidation of GA. The study explores the pseudo-first-order kinetics of GA degradation ($1.47 \cdot 10^{-4}$ M), examining variables such as [$Fe^{2+}$], [$H_2O_2$], pH, and temperature. Results indicate 50% degradation and 30% mineralization after 5 min, with improved COD removal over 60 min. Optimal conditions were identified as [$H_2O_2$] = $5 \cdot 10^{-4}$ M, [$Fe^{2+}$] = $1 \cdot 10^{-5}$ M, pH=3.0. The average rate constant ($k_{av}g$) was $5.7 \cdot 10^{-3}$ $s^{-1}$. Intermediate byproducts include hydroxylated derivatives and carboxylic acids. These findings highlight the relevance of advanced oxidation for environmental biotechnology.*

***Keywords: Fenton, gallic acid, kinetics, oxidation, wastewater treatment.***

## INTRODUCTION

Gallic acid (GA), a trihydroxybenzoic acid prevalent in various plant materials and industrial effluents, is widely utilized in pharmaceutical, cosmetic, and food industries due to its antioxidant, antimicrobial, and anti-inflammatory properties [1]. However, its release into aquatic systems contributes to environmental contamination and necessitates effective treatment technologies [2].

Traditional wastewater treatment methods often fail to degrade such recalcitrant phenolic compounds effectively. Advanced oxidation processes (AOPs), particularly the Fenton and photo-Fenton reactions, have emerged as promising alternatives. These methods rely on the in situ generation of hydroxyl radicals (•OH), which are highly reactive and capable of mineralizing a wide spectrum of organic pollutants [3].

This study aims to investigate the kinetics, efficiency, and degradation mechanisms of GA oxidation via Fenton and Fenton/UV processes, with an emphasis on intermediate byproduct formation and potential integration with microbiological

treatments.

## MATERIALS AND METHODS

*Reagents and Reaction Conditions*

Gallic acid ($C_7H_6O_5$) degradation was studied using Fenton ($Fe^{2+}/H_2O_2$) and photo-Fenton ($Fe^{2+}/H_2O_2$/UV) systems. Gallic acid (GA) solutions at an initial concentration of $1.47 \cdot 10^{-4}$ M were prepared using deionized water. Hydrogen peroxide ($H_2O_2$) served as the oxidizing agent, and ammonium iron(II) sulfate ($(NH_4)_2SO_4 \cdot Fe(SO_4) \cdot 6H_2O$) provided the ferrous ions. Hydroxyl radicals were generated through the classical Fenton reaction:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \bullet OH$$

Experiments were conducted at room temperature (25 ± 1 °C) in a batch reactor. The solution pH was adjusted to 3.0 using sulfuric acid ($H_2SO_4$), based on the optimal acidic condition for Fenton and Fenton/UV reactions (Bautista et al., 2008). For the Fenton/UV process, a UV lamp emitting at 254 nm was used to irradiatethe reaction mixture to enhance •OH radical generation. Samples were withdrawn at fixed time intervals for analysis.

The concentration of gallic acid was monitored by UV-Vis spectrophotometry at 265 nm. Chemical oxygen demand (COD) was measured according to standard methods to assess the mineralization degree.

*Analytical Methods*

The degradation of GA was monitored via UV–Vis spectrophotometry at 265 nm. Mineralization was evaluated by chemical oxygen demand (COD) analysis, and byproduct identification was performed using high-performance liquid chromatography (HPLC), following methods reported in prior studies [4].

*Kinetic Modeling*

The degradation of gallic acid was modeled by pseudo-first-order kinetics, with the rate equation:

$$d[C]/dt = -k_{obs}\,[C]$$

where:

[C] is the concentration of gallic acid at time $t$

$k_{obs}$ is the observed pseudo-first-order rate constant ($s^{-1}$)

Integration between $t = 0$ and $t = t$ yields:

$$\ln([C_0]/[C_t]) = k_{obs} \cdot t$$

where:

$[C_0]$ and $[C_t]$ are the initial and time-dependent concentrations of GA, respectively.

The half-life $\tau_{1/2}$ of GA under these conditions is given by:

$$\tau_{1/2} = \ln(2) / k_{obs}$$

The average reaction rate during the half-life was calculated as:
$r_{avg} = \Delta C / \Delta t = ([C_0] - [C\tau_{1/2}]) / \tau_{1/2}$

Average reaction rates were derived from concentration versus time data, enabling assessment of overall degradation efficiency.

## RESULTS AND DISCUSSIONS

*Gallic Acid Degradation Kinetics*

The oxidation of gallic acid (GA) by Fenton and Fenton/UV processes resulted in rapid degradation. Under optimized conditions, approximately 50% of GA was degraded and 30% mineralized within the first 300 s. After 60 min, the chemical oxygen demand (COD) removal increased slightly, indicating prolonged oxidation of intermediates The average reaction rate was calculated as $6.7 \cdot 10^{-7}$ $M \cdot s^{-1}$, and the pseudo-first-order rate constant was determined to be $5.7 \cdot 10^{-3}$ $s^{-1}$, with a corresponding half-life ($\tau_{1/2}$) of 118 s.

These results are consistent with earlier studies reporting that optimal Fenton oxidation occurs at low pH values (around 3.0) due to the maximum stability of $Fe^{2+}$ ions and effective generation of •OH radicals [3]. UV irradiation in the photo-Fenton process further enhanced the oxidation efficiency by photoreducing $Fe^{3+}$ back to $Fe^{2+}$ and generating additional •OH radicals through photolysis of $H_2O_2$ [5].

The intermediates detected included hydroxylated compounds and low molecular weight acids such as oxalic acid, formic acid, and acetic acid. These findings suggest both hydroxylation and ring cleavage mechanisms are active. The table below summarizes the kinetic parameters, which were obtained by plotting $\ln([C_0]/[C_t])$ versus time and fitting a linear regression to experimental data.

Table 1. Kinetic parameters and experimental conditions

| Parameter | Value | Units | Notes |
|---|---|---|---|
| Initial GA concentration ($C_0$) | $1.47 \cdot 10^{-4}$ | M | Set experimentally |
| $[H_2O_2]$ | $5 \cdot 10^{-4}$ | M | Optimal oxidant dose |
| $[Fe^{2+}]$ | $1 \cdot 10^{-5}$ | M | Catalyst |
| pH | 3.0 | - | Optimal acidic condition |
| Rate constant ($k_{av}g$) | $5.7 \cdot 10^{-3}$ | $s^{-1}$ | Pseudo-first-order |
| Half-life ($\tau_{1/2}$) | 118 | s | Calculated via ln(2)/k |
| Average reaction rate ($\Delta c/\Delta t$) | $6.7 \cdot 10^{-7}$ | $M \cdot s^{-1}$ | Degradation velocity |
| Degradation at 5 min | 50% | % | Spectrophotometric analysis |
| Mineralization at 5 min | 30% | % | COD analysis |
| COD removal at 60 min | ~55% | % | Final removal efficiency |

*Byproduct Formation and Oxidation Mechanism*

Analysis of the reaction intermediates revealed the formation of hydroxylated aromatic compounds and short-chain aliphatic acids, including oxalic, formic, and acetic acids. These findings indicate a sequential degradation pathway involving initial hydroxylation of the aromatic ring, followed by oxidative ring cleavage and

mineralization to $CO_2$ and $H_2O$ [4].

The regeneration of $Fe^{2+}$ ions by GA and its oxidized products, such as quinones and semiquinone radicals, suggests a self-sustaining mechanism where GA acts both as a substrate and a reducing agent. This process not only sustains the catalytic cycle but also enhances pollutant degradation efficiency [4,5]. Figure 1 illustrates the proposed mechanism of GA oxidation under Fenton conditions, highlighting the formation of hydroxylated intermediates, ring-opening reactions, and mineralization steps. This mechanism aligns with prior research on phenolic compound degradation via Fenton chemistry [5].

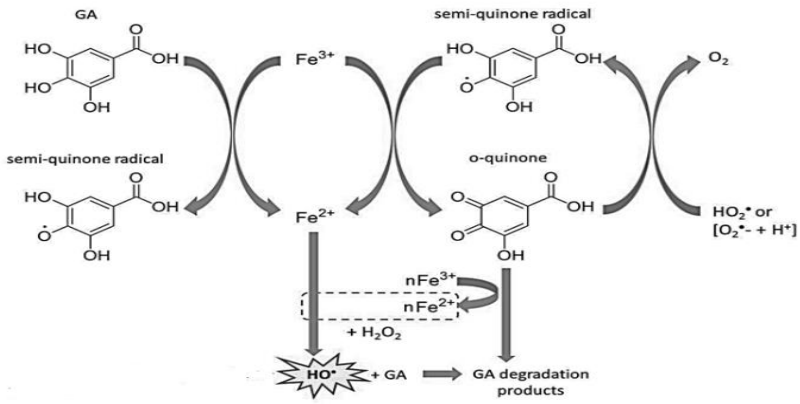


Figure 1. Oxidation mechanism of gallic acid under Fenton conditions

The mechanism behind involves initial hydroxylation of the aromatic ring, followed by ring opening and formation of short-chain organic acids. Complete mineralization results in the production of $CO_2$ and $H_2O$.

## MICROBIOLOGICAL ASPECTS AND FUTURE PERSPECTIVES

While Fenton-based processes provide rapid and efficient chemical degradation, they may not achieve complete mineralization of all byproducts. Consequently, integrating biological post-treatment can significantly enhance the overall treatment performance. Microorganisms such as Pseudomonas putida, Aspergillus niger, and Bacillus subtilis are known to degrade GA and its oxidation products through enzymatic systems involving laccases, tannases, and dioxygenases [6,7]. Studies have shown that low-molecular-weight acids, such as those formed during Fenton oxidation, are readily biodegradable under aerobic conditions by heterotrophic bacteria commonly found in activated sludge systems [7,8]. In this context, hybrid chemical-biological systems emerge as sustainable solutions for industrial wastewater treatment.

The integration of advanced oxidation processes with biological treatment has already been successfully applied in various studies, demonstrating improved

mineralization rates and reduced toxicity of effluents [7]. Future work should focus on optimizing operational parameters, selecting robust microbial consortia, and evaluating potential toxicity of intermediates to ensure safe and effective full-scale application.

## CONCLUSIONS

This study demonstrated that Fenton and photo-Fenton processes are effective for the degradation of gallic acid in aqueous systems. The oxidation followed pseudo-first-order kinetics, with rapid initial degradation and significant mineralization observed under optimal conditions. Identified intermediates included hydroxylated aromatic compounds and short-chain acids, which can serve as substrates for subsequent microbial degradation.

The findings highlight the potential for integrating chemical oxidation with biological treatment in wastewater remediation strategies. Such hybrid systems can maximize removal efficiency while aligning with principles of environmental sustainability and green chemistry.

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# STUDY OF THE EFFICIENCY OF SUNFLOWER BEETLE CONTROL METHODS IN THE CONTEXT OF INTENSIFICATION AND EXPANSION OF CULTIVATION AREAS

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***Abstract.*** *The sources propose a multitude of strategies within the control and combat methods of sunflower blight. Some of them emphasize the valorization of the genetic potential of resistance, giving it major importance. Just as the strategies for preventing and controlling sunflower blight in the organic farming system differ, so do those within the conventional system. Research conducted during the agricultural years 2022-2024 in repetition of 3 consecutive years on the same land in the southern area of the Republic of Moldova, Cahul district, Iujnoe village. During the vegetation period, the development and evolution of the crop as well as diseases, including sunflower blight, were recorded. For each variant, one hundred plants were chosen on the diagonal of the plot. The recording and analysis of the manifestation of sunflower blight consisted of collecting data in order to calculate the intensity and frequency of the attack generated. The tendency of increasing the frequency values and the intensity of the pathogen attack is due to the intensification of the sunflower cultivation process. The organic preparation Trichodermin-SC shows high biological efficacy in controlling sunflower scab and is considered to be suitable for use in an organic protection system. The fungicide Unical,SC, showing high biological efficacy in controlling sunflower powdery mildew.*

***Keywords: sunflower, phytosanitary status, genetic factor, Trichodermin-SC, Unical,SC.***

## INTRODUCTION

Sunflower blight caused by the pathogen *Plasmopara halstedii* is considered to be the most widespread disease throughout the sunflower cultivation area. It was first reported in our country in 1948 [1]. Source [2] is of a similar opinion, determining it as the main and most widespread disease in the country, and in rainy years the development of the disease becomes vertiginous. The background of the increase in the average annual temperature by +1°C causes the reduction of the attack frequency from 56.8% to 32.3% and the intensity from 5.4% to 4.3%. The same influence on the spread of the disease is also exerted by the temperature fluctuations within each season, in summer the increase in the average temperature from +19.15°C to 21.02°C causes the reduction of the attack frequency by two times. The increase in the amount of precipitation from 405 mm to 560 mm causes

the increase of the attack frequency from 25% to 51.7% and the intensity of the attack from 4.9% to 6.1% [3].

Although five forms of sunflower mange development are known, the second and fifth forms pose the greatest danger [1]. In the case of the first form of development, the plants remain dwarf, rarely exceeding 100 cm, with thick nodes and short internodes. The foliar apparatus in comparison with the plant's height has a luxuriant appearance, with the presence of discoloration spots on the upper side and felt-like conidial fructification on the lower side. The attacked plants form small calatidia with rare insertion of the achenes. The fifth form is characterized by partial necrosis of the formed calatidia or total necrosis of the floral bud [4]. The use of infected seed material and the neglect of effective weevil control are considered to be the decisive factors in field contamination and crop compromise[5].

The sources propose a multitude of strategies within the control and combat methods of sunflower blight. Among these sources, various strategies within the ecological methods based on the use of environmentally friendly biopreparations and strategies within the chemical methods based on the application of chemical products can be identified. Some of them emphasize the valorization of the genetic potential of resistance, giving it major importance.

Major importance in the protection of sunflower from mange plays the genetic resistance of many hybrids induced by selection [6]. Highlighting the resistance genes was the basis for the creation of resistant hybrids, allowing the control of the disease by genetic method [7]. Currently, every latest generation hybrid in its portfolio of characteristics has resistance to the latest races of sunflower mange [8]. This leads [1] to state that sunflower mange is of no practical interest in the Republic of Moldova in recent years. Source [9] is more skeptical in this regard, considering that sowing large areas based only on the resistance of the hybrid may have unpredictable effects.

The strategies proposed within the ecological method of control and protection of sunflower blight are diverse. In his work [10] noting the effectiveness of biopreparations based on hyperparasitic fungi *Coniothyrium minitans* and *Gliocladium catenulatum* on the sclerotia of the fungus *Plasmophara halstedii*, he considers their use in phytosanitary treatments to be effective. The effectiveness of biopreparations based on the fungus *Coniothyrium minitans* is also mentioned in the source [1]. A strategy for controlling sunflower blight in vegetation according to [11] consists of using the preparation Trichodermin-SC in a dose of 0.5 l/ha, especially when treating seed material and in the form of treatment in vegetation. The effect of this preparation on a number of sunflower diseases, including blight, is demonstrated. As mentioned by [6], the high efficacy of organic preparations based on the fungus *Trichoderma spp.* in the organic control of the fungus Plasmophara halstedii is worth exploiting. According to [12], in addition to exploiting the potential of the fungi *Trichoderma spp.*, the potential of the

bacterium *Bacillus subtilis* can also be exploited in the control of parasitic fungi.

Agrotechnical and phytotechnical disease prevention and control strategies also differ depending on the source. As mentioned by [9], the cheapest method of preventing and combating sunflower blight is to strictly observe the rotation, the time interval between two sunflower crops of at least 5 years. As mentioned by [13], the presence of blight-resistant hybrids on the market is not a valid reason for ignorance of the principles of crop rotation and rotation. As a measure to prevent and combat the disease, [6] pays attention to the location of the crop, avoiding low and over-wetted lands and soil texture, in addition to these, mentioning the need to maintain the field in an impeccable condition from an agrotechnical point of view.

Just as the strategies for preventing and controlling sunflower blight in the organic farming system differ, so do those within the conventional system. As mentioned [8], products based on azoxystrobin, difenoconazole, cyproconazole, and tebuconazole are considered effective. High efficiency is shown by the use of the products Apron Gold 35 EW, Sumilex 50 WP, Protexil 350 FS for seed treatment and the fungicides Aliette 80 WP, Ronilan WG for vegetation treatments [3]. In the control of sunflower blight, it is considered reasonable to comparative test the efficiency of the preparations Trichodermin-SC and Unical, SC under the conditions of intensification of sunflower cultivation processes in the southern part of the country. The agro-climatic and economic situation in which agricultural producers are placed requires the identification of cheap and effective solutions in order to be able to face the challenges.

## MATERIALS AND METHODS

Research conducted during the agricultural years 2022-2024 in repetition of 3 consecutive years on the same land in the southern area of the Republic of Moldova, Cahul district, Iujnoe village. Observations were made within the framework of the trifactorial experience which in turn includes three variants in three repetitions. The first factor is considered the ecological protection scheme in which the Trichodermin-SC preparation was used, the second factor is considered the chemical protection scheme in which the Unical,SC preparation was used and the third factor is represented by the genetic resistance of hybrids to diseases including sunflower blight.

The ecofungicide Trichodermin-SC 1% suspension based on the fungus Trichoderma lignorum was used within the framework of the ecological protection scheme, administered for seed treatment, spraying of plants in the 6-leaf phase and in the phase of floral bud emergence. The fungicide Unical,SC based on tebuconazole 250 g/l was used in the chemical protection variant at a dose of 0.5 l/ha administered in identical terms to the application of the ecological product. The study of the genetic factor consisted of testing three sunflower hybrids, considered

to be the most current on the market, the hybrids P64LE25, P64LP130, LG5377, in the description of which the manufacturer mentions the presence of a high degree of resistance to diseases, including sunflower blight.

During the vegetation period, the development and evolution of the crop as well as diseases, including sunflower blight, were recorded. For each variant, one hundred plants were chosen on the diagonal of the plot. The recording and analysis of the manifestation of sunflower blight consisted of collecting data in order to calculate the intensity and frequency of the attack generated.

As mentioned by [13], the application of a plant protection product requires the achievement of three indicators, including biological efficiency.

## RESULTS AND DISCUSSIONS

Table 1 shows the dynamics of sunflower blight over three years, two of which were in monoculture conditions. It shows both the dynamics of the frequency and intensity of the attack and the biological efficacy of the preparations Trichodermin-SC and Unical,SC in controlling sunflower blight.

Table 1. Efficacy of Trichodermin-SC and Unical,SC preparations in controlling sunflower blight.

| **Variant** | **Repetition** | **2022** | | | **2023** | | | **2024** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F,(%) | I,(%) | E,(%) | F,(%) | I,(%) | E,(%) | F,(%) | I,(%) | E,(%) |
| **Control** | **$R_1$** P64LE25 | 4,4 | 3,8 | - | 13,0 | 4,25 | - | 26,0 | 7,5 | - |
| | **$R_2$** P64LP130 | 5,2 | 4,25 | - | 10,0 | 3,25 | - | 22,0 | 6,5 | - |
| | **$R_3$** LG5377 | 4,5 | 2,84 | - | 14,0 | 4,5 | - | 28,0 | 8,0 | - |
| **Unical,SC** | **$R_1$** P64LE25 | 2,8 | 1,67 | **56,05** | 6,0 | 2,25 | **47,05** | 12,0 | 4,25 | **43,33** |
| | **$R_2$** P64LP130 | 2,0 | 1,48 | **65,17** | 5,0 | 1,75 | **46,15** | 10,0 | 3,25 | **50,0** |
| | **$R_3$** LG5377 | 4,0 | 1,8 | **36,61** | 7,0 | 2,75 | **38,88** | 14,0 | 4,5 | **43,75** |
| **Trichodermin-SC** | **$R_1$** P64LE25 | 3,0 | 1,63 | **57,1** | 7,0 | 2,5 | **41,17** | 19,0 | 5,75 | **23,33** |
| | **$R_2$** P64LP130 | 2,7 | 1,61 | **62,11** | 7,0 | 2,5 | **23,07** | 19,0 | 5,5 | **15,38** |
| | **$R_3$** LG5377 | 4,2 | 2,01 | **29,22** | 10,0 | 3,5 | **22,22** | 25,0 | 7,25 | **9,37** |

From the analysis of the data in the table, it is found that the directly proportional influence of the process of intensification of sunflower cultivation on the degree of spread of the pathogen Plasmophara halstedii is observed. The tendency of increasing the frequency values and the intensity of the pathogen attack is due to the intensification of the sunflower cultivation process.

In general, experience shows a 14-fold increase in attack frequency from the minimum value per experience of 2.0% in 2022 to the maximum value per experience of 28% in 2024. The intensity of the attack also increases significantly by about 5 times from the minimum value of 1.48% to the maximum value of 8.0%.

The results obtained in the experiment demonstrate the importance of the

genetic factor in the control of sunflower blight. In the present case, the most resistant hybrid to blight is P64LP130, showing itself superior to the hybrids P64LE25 and LG5377 over the years of experience. The identification in the experiment of a different degree of resistance of sunflower hybrids to blight leads to agreement with the sources [6; 8] in what concerns the rational use of the genetic factor. On the other hand, the results of the experiment partially contradict the source [1] in what concerns the statement regarding the unimportance of sunflower blight in the context of hybrid resistance. Because analyzing the hybrid P64LP130, we agree, and analyzing the hybrid LG5377, we reach a dissonance with the statement of the previously mentioned source.

In the agricultural year 2022, the variant in which the Trichodermin-SC product was applied, the lowest values of the intensity and frequency of the disease are observed in the P64LP130 hybrid, the product shows a biological efficacy of 62.11%. The biological efficacy of 57.1% is also shown by the preparation in the P64LE25 hybrid. The lower biological efficacy of the preparation is manifested in the LG5377 hybrid in the amount of 29.22%, this hybrid also shows itself to be the most subject to attack. In the agricultural year 2023, the presence of a high background of infection determines the increase in the values of the frequency and intensity of the attack in all hybrids, reduces the biological efficacy which oscillates between the maximum value of 41.17% in the P64LE25 hybrid and the minimum value of 22.22% in the LG5377 hybrid.

The agricultural year 2024, in which the even more intensive background of infection determines the increase in the frequency and intensity of the attack values compared to the values of the previous year, the biological efficacy of the preparation decreases considerably in all three hybrids, it oscillates between the maximum value of 23.33% obtained in the P64LE25 hybrid and the minimum value of 9.37% obtained in the LG5377 hybrid. In general, a good biological efficacy of the Trichodermin-SC preparation in the control of sunflower blight is observed. In the context of intensification of sunflower cultivation, the biological efficacy of the preparation decreases considerably. The research results demonstrate the efficacy of the Trichodermin-SC product and determine it as suitable for the control of sunflower blight within the ecological system. It also reaches agreement with the sources [6; 11] regarding the effectiveness of products based on the fungus *Trichoderma spp.*, in the effective control of sunflower blight.

In 2022, when applying the Unical,SC product, a biological efficiency that is not significantly higher than the level of effectiveness of the organic product is observed. The highest value of biological efficiency of 65.17% was obtained for the P64LP130 hybrid, followed by the P64LE25 hybrid with a value of 56.05% and the LG5377 hybrid with 36.61%. In 2023, the given preparation shows superior biological efficiency compared to that of the organic product, this being the value of 47.05% obtained for the P64LE25 hybrid, followed by 46.15% for the P64LP130

hybrid and the minimum value of 38.88% obtained for the LG5377 hybrid. In the agricultural year 2024, on an even higher background of infection, the Unical,SC fungicide shows significantly superior biological efficiency compared to the efficiency of the Trichodermin-SC preparation. The highest value of biological efficacy is found in the hybrid P64LP130 in the amount of 50.0% followed by the hybrids LG5377 and P64LE25 with almost identical values of 43.75% and 43.33%. It can be noted that the biological efficacy of the product Unical,SC is observed under conditions of intensification of crop cultivation. The increase in the infection background shows maximum efficiency in 2024 in all three hybrids. The application of the preparation significantly reduces the frequency and intensity of the attack compared to the values in the control and organic variants.

Throughout the experiment, an increase in the aggressiveness of sunflower blight is observed in the context of intensification of crop cultivation. Although the years 2022, 2023, 2024 were considered dry, arid and unfavorable for the spread of diseases, sunflower blight did not reduce the degree of spread. The results of the experiment contradict the findings of the source [3], such as increasing temperatures and decreasing precipitation in the summer season significantly reduce the risk of spreading the disease. That is, it can be firmly said that the aridification of the climate against the background of high infection does not reduce the degree of spread of the *Plasmopara halstedii* fungus.

## CONCLUSIONS

Within the experience of the hybrid P64LP130, P64LE25 demonstrates increased resistance to sunflower blight, considering themselves suitable for cultivation in both conventional and organic systems. The hybrid LG5377 in the context of intensification of sunflower cultivation is considered less suitable. The aggressive phytopathological background demonstrated the importance of correctly exploiting the genetic factor in improving the phytosanitary status of sunflower crops.

The organic preparation Trichodermin-SC shows high biological efficacy in controlling sunflower scab and is considered to be suitable for use in an organic protection system. The aggressive phytopathological background generated by the intensification of crop cultivation makes it less suitable. In order to reduce the chemical load in agriculture, it is reasonable to integrate the preparation Trichodermin-SC into the conventional sunflower protection system.

The fungicide Unical,SC, showing high biological efficacy in controlling sunflower powdery mildew, can be considered suitable for use in conditions of intensification of sunflower cultivation in a conventional system. The high biological efficacy, low cost and accessibility position this fungicide among the most promising fungicides in the current economic context.

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# THE INFLUENCE OF UV PROTECTANTS ON THE BACTERIOPHAGE ABILITY TO SUPPRESS FIRE BLIGHT DEVELOPMENT IN THE PLANTS TISSUES

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***Abstract***. *Bacteriophages are considered to play an important role in the fire blight management as the natural habitants of the pome orchards and highly specific bacterial parasites. The main problem of the phage therapy application against fire blight is the necessity to suppress the pathogen development in the phyllosphere were they are easily inactivated by unfavorable environmental factors such as changes of temperature, pH and sunlight irradiation. UV irradiation is critical for bacteriophages and serious efforts are being made for to find effective and economically acceptable UV protectants of the bacterial viruses' particles. In this study it has been shown that casein, soy peptone and carrot juice protected bacteriophages from the UV irradiation damage and phages were able to suppress development of the fire blight causative agent in plant tissues.*

***Keywords: bacteriophages, Erwinia amylovora, phage therapy, UV protectants, UV radiation.***

## INTRODUCTION

Microbial pathogens damage approximately a quarter of the world's plant crops [1]. The causative agent of fire blight disease, bacteria *Erwinia amylovora*, is among the pathogens, responsible for the pome cultures crops losses. In 2012 it was included in the Top 10 bacterial plant pathogen list as voted for by plant bacteriologists associated with the journal Molecular Plant Pathology [2]. Bacteriophages (phages), i.e. bacterial viruses are considered to play an important role in the fire blight management as the natural habitants of the pome orchards and highly specific bacterial parasites. The main problem of the phage therapy application against fire blight is the necessity to suppress the pathogen development in the aerial parts of the plants. It means that the disease control agents have to be able to survive in the phyllosphere which permanently exists under the pressure of such unfavorable factors as changes of temperature, pH and sunlight irradiation, especially of its UV spectrum.It has been demonstrated that bacteriophages are easily inactivated by all factors mentioned above [3].

By now there are very few commercially available preparations on the basis

of bacteriophages against the most phyllosphere bacterial pathogens, including *Erwinia amylovora,* due to the high vulnerability of the phage particles to the aggressive abiotic factors. Since UV irradiation is critical for bacteriophage application in the plants disease control, serious efforts are being made for to find effective and economically acceptable UV protectants of the bacterial viruses' particles. Substances of different nature, able to minimize the negative effect of UV rays were tested in laboratory and field conditions [4, 5, 6]. Natural, organic compounds such as lignin, black and green tea, coffee and cocoa extracts, have shown high level of the protection of the virus particles from the UV- induced damage [7].

The aim of this work was to study the effect of several UV protectants on the ability of bacteriophages to suppress development of bacteria *E. amylovora* in immature pome fruits under the controlled conditions.

## MATERIALS AND METHODS

Bacteria *Erwinia amylovora* were cultivated on the LB agar (10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl, 20 g/L agar) at 28˚C. Bacteriophages *E. amylovora* were cultivated by a standard double agar overlay method on the LB agar (10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl, 20 g/L agar) and soft LB agar (0.7% agar). Liquid cultures were grown in LB medium (10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl) at 28˚C. Immature pear fruits were inoculated with suspension of *E. amylovora* EaM contained $10^4$ CFU/ml and treated with $10^7$ PFU/ml of bacteriophage isolates with casein, soy peptone and carrot juice, prepared as described by Born et al [4].

Immature pear fruits infected with bacteria and treated with bacteriophages with different UV protectants were exposed to UV light, wavelength 254 nm, for 1, 3 and 5 minutes. Immature pear fruits, treated with sterile distilled water and immature pears, infected with bacteria *E. amylovora* and treated with bacteriophage lysates without UV protectants were used as the controls. The experimental pear fruits were incubated in the wet chambers at the temperature +28˚C. Symptoms of the disease were recorded at 1, 2, 3, 4 and 7 days after inoculation. For each bacterium/phage with UV protectants' combination tested pears were assayed in triplicate and each experiment was repeated at least two times. The assessment of the degree of *E. amylovora* infection on the immature pear fruits was carried out according to the following scale: 0 – absence of the necrosis; 1– necrotic spots on the 5% of the fruit's surface; 2– necrotic spots on the 25% of the fruit's surface; 3 – necrotic spots on the 50% of the fruit's surface; 4 – necrotic spots on the 75-100% of the fruit's surface and/or ooze formation.

## RESULTS AND DISCUSSIONS

In the natural conditions all participants of the system "plant - pathogen - biocontrol agent" are under the influence of the environmental biotic and abiotic factors. In the pome orchards plants affected with bacteria, fire blight causative agent bacteria *Erwinia amylovora*, and bacterial viruses are UV irradiated at the same time. Experiments, carried out in the controlled conditions can present information concerning the ability of the UV protectants to provide such protection of bacteriophages that they were able to suppress development of the pathogen in the plant tissues.

Three substances: casein, soy peptone and carrot juice, known for their ability to absorb UV waves [4, 5, 6] were used in the assays (Figure 1.).

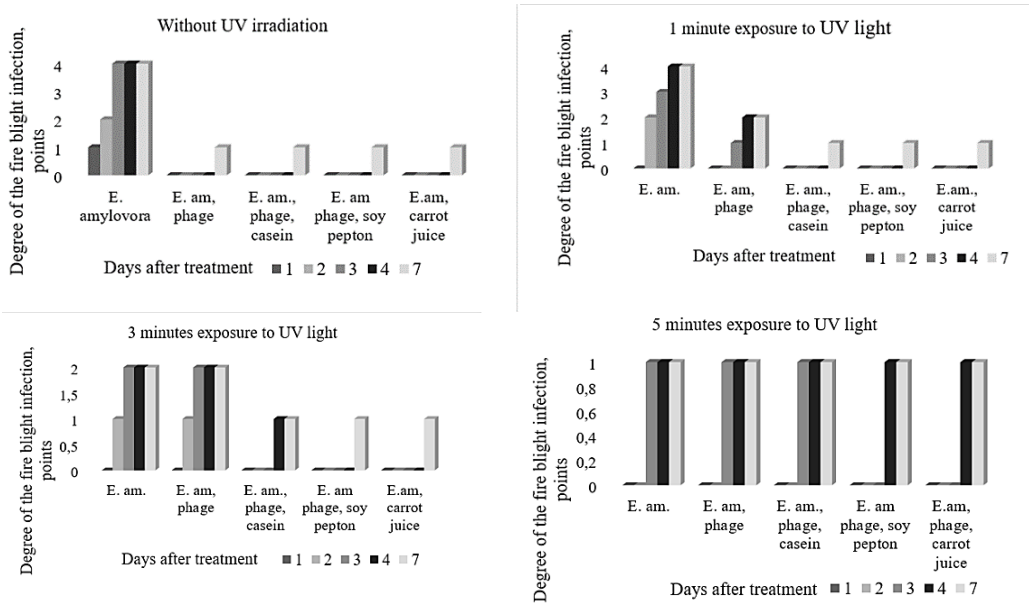


Figure1. Degree of *Erwinia amylovora* infection on the immature pear fruits treated with phage lysates with UV protectants and exposed to UV light.

In the absence of UV irradiation phage lysates without UV protectants and with UV protectants successfully destroyed bacterial cells in the pear tissues. On the fourth day of experiment, when pear fruits, infected with pathogenic bacteria, produced typical milky white ooze, there were single necrotic spots on the bacteriophages treated pear fruits in all variants with phage lysates treatment.

As expected, with increased exposure to UV light the amount of bacteria and bacteriophages decreased. After one-minute exposure to UV light the first necrotic spots on the immature pear fruits infected with bacteria appeared in 24

hours after infection. Bacterial population, exposed to UV light for 1 minutes, recovered virulence on the fourth day of the experiment. On the pear fruits, infected with bacteria and treated with phage lysate without UV protectant 50% of the surface was covered with necrotic spots. At the same time, no more than 5% of the pear fruits surface, infected with bacteria and treated with phage lysates contained UV protectants, were covered with necrotic spots.

Three minutes' exposure to UV light partially destroyed bacterial cells, so that on the fourth day of the experiment the 50% of the immature pear fruits surface, infected with bacteria *Erwinia amylovora,* were covered with necrotic spots. The same degree of the pathogen infection was revealed on the pear fruits, treated with phage lysate without UV protectants. Pear fruits, infected with bacteria and treated with bacteriophage lysates contained UV protectants, demonstrated 5% necrosis on their surface.

After five minutes' exposure to UV light the single necrotic spots on the immature pear fruits were recorded in all variants. It can be assumed that in this case just a single bacterial cells were alive and bacteriophages in all phage lysates were inactivated.

In this study the substances, which absorb UV light due to the presence of aromatic amino acids - casein, aromatic amino acids tryptophan, tyrosine, and phenylalanine within its peptide structure – soy peptone, carotenoids – carrot juice were able to provide protection of the bacteriophage particles in the plants tissues at least for 96 hours after three minutes' exposure to UV light. Phage lysates without UV protectants suppressed the development of the pathogen in the plant tissues at the same level as phage lysates with UV protectants when they were not irradiated by UV rays. There were no significant differences in the action durability of the UV protectants used in the experiment.

The control efficacy of bacteriophage-based treatment in agriculture can be influenced by numerous biotic and abiotic factors as well as by the way of treatment such as soil drenching, foliar spraying, infiltration etc. [8]. Besides, bacteriophages can be destroyed by chemical preparations used for the pests control. However, bacteriophages advantages as biological control agents are worthy of all these limitations being overcome. Phage formulations for commercial purposes should be effective, economically attractive and easy to use. That is why it is important to select the most effective phage-based preparations for the target bacterial pathogen.

## CONCLUSIONS

The selection of the most biological and cost effective UV protectant of the bacterial virus's particles is necessary for to use the high potential of phage therapy. The results of the experiments have shown that tested UV protectants casein, soy peptone and carrot juice demonstrated ability to protect phage particles from UV

light. Phage particles, treated with the studied UV protectants kept the degree of the *Erwinia amylovora* infection on the immature pear fruits on the low level after exposure to UV light.

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# TESTING OF A BIOPREPARATION BASED ON *BACILLUS AMYLOLIQUEFACIENS* FOR WINTER WHEAT PROTECTION AGAINST DISEASES

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***Abstract.*** *The results of the testing of the biological fungicide based on the bacteria Bacillus amyloliquefaciens, strain FZB24 to protect the winter wheat variety Moldova-614 from the complex of pathogens in the conditions of the Republic of Moldova. The research was carried out on the experimental field of the IGFPP of SUM. Two preparation consumption norms were tested: 2 kg/ha and 2,5 kg/ha. Used once by the spraying method. As a result, the biological efficiency against Blumeria graminis was 89,0% and 94,7%, Septoria tritici – 85,0% and 91,7%, respectively, Puccinia recondita – 94,7% and 94.9%. Reduced development of other wheat diseases may be possible.*

***Keywords: Bacillus amyloliquefaciens, biological efficacy, pathogen complex, testing, winter wheat.***

## INTRODUCTION

Wheat (*Tríticum* L.) is the leading cereal crop in many countries, feeding more than half of the world's population. It is cultivated all over the world and ranks second after corn in terms of cultivated area. The world leaders in wheat grain production are the European Union, China and India. Also in the top five leaders are Russia and the United States. These countries account for 65% of global grain production. The world leaders in wheat yield per unit area are Germany - 7.53 t/ha, France - 6.8 t/ha, Egypt - 6.4 t/ha, China - 5.7 t/ha [1].

In Republic of Moldova, wheat sown areas in the last two decades make from 311,4 to 377,0 ha, or 20,3 - 23,6% of the total sown areas of agricultural crops. The gross grain harvest ranges from 727.7 tons in 2000 to 1 million 555 thousand tons in

2023. Wheat yield in Republic of Moldova in farms of all category's ranges from 1.9 to 4.6 t/ha [2].

Wheat is considered an unpretentious crop, preferring a moderately continental, rather warm climate. In regions that are too hot, yields are low. Gross grain yield depends on a large number of factors, including the competitiveness of the variety, weather conditions of the growing season, soil composition, agrotechnical practices, crop protection measures and others. Infection of plants with pathogenic microorganisms leads to significant yield losses. Root rots affecting plants during the whole vegetation period, powdery mildew, leaf spots, spikelet fusarium, etc. are widespread in grain crops cultivation regions. [3]. Plant protection against pests is the most important reserve for increasing production and improving its quality.

The purpose of this test is to establish the biological efficacy of biological fungicide based on the bacterium *Bacillus amyloliquefaciens*, strain FZB24 in the protection of winter wheat against a complex of diseases in the conditions of the Republic of Moldova.

## MATERIALS AND METHODS

Studies were conducted in 2024 at the experimental field of the Institute of Genetics, Physiology and Plant Protection of the MSU. The object of research was a biological fungicide based on the bacterium *Bacillus amyloliquefaciens*, strain FZB24 with a titer of $1\times10^{13}$ CFU/ml. It was used to protect winter wheat (*Tríticum aestivum* L.), variety Moldova-614 from a complex of phytopathogens: 1 - wheat mildew (*Blumeria graminis*, syn. *Erysiphe graminis*), 2 - Septoria leaf blotch (*Septoria tritici*), 3 - Brown rust (*Puccinia recondita*), 4 – Helminthosporium (*Helminthosporium* spp.), 5 - pink snow mold (*Microdochium nivale*), 6 - blight of cereals (*Fusarium graminearum*). Application is a single spraying method during the growing season.

Sowing of winter wheat was carried out in the second decade of October 2023. Laying of variants was carried out in April 2024, each of the variants in three repetitions. The area of one repetition was $25m^2$ (1m×25m), randomization method was used in placing the variants of the experiment. The testing scheme included 4 variants: 1) control - no treatment; 2) reference - Bio Force, 3 l/ha; 3) test preparation, rate of consumption 2 kg/ha; 4) test preparation, rate of consumption 2.5 kg/ha.

Prior to testing, samples were taken from different areas of the experimental field to determine the extent to which the wheat was infested by the indicated pathogens. The plants were in development phase 31-37 (BBCH) and had reached the trumpeting stage. Ten samples, each consisting of ten plants, were taken. One hundred stems were examined for snow mold, root rot, and powdery mildew. Three hundred leaves were examined to determine the presence of spot blight and brown

rust.

To determine the biological efficiency of the tested preparation in restraining the above-mentioned diseases, 3 samples of 10 plants from each repetition of the variant were taken. Plant lesion counts for spot blight, brown rust, and ear fusarium were carried out using percentage scales [4].

The obtained results were processed mathematically using one-factor analysis of variance and computer data processing methods on the ABC Pascal platform.

## RESULTS AND DISCUSSIONS

Weather conditions in early autumn 2023 were not favorable for sowing and autumn vegetation of winter wheat due to insufficient moisture supply and high temperatures. In September and October, precipitation amounts amounted to 30% and 20% of the norm, respectively. The average monthly air temperature deviated from the norm by +4.0°C and +4.3°C, respectively, with anomalously high maximum temperatures of 21-31°C in the third decade of October. However, the total precipitation from January to May 2024 was 265.9 mm, significantly exceeding the norm of 194 mm (137% of the norm), which increased soil moisture reserves. This ensured the normal growth and development of wheat plants during the trumpeting phase and the beginning of the earing phase. Precipitation in June, at 65 mm (162% of the norm), contributed to the normal ripening of winter wheat and the completion of its growth cycle [5].

According to the results of surveys conducted before wheat treatment with fugicides it was found that the degree of spread of powdery mildew *Blumeria graminis* was 21.6%, the intensity of development - 4.1%. The spread of Septoria tritici septoriosis was 28.0% and the disease development was 2.3%. The spread of helminthosporiosis spot disease *Helminthosporium* sativum was weak - 5.3%, development - 0.06%. Snow mold, brown rust and helminthosporiosis root rot were not observed (Table 1).

Table 1. Infestation of wheat by pathogens before fungicide treatment

| The causative agent | Disease progress, % | Disease intensity, % |
|---|---|---|
| *Blumeria graminis* | 21,6 | 4,1 |
| *Septoria tritici* | 28,0 | 2,3 |
| *Puccinia recondita* | 0 | 0 |
| *Helminthosporium sativum* | 5,3 | 0,06 |
| *Microdochium nivale* | 0 | 0 |
| *Fusarium graminearum* | 0 | 0 |

Winter wheat crops were treated with the tested preparation immediately

after the first disease survey - in the BBCH 31-37 trumpeting stage of plant development. The surveys were conducted three weeks after the treatment in the BBCH 50-59 earing stage of plant development.

The surveys revealed that, over the course of three weeks after the spring disease survey, the indices of powdery mildew infestation in the control group increased significantly. The degree of spread of *B. graminis* increased from 21.4 to 21.5 tons, the percentage increased from 21.6% to 53.8%, and the intensity of development increased from 4.1% to 24.6%. Treating wheat with the tested fungicide decreased these indicators (Table 2).

Table 2. Biological efficacy of fungicide based on the bacterium *Bacillus amyloliquefaciens* in protecting winter wheat from wheat mildew *Blumeria graminis*

| № | Variant | Dosa, litres, kg/ha | Disease progress, % | Disease intensity, % | Biological efficiency, % |
|---|---|---|---|---|---|
| 1 | Control | - | 53,8 | 24,6 | - |
| 2 | Etalon *"Bio Force"* | 3,0 | 42,1 | 0,7 | 97,1 |
| 3 | Test preparation | 2,0 | 42,6 | 2,7 | 89,0 |
| 4 | Test preparation | 2,5 | 42,1 | 1,3 | 94,7 |
| $HCP_{0,05}$ | | | 17,2 | 11,4 | 5,0 |

Therefore, our results attest that the effective treatment solution when using the rate of 2.0 kg/ha, decreased the degree of powdery mildew spread decreased to 42.6%, and the intensity of disease development to 2.7%. The biological efficiency amounted to 89.0%. Using a rate of 2.5 kg/ha resulted in a 42.1% spread and 1.3% development of the disease. The biological efficiency was 94.7%. The biological efficiency of the "Bio Force" reference material was 97.1% (Table 2).

To determine the effect of the biopreparation based on the bacterium *Bacillus amyloliquefaciens* on reducing leaf septoria, the number of infected leaves was counted in samples of ten plants. Over the course of three weeks of winter wheat vegetation after the initial disease count in the control group, the degree of septoriosis increased from 28.0% to 45.0%, and the development intensity increased from 2.3% to 13.3%. When wheat was treated with a biofungicide at a rate of 2.0 kg/ha, the spread rate of the disease was 33.2%, and the development intensity was 2.0%, with a biological efficiency of 85.0%. At a consumption rate of 2.5 kg/ha, the degree of septoriosis spread was 30.7%, the development intensity was 1.1%, and the biofungicide's biological efficiency was 91.7%. The biological efficiency of the "Bio Force" reference was 92.9 % (Table 3).

Table 3. Biological efficacy of biofungicide based on *B. amyloliquefaciens* in protecting winter wheat against Septoria leaf blotch (*Septoria tritici*)

| № | Variant | Dosa, litres, kg/ha | Disease progress, % | Disease intensity, % | Biological efficiency, % |
|---|---|---|---|---|---|
| 1 | Control | - | 45,0 | 13,3 | - |
| 2 | Etalon *"Bio Force"* | 3,0 | 41,6 | 0,94 | 92,9 |
| 3 | Test preparation | 2,0 | 33,2 | 2,0 | 85,0 |
| 4 | Test preparation | 2,5 | 30,7 | 1,1 | 91,7 |
| $HCP_{0,05}$ | | | 4,9 | 3,1 | 6,1 |

As a result, it can be observed that no wheat brown rust (*Puccinia recondita*) infestation was detected during the first survey. Over the next three weeks of winter wheat growth, the disease spread by 33.5% in the control group and developed to an intensity of 8.1%. When wheat was treated with the tested biofungicide at a rate of 2.0 kg/ha, the spread rate of the disease was 22.6% and the development intensity was 0.43%, with a biological efficiency of 94.7%. At a consumption rate of 2.5 kg/ha, the spread rate of brown rust was 19.9%, and the development intensity was 0.41%, with a biological efficiency of 94.9% for the biofungicide. The biological efficiency of the "Bio Force" reference material was 96.3% (Table 4).

Table 4. Biological efficacy of biofungicide based on *B. amyloliquefaciens* in protecting winter wheat from brown rust (*Puccinia recondita*)

| № | Variant | Dosa, litres, kg/ha | Disease progress, % | Disease intensity, % | Biological efficiency, % |
|---|---|---|---|---|---|
| 1 | Control | - | 33,5 | 8,1 | - |
| 2 | Etalon *"Bio Force"* | 3,0 | 23,5 | 0,3 | 96,3 |
| 3 | Test preparation | 2,0 | 22,6 | 0,43 | 94,7 |
| 4 | Test preparation | 2,5 | 19,9 | 0,41 | 94,9 |
| $HCP_{0,05}$ | | | 19,3 | 2,02 | 2,8 |

In our studies, helminthosporiosis of wheat was observed on leaves in the form of a dark brown spot caused by the pathogen Helminthosporium sativum. In the initial survey, before treatment, plant infestation by this pathogen was minimal: the degree of disease spread was 5.3%, and the intensity of development was 0.06%. After three weeks, the infestation indices remained low in the control: the degree of disease spread was 8.4%, and the intensity of development was 0.15%, both of which are below the economic threshold of harmfulness. In the treatment variants with the tested biofungicide and the "Bio Force" reference, the disease was not observed.

No snow mold (*Microdochium nivale*) or spikelet Fusarium (*F. graminearum*) pathogens were detected throughout the entire vegetation period.

## CONCLUSIONS

Testing of biological fungicide based on the bacterium *Bacillus amyloliquefaciens* strain FZB24 against a complex of diseases of winter wheat under the conditions of the Republic of Moldova revealed:

1) The biopreparation significantly reduces the development of powdery mildew (*Blumeria graminis*). At a rate of 2.0 kg/ha, the biological efficiency was 89.0%; at a rate of 2.5 kg/ha, it was 94.7%.

2) The biological efficiency of the biofungicide in reducing wheat septoria (*Septoria tritici*) was 85.0% at a rate of 2.0 kg/ha and 91.7% at a rate of 2.5 kg/ha.

3) The biological efficiency of the tested biofungicide in protecting winter wheat from brown rust (*Puccinia recondita*) was 94.7% at a rate of 2.0 kg/ha and 94.9% at a rate of 2.5 kg/ha. It is possible to reduce the development of other wheat diseases.

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# INFLUENCE OF THE PREPATATION ECOSTIM ON THE PARAMETERS OF PHOTOSYNTHETIC ACTIVITY AND PRODUCTIVITY OF WINTER BARLEY VARIETIES

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**Abstract:** The results of studies by the action of the drug Ecostim on the growth parameters, photosynthetic activity and productivity of Czech winter barley varieties Luran and Lancelot in the conditions of the South of the Republic of Moldova are presented. It has been shown that spraying plants in the tillering phase - the beginning of their emergence into the tube with a growth regulator of a steroid nature - stimulates plant growth, accumulation of plastid pigments in assimilation organs, the formation of raw and absolutely dry biomass and an increase in the yield of varieties. It was shown that the response to processing depends on the varietal characteristics, manifests itself to a greater extent in the Lancelot variety, which is characterized by lower parameters of photosynthetic activity and productivity, compared to the Luran variety.

***Keywords: growth regulator, Ecostim preparation, winter barley varieties, plastid pigments, productivity.***

## INTRODUCTION

Barley crop is the second most important and produced grain crop in the Republic of Moldova (RM). Due to global climate change, as well as weakening economic relations in the RM and increasing costs for fertilizers, plant protection products, etc., it is necessary to develop such technology models that would allow increasing the yield of winter barley at the lowest cost.

In recent years, new generation growth regulators *of steroid nature* have become widely used in plant growing practice [9; 11]. Interest in this group of compounds is due to the broad spectrum of their effects on plants, the ability to specifically regulate individual stages of plant growth and development, with the aim of mobilizing potential opportunities and increasing the yield of agricultural crops. They have a mild type of action, give the desired effect in endogenous concentrations. Such substances of secondary origin as steroid glycosides meet these requirements. They are widely distributed in higher plants and have biological activity [7; 1]. The aim of the research was to study the effect of a steroid growth regulator the preparation Ecostim (ES) on the growth parameters, photosynthetic activity and productivity of winter barley varieties in the conditions of the southern region of the Republic of Moldova. The prepation ES (*tomatoside*) of natural origin

is included in the list of chemical and biological preparations approved for use in agriculture of the Republic of Moldova. [2].

## MATERIALS AND METODS

The experiment was carried out on the varieties of Czech selection Luran and Lancelot (created at the Institute of Selection Luzan, "SELEGEN" of the Czech Republic), intensive type, forage direction of use, in agrophytocenoses of the farm SRL "Terra-Vitis", Burlaku village, Kahul district. The varieties are characterized by resistance to lodging and seed fall, low temperatures, drought, some viral and fungal diseases. [12; 13]**.** Winter barley plants were sprayed with a solution of Ecostim (ES) at a dose of 25 mg/l in the phase of late tillering – beginning of tube emergence [7]. Plants not treated with the growth regulator served as a control.

The characteristics of growth processes and parameters of photosynthetic activity in the studied varieties of winter barley were carried out in the main phases of ontogenesis - emergence into the tube and earing, taking into account that in grain crops the period of highly effective work of a unit of sowing area is very short, associated with the death of lower leaves and the transfer of photosynthetic activity to the upper leaves and ear.

The growth parameters of shoots and leaf surface were determined by the method of linear changes; accumulation of raw and absolute dry biomass in plant organs by weighing; the content of plastid pigments (chlorophylls a, b and carotenoids) - in an alcohol extract on SF-26, calculated by the Halm-Wettstein formula [3], expressed in mg/g absolute dry matter; productivity of varieties according to [4]; mathematical processing of research data according to Dospekhov B.A. [6].

## RESULTS AND DISCUSSIONS

It was found that in the Lancelot variety, in the tube emergence phase, the plant height is 62,0 cm, the stem length is 56,4 cm, the diameter in the middle part is 0,44 cm, the leaf area is 58,7 $cm^2$; in the Luran variety, these indicators are higher and are, respectively, 86,4, 64,8 and 0,46 cm, the leaf area is 66,0 $cm^2$.

In the earing phase, the growth indicators of the studied varieties increase and are: plant height – 80,8 and 97,9 cm; stem length – 62,3 and 69,6 cm; diameter in the middle part of the stem – 0,48 and 0,47 cm; leaf area – 82,2 and 94,7 $cm^2$.

Treatment of vegetative plants with the ES preparation solution leads to an increase in growth parameters. A general pattern of an increase in plant height, length and stem diameter by 1,1 times, regardless of varietal characteristics, is observed; the leaf surface area increases by 1,5 (*Lancelot*) and 1,1 times (*Luran*) (Table 1).

Table 1. Growth parameters of winter barley plants, depending on varietal characteristics and treatment with Ecostim, *Earing phase*

| Variety | Experience options | Plant height, cm | Stem length, cm | Stem thickness, cm | Leaf surface area, $cm^2$/plant |
|---|---|---|---|---|---|
| Lancelot | Control | 80,8 | 62,3 | 0,48 | 82,2 |
| | Ecostim | 86,2 | 69,9 | 0,53 | 126,2 |
| Luran | Control | 97,9 | 69,6 | 0,47 | 94,7 |
| | Ecostim | 99,8 | 74,4 | 0,56 | 100,5 |

During ontogenesis, accumulation of raw and absolute-dry biomass by winter barley plants depends on the vegetation phases and the action of the growth regulator. Thus, in the earing phase, the contribution of individual organs to their accumulation changes in winter barley plants. Due to the drying of the lower leaves and the outflow of assimilates to the reproductive organs, the size of the leaf surface decreases.

The absolute values of green leaf biomass in the control variants, compared to the tube emergence phase, decrease, but the proportion of shoots with leaf sheaths and ears increases. The level of total absolute dry biomass in the control variants for the Lancelot variety is 39,17 g, for Luran – 44,07 g/10 plants.

Under the influence of the ES preparation, the level of total abs.-dry biomass of plants increases by 12,4% in the Lancelot variety and by 7,1% in the Luran variety (Table 2).

Table 2. Content of absolute dry biomass in winter barley plant organs, depending on varietal characteristics and treatment with Ecostim, g/10 plants. *Earing phase.*

| Variety | Experience options | Leaves | | Stems | Ears of corn | Total | % to Control |
|---|---|---|---|---|---|---|---|
| | | yellow | green | | | | |
| Lancelot | Control | 0,91 | 3,77 | 21,64 | 12,85 | 39,17 | 100,0 |
| | Ecostim | 0,65 | 6,51 | 22,41 | 14,44 | 44,01 | 112,4 |
| Luran | Control | 0,46 | 6,89 | 22,89 | 13,83 | 44,07 | 100,0 |
| | Ecostim | 0,34 | 7,29 | 24,24 | 15,33 | 47,20 | 107,1 |

According to Josan-Secrieru S. [7] the formation of the assimilation apparatus, the content of plastid pigments in it, depends on the varietal characteristics of plants and is closely related to the response of the variety to treatment with growth regulators.

In variants with the use of steroid glycosides in the assimilating organs of plants, the content of chlorophylls *a, b* and carotenoids increases, the chlorophyll index (*chl. a/b*) and the pigment index (*chl. a+b/carot.*) change. The author notes the greatest differences in the concentration of plastid pigments in plants in the earing phase. A similar pattern was found in the conditions of our experiment.

It was found that in the control variant of the Lancelot variety, the sum of

chlorophylls *(a + b)* is: in the leaves 5,29; in the shoots – 0,97 and in the ears – 0,37 mg/g of absolute dry matter; in the Luran variety – 4,58; 1,03 and 0,32 mg/g of absolute dry matter. When treating vegetative plants with the ES preparation, the chlorophyll content of the Lancelot variety increases: in the leaves - by 0,44, in the shoots - by 0,92 and in the ears - by 0,30 mg / g of absolute dry matter. in the Luran variety - by 0,62; 0,08 and 0,39 mg/g of absolute dry matter. A similar pattern is observed for the level of carotenoids.

In the figurative expression of Rubin A.B. [10] chlorophyll is a "*natural sensor*" of the state of algae and higher plant cells. Calculations of the total chlorophyll content in mg/10 plants (taking into account the absolute dry biomass) indicate significant differences in the chlorophyll content of the studied varieties.

Thus, in the control variants, the chlorophyll content of the Lancelot variety is 45,7 mg, and of the Luran variety – 59,6 mg. When treated with the ES preparation, the chlorophyll content of the Lancelot variety increases up to 2 times, and of the Luran variety - by 27,1% (Table 3).

Table 3. Chlorophyll content (a+b) in assimilating organs of winter barley plants, depending on varietal characteristics and treatment with Ecostim, mg/10 plants. *Earing phase*

| Variety | Experience options | Leaves | Stems | Ears of corn | Total | In % to to control |
|---|---|---|---|---|---|---|
| Lancelot | Control | 19,9 | 21, 0 | 4,8 | 45,7 | 100,0 |
| | Ecostim | 37,3 | 42,4 | 9,7 | 89,3 | 195,5 |
| Luran | Control | 31,6 | 23,6 | 4,4 | 59,6 | 100,0 |
| | Ecostim | 37,9 | 26,9 | 10,9 | 75,7 | 127,1 |

To characteristics the condition of barley crops, the density of plant standing, as well as the density of total and productive stem stand were determined. It was found that for the Lancelot and Luran varieties, per 1 $m^2$ there are: total number of plants - 220 and 285, total number of stems - 455 and 580, including productive - 443 and 560 things. The Luran variety has an increase in stem stand density (total and productive tillering) by 1,3 times, compared to Lancelot. The tillering coefficient of the studied varieties is 2,0.

The determining parameters of the production process are the mass of the ear, the number of grains in the ear and their mass. It has been established that the indicators of productivity elements change depending on the varietal characteristics (Figura 1).

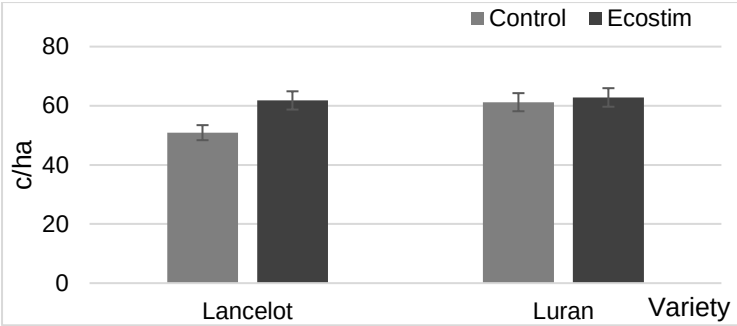


Figure 1. The effect of the Ecostim preparation on the potential yield of winter barley varieties, *SRL "Terra-Vitis"*.

Thus, the Luran variety, compared to Lancelot, has an increased ear weight, grain weight in an ear and the number of grains in them, regardless of the size of the ears. Treatment of vegetative plants with the ES preparation leads to an increase in the productivity elements and yield of varieties. It has been shown that in the control variants, the Lancelot variety yield is 50,9 c/he; for the Luran variety – 61,2 c/he. Treatment with the Ecostim preparation leads to an increase in the Lancelot variety yield by 1,2 times, while for the Luran variety it remains at the control level – 62,8 c/he.

## CONCLUSIONS

1.It has been shown that spraying winter barley plants of Czech selection *Lancelot* and *Luran* in the tillering-early tube-forming phase with a steroid growth regulator stimulates plant growth, accumulation of plastid pigments in plant organs, formation of raw and absolutely dry biomass and an increase in plant productivity.

2.The reaction to treatment depends on the varietal characteristics and is more pronounced in the *Lancelot* variety, which is characterized by lower rates of photosynthetic activity and economic productivity, compared to the *Luran* variety.

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# AQUATIC BASINS — NATURAL SOURCE OF FUNGI WITH ANTIFUNGAL POTENTIAL

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**Abstract.***The study of aquatic fungi is an area of major interest in microbiology, due to their essential ecological role and valuable biotechnological potential. In this context, the purpose of the paper was to test 71 strains of aquatic micromycetes isolated from the water basins of Chisinau municipality, in order to evaluate their antifungal activity against phytopathogens of agricultural importance. The results showed that over 80% of the analysed strains showed high antifungal activity, the most effective being against Fusarium solani (93%) and Botrytis cinerea (85.9%). The Trichoderma genus was shown to have remarkable antifungal potential, with inhibition areas ranging from 16 to 40 mm. Following the tests, two strains - T. atrobruneum CNMN FD 25 and T. longibrachiatum CNMN FD 27 - were selected to obtain biopreparations, which demonstrated increased efficiency in inhibiting the development of phytopathogenic fungi.*

***Keywords: aquatic fungi, biotechnology, durable agriculture, antifungal activity, biopesticides.***

## INTRODUCTION

The aquatic microbiota encompasses a wide range of microorganisms belonging to diverse taxonomic groups. Among them, filamentous fungi play a pivotal role in aquatic ecosystems, contributing significantly to nutrient cycling and the decomposition of organic matter, as well as to the formation of subsurface mycelial networks. Numerous fungal genera—such as *Aspergillus*, *Penicillium*, *Talaromyces*, and *Trichoderma*—are commonly found in aquatic environments and are represented by a variety of species.

These fungi have garnered considerable attention due to their ecological significance, as well as their capacity to produce energy and serve as raw materials in biotechnological applications, including the production of bioactive compounds of high relevance. In particular, filamentous fungi from the genus *Trichoderma* have been extensively employed in sustainable agriculture, where they contribute to soil improvement and are incorporated into the formulation of biopesticides and biofertilizers [1–5].

The genus *Trichoderma* is cosmopolitan, occurring ubiquitously in soil, water, and air. These fungi are well recognized for their ability to enhance plant growth and suppress phytopathogens through mechanisms of biological control. Notable species include: *T. harzianum*, which promotes plant growth by colonizing root systems and facilitating nutrient uptake, while also acting as an effective biocontrol agent through competition and the secretion of hydrolytic enzymes; *T. asperellum*, which provides plant protection via the production of antimicrobial compounds and the induction of systemic resistance; *T. viride* and *T. virens*, noted for their synergistic interactions in commercial formulations of biofertilizers and biopesticides; *T. longibrachiatum* and *T. saturnisporum*, which enhance plant tolerance to abiotic stressors such as salinity and drought [6–11].

These fungi also contribute to improved soil fertility and the suppression of soilborne pathogens. All higher plants form symbiotic associations with micromycetes that establish mycorrhizal networks on root surfaces. In agricultural systems, technological interventions are employed to create conditions that favor plant development, modulate soil microbial communities, and aid in the management of infectious soil diseases [12–14].

In light of these findings, the investigation of aquatic fungal strains and their antifungal potential constitutes a research area of high importance, given their critical ecological functions and the growing interest in their exploitation for biotechnological innovations, sustainable agricultural practices, and the development of bioactive molecules with broad-spectrum applications.

## MATERIALS AND METHODS

A total of 71 micellar fungal strains isolated from aquatic basins located in the municipality of Chișinău—specifically “La Izvor”, “Valea Morilor”, and “Valea Trandafirilor”—were used as objects of study. These isolates belong to the genera *Trichoderma*, *Talaromyces*, and *Penicillium*. The fungal strains were evaluated for their antifungal activity against five phytopathogenic fungi.

Antifungal activity was assessed using the agar block diffusion method, as described by Egorov (2004). The following phytopathogenic test strains were employed: *Alternaria alternata* CNMN FF 09, *Aspergillus niger* CNMN FD 01, *Botrytis cinerea* CNMN FF 10, *Fusarium oxysporum* CNMN FF 06, and *F. solani* CNMN FF 07.

The selected fungal strains were cultivated on malt agar medium in Petri dishes for 96 hours at 28–30°C. Agar blocks (8 mm in diameter) were excised using a sterile cork borer from areas of dense fungal growth. These blocks were then transferred into wells prepared in Petri dishes containing solidified nutrient medium inoculated with freshly subcultured test organisms. The Petri dishes were kept at low temperature for 24 hours prior to incubation to allow for the diffusion of

bioactive compounds.

The diameter of the inhibition zones was measured after incubation at 28–30°C for 96 hours for fungal pathogens and at 37°C for 24 hours for bacterial strains, respectively.

Among the analyzed strains, *T. atrobruneum* CNMN FD 25 and *T. longibrachiatum* CNMN FD 27 exhibited high antifungal potential. These strains were subsequently cultivated under submerged conditions to obtain the cultural filtrate. The composition of the liquid medium (g/L) was as follows: glucose – 30.0; $NaNO_3$ – 1.0; $KH_2PO_4$ – 1.0; $MgSO_4 \cdot 7H_2O$ – 1.0; $CaCO_3$ – 1.0; pH – 6.0, with distilled water up to 1 liter. The medium was supplemented with 10 mg/L yeast extract for *T. atrobruneum* CNMN FD 25 and 20 mg/L corn extract for *T. longibrachiatum* CNMN FD 27.

The strains were cultivated in 1 L Erlenmeyer flasks containing 200 mL of inoculated liquid medium ($1 \times 10^7$ spores/mL), at 28–30°C, under continuous agitation (160 rpm), for 6 days (*T. atrobruneum*) and 5 days (*T. longibrachiatum*). The obtained culture supernatant was subsequently tested for antifungal activity using the agar block diffusion technique.

Experimental results were subjected to statistical analysis. Statistical indicators were calculated using Microsoft Office Excel 2021.

## RESULTS AND DISCUSSIONS

Testing the antifungal properties of 71 strains against the studied phytopathogens showed that over 80% of them exhibit high antifungal activity. Thus, out of the total number of 71 tested strains, 57.75% showed antagonism against A. niger, 84.5% against A. alternata, 85.9% against B. cinerea, 93% against Fusarium solani, and 81.7% against F. oxysporum (Figure 1).

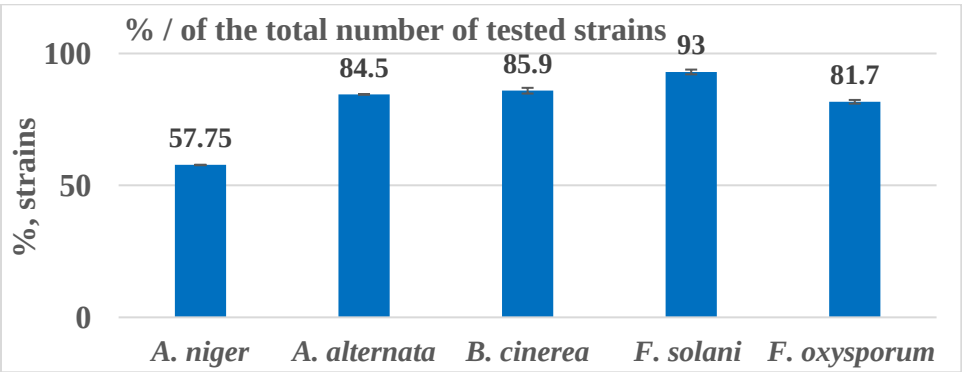


Figure 1. Stems (% of the total number of 71) that have shown antagonism towards some phytopathogens.

It was demonstrated that certain strains of aquatic micromycetes possess

significant inhibitory activity against the tested pathogens, with the largest inhibition zones observed in the case of *Trichoderma* strains. The diameter of the inhibition zones ranged from 16 to 40 mm (Figure 2).

Among all tested genera of micromycetes, the genus *Trichoderma* proved to have remarkable antifungal and antibacterial potential, which is why two strains were selected to be tested on wheat cultures. *Trichoderma* is a fungal genus that includes species widely used in the biocontrol of plant diseases, due to their ability to inhibit the growth of other phytopathogenic fungi through physical and chemical mechanisms, such as enzyme production.

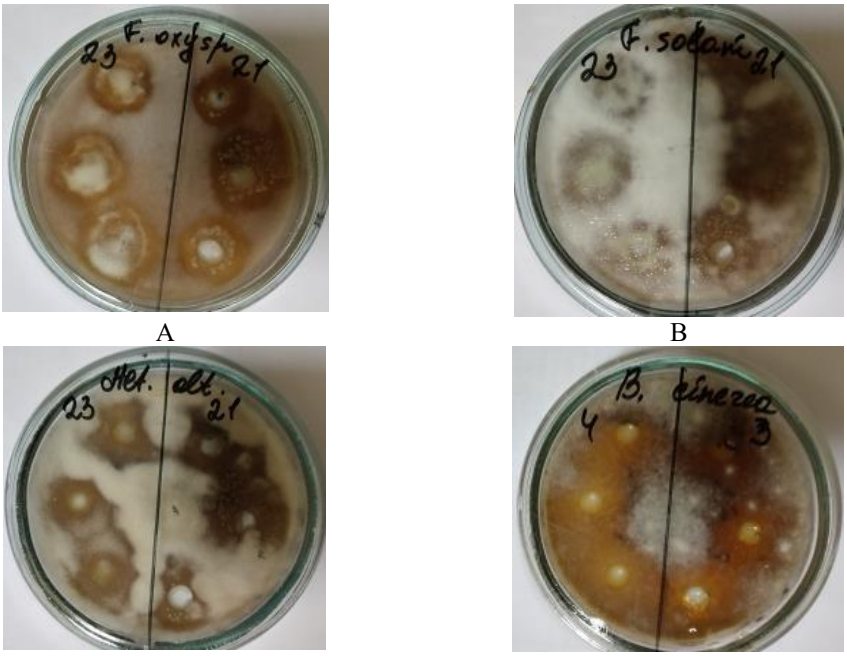


Figure 2. Areas of inhibition of some strains of fungi of the genus *Trichoderma* against phytoppathogens:

*a) Fusarium oxysporum; Fusarium solani; Alternaria alternata; Botrytis cinerea.*

Of all the genera of micromycetes tested, the genus *Trichoderma* was found to have remarkable antifungal potential, which is why 2 strains were selected, which were grown submersed in the liquid medium and obtained biopreparations. The biopreparations obtained have been tested against phytopathogens and the results obtained are shown in Figure 3.

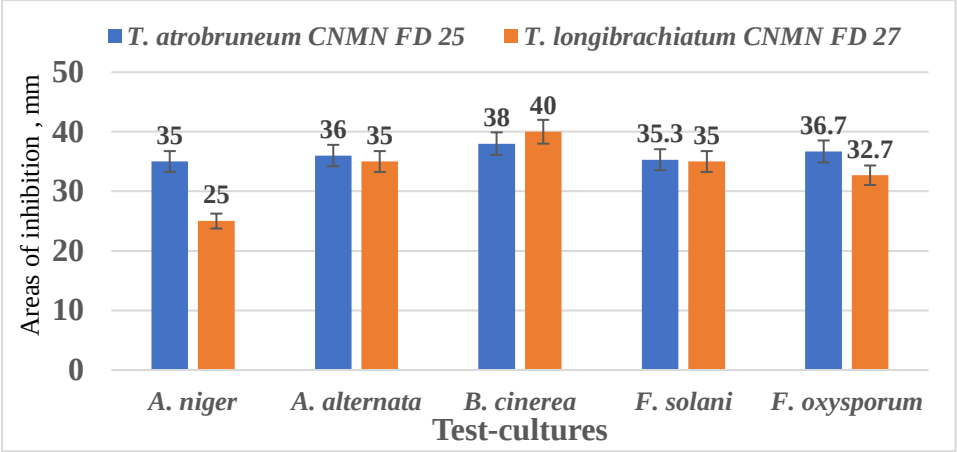


Figure 3. Diameter of phytopathogenic inhibition zones under the action of biopreparations obtained from *Trichoderma* strains

The biopreparation obtained from the strain *T. atrobruneum* CNMN FD 25 exhibited high sensitivity against phytopathogenic fungi, with inhibition zone diameters ranging from 35.3 mm (*F. solani*) to 38 mm (*B. cinerea*). The inhibition zones of phytopathogenic fungi under the action of the biopreparation derived from the strain *T. longibrachiatum* CNMN FD 27 varied from 25 mm (*A. niger*) to 40 mm (*B. cinerea*).

## CONCLUSIONS

Aquatic basins can serve as a source of fungi with antifungal potential applicable in the agricultural field. Over 80% of the studied strains exhibited antifungal activity against five phytopathogenic fungi. Fungi of the genus *Trichoderma* demonstrated the highest antifungal potential, with inhibition zones ranging from 16 to 40 mm. Biopreparations obtained from the strains *T. atrobruneum* CNMN FD 25 and *T. longibrachiatum* CNMN FD 27 showed high antifungal activity against phytopathogens and could be used as potential producers of antifungal substances in agriculture.

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# VARIABILITY AND EQUALITY OF BIOCHEMICAL QUALITATIVE CHARACTERISTICS VARIETIES AND LINES NEW TOMATO OBTAINED *IN VITRO*

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***Abstract.*** *In the agrarian sector, lines, varieties, which had high uniformity, distinctiveness and stability of fruit productivity and quality in different environmental conditions, were of particular importance. The most important trend in the strategy of plant breeding at the stage of adaptive intensification in agriculture was combining the resistance of the varieties to the stress-producing abiotic factors of the environment with high levels of fruit productivity and quality. The determination of the genetic complex of quantitative characters, including drought resistance, hindered the success of the breeding process, the purpose of which was to create genotypes combining many valuable characters. As a result of distance hybridization, interest for the breeder also presents the spectrum of genetic variability, which increases the efficiency of genotypes selection with high productivity and increased resistance to environmental stressors. One of the main tasks of amelioration was grandiose blending in the same variety of high productivity and resistance to stress factors (drought).*

***Keywords: tomato, lines, varieties, variability, productivity.***

## INTRODUCTION

The climatic conditions of the Republic of Moldova allow for assured harvests of tomatoes grown in unprotected fields (seed crop) after rotation once every 3-4 years. Obviously, special attention is paid to adaptive breeding, which allows the creation of new varieties with increased productivity and resistance to stress factors. Of particular importance in the agricultural sector are varieties that have high uniformity, distinctiveness and stability of productivity and fruit quality in different environmental conditions. The most priority direction in the plant breeding strategy at the stage of adaptive intensification in agriculture is the

combination of resistance of varieties to abiotic stress factors of the environment with a high level of productivity and fruit quality.

The complex genetic determinism of quantitative traits, including drought resistance [3], hinders the success of the breeding process, the purpose of which is to create genotypes that combine several valuable traits. As a result of distant hybridization, the breeder is also interested in highlighting the spectrum of genetic variability, which increases the efficiency of selection of genotypes with high productivity and increased resistance to environmental stress factors [1]. One of the main tasks of breeding is the grandiose combination of high productivity and resistance to stress factors (drought) in the same variety. From the literature data it has been demonstrated that the prolonged action of high temperatures 30–35°C on tomatoes during fruit setting leads to plant sterility. The most sensitive phase of tomato development at high temperatures is from the beginning of flowering to fruit setting. Using the method of embryo culture and distant hybridization [4,5,6,7,8] a vast number of simple and compound interspecific hybrids were obtained between Solanum esculentum L. (cultivated tomatoes with large red fruits) and the spontaneous forms Lycopersicon hirsutum, Lycopersicon peruvianum and Lycopersicon chilense with very small, purple and green fruits) [4, 5, 6, 7, 8]. In the segregable generations a valuable material with a diverse soma clonal variability was obtained.

## MATERIALS AND METHODS

The investigations were carried out in the laboratory "Genetics of Plant Resistance" and on the experimental lot of IGFPP. As the initial biological material in the study, 8 tomato varieties obtained by *in vitro* culture were used, for comparative research, control forms were cultivated (Peto 86, Elvira, Novinca Pridnestrovia.

Lines: L.20, L.25, L.47, L.55, L.63, L.64 and L.317, obtained based on interspecific hybridization and *in vitro* culture and standard forms Victorina, Kecskemet, Flacăra, Mia, Iulihirsutian, Anatolie, Cerrydani, Cerry brown pruniforme, Cerry red round. They were sown in the greenhouse (seedling culture) and planted in the field in the nursery for the multiplication of tomato varieties and lines.

Field planting scheme: (70 x70) x 30cm plant spacing, in 5m rows, 30 plants per variant. The placement of tomato forms was randomized in 2 replicates. All mentioned genotypes were evaluated for a complex of valuable traits. During the vegetation period, phenological evidence was carried out from the emergence of seedlings to fruit maturity (emergence of seedlings, flowering, fruiting, single and mass ripening). Morphological and biometric evidence: plant height, number of shoots on the main stem, number of nodes on the main stem, leaf length and width, number of inflorescences on the main stem, number of flowers and fruits on the first three clusters, number of flowers and fruits per plant. The results obtained were statistically processed as an average for two

repetitions. The statistical analysis was performed according to the STATGRAPHICS PLUS 5.0 software packages and EXCEL.

## RESULTS AND DISCUSSIONS

Literature data demonstrated that the long-term impact of 30-35°C on tomatoes during fruit forming resulted in plant sterility. The most sensitive phase of developing tomatoes at high temperatures is from the beginning of flowering to the fruit forming. Thus, the research goal consisted in obtaining the new tomato genotypes with valuable characters, useful for the breeding process. The research was carried out within the Plant Resistance Genetics laboratory of the Institute of Genetics, Plant Physiology and Plant Protection. As a subject of study, 7 varieties of tomato were produced, created through interspecific crosses and application of *in vitro* culture. *Iulihirsutian, Anatolie, CerryDani, Jacot)* approved in the Republic of Moldova, (*Flacara and Mia*) were tested at the State Commission for Testing Plant Varieties. The evaluation of the native varieties was carried out according to the most valuable biochemical qualitative indices (dry matter content, sugar content, ascorbic acid accidie content, titratable acidity and sugar/acidity ratio). As a control variety, the varieties (*Solaris, Elvira, Peto-86 and Novinca pridnestrovia*) were used. At the assembly of experiments there were used seeds and seed culture. Estimating the dry matter content and the percentage of sugar in tomatoes was performed using the refractometer. As a result of the application of remote hybrids and *in vitro* culture, varieties *(Iulihirsutian, Anatolie*', *CerryDani, Jacot, Flacara and Mia*). The results of the investigations showed that local tomato varieties surpassed the check varieties (*Solearis, Elvira, Peto 86 and Novinca pridnestrovia*) by the following characters – the dry matter content, sugar content and total acidity. As a result of the remote hybridization the interest for the breeders also provided the spectrum of genetic heredity variability, which increases the efficiency of selection of genotypes with high productivity and increased resistance to environmental stressors. One of the main tasks of the improvement was the gorgeous ffixing of several characters (productivity, precocity and high quality of fruit to stress factors (drought and low positive temperatures). The study of the obtained results demonstrated that the limits of variability of biochemical components content, which determined the quality of the fruits, were very large. Of particular interest was the early average varieties characterized by an increase in the dry matter content eg. the latter in the variety increased by 17 % compared to the check variety *Solaris* and *Iulihirsutian* by 20 % against the check variety *Elvira*). These varieties possessed superior qualities of the dry matter content with the variability of 13.33 % and 14.13 %. In late varieties (Anatolie and Jacot) the variation coefficient oscillated between 12.03 to 8.14 %. The early variety (*CerryDani*) exceeded the check variety (*Peto-86*) by 14.24 %. Concerning the

percentage of sugar content the maximum values against the check varieties accumulated the early average varieties.

The sugar content of the tomato varieties constituted 5.32 to 7.10 % with different values of the variation from 5.86 to 10.63 % compared to the check varieties of 4.89 to 6.39 % as well as the coefficient of variation 5.87 to 10.79 %. The use of the genetic potential of spontaneous forms was also of great importance for increasing the size, shape and quality of the fruit with high vitamin C content. Biochemical determinations of ascorbic acid (vitamin C) were also of great importance because they determined the quality of fruits that had high taste qualities. The biochemical content of ascorbic acid in tomato fruits showed a very high variability. Noteworthy that the indices of vitamin C content in tomato varieties formed the limit of variability of 9.66 to 34.15 % compared to check varieties of 8.43 to 24.03 %.

In assessing new tomatoes varieties, the ratio of sugars to acidity was a very important one, because this index determined the taste properties of the fruits. Based on the data obtained previously and taking into account the specific biochemical properties characterized by the accumulation capacity of the maximum volume of dry matter, sugar and, in particular, ascorbic acid (vitamin C), the varieties mentioned were to be used in the improvement programs as donors of valuable biochemical peculiarities of the fruits.

According to the investigations carried out by the application of *in vitro* culture, there was a harmonization of recombinant genes, possessing some precious qualities that allowed creating high performance varieties, fruits with high taste qualities, distinct technological qualities and increased resistance to drought, low positive temperatures and diseases. As a result, new lines with valuable economic characters were created, producing high yields of fruits with lower vegetation periods compared to the check variants. As a source of initial research material, 10 performance lines of tomatoes were used (L.20, L.25, L.47, L.55, L.63, L.64 and L.317) created in the plant resistance genetics laboratory and three varieties as a check (*Peto-86*', *Novinca Pridnestrovia and Elvira*). The evaluation of the lines was carried out according to the most phenol phasic valuable indices of the phases which determined the period of vegetation (emergence – single blooming, blooming-blooming in the mass, single rising- fructification, baking-baking single breading-baking, morphological characters fructus, fruit shape, pericarp and mesocarp thickness, pedicle length and biochemical characters (fruit acidity, dry matter content, sugar content and total harvest). It was necessary to emphasize that the selective analysis of the most successful tomato lines showed that during the study period, recombinant lines with various types of precocity (early, early and late average) were revealed. The results obtained showed that the early tomato lines (L.63 and L.64) exhibited a lower precocity period compared to the (11–7) days of the check variety (Peto-86). Investigations performed on early medium-grain

tomato lines (L.20 and L.47) demonstrated the longevity of the reduced maturity period (11 and 10) days compared to the check variant (*Novinca Pridnestrovia*). For the medium-term lines (L.20, L.25), the precocity ratio was lower than the control (Elvira') with (12.4, 10.4) days. Two lines (L.55 and L.317') were at the check level. Analyzing the preliminary results, it was concluded that the performance of tomato obtained *in vitro* with various types of precocity showed a lower fruit maturity period compared to the check varieties Based on the experimental data obtained, it was apparent that in tomato promising lines the plant's height varied within the limits of the values (45.1–77.4) cm, the blank forms (55.4–67.5) cm. The plants of the studied lines were characterized by a high vigor, with higher level, which resulted in a better adaptation of the fruit in the unfavorable conditions of the environment. In most *in vitro* lines, the descendants of the populations were genotypes of the native type, inclined towards the characters of the parent plant, at the same time another set of genotypes was composed of paternal descendants where most of the morphological characters predominated from the plant and a source of genotypes that predominated intermediate inheritance characters from both genitors.

This mode of heredity of the plant's height occupied an intermediate position and played the leading role both in plant evolution and in the amelioration of a certain character, which was defined as an attribute of an individual in a population by which it differed from other individuals in another population or taxon, any morphological, physiological or biochemical particularity of an individual or group of individuals that was determined by a gene or group of genes in interaction with environmental conditions. The preliminary analysis of the performance lines showed that during the research period the lines (L.20, L.25 and the control variety (Elvira') were highlighted with maximum values of the fruit mass (123.4, 112.7 and 125.0 grams respectively). The smallest values were accumulated in the lines (L.317, L.63, L.64) compared to check varieties (*Peto- 86' and-Novinca Pridnestrovia*). The shape of the fruit varied according to the genotype from round to cylindrical. From the bibliographic data it was known that the thickness of the pericarp was an important feature that depended on the quality of the fruits. In this contest, the tomato lines had more pronounced values (0.6–0.8mm) than the check (0.4–0.7mm). According to the results of the mesocarp thickness, there was an increase compared to the check variant (L.20, L.25) compared to the check variant *(Peto-86, Novinca Pridnestrovia and Elvira*). It was found that tomato lines with thickness indices of pericarp greater than 6 mm and the thickness of the 8 mm mesocarp were destined for the transport of the fruits. An especially important role was for the character of the fruit pedicel. The pedicel was without and with a geniculate joint where some fruits were easily detached from it while other were detached more difficult. Due to the data obtained on the biochemical quality of the fruit, it was clear that the content of dry substances was

largely dependent on the conditions of the year. Under dry conditions, the dry matter content was high and in the rainy weather dry matter ways low in fruit. However, in some lines the level of dry matter was greater than that of the check varieties. Concomitantly, the fruit sugar content in some lines showed an advantage of 6.23–6.43 % compared to the check varieties of 5.23–5.63 %. Productivity on promising lines was peaked at 49.5–58.7 t/ha. compared to 33.9–50.7 t/ ha. Analysis of *in vitro* tomato populations demonstrated that remote hybridization was indeed a method incompatible with other methods in the transformation of nature into heredity of crop plants. 0ne of the most important issues of breeding is development of new varieties in which to assemble more valuable characters, high productivity, increased resistance to unfavorable environmental factors and precocity with large and qualitative fruits. However, to accelerate the process of creating such varieties, it is necessary to apply new selection methods and technologies. We mentioned that a real perspective in this aspect was presented by the modern biotechnology methods and techniques, based on the principles of molecular genetics, genetic and cellular engineering, cell cultures and vegetal tissues, accelerated micro-propagation, induced variability and soma clonal variability, remote hybrids. Remote hybridization methods with the mobilization of the spontaneous Geno fond and the application of the *in vitro* culture allowed us to settle many issues in the amelioration process. Through classical hybrids, efficient capacities have been transferred to the activity of plant regeneration by crossing spontaneous species with the crop species through the embryo callus culture. The hybridization of different genetically-stable species varied according to the degree of kinship between them, with the transmission of genetic information that results in some valuable economic carcasses from spontaneous species to culture species. Thus, the emergence of biotechnologies, especially *in vitro* culture, produced a real revolution in the process of improving vegetable crops and other agricultural species.

## CONCLUSIONS

1.The varieties (Iulihirsutia, Anatolie, Jacotă and CerryDani) exceeded the control varieties (Elvira and Peto -86), both in terms of overall yield and commodity, precocity and fruit quality.

2.The varieties (Iulihirsutia, Anatolie, Jacotă and CerryDani) exceeded the control varieties (Elvira and Peto -86), in terms of biochemical composition of the fruits.

3.The varieties (Iulihirsutia, Anatolie, Jacotă and CerryDani) exceeded the control varieties (Elvira and Peto -86), in terms of drought and disease resistance.

4.Concomitantly, the fruit sugar content in some lines showed an advantage of 6.23–6.43 % compared to the check varieties of 5.23–5.63 %. Productivity on

promising lines was peaked at 49.5–58.7 t/ha. compared to 33.9–50.7 t/ ha. Varieties Agaphia and Vladigen approved in the Republic of Moldova were tested at the State Commission for Testing of Plant Varieties.

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# ANTIBACTERIAL ACTIVITY OF SOME *BACILLUS* STRAINS IN DEPENDENCE ON THE CULTURE MEDIUM

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**Abstract**: *Biological control of plant pathogenic bacteria, which includes the use of antagonistic microorganisms, is an alternative method to the application of chemicals. Bacteria belonging to the Bacillus spp. have demonstrated antimicrobial activity against plant pathogenic microorganisms due to the produced secondary metabolites. Thus, it is of particular interest to study and to identify new microorganisms, in particular Bacillus spp., possessing pronounced antimicrobial activity against phytopathogens that can be used as biotechnological alternatives for agriculture, with the production of biologically active preparations based on the microorganism strains or metabolites. From 12 strains of Bacillus spp. three strains, grown on liquid media, were selected and characterized by high antimicrobial activity against pathogenic plant bacteria, both bacterial suspension and cell-free supernatant showed. The selected strains can be proposed as potential bioactive antagonists against phytopathogens, which can be used to obtain bioactive preparations useful for agriculture in the field of plant protection and cell-free supernatant may have prospects for use in the medical field to obtain bioactive preparations due to the ability to produce various secondary metabolites such as antibiotics.*

***Keywords: antibacterial activity, Bacillus, culture medium.***

## INTRODUCTION

Fungal and bacterial phytopathogens are responsible for significant losses or decrease in the quality and safety of agricultural products by damaging crops. Among the plant pathogenic bacteria causing numerous crop plant diseases are those of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Xanthomonas* and so on and among pathogenic fungi the most common are from *Alternaria*, *Botrytis*, *Fusarium*, *Aspergillus* and *Penicillium* [1-3].

Chemical pesticides are mainly used to control diseases caused by plant pathogens. Besides the fact, that chemical pesticides have a number of side effects on both the environment and human health, pathogens develop resistance to them [1, 2]. Biological control of plant pathogenic bacteria, which includes the use of antagonistic microorganisms, is an alternative method to the application of chemicals, which can be achieved by destroying pathogens, and is a safe method

for an effective and integrated disease management system that is environmentally friendly and non-toxic to human health [1, 4].

Most species of the genus *Bacillus* are considered safe microorganisms and possess remarkable abilities to synthesize numerous substances that have been successfully used in agriculture and for industrial purposes [3, 5]. Bacteria of the genus *Bacillus* have demonstrated antimicrobial activity against phytopathogenic microorganisms due to the secondary metabolites produced by them through a variety of mechanisms of action, such as competition, induction of systemic resistance and antibiotic production [6-10]. *Bacillus* spp. strains are already adapted to the environment and can be applied for a biological control method, due to the large number of advantages such as the fact that most of them are isolated from the environment, grow rapidly in liquid media, form extremely resistant spores with high thermal tolerance, which allows them to be considered prioritizing candidate agents for the biological control of plant diseases [1, 3, 11].

All of the above points to the necessity of studying and identification of new microorganisms, especially *Bacillus* spp. possessing pronounced antimicrobial activity on phytopathogens that can be used as alternative biotechnological solutions for agriculture, with the production of biologically active antimicrobial preparations based on the strains of microorganisms or their metabolites.

Thus, the aim of this research focused on the identification of strains of *Bacillus* spp. possessing pronounced antibacterial activity against phytopathogenic bacteria, depending on the culture medium.

## MATERIALS AND METHODS

The study included 12 bacterial strains from *Bacillus* spp.: *Bacillus subtilis* CNMN-BB-01, *Bacillus subtilis* CNMN-BB-06, *Bacillus subtilis* CNMN-BB-08, *Bacillus subtilis* CNMN-BB-09, *Bacillus subtilis* CNMN-BB-10, *Bacillus velezensis* CNMN-BB-12, *Bacillus velezensis* CNMN-BB-13, *Bacillus velezensis* CNMN-BB-14, *Bacillus velezensis* CNMN-BB-15, *Bacillus velezensis* CNMN-BB-16, *Bacillus velezensis* CNMN-BB-17 and *Bacillus velezensis* CNMN-BB-18, stored and deposited in the National Collection of Non-Pathogenic Microorganisms (NCNM) at the Institute of Microbiology and Biotechnology of Technical University of Moldova.

As growing media, served Nutrient Agar (NA) with the following composition (g/L): Meat Extract – 1.0; Yeast extract – 2.0; Peptone – 5.0; Sodium chloride – 5.0; Agar – 15.0 and Mueller Hinton Agar (MHA) with the following composition (g/L): Meat infusion – 2.0; Casein hydrolysate – 17.5; Starch – 1.5; Agar-agar – 13.0. The bacterial cultures were grown on the whole Petri dish surface, concentration was adjusted to 0.5 McFarland standard. Cultivation was carried out at a temperature of 35±1°C, 48 hours.

The bacterial cultures were also grown on liquid medium Nutrient Broth (NB) with the following composition (g/L): Peptic digest of animal tissue – 5.0; Sodium chloride – 5.0; Beef extract – 1.5; Yeast extract – 1.5. Experiments were carried out in 250 mL Erlenmeyer flasks with a working volume of 50 mL. The inoculum concentration was adjusted to 0.5 McFarland standard. Optimal cultivation conditions include a temperature of 30±1°C, continuous illumination. Cultures were agitated continuously at 200 rpm for 48 hours.

At the end of the cultivation cycle, the bacterial biomass was separated from the culture liquid by centrifugation to obtain the cell-free supernatant (CFS), then the dry mass of the supernatant was determined, which was on average 10 mg/mL.

The antimicrobial activity was tested against *Agrobacterium tumefaciens* CNMN-AB-01, *Corynebacterium michiganense* CNMN-CrB-01, *Erwinia carotovora* CNMN-BE-01 and *Xanthomonas campestris* CNMN-BX-01 – bacteria that cause various crop plant diseases. The assessment of antibacterial activity was determined by disc diffusion method and well plate method [12]. For disc diffusion method, antibacterial activity was defined by using agar blocks. For well diffusion method, a quantity of 100 µL (bacterial suspension (BS) or cell-free supernatant (CFS)) obtained after cultivation in liquid media was dosed into wells of 8.0 mm diameter in Petri plates with medium already containing seeded test-bacterial strains. Plates were left at room temperature for 1 hour to allow diffusion, then incubated at +35±1°C. Inhibition zone diameters were measured after 24 hours of incubation.

Statistical analysis of data was performed using Microsoft Excel 2016, there were three replications for each test and the biocidal assessment was performed twice. The statistical significance threshold was $p \leq 0.05$.

## RESULTS AND DISCUSSIONS

The results obtained on the antibacterial activity of *Bacillus* spp. strains, cultivated on agarized media, demonstrated that out of five investigated strains of *B. subtilis*, only *B. subtilis* CNMN-BB-10 showed activity against the four strains of phytopathogenic bacteria, regardless of the cultivation medium NA or MHA, the inhibition zones varying between 14.7–27.0 mm and 14.3–25.7 mm, respectively (Table 1).

In contrast, the *B. velezensis* strains all had antibacterial activity against phytopathogens, the zones of inhibition being dependent on the strain or medium tested. The most sensitive was the *A. tumefaciens* CNMN-AB-01 strain, with zones of inhibition of growth and development ranging from 19.7-27.3 mm, depending on the growth medium used.

Table 1. Antibacterial activity of some *Bacillus* strains in dependence on the culture medium, by disc diffusion method, zone of inhibition, mm

| | ***A. tumefaciens* CNMN-AB-01** | | ***C. michiganense* CNMN-CrB-01** | | ***E. carotovora* CNMN-BE-01** | | ***X. campestris* CNMN-BX-01** | |
|---|---|---|---|---|---|---|---|---|
| | **NA** | **MHA** | **NA** | **MHA** | **NA** | **MHA** | **NA** | **MHA** |
| ***B. subtilis* CNMN-BB-01** | Ni | Ni | Ni | Ni | Ni | 10.7±0.7 | Ni | 10.3±0.7 |
| ***B. subtilis* CNMN-BB-06** | Ni | Ni | Ni | Ni | Ni | Ni | Ni | 11.7±1.7 |
| ***B. subtilis* CNMN-BB-08** | Ni | Ni | Ni | Ni | Ni | Ni | Ni | Ni |
| ***B. subtilis* CNMN-BB-09** | Ni | Ni | Ni | Ni | Ni | Ni | Ni | 12.0±1.1 |
| ***B. subtilis* CNMN-BB-10** | 23.3±1.3 | 24.0±1.1 | 27.0±1.1 | 25.7±1.7 | 17.0±1.1 | 14.7±0.7 | 14.7±0.7 | 14.3±0.7 |
| ***B. velezensis* CNMN-BB-12** | 21.7±0.7 | 27.3±0.7 | 15.0±1.1 | 20.7±2.6 | 16.3±0.7 | 23.3±1.3 | 19.3±1.3 | 23.7±0.7 |
| ***B. velezensis* CNMN-BB-13** | 24.0±1.1 | 20.3±1.7 | 27.7±0.7 | 25.7±0.7 | 16.3±0.7 | 16.3±0.7 | 15.7±0.7 | 15.7±0.7 |
| ***B. velezensis* CNMN-BB-14** | 20.7±0.7 | 24.3±0.7 | 22.0±1.1 | 24.7±0.7 | 14.7±0.7 | 14.0±1.1 | 14.3±0.7 | 14.3±0.7 |
| ***B. velezensis* CNMN-BB-15** | 19.7±0.7 | 21.3±0.7 | 12.3±0.7 | 12.7±0.7 | 19.7±2.4 | 22.3±0.7 | 17.0±1.1 | 23.7±0.7 |
| ***B. velezensis* CNMN-BB-16** | 26.3±0.7 | 27.0±1.1 | 14.3±0.7 | 13.3±0.7 | 22.3±0.7 | 24.7±0.7 | 22.0±1.1 | 23.3±0.7 |
| ***B. velezensis* CNMN-BB-17** | 21.0±1.1 | 25.3±0.7 | 15.3±0.7 | 13.7±0.7 | 18.7±1.7 | 22.0±2.0 | 18.7±1.3 | 21.7±1.7 |
| ***B. velezensis* CNMN-BB-18** | 20.3±0.7 | 25.0±2.0 | 13.3±0.7 | 13.7±0.7 | 22.3±0.7 | 23.7±0.7 | 18.3±0.7 | 21.7±0.7 |

*Note: NA – Nutrient Agar, MHA – Mueller Hinton Agar, Ni – no inhibition, $p \leq 0.05$.*

The use of disc diffusion method is a rapid method, useful both for screening of strains that possess antimicrobial activity and for obtaining of biologically active preparations as a result of the cultivation of microorganisms of biotechnological interest for various use. It is more efficient to grow them in a liquid medium, which offers wider possibilities of manipulation and obtaining biopreparations from microbial biomass and the resulting culture liquid due to the synthesis of different secondary metabolites with various actions.

Thus, as a result of the screening of *Bacillus* strains, were selected three most active strains *B. subtilis* CNMN-BB-10, *B. velezensis* CNMN-BB-12 and *B. velezensis* CNMN-BB-16, cultured on liquid Nutrient Broth medium. The antibacterial activity of both the bacterial suspension and the cell-free supernatant, following centrifugation and separation from the microbial biomass was determined after 48 hours of cultivation. The results have demonstrated that the selected *Bacillus* spp., *B. subtilis* CNMN-BB-10, *B. velezensis* CNMN-BB-12 and *B. velezensis* CNMN-BB-16 strains, maintained their antibacterial activity against phytopathogens (Table 2).

Table 2. Antibacterial activity of some *Bacillus* strains cultivated on Nutrient Broth medium, by well plate method, zone of inhibition, mm

| | ***A. tumefaciens* CNMN-AB-01** | | ***C. michiganense* CNMN-CrB-01** | | ***E. carotovora* CNMN-BE-01** | | ***X. campestris* CNMN-BX-01** | |
|---|---|---|---|---|---|---|---|---|
| | **BS** | **CFS** | **BS** | **CFS** | **BS** | **CFS** | **BS** | **CFS** |
| ***B. subtilis* CNMN-BB-10** | 24.4±0.7 | 22.1±0.9 | 25.0±1.1 | 22.5±1.6 | 13.8±0.7 | 11.7±0.4 | 14.7±0.7 | 11.5±0.6 |
| ***B. velezensis* CNMN-BB-12** | 25.4±0.7 | 23.7±0.7 | 14.7±0.7 | 12.7±0.7 | 20.0±1.0 | 13.0±0.4 | 20.8±0.5 | 12.8±0.7 |
| ***B. velezensis* CNMN-BB-16** | 25.0±0.5 | 19.9±0.9 | 25.3±1.4 | 24.3±1.1 | 15.7±0.4 | 13.9±0.5 | 19.7±1.3 | Ni |

*Note: BS – Bacterial Suspension, CFS – Cell-free supernatant, Ni – no inhibition, $p \leq 0.05$.*

Analysing the obtained data, both the BS and CFS show inhibitory action on the growth and development of phytopathogenic bacteria, with growth inhibition values ranging from 13.8 – 25.4 mm and 11.5 – 24.3 mm, respectively. The exception is only the CFS obtained from the cultivation of *B. velezensis* CNMN-BB-16, which lost its activity against *X. campestris* CNMN-BX-01.

As in the case of cultivation on agarized media, the *A. tumefacies* CNMN-AB-01 strain proved to be the most sensitive strain, growth being inhibited over areas between 24.4 – 25.4 mm when using bacterial suspension and between 19.9 – 23.7 mm when using CFS.

Therefore, following the analysis of the obtained data, it was found that the exometabolites synthesized in the medium by *B. subtilis* CNMN-BB-10, *B. velezensis* CNMN-BB-12 and *B. velezensis* CNMN-BB-16 have antibacterial action on plant pathogenic bacteria, and the given strains can be proposed as potential bioactive antagonists against phytopathogens, which can be used to obtain bioactive preparations useful for agriculture in the field of plant protection.

The obtained results on the antimicrobial activity of the CFS obtained from the cultivation of *Bacillus* spp. bacteria may have prospects for use in the medical field to obtain bioactive preparations based on it. According to literature data, it is known that *Bacillus* spp. strains are capable of producing in the medium various secondary metabolites, diverse in function and structure, such as peptide antibiotics as: gramicidins, bacitracin and thyrocidins that possess pronounced antimicrobial activity [13-16].

## CONCLUSIONS

Results obtained on the antibacterial activity of bacterial strains of *Bacillus spp*., cultivated on agarized media, showed that out of five *B. subtilis* strains investigated only *B. subtilis* CNMN-BB-10 showed activity against the four phytopathogenic bacterial strains, while those of *B. velezensis*, all seven had

activity, regardless of the culture medium Nutrient Agar or Mueller Hinton Agar, zones of inhibition ranging from 13.3-27.7 mm and 12.7-27.3 mm, respectively.

Cultivation of *B. subtilis* CNMN-BB-10, *B. velezensis* CNMN-BB-12 and *B. velezensis* CNMN-BB-16 strains on liquid Nutrient Broth medium demonstrated that both the bacterial suspension and the cell-free supernatant possessed quite high antibacterial activity on test cultures of phytopathogenic bacteria, in some cases the zones of inhibition being larger than those grown on agarized media.

The selected strains can be proposed as potential bioactive antagonists against phytopathogens, which can be used to obtain bioactive preparations useful for agriculture in the field of plant protection and cell-free supernatant may have prospects for use in the medical field to obtain bioactive preparations due to the ability to produce various secondary metabolites such as antibiotics.

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# PHYTOSTIMULATORY POTENTIAL OF SOME BACTERIA FOR WHEAT AND MAIZE

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**Abstract**: *Increasing plant productivity and improving crop quality in agriculture is one of the main tasks of the biological and agronomic sciences. Since wheat and maize are important for the Moldovan agriculture, our goal was to identify strains of microorganisms that could be beneficiary for these crops. Pseudomonas fluorescens CNMN-PsB-01 and P. fluorescens CNMN-PsB-02 were selected for their ability to stimulate wheat and maize by causing 10.8-22.6% and 9.2-26.2% increases in plant root length and seedling dry mass respectively.*

***Keywords: bacterial strains, phytostimulator, wheat, maize.***

## INTRODUCTION

Increasing plant productivity and improving crop quality in agriculture is one of the main tasks of the biological and agronomic sciences. An important role in these matters can be played by the plant growth promoting micro-organisms [1-6]. The latter are known for their ability to stimulate plants and to suppress the phytopathogen activity in soil without substantial risk for the environment and/or human health [3, 7-11]. For example, the representatives of *Bacillus*, *Pseudomonas*, *Azotobacter*, *Paenibacillus*, *Streptomyces*, and other genera can produce such secondary metabolites as phytohormones, siderophores, antibiotics, etc. Their application increased seed germination, accelerated the growth, development, and productivity of plants [3, 8, 10, 11].

Our goal was to identify the microbial strains that could be used for plant growth promoting in the Republic of Moldova. Our focus was on those which could be beneficial for wheat and maize, since these crops are of particular importance for the Moldovan agriculture.

## MATERIALS AND METHODS

The study included the following bacterial strains: *Bacillus cereus* var. *fluorescens* CNMN-BB-07, *Bacillus velezensis* CNMN-BB-31, *Pseudomonas fluorescens* CNMN-PsB-01, *Pseudomonas fluorescens* CNMN-PsB-02 and *Pseudomonas fluorescens* CNMN-PsB-12 stored and deposited in the National Collection of Non-Pathogenic Microorganisms (NCNM) at the Institute of Microbiology and Biotechnology of the Technical University of Moldova.

The media for *Bacillus sp.* Was Nutrient Broth (NB) with the following composition (g/L): Peptic digest of animal tissue – 5.0; Sodium chloride – 5.0; Beef extract – 1.5; Yeast extract – 1.5. For *Pseudomonas* spp. – liquid media King B with Peptone – 20.0; Glycerine – 10.0; $K_2HPO_4$ – 1.5; $MgSO_4 \cdot 7H_2O$ – 1.5. The microorganisms were cultivated in 250 mL Erlenmeyer flasks with a working volume of 50 mL. The inoculum concentration was adjusted to the 0.5 McFarland standard. Optimal cultivation conditions included a temperature of 30±1°C and continuous illumination. Cultures were agitated continuously at 200 rpm for 48 hours.

Winter wheat MOLDOVA 16 and maize hybrid MOLDAVSCHII 450 were used as crop plants. The vegetation experiments were done under laboratory conditions in Petri dishes and in pots with non-sterile soil and included three replicas for each test case and the controls. The stimulatory activity of the bacteria was assessed via measuring the changes in germination capacity of seeds and in plant growth parameters [12]. The tests were done in Petri dishes and in pots with soil. Immediately before the tests the plant seeds were treated for 18-20 hours by microbial suspensions in the concentration of 0.2, 0.3, and 1.0%.

Statistical analysis of data was performed using Microsoft Excel. The statistical significance threshold was $p \leq 0.05$ [10].

## RESULTS AND DISCUSSIONS

Under the condition of Petri dishes, the best impacts on wheat were observed from *Bacillus cereus* var. *fluorescens* CNMN-BB-07 and *Pseudomonas fluorescens* CNMN-PsB-12, which when used at concentration 0.3% caused 11.8% and 20.6% increases in dry mass of the seedlings respectively. *Pseudomonas fluorescens* CNMN-PsB-01 and *Pseudomonas fluorescens* CNMN-PsB-02 when used in concentrations 0.3 and 0.2% respectively increased the maize germination up to 100% and the maize seedling dry mass by 1.41 – 1.44 times (tab. 1).

Table 1. The best impacts on germination and growth of wheat and maize in Petri dishes

| **Variant** | **Germination, %** | **Seedling gross mass, g± c.i.*** | **Seedling dry mass, g ± c.i.** |
|---|---|---|---|
| **Moldova 16 winter wheat** | | | |
| Control (distilled water) | 98.3 | 8.8 ±0.02 | 1.02±0.03 |
| *B. cereus* var. *fluorescens* CNMN-BB-07 | 98.3 | 10.2±0.17 | 1.14±0.01 |
| *P. fluorescens* CNMN-PsB-12 | 93.3 | 10.4±0.65 | 1.23±0.05 |
| **Moldavschii 450 maize hybrid** | | | |
| Control (distilled water) | 96.7 | 14.8±2.5 | 2.04±0.1 |
| *B. cereus* var. *fluorescens* CNMN-BB-07 | 96.6 | 17.4±1.1 | 2.18±0.2 |
| *P. fluorescens* CNMN-PsB-01 | 100.0 | 29.5±4.6 | 2.94±0.3 |
| *P. fluorescens* CNMN-PsB-02 | 100.0 | 26.6±3.0 | 2.88±0.2 |
| *P. fluorescens* CNMN-PsB-12 | 93.3 | 18.3±0.6 | 2.08±0.1 |

** Average data for 100 seedlings ± confidence interval at p≤0.05.*

In the experiments with wheat grown in pots with non-sterile soil *Bacillus velezensis* CNMN-BB-31 increased the seedling height and root length by 10.9% and 14.9% respectively. *Bacillus cereus* var. *fluorescens* CNMN-BB-07 caused a 23.3% increase of the maize root length. The greatest stimulation of maize growth was observed from *Pseudomonas fluorescens* CNMN-PsB-01 and *Pseudomonas fluorescens* CNMN-PsB-02 and constituted 10.8–22.6% increases in root length and by 9.2–26.2% increases of the seedling dry mass (tab. 2).

Table 2. The best impacts on growth of wheat and maize in pots with non-sterile soil

| **Variant** | **Seedling height, cm ± c.i.*** | **Root length, cm ± c.i.** | **Seedling dry mass, g ± c.i.** |
|---|---|---|---|
| **Moldova 16 winter wheat** | | | |
| Control (distilled water) | 26.5±0.3 | 15.4±0.2 | 0.79±0.05 |
| *B. velezensis* CNMN-BB-31 | 29.4±1.1 | 17.7 ±0.3 | 0.82±0.02 |
| *P. fluorescens* CNMN-PsB-12 | 27.5±0.5 | 16.2 ±1.1 | 0.75±0.01 |
| **Moldavschii 450 maize hybrid** | | | |
| Control (distilled water) | 45.0±1.6 | 28.8±3.5 | 0.65±0.08 |
| *B. cereus* var. *fluorescens* CNMN-BB-07 | 44.5±2.4 | 35.5±2.5 | 0.63±0.07 |
| *P. fluorescens* CNMN-PsB-01 | 47.4±0.9 | 31.9±3.7 | 0.82±0.05 |
| *P. fluorescens* CNMN-PsB-02 | 45.8±1.2 | 35.3±3.4 | 0.71±0.09 |
| *P. fluorescens* CNMN-PsB-12 | 45.5±1.9 | 30.5±2.4 | 0.63±0.03 |

** Average ± confidence interval at p≤0.05.*

## CONCLUSION

Based on the testing under conditions of Petri dishes and pots with non-sterile soil the bacterial strains *Pseudomonas fluorescens* CNMN-PsB-01 and *Pseudomonas fluorescens* CNMN-PsB-02 were selected for their potential to stimulate the growth of maize. Further research is necessary to confirm this potential under field conditions. Further screening of efficient stimulators of wheat growth is needed.

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# TRENDS IN COMBATING VINE PHYTOPATHOGENS WITH THE APPLICATION OF EPIPHYTIC MICROORGANISMS

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**Abstract.** *The management of phytopathogens plays a decisive role in the sustainable development of agriculture, especially in viticulture. A particularly serious impact is recorded in the development of pathogens of the vine, especially of powdery mildew (Uncinula necator (Schwein) Burrill). In addition to measures promoted by modern agricultural biotechnologies the particular interest are the epiphytic microorganisms, which populate the leaf surface of plants, constituting complex heterogeneous habitats from various extended communities and numerous transients from various systematic entities. Through effective management and control of plant diseases, crop losses can be reduced and the quality and quantity of agricultural products can be improved. The article includes the analysis of the role of different biotic and abiotic factors, which change the relationships within these communities, as well as the adaptation processes of some entities of epiphytic and phytopathogenic microorganisms on the leaf surface of the vine.*

***Keywords**: **biocontrol, ecological agriculture, epiphyte, phytopathogen agent***

## INTRODUCTION

The worsening of phytosanitary problems against the background of climate change lies in the modification of the epidemiological particularities of phytopathogenic agents and the increase in the prevalence of pests by manifesting various difficulties in carrying out technological processes for the production of agricultural crops [1]. The main problem of contemporary viticulture lies in increasing the receptivity of existing varieties to the attack of phytopathogens and specific harmful arthropods, which reduce grape harvests by 30-35% or cause total compromise of wine production.

From the great diversity of pathogens, a special role is played by diseases of cryptogamic etiology, and during the last decades there has been an increase in the pathogenicity of grapevine powdery mildew (*Uncinula necator (*Schwein*)* Burrill). Being widespread everywhere, powdery mildew in the conditions of climate change and the predominance of dry summers, and the pathogen attacks all the vegetative and generative organs of the plant. The manifestation of the disease is recorded by browning and falling of young bunches and abortion of flowers, as well as necrotization of berries at different stages of development, which requires the development and application of protection systems [2].

Ensuring the biological efficacy of the measures to reduce the impact of the pathogen requires increasing the efficiency of the protection systems oriented to the application of natural mechanisms by using the principles and techniques in the research of the efficiency of the action of the biological preparations proposed for the elaboration of the concept of synergism aimed at increasing the effectiveness of the elements proposed for conventional and organic farming systems [3].

Measures to reduce the impact of pathogens on agricultural crops are carried out by investigating the relationships between agricultural plants and microorganisms, which populate their leaf area, which largely determine the health status of agricultural crops. This is explained by the ubiquity of microscopic entities in natural environments (soil biota, aquatic environment and bioaerosols). Unlike the relationships of agricultural plants with pathogens in soil, water and air, epiphytic communities are not mechanisms for triggering pathogenic processes, and some interactions between the plant and epiphytic microorganisms are symbiotic, in which various synergistic mechanisms are manifested [4]. These include closely co-evolved partnerships between plants and mycorrhizal fungi or nitrogen-fixing bacteria. Most plant species are populated by numerous microorganisms, gathered within different microbiomes, consisting of microscopic entities with a lower degree of association and are influenced by the organisms that inhabit that part of the soil, and whose development is determined by the functionality of the plant rhizosphere. The manifestation of these microorganisms is recorded by the activity of rhizobacteria, which promote the growth of endophytic plants and fungi, which

participate in increasing resistance to pathogens and stressful conditions.

Plants have various microbial niches in the form of rhizosphere and phyllosphere, the representatives of which host representatives of various mutual, commensal and pathogenic microorganisms. They influence microbial communities in a species- and genotype-dependent manner, through secondary metabolites, exudates, and immune responses, all of which vary depending on developmental stage, plant compartment, and biotic interactions. Numerous species of bacteria, microscopic fungi, algae, viruses and protozoa have been isolated and identified on the surface of the photosphere [5], which represent a perfect model for analyzing the evolution of microflora and genetic exchange, as well as being a significant source of microorganisms, which diffuse to other spheres.

The contact of microorganisms with the surface of the leaves occurs with the help of raindrops and through the movement of air, as there are large fluxes of microorganisms in the atmosphere and at nearby surfaces. Landing on the leaf, microscopic organisms form aggregates of different sizes, separate individuals, as well as microcolonies. The peculiarities of the leaf surfaces lead to the formation of heterogeneous microhabitats covered by cuticle consisting of crystalline wax structures located on the protective tissues with a network of grooves, as well as the guard cell complexes around the stomata, which determine the permeability of the leaf apparatus. Penetrating into the essence of the mechanisms of the functioning of epiphytic communities and the relationships between the ecestora components and the phytopathogenic agents of plants represents a prospective way in reducing the impact of harmful organisms on agricultural crops.

## RESEARCH MATERIALS AND METHODS

Various strains of bacteria, fungi and other pathogens of vine diseases were used for research, their control was carried out by testing the means of combating 4 randomized repetitions, in accordance with the general requirements of experiments of this kind [1, 6]. The protection programs against pathogenic organisms were developed based on the massive information recorded within the Laboratory of Phytopathology and Biotechnology.

The identification of biological products to combat biological agents will allow the improvement of integrated protection programs in conventional and organic farming systems. The situation in the field of plant protection and the environment was analyzed by applying the methodology of systemic approaches, as a tool of complexity management and as one of the essential paradigms of the future.

Laboratory and field experiment testing was carried out in 4 randomized trials, respectively, in accordance with the general requirements of experiments of this kind [7].

## RESULTS AND DISCUSSIONS

### Peculiarities of the pathogen of vine powdery mildew

Powdery mildew (*Uncinula necator)* is one of the most contagious diseases, affecting all vegetative and generative aerial organs and causing serious technological, economic and ecological problems. From the moment of initiation of the pathogenesis process, significant damage is recorded, which determines the need to apply effective means of prevention and control.

On the leaves there is a fine, spider's web-like mycelium, with a dusty appearance, which spreads forming whitish spots on both surfaces of the blade. Young shoots covered with whitish, and then brown, spots are stopped from growing. The bunches are browned and then dried. The berries attacked by mycelium crack, providing an optimal environment for the development of other fungal pathogens, and in rainy autumns they are covered with gray rot (*Botrytis cinerea*), which causes the quality of the grapes to depreciate.

The primary infection occurs through attacked shoots and cleistothecia (fig.1). The conidiospores, being spread by the wind, land on the foliar apparatus where they germinate and infect the vine by penetrating the haustors into the epidermal cells. The germination, infection and development of ascospores and conidiospores take 5-12 days, depending on the temperature, developing several cycles of infection throughout the growing season. It is worth mentioning that the development of the pathogen is favored by mild cloudy weather, moderate degree of lighting, optimal temperatures of 22-28°C with a range of 6-33°C, humid conditions necessary for sporulation [8].

Figure 1. Manifestation of *U.necator* symptoms on vine leaves and shoots

The phenomenon of climate change contributed to the early development of the pathogen. Weather conditions at the beginning of the season will affect management decisions to prevent outbreaks that occur later in the season (fig. 2). Maintaining wine plantations requires good air circulation, spray penetration, and exposure to sunlight.

Figure 2. Manifestation of *U. necator* symptoms on the generative organs of the vine

In order to achieve better spray coverage and prevent further development of the disease, the following practices are recommended: carrying out the first treatment at the stage of 2-3 true leaves; cutting shoots for aerating bushes; lifting threads to expose the bunches; removal of 2-3 leaves immediately above and below the bunches; adjustment of spray nozzles and direct airflow of spray machines; the use of large spray volumes and the highest rate of wetting agent; applying 2-3 sprays every seven to ten days to ensure maximum coverage of leaves and bunches [9].

Among the prevention measures, it is necessary to comply with the regulations for the correct application of maintenance works (cutting, tying, fertilizing, pinching, weed control), as well as a complex of prospective measures to reduce the impact of harmful organisms [1, 6].

Activities to expand the implementation of regenerative agriculture are based on the application of the agroecological principles of sustainable and resilient soil management, which are analogous to those of natural ecosystems and include: the permanent presence of living roots in the soil, permanent soil cover with plant debris, the great diversity of main and successive crops in rotation at the level of each individual lot and landshaft.

Synergistic phenomena – direction of increasing the effectiveness of the means of plant protection as natural sources of intensification of actions aimed at obtaining the integral effect of the joint application of several factors, which is greater than the simple sum of its parts and signifies "working together" and defines the increased effect that can be obtained by the simultaneous action of several physical elements, biological, economic, social.

The application of prophylactic measures remains as effective levers to reduce the impact of pathogens on the vine (selection of immune, resistant and tolerant varieties, choice of rootstock and stump shape, fertilizers, application of technological operations in green.

Development and application of technological programs for integrated protection of vines with the use of specific powdery mildew protection products, as well as the implementation of ecological agriculture.

**Relationships between pathogens and epiphytic community elements on leaf surface**

Starting from the needs of reducing biotic and abiotic stresses associated with leaf surface, epiphytic microorganisms are oriented towards the establishment of microhabitats capable of protecting host plants. The ability to navigate and stay in those niches in the leaf surface that are the richest in nutrients and water available is key to the epiphytic survival of both bacteria and fungi. To survive abiotic stress on the leaf surface, many microbes form biofilms composed of extracellular polysaccharides for cell aggregation. Biofilms are resistant to drying, antimicrobials and reactive oxygen species, which are important for plant protection. Biofilms can also be formed from yeasts and filamentous fungi, including a number of plant pathogens. The physical proximity of different microbial species in a stressful environment supports a high rate of horizontal gene transfer, especially for the exchange of plasmids and genes. The in-depth analysis of the relationships within epiphytic communities with the participation of bacteria is presented by [10], and filamentous microorganisms by [11].

Particularly important is the secretion of enzymes such as chitinases, esterases and lipases, which can release nutrients from the cuticle and, by degrading the cuticle components, increase its permeability to nutrients and the susceptibility of plants to infection. Epiphytic microorganisms produce siderophores containing iron chelates to increase iron absorption. The production of siderophores is also recorded in epiphytic yeasts, which have capacities to combat fungal pathogens.

In order to apply the capacities of epiphytic communities, it becomes necessary to highlight the similarities between epiphytic and phytopathogenic adaptations manifested in the production of plant hormones, the production of surfactants and the suppression of defenses, which cause the environment around pathogens to change, reducing penetration capacities and behaving as epiphytes during early leaf colonization [4]. Most leaf surface microorganisms occur in the same suitable micro habitats and can collaborate closely in biofilm formation with several species [12]. The interactions between them are not always oriented towards collaboration, becoming competitive in the conditions of limited nutritional resources. These interactions and their potential exploitation for the biological control of phytopathogens represent perspectives in order to ensure the high degree of plant health.

## CONCLUSIONS

The extension of the phenomenon of climate change causes a series of changes in the phytosanitary status of agricultural crops, considerably increasing the impact of phytopathogens. There has been an increase in the virulence and aggressiveness of the pathogen *Uncinula necator* in the vineyard, which currently has a high degree of pathogenicity, and in hybrid varieties, which previously showed immunity to this pathogen.

The leaf surface represents an important habitat consisting of microorganisms with considerable potential for control of phytopathogens. Epiphytic communities are constituted by the host plant and under the influence of neighboring plants, soil, water, and air, which cause the formation of adaptations that manipulate the host plant and improve access to nutrients.

The application of the capacities of epiphytic communities requires the development and investigation of research models of interactions between microorganisms and the host plant, as well as the opening of potential pathways for the biological control of phytopathogens for the extrapolation of results aimed at preserving plant health.

Reducing the impact of the vine requires the application of the integral control system with the application of effective prophylactic and therapeutic means based on the achievements of modern agricultural biotechnologies.

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