

EFFECT OF BIODEGRADABLE COMPOSITION FOR SEED COATING ON EARLY STAGE OF CORN GROWTH

Elena LUTCAN, Raisa IVANOVA, Ala BOROVSKAIA, Dina ELISOVETCAIA

Moldova State University, Institute of Genetics, Physiology and Plant Protection,
20 Pădurii Street, Chişinău, MD-2002, Republic of Moldova

Corresponding author email: lutcanelena322@gmail.com

Abstract

The objective of this research was to evaluate the influence of biodegradable carboxymethylcellulose-based composition on the growth characteristics of coated corn seeds during storage. The seeds of four corn hybrids (P280, P300, P398, P402) were coated with a carboxymethylcellulose-based composition containing an extract of phenolic compounds from Juniperus sabina and natural dye obtained from Phytolacca americana berries. Early growth characteristics (germination, root/shoot lengths, root/shoot biomass, root/seedling vigour, metabolic efficiency) of coated seeds were studied before storage and during four months of storage. During storage, the overall germination of seeds remained at the level of 94-98%, while the length of the roots and seedlings of the germinated seeds increased by 1.2-1.9 and 1.4-1.8 times, respectively. After storage, the coated seeds also showed an increase in the vigour of roots and seedlings (by 1.5-2.1 times). Thus, the developed composition contributes to the improvement of the growth characteristics of corn seeds and, due to the original dye, allows for easy identification of coated seeds.

Key words: corn, seed coating, biodegradable composition, growth characteristics.

INTRODUCTION

Corn grain production, which in 2023 accounted for 41.9% of the total grain production, is an important component of the Republic of Moldova economy (Statistica RM, 2023). Increasing gross harvests of corn remains a priority task for agricultural production in republic.

The use of high-quality seeds for sowing is the basis for increasing the productivity of corn, which is carried out using various types of highly productive varieties and pre-sowing treatments of corn seeds. However, by protecting seeds through coating with synthetic components (fungicides, pesticides, colorants), in most cases, could be provide an inhibitory effect on the seed germination and the plant growth as a whole. However, adverse environmental impacts caused by using non-renewable and non-biodegradable polymers are driving the search for alternatives to overcome these effects, such as natural-based polymers.

At present, much attention is paid to the search for new environmentally friendly methods of stimulating germination, growth and protection of plants from diseases, pests, abiotic stresses, based on increasing resistance by inducing

internal reserves of the seed itself (Ivanova & Makarov, 2020; Dziwulska-Hunek et al., 2023). In addition, the natural substances and biological products are used to obtain environmentally friendly and healthy products as well as to maintain of soil fertility.

It is relevant to use biologically active compounds, secondary metabolites of higher plants, in the composition of a complex cover mixture for seed protection, which in small quantities have the ability to influence many physiological processes of seed germination and plant growth.

When using natural growth regulators, the degree and some nature of the metabolic processes of seed germination change. Activation of metabolic processes and mobilization of reserves substances are key steps to maintain the growth of seedlings and roots before the mechanisms of photosynthesis are initiated (Zhang et al., 2018; Silva & Carvalho, 2023).

Significant variability in emergence and plant vigour existed in field condition by influence of abiotic factors, namely cold temperatures and high soil moisture (Ali et al., 2018; Khaeim et al., 2022; Beegum et al., 2023).

To protect corn seeds from unfavourable environmental factors and ensure uniform

emergence of seedlings, which leads to increased yields, pre-sowing seed treatment with natural plant growth regulators and coating with biodegradable polymers are studied.

Biodegradable polymers, such as, starch, pectin, alginate, chitosan, gelatine gum arabic, polyethylene glycol, cellulose and its derivatives (hydroxyethylcellulose, carboxymethylcellulose) use as substitutes to synthetic materials owing to their environment friendly and nontoxic characteristics, as well as good adherence to the seeds (Pirzada et al., 2020; Rahman et al., 2021; Vinzant et al., 2023; Zacharias et al., 2024).

Carboxymethylcellulose is one of the most promising biopolymers due to its characteristic of surface properties, mechanical strength, viscous properties, availability and low-cost (Rahman et al., 2021). Carboxymethylcellulose-based seed coat has developed and tested for soybean (de Camargo et al., 2017), wheat (Ren et al., 2019) and sweet corn (Pallaoro et al., 2016; Mahisanon et al., 2021) and parental form of corn (Borovskaia et al., 2022) seeds using various fungicides, plant growth regulators and additives; and showed the effect of seed coating on germination, root/shoot growth and vigour.

The natural plant growth regulators with suitable concentrations incorporated in coating compositions improved seed early growth characteristics of sweet corn (Suo et al., 2017) and corn for grain and silage (Borovskaia et al., 2023). The positive effect of presowing seed coating of corn parental form with composition on carboxymethylcellulose containing natural bioregulator (genistifolioside) was confirmed by experimental data obtained in the field, which was expressed in improving the development of the root system and increasing the mass of both the green aerial part of plants and 1000 grains (Borovskaia et al., 2022).

This study is a continuation of the ongoing research concerning the influence of seed coating with composition containing bioregulator JS extracted from aboveground parts of *Juniperus sabina* L. plants and natural dye from berries of *Phytolacca americana* L. on the bio-morphological and physiological characters of coated corn seeds during storage. It is well known that extracts from *J. sabina* contains terpenoids, flavonoids, coumarins, lignans, reducing sugars, etc. and possess increased antioxidant activity, have the ability to

increase plant immunity and resistance to pathogens and unfavorable environmental factors (Orhana et al., 2017; Elisovetcaia et al., 2019; Zazharskyi et al., 2020; Pan et al., 2024). Early it was showed that extract from *J. sabina* exhibit biological activity on beech seed by increasing the mean daily germination (1.33) in comparison with control by 1.4 times (Elisovetcaia et al., 2024).

Natural dye from the berries of the American pokeweed (*Phytolacca americana* L.) contains red betacyanins, which are betalain compounds and also have biological activity, such as antimicrobial, antioxidant, and immunomodulatory effects (Mchedlishvili et al., 2014; Rahimi et al., 2019; Sadowska-Bartosz & Bartosz, 2021; Martínez-Rodríguez et al., 2022). Betacyanins were extracted and analyzed from different plant, namely *Opuntia* spp. (Castellar et al., 2003; Sanchez-Gonzalez et al., 2013); cactus pear (Ruiz-Gutierrez et al., 2015), red pitaya (Low Pinn Yee et al., 2017), *Amaranthus* spp. (Chong et al., 2014; Das et al., 2019), red dragon fruits (Woo et al., 2011), red beetroot (Lazar et al., 2021). The results of research concerning to application of betacyanins as dye in food and textile industries wide reported (Baaka et al., 2019; Singh et al., 2020; Khan, 2022; Rocha et al., 2022; Roriz et al., 2023). However, no information was found on the use of betacyanins in seed coatings and their biological effect on seed germination and plant growth.

The objective of this study was to evaluate the effect of coating composition based on biodegradable carboxymethylcellulose and containing a natural plant growth regulator from *J. sabina* and natural dye betacyanins on the early growth characteristics of coated corn seeds.

MATERIALS AND METHODS

The studies were carried out in 2024 year in the Institute of Genetics, Physiology and Plant Protection (IGPPP), Moldova State University (MSU), Republic of Moldova.

Corn seeds. The National Center of Research and Seed Production, Institute of Crop Science „Porumbeni” generously contributed the seeds of food corn hybrids, which have different ripening periods and differ according to the structure of the endosperm:

Porumbeni 280su (P280) is mid-season sweet corn hybrid, FAO 280. In the technical ripening phase, the grains contain 14.0% total sugar and 31.5% starch. Light yellow grain with fine pericarp. Porumbeni 300 sh-2 (P300) is a mid-season synthetic population, super-sweet corn, FAO 300. At the technical maturity stage, the grains contain 14.0-16.0% total sugar and 31.4% starch, dextrin does not contain. A distinctive feature is the large, angular, wrinkled pericarp grain, consisting of floury endosperm.

Porumbeni 398 (P398) is a mid-early maturing corn hybrid that belongs to the popcorn group, FAO 400. The grains have a glassy consistency and are characterized by a high specific gravity of the endosperm. The floury part of the endosperm is present only near the embryo. The grains have a high protein content (16%).

Porumbeni 402 (P402) is mid-late-ripening corn hybrid with toothed-glassy, orange grain, semi-flint corn, FAO 400. The grains contain 70–75% starch, up to 15% protein and 3–6% fat.

Coating composition. The water solution of sodium salt of carboxymethylcellulose (CMC) in concentration 1.0-1.2% was used as a film former support. Natural plant growth regulator as concentrated extract from aboveground parts of *Juniperus sabina* (bioregulator JS), was obtained in laboratory Natural Bioregulators, IGPPP. Extract contents the total polyphenolic substances 38.89 ± 1.67 mgGAE*/g; flavonoids 8.55 ± 0.09 mgQE**/g; and phenolic acids 13.25 ± 0.25 mgCAE***g. (Note: *GAE - gallic acid equivalent; **QE - quercetin equivalent; ***CAE- caffeic acid equivalent). Antioxidant activity of extract is equal $IC_{50} = 59.19 \pm 9.77$ µg/ml was evaluated by potentiometric procedure (Ivanova, 2016; Elisovetcaia et al., 2018). Bioregulator JS diluted to a concentration of 0.001% was used to coat the corn seeds.

Natural dye of red color containing betacyanins was extracted from berries of *Phytolacca americana*. Procedure for obtaining of this natural dye in powder form was patented (Patent MD-1817). Antioxidant activity of obtained natural dye evaluated by potentiometric procedure and expressed in gallic acid equivalent is 129.72 ± 2.59 µMGAE/g. In coating composition, the natural dye was used in concentration of 0.3%.

Germination testing. Germination of control seeds and coated seeds was carried out in accordance with the provisions of international

rules (ISTA, 2017). After germination, the overall seed germination, root and seedling length, root and seedling biomass, root and seedling vigor were assessed (Kerecki et al., 2021). The biomass of reserve substances that was spent for energetic support of physiological processes and respiration during seed germination as well as metabolic efficiency were calculated (Sikder et al., 2009; Dascalciuc et al., 2020; Borovskaia et al., 2023).

Statistical analysis. The obtained experimental data were processed by the statistical methods using the software package Statgraphics Plus 5.0. The ANOVA test was applied for variance analysis of characters, Student test in assessment of statistically significant differences between plots (Raudonius, 2017).

RESULTS AND DISCUSSIONS

The overall germination of corn seeds of the studied hybrids before coating and storage was 92-97%, which is within the error limits of the method. However, the lengths of roots and seedlings differed significantly (Figure 1). Hybrids were divided into two homogeneous groups by root length: 1) P280 and P398 with an average root length of 5.78-5.95 cm; and 2) P300 and P402 (4.24-4.48 cm). The root lengths of these groups of hybrids had statistically significant differences at $p \leq 0.001$. The length of the embryonic roots in corn affects the development and growth of the aboveground organs of the plant, as well as its overall productivity. During germination of the corn seeds, the embryonic root quickly goes deep into the soil by 30-40 cm and, together with the lateral hypocotyl roots, forms a system of primary roots that provide the plant with nutrients and water for about 2-3 weeks.

The length of the seedlings is also important, since due to the elongation of the epicotyl, corn seeds can germinate from a great depth, bringing the first stem node to the soil surface. The distribution of hybrids by seedling length was as follows: one group P280, P300, P398 and the other P402. The differences in seedling length between these two groups were at $p \leq 0.01$. Nevertheless, the P402 hybrid had statistically significantly lower seedling lengths ($p \leq 0.001$) than the P280 and P398 hybrids. Thus, the studied hybrids had the various early growth characteristics.

The early growth characteristics of coated seeds of studied corn hybrids before storage were not affected by the coating procedure.

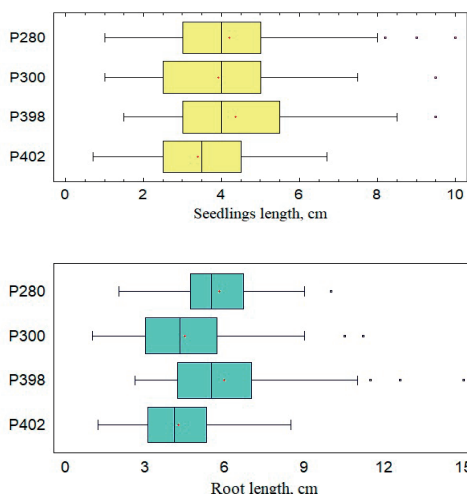


Figure 1. Roots and seedlings length of control corn seeds

The mean values of root/seedling length of coated seeds during germination changed, but not statistically significantly, for hybrids P280, P300 and P398 (Figures 2-4).

However, for hybrid P402 the root length of coated seeds before storage increased significantly ($p \leq 0.001$) to 5.92 ± 2.28 cm from 4.24 ± 1.54 cm in control seeds (Figure 5). The seedlings length of coated seeds also changed significantly ($p \leq 0.001$), but their growth was suppressed by an average of 0.88 cm after coating procedure.

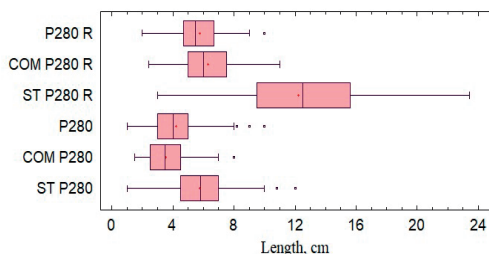


Figure 2. Roots and seedlings length of coated corn seeds of P280 hybrid: P280 R - root control; COM P280 R - root of coated seeds before storage; ST P280 R - root of coated seeds after 4 months of storage coated seeds; P280 - seedlings control; COM P280 - seedlings of coated seeds before storage; ST P280 - seedlings of coated seeds after 4 months of storage

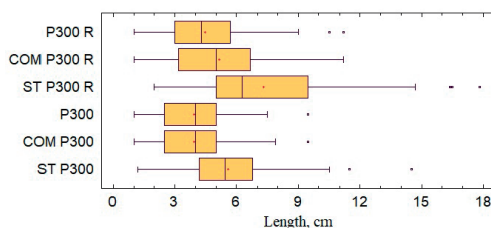


Figure 3. Roots and seedlings length of coated corn seeds of P300 hybrid: P300 R - root control; COM P300 R - root of coated seeds before storage; ST P300 R - root of coated seeds after 4 months of storage coated seeds; P300 - seedlings control; COM P300 - seedlings of coated seeds before storage; ST P300 - seedlings of coated seeds after 4 months of storage

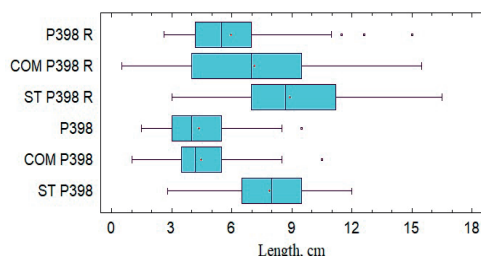


Figure 4. Roots and seedlings length of coated corn seeds of P398 hybrid: P398 R - root control; COM P398 R - root of coated seeds before storage; ST P398 R - root of coated seeds after 4 months of storage coated seeds; P398 - seedlings control; COM P398 - seedlings of coated seeds before storage; ST P398 - seedlings of coated seeds after 4 months of storage

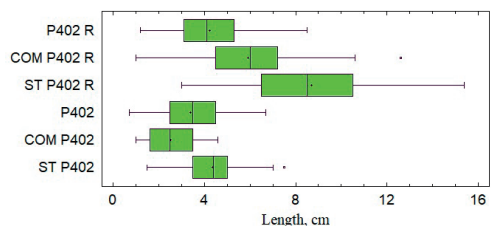


Figure 5. Roots and seedlings length of coated corn seeds of P402 hybrid: P402 R - root control; COM P402 R - root of coated seeds before storage; ST P402 R - root of coated seeds after 4 months of storage coated seeds; P402 - seedlings control; COM P402 - seedlings of coated seeds before storage; ST P402 - seedlings of coated seeds after 4 months of storage

After 4 months of storage, the coated seeds were germinated under optimal conditions. During storage, the overall germination rate of seeds did not change significantly, remaining at the level of 94-98%. The effect of the bioregulator from *J. sabina* and natural dye with antioxidant

activity during storage period was positive and significant ($p \leq 0.001$) on the growth of roots and seedlings for all the studied hybrids (Figures 2-5). Storage of coated seeds contributed to an increase in the root length an average of 1.2-1.9 times, and seedlings - 1.4-1.8 times compared to these indexes before storage. On average, the root length increased by 1.79 cm for seeds of the P398 hybrid, by 2.14 cm for P300, by 2.77 cm for P402, and by 5.94 cm for the P280 hybrid. The length of seedlings elongated by 1.64 cm for P300, by 1.85 cm for P402, by 2.22 cm for P280, and by 3.42 cm for P398. Considering the fact that the root/seedling length may indicate corn tolerance to abiotic factors, the investigated coating composition can contribute to seed resistance to adverse environmental conditions. The procedure of coating corn seeds and storing them for four months had a positive effect not only on the length of roots and seedlings, but also on their vigour (Figures 6-9). It should be noted that before storage, the coated seeds changed the root/seedling vigour compared to control seeds according to the above-described pattern relative to the length of the roots/seedlings. Namely, the corn of the P280, P300, and P398 hybrids showed root/seedling vigour that was not significantly different from control seeds (Figures 6-8). However, the root vigour of coated seeds of the P402 hybrid before storage fortified significantly ($p \leq 0.001$), while the seedling vigour decreased significantly ($p \leq 0.001$) compared with control seeds (Figure 9).

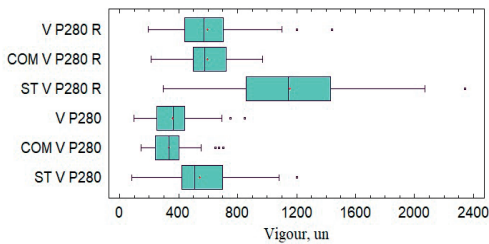


Figure 6. Vigour of roots and seedlings of P280 hybrid: V P280 R - root control; COM V P280 R - root of coated seeds before storage; ST V P280 R - root of coated seeds after 4 months of storage; V P280 - seedlings control; COM V P280 - seedlings of coated seeds before storage; ST V P280 - seedlings of coated seeds after 4 months of storage

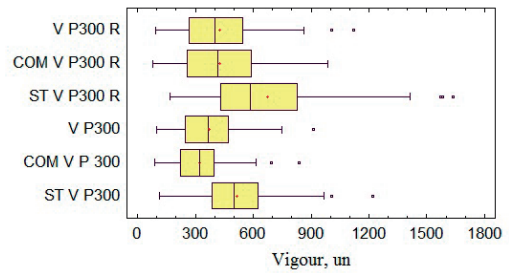


Figure 7. Vigour of roots and seedlings of P300 hybrid: V P300 R - root control; COM V P300 R - root of coated seeds before storage; ST V P300 R - root of coated seeds after 4 months of storage; V P300 - seedlings control; COM V P300 - seedlings of coated seeds before storage; ST V P300 - seedlings of coated seeds after 4 months of storage

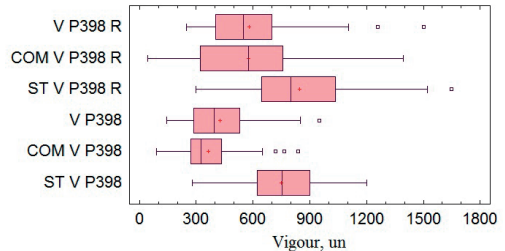


Figure 8. Vigour of roots and seedlings of P398 hybrid: V P398 R - root control; COM V P398 R - root of coated seeds before storage; ST V P398 R - root of coated seeds after 4 months of storage; V P398 - seedlings control; COM V P398 - seedlings of coated seeds before storage; ST V P398 - seedlings of coated seeds after 4 months of storage

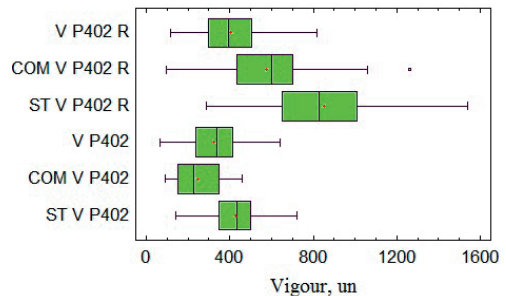


Figure 9. Vigour of roots and seedlings of P402 hybrid: V P402 R - root control; COM V P402 R - root of coated seeds before storage; ST V P402 R - root of coated seeds after 4 months of storage; V P402 - seedlings control; COM V P402 - seedlings of coated seeds before storage; ST V P402 - seedlings of coated seeds after 4 months of storage

The seedling vigour of coated seeds after storage was higher than vigour of coated seed before storage by 183.34 (P402); 208.67 (P280); 353.50 (P300) and 387.11 (P398). Compared with control seeds, the seedlings vigour of coated and stored seeds increased 1.33 (P402) - 1.71 (P398) times.

Application of coating procedure on corn seeds and storing them during 4 months led to a significant increasing of early growth characteristic such as root/seedling length and vigour. In addition, the biomass of roots and seedlings of coated and stored corn seeds was more 1.5-2.0 times than root/seedling biomass of control seeds. However, the proportion of reserve substances used for the growth of roots and seedlings from the total amount of reserve substances spent on germination was different (Figures 10-11).

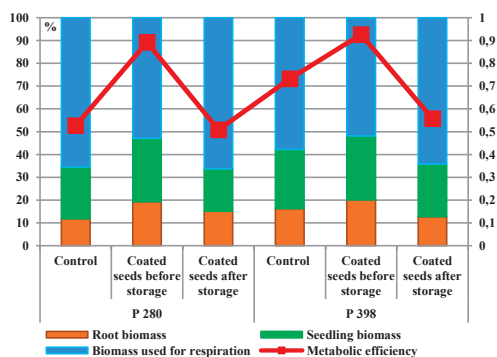


Figure 10. Proportion of reserve substances eliminated for growth of root and seedlings, and spent for respiration, metabolic efficiency of hybrids P280 and P398

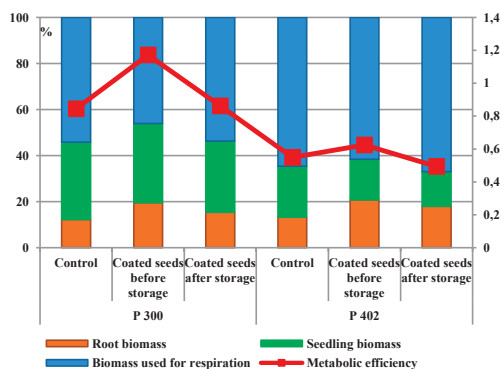


Figure 11. Proportion of reserve substances eliminated for growth of root and seedlings, and spent for respiration, metabolic efficiency of hybrids P300 and P402

This is explained by the fact that the proportion of reserve substances spent on energy support of the physiological processes of germination and respiration varied also, which naturally affected the values of metabolic efficiency in the studied variants (Figures 10-11). Early it was showed the high negative correlation between the biomass of reserve substances eliminated for respiration and the metabolic efficiency of the P427 and P458 hybrids, Pearson correlation coefficients were equal to -0.9557 and -0.9457, respectively (Borovskaia et al, 2023).

The studied hybrids showed a characteristic feature of changes in the metabolic efficiency of germination of coated seeds (Figures 10-11): before storage, it increased compared to the control, and after 4 months of storage, it practically returned to the values of the control seeds.

It can be assumed that coated corn seeds during storage occur balancing of the initial early growth characteristics concerning distribution of eliminated reserve substances, but at the same time the coating composition contributes to increase the root/seedling length, biomass and vigour.

Similar results were obtained on coated seeds of hybrids P427 and P458, which were coated with a composition containing biologically active substances of natural origin, genistifoliosides, and a biodegradable polymer, sodium salt of carboxymethylcellulose. However, long-term storage of coated corn seeds from four to 12 months did not lead to a further increase in root/seedling vigour (Borovskaia et al, 2023).

Thus, the coating composition based on carboxymethylcellulose containing an extract of phenolic compounds from *J. sabina* and a natural dye obtained from *P. americana* berries with antioxidant activity contributed to improve the early growth characteristics of coated corn seeds of different hybrids after four months of storage. Storage during four months is the most practicable period for pre-sowing treatment of corn seeds by coating procedure.

CONCLUSIONS

The obtained results of experimental studies provide grounds to assert that pre-sowing coating of corn seeds with a biodegradable composition containing phenolic compounds

from *J. sabina* and a natural dye obtained from *P. americana* berries contributes to preserve the sowing qualities of seeds and improve the growth characteristics of corn seeds over a storage period. Due to the original dye, this composition allows for easy identification of coated seeds. Studied coating composition showed promising results in terms of physical aspects of corn seeds and their physiological potential.

ACKNOWLEDGEMENTS

Research was carried out within the Subprogramme 011101 “Genetic and biotechnological approaches to agroecosystem management under climate change conditions” for 2024-2027, financed by the Ministry of Education and Research of the Republic of Moldova.

The research on obtaining a natural dye from the berries of the American pokeweed and extract from *Juniperus sabina* were carried out thanks to the support of the Ministry of Education, Science, Research and Sport of the Slovak Republic for the mobility of PhD Raisa Ivanova and PhD Dina Elisovetcaia within the framework of the bilateral scholarship programs for 2023-2024 and 2024-2025.

The authors gratefully acknowledge Nicolai Vanicovici, PhD, head of laboratory of the National Center of Research and Seed Production, Institute of Crop Science “Porumbeni”, who kindly provided us with the corn seeds of all studied hybrids.

REFERENCES

- Ali, O.N., Whittenton, J.B., Williams, J.J., Watts F. & Henry, W.B. (2018). Sub-optimal temperature effects on hybrid corn seed and seedling performance. *Seed Technology*, 39(1/2). 129–142.
- Baaka, N., Ticha, M.B. & Guesmi, A. (2019). Valorization of anthocyanin pigments extracted from *Phytolacca americana* L. fruits as bio-colorant to dye wool yarns. *Fibers and Polymers*, 29(12). 2522–2528.
- Beegum, S., Walne, C.H., Reddy, K.N., Reddy, V. & Reddy, K.R. (2023). Examining the corn seedling emergence – temperature relationship for recent hybrids: insights from experimental studies. *Plants*, 12. 3699.
- Borovskaia, A., Lutcan, E., Ivanova, R. & Vanicovici, N. (2023). Influence of storage time on quality of encrusted maize seeds. *AGROFOR International Journal*, 8(3). 69–76.
- Borovskaia, A.D., Ivanova, R.A., Mascenco, N.E. & Mistret, S.I. (2022). Application of natural biologically active substances for seed encrustation. *Proceedings of the International Scientific Conference «Agrophysical institute: 90 years in the service of arable farming and crop production»*, April 14–15, 2022, Agrophysical Research Institute, St. Petersburg, Russia, 205–213.
- Castellar, R., Obon, J.M., Alacid, M. & Fernandes-Lopez, J.A. (2003). Color properties and stability of betacyanins from *Opuntia* fruits. *Agricultural and Food Chemistry*, 51(9). 2772–2776.
- Chong, P.H., Yusof, Y.A., Aziz, M.G., Nazli, M.N., Chin, N.L. & Syed, M.S.K. (2014). Evaluation of solvent extraction of *Amaranth* betacyanins using multivariate analysis. *International Food Research Journal*, 21(4). 1569–1573.
- Das, M., Saeid, A., Md. Hossain, F., Juang, G.-H., Eun, J.B. & Ahmed, M. (2019). Influence of extraction parameters and stability of betacyanins extracted from red amaranth during storage. *Journal of Food Science and Technology*, 56(2). 643–653.
- Dascaliuc, A., Jeleu, N., Ralea, T., Parii, Ia. & Parii Iu. (2020). Mobilization of reserve substances of seeds for germination and growth of seedlings in wheat varieties with different frost resistance. *Buletinul Academiei de Stiinta a Moldovei. Stiintele Vietii*, 2(341). 67–72.
- de Camargo, F.R.T., Silva, I.L., Barros, P.J.R., Ascheri, D.P.R., Rodovalho, R.S., Bellizzi, N.C., Ascheri, J.L.R., Teixeira, I.R., Devilla I.A. & de Campos A.J. (2017). Physiological quality of soybean seeds treated with carboxymethylcellulose and fungicide. *American Journal of Plant Sciences*, 8. 2748–2757.
- Dziwulska-Hunek, A., Niemczynowicz, A., Kycia, R.A., Matwijczuk, A., Kornarzyński, K., Stadnik, J. & Szymanek M. (2023). Stimulation of soy seeds using environmentally friendly magnetic and electric fields. *Scientific Reports*, 13(1). 18085.
- Elisovetcaia, D., Ivanova, R. & Brindza, J. (2024). Influence of *Juniperus sabina*, alginite and bioregulators on the germination kinetics of beech seeds of Roznava origin. *AGROSYM-2024. Proceedings of XV International Scientific Agriculture Symposium*, Jahorina, October 10-13, [editor in chief Dusan Kovacevic]. East Sarajevo: Faculty of Agriculture, Bosnia and Herzegovina, 1451–1456.
- Elisovetcaia, D., Ivanova, R., Gladei, D., Simkova, J. & Brindza J. (2019). Biological activity of extracts from some species of Coniferous plants. *Agrobiodiversity for improving nutrition, health and life quality*, Nitra, Slovakia, 3. 66–80.
- Elisovetscaia, D., Ivanova, R., Ivanisova, E., Vergun, E., Simkova, J. & Brindza, J. (2018). Evaluation of antioxidant activity and phenolic compounds of *Juniperus sabina* from the Republic of Moldova and Slovakia. *Program and abstracts of 2nd International Conference on the Scientific Actualities and Innovations in Horticulture SAIH-2018 “Development and technology”*, Kaunas, 04-06 June 2018, 28–29.
- ISTA (2017). International rules for seed testing. Chapter 5: The germination test. 2017 (1).
- Ivanova, E.N. & Makarov, D.V. (2020). Stimulation of secondary metabolite biosynthesis using yeast extract

- as a biotic elicitor. *Journal of Plant Biotechnology*, 28(2). 97–105.
- Ivanova, R. (2016). Antiradical capacity of seed extracts evaluated by potentiometric procedure. *Agrobiodiversity for Improving Nutrition, Health and Life Quality*, Nitra, Slovakia, 140–144.
- Kerecki, S., Jovicic-Petrovic, J., Kljujev, I., Lalevic, B., Karlicic, V., Petrovic, I. & Raicevic V. (2021). Biopriming: a sustainable support for crop establishment. *AGROSYM-2021. Proceedings of XII International Scientific Agriculture Symposium*, Jahorina, October 10-13, [editor in chief Dusan Kovacevic]. East Sarajevo: Faculty of Agriculture, Bosnia and Herzegovina, 188–194.
- Khacim, H., Kende, Z., Jolánkai, M., Kovács, G.P., Gyuricza, C. & Tarnawa, Á. (2022). Impact of temperature and water on seed germination and seedling growth of maize (*Zea mays* L.). *Agronomy*, 12(2). 397.
- Khan, M.I. (2022). Plant betalains: Recent application in food freshness monitoring films. *Food Packaging and Shelf Life*, 30. 100921.
- Lazar, S., Constantin, O.E., Stanciuc, N., Aprodu, I., Croitoru, C. & Rapeanu, G. (2021). Optimization of betalain pigments extraction using beetroot by-products as a valuable source. *Inventions*, 6(3). 50.
- Low Pinn Yee, Tan Chin Ping, Lim Pek Kui & Chan Sook Wah. (2017). Application of red pitaya powder as a natural food colorant in fruit pastille. *Jurnal Gizi Klinik Indonesia*, 13(3). 111–120.
- Mahisanon, N., Liangsakul, P., Jarussophon, N., Chunhachart, O. & Teingtham K. (2021). Effect of seed coating with biopolymer and coumarin derivative on water uptake and germination of sweet corn seed. *Resent Science and Technology. Rajamangala University of Technology Srivijaya Research Journal*, 15(1). 14–23.
- Martínez-Rodríguez, P., Guerrero-Rubio, M.A., Henarejos-Escudero, P., García-Carmona, F. & Gandía-Herrero, F. (2022). Health-promoting potential of betalains in vivo and their relevance as functional ingredients: A review. *Trends in Food Science & Technology*, 122. 66–82.
- Mchedlishvili, N.I., Omiadze, N.T., Abutidze, M.O., Rodriguez-Lope, J.N., Sadunishvili, T.A., Gurielidze, M.A. & Kvesitadze, G.I. (2014). Investigation of phenolic content, antioxidant and antimicrobial activities of natural food red colorant from *Phytolacca americana* L. fruits. *Annals of Agricultural Sciences*, 12(3). 71–75.
- Orhana, N., Orhana, D.D., Gökbultut, A., Aslana, M. & Erguna F. (2017). Comparative analysis of chemical profile, antioxidant, in-vitro and in-vivo antidiabetic activities of *Juniperus foetidissima* Willd. and *Juniperus sabina* L. *Iranian Journal of Pharmaceutical Research*, 16 (Special Issue). 64–74.
- Pallaoro, D.S., Avelino, A.C.D., Camili, E.C., Guimaraes, S.C. & Albuquerque, M.C.D.F.E. (2016). Priming corn seeds with plant growth regulator. *Journal of Seed Science*, 38(3). 227–232.
- Pan, L., Zhou, T., Chen, C., Xu, H., & Wang, W. (2024). Phytochemistry, pharmacology, and traditional medicine applications of *Juniperus sabina* L.: A comprehensive overview. *Molecules*, 29(24). 5876.
- Patent MD-1817. (2025). Process for obtaining the red dye betacyanin from the berries of American pokeweed (*Phytolacca americana* L.). Application s2024 0046, 2024.04.30. Authors: Ivanova, R., Casian, I. & Casian, A. *BOPI*, 2025, 1. 41.
- Pirzada, T., de Farias, B.V., Mathew, R., Guenther, R.H., Byrd, M.V., Sit, T.L., Pal, L., Opperman, C.H. & Khan S.A. (2020). Recent advances in biodegradable matrices for active ingredient release in crop protection: Towards attaining sustainability in agriculture. *Current Opinion in Colloid and Interface Science*, 48. 121–136.
- Rahimi, P., Abedimanesh, S., Mesbah-Namin, S.A. & Ostadrahimi, A. (2019). Betalains, the nature-inspired pigments, in health and diseases. *Critical Reviews in Food Science and Nutrition*, 59(18). 2949–2978.
- Rahman, M.S., Hasan, M.S., Nitai, A.S., Nam, S., Karmakar, A.K., Ahsan, M.S., Shiddiky, M.J.A. & Ahmed, M.B. (2021). Recent developments of carboxymethylcellulose. *Polymers*, 13. 1345.
- Raudonius, S. (2017). Application of statistics in plant and crop research: important issues. *Zemdirbyste-Agriculture*, 10(4). 377–382.
- Ren, X.X., Chen, C., Ye, Z.H., Su, X.Y., Xiao, J.J. & Liao M. (2019). Development and application of seed coating agent for the control of major soil-borne diseases infecting wheat. *Agronomy*, 9(8). 413.
- Rocha, F., Marques, C.S., de Sousa, L.S., Minim, V.P.R., Pires, A.C.S., Minim, L.A., Stringheta, P.C., Jones, O.G. & Vidigal M.C.T.R. (2022). Betalains nanodispersions: Effects on betalains stability and on rheological properties of Greek yogurt. *Food Research International*, 159. 111583.
- Roriz, C.L., Carocho, M., Alves, M.J., Rodrigues, P., Morales, P., Ferreira, I.C.F.R., Heleno, S.A. & Barros, L. (2023). Betacyanins obtained from alternative novel sources as natural food colorant additives: Incorporated in savory and sweet food products. *Food Function*, 14. 8775–8784.
- Ruiz-Gutierrez, M.G., Amaya-Guerra, C.A., Quintero-Ramos, A., Perez-Carrillo, E., Ruiz-Anchondo, T.J., Baez-Gonzalez, J.G. & Melendez-Pizarro, C.O. (2015). Effect of extrusion cooking on bioactive compounds in encapsulated red cactus pear powder. *Molecules*, 20(5). 8875–8892.
- Sadowska-Bartos, I. & Bartosz, G. (2021). Biological properties and application of betalains. *Molecules*, 26(9). 2520.
- Sanchez-Gonzalez, N., Jaime-Fonseca, M.R., Martin-Martinez, E.S. & Zepeda, L.G. (2013). Extraction, stability, and separation of betalains from *Opuntia joconostle* cv. using response surface methodology. *Journal of Agricultural and Food Chemistry*, 61(49). 11995–12004.
- Sikder, S., Hasan M.A. & Hossain M.S. (2009). Germination characteristics and mobilization of seed reserves in maize varieties as influenced by temperature regimes. *Journal of Agriculture and Rural Development*, 7(1-2). 51–56.
- Silva, J.A. & Carvalho, M.A. (2023). Mobilization of seed reserves and secondary metabolites during

- germination: A metabolic perspective. *Revista de Biología Tropical*, 71(1). 43–56.
- Singh, K., Kumar, P. & Singh, N.V. (2020). Natural dyes: an emerging ecofriendly solution for textile industries. *Pollution Research*, 39 (Suppl.). 80–86.
- Statistica RM, (2023). Available on: https://statistica.gov.md/ro/activitatea-agricola-in-anul-2023-9515_60969.html
- Suo, H.C., Li, W.I., Wang, K.H., Ashraf, U., Liu, J.H., Hu, J.G., Li, Z.J., Zhang, X.L., Xie, J. & Zheng J.R. (2017). Plant growth regulators in seed coating agent affect seed germination and seedling growth of sweet corn. *Applied Ecology and Environmental Research*, 15(4). 829–839.
- Vinzant, K., Rashid, M. & Khodakovskaya M.V. (2023). Advanced applications of sustainable and biological nano-polymers in agricultural production. *Technical Advances in Plant Science, Frontier in Plant Science*, 13. 1081165.
- Woo, K.K., Nigou, F.H., Ngo, L.S., Soong, W.K. & Tang, P.Y. (2011). Stability of betalain pigment from red dragon fruit (*Hylocereus polyrhizus*). *American Journal of Food Technology*, 6(2). 140–148.
- Zacharias, M.B., Forti, V.A. & da Silva, M.A. (2024). Physiological potential and health quality of corn seeds coated with chitosan. *Scientia Agricola*, 81. e20230121
- Zazharskyi, V.V., Davydenko, P.O., Kulishenko, O.M., Borovik, I.V., Kabar, A.M. & Brygadyrenko, V.V. (2020). Antibacterial and fungicidal effect of ethanol extracts from *Juniperus sabina*, *Chamaecyparis lawsoniana*, *Pseudotsuga menziesii* and *Cephalotaxus harringtonia*. *Regulatory Mechanisms in Biosystems*, 11(1). 105–109.
- Zhang, H., Wang, X., Pan, Y., Wang, Y., Ma, J. & Zhang, X. (2018). Mobilization of starch, proteins, and lipid reserves during seed germination of six grassland species. *Frontiers in Plant Science*, 9. 234.