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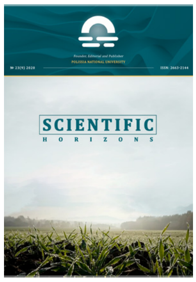

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Formation of adaptive immunity against salmonellosis in cows using effector memory cells

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Abstract. The study aimed to investigate how the number of effector memory cells changes under the influence of a *Salmonella* vaccine antigen in cows. A homogeneous group of 100 Holstein-Friesian cows, kept under the same conditions, had blood samples taken. The blood was collected at four time points: before the first vaccination, and 7, 45, and 56 days after. The cows also received a booster vaccination on days 8-10. They were immunised with a polyvalent vaccine against livestock salmonellosis in

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Kazakhstan. Blood was separated into plasma and cellular fractions by centrifugation at 1,500 rpm for 10 minutes. The cellular fraction was then analysed by flow cytometry to determine the number of CD4+, CD8+, and γδ T-cell subpopulations at the four time points: before vaccination, and at 7-, 45-, and 56-days post-vaccination. Analysis of over 10,000 cells from each sample was conducted using FlowJo software. The data showed that the population of CD4+ and CD8+ T-lymphocytes and γδ T-cells increased 1.5 times by day 7 after the initial vaccination. The cows then received a booster dose on days 8-10, and by day 45 after the first vaccination, the CD4+ and CD8+ T-lymphocyte and γδ T-cell populations had increased threefold. CD45RA+ T-lymphocytes and γδ T-cells demonstrated a steady increase by day 45, followed by a decline in the numbers of T-cells across all phylogenetic groups. Thus, it can be concluded that the primary vaccination stimulates the development of long-term immune memory, while the booster dose triples the number of CD4+, CD8+, and γδ T-cell subpopulations. The findings provide insights into the mechanism of adaptive immunity formation in cows against salmonellosis through the use of effector memory cells and may be applied in developing vaccination strategies for cattle

Keywords: CD4+ and CD8+ T-lymphocytes; CD45RA+ T-lymphocytes; immunological memory; vaccination; cellular immunity; humoral immunity

INTRODUCTION

The relevance of this study lies in the fact that salmonellosis is a highly contagious zoonotic infection, and the formation of immune memory against this disease in cows has not been sufficiently studied. This research is particularly important due to certain characteristics of *Salmonella*, such as its potential for reservoir hosts, cross-species transmission, latent disease progression, and both vertical and horizontal pathogen transmission.

According to C.L. Holschbach and S.F. Peek (2019), over 2500 serovars of *Salmonella enterica* pose a significant threat as zoonotic pathogens, causing diseases in poultry, cattle, and humans. M. Suar *et al.* (2019) highlighted the particular problem of *S. enterica ser. Dublin* in cattle, leading to decreased milk production, spontaneous abortions, and the potential for human infection through contaminated milk. Moreover, infected cows often experience stress and fever. The pathogenicity of this serovar is often underestimated by international veterinary organisations due to the possibility of asymptomatic infections and latent carriers (Liasota *et al.*, 2022). *Salmonella typhimurium* is a zoonotic pathogen capable of spreading between poultry flocks (making it a target serotype for testing and control in the poultry industry), as well as nearby dairy herds and pig farms. These agricultural settings can serve as reservoirs for resistant strains or resistance genes, which can then spread to human populations. Furthermore, as reported by N. McLeod *et al.* (2019), reptiles and amphibians can also act as reservoirs for *Salmonella*. According to *Salmonella* in animals and feed in Great Britain (2022), in 2021, this serovar was detected in 23% of identified human cases and 12.5% of cattle serovars. Different serotypes appear to be associated with specific transmission pathways, depending on their role in natural ecological systems. Rapid colonisation of strains occurs due to asymptomatic persistence in the bovine gut. At the same time, researchers noted that vaccinating cows during the dry period and using their colostrum in calves helps to halt outbreaks of the infection. As B. Menichetti *et al.* (2021) point out, the quality of colostrum is partly dependent on vaccinating cows during pregnancy.

A particular issue is the spread of *Salmonella* and *Campylobacter* into the environment through animal faeces. A meta-analysis of studies published by F.D. Gutema *et al.* (2019), covering the period from 2000 to 2017, resolved long-standing debates by showing that 9% of clinically healthy cattle are carriers of *Salmonella*. According to L. Guillier *et al.* (2021), the pathogen can survive for varying periods depending on environmental factors – up to three months in manure and up to one month in soil. This significantly increases the risk of infection spread during summer and over the long term. Therefore, the development of immunity to salmonellosis in cows occurs continuously, even without the use of vaccine strains. Field virus pressure was present with varying intensity throughout the study by L. Mughini-Gras *et al.* (2021), although small ruminants showed a significant negative association with salmonellosis in cattle.

The emergence of multidrug-resistant strains could become a serious issue in Kazakhstan and Turkey, as both countries have yet to fully discontinue the use of antibiotics. By 2019, Turkey's cattle production reached 600,000 head per year, with losses due to calf mortality amounting to EUR 525 million in 2018. Diarrhoea is the main cause of calf losses in the postnatal period, and its occurrence is closely linked to colostral immunity (Nyzhnyk *et al.*, 2024). Additionally, bacteria causing salmonellosis are periodically detected in meat (Pyatkovskyy, 2023). For example, on 21 August 2024, Latvia's Food and Veterinary Service identified this pathogen in chicken meat imported from Poland.

The necessity of vaccinating cattle against salmonellosis remains a topic of debate. Research on this issue has mainly focused on calves, yet none of the field trials have identified significant discrepancies between vaccinated and control calves in the outcomes assessed.

Additionally, lower levels of colostral immunity have been noted in beef cattle breeds. Key challenges in addressing the vaccination issue are the substantial antigenic diversity of *Salmonella* and the inability of inactivated vaccines to stimulate a cell-mediated immune response, which, alongside the humoral response, plays a crucial role in overall immunity formation. Live vaccines, on the other hand, result in the shedding of the pathogen in faeces, which raises concerns within veterinary services and limits their use. However, attenuated live pathogens offer the advantage of presenting multiple heterogeneous antigens, as they induce a complex immune response that includes mucosal, humoral, and cellular immunity. This study aimed to examine changes in the number of central and effector memory cells, as well as their subpopulations, over time following antigen administration.

LITERATURE REVIEW

The diversity of salmonellosis vaccine strains and the lack of cost-effectiveness in vaccinating calves suggest that the only way to achieve epizootic security in a herd is through the development of long-term immunity in cows via vaccination. According to H.O. Zinko (2017), two factors are essential for the formation of a lasting immune response: a sufficient amount of antigen and stimulation of the innate immune system. This process has been described by O.B. Kyrychko *et al.* (2021), and I. Azaldegui *et al.* (2024). Cellular immunity is maintained by macrophages and neutrophils, while dendritic cells detect pathogen components through simple mechanisms. Macrophages and neutrophils, when affected by aggressive pathogens, respond by producing substances that combat the pathogen. C.C. Chase *et al.* (2019) highlight the potential of *Salmonella* to enhance the immune response to heterologous antigenic proteins during the body's direct response to the pathogen. This simplifies the development and use of vaccines against salmonellosis without the need for adjuvants. K.J. Cummings *et al.* (2018), R.R. Harvey *et al.* (2017), and E.L. Cuttance *et al.* (2019) note that cattle's adaptability to *S. enterica ser. Dublin* presents a challenge for understanding preventative measures against this pathogen. Based on this, it can be concluded that studying T-lymphocytes is essential for understanding the formation of immunological memory.

For instance, research conducted by A. Facciuolo *et al.* (2020) and A. Kandel *et al.* (2022) on CD4+ and CD8+ T lymphocytes has demonstrated that these cells perform various functions, including coordinating immune cells, eliminating infected cells, activating other immune cells, producing antibodies, and localising the spread of infection. Furthermore, $\gamma\delta$ T-cells play a significant role in the initial immune response to a wide range of antigens. CD45RA+ effector cells and CD45RA- memory cells are crucial for both immunological memory and the development of primary immunity. Data on cellular and humoral immunity improve following vaccination, regardless of whether it is administered before or after calving. It is important to note that simple longitudinal studies and assessments of immunity levels are often questioned. The cow's body changes immune system parameters due to stress not caused by vaccination. For example, R. Pamplona and D. Costantini (2011) reported a normal level of leukocytes, but a significant reduction in lymphocyte concentration (75% of the normal level).

A more detailed analysis by N. Husseini *et al.* (2022) of lymphocyte populations revealed that while T-lymphocyte levels were within the normal range, B-lymphocyte levels were 27% below normal, and O-cells were at the upper limit of the normal range. This creates a foundation for the risk of disease development. Furthermore, the study by A.Y. Kraevsky *et al.* (2020) indicated that Tlymphocytes with immunological memory play a dominant role in maintaining the health of cows under stress conditions, as their levels remain unchanged, protecting against long-term threats. Additionally, adaptive mechanisms involving T-lymphocytes can be activated even under relatively comfortable conditions. Immunodeficiency can arise due to alterations in the numbers of phagocytic cells, polymorphonuclear monocytes, and lymphocytes. Prolonged intoxication, even without apparent clinical signs, can disrupt these adaptive mechanisms, leading to decreased numbers of T- and B-lymphocytes and an increase in immature lymphocytes.

Immunosuppression can also result from natural processes, as documented in the study of A. Heiser *et al.* (2015) regarding cows that have recently calved. A reduction in cellular immune activity is observed during the dry period, progressing in the first two weeks post-calving. Consequently, the decrease in both cellular and humoral immune activity in cows during this critical period for producing healthy offspring (natal and prenatal stages) underscores the necessity for vaccination against salmonellosis to alter this situation and stimulate the immune activity of the cows' bodies. The assessment of antibody levels is typically conducted through two methods: neutralisation assays or ELISA. These approaches provide insight into short-term immunity levels, whereas T-cell responses induced by salmonellosis vaccines in cows offer long-term potential for analysing the herd's protection against this pathogen. M. Meyer-Hermann (2019), J.D. Neill *et al.* (2019) have highlighted a paradox for practising veterinary surgeons: serological analyses indicate that animals with low levels or no neutralising antibodies can still be protected upon infection. In other words, clinically healthy animals may present with low antibody levels, while data also exists showing high antibody levels in sick animals. To gain a deeper understanding of the long-term effects of vaccination, J. Pinheiro and D. Bates (2024), E.O. Roos *et al.* (2023), and S. Saravanan *et al.* (2020) highlight the importance of studying dynamic changes in T-cell subpopulations within the peripheral blood of cattle. Notably, an increase in the percentage of CD4+CD8+ T-cells in the peripheral blood of cows post-vaccination has been observed, serving as an indicator of vaccine efficacy. According to F.N. Souza *et al.* (2023), the expression of memory cell surface markers CD45RO and CD27 is directly linked to the efficient differentiation of T-cell memory subsets in cows. Each marker serves a distinct diagnostic purpose: CD27 identifies stages of T-cell differentiation, CD45RO identifies memory T-cells, and the presence of central memory T-cells within CD4 and CD8 T-cells serves to identify the vaccine response.

Thus, prolonged T-cell responses are essential for the clearance of pathogens and the development of robust memory reactions. In addition to the aforementioned points, it is crucial to acknowledge that a proper assessment of vaccine immunogenicity relies on studying the dynamic changes in T-cell subpopulations in the peripheral blood of cattle, while also ensuring a sufficient level of antibodies is present. However, in practice, veterinarians often depend on their experience, recognising that antibody-mediated immune responses and cell-mediated immune responses are interconnected. Therefore, analyses should be comprehensive, incorporating both serological studies and investigations into the dynamic changes in T-cell subpopulations. Furthermore, it is important to note that salmonellosis (unless it occurs as a polyinfection) does not, in itself, cause lymphopenia or other mechanisms by which the pathogen evades the host's immune response, thereby leading to immune suppression. According to the findings from the review, vaccination serves as a catalyst for the development of immune protection in cattle herds and the formation of memory T-cells.

MATERIALS AND METHODS

The research was conducted based on the peasant farm "Kyran" located in the Turkestan Region, Kazygurt District of the Republic of Kazakhstan. The study period spanned 56 days. For this research, 100 clinically healthy cows from this farm were selected. The cows are housed in a single facility, constituting a homogeneous herd of Holstein-Friesian cattle. Typically, the number of lactating cows fluctuates throughout the year, as determined by the farm's director about educational and scientific inquiries; however, consistent housing and feeding conditions are always maintained in one location for the convenience of research. The herd is kept in stalls and is housed in a loose system. The cows are milked twice daily. All animals were vaccinated with a polyvalent vaccine against livestock salmonellosis (Kazakhstan). The vaccine was administered subcutaneously in the middle third of the animal's neck. The vaccination was administered twice: the first dose was given to pregnant cows 50 to 60 days before calving, followed by a booster dose 8 to 10 days after the initial injection, at a volume of 10 to 15 cm^3. Flow cytometry methods were employed for blood analysis. Blood samples were collected four times for analysis: before vaccination and at 7, 45, and 56 days after the first vaccination. Samples were obtained from the jugular vein using sterile vacutainers. Throughout the study, a standard clinical examination of the cows was conducted by the staff under the supervision of a veterinary physician. No post-vaccination reactions were observed. At the laboratory of the Department of Microbiology and Virology at Kazakh National Agrarian Research University, the collected blood was separated into plasma and cellular fractions by centrifugation at 1,500 rpm for 10 minutes. The cellular fraction served as the primary subject of the study for the analysis of T-lymphocyte phenotypes.

Flow cytometry was employed to determine the quantity of CD4+, CD8+, and $\gamma\delta$ T-cell subpopulations at four-time points: before vaccination, and at 7, 45, and 56 days after the first vaccination. Most $\gamma\delta$ T-cells negatively interact with the expression of CD4 and CD8; however, some are capable of expressing the co-receptor CD8, making the dynamics of their changes following vaccination informative. After incubation, the obtained cells underwent the following stages: washing, resuspension in PBS (phosphate-buffered saline), and analysis using a flow cytometer (BD FACSCalibur, USA). More than 10,000 cells from each sample were analysed on the flow cytometer, with the data processed using FlowJo software.

Descriptive and inferential statistics, including t-tests, ANOVA, and significance testing, were employed to analyse the digital data obtained from the study. Results are presented as mean values with standard deviations, and differences were considered significant at $p < 0.05$. All experimental procedures were conducted following modern methodological approaches and adhered to relevant standards, specifically ISO/IEC 17025:2005 (2006). Animal care and experimental manipulations were performed in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986).

RESULTS AND DISCUSSION

The protection of cows against infectious diseases is achieved through the formation of immunological memory. This mechanism operates on the principle that when an animal encounters a pathogen a second time, there is a rapid and effective response from the immune system. This is particularly crucial for highly contagious zoonotic diseases such as salmonellosis. Additionally, it is important to note that the cow serves as a source of colostral immunity for calves.

The adaptive immune response is directly dependent on T-lymphocytes. A key indicator of these processes is the presence of effector cells, which can be classified based on the expression of the CD45RA marker into effector cells from the first encounter with an antigen (CD45RA+) and those from a subsequent encounter (CD45RA-). Therefore, it is possible to assess the activity of immunological memory; although the immune response in cows has been extensively studied, the role of T-lymphocyte phenotypes remains inadequately explored. The formation of the immune response is based on the adaptation of the organism and long-term protection. This protection relies on the fact that, upon receiving an antigen during vaccination, the organism already possesses a ready-made protective response in the event of reinfection, thus reducing the likelihood of infection or the severity of the disease. Vaccination is the simplest method for studying the mechanisms of immunological memory formation. However, its use also presents numerous potential risks for the practising veterinary physician, including the selection of the appropriate timing for vaccination and the correct choice of vaccine. To investigate this mechanism, the subpopulations of CD4+ and CD8+ T-lymphocytes were studied at three-time points (Table 1).

***Table 1**. Phylogenetic data of CD4+ and CD8+ T-lymphocyte populations in the peasant farm "Kyran" of Turkestan Region, Kazygurt District of the Republic of Kazakhstan*

Time after the first vaccination (days)	CD4+ cells	CD8+ cells
0	340	270
7	650	570
45	1,200	1,200
56	1,400	1,100

***Source:** created by the authors*

The organism's response to the first vaccination can be observed on the 7th day of the study. The booster vaccination was administered on days 8 to 10, and the formation of immunity following the booster dose can be seen on the 45th day of the study. As illustrated in Table 1, the populations of CD4+ and CD8+ T-lymphocytes increased by nearly twofold on the 7th day post-vaccination. However, a decrease in these values is noted on the 56th day, which reflects a natural process: the pathogen ceased to affect the immune system, and the immune response stabilised while maintaining the protective mechanism against subsequent exposures. A significant increase in $\gamma\delta$ T-cells was recorded during the post-vaccination period (Table 2).

***Table 2**. Changes in $\gamma\delta$ T-cell count in response to vaccination in the peasant farm "Kyran" of Turkestan Region, Kazygurt District of the Republic of Kazakhstan*

Time after the first vaccination (days)	Number of $\gamma\delta$ T-cells
0	100
7	250
45	550
56	400

***Source:** created by the authors*

Early immune activation and an increase in $\gamma\delta$ T-cells strongly suggest that this response was triggered by the vaccination. Additionally, $\gamma\delta$ T-cells express the CD8 co-receptor, despite being phylogenetically a complex of histocompatibility-unrestricted T-lymphocytes. Changes in the number of CD45RA+ effector cells serve as an indicator of the primary immune response (Table 3).

***Table 3**. Changes in CD45RA+ T-lymphocytes count in response to vaccination in the peasant farm "Kyran" of Turkestan Region, Kazygurt District of the Republic of Kazakhstan*

Time after the first vaccination (days)	Number of CD45RA+ cells
0	500
7	850
45	1,800
56	1,700

***Source:** created by the authors*

Seven days post-vaccination, CD45RA+ cells began to accumulate, demonstrating a steady increase by day 45. Notably, the booster vaccination led to a more than threefold increase in these cells compared to pre-vaccination levels, and a 1.5-fold increase compared to the post-primary vaccination level. This indicates a rapid and sustained antigen-specific response, whereby each vaccination stimulates the production of effector cells. This serves as direct evidence of the body's long-term protective response. Interestingly, the analysis of CD45RA+ T-lymphocytes on day 56 post-primary vaccination revealed a decline. Effector CD45RA+ cells reached their peak at day 45, after which they either transitioned to a resting state or differentiated into memory cells. Data from Tables 1 and 2 show a decrease in both CD4+ and CD8+ T-lymphocytes, as well as CD45RA+ T-lymphocytes, by day 56. These data confirm that cows maintain protective mechanisms against *Salmonella* pathogens, although the number of effector memory cells decreases over time. Thus, effector memory CD4+ and CD8+ cells accumulate only modestly after the primary immunisation, but a threefold increase in these cells is observed after the booster vaccination compared to the pre-vaccination level. This indicates that the primary immune response to the antigen occurs between the first and second vaccinations, specifically between days 7 and 45. Rapid differentiation of specific memory cells occurs during the secondary immune response to the antigen. The first vaccination primes the cows' immune system to respond to the antigen, while the second vaccination demonstrates that the body, through immunological memory, has developed a rapid response to the presence of the antigen in the blood, increasing the number of effector cells threefold.

The findings regarding $\gamma\delta$ T-cell populations are unequivocal: their numbers increased with each vaccination but decreased by day 56. These cells are integral to the primary cellular immune response, activating macrophages and dendritic cells. Their significant change confirms the development of immunological memory following the vaccine response. These cells exert a particularly strong influence on the CD8 phylogenetic group, potentially leading to an enhanced immune response as a component of antigen-presenting activity. This is manifested by a rapid and immediate cellular immune response, stimulating the production of active substances involved in combating pathogens (chemokines and other substances), and stimulating the production of a variety of cells directly involved in the formation of immunity (monocytes, neutrophils). From this, it can be concluded that the correlated increase in $\gamma\delta$ T-cells with other T-cell phylogenetic groups indicates a direct primary response to the introduction of the vaccine pathogen and the formation of immunological memory. The study of changes in $\gamma\delta$ T-cell numbers has proven to be fundamentally important, as these changes are directly linked to other phylogenetic groups.

Understanding how the mechanism of immunological memory functions after the administration of vaccine antigens against *Salmonella* allows for the creation and modification of preventive veterinary treatment schemes for cows within a farm setting. By recognising the roles of various T-cell populations in the immune response, it becomes possible to develop more effective vaccines. When designing vaccination protocols, it is essential to consider the processes involved in the formation of long-term immunological memory following both primary and booster vaccinations. The initial vaccination stimulates a rapid accumulation of effector cells, while the subsequent vaccination enhances the number of memory cells (Kondibaeva *et al.*, 2021). Based on these observations, it can be concluded that the use of combination vaccines will yield greater efficacy. The mechanism of immunological memory formation, characterised by a modest increase in CD4+ and CD8+ T-lymphocytes following the first vaccination and a significant increase after the booster vaccination, is corroborated by the research of K. Anmol *et al.* (2022).

The increase in CD4+ and CD8+ T-lymphocytes with each vaccination is linked to the fact that, when memory cells already exist, the cow's organism responds more rapidly to a known antigen, as confirmed by the study of M. Suar *et al.* (2019). The results obtained in this study align with their findings. These results demonstrate that the introduction of *Salmonella* antigen into a cow's body stimulates the activation and proliferation of CD4+ T helper cells. These cells play a crucial role in coordinating the immune response. Similar processes, namely activation and proliferation, occur with CD8+ cytotoxic T-lymphocytes, which are responsible for destroying infected cells.

Effector CD45RA+ cells serve as indicators of the primary immune response, as confirmed by the research of N. McLeod *et al.* (2019). The mechanism of progressive growth in the number of these cells begins after the first vaccination, followed by a stable increase, which is significantly stimulated by the booster vaccination. At 45 days, the number of effectors CD45RA+ cells reach its peak, and then the results of the study conducted on day 56 after the primary vaccination showed a slight decrease in the number of these cells. Memory effector cells with CD4+ and CD8+ markers demonstrated an increase in their numbers even after some time post-vaccination. This suggests that the immune system produces specific memory cells that can rapidly proliferate during a secondary immune response to the antigen. Effector CD45RA+ cells and CD45RA memory cells, as this study has shown, provide the initial impetus for the development of sustained immunological memory, and when a booster antigen enters the cow's body, an immediate response of the cow's immune system occurs.

The obtained data can be used practically in planning the timing of both primary and booster vaccine doses, as well as in the use of complex vaccines. Additionally, such data can allow for studies on the practical application of the vaccine itself, including different dosages, experiments with intervals between the first and second vaccinations, experiments with the development of *Salmonella* vaccines with various adjuvants, and experiments with different methods of administering the *Salmonella* antigen. A separate parameter for research could be the study of the influence of differences in genetic background and feeding and housing conditions. Genetic factors influencing the effectiveness of immune memory could be useful for developing a personalised approach to immunisation on large livestock farms (Mussayeva *et al.*, 2023).

The use of a booster vaccine reinforced the effect of stimulating immunological memory obtained as a result of the primary immunisation. This provides a prolonged immune effect. Thus, it is only possible to protect the herd after a double application of the *Salmonella* vaccine according to the instructions for the vaccine used. However, further research into the duration of immunological memory remains promising. This study examined the mechanisms of immunological memory formation, but how long the organism remains protected from *Salmonella* pathogens after two vaccinations remains a subject for further study. This study should also address questions about the duration of immunological memory and the factors that can influence this duration, namely data on the persistence of memory cells and their ability to reactivate upon repeated exposure to antigens. Memory T-cells with CD4/5RO markers and homing molecules are associated with a specific recirculation pathway. There is no data on how long the immune memory in cows remains active after the first vaccination. However, if *Salmonella* is not a chronic pathogen in cows and there is no constant factor stimulating the activity of memory T-cells, the duration of immune memory depends directly on the effectiveness of the vaccination (Mussayeva *et al.*, 2021). At the same time, vaccination prevents the appearance of clinically apparent forms of salmonellosis, but there is no data on how the mechanisms of immunological memory formation in cows work if the animals are *Salmonella* carriers or carriers of a subclinical form of the disease.

When studying the pathogenicity of *Salmonella*, F.D. Gutema *et al.* (2019) revealed that the pathogen is a reservoir in various species of farm animals and poultry. Therefore, an important area of future research is the specificity of vaccines and the mechanisms of the immune response in different species of farm animals and poultry. This could potentially lead to the development of universal interspecies vaccines, which has significant practical implications when two species of animals are kept on the same farm, or when two farms with different species are located in close proximity. The question of the influence of feeding regimes and quality, housing conditions, climatic conditions, and stress levels on the development of adaptive immunity against *Salmonella* in cows using memory effector cells is also a promising area of study. Feeding and housing conditions, as well as stress prevention during the cow's response to vaccination, are factors that are often underestimated in practice. It is known that 2/3 of the body's lymphoid tissue and 80% of antibodyproducing cells are located in the cow's intestine, therefore, feeding conditions undoubtedly influence the quality of vaccination.

Stress is an immunosuppressive factor. B.I. Cappellozza and R.F. Cook (2022) considered stress as a reaction that has a significant impact on the immune system and leads to immunosuppression. How this factor affects the formation of adaptive immunity in cows during vaccination against salmonellosis is a question that has not been sufficiently studied. Cortisol, which is produced during stress, affects the immune system in several ways, including reducing the proliferation and differentiation of immune cells, effector cell function, and increasing cytokine expression. K.M. Schubach *et al.* (2020) believe that dietary supplements that improve metabolism or calm animals during various technological and veterinary procedures can reduce the level of cortisol produced and reduce stress during these actions and their impact on the quality of vaccination. As a rule, after experiencing stress, the concentration of cortisol in the blood serum decreases after 72 hours, which means that vaccination can be planned so that stress factors (such as weighing animals or moving them to a different room, the first grazing, etc.) and the planned vaccination occur with an interval of three days. However, unfortunately, how immunity will develop in cows if it is impossible to apply both of these stress prevention methods remains unexplored.

During the research, it was found that although flow cytometry is a modern method, it may prove costly and laborious for regular use on farms. Therefore, simpler yet effective methods for diagnosing and monitoring the immune response are required. These methods should not only be straightforward but should also be accompanied by the development of bioinformatic systems for analysing the collected data. The primary requirements for such methods of analysis should include ease of use, rapid results, efficiency, and affordability.

CONCLUSIONS

A dynamic increase in the number of CD4+, CD8+, and $\gamma\delta$ T-cell subpopulations was recorded in both memory cell pools. All these phylogenetic groups are interconnected in a complex immune mechanism, and the correlative increase in their number directly indicates the formation of a primary immune response and the subsequent formation of immunological memory. Additionally, the research revealed a quantitative difference in the various phenotypes of memory cells in animals after primary immunisation compared to booster immunisation. This is explained by the fact that the primary immune response to the initial antigen introduction does not demonstrate a significant accumulation of memory cells, which appear only after some time, as recorded in experiments conducted between

7 and 45 days after primary immunisation. In contrast, the number of memory cells in laboratory animals after revaccination increased rapidly immediately after antigen administration, indicating the preservation of a certain number of effector subpopulation pools after the initial encounter with the antigen. It should also be noted that the level of such cells decreases over time but remains higher than the initial level, even after antigen elimination.

The reviewed literature highlights that effector Tm cells with CD45RA+ markers serve as an indicator of the immune response during the first encounter with an antigen. The formation of memory cells marked by CD45RA-, which respond to antigens, is observed during the immune system's subsequent exposure to the same antigen. This phenotype is considered true memory cells, which are activated upon contact with the antigen, aligning with the findings of the current studies. Data indicate that effector subpopulations accumulate only minimally during the primary response to the antigen, with a significant increase occurring between days 7 and 45. Effector memory cells with CD4+, CD8+, and $\gamma\delta$ T-cell markers demonstrated considerable changes following two rounds of immunisation. The use of polyvalent vaccines offers several advantages. It simplifies the work of practising veterinary professionals by reducing the need to plan and administer multiple vaccinations, as a single immunisation would cover several infectious diseases. Additionally, the potential efficiency of immunisation improves, as the use of polyvalent vaccines would not overwhelm the immune system's long-term memory mechanisms.

Another promising area of research into the formation of immunological memory could be the investigation of whether genetic factors and the conditions in which cows are kept influence changes in the number of CD45RA+ T-lymphocytes in response to vaccination. Thus, the results of the studies did not take into account stress factors, which could have affected the production and differentiation of effector cells. These studies, as well as the study of various vaccination methods, vaccine production methods, the use of combination vaccines, and the influence of all these factors on the formation of immunity, are promising. Further research could be directed towards a variety of vaccination methods, as well as the use of vaccines against different diseases. The question of research on other animal species also remains open. All these data will help to more fully explain the workings of the immune system in living organisms.

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CONFLICT OF INTEREST

None.

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Формування адаптивного імунітету проти сальмонельозу у корів з використанням ефекторних клітин пам'яті

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Анотація. Метою дослідження було з'ясувати як відбувається розвиток зміни кількості ефекторних клітин пам'яті під впливом вакцинного антигену сальмонельозу корів. У однорідної групи 100 голів голштино-фризьких корів, що утримуються в однакових умовах, була взята кров. Кров було взято чотири рази: до першої вакцинації, через 7, 45 і 56 днів після. Також корови отримали на 8-10 день бустерну вакцинацію. Корови були щеплені полівалентною вакциною проти сальмонельозу сільськогосподарських тварин у Казахстані. Кров розділяли на плазмову і клітинну фракції центрифугуванням за 1500 об/хв протягом 10 хвилин, потім клітинну фракцію за допомогою методу проточної цитометрії досліджували для визначення кількості субпопуляцій CD4+ і CD8+ та $\gamma\delta$ Т-клітин у чотирьох часових точках: до вакцинації, за 7, 45 і 56 днів після першої вакцинації. Аналіз понад 10000 клітин кожного зразка на проточному цитофлуориметрі обробляли за допомогою програмного забезпечення FlowJo. Отримано дані: популяція CD4+ і CD8+ Т-лімфоцитів і $\gamma\delta$ Т-клітин на 7 день після першої вакцинації зросла в 1,5 раза, потім на 8-10 день корови отримали бустерну дозу вакцини і на 45 день після першої вакцинації популяція CD4+ і CD8+ Т-лімфоцитів і $\gamma\delta$ Т-клітин зросла в 3 рази. CD45RA+ Т-лімфоцити і $\gamma\delta$ Т-клітини демонстрували стабільний ріст до 45 дня, з подальшим зниженням кількості клітин усіх філогенетичних груп Т-клітин. Таким чином можна зробити висновок, що первинна вакцинація дає поштовх до формування довгострокової імунної пам'яті, бустерна доза вакцини збільшує кількість субпопуляцій CD4+ і CD8+ та $\gamma\delta$ Т-клітин у 3 рази. Отримані дані засвідчили механізм формування адаптивного імунітету в корів проти сальмонельозу з використанням ефекторних клітин пам'яті та можуть бути використані на практиці при складанні схем вакцинації корів

Ключові слова: CD4+ і CD8+ Т-лімфоцити; CD45RA+ Т-лімфоцити імунологічна пам'ять; вакцинація; клітинний імунітет; гуморальний імунітет

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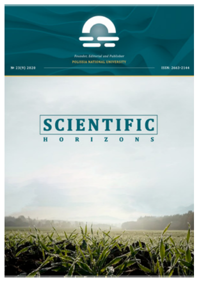

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Chromatographic purification technology optimisation of immunoglobulin G (IgG) from horse serum for animal chlamydia diagnostics

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Abstract. The study aimed to develop and evaluate an antibody quality improvement method to improve the accuracy and efficiency of chlamydia diagnosis in horses. The study was conducted in Kazakhstan and included 100 horses of the Kazakh breed infected with chlamydia, which was divided into two groups: experimental and control. In the experimental group, affinity chromatography was used to purify

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Tyrsynbayev, N., Akhmetsadykov, N., Krykbayev, Ye., Khussainov, D., & Mendybayeva, A. (2024).Chromatographic purification technology optimisation of immunoglobulin G (IgG) from horse serum for animal chlamydia diagnostics. *Scientific Horizons*, 27(10), 20-30. doi: 10.48077/scihor10.2024.20

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immunoglobulin G (IgG), which allowed for a 95% purity of the antibodies. The control group used a traditional diagnostic method without preliminary purification, which ensured IgG purity of only 60-65%. The data showed that the purified antibodies demonstrated improved diagnostic performance, including an increase in sensitivity of up to 92% and specificity of up to 95%. The purified antibodies provided effective binding to chlamydial antigens even at low concentrations (0.2cμg/ml), which is 2.5 times better than in the control group. The time to obtain a stable diagnostic signal was reduced by 33% and amounted to 20 minutes in the experimental group versus 30 minutes in the control group. The frequency of false-positive results in the experimental group was reduced to 5% and false-negative results to 4%, which significantly increases the overall reliability of diagnostics. The purified antibodies retained their activity for 12 months, demonstrating high stability and durability. These results highlighted the importance of using chromatographic purification to improve the quality of antibodies used for diagnostic purposes and offer a reliable approach for the accurate detection of chlamydia as well as other infectious diseases in animals. The introduction of such methods can significantly improve the efficiency of veterinary diagnostics and contribute to more timely and adequate treatment of animals

Keywords: Kazakh breed; antibody stability; parasitology; predictive value; specificity

INTRODUCTION

Chlamydia remains a significant issue in the Kazakh horse breeding industry, causing economic losses and reducing productivity. Chlamydia infection leads to a variety of clinical manifestations, including reproductive problems, conjunctivitis and respiratory diseases, making it important to develop accurate diagnostic methods to control and prevent the spread of infection. Horse breeding in Kazakhstan is not only an economically important industry but also part of the national cultural heritage, which further emphasises the need for high-quality diagnostic tools to maintain the health of horses. Existing methods for diagnosing chlamydia include serological tests such as enzyme-linked immunosorbent assay (ELISA), which are widely used to detect specific antibodies against chlamydia. However, conventional approaches are often limited by the quality of the antibodies used, which leads to a decrease in test accuracy. Serum impurities and insufficient antibody purification can cause nonspecific reactions and lead to false-positive and false-negative results. In an environment where diagnostic accuracy is critical, there is a need to improve antibody purification methods to increase their specificity and sensitivity.

Studies by Kazakh scientists have repeatedly raised issues related to the diagnosis of chlamydia in animals. V. Ulyanov *et al.* (2024) noted that microscopy and standard serological tests do not provide sufficient accuracy, especially in the early stages of infection. Their study emphasised the need to develop new diagnostic approaches that would provide higher sensitivity. However, Amangeldiev's study did not cover possible ways to improve antibody purification methods, leaving this aspect undisclosed. K. Tabynov *et al.* (2023), in turn, conducted a study analysing the effectiveness of standard diagnostic tests for chlamydia. They determined that serological tests often give inconsistent results, making diagnosis difficult. Their study highlighted the importance of developing new approaches to improve diagnostic accuracy. However, the study did not include an analysis of modern methods of antibody purification, which could have improved their performance.

The use of affinity chromatography for the purification of antibodies shows significant potential to improve the quality of chlamydia diagnosis. B. Faye *et al.* (2023) showed that the use of affinity chromatography can significantly improve the purity of antibodies used in the diagnosis of infections. They demonstrated that the purified antibodies exhibit improved characteristics, such as increased specificity and a reduction in the number of false-positive reactions. However, authors did not address the stability of purified antibodies during long-term storage, which is important for practical use in veterinary clinics.

Serological tests, despite their widespread use, often face the problem of insufficient specificity and sensitivity, which leads to false results (Abutalip *et al.*, 2024). This phenomenon was discussed in detail by Z. Bermukhametov *et al.* (2024) and N. Akhmetsadykov *et al.* (2024), who found inconsistent results when using standard serological tests to diagnose chlamydia. They pointed to the importance of improving diagnostic methods to increase the accuracy of infection detection, but their study did not address the issue of antibody purification as a means of improving accuracy. Modern methods such as affinity chromatography offer significant opportunities to improve the quality of antibodies used in diagnostics (Egorov *et al.*, 2022). Research in this area has shown that the use of affinity chromatography can significantly improve the purity of antibodies. A.A. Smagulova *et al.* (2022) demonstrated that purified antibodies have increased specificity and reduce the number of false-positive reactions. However, their study did not cover the issue of antibody durability and stability during long-term storage, which is an important aspect for use in veterinary clinics.

One of the key aspects of infection diagnosis is not only the accuracy but also the stability of the diagnostic reagents used. K.T. Sultankulova *et al.* (2022)

investigated the effect of chromatographic purification on the functional properties of antibodies and confirmed that such purification improves their diagnostic performance. However, their work was limited to laboratory conditions and did not consider the use of these methods in real-life veterinary practice, which leaves open the question of their practical relevance. The lack of stability of diagnostic antibodies and their ability to remain active for long periods is also a problem, especially in Kazakhstan, where access to regular supplies of diagnostic reagents may be limited. In this context, I.S. Beishova *et al*. (2022) pointed out the need to study the stability of antibodies during long-term storage but did not propose specific solutions to improve stability, which leaves this topic open for further research.

The study aimed to investigate the effect of chromatographic purification of immunoglobulin G (IgG) from horse serum on its diagnostic efficiency in the detection of chlamydia. The objectives of the study were assessment of the specificity and sensitivity of purified antibodies, analysis of their ability to bind effectively to chlamydial antigens even at low concentrations, study of the stability and durability of purified antibodies under conditions of long-term storage, and comparison of results obtained using purified and non-purified antibodies to identify the advantages of chromatographic purification in veterinary diagnostics.

MATERIALS AND METHODS

The study to optimise the technology of chromatographic purification of IgG from horse serum for the diagnosis of chlamydia in animals was conducted at the LLP Research and Production Enterprise "Antigen" in Almaty, Kazakhstan, from January to June 2014. The study was part of a programme to improve the diagnosis of infectious animal diseases in the country. For the study, 100 Kazakh horses infected with chlamydia were selected. The presence of chlamydia was confirmed using serological tests and polymerase chain reaction. The horses were aged between 4 and 10 years and were divided into two equal groups of 50 animals: experimental and control. Each group included an equal number of mares and stallions to ensure gender balance. The animals were kept in standard conditions, including free-range housing, daily access to pasture and constant access to water. The diet consisted of hay and mixed fodder, which provided the necessary level of nutrition to maintain health.

Blood was collected by venipuncture of the jugular vein, using sterile disposable syringes and needles, to minimise stress and ensure serum quality. The blood was centrifuged at 2000 x g for 15 minutes to separate the serum. Approximately 50 ml of serum was collected for each animal, which provided sufficient material for further purification and analysis. The experimental group consisted of 50 horses in which chlamydia was diagnosed using a new method based on chromatographic purification of IgG. IgG purification from serum was performed using affinity chromatography on A-separose protein columns (GE Healthcare). The columns were equilibrated with 0.1 M phosphate buffer at pH 7.4. After application of the serum to the column, the bound IgG was eluted with 0.1 M glycine-HCl buffer at pH 2.7. The eluate was immediately neutralised by adding 1 M Tris-HCl buffer at pH 9. A NanoDrop 2000 spectrophotometer was used to determine the concentration of IgG, and the optical density (OD) was measured at 280 nm.

The purity of the obtained IgG was analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). To evaluate the functional activity of the antibody, commercial ELISA kits "Chlamydia trachomatis IgG ELISA" (Abcam) were used. The kits were adapted for use with horse serum by modifying the concentration of reagents and sample volumes. In particular, the concentration of primary and secondary antibodies was reduced by 50% to prevent signal saturation, and sample and reagent volumes were adjusted to 50 μl instead of the standard 100 μl to reduce serum consumption. All incubations were performed at room temperature for 1 hour, except for the last step when substrate was added and incubated for 30 minutes to increase the sensitivity of the assay.

The control group of 50 horses used the traditional method of diagnosing chlamydia without IgG purification. The blood of these animals was also drawn by venipuncture and centrifuged to obtain serum, but the serum was then used without further purification. Diagnostics were carried out using a standard ELISA kit, which was used to detect specific antibodies against chlamydia. This approach was used to evaluate how the traditional diagnostic methodology differs in efficiency from the improved methodology based on chromatographic purification of IgG. The data was analysed using the IBM SPSS Statistics statistical package. Student's t-test was used to assess the differences between the groups, with the significance level set at $p < 0.05$, which was used to identify statistically significant differences. All procedures were carried out in strict compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and the Universal Declaration on Animal Welfare (2007).

RESULTS

The results of the IgG purity assay showed a significant advantage of the new chlamydia diagnostic method based on chromatographic purification compared to the traditional method. In the experimental group, where chromatographic purification was used, electropherograms showed the presence of one clear and intense band corresponding to IgG with a molecular weight of about 150 kDa (Table 1). This indicated that most of the impurities had been removed, and the main component in the purified samples was the target IgG.

It is important to note that the almost complete absence of additional bands indicated a high degree of purity of the obtained IgG. This result was achieved due to affinity chromatography using A-sepharose protein, which has a high specificity for the Fc fragment of IgG. This allows for efficient binding and isolation of IgG while removing impurity proteins and other undesirable components of the serum.

***Table 1**. Comparison of horse IgG characteristics in the experimental and control groups*

Metric	Experimental group (with cleaning)	Control group (no treatment)
Average IgG concentration (mg/ml)	4.8 ± 0.9	10.2 ± 2.1
IgG purity (%)	95 ± 3	60 ± 5
Number of impurities (number of bands)	1	8-12
Main IgG band (intensity)	High	Average
Molecular weight of IgG (kDa)	~150	~150
Level of non-specific binding	Low	High
Potential for cross-reactions	Low	High
Stability of antibodies (retention time of activity)	High (up to 12 months)	Medium (up to 6 months)
Average OD at 450 nm	1.20 ± 0.1	0.85 ± 0.12

***Source:** compiled by the authors*

In the control group, where the traditional diagnostic methodology without IgG purification was used, electropherograms showed a more complex picture. In addition to the band corresponding to IgG, many additional bands were observed on the gels, indicating the presence of various proteins and impurities. This indicates that serum without preliminary purification contains a significant amount of other protein components that may affect the specificity and accuracy of the diagnosis. The presence of these impurities can lead to false-positive and false-negative results, reducing the efficiency and reliability of chlamydia diagnostics.

To quantify the purity of IgG, the concentration of antibodies in both groups was measured. In the experimental group, the average concentration of purified IgG was 4.8±0.9 mg/ml. Although this value is 54% lower than the initial concentration (10.5 ± 2.3 mg/ml), the achieved purity compensated for the decrease in concentration. The chromatographic purification process allowed the removal of non-target proteins and other impurities that could interfere with the specific binding of IgG to antigens. The removal of such components reduces the risk of nonspecific reactions, which is important for ensuring the accuracy and specificity of diagnostic tests. The high purity of the purified IgG means that most of the protein is the target antibody that can effectively interact with chlamydial antigens, providing accurate diagnostic results. After chromatographic purification, the purity of the IgG increased from 65% to 95%, a significant improvement. This data confirms that affinity chromatography effectively removes protein impurities, leaving pure antibodies.

In contrast, the mean IgG concentration in the control group was 10.2 ± 2.1 mg/ml, which was the same as the baseline concentration. However, the purity of IgG remained at the level of 60-65%, indicating the presence of a significant number of impurities. These impurities may include various serum proteins, such as albumin, globulins and other immunoglobulins, which can reduce the specificity of the reaction and cause non-specific binding in diagnostic tests. The increase in IgG purity in the experimental group has a positive impact on all subsequent stages of the diagnostic process. Higher purity antibodies reduce the risk of cross-reactions, leading to more accurate and reproducible diagnostic results. This is especially important in the diagnosis of infectious diseases such as chlamydia, where high test accuracy and specificity are essential for correct diagnosis and timely treatment. The use of purified IgG reduces the likelihood of false-positive results, which can lead to unnecessary treatment and stress for animals.

In addition, a high degree of IgG purity contributes to the stability of antibodies. Impurities present in untreated serum can interact with IgG and cause its degradation, which reduces the effectiveness of antibodies over time. In the experimental group, due to their high purity, the antibodies retain their activity and stability for longer periods of time. This is important for the storage of diagnostic kits, especially in conditions with limited access to refrigeration or other special storage conditions. Thus, the results of the study confirm that chromatographic purification of IgG significantly increases the purity of antibodies, which leads to improved diagnostic performance. These findings highlight the importance of introducing improved purification techniques into diagnostic processes to ensure the high accuracy and reliability of tests used to detect chlamydia and other infectious diseases in animals.

The study demonstrated that the use of chromatographic purification of IgG from equine serum significantly improves the functional activity of antibodies in the diagnosis of chlamydia. When assessing the functional activity using ELISA, tests showed that the purified antibodies had a higher ability to bind specifically to chlamydial antigens, which was reflected in the OD values. In the experimental group, where purified antibodies were used, the average OD value at 450 nm was 1.2 ± 0.1, indicating a high level of specific binding of antibodies to antigens. In the control group, where serum without purification was used, the OD was 0.85 ± 0.12, indicating less efficient binding. This 41.2% increase in the experimental group confirms that the antibodies became more active and efficient after purification, as well as more sensitive to chlamydial antigens. To further analyse the effectiveness of the diagnostic methods, the number of antibodies capable of specific binding to chlamydial antigens was assessed. The results showed that in the experimental group, where purified antibodies were used, up to 95% of the antibodies demonstrated the ability to interact specifically with chlamydial antigens. This means that most antibodies are effectively recognised and bound to the target antigens, ensuring high specificity and sensitivity of the diagnostic test.

In the control group, where untreated antibodies were used, this figure was about 70%. This means that a significant proportion of the antibodies in the control group could not effectively bind to chlamydial antigens, which reduced the overall diagnostic efficiency. The presence of impurities and other proteins that were not removed from the serum interfered with the specific binding of antibodies to antigens, creating competition for binding and reducing the likelihood of correct recognition of the infection. The 25% difference between the experimental and control groups in terms of antibody-specific binding capacity highlights the critical importance of antibody purification in improving diagnostic accuracy. The high binding efficiency in the experimental group not only reduces the risk of false-positive or false-negative results but also increases the likelihood of detecting infection at an early stage. Early diagnosis plays a key role in the successful treatment of chlamydia, as it allows for the timely initiation of therapy and prevents the development of complications and the spread of infection to other animals (Nyzhnyk *et al.*, 2024).

Additionally, the high specific binding capacity of the purified antibodies ensures better reproducibility of test results. When all antibodies in a diagnostic kit interact effectively with antigens, test results become more predictable and stable. This is relevant for veterinary laboratories, where reliability and accuracy are required in daily diagnostic practice. Improved binding efficiency also helps to reduce variability between batches of diagnostic kits, making them more reliable in mass production. It is also worth noting that the high degree of specific binding in the experimental group allows for more accurate calibration and standardisation of diagnostic tests. This makes it easier to adjust tests to define specific thresholds at which results can be considered positive or negative. Calibration accuracy is critical to obtaining unambiguous diagnostic results, which is important not only for detecting infection but also for monitoring the effectiveness of treatment (Yespembetov *et al.*, 2019). In addition, the time to obtain a stable signal in the ELISA was measured. In the experimental group, the time to reach a stable signal was on average 20 minutes, while in the control group, it took up to 35 minutes to achieve similar results. This indicates that the purified antibodies bind to antigens faster and more efficiently, which reduces the diagnostic time and increases the speed of results.

The reproducibility of the results was also significantly higher in the experimental group. Reproducibility was measured as the degree of variation in OD between test repetitions. In the experimental group, the coefficient of variation was only 5%, while in the control group, it reached 15%. The low coefficient of variation indicates the high stability and reliability of the purified antibodies, which makes them preferable for mass production and use in mass diagnostics. The reduction in the rate of false-positive and false-negative results was another important indicator identified in the study. In the experimental group, the false-positive rate was 30% lower than in the control group, which confirms the high specificity of the purified antibodies. There was also a 25% reduction in the false-negative rate, indicating a higher diagnostic sensitivity when using purified antibodies. These data emphasise the importance of IgG purification for improving the accuracy of chlamydia diagnosis.

In the experimental group, the antibodies retained their activity when stored for 12 months at +4°C, while the activity of the antibodies in the control group began to decline after 6 months. This demonstrates that the purified antibodies are more stable and retain their diagnostic activity for longer periods, which is important for the development of diagnostic kits with a long shelf life. These results emphasise that the use of chromatographic purification significantly increases the functional activity of IgG, making the antibodies more effective and accurate in the diagnosis of chlamydia. The increased specific activity and improved stability of the purified antibodies provide more reliable and reproducible results, reducing the likelihood of diagnostic errors. Thus, the introduction of chromatographic purification into the diagnostic process can significantly improve the quality and accuracy of chlamydia diagnosis, which contributes to the improvement of veterinary practice and animal health. The results of the study showed that the introduction of the method of chromatographic purification of IgG from horse serum significantly improves the accuracy of chlamydia diagnosis (Table 2). To assess the diagnostic accuracy, the sensitivity, specificity, and false-positive and false-negative rates in the experimental and control groups were analysed.

***Table 2**. Chlamydia diagnostic accuracy rates in the experimental and control groups*

Metric	Experimental group (with cleaning)	Control group (no treatment)
Sensitivity (%)	92±2	75±4
Specificity (%)	95±2	80±3
False positive rate (%)	5±2	20±3
False negative rate (%)	8±2	25±4
Positive predictive value (%)	93	78
Negative predictive value (%)	94	82

***Source:** compiled by the authors*

In the experimental group, where purified antibodies were used, the sensitivity of the diagnostic test was 92 ± 2%. This means that the test correctly detected 92% of all cases of chlamydia, indicating that the purified antibodies are highly capable of detecting even low concentrations of chlamydial antigens. The ELISA test revealed that the minimum antigen concentration at which the diagnostic test confidently recognised the presence of chlamydia was 0.2 μg/ml in the experimental group. At the same time, in the control group, the minimum concentration for confident detection was 0.5 μg/ml. This 2.5-fold difference confirms that the purified antibodies have significantly higher sensitivity, allowing for the diagnosis of infection at earlier stages when the antigen concentration is still low. Early diagnosis is extremely important for preventing the spread of infection, as it enables earlier treatment and more effective control of the outbreak. In the control group, the sensitivity was 75 ± 4%, which is significantly lower than in the experimental group. This difference emphasises that the presence of impurities in the untreated serum reduces the ability of antibodies to recognise antigens effectively, which can lead to an underestimation of the number of infected animals.

The purified antibodies were more reliable in making a diagnosis at borderline antigen levels. In situations where the antigen level was close to the detection threshold, the purified antibodies correctly identified the infection in 87% of cases. In the control group, this figure was only 67%. This 20% higher accuracy in the experimental group suggests that the purified antibodies can be used effectively in diagnosis even at minimal levels of infectious material, which is particularly useful in cases of chronic or subclinical infection where symptoms may not be apparent. The specificity of the test in the experimental group was also high, reaching 95 ± 2%. This means that the diagnostic test correctly identified 95% of healthy animals as not infected, minimising the number of false-positive results. In the control group, the specificity was 80±3%, indicating a higher probability of false-positive reactions when using untreated serum. The increase in specificity in the experimental group is since purified antibodies bind exclusively to target antigens, while impurities can cause nonspecific reactions.

The false-positive rate in the experimental group was 30% lower than in the control group. This means that tests using purified antibodies were less likely to falsely indicate the presence of infection in healthy animals. Reducing false-positive results is important not only for diagnostic accuracy but also for preventing unnecessary treatment, which reduces stress for animals and saves resources. The frequency of false-negative results, in which infected animals were not recognised, was also significantly lower in the experimental group. False negatives were 25% more common in the control group than in the experimental group. This highlights the importance of antibody purification to improve the sensitivity of the test and the timely detection of infections, which is critical to preventing the spread of chlamydia and improving animal health control. The results of statistical analysis using Student's t-test confirmed that the differences between the experimental and control groups were statistically significant ($p < 0.05$). This confirms that the improvement in the accuracy of chlamydia diagnosis is due to the use of IgG chromatographic purification (Table 3).

***Table 3**. Statistical analysis of differences between the experimental and control groups*

Metric	Mean value (experimental group)	Mean value (control group)	t-value	p-value
Sensitivity	92	75	5.67	<0.05
Specificity	95	80	4.89	<0.05
False positive rate	5	20	5.12	<0.05
False negative rate	8	25	6.34	<0.05
Positive predictive value	93	78	5.45	<0.05
Negative predictive value	94	82	4.76	<0.05

***Source:** compiled by the authors*

The use of purified antibodies also improved the predictive value of the tests. The positive predictive value (the likelihood that a positive test result indicates an infection) in the experimental group reached 93%, while in the control group, it was 78%. The negative predictive value (the probability that a negative test result indicates the absence of infection) in the experimental group was 94%, compared to 82% in the control group. These data emphasise that purified antibodies provide more reliable diagnostic results, allowing accurate conclusions to be drawn about the health status of animals.

DISCUSSION

The above study demonstrated that chromatographic purification of IgG significantly increases the purity of antibodies, which improves their diagnostic value. An increase in purity of up to 95% after affinity chromatography confirms that the technique effectively removes impurities that can cause nonspecific reactions. A. Ramos *et al*. (2022) and V. Herb *et al*. (2022) showed that the use of chromatographic purification also increases the specificity of antibodies, which is consistent with the results obtained here. A. Ramos *et al*. emphasised that impurities could reduce the effectiveness of antibodies by causing undesirable interactions, which is similar to the observations in the Kazakh study.

Another important observation concerned the functional activity of antibodies. The use of purified antibodies in diagnostic tests increased their sensitivity to 92% and specificity to 95%. These figures are significantly higher than those achieved in the control group, where the antibodies were not purified. Similar results were presented in the study by P. Liang *et al*. (2022), where purified antibodies demonstrated an increased ability to bind to antigens and more accurately distinguish between infected samples. Authors emphasised that antibody purity is a critical factor in improving diagnostic accuracy, which was also confirmed in the Kazakh study.

In addition, the reduction in the false-positive and false-negative rates to 5% and 4%, respectively, in the experimental group emphasises the importance of using purified antibodies to minimise diagnostic errors. This is consistent with the results obtained by I. Trbojević-Akmačić *et al*. (2022) and L. Rumokoy and W. Toar (2022), who noted that the use of highly purified antibodies reduces the likelihood of diagnostic errors, which is especially important for infectious diseases requiring high accuracy. I. Trbojević-Akmačić *et al*. (2022) also noted that purified antibodies help to improve the quality of diagnosis by reducing background signals, which is similar to the findings of the Kazakh study.

An important comparison can be made with the results of F. Ghalamfarsa *et al*. (2023) and C. Li *et al*. (2022), where less specific methods of antibody purification were used. In their study, the sensitivity and specificity were only 75% and 80%, respectively, which is significantly lower than the values achieved using affinity chromatography. This difference confirms that highly efficient purification methods, such as affinity chromatography, are preferred to ensure high accuracy of diagnostic tests. It has also been noted that the presence of impurities in antibodies can lead to cross-reactions, which reduces the accuracy of diagnosis. L. Athanasiou *et al*. (2023) and C. El-Hage *et al*. (2023) demonstrated similar efficacy of affinity chromatography to increase the sensitivity of antibodies. They noted that purified antibodies can detect low concentrations of antigens, which is important for early diagnosis. This is in line with the Kazakh study, which showed that the purified antibodies could detect chlamydial antigens at a concentration of only 0.2 μg/ml, while the minimum detectable concentration in the control group was 0.5 μg/ml. This level of sensitivity is critical for detecting infection at an early stage and preventing its spread.

The data also demonstrated that the purified antibodies showed not only higher specificity and sensitivity but also a faster response in diagnostic tests. This phenomenon is important for rapid diagnostics, where the speed of obtaining results is crucial. In the experimental group, the time to obtain a stable diagnostic signal was on average 20 minutes, which is 33% faster than in the control group. This improvement is in line with the findings of H. Ali and L. Al-Bayati (2022) and S. Doff *et al*. (2022), who also showed that increasing antibody purity through chromatographic purification reduces reaction time, providing a fast and accurate result. This reduction in diagnostic time is particularly useful in disease outbreaks, where rapid decision-making is required to prevent the spread of infection. These observations highlight the importance of introducing chromatographic purification not only to improve accuracy but also to speed up diagnostic processes in clinical practice (Berezin *et al.*, 2008).

The results from the Kazakh study also highlight the importance of antibody stability for long-term use. In the experimental group, the purified antibodies retained their activity for 12 months, which is consistent with the findings of C. Thurston *et al*. (2022) and R. White *et al*. (2022), who emphasised that affinity chromatography increases the stability of antibodies, allowing them to remain active for long periods. This quality is particularly important for the development of long-life diagnostic kits, which is crucial for their use in the field. R. Ricard *et al*. (2023) also noted that the high purity of antibodies increases their resistance to denaturation and degradation, which allows them to retain their activity under different storage conditions. This is in line with the data obtained in Kazakhstan, where purified antibodies demonstrated high stability even after long-term storage. Such results confirm the importance of using affinity chromatography to improve the quality of antibodies and their suitability for widespread use in diagnostics.

Y. Minamijima *et al.* (2022) provide an interesting contrast to the Kazakh data. In their studies, it was argued that certain impurities can contribute to an increase in antibody activity. However, the results of the Kazakh study show that a high degree of purity, on the contrary, improves specificity and sensitivity, reducing the number of false positives. This highlights the need for further research to understand the interaction between impurities and antibodies and their impact on diagnostics. Additionally, an important aspect confirmed by the Kazakhstan study is the ability of purified antibodies to effectively recognise antigens at low concentrations. This ability is crucial for the early diagnosis of infectious diseases, allowing the infection to be detected at the initial stages. This not only facilitates the timely initiation of treatment but also helps to prevent the spread of infection to other animals. Comparison with the study by X. Chen *et al.* (2023) also confirms the importance of antibody purity. They showed that the use of purified antibodies reduces false signals, improving diagnostic accuracy. This is in complete agreement with the findings of the Kazakhstani study, where the use of purified antibodies resulted in a significant reduction in the incidence of false positives and false negatives. R. Piccoli *et al.* (2023) emphasised that highly purified antibodies help to achieve higher diagnostic accuracy, which is particularly important for infectious diseases where accuracy is crucial.

Analysing the data obtained, attention should be paid to the observed increase in the specific activity of the purified antibodies, which is directly related to their improved ability to bind specifically to Chlamydia antigens. This phenomenon indicates a reduction in the influence of non-specific proteins and other impurities that may interfere with binding (Alhindy *et al.*, 2024). Such findings are supported by X. Chen *et al.* (2022) and S. Albini *et al.* (2023), who showed that impurities in serum can bind to antibodies, reducing their ability to specifically interact with target antigens. Purified antibodies show more efficient binding, as evidenced by both increased sensitivity and increased specificity of diagnostic tests, as shown by similar studies by K. Aaron *et al.* (2023) also demonstrated the advantages of using highly purified antibodies for the diagnosis of infectious diseases. They observed that the use of affinity chromatography improved the performance of antibodies by increasing their ability to bind specifically to antigens. This is similar to the results obtained in Kazakhstan and confirms that chromatographic purification is an effective method to improve the quality of diagnostic tests.

Thus, the analysis of the data obtained in the context of global research shows that chromatographic purification of IgG is a key factor in improving the quality of antibodies and their diagnostic value. Comparison with the work of other authors confirms the importance of high purity and specificity of antibodies to ensure the accuracy and reliability of diagnostic tests, which is important for veterinary medicine and infectious disease control.

CONCLUSIONS

A study aimed at improving the diagnosis of chlamydia in horses through chromatographic purification of IgG has confirmed that the use of affinity chromatography significantly improves the purity and efficiency of antibodies. The results showed that the purified antibodies reached a purity of 95%, which is 30-35% higher than the control group using the traditional diagnostic method. This improvement resulted in an increase in the sensitivity of the diagnostic tests to 92% and specificity to 95%, providing more accurate and reliable detection of chlamydia. The study also determined that the purified antibodies can effectively bind to antigens at low concentrations (0.2 μg/ml), which significantly increases their diagnostic value, especially for early detection of infection.

A 33% reduction in the time to stable diagnostic signal allows for faster diagnosis, which is critical in the context of disease outbreaks. In addition, there was a significant reduction in the false-positive and false-negative rates to 5% and 4%, respectively, which minimises the risk of misdiagnosis and unnecessary treatment. The high stability of the purified antibodies, which retain their activity for 12 months, underlines their suitability for long-term storage and use, which is especially relevant for the development of diagnostic kits with a long shelf life.

These results emphasise the importance of using IgG chromatographic purification in veterinary diagnostics, especially when accurate and rapid detection of infectious diseases is required. These data may be useful for the development of improved diagnostic tests not only for chlamydia but also for other infections requiring high accuracy and reliability. Future research may be aimed at optimising the chromatographic purification conditions for different types of antibodies and extending the application of this technique to other infectious diseases. It is also possible to address the interaction of purified antibodies with different antigens to develop multiplex diagnostic tests capable of simultaneously detecting several pathogens.

Despite the positive results, some limitations of the study should be addressed. The study was conducted on only one breed of horse, which may limit the extrapolation of the results to other breeds and species. The use of only one method of purification may not account for all variations and peculiarities of other approaches to antibody purification. These limitations could be addressed in future studies by including a greater variety of animals and comparing different antibody purification methods. The development and implementation of more effective diagnostic approaches will contribute to improved control of infectious diseases and animal health.

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CONFLICT OF INTEREST

None.

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Оптимізація технології хроматографічного очищення імуноглобуліну G (IgG) із сироватки коней для діагностики хламідіозу тварин

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Анотація. Метою даного дослідження було розробити та оцінити метод поліпшення якості антитіл для підвищення точності та ефективності діагностики хламідіозу у коней. Дослідження проводили в Казахстані, воно включало 100 коней казахської породи, інфікованих хламідіями, яких було розділено на дві групи: експериментальну та контрольну. В експериментальній групі застосовували афінну хроматографію для очищення імуноглобуліну G (IgG), що дало змогу домогтися чистоти антитіл на рівні 95 %. У контрольній групі використовували традиційну методику діагностики без попереднього очищення, що забезпечувало чистоту IgG лише на рівні 60-65 %. Отримані дані засвідчили, що очищені антитіла демонстрували поліпшені діагностичні характеристики, включно з підвищенням чутливості до 92 % і специфічності до 95%. Очищені антитіла забезпечували ефективне зв'язування з антигенами хламідій навіть за низьких концентрацій (0,2 мкг/мл), що в 2,5 разик раще, ніж у контрольній групі. Час до отримання стабільного діагностичного сигналу було скорочено на 33 % і становив 20 хвилин в експериментальній групі проти 30 хвилин у контрольній групі. Частота хибнопозитивних результатів в експериментальній групі була знижена до 5 %, а хибнонегативних – до 4 %, що значно підвищує загальну надійність діагностики. Очищені антитіла зберігали свою активність протягом 12 місяців, демонструючи високу стабільність і довговічність. Ці результати підкреслюють важливість використання хроматографічного очищення для поліпшення якості антитіл, що застосовуються в діагностичних цілях, і пропонують надійний підхід для точного виявлення хламідіозу, а також інших інфекційних захворювань у тварин. Впровадження таких методів може значно підвищити ефективність ветеринарної діагностики та сприяти більш своєчасному й адекватному лікуванню тварин

Ключові слова: казахська порода; стабільність антитіл; паразитологія; передбачувальна цінність; специфічність

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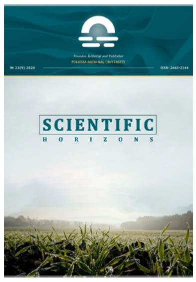

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Ecological plasticity of soft winter wheat varieties and resistance to snow mould pathogen (*Microdochium nivale* (Fr.) Samuels & I.C. Hallett)

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Abstract. Identifying superior genotypes of soft winter wheat with high ecological plasticity to soil and climatic conditions, combined with tolerance to common diseases caused by fungal pathogens, including snow mould, is becoming increasingly important for both production and breeding. This is driven by the global demand for

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environmentally safe products. The preliminary selection of the most valuable initial material with high-stress resistance to periodic climate changes and deteriorating phytosanitary conditions is essential for the success of the breeding process in addressing current challenges. This study aimed to determine the level of ecological plasticity in soft winter wheat varieties, differentiated by their resistance to the snow mould pathogen, through the analysis of genotype effect ranks and yield regression coefficients. Additionally, high-yielding sources were identified for use in breeding for adaptability. The research was conducted according to commonly accepted methodologies for evaluating materials based on disease resistance and adaptive traits, ensuring a highly objective interpretation of the results obtained. It was determined that the highest ecological plasticity among the studied material was differentiated by resistance to *Microdochium nivale* (Fr.) Samuels & I.C. Hallett, was exhibited by five varieties (16.7%) that achieved yields ranging from 118% to 129% of the standard. These include the varieties Muza Bilotserkivska, Askaniiska Berehynia, Hetmanska, Malovanka (UKR), and Nordika (CZE), as evidenced by the lowest sum of ranks (sum of ranks 2) for the genotypic effect (ε_i) and regression coefficient (R_i). New sources of high and stable resistance in soft winter wheat to the snow mould pathogen were identified, specifically: Sanzhara, Malovanka, Svitiaz, Muza Bilotserkivska (UKR); Smuga (POL), and Nordika (CZE). It was found that, in soft winter wheat varieties, resistance to *Microdochium nivale* (Fr.) Samuels & I.C. Hallett significantly negatively correlates with the sum of ranks for genotypic effect and yield plasticity, with r =-0.69, P < 0.01. The systematisation of the results obtained and the identification of varieties with the highest ecological plasticity under variable weather conditions within the agroecological zone allows for the identification of the best gene pool for adaptive breeding

Keywords: adaptability; homeostasis; epiphytotics; genotypic effect; variability; yield

INTRODUCTION

Periodic droughts, increasingly common in Ukraine due to global warming, along with the variability of prevalent pathogens affecting soft winter wheat, including snow mould (*Microdochium nivale* (Fr.) Samuels & I.C. Hallett) (syn. *Fusarium nivale* Ces. ex Berl. & Voglino), which is accompanied by the emergence of virulent and aggressive pathogens, highlight the priority of breeding for adaptability and the need to select valuable initial material based on these traits. Determining the ecological plasticity of soft winter wheat (*Triticum aestivum* L.) varieties and identifying sources of resistance to *M. nivale* (Fr.) Samuels & I.C. Hallett that exhibit high levels of adaptability to environmental stress are primary tasks in this endeavour. The successful resolution of these tasks supports the breeding process aimed at increasing total grain yield and stabilising production, which is of significant nutritional and economic importance. Soft winter wheat (*Triticum aestivum* L.) is a leading cereal crop in various countries, including Ukraine, due to its wide range of food products and high export potential. However, various biotic and abiotic environmental stress factors limit the genetic potential for yield and adaptability in this crop.

A Turkish scientist, F. Ünal (2024), in his research on the pathogenicity of *Microdochium* fungi in cereal crops, concluded that the level of infection by various snow mould pathogens in *T. aestivum* ranged from 83.29% to 91.52% and indicated a strong correlation with yield. Ukrainian researchers, O. Afanasyeva *et al.* (2023), emphasise the importance of cultivating disease-resistant plant varieties as a means of suppressing phytopathogens and preventing epidemics. Ukrainian scientists L. Murashko *et al.* (2022) note that among the various diseases of soft winter wheat in Ukraine, those caused by highly virulent fungi are becoming increasingly common. One such disease is snow mould, caused by the pathogen *Microdochium nivale* (Fr.) Samuels & I.C. Hallett (syn. *Fusarium nivale* Ces. ex Berl. & Voglino), which, due to its mutational, combinatorial, and population variability, is capable of overcoming the protective action of resistance genes in plants.

Researchers from China, S. Huang *et al.* (2020), assert that the snow mould pathogen, *M. nivale*, is a facultative parasite with distinctly expressed saprophytic properties. According to I. Furtat *et al.* (2020), this pathogen, when the infection is severe, leads to the death of the tillering node, roots, and the entire plant. K. Hockemeyer and P.L. Koch (2022) noted that snow mould manifests in the spring, immediately after the snow melts, with a white or pinkish coating appearing on the tillering node of infected plants. By studying the pathogenicity of 15 isolates of *M. nivale* through sequencing four genetic regions: elongation factor 1-alpha (*EF-1α*), β-tubulin, RNA polymerase II (*RPB2*), and internal transcribed spacer (ITS), Norwegian scientists – M. Abdelhalim *et al.* (2020) – concluded that the high heterogeneity of this pathogen is the reason for the widespread occurrence of snow mould in temperate climates, which, combined with provoking factors (snowfall on unfrozen soil, significant amount and duration of snow cover, presence of an ice crust, and increased doses of nitrogen fertilisers), significantly reduces plant immunity.

Several researchers, including A.V. Tyshchenko *et al.* (2023), note that the level of a genotype's resistance to environmental stressors characterises its stability. Plasticity is understood as the ability to adapt to a range of varying environmental conditions (Lenoir *et* *al*., 2023). The ecological plasticity of a genotype, observed in its interaction with the environment, is one of the main factors that has the greatest influence on the variability and formation of yield. Ukrainian scientists M. Samoilyk *et al*. (2023) emphasise that for the successful introduction of new *T. aestivum* varieties into production, it is essential to assess both yield and adaptability.

Great attention is paid to optimal sowing dates as one of the elements of cultivation technologies necessary for the formation of high yields. Chinese researchers J. Liu *et al*. (2023) found that wheat yield and yield stability were highest when the photothermal potential was 16.0 t·ha^{-1} and the pre-winter active accumulated temperature regime was 400°C·d, which was observed when winter wheat was sown at the Gucheng station from 1 to 15 October during the 1997-2021 research period. M. Babar *et al*. (2022) note that the selection of wheat varieties with the necessary genetic variability of the traits under study contributes to the creation of stress-resistant genotypes as one of the important directions of modern breeding.

Therefore, the identification of soft winter wheat varieties with high ecological plasticity and resistance to *M. nivale* remains a relevant task. Successful resolution of this issue contributes to the creation of new genotypes with high adaptive capabilities. This study aimed to determine the ecological plasticity of soft winter wheat varieties by analysing the sum of genotypic effect ranks and the yield regression coefficient, and to identify sources with a high level of plasticity and resistance to *Microdochium nivale* (Fr.) Samuels & I.C. Hallett, which are well adapted to the limiting effects of prevalent stress factors.

MATERIALS AND METHODS

The research was conducted between 2020 and 2023 in an 8-field crop rotation at the Yuriev Plant Production Institute of the National Academy of Agrarian Sciences of Ukraine (NAAS), located in the eastern region of the Forest-Steppe zone. Standard agronomic practices for this ecological zone were applied. The study involved 30 soft winter wheat varieties developed in 12 countries, including 13 varieties of Ukrainian origin: Akademichna 100, Askaniiska Berehynia, Hetmanska, Kvitka Poliv, Liryka Bilotserkivska, Muza Bilotserkivska, Malovanka, Melashka, Merezhka, Musii, Sanzhara, Svitiaz, and Yuvileina Patona. The study also included three varieties from Austria – Aurelius, Dominikus, Turanus; three from the United States of America – Vanguard, Northern, Warhorse; two from Poland – Natula, Smuga; two from Azerbaijan – Gobustan, Ruzi 84; and one each from the Czech Republic – Nordika, the Netherlands – Manella, Romania – Armura, Germany – Rumor, Kazakhstan – Mida, Tajikistan – Sarvar, and Turkey – Yayla 302.

The experiments were conducted according to the methodology of qualification examination (Tkachyk, 2016). The accounting area of each plot was 5 m^2, with three replications. The standard variety used was Podolianka. The varieties were assessed following the methodology of S.O. Tkachyk (2016) and the methodology for evaluating the resistance of wheat varieties to pests and pathogens (Trybel *et al*., 2010). The levels of resistance to *M. nivale* were rated using a nine-point scale, where 1 point indicated very low resistance (very severe damage, >70%), 3 points indicated low resistance (severe damage, 51-70%), 5 points indicated moderate resistance (average damage, 31-50%), 7 points indicated high resistance (slight damage, 10-30%), and 9 points indicated very high resistance (negligible damage, <10%).

The level of homeostasis (Hom) in soft winter wheat varieties regarding their resistance to *M. nivale* (Fr.) Samuels & I.C. Hallett was determined according to the methodology established by V.V. Khangildin and N.A. Lytvynenko (1981). This approach is based on the observed patterns of lower variability in the studied traits and a reduced decline in adverse conditions for samples exhibiting high homeostasis. The selection value (Sc) was also calculated using this methodology, which allows for the identification of genotypes that combine a high or moderate level of the trait in question with its stable expression under varying cultivation conditions (Khangildin & Lytvynenko, 1981).

The determination of ecological plasticity was conducted according to the methodology of B.P. Hurev *et al*. (1981). In this methodology, the genotypic effect (ε_i) indicates the level of overall adaptive capacity of the studied genotype, while the regression coefficient (R_i) reflects the degree of plasticity. Specifically, varieties with a high genotypic effect and a low regression coefficient occupy the highest rank (rank 1 – high), whereas the reverse scenario results in the lowest rank (rank 3 – low), and intermediate cases yield a medium rank (rank 2). Samples that achieve the lowest sum of ranks (between 2 and 3) during the study are considered the most valuable, as they combine a high potential with stability in the trait being investigated (Hurev *et al*., 1981). For a qualitative assessment of the correlation coefficients, the R.E. Chaddock (1952) scale was employed. Statistical analysis of the experimental data was carried out using MS Excel 2007 and Statistica 6.0 software. The authors adhered to the standards outlined in the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1979).

RESULTS

The contrast in moisture availability across the different years of study, along with the prevalence of snow mould, enabled an assessment of the study material regarding ecological plasticity in yield and resistance to *M. nivale*. The hydrothermal coefficient (HTC) varied significantly by month and characterised the range of meteorological conditions, primarily from very dry to excessively moist. For example, the HTC for September varied from 0.05 to 2.14; October – from 0.16 to 3.53; April – from 0.61 to 2.55; May – from 0.57 to 1.29; June – from 0.22 to 1.25, and July – from 0.09 to 2.24. (Table 1).

***Table 1**. Hydrothermal coefficient during the growing seasons of the study, 2020-2023*

Year	Month					
	April	May	June	July	September	October
2020	-	-	-	-	0.05	0.87
2021	1.62	1.29	1.19	0.09	0.55	0.16
2022	0.61	0.57	1.25	1.09	2.14	3.53
2023	2.55	0.66	0.22	2.24	-	-
Mean annual HTC	1.2	0.88	1.04	1.1	1.0	1.67

***Source:** compiled by the authors*

To identify sources of resistance to *Microdochium nivale* (Fr.) Samuels & I.C. Hallett, the years 2021 and 2023 were favourable. The year 2022 was most favourable for achieving high yields. In other years of the study, the levels of its manifestation were generally lower. Over the period 2020-2023, the resistance of the studied soft winter wheat varieties to the snow mould pathogen varied from 1 to 9 points. As a result of the study, ten sources (33.3%) of soft winter wheat with a high level of resistance to *M. nivale* (Fr.) Samuels & I.C. Hallett (from 7 to 9 points) were identified: Sanzhara, Malovanka, Muza Bilotserkivska, Liryka Bilotserkivska, Melashka, Svitiaz (UKR); Smuga (POL); Nordika (CZE); Aurelius (AUT); Manella (NLD), and the standard variety Podolianka – 7 points (Table 2).

***Table 2**. Resistance of soft winter wheat varieties to the snow mould pathogen (Microdochium nivale (Fr.) Samuels & I.C. Hallett), 2020-2023*

Sample name	Country of origin	Resistance to snow mould pathogen, points			
		Range of variation		X_{med}	CV, %
		max	min		
Podolianka, standard	UKR	8.0	6.5	7.0	12.4
Sanzhara	UKR	9.0	8.0	8.5	5.9
Malovanka	UKR	9.0	8.0	8.3	6.9
Muza bilotserkivska	UKR	9.0	7.0	7.7	7.5
Liryka bilotserkivska	UKR	8.0	6.0	7.0	14.3
Melashka	UKR	8.0	6.5	7.0	12.4
Svitiaz	UKR	7.5	6.5	7.0	7.1
Akademichna 100	UKR	7.0	5.5	6.2	12.4
Kvitka poliv	UKR	7.0	5.5	6.2	12.4
Hetmanska	UKR	7.0	5.0	6.0	16.7
Askaniiska berehynia	UKR	6.0	4.0	5.2	20.2
Merezhka	UKR	6.0	4.0	5.2	20.2
Yuvileina Patona	UKR	6.0	4.0	5.0	20.0
Musii	UKR	5.0	3.0	4.3	26.7
Smuga	POL	9.0	7.0	7.7	7.5
Natula	POL	6.0	3.0	4.0	43.3
Nordika	CZE	9.0	7.5	8.2	9.4
Armura	ROU	4.0	3.0	3.3	17.3
Rumor	DEU	5.0	3.0	4.3	26.7
Aurelius	AUT	9.0	6.0	7.3	20.8
Turanus	AUT	6.0	3.0	4.0	43.3
Dominikus	AUT	3.5	1.0	2.2	58.1
Manella	NLD	9.0	5.0	7.0	28.6
Mida	KAZ	3.5	1.0	2.2	58.1

Table 2. Continued

Sample name	Country of origin	Resistance to snow mould pathogen, points			
		Range of variation		X_{med}	CV, %
		max	min		
Sarvar	TJK	4.0	1.0	2.3	65.5
Gobustan	AZE	4.0	2.0	2.7	43.3
Ruzi 84	AZE	4.0	1.0	2.3	65.5
Yayla 302	TUR	4.0	2.0	3.0	33.3
Vanguard	USA	6.0	4.0	5.0	20.0
Northern	USA	5.0	2.0	3.0	57.7
Warhorse	USA	4.0	2.0	3.0	33.3
$LSD_{0.05}$		–	–	0.8	–

Source: *compiled by the authors*

The following genotypes exhibited resistance to the snow mould pathogen at levels ranging from 4 to 6 points: Kvitka Poliv, Akademichna 100, Hetmanska, Merezhka, Askaniiska Berehynia, Yuvileina Patona, and Musii (UKR); Natula (POL); Turanus (AUT); Rumor (DEU); and Vanguard (USA), accounting for 36.7% of the varieties studied. Nine varieties (33.0%) demonstrated low levels of resistance to *M. nivale* (Fr.) Samuels & I.C. Hallett, namely: Armura (ROU); Dominikus (AUT); Mida (KAZ); Sarvar (TJK); Ruzi 84 and Gobustan (AZE); Yayla 302 (TUR); Northern and Warhorse (USA).

The coefficient of variation (CV, %) indicated that the variability of resistance to *M. nivale* (Fr.) Samuels & I.C. Hallett ranged from 5.9% to 65.5%. An analysis of the variation series revealed that six varieties – Sanzhara, Malovanka, Svitiaz, Muza Bilotserkivska (UKR), Smuga (POL), and Nordika (CZE) – exhibited low variability (CV ⩽ 10.0%), comprising 20.0% of the samples. Eight samples, accounting for 26.7%, displayed medium variability (CV = 11.0% – 20.0%), including Melashka, Akademichna 100, Kvitka Poliv, Liryka Bilotserkivska, and Hetmanska (UKR); Armura (ROU); and Vanguard (USA). High variability (CV > 20.0%) in resistance to *M. nivale* was observed in the following varieties: Askaniiska Berehynia, Merezhka, Musii (UKR); Natula (POL); Aurelius, Turanus, and Dominikus (AUT); Rumor (DEU); Manella (NLD); Mida (KAZ); Sarvar (TJK); Gobustan and Ruzi 84 (AZE). It was determined that the proportion of samples exhibiting significant variability in resistance to the snow mould pathogen constituted 53.3%. The resistance of the standard variety, Podolianka, showed medium variability with a CV of 12.4%. The analysis indicated that the selection value (Sc) of the soft winter wheat varieties for resistance to *M. nivale* (Fr.) Samuels & I.C. Hallett varied from 0.6 to 7.6, while homeostasis (Hom) ranged from 3.6 to 144.5 (Fig. 1).

The study of selection value in soft winter wheat varieties for resistance to *M. nivale* (Fr.) Samuels & I.C. Hallett identified sources that exceeded the average value observed in the experiment (Sc = 3.7). These include 13 varieties (43.3%): Sanzhara (Sc = 7.6), Malovanka (Sc = 7.4), Muza Bilotserkivska (Sc = 6.2), Svitiaz (Sc = 6.1), Melashka (Sc = 5.7), Liryka Bilotserkivska (Sc = 5.3), Kvitka Poliv (Sc = 4.9), Akademichna 100 (Sc = 4.9), Hetmanska (Sc = 4.3) (UKR); Smuga (Sc = 6.2) (POL); Nordika (Sc = 6.8) (CZE); Aurelius (Sc = 4.9) (AUT); Manella (Sc = 3.9) (NLD); with the standard variety Podolianka (Sc = 5.7).

The criterion for homeostasis is the ability of genotypes to maintain a low level of variability for a trait over the study period. Among the material examined, genotypes exhibiting high homeostasis (Hom) and low variability (CV ⩽ 10.0%) in resistance to *M. nivale* (Fr.) Samuels & I.C. Hallett were identified, specifically: Sanzhara (Hom = 144.5), Malovanka (Hom = 120.3), Svitiaz (Hom = 98.0), and Muza Bilotserkivska (Hom = 64.0) (UKR); Smuga (Hom = 64.0) (POL); and Nordika (Hom = 87.3) (CZE). Thus, the varieties Sanzhara (Sc = 7.6; Hom = 144.5), Malovanka (Sc = 7.4; Hom = 120.3), Svitiaz (Sc = 6.1; Hom = 98.0), Muza Bilotserkivska (Sc = 6.2; Hom = 64.0) (UKR); Smuga (Sc = 6.2; Hom = 64.0) (POL); and Nordika (Sc = 6.8; Hom = 87.3) (CZE) are valuable genotypes distinguished by their high selection value and homeostasis in resistance to *M. nivale* (Fr.) Samuels & I.C. Hallett.

To determine the ecological plasticity of winter soft wheat varieties differentiated by their resistance to *M. nivale* (Fr.) Samuels & I.C. Hallett, the research material was assessed based on the ranks of genotype effect and the regression coefficient of yield. Over the period from 2020 to 2023, sources were identified among the various eco-geographical origins of soft winter wheat that exhibited a high yield formation relative to the standard, specifically ranging from 118% to 129%. These include Svitiaz, Liryka Bilotserkivska, Yuvileina Patona, Muza Bilotserkivska, Askaniiska Berehynia, Hetmanska, and Malovanka (UKR); Nordika (CZE); and the standard Podolianka (UKR) with a yield of 5.73 t/ha (Table 3).

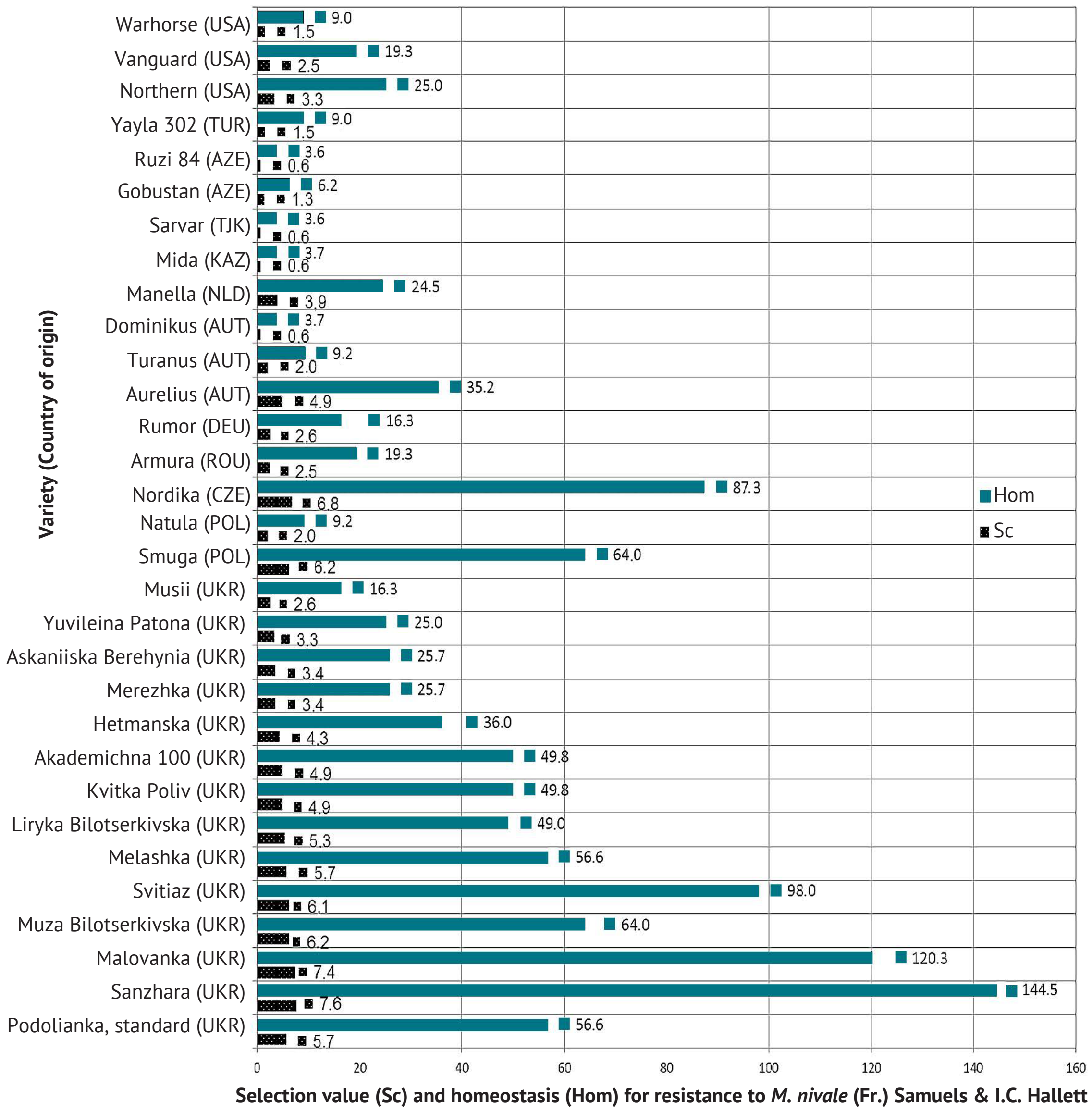

***Figure 2**. Parameters of selection value and homeostasis in soft winter wheat varieties for resistance to the snow mould pathogen (Microdochium nivale (Fr.) Samuels & I.C. Hallett), 2020-2023*
***Source:** compiled by the authors*

***Table 3**. Ecological plasticity of soft winter wheat varieties differentiated by resistance to Microdochium nivale (Fr.) Samuels & I.C. Hallett and yield, 2020-2023*

Variety name	Country of origin	Yield, t/ha			Genotype effect		Regression coefficient (degree of plasticity)		Ecological plasticity, the sum of ranks
		max	min	med	ε_i	rank	R_i	rank	
Podolianka, standard	UKR	7.15	4.78	5.73	-0.30	2	0.94	2	4
Svitiaz	UKR	8.35	6.12	7.42	1.39	1	0.88	2	3
Liryka Bilotserkivska	UKR	9.04	5.74	7.37	1.34	1	1.31	3	4
Yuvileina Patona	UKR	9.22	5.35	7.29	1.26	1	1.54	3	4
Muza Bilotserkivska	UKR	7.82	6.45	7.17	1.14	1	0.54	1	2
Askaniiska berehynia	UKR	7.65	6.25	6.86	0.83	1	0.56	1	2
Hetmanska	UKR	7.50	6.30	6.84	0.81	1	0.31	1	2
Malovanka	UKR	7.95	5.93	6.74	0.71	1	0.80	1	2
Melashka	UKR	7.80	5.54	6.62	0.59	1	0.90	2	3
Kvitka Poliv	UKR	7.85	5.64	6.62	0.59	1	0.88	2	3

Table 3. Continued

Variety name	Country of origin	Yield, t/ha			Genotype effect		Regression coefficient (degree of plasticity)		Ecological plasticity, the sum of ranks
		max	min	med	ε_i	rank	R_i	rank	
Musii	UKR	7.10	5.78	6.48	0.45	1	0.52	1	2
Merezhka	UKR	7.90	5.08	6.41	0.38	1	1.12	2	3
Akademichna 100	UKR	7.57	5.34	6.32	0.29	2	0.89	2	4
Sanzhara	UKR	7.40	5.5	6.22	0.19	2	0.76	1	3
Natula	POL	6.80	6.25	6.52	0.49	1	0.22	1	2
Smuga	POL	6.74	5.33	5.97	-0.06	2	0.56	1	3
Nordika	CZE	7.50	6.26	7.02	0.99	1	0.49	1	2
Armura	ROU	7.86	4.42	6.01	-0.02	2	1.37	3	5
Rumor	DEU	7.42	4.26	5.83	-0.20	2	1.26	3	5
Turanus	AUT	7.95	4.33	6.08	0.05	2	1.44	3	5
Aurelius	AUT	6.90	4.85	5.93	-0.10	2	0.81	1	3
Dominikus	AUT	7.76	3.27	5.90	-0.13	2	1.78	3	5
Manella	NLD	7.36	4.10	5.97	-0.06	2	1.29	3	5
Mida	KAZ	6.30	2.60	4.48	-1.55	3	1.47	3	6
Sarvar	TJK	7.26	3.50	5.39	-0.64	3	1.49	3	6
Ruzi 84	AZE	5.71	3.21	4.62	-1.41	3	0.99	2	5
Gobustan	AZE	5.65	2.95	4.57	-1.46	3	1.07	2	5
Yayla 302	TUR	6.44	2.75	4.55	-1.48	3	1.47	3	6
Vanguard	USA	7.25	3.42	5.31	-0.72	3	1.52	3	6
Warhorse	USA	5.62	3.15	4.37	-1.66	3	0.98	2	5
Northern	USA	5.30	3.25	4.35	-1.68	3	0.81	1	4
$LSD_{0.05}$		–	–	0.35	–	–	–	–	–
min		5.30	2.60	4.35	-1.68	1	0.22	1	2
max		9.22	6.45	7.42	1.39	3	1.78	3	6
med		7.29	4.76	6.03	0.00	–	1.00	–	–

Source: *compiled by the authors*

Thirteen varieties, constituting 43.3%, were distinguished by high values of genotype effect (rank 1) for yield. These included the following samples: Svitiaz, Liryka Bilotserkivska, Yuvileina Patona, Muza Bilotserkivska, Askaniiska Berehynia, Hetmanska, Malovanka, Melashka, Kvitka Poliv, Musii, and Merezhka (UKR); Natula (POL); and Nordika (CZE). The varieties Akademichna 100 and Sanzhara (UKR); Smuga (POL); Armura (ROU); Rumor (DEU); Turanus, Aurelius, and Dominikus (AUT); and Manella (NLD) exhibited an average genotype effect (rank 2), representing 30.0% of the samples. A low level of genotype effect (rank 3) was characteristic of eight varieties (26.7%), namely Mida (KAZ); Sarvar (TJK); Ruzi 84, Gobustan (AZE); Yayla 302 (TUR); and Vanguard, Warhorse, and Northern (USA). The standard Podolianka (UKR) demonstrated an average level of genotype effect for yield (rank 2). The conducted research established that the genotype effect (ε_i) for yield among the differentiated varieties resistant to *M. nivale* ranged from 1.68 to 1.39.

Based on the degree of plasticity (R_i), it was determined that the number of homeostatic samples exhibiting high stability in yield performance (rank 1) included 11 varieties, accounting for 36.7%. The following varieties were characterised by this genotype capability: Muza Bilotserkivska, Askaniiska Berehynia, Hetmanska, Malovanka, Musii, and Sanzhara (UKR); Natula and Smuga (POL); Nordika (CZE); Aurelius (AUT); and Northern (USA). Eight varieties (26.7%), including Svitiaz, Melashka, Kvitka Poliv, Merezhka, and Akademichna 100 (UKR); Ruzi 84 and Gobustan (AZE); and Warhorse (USA), were classified as moderately sensitive genotypes for yield in variable growing conditions (rank 2). The number of intensive, or sensitive, genotypes that respond to both improved and worsened growing conditions (rank 3) totalled 11 varieties, also constituting 36.7%. These included Liryka Bilotserkivska, Yuvileina Patona (UKR); Armura (ROU); Rumor (DEU); Turanus and Dominikus (AUT); Manella (NLD); Mida (KAZ); Sarvar (TJK); Yayla 302 (TUR); and Vanguard (USA). The standard Podolianka was characterised by average sensitivity to variable growing conditions (rank 2).

In investigating the ecological plasticity of soft winter wheat varieties differentiated by their resistance to the snow mould pathogen, based on an analysis of the sum of the ranks of the genotype effect (ε_i) and the regression coefficient (R_i) for yield, seven varieties (23.3%) were identified as having the highest genetic potential for adaptability, and consequently the greatest breeding value, as indicated by the lowest sum of ranks (sum of ranks 2). These varieties are: Muza Bilotserkivska (ε_i = 1.14; R_i = 0.54), Askaniiska Berehynia (ε_i = 0.83; Ri = 0.56), Hetmanska (ε_i = 0.81; R_i = 0.31), Malovanka (ε_i = 0.71; R_i = 0.80), Musii (ε_i = 0.45; R_i = 0.52) (UKR); Natula (ε_i = 0.49; R_i = 0.22) (POL); and Nordika (ε_i = 0.99; R_i = 0.49) (CZE). The samples Sanzhara (ε_i = 0.19; R_i = 0.76), Svitiaz (ε_i = 1.39; R_i = 0.88), Melashka (ε_i = 0.59; R_i = 0.90), Kvitka Poliv (ε_i = 0.59; R_i = 0.88), and Merezhka (ε_i = 0.38; R_i = 1.12) (UKR); Smuga (ε_i = 0.06; R_i = 0.56) (POL); and Aurelius (ε_i = 0.10; R_i = 0.81) (AUT) were somewhat less notable in terms of total rank (sum of ranks 3). 13.3 % of varieties achieved a total rank of 4: Akademichna 100 (ε_i = 0.29; R_i = 0.89), Liryka Bilotserkivska (ε_i = 1.34; R_i = 1.31), Yuvileina Patona (ε_i = 1.26; R_i = 1.54) (UKR); and Northern (ε_i = 1.68; R_i = 0.81) (USA). A total rank of 5 was assigned to varieties of foreign breeding, specifically: Armura (ε_i = 0.02; R_i = 1.37) (ROU); Rumor (ε_i = 0.20; R_i = 1.26) (DEU); Turanus (ε_i = 0.05; R_i = 1.44), Dominikus (ε_i = 0.13; R_i = 1.78) (AUT); Manella (ε_i = 0.06; R_i = 1.29) (NLD); Ruzi 84 (ε_i = 1.41; R_i = 0.99), Gobustan (ε_i = 1.46; R_i = 1.07) (AZE); and Warhorse (ε_i = 1.66; R_i = 0.98) (USA), which constitute 26.7%. The four varieties with the lowest adaptability potential, as assessed by genotype effect and degree of plasticity (sum of ranks 6), include Mida (ε_i = 1.55; R_i = 1.47) (KAZ); Sarvar (ε_i = 0.64; R_i = 1.49) (TJK); Yayla 302 (ε_i = 1.48; R_i = 1.47) (TUR); and Vanguard (ε_i = 0.72; R_i = 1.52) (USA). The standard Podolianka (ε_i = 0.30; R_i = 0.94) (UKR) was characterised by a sum of ranks of 4 regarding ecological plasticity. The identified high-yielding genotypes, which exhibit tolerance to the snow mould pathogen, provide a foundation for developing the most suitable and adaptive varieties based on them.

An analysis of the relationships between the studied traits revealed that, in soft winter wheat varieties, resistance to the snow mould pathogen (*Microdochium nivale* (Fr.) Samuels & I.C. Hallett) correlates negatively and significantly with the sum of ranks of the genotype effect and degree of yield plasticity, with a correlation coefficient of r=0.69. Additionally, the selection value and homeostasis regarding resistance to *M. nivale* (Fr.) Samuels & I.C. Hallett also correlated negatively and significantly with the sum of ranks (genotype effect and degree of yield plasticity), specifically: r = 0.68 and r = 0.56, respectively. The significance of these correlations was found to be 99% (P < 0.01) (Table 4).

Table 4. *Correlation (r) of the sum of the ranks of the genotype effect and degree of yield plasticity in soft winter wheat varieties with adaptive parameters for resistance to Microdochium nivale (Fr.) Samuels & I.C. Hallett, 2020-2023*

Trait	The sum of ranks of genotype effect (ε_i) and degree of plasticity (R_i) for yield, t/ha
Resistance to *M. nivale* (Fr.) Samuels & I.C. Hallett, score	-0.69 [1)]
Selection value for resistance to *M. nivale* (Fr.) Samuels & I.C. Hallett	-0.68 [1)]
Homeostasis of resistance to *M. nivale* (Fr.) Samuels & I.C. Hallett, Hom	-0.56 [1)]

Note: [1)] *– P < 0.01*
Source: *compiled by the authors*

Thus, among the biotic factors that act as environmental stressors, the snow mould pathogen (*Microdochium nivale* (Fr.) Samuels & I.C. Hallett) has a significant negative impact on the potential and stability of yield in soft winter wheat varieties. This necessitates the compulsory inclusion of sources exhibiting high resistance to this phytopathogen in the breeding process. Based on the obtained research results, it can be asserted that within the genetic diversity of the studied soft winter wheat material, there exist genotypes characterised by a combination of high levels of resistance to *M. nivale* (Fr.) Samuels & I.C. Hallett, yield, and adaptability, among which varieties of Ukrainian selection predominate in terms of quantity. An extensive search for these sources, along with investigations into the correlations between environmental stress factors and the adaptive traits of soft winter wheat varieties, enables the identification of superior genetic material for selection focused on adaptability and helps determine priority approaches for developing well-adapted genotypes.

DISCUSSION

Several authors, including T. Yurchenko *et al*. (2020), note that the increased impact of various environmental stressors on the genetic yield potential and adaptive traits of soft winter wheat makes it necessary to conduct research aimed at determining ecological plasticity and stress resistance, which are a preliminary and mandatory stage for the selection of valuable genotypes as a necessary basis for selection for adaptability.

There are many aspects to the importance of factors that can influence the realisation of a high and stable yield genotype. Significant attention is paid to weather conditions (Parvez & Chowdhury, 2020). Researchers P. Langridge and M. Reynolds (2021) note that the main limiting factor that wheat plants suffer from in the environment is water deficit. Therefore, the identification and selection of highly effective sources and donors of drought resistance, through the use of wide genetic analysis strategies and modification of gene expression among diverse varieties, is the basis for identifying adapted genotypes for specific agroecological growing conditions.

Ukrainian scientists M.V. Tarasiuk and O.O. Stasik (2022), studying the impact of drought on the dynamics of accumulation and remobilisation of reserve water-soluble carbohydrates (WSC), which are an important source of assimilates for grain filling, established that the content of WSC in the second and lower internodes correlated most strongly and positively with grain weight per plant (r=0.534...0.693) and with the weight of 1000 grains (r=0.778...0.897). Iranian researchers S. Mahdavi *et al.* (2022), investigating 64 wheat genotypes under thermal stress caused by delayed sowing, concluded that thermal stress led to a significant reduction in grain density due to a decrease in starch content (SC) and a non-compensatory increase in protein content (PC), resulting in reduced yield. The researchers also noted a significant increase in the water absorption capacity of flour (WA), ash content (AC), lipid content (LC), wet gluten content (WG), dry gluten content (DG), gluten index (GI), and amylopectin content (APC) following thermal stress, which improved certain baking properties.

Researchers S.P. Lyfenko *et al.* (2021) argue that to increase the homeostasis of modern wheat varieties and their resistance to sudden abiotic stressors, it is necessary to create varieties with greater sensitivity to photoperiod and to include drought-tolerant material in breeding programs. Researchers X. Wu *et al.* (2021) emphasise the importance of introducing new varieties with reliable genetic protection against pathogens, which is associated with the undeniable economic and environmental advantages of alternative plant protection. Chinese scientists D.-X. Tang *et al.* (2022) note that a detailed study of the species composition and toxicity of common fungi of the genus *Microdochium* contributes to the effectiveness of selection for immunity and increased adaptability in new varieties.

Ukrainian scientists A.V. Pirych *et al.* (2021) indicate that a significant component of the overall adaptive potential of soft winter wheat is its winter hardiness, which is determined by a complex of traits that ensure plant survival during winter. These traits include frost resistance, duration of vernalisation, photoperiod sensitivity, and resistance to the pathogen causing snow mould. As a result of a targeted search by Ukrainian scientists for genotypes with high adaptive properties, soft winter wheat varieties have been identified that are characterised by increased yield potential and ecological plasticity to the agro-ecological conditions of the central Forest-Steppe of Ukraine – Estafeta Myronivska ta Hratsiia Myronivska, MIP Assol and Balada Myronivska.

In their study of new varieties of soft winter wheat across various soil and climatic zones in Ukraine, A.M. Kyrylchuk *et al.* (2024) identified genotypes with high homeostasis and low yield variability (V≤10.0%), among which Yevraziia (Hom=15.6) and Dekaster (Hom=15.2) stood out as the best. The researchers noted that the varieties LG Optimist and Dekaster exhibited the highest selection value for yield – Sc=6.9 and Sc=6.6, respectively. Additionally, in their analysis of yield variability and quality parameters of *T. aestivum*, as well as their relationship with natural and anthropogenic factors, O. Demydov *et al.* (2022) established a significant (P≤0.01) influence of the growing period on the yield of this crop (66.2%), the mass of 1000 grains (63.2%), and grain density (58.8%).

Despite the variety of approaches to realising a high and stable yield genotype, several scientists, including T. Miedaner and P. Juroszek (2021), have concluded that due to the trend towards a changing climate towards global warming, droughts, which are becoming increasingly frequent, have a detrimental impact on the formation of yield levels and stability. Therefore, the identification and creation of material with simultaneous resistance to phytopathogens and water deficit is becoming increasingly relevant, while it is important to focus on quantitative trait loci (QTL) that reduce temperature sensitivity.

Thus, the results of determining the ecological plasticity of soft winter wheat varieties differentiated by resistance to the snow mould pathogen, establishing correlations between the sum of ranks of the genotypic effect and the degree of yield plasticity with the parameters of adaptability for resistance to *M. nivale* indicate the uniqueness of the study.

CONCLUSIONS

Based on the sum of ranks for genotype effect (ε_i) and the regression coefficient (R_i) for yield among soft winter wheat varieties differentiated by their resistance to the snow mould pathogen (*Microdochium nivale* (Fr.) Samuels & I.C. Hallett), genotypes have been identified that exhibit the highest genetic potential for adaptability. This is evidenced by the lowest sum of ranks (sum of ranks 2), which results in a yield exceeding the standard by more than 16%. The identified varieties include Muza Bilotserkivska (ε_i = 1.14; R_i = 0.54), Askaniiska Berehynia (ε_i = 0.83; R_i = 0.56), Hetmanska (ε_i = 0.81; R_i = 0.31), Malovanka (ε_i = 0.71; R_i = 0.80) (UKR), and Nordika (ε_i = 0.99; R_i = 0.49) (CZE), collectively accounting for 16.7%. The variety Svitiaz (sum of ranks 3) was slightly behind these, yet also exhibited a high yield with ε_i = 1.39 and R_i = 0.88. The lowest ecological plasticity, based on genotype effect and plasticity degree (sum of ranks 6), was observed in the varieties Mida (ε_i = 1.55; R_i = 1.47) (KAZ), Sarvar (ε_i = 0.64; R_i = 1.49) (TJK), Yayla 302 (ε_i = 1.48; R_i = 1.47) (TUR), and Vanguard (ε_i = 0.72; R_i = 1.52) (USA), which comprised 13.3% of the total research sample.

New sources of high and stable resistance in soft winter wheat to *M. nivale* (Fr.) have been identified, specifically: Sanzhara (Hom = 144.5), Malovanka

(Hom = 120.3), Svitiaz (Hom = 98.0), and Muza Bilotserkivska (Hom = 64.0) (UKR); Smuga (Hom = 64.0) (POL); and Nordika (Hom = 87.3) (CZE). It has been determined that among the soft winter wheat varieties differentiated by their resistance to *M. nivale* (Fr.) Samuels & I.C. Hallett, the genotype effect (ε_i) for yield ranged from 1.68 to 1.39, while the regression coefficient (R_i) varied from 0.22 to 1.78. This significantly influenced the level of ecological plasticity, with the sum of ranks ranging from 2 to 6. The proportion of samples exhibiting high values of the genotype effect constituted 43.3%, while those with high yield stability accounted for 36.7%. It was established that for the soft winter wheat varieties, resistance to *M. nivale* negatively correlated at a significant level with the sum of ranks for genotype effect and yield plasticity degree, with $r = 0.69$, $P < 0.01$.

The identified sources of high levels of group resistance to *M. nivale* (Fr.) Samuels & I.C. Hallett, high potential and yield stability expand the information content about the genetic diversity of existing varieties and open up additional opportunities in adaptive selection. The study of physiological and biochemical processes of adaptation, features of transcriptomics and proteomics in material with different ecological plasticity is promising for future research, which will open up more opportunities for generalising and systematising the results of the study.

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CONFLICT OF INTEREST

None.

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Екологічна пластичність сортів пшениці м'якої озимої та стійкість до збудника снігової плісняви (*Microdochium nivale* (Fr.) Samuels & I.C. Hallett)

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Анотація. Визначення кращих генотипів пшениці м'якої озимої з високою екологічною пластичністю до ґрунтово-кліматичних умов вирощування у поєднанні з толерантністю до поширених хвороб, спричинених грибними збудниками, в тому числі і снігової плісняви, набуває все більшої актуальності для виробництва та селекції, яка зумовлена попитом на світовому ринку екологічно-безпечної продукції. Попереднє виділення найбільш цінного вихідного матеріалу з високою стресостійкістю до періодичних змін у кліматі та погіршеного фітосанітарного стану передує успішності селекційного процесу щодо актуальних викликів сьогодення. Метою досліджень було визначення рівня екологічної пластичності в сортів пшениці м'якої озимої, диференційованих за стійкістю до збудника снігової плісняви, шляхом аналізу рангів генотипового ефекту та коефіцієнту регресії врожайності, а також виділення високоврожайних джерел для використання в селекції на адаптивність. Дослідження проведені згідно загальноприйнятих методик для оцінки матеріалу вивчення за стійкістю до збудників хвороб та адаптивних властивостей, які забезпечили інтерпретацію одержаних результатів високою об'єктивністю. Визначено, що найвищою екологічною пластичність серед диференційованого матеріалу вивчення за стійкістю до *Microdochium nivale* (Fr.) Samuels & I.C. Hallett відзначилися п'ять сортів (16,7 %), які вирізнялися формуванням врожайності від 118 % до 129 % до стандарту. До них відносяться сорти Муза білоцерківська, Асканійська берегиня, Гетьманська, Мальованка (UKR) та Nordika (CZE), про що свідчить найменша сума рангів (сума рангів 2) генотипового ефекту (ε_i) та коефіцієнту регресії (R_i). Виділено нові джерела високої та стабільної стійкості пшениці м'якої озимої до збудника снігової плісняви, а саме: Санжара, Мальованка, Світязь, Муза білоцерківська (UKR); Smuga (POL) та Nordika (CZE). Встановлено, що у сортів пшениці м'якої озимої стійкість до *Microdochium nivale* (Fr.) Samuels & I.C. Hallett на значному негативному рівні корелює із сумою рангів генотипового ефекту і ступеня пластичності врожайності – $r = -0,69$, $P < 0,01$. Систематизація отриманих результатів вивчення, виділення сортів з найвищою екологічною пластичністю в мінливих погодних умовах агроекологічної зони, дають можливість ідентифікувати кращий генофонд для адаптивної селекції

Ключові слова: адаптивність; гомеостатичність; епіфітотії; генотиповий ефект; мінливість; урожайність

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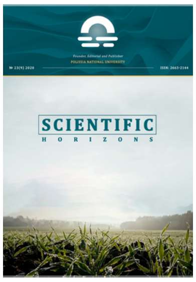

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Biochemical value of table grape varieties when grown in the Northern Steppe of Ukraine

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Abstract. The study of the possibilities of using the existing biodiversity of table grape varieties to meet the usefulness of the human diet is a key component of improving the existing cultivation of fruit products within the framework of modern agricultural development strategies. The purpose of the study was to establish the possibilities of meeting the needs of the population for valuable nutritional elements based on traditional table grape varieties, which are mainly introduced in the region at the level of small farms and household plots. Five table grape varieties – Arcadia, Nadezhda AZOS, Preobrazhenie, Rumeyka, Dubovsky pink – were investigated for the content of calcium, phosphorus, sulphur, magnesium, potassium, zinc, copper, selenium, manganese, glucose, dietary fibre, vitamins A, C, E, PP, and the dependence on the factors of the year and variety, their interaction, and the classifying ability of features in the space of canonical functions were calculated. The presence of minerals was analysed by atomic emission spectrometry, glucose – by sugar meter, dietary fibre – by enzymatic gravimetric method, vitamins – by fluorometric method, and vitamin C – by titrometric analysis. The possibilities of traditional table grape varieties in meeting the needs for basic valuable food elements are shown and a conclusion is made about the possibilities of selecting appropriate forms, which in the complex are quite capable of meeting modern requirements in this aspect of agricultural development. A predominantly low variability of the studied traits was revealed, which indicates the good reproducibility of the obtained result and the predominant influence of the genotype on the development of the corresponding trait in conditions of contrasting environmental characteristics of years. It was found that improvement in only one of the signs – the content of vitamin PP – is problematic. The low differentiating ability

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of varieties of the Dubovsky pink, Preobrazhenie, and Rumeyka group leads to the need for further clarification of the ability of only the first of the varieties to be a component in the proposed complex. It is recommended to grow a complex of varieties Nadezhda AZOS and Dubovsky pink

Keywords: viticulture; genotype; nutritional value; trace elements; product quality; biologically active substances

INTRODUCTION

Production variety testing of grapes is a long process that takes approximately ten years from the initial stages to the introduction of a promising adapted genotype. Although certain goals, such as yield and basic product quality, are always important, general consumption characteristics, environment, marketing, and production trends influence the strategy that is used as a basis in research programmes in this area, trying to anticipate the future needs of the fruit industry. The importance of each trend depends on the level of performance and the environment. The main trends are the introduction of varieties that simplify production technology, have increased resistance to biotic and abiotic stresses, expand the adaptation zones of the crop, create new types of fruits, primarily more attractive in shape and colour, grow grape varieties with increased health benefits, and ensure stable high quality (Cichi *et al.*, 2023).

Breeders in the fruit growing industry should anticipate the need for varieties at least 10 years in the future, as this is the minimum time that most grape varieties need for the maturation process from the first pollination to zoning. This aspect examines general trends such as environmental issues, healthy eating, consumer lifestyle trends (Farvid *et al.,* 2021), and the expectations and needs of producers to determine how they will affect the future strategy of consumption of manufactured products (Ağızan *et al.,* 2024). Much attention has been paid to the health benefits of grapes and grape products, primarily due to the antioxidant activity of various phenolic compounds, especially flavonoids, and the high content of beneficial trace elements that are deficient in traditional foods. Manufacturers have widely launched programmes to introduce new and increase the introduction of traditional products that are more complete for human biochemistry, with increased levels of health benefits in grapes and processed products (Koyama *et al.,* 2020).

Constant efforts to introduce new varieties with different parameters of valuable elements, different colours with an attractive berry shape, texture from hard to crisp, attractive clusters of acceptable size for packaging and ripening at different points in time (Navarro-Caldero *et al.,* 2023) during the growing season led to the creation of a fundamentally new cluster of products. Producers in important table grape growing areas focus their efforts on grape (Cameron *et al.,* 2022) that is suitable for different market niches based on availability at a unique time of year, with quality characteristics that exceed varieties that may already be on the market. Suitability for storage, and avoiding problems after harvesting (grinding, rot, market damage) is also important, especially for varieties of later maturation.

The objective of the study was to identify the limits of variability of different varieties of table grapes in terms of the content of valuable food elements that determine the consumer usefulness of products, provided that these varieties are widely distributed in the production of the region, mainly at the level of small farms, to identify genotypic and annual variations, and their interaction. These varietal resources are a local source of biodiversity and enrichment of the human diet.

MATERIALS AND METHODS

The study was conducted at the LLC "Agrosilprom", Znamenivka village, Novomoskovsk District of the Dnipropetrovsk Oblast in 2021-2023. Five table grape varieties – Arcadia, Nadezhda AZOS, Preobrazhenie, Rumeyka, and Dubovsky pink – were studied by biochemical analysis methods (an average sample of 1 kg with each repetition, three repetitions for each variety at full ripeness). The cultivation technology was recommended for the region, with drip irrigation.

The content of such valuable elements as calcium, phosphorus, sulphur, magnesium, potassium, zinc, copper, selenium, and manganese was studied in the laboratory of the Research Centre for Biosafety and Environmental Control of Agricultural Resources of the Dnipro State Agrarian and Economic University. The sample preparation protocol provided for mineralisation using the MultiWave Go Plus microwave decomposition system (manufacturer Anton Paar, Austria), with the addition of 0.5 g of 10 ml of 65% nitric acid and 1 ml of concentrated hydrochloric acid to the resulting suspension of each sample. The mineralisation time (together with the cooling time) was 45 minutes at a temperature of 185°C. The presence of minerals was analysed using an atomic emission spectrometer with inductively coupled Agilent 5110 plasma at the emission intensity of the light flux at the wavelengths corresponding to each element. Multi-element solutions produced by Agilent (Ca, S, Mg, K, P, Zn, Se, Mn, Cu) were used as reference standards.

The content (per 100 g) of the following substances: glucose, dietary fibre in berries, vitamins A, E, C, PP was determined in the Laboratory of Biochemistry and Plant Physiology of the Department of Plant Physiology and Introduction of O. Honchar State University (DSTU EN 12822:2005, 2006; DSTU EN 12823-

2:2006, 2006; DSTU EN 7803:2015, 2016; DSTU EN 12630:2019, 2019). To determine the glucose content, extraction was performed and a VPCH-17 sugar meter (manufactured by Elcantr (Spain)) was used. Extraction of the solution to determine the percentage of glucose was performed by the standard method. Dietary fibre was investigated by enzymatic-gravimetric methods. The presence of A, E (tocopherol), PP (nicotinamide) content in the samples was studied using a standardised fluorometric method at the corresponding light wavelengths using an ULAB 102UV spectrophotometer, and vitamin C was detected by titrometric method through oxidation in dehydroascorbic acid of grape samples (5 g sample) (Badenes & Byrne, 2012).

Mathematical and statistical analysis of the obtained values was investigated by Anova method, grouping, and estimation of parameters were performed using discriminant and cluster analysis modules (Euclidean distance, single links) (Statistica 10.0, multivariate statistics module, TIBCO, Palo Alto, USA). The compliance of the obtained data with the normal distribution was established by calculating the Shapiro-Wilk W test. Pairwise comparison between varieties and years of variety testing was performed using the Tukey' range test (factor analysis package). Experimental studies of plants (both cultivated and wild), including the collection of plant material, were in accordance with institutional, national or international guidelines. The authors adhered to the standards of the Convention for the Protection of Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1979).

RESULTS AND DISCUSSION

The study of the obtained material took place in several stages. During the first stage, the content of key valuable macronutrients – calcium, phosphorus, sulphur, magnesium, and potassium – was determined. Usually, problems can be caused by the low presence primarily of sulphur and magnesium, sources of other elements are usually sufficient. Also significant was the fact that the content of these elements depended on both the variety and the growing conditions (year). In total, three samples were examined for variants (varieties) during three years of cultivation. According to the results of factor analysis, the calcium content was significantly affected as a Variety factor ($F = 257.19$; $F_{0.05} = 2.66$; $P = 3.47*10^{-32}$), and the Year factor ($F = 13.62$; $F_{0.05} = 2.44$; $P = 2.33*10^{-6}$), although with a very significant difference. The interaction of factors by influence was not significant ($F = 3.22$; $F_{0.05} = 3.50$; $P = 0.06$). By pairwise comparison (Tukey's range test), the Nadezhda AZOS variety was ahead with a high calcium content, then Preobrazhenie and Rumeyka (were at the same level), then Dubovsky pink, and the lowest content was in the Arcadia variety. The trait varies very significantly between varieties, but within the same variety it is considered low-varietal (less than 5%). The results are presented in Table 1.

***Table 1**. Content of table macronutrients in grapes depending on genotype (2021-2023), (x = 27, ±SD)*

Indicators	Arcadia	Nadezhda AZOS	Preobrazhenie	Rumeyka	Dubovsky pink
Calcium, mg/kg	22.78 ± 1.24^{a}	86.93 ± 2.09^{b}	43.32 ± 1.39^{c}	40.11 ± 1.11^{c}	35.67 ± 1.18^{d}
Phosphorus, mg/kg	24.70 ± 1.74^{a}	85.10 ± 2.92^{b}	29.20 ± 2.04^{c}	32.90 ± 2.05^{c}	33.80 ± 2.05^{cd}
Sulphur, g/kg	0.31 ± 0.06^{ab}	0.40 ± 0.07^{a}	0.21 ± 0.05^{ab}	0.19 ± 0.04^{b}	0.29 ± 0.07^{ab}
Magnesium, mg/kg	17.23 ± 1.47^{a}	41.22 ± 1.65^{b}	29.34 ± 1.16^{c}	35.67 ± 1.25^{d}	32.56 ± 1.57^{d}
Potassium, g/kg	2.13 ± 0.19^{a}	8.92 ± 0.31^{b}	3.12 ± 0.25^{c}	3.09 ± 0.23^{c}	3.49 ± 0.21^{c}

***Note:** indicates a significant difference at P < 0.05 for the Tukey's range test with Bonferroni correction. Comparison within varieties*
***Source:** compiled by the authors*

According to the results of the factor analysis, the phosphorus content was significantly influenced by the variety factor ($F = 112.14$, $F_{0.05} = 2.66$, $P = 7.17*10^{-14}$), but not by the year factor ($F = 2.42$, $F_{0.05} = 2.44$, $P = 0.06$). The interaction of factors by influence was also not significant ($F = 2.65$; $F_{0.05} = 3.50$; $P = 0.08$). By pairwise comparison (Tukey's range test), the Nadezhda AZOS variety was ahead with a high phosphorus content, Dubovsky Pink was on the same level as the Rumeyka variety, but significantly higher content than the Preobrazhenie variety (which was on the same level as the Rumeyka variety), significantly lower content was in the Arcadia variety, as in the previous case. The trait again varies very significantly between varieties, and within one variety it is considered medium-variable (at the level of 7-9%, except for the Rumeyka variety, where it showed itself as weakly variable). Thus, a set of varieties can have a fairly significant polymorphism on this feature in most cases.

The sulphur content is particularly interesting, since the sources of this element in the diet are more limited. According to the results of factor analysis, the variety factor was significantly affected ($F = 34.90$; $F_{0.05} = 2.66$; $P = 6.22*10^{-6}$), the Year factor was not significant ($F = 2.02$; $F_{0.05} = 2.44$; $P = 0.08$). The genotype-environment interaction was also not significant ($F = 2.11$; $F_{0.05} = 3.50$; $P = 0.09$). By pairwise comparison (Tukey's range test), the Nadezhda AZOS variety was ahead with high sulphur content, Arcadia was on par with the Nadezhda AZOS and Dubovsky pink varieties (which was already inferior to the best), in turn, the Dubovsky pink variety was on par with the Preobrazhenie variety, which was statistically significantly inferior in sulphur content to Arcadia and Nadezhda AZOS. The Rumeyka variety was worse, but the sulphur content in it did not differ statistically from the Preobrazhenie variety. The attribute varies less significantly between varieties, and within the same variety, it is considered slightly variable (at the level of 3-4%). The studied varieties show the absence of significant polymorphism in this trait, but it is quite difficult to manifest.

For magnesium content according to the results of factor analysis, the variety factor significantly affected ($F = 119.19$; $F_{0.05} = 2.66$; $P = 3.22*10^{-15}$), but not the Year factor ($F = 2.34$; $F_{0.05} = 2.44$; $P = 0.06$). The interaction of factors by influence was also not significant ($F = 2.87$; $F_{0.05} = 3.50$; $P = 0.07$). By pairwise comparison (Tukey's range test), the Nadezhda AZOS variety was ahead with a high magnesium content, then the Dubovsky pink and Rumeyka varieties (without significant differences between the varieties), the Preobrazhenie variety had a significantly lower content, and the Arcadia variety had the lowest content. The trait varies significantly between varieties, and within the same variety it is considered slightly variable (at the level of 3-5%). Thus, the set of varieties does not have a significant polymorphism in this trait.

According to the results of the factor analysis, potassium content significantly depended on the Variety factor ($F = 93.10$; $F_{0.05} = 2.66$; $P = 1.34*10^{-12}$), but not from the Year factor ($F = 2.31$; $F_{0.05} = 2.44$; $P = 0.06$). The interaction of factors by influence was also not significant ($F = 2.11$; $F_{0.05} = 3.50$; $P = 0.09$). By pairwise comparison (Tukey's range test), the Nadezhda AZOS variety was ahead with a high potassium content, followed by the Dubovsky pink, Preobrazhenie and Rumeyka varieties (they were at the same level in the trait), and the Arcadia variety had a significantly lower content. The trait varies significantly between varieties, and within the same variety, it is considered slightly variable (at the level of 2-4%). Thus, the set of varieties does not show significant polymorphism in this trait. The results of the discriminant analysis showed that the content of sulphur and potassium is less variable in the component determined by the variety, and the variability in the content of calcium, phosphorus, and magnesium is much more determined (Table 2).

***Table 2**. Results of discriminant analysis of the reliability of individual parameters by macronutrient content*

Indicators	Wilks' criterion	Partial	F	p-level
By varieties ($F_{critical} = 4.36$)				
Calcium, mg/kg	0.01	0.01	2,957.20	<0.01
Phosphorus, mg/kg	0.01	0.01	1,200.63	<0.01
Sulphur, g/kg	0.01	0.64	5.09	0.03
Magnesium, mg/kg	0.01	0.01	1,616.17	<0.01
Potassium, g/kg	0.01	0.36	15.90	<0.01
By year ($F_{critical} = 2.38$)				
Calcium, mg/kg	0.08	0.74	3.78	0.04
Phosphorus, mg/kg	0.98	0.997	0.02	0.97
Sulphur, g/kg	0.99	0.99	0.18	0.83
Magnesium, mg/kg	0.99	0.99	0.16	0.84
Potassium, g/kg	0.98	0.99	0.09	0.90

***Source:** compiled by the authors*

The Year factor, i.e., the environment, was significant only for the calcium content. It can be concluded that the content of the relevant substances is mediated only by genotypic features, given that the years were quite contrasting in their conditions according to the results of the calculated centroid distances (Fig. 1).

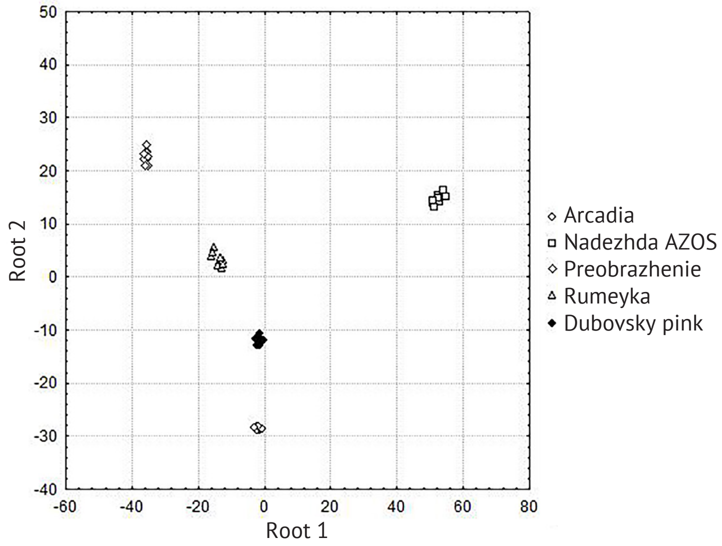

***Figure 1**. Identification of the influence of individual indicators by Variety*
***Source:** compiled by the authors*

The obtained characteristics of clusters in the factor space by canonical functions show a clear differentiation and differentiation of each of the varieties, significantly lower variability in the group than between genotypes. Each variety had its own significant features in the complex, a slight sparsity is observed only for the Rumeyka variety. Thus, with the exception of the phosphorus content, the studied features are mainly slightly variable, which indicates a significant uniformity of the studied material. In terms of calcium, magnesium, phosphorus and potassium, the Nadezhda AZOS variety prevailed, while the Arcadia variety, which showed the lowest performance in all other parameters, was also at its level in terms of sulphur content. According to factor analysis, the Variety factor was always significant, the Year factor was only in terms of calcium content, and the genotype-environment interaction was always unreliable. At the next stage, the content of valuable trace elements zinc, copper, selenium, and manganese for all five table grape varieties was analysed (Table 3).

***Table 3**. Content of table trace elements in grape berries depending on genotype (2021-2023), (x = 27, ±SD)*

Indicators	Arcadia	Nadezhda AZOS	Preobrazhenie	Rumeyka	Dubovsky pink
Zinc, mg/kg	0.17 ± 0.04^{a}	0.66 ± 0.09^{b}	0.12 ± 0.03^{a}	0.06 ± 0.01^{c}	0.07 ± 0.02^{c}
Copper, mg/kg	0.34 ± 0.04^{a}	0.47 ± 0.02^{b}	0.39 ± 0.04^{a}	0.38 ± 0.05^{a}	0.42 ± 0.05^{ab}
Selenium, µg/kg	0.12 ± 0.02^{a}	0.19 ± 0.03^{b}	0.21 ± 0.03^{b}	0.20 ± 0.03^{b}	0.28 ± 0.04^{bc}
Manganese, mg/kg	0.12 ± 0.02^{a}	0.47 ± 0.04^{b}	0.41 ± 0.05^{b}	0.38 ± 0.04^{b}	0.35 ± 0.04^{bc}

***Note:** indicates a significant difference at P < 0.05 for the Tukey's range test with Bonferroni correction. Comparison within varieties*
***Source:** compiled by the authors*

According to the results of factor analysis, the zinc content was significantly affected by the Variety factor ($F = 311.45$; $F_{0.05} = 2.66$; $P = 2.66*10^{-36}$), the Year factor was not significant ($F = 1.65$; $F_{0.05} = 2.44$; $P = 0.09$). The interaction of factors by influence was not significant ($F = 1.11$; $F_{0.05} = 3.50$; $P = 0.11$). By pairwise comparison (Tukey's range test), the Nadezhda AZOS variety was ahead with a high zinc content, next Arcadia and Preobrazhenie (they were on the same level), then Dubovsky pink and Rumeyka. The trait varies very significantly between varieties, but within the same variety it is considered low-varietal (at the level of 2-3%).

As for copper content, according to the results of factor analysis, this trait was significantly influenced by the Variety factor ($F = 27.17$; $F_{0.05} = 2.66$; $P = 7.11*10^{-5}$), the Year factor was not significant ($F = 1.87$; $F_{0.05} = 2.44$; $P = 0.08$). The interaction of factors by influence was not significant ($F = 1.42$; $F_{0.05} = 3.50$; $P = 0.09$). By pairwise comparison (Tukey's range test), the Nadezhda AZOS variety was ahead with a high copper content, at its level, but with an unreliable difference from the following varieties: Dubovsky pink, then Arcadia, Rumeyka, and Preobrazhenie (they were on the same level). The attribute varies less significantly between varieties, within the same variety, it is classified as low-varietal (at the level of 2-3%).

According to the selenium content according to the results of factor analysis, this trait was significantly affected by the variety factor ($F = 35.12$; $F_{0.05} = 2.66$; $P = 5.98*10^{-7}$), the Year factor was not significant ($F = 1.91$; $F_{0.05} = 2.44$; $P = 0.08$). The interaction of factors by influence was not significant ($F = 1.48$; $F_{0.05} = 3.50$; $P = 0.09$). By pairwise comparison (Tukey's range test), the high selenium variety Dubovsky pink was ahead of the Preobrazhenie variety, which was at its level, but with a not significant difference from the next varieties, Nadezhda AZOS and Rumeyka (which were at the same level), and Arcadia, which was significantly inferior. The attribute varies slightly between varieties, within the same variety, it is classified as low-varietal (at the level of 2-3%). It can be concluded that there is a slight polymorphism of the variety and a slight variability of the trait.

According to the results of factor analysis, the manganese content was significantly affected by the Variety factor ($F = 113.12$; $F_{0.05} = 2.66$; $P = 4.43*10^{-14}$), the Year factor was not significant ($F = 1.73$; $F_{0.05} = 2.44$; $P = 0.09$). The interaction of factors by influence was not significant ($F = 1.10$; $F_{0.05} = 3.50$; $P = 0.11$). In the pairwise comparison (Tukey's range test), Nadezhda AZOS, Preobrazhenie and Rumeyka were ahead of the high manganese varieties (at the same level), while the first two varieties were inferior to Rumeyka, but not to Dubovsky pink, and Arcadia was the worst. The attribute varies slightly between varieties, and within one variety it is considered low-varietal (at the level of 2-3 %). The results of the discriminant analysis showed that the copper and selenium content is less variable in terms of the component caused by the variety, while the variation in the zinc and manganese content is much more conditioned (Table 4).

***Table 4**. Results of discriminant analysis of the reliability of individual parameters for the content of trace elements*

Indicators	Wilks' criterion	Partial	F	p-level
		By varieties ($F_{critical} = 4.37$)		
Zinc, mg/kg	0.01	0.02	311.44	P < 0.01
Copper, mg/kg	0.01	0.55	7.54	P < 0.01
Selenium, µg/kg	0.01	0.40	13.40	P < 0.01
Manganese, mg/kg	0.01	0.07	115.47	P < 0.01
		By year ($F_{critical} = 2.39$)		
Zinc, mg/kg	0.98	0.99	0.02	0.98
Copper, mg/kg	0.99	0.98	0.23	0.79
Selenium, µg/kg	0.98	0.99	0.01	0.99
Manganese, mg/kg	0.98	0.99	0.01	0.98

***Source:** compiled by the authors*

The Year factor, i.e., the environment, was not significant for any of the indicators. It can be concluded that the content of the relevant substances is mediated only by genotypic features, given that the years were quite contrasting in their conditions according to the results of the calculated centroid distances (Fig. 2).

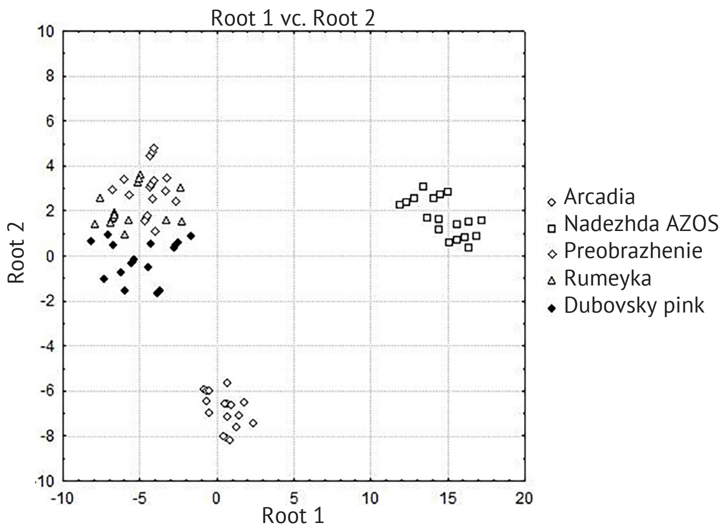

***Figure 2**. Identification of the influence of individual indicators by Variety*

***Source:** compiled by the authors*

The obtained characteristics of clusters in the factor space according to canonical functions show a clear differentiation of Arcadia and Nadezhda AZOS varieties, a fairly significant variability in the group, varieties Preobrazhenie, Dubovsky pink and Rumeyka form one group. Clusters of varieties, in contrast to the previous group of traits, are quite sparse. Thus, the studied features are mainly weakly variable, which indicates a significant uniformity of the studied material. Complex in terms of the best content of zinc (the first with a significant difference), copper and manganese, the Nadezhda AZOS variety prevailed, but with some problems regarding the selenium content, which was preceded by the Dubovsky pink variety, which is a valuable source of this trace element. According to factor analysis, the variety factor was always significant, the Year factor was never significant, and the genotype-environmental interaction was always not significant. The group of traits is characterised by a significantly lower differentiating ability. It is difficult to note a definite better option, only the shortcomings of the Arcadia variety can be noted. At the last stage, the content of biologically active components – glucose, dietary fibre, vitamins A, E, C, PP, mg – was analysed for all five table grape varieties (Table 5).

Table 5*. Content of biologically active components in table grapes depending on the genotype, recalculated per 100 g (2021-2023), (x = 27, ±SD)*

Indicators	Arcadia	Nadezhda AZOS	Preobrazhenie	Rumeyka	Dubovsky pink
Glucose, g	15.05 ± 0.34[a]	16.55 ± 0.39[b]	17.12 ± 0.43[b]	12.53 ± 0.31[c]	13.61 ± 0.32[d]
Dietary fibre, g	1.91 ± 0.24[a]	6.77 ± 0.82[b]	1.11 ± 0.24[c]	1.34 ± 0.25[as]	1.25 ± 0.25[ac]
Vitamin A, μg	3.12 ± 0.12[a]	4.34 ± 0.17[b]	3.09 ± 0.13[a]	3.45 ± 0.14[a]	3.12 ± 0.14[a]
Vitamin E, mg	0.17 ± 0.02[a]	0.11 ± 0.01[b]	0.15 ± 0.01[ab]	0.13 ± 0.02[b]	0.16 ± 0.01[a]
Vitamin C, mg	10.20 ± 0.24[a]	4.70 ± 0.11[b]	8.91 ± 0.25[c]	9.67 ± 0.22[c]	10.21 ± 0.27[a]
PP, mg	0.189 ± 0.04[a]	0.143 ± 0.03[b]	0.182 ± 0.03[a]	0.179 ± 0.04[a]	0.188 ± 0.05[a]

Note: *indicates a significant difference at P < 0.05 for the Tukey's range test with Bonferroni correction. Comparison within varieties*
Source: *compiled by the authors*

According to the results of factor analysis, the glucose content was significantly affected by the Variety factor ($F = 111.45$; $F_{0.05} = 2.66$; $P = 1.33*10^{-14}$), the Year factor was also significant ($F = 32.15$; $F_{0.05} = 2.44$; $P = 3.18*10^{-8}$). The interaction of factors by influence was significant ($F = 6.34$; $F_{0.05} = 3.50$; $P = 0.01$). By pairwise comparison (Tukey's range test), Preobrazhenie and Nadezhda AZOS were ahead of the high-glucose varieties (they were at the same level), followed by Arcadia, then Dubovsky pink, and the worst was Rumeyka. The trait varies quite significantly between varieties, within the same variety, it is classified as medium-varietal (at the level of 7-8 %, except for the Arcadia variety – 5%).

According to the results of factor analysis, the content of dietary fibre was also significantly affected by the Variety factor ($F = 345.15$; $F_{0.05} = 2.66$; $P = 6.67*10^{-43}$), the Year factor was not significant ($F = 2.25$; $F_{0.05} = 2.44$; $P = 0.06$). The interaction of factors by influence was not reliable ($F = 3.35$; $F_{0.05} = 3.50$; $P = 0.06$). By pairwise comparison (Tukey's range test), the Nadezhda AZOS variety was ahead with a higher content of dietary fibre, then Arcadia, then Dubovsky pink and Rumeyka (at the same level), and the Preobrazhenie variety was worse. The trait varies quite significantly between varieties, within the same variety, it is classified as medium-varietal (at the level of 7-10%, which indicates a significant hidden polymorphism in this parameter).

According to the results of factor analysis, the content of vitamin A was also significantly affected by the variety factor ($F = 25.17$; $F_{0.05} = 2.66$; $P = 3.47*10^{-6}$), the Year factor was more significant ($F = 52.15$; $F_{0.05} = 2.44$; $P = 1.1*10^{-18}$). The interaction of factors by influence was also significant ($F = 9.90$; $F_{0.05} = 3.50$; $P = 0.002$). By pairwise comparison (Tukey's range test), the Nadezhda AZOS variety was ahead with a higher vitamin A content, followed by all other varieties (they were at the same level). The trait varies slightly between varieties, within the same variety, it is considered slightly variable (at the level of 2-4 %, which indicates that there is no hidden polymorphism in this parameter). All this is shown in Table 6.

Table 6*. Results of discriminant analysis of the reliability of individual parameters for the content of biologically active components*

Indicators	Wilks' criterion	Partial	F	p-level
By varieties ($F_{critical}$ =4.35)				
Glucose, g	0.01	0.07	119.25	P < 0.01
Dietary fibre, g	0.01	0.02	445.69	P < 0.01
Vitamin A, μg	0.01	0.24	27.45	P < 0.01
Vitamin E, mg	0.01	0.34	16.50	P < 0.01
Vitamin C, mg	0.01	0.36	14.98	P < 0.01

Table 6. Continued

Indicators	Wilks' criterion	Partial	F	p-level
PP, mg	0.01	0.88	1.09	0.37
By year ($F_{critical}$ = 2.37)				
Glucose, g	0.58	0.37	31.36	P < 0.01
Dietary fibre, g	0.24	0.86	2.80	0.07
Vitamin A, µg	0.85	0.25	54.78	P < 0.01
Vitamin E, mg	0.22	0.95	0.95	0.39
Vitamin C, mg	0.41	0.51	17.18	P < 0.01
PP, mg	0.32	0.66	9.32	P < 0.01

Source: *compiled by the authors*

The content of vitamin E was significantly affected by the Variety factor ($F = 15.47$; $F_{0.05} = 2.66$; $P = 2.12*10^{-5}$), the Year factor was not significant ($F = 1.00$; $F_{0.05} = 2.44$; $P = 0.12$). The interaction of factors by influence was also not significant ($F = 0.71$; $F_{0.05} = 3.50$; $P = 0.22$). According to pairwise comparisons (Tukey's range test), Arcadia and Dubovsky pink varieties were ahead with a high vitamin E content, Nadezhda AZOS and Rumeyka varieties were significantly worse, and the Preobrazhenie variety occupied an intermediate position. The trait varies slightly between varieties, within the same variety, it is considered slightly variable (at the level of 2-3 %, which indicates that there is no hidden polymorphism in this parameter).

The content of vitamin C was significantly affected by the Variety factor ($F = 15.07$; $F_{0.05} = 2.66$; $P = 1.14*10^{-5}$), the Year factor was more significant ($F = 17.10$; $F_{0.05} = 2.44$; $P = 5.64*10^{-8}$). The interaction of factors by influence was also significant ($F = 9.12$; $F_{0.05} = 3.50$; $P = 0.004$). By pairwise comparison (Tukey's range test), the Arcadia and Dubovsky pink varieties were ahead with a higher vitamin C content, then there were Preobrazhenie and Rumeyka varieties, and the Nadezhda AZOS variety was worse. The trait varies slightly between varieties, within the same variety, it is considered slightly variable (at the level of 4-5%, which indicates that there is no hidden polymorphism in this parameter).

The content of vitamin PP was not significantly affected by the Variety factor ($F = 2.07$; $F_{0.05} = 2.66$; $P = 0.07$), the Year factor was significant ($F = 7.80$; $F_{0.05} = 2.44$; $P = 0.002$). The interaction of factors by influence was not reliable ($F = 2.92$; $F_{0.05} = 3.50$; $P = 0.07$). According to pairwise comparisons (Tukey's range test), the varieties Arcadia and Dubovsky pink, Preobrazhenie, and Rumeyka were ahead with a high content of vitamin PP, while the variety Nadezhda AZOS was worse. The trait varies slightly between varieties, within the same variety, it is considered slightly variable (at the level of 1-2 %, which indicates that there is no hidden polymorphism in this parameter).

Results of the discriminant analysis performed in Table 6 showed that the content of vitamins is less variable in the component due to the variety, the genotype factor did not affect the content of vitamin PP at all, and the variability in the content of glucose and dietary fibre is much more determined. The Year factor, i.e., the environment, was significant for most indicators (except for the content of dietary fibre and vitamin E). It can be concluded that the content of the corresponding substances is mediated by both varietal diversity and environmental effect, especially for the content of glucose and dietary fibre traits, given that according to the results of the calculated centroid distances, the years were quite contrasting in their conditions (Fig. 3).

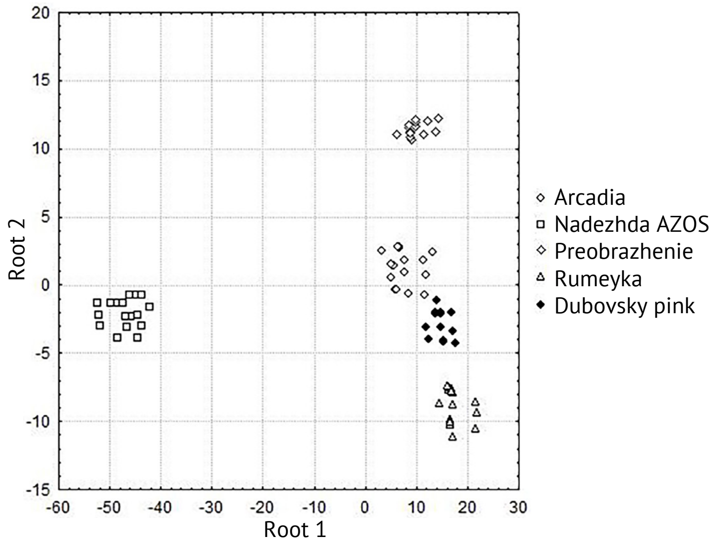

Figure 3*. Identification of the influence of individual indicators by Variety*
Source: *compiled by the authors*

However, the obtained characteristics of the clusters in the factor space by canonical functions show a clear differentiation and separation of varieties Arcadia and Nadezhda AZOS, less so of the variety Rumeyka, quite significant variability in the group, varieties Preobrazhenie and Dubovsky pink form one group. Clusters of varieties of this group of traits are quite sparse, which indicates a significant polymorphism of traits.

It was found that local material can also have a sufficient level of latent polymorphism to be used as a source of primary selection for improving certain traits, which was also partially noted earlier (mainly the first and second groups, glucose and dietary fibre traits) (Nazarenko & Simchenko, 2023). High varietal differentiation (the first group of traits) indicates the possibility of impoverishment of biodiversity with careful selection of more positive forms for full nutrition, while the lower (second-third group of traits) leads to a significant complication in the selection of more useful varieties, although in these studies it can be considered quite optimal for the second group of traits (Eyiz *et al.,* 2020).

In turn, higher variability in the variety itself by feature can lead to a more significant variation in the parameter depending on environmental conditions, which is not always positive for the producer. However, given the latest trends in the promotion of culture to the south due to global climate change, this problem can be ignored for some of the studied features (Leão *et al.,* 2020; Petretto *et al.,* 2021). The possibility of finding a variety with a high level of key characteristics and the need to supplement with other material is proved, primarily in terms of the content of necessary vitamins (Alem *et al*., 2019; Klimek *et al.,* 2022).

New varieties are being introduced faster in the raisin and table grape production sectors, but according to N. Khan *et al*. (2020), A. Atak (2024), the importance of local varietal resources for these areas cannot be ignored. There are also less valuable varieties in production, which may be appropriate to use according to marketing preferences, but they can significantly worsen the level of consumption of valuable food elements (mainly in this study this concerned the Arcadia variety). But any final recommendation needs to be clarified from the standpoint of such important features for table grapes as taste, preferences for the shape and colour of berries, processing capabilities and technological qualities of products (de Oliveira *et al.,* 2024). The consumption strategy changes significantly and promotes the introduction of more fruits in the diet. In eastern North America and Asia, fruit and aromatic flavours of V. Labrusca were actively used. Some American breeders also use this variety as a source of valuable food elements, which may be promising for local improvement (Arnold & Schnitzler, 2020).

The problem of preserving the corresponding biodiversity of the main cultivated plants is largely based on the possibility of using local varietal resources, in this case, traditional grape varieties for growing in farms in the region, which, mainly based on conservative trends in viticulture, can be created decades ago (Nazarenko *et al.,* 2022). These varieties can be the basis for improving the nutritional value of the diet and contain a high level of relevant nutrients for consumption. Moreover, as already partially noted earlier, such an advantage can be either complex or tied to the use of several varieties in production, which is all the more relevant, given the need for grapes as a crop to form a conveyor belt according to the maturation period and external characteristics (colour and shape of the berry) of its kind during the corresponding marketing period (De Palma *et al.,* 2020; Wang *et al.,* 2023).

Global warming significantly affects the strategy of agricultural development, as this industry negatively affects the intensity of forest stands and significantly increases the carbon footprint that is created during the production and sale of fruit. The system of long-term fruit production is more sustainable than the system of annual cultivation of crops, but in both cases, natural vegetation is replaced by cultivated plants, which significantly impoverishes biodiversity (Costa *et al.,* 2020; Pisciotta *et al.,* 2022).

The studied traits of vitamin content are mainly low-variable, which indicates a significant homogeneity of the studied material, the traits of glucose and dietary fibre content are medium-variable, i.e., there is a significant varietal polymorphism, which is typical for older varieties. Collectively, the best content of glucose, dietary fibre, and vitamin A was dominated by the Nadezhda AZOS variety, but there are problems with the other three vitamins. In view of this, it is a good idea to introduce the Dubovsky pink variety for this group of traits. In addition to the content of vitamin PP, the Year factor was not significant for the content of dietary fibre and vitamin E, and the genotype-environment interaction was significant for the content of glucose, vitamins A and C. The group of traits is characterised by a significant intermediate differentiating ability between the first and second. The best option is to use the Nadezhda AZOS variety in combination with Dubovsky pink.

CONCLUSIONS

The studied traits indicate a significant homogeneity of the varietal set. Only three medium-variable features can be used for primary selection to improve the source material. In terms of calcium, magnesium, phosphorus, potassium, zinc, copper, manganese, glucose, dietary fibre, and vitamin A, the Nadezhda AZOS variety prevailed, but only together with the cultivation of the Dubovsky pink variety, a comprehensive supply of useful food elements at the appropriate level is possible. The classification shows that Nadezhda AZOS and Arcadia are more unique, which indicates complex problems of Arcadia in terms of the content of necessary substances and undesirability of its use. In fact, the content of vitamin PP is not determined by the genotype, which indicates the need to introduce additional sources of this trait to local biodiversity. The characteristics of the medium were significant for the content of calcium, glucose, vitamins A, C, and PP. The genotype-environment interaction was factually derivative for traits where the influence of the environment was significant. In the future, it is planned to compare more modern and intensive varieties of table grapes according to this set of characteristics, to establish the variability of these forms in comparison and the level of necessary provision of the usefulness of the diet with varieties that are more traditional for growing by small agricultural firms by farms in the region, which mainly provide the production of table grapes for the region.

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CONFLICT OF INTEREST

None.

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Біохімічна цінність сортів винограду столового при вирощуванні в умовах Півночі Степу України

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Анотація. Дослідження можливостей використання існуючого біорізноманіття сортів винограду столового для задоволення повноцінності раціону людини є ключовим компонентом вдосконалення існуючого виробництва плодової продукції в рамках сучасних стратегій розвитку сільського господарства. Метою було встановити можливості задоволення в цінних елементах харчування потреб населення на базі традиційних сортів винограду столового, котрі переважно впроваджені у регіоні на рівні невеликих господарств та на присадибних ділянках. Досліджували п'ять сортів винограду столового Аркадія, Надєжда АЗОС, Преображеніє, Румейка, Дубовський розовий на вміст кальцію, фосфору, сірки, магнію, калію, цинку, міді, селену, марганецю, глюкози, харчових волокон, вітамінів А, С, Е, PP, обраховували залежність від факторів року та сорту, їх взаємодії, класифікуючої спроможності ознак у просторі канонічних функцій. Наявність мінеральних речовин досліджували атомно-емісійною спектрометрією, глюкози цукрометром, харчові волокна ферментативно-гравіметричними методом, вітаміни флюорометричним, вітамін С титрометричним методами. Показано можливості традиційних сортів винограду столового у задоволенню потреб у основних цінних харчових елементах та зроблено висновок щодо можливостей добору відповідних форм, котрі у комплексі цілком спроможні вдовольнити сучасні вимоги в даному аспекті розвитку сільського господарства. Виявлена переважно низька мінливість досліджених ознак, що дозволяє зробити висновок щодо гарно відтворюваності отриманого результату та переважний влив генотипу на формування відповідної ознаки в умовах контрастних за середовищними характеристиками років. Встановлено, що проблемним є поліпшення лише за однією з ознак – вмісту вітаміну PP. Низька диференціююча здатність сортів групи Дубовський розовий, Преображеніє, Румейка призводить до необхідності подальшого уточнення спроможності лише першого з сортів бути складовою у запропонованому комплексі. Рекомендовано вирощування комплексу сортів Надєжда АЗОС та Дубовський розовий

Ключові слова: виноградарство; генотип; харчова повноцінність; мікроелементи; якість продукції; біологічно-активні речовини

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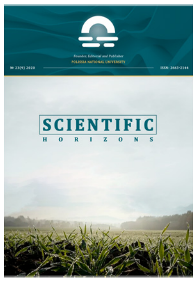

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Yield and quality of winter wheat depending on sowing dates in the Southern Steppe of Ukraine

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Abstract. Changing climatic conditions necessitate continued research to refine sowing dates for new winter wheat varieties and determine the level of their influence on productivity indicators, taking into account the specific weather conditions of the year. Therefore, the study of sowing dates, especially for innovative varieties with

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intensive initial growth, remains a relevant issue. This research aimed to determine and adapt to the conditions of the Southern Steppe of Ukraine the optimal and permissible sowing dates for new winter wheat varieties to achieve the highest possible level of realisation of their genetic potential in terms of yield and grain quality. Field experiments were conducted in 2021-2023 under the conditions of the Odesa Region. The main method used in the experiment was field research, which was supplemented by analytical studies, measurements, calculations, and observations. The harvest was recorded using a continuous threshing method with a SAMPO selection combine. It was established that the highest grain yield of winter wheat over the three-year study was achieved with sowing on 5 October. In 2021-2022, the average yield was 3.60 t/ha, while in 2022/2023, it increased to 3.90 t/ha. The highest yields were recorded for the following winter wheat varieties sown on 5 October (average for 2021-2022): Dovira Odeska – 4.03 t/ha, Katrusia Odeska – 5.16 t/ha, Pokrovska – 4.48 t/ha, Hospodarka Odeska – 4.28 t/ha, and Udacha Odeska – 4.08 t/ha (2022/2023). It was found that the grain quality of winter cereals largely depends on the year's weather conditions, variety, and sowing dates. The best results for these indicators were obtained with sowing on 5 October. However, for all winter wheat varieties, there was a tendency towards an increase in protein and gluten content in the grain when sown on 15 October. Thus, the practical value of this research lies in identifying the adaptability of new-generation winter wheat varieties to unfavourable growing conditions, realising their potential genetic yield, and determining the optimal and permissible sowing dates in the soil and climatic conditions of the Southern Steppe of Ukraine

Keywords: winter cereals; weather conditions; sowing dates; productivity; physical and chemical quality indicators

INTRODUCTION

Modern technology for growing winter cereals requires the development and implementation of new innovative solutions aimed at mitigating the negative impact of environmental factors on plant growth, development, and productivity, especially in light of the increasing challenges posed by climate change. Therefore, the response of promising and new winter wheat varieties to various abiotic conditions must be clarified, and research in this direction holds both scientific and practical interest, highlighting the relevance of this study.

O. Shevchenko (2023) in their studies highlights the threats of the climate crisis for the entire world and, in particular, for Ukraine. The author noted that in Ukraine, changes in climatic conditions are primarily manifested as an increase in average temperature, changes in precipitation, and an intensification of extreme weather events. Climate change can lead to instability in agriculture due to its impact on crop yields, the risks of extreme weather events, infrastructure, and water resources. Research into the impact of climate change on agricultural land use in Ukraine is crucial for developing effective adaptation strategies and implementing new technologies that will allow agriculture in Ukraine to maintain sustainable growth in a changing climate.

J. Dong *et al.* (2020) highlight the significant importance of winter wheat (*Triticum aestivum* L.) globally. It occupies the largest sown areas and has the highest grain production and trade. According to their findings, winter wheat accounts for 20% of human caloric needs and plays a major role in ensuring food security. Therefore, to achieve a significant increase in winter wheat yield, reduce the anthropogenic load, optimise water and resource provision, improve the environmental condition, and reduce production costs, the correct choice of sowing time is crucial.

Timely sowing is an important agronomic practice that ensures proper seed germination, stable seedling establishment, and the formation of the final winter wheat (*Triticum aestivum* L.) yield. However, delays in sowing often occur due to uncontrollable constraints, especially in repeated sowing. Long-term studies by D. Sun *et al.* (2018) have shown that climate change negatively affects winter wheat grain yields. Therefore, scientific adjustment of sowing dates can help mitigate these effects. S. Ma *et al.* (2018) and A. Ren *et al.* (2019) argue that regulating sowing dates allows for the effective utilisation of natural resources and helps prevent factors such as frost, lodging, and premature plant ageing, which can reduce yields. Research by F. Shah *et al.* (2020) found that delaying sowing beyond the optimal time may become a major obstacle to realising the genetic yield potential of winter wheat. In experiments conducted at the Sanyuan Experimental Station (34°36′N, 108°52′E) of Northwest A&F University in Shaanxi province of China, grain yield decreased by 1% for each day of delay beyond the optimal sowing date.

Sowing dates are one of the key factors in winter wheat cultivation technology, particularly in the context of climate change. Selecting optimal sowing dates reduces the risks associated with unstable weather conditions, such as droughts, frosts, or excessive rainfall, and provides plants with the necessary time to develop before the onset of winter. Early sowing dates come with their own risks, such as plant overgrowth, susceptibility to diseases, and reduced frost resistance, while late sowing dates may lead to insufficient plant development and lower yields. The optimal sowing dates should ensure that plants reach the tillering stage before winter without overgrowing, striking the best balance between winter hardiness and maximum yield. This is particularly important in the context of climate change, where weather conditions can be unpredictable. According to O. Vinyukov *et al.* (2021), deviations from the optimal sowing dates for winter wheat can significantly affect several critical aspects of its development, including plant growth, frost and winter hardiness, resistance to adverse environmental factors, plant survival, density of productive stems, and yield reduction. These factors collectively lead to yield decline. Optimal sowing dates offer the best balance between plant development and resilience to adverse conditions, which is crucial for ensuring high yields. Thus, precise selection of sowing dates is a decisive factor in ensuring winter wheat's resilience to winter conditions and optimising its productivity.

The quality of winter wheat grain is of significant importance. L. Guerrini *et al.* (2020) and D. Dilmurodovich *et al.* (2022) note that winter wheat should be characterised by high yield and meet technological quality requirements, particularly in terms of protein content, gluten content, and baking properties, which ensures its suitability for processing and consumption. K. Mitura *et al.* (2023) point out that the suitability of wheat for processing is determined by grain quality indicators, including both physical and chemical properties such as 1,000-grain weight, falling number, protein content, and crude gluten content, which directly affect the quality of the milling process and flour yield. Protein content in the grain also significantly affects the quality of the flour.

Given the analysis presented above, this study aimed to test and adapt innovative technologies for winter wheat production of modern varieties to regional conditions, to maximise the realisation of their genetic potential in terms of grain yield and quality.

MATERIALS AND METHODS

Weather conditions during the research years. Odesa Region is located in the southwestern part of Ukraine, in the steppe zone, which is characterised by a warm climate, a relatively mild winter with little snow, hot summers, and frequent dry winds. The average annual temperature is 9.0-11.0°C. The average annual precipitation is 491 mm, with about 70% falling during the warm period. The amount of precipitation varies from year to year, ranging from 263 mm to 766 mm. Weather conditions during the research years were typical for the region but varied significantly from year to year in terms of temperature regime, amount of precipitation, and its uneven distribution throughout the growing season, soil moisture reserves in the meter layer, and the presence of dry winds, which have already reached threatening levels and met the criteria for hazardous phenomena. These factors collectively influence plant growth, development, and the formation of winter wheat yield and grain quality throughout the growing season.

Hydrothermal regime indicators such as air temperature and precipitation were analysed based on data from the Odesa State Agricultural Experimental Station of the Institute of Climate-Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine. Throughout all years of the study, an elevated temperature regime was observed during the growing season, exceeding the long-term average (Table 1), which confirms the ongoing climate change towards warming. Particularly high temperatures were observed in the 2022/2023 agricultural year. In this year, the average annual temperature exceeded the long-term average by 4.3°C.

***Table 1**. Average monthly air temperature during the research years compared to the long-term average, °C*

Month	Average air temperature for the month			Long-term average
	Year of research			
	2020/2021	2021/2022	2022/2023	
September	20.7	16.1	19.6	16.6
October	16.2	10.7	16.1	10.9
November	6.0	6.5	9.8	4.4
December	4.1	2.7	6.3	1.4
January	-2.5	1.1	0.8	-3.1
February	5.1	3.8	3.0	-2.8
March	4.1	3.2	7.6	2.6
April	8.6	10.9	11.2	9.8
May	15.7	16.0	18.4	15.8
June	20.1	22.3	25.1	19.4
Average for the growing season	9.8	9.3	11.8	7.5

***Source:** data from the meteorological station of the Odesa SAES, compiled and processed by the group of authors*

The winter temperature regime did not cause the death of winter wheat plants. During this period, the air temperature was higher than the long-term average. The presence of soil moisture before sowing and in autumn, when winter wheat plants are in their growing phase, is a crucial factor in obtaining high yields. However, in recent years, after sowing winter crops in the Odesa Region, a prolonged period of high temperatures and lack of precipitation has often been observed (Table 2).

***Table 2**. Amount of precipitation over the years of research compared to the long-term average, mm*

Month	Amount of precipitation per month			Long-term average
	Year of research			
	2020/2021	2021/2022	2022/2023	
September	31.0	18.0	45.0	31.8
October	7.5	26.0	18.5	23.1
November	32.0	28.0	37.0	41.8
December	34.0	50.1	47.5	32.0
January	24.0	10.8	19.0	25.0
February	39.8	5.0	6.0	22.0
March	41.0	12.0	36.0	32.1
April	84.0	8.1	85.5	33.3
May	77.2	26.0	45.0	36.6
June	62.6	28.0	32.0	55.0
Average for the growing season	434.1	211.0	375.5	332.7

***Source:** data from the meteorological station of the Odesa SAES, compiled and processed by the group of authors*

In terms of the complex hydrothermal conditions, the 2021/2022 agricultural year was the most unfavourable for the growth, development, grain filling, and yield formation of winter wheat. During the spring-summer period, when generative organs were laid, and grain was formed and matured, there was a severe moisture deficit. The autumn of 2020/2021 and 2022/2023 agricultural years was characterised by insufficient moisture supply and elevated temperatures, which affected the growth and development of winter wheat plants. The winter period was warm, without pronounced contrasts and with uneven precipitation distribution. The spring period was characterised by abundant rains, which significantly replenished soil moisture reserves, positively influencing plant growth and development. In these years, the total amount of precipitation exceeded the long-term average by 101.4 mm (130.5% of the total) and 42.8 mm (112.9% of the total), respectively. The experimental field where the research was conducted is located on typical zonal soils – southern, non-leached, heavy loamy chernozems. The humus horizon is 50-55 cm thick, with a humus content of 3.71%. The arable layer of the soil (25 cm) has the following agrochemical characteristics: content of easily hydrolysable nitrogen – 113-138 mg/kg of soil, mobile phosphorus (according to Chirikov) – 114-131 mg/kg of soil, exchangeable potassium (according to Chirikov) – 161-184 mg/kg of soil (high and high levels of provision), sum of exchangeable bases – 300-341 mg/kg of soil, nitrification capacity according to Kravkov – 11.4 mg/kg. The soil pH in water is 7.8. In the experiment, 18 promising soft winter wheat varieties (Tables 3 and 4) developed by the Plant Breeding & Genetics Institute – National Centre of Seeds and Cultivar Investigation were used in different years.

***Table 3**. Experimental design (2021-2022)*

Variety	Year of registration	Sowing dates		
		25 September	5 October	15 October
		Plot No.		
Zhytnytsia Odeska (soft)	2016	1	11	21
Lira Odeska (soft)	2013	2	12	22
Mudrist Odeska (soft)	2015	3	13	23
Kantata Odeska(soft)	2016	4	14	24
Oranta Odeska (soft)	2017	5	15	25
Nasnaha (soft)	2015	6	16	26
Perepilka (soft)	2016	7	17	27
Knopa (soft)	2014	8	18	28
Shliakhetnyi (hard)	2017	9	19	29
Blyskuchyi (hard)	2018	10	20	30

***Source:** developed by the group of authors*

***Table 4**. Experimental design (2023)*

Variety	Year of registration	Sowing date		
		25 September	5 October	15 October
		Plot No.		
Katrusia Odeska (soft)	2016	1	11	21
Mudrist Odeska (soft)	2015	2	12	22
Fortetsia (soft)	2019	3	13	23
Udacha Odeska (soft)	2021	4	14	24
Hospodarka Odeska (soft)	2022	5	15	25
Oranta Odeska (soft)	2017	6	16	26
Peremoha Odeska (soft)	2018	7	17	27
Pokrovska (soft)	2014	8	18	28
Vyhoda Odeska (soft)	2021	9	19	29
Dovira Odeska (soft)	2020	10	20	30

***Source:** developed by the group of authors*

The experiment was replicated three times, with a total plot area of 12 m^2 and a recorded area of 10 m^2. The experiments were laid out using a randomised complete block design. Wheat was sown in all experiments from 25 September to 15 October at a seeding rate of 4.5 million viable seeds per hectare, with a sowing depth of 6-7 cm.

For the winter wheat following short fallow, surface tillage was performed using a disc harrow and cultivators with arrow-shaped tines. Harvesting was carried out using a direct combine harvester in the full maturity phase. The yield of winter wheat was determined by weighing and then adjusting to a standard moisture content of 14% and purity of 100%. The combine harvester thresher was switched off after harvesting each plot when all the grain had been completely transferred to the bag. Bags of grain were weighed, and samples were taken to determine the 1000-grain weight, hectolitre weight, protein content, and gluten content. The grain yield was weighed to an accuracy of 0.1 kg. The protein content in winter wheat grain was determined by infrared spectroscopy using a Spektran-119M device (DSTU 4117-2007, 2007). To determine the 1000-grain weight, DSTU 4138-2002 (2002) was used. The hectolitre weight of the grain was determined using a hectolitre meter (DSTU 10840-2019, 2020). The quantity and quality of gluten in the wheat grain were determined according to DSTU ISO 21415-1:2009 (2011).

Statistical processing of the research results was carried out using generally accepted methods in the field of crop science (Ermantraut *et al.,* 2007; Ushkarenko *et al.,* 2008). To analyse the results, mathematical and statistical methods were used, including analysis of variance, correlation analysis, and regression analysis, as well as logical-theoretical approaches to interpreting the obtained data. During the study, the authors also used their previous findings (Pochkolina *et al.,* 2023).

RESULTS AND DISCUSSION

Yield of winter wheat under different sowing dates. Research shows that sowing dates undoubtedly affect the yield level of winter wheat. Over three years, only three winter wheat varieties were studied (Table 5).

***Table 5**. Grain yield of winter wheat varieties depending on sowing dates, t/ha (average 2021-2023)*

Variety (A)	Sowing date (B)			Average
	25 September	5 October	15 October	
Fortetsia	2.92	3.21	3.25	3.13
Pokrovska	3.15	3.87	2.93	3.32
Dovira Odeska	3.72	3.99	3.71	3.81
Average	3.26	3.69	3.30	3.42
%, before sowing on 25 September	100.0	113.2	101.2	
$LSD_{0.95}$, t/ha	**A**-0.09; B-0.09			

***Source:** developed by the group of authors based on the conducted research*

Analysis of the research results shows that the highest yield – 3.69 t/ha – was obtained with the second sowing date (5 October). With sowing on 25 September and 15 October, almost the same yield was harvested, and the difference in yield was insignificant. On average over 3 years, the highest yield was formed with sowing on 5 October for the variety Dovira Odeska – 3.99 t/ha. With sowing on 25 September, the grain yield of winter wheat was 13.2% lower and with sowing on 15 October – 11.8% lower compared to sowing on 5 October. The averaged data on the yield of 10 winter wheat varieties for 2021-2022 shows that the optimal weather conditions for plant growth and development, for grain formation, were formed with sowing on 5 October for winter wheat (Table 6).

***Table 6**. Grain yield of winter wheat varieties depending on sowing dates, t/ha (average 2021-2022)*

Variety (A)	Sowing date (B)			Average
	25 September	5 October	15 October	
Zhytnytsia Odeska	3.66	3.68	3.46	3.60
Lira Odeska	2.82	3.33	3.15	3.10
Fortetsia	2.83	3.28	3.24	3.12
Palitra	3.65	3.80	3.57	3.67
Liha Odeska	3.10	3.65	2.97	3.24
Nasnaha	3.31	3.50	3.21	3.34
Veteran	3.08	3.24	2.84	3.05
Pokrovska	3.04	3.57	2.68	3.10
Storytsia	3.79	3.90	3.47	3.72
Dovira Odeska	3.84	4.03	3.78	3.88
Average	3.31	3.60	3.24	3.38
%, before sowing on 25 September	100.0	110.9	97.0	
$LSD_{0.95}$, t/ha	**A**-0.08; B-0.08			

***Source:** developed by the group of authors based on the conducted research*

With a sowing date of 5 October, the highest average yield of 3.60 t/ha was obtained across the 10 varieties studied. This represents a 10.9% increase compared to the first sowing date (25 September) and a 1% increase compared to the third sowing date. Earlier and later sowing dates (25 September and 15 October) can be considered acceptable. The difference in yields obtained with sowing on 25 September (3.31 t/ha) and 15 October (3.24 t/ha) is insignificant, meaning that the yield for these sowing dates is the same. The highest yield was formed with sowing on 5 October for the following varieties: Dovira Odeska (4.03 t/ha), Storytsia (3.90 t/ha), Palitra (3.80 t/ha), and Zhytnytsia Odeska (3.68 t/ha). The lowest yield was formed in the wheat variety Veteran (3.24 t/ha). The research shows that different winter wheat varieties, registered at different times and requiring different vernalisation and photoperiod conditions, respond differently to the same abiotic conditions within each sowing date.

***Table 7**. Grain yield of winter wheat varieties depending on sowing dates, t/ha (2022)*

Variety (A)	Sowing date (B)			Average
	25 September	5 October	15 October	
Katrusia Odeska	4.73	5.16	4.58	4.82
Mudrist Odeska	3.29	3.28	3.26	3.28
Fortetsia	3.10	3.09	3.26	3.15
Udacha Odeska	3.67	4.08	3.88	3.88
Hospodarka Odeska	3.89	4.28	3.90	4.2
Oranta Odeska	3.62	3.81	3.72	3.72
Peremoha Odeska	3.39	3.69	3.67	3.58
Pokrovska	3.37	4.48	3.43	3.76
Vyhoda Odeska	2.84	3.16	2.76	9.2
Dovira Odeska	3.50	3.92	3.58	3.67
Average	3.54	3.90	3.60	3.68
%, before sowing on 25 September	100.0	110.2	101.7	
$LSD_{0.95}$, t/ha	**A**-0.09; B-0.09			

***Source:** developed by the group of authors based on the conducted research*

The data in the table indicate that the highest yield of 3.90 t/ha was also achieved with the second sowing date (5 October). The difference in yield between the sowing dates of 15 October and 5 October across the ten varieties is 0.30 t. Compared to the sowing on 25 September, the increase in yield is 0.36 t. The difference in yield between the sowing dates of 25 September and 15 October was not 2% compared to the 25 September sowing, but was higher by 8.3% compared to the 15 October sowing, as mathematically demonstrated. The yield differences among certain varieties averaged across all sowing dates are not significant (Oranta Odeska – 3.72 t/ha and Pokrovska – 3.76 t/ha; Oranta Odeska – 3.72 t/ha and Dovira Odeska – 3.67 t/ha). The highest yields on 5 October were produced by the following varieties: Katrusia Odeska (5.16 t/ha), Pokrovska (4.48 t/ha), Hospodarka Odeska (4.28 t/ha), and Udacha Odeska (4.08 t/ha). The minimum yield was recorded for the wheat variety Fortetsia at 3.09 t/ha.

Among scientists, there are conflicting conclusions regarding sowing dates. V. Tkachuk and T. Timoshchuk (2020) demonstrated that under the conditions of Polissia, the maximum yield of winter wheat (3.56 t/ha) was achieved when sown on 10 September. In contrast, sowing on 10 October resulted in a decrease in grain yield by 1.02 t/ha. According to S. Shakaliy *et al.* (2020), in the Poltava region, the highest yield (5.36 t/ha) was provided by the variety Bohemiia when sown on 20 September, and the variety Kosovytsia yielded 5.29 t/ha when sown on 30 September, due to differing levels of weather conditions and sowing dates. Furthermore, the sowing dates influenced both the total and productive number of stems, with the highest figures observed in the experimental varieties sown on 30 September, while the lowest were recorded for sowing on 10 September.

According to the research conducted by S. Yaroshenko (2020) in the northern subzone of the Steppe, shifting sowing dates both earlier (5 September) and later (10 October) resulted in an average decrease in winter wheat yield by 0.36 t/ha and 0.56 t/ha, respectively. In analysing the impact of sowing dates, H. Chuhrii (2021) emphasises that despite the varying duration of autumn vegetation, the highest yields were achieved by plants sown on the same date – 25 September. For instance, in the unfavourable year of 2016, the average yield across three varieties was 35.8 centner/ha, while in another unfavourable year, 2020, it was 34.1 centner/ha. In contrast, the favourable year of 2018 yielded an average of 45.8 centner/ha, and 44.3 centner/ha in 2019, which was also favourable. V. Petrychenko *et al.* (2021), who conducted experiments at the "AgroExpressService" farm in the Mlyniv District of the Rivne Region, noted that the highest yields (9.22 t/ha and 9.43 t/ha) for the winter wheat variety Kubus were achieved with sowing on 30 September. High yield figures were also observed when sowing occurred between 20 September and 10 October. When sowing is delayed until November-December, yields of 7–8 t/ha can still be obtained. These findings are corroborated by data presented in the studies of other researchers, indicating their reliability and consistency with existing scientific principles.

A. Krivenko and S. Pochkolina (2021) concluded from their experiments that the highest yields were obtained from sowing on 5 October for all varieties of winter wheat under study. In this case, the yield was 15.5% higher compared to sowing on 25 September, 19.1% higher than on 15 October, and 27.5% higher than on 25 October. Based on the results of their research, I. Pravdziva *et al.* (2020) established a general trend of decreasing yield with the shift of sowing dates from 26 September to 16 October. However, regarding the number of genotypes for specific predecessors, the optimal sowing date was identified as 5 October following green manure fallow for the varieties Trudivnytsia Myronivska, MIP Assol, and MIP Dniprianka, after mustard for Vezha Myronivska, after sunflower for MIP Fortuna, and after maize for both MIP Fortuna and Podolianka. Using the GGE biplot, it was established that the second sowing date after a green manure predecessor was closest to the "ideal environment" for realising the yield level of most genotypes.

J. Liu *et al.* (2023) from the Hebei Gucheng Agricultural Meteorology National Observation and Research Station in Baoding, Hebei Province (China), state that to ensure high and sustainable yields of winter wheat, the optimal sowing period varied from 1 to 10 October. V. Kyrylenko *et al.* (2023) conducted experiments at the V.M. Remeslo Myronivka Institute of Wheat of the NAAS of Ukraine from 2018/19 to 2020/21. The results indicated that the variety MIP Yuvileina produced the highest yield for the first sowing date (5.52 t/ha and 6.24 t/ha after preceding sunflower and soybean, respectively), while the variety MIP Fortuna yielded the highest for the second sowing date (5.46 t/ha after preceding soybean). The average yield of winter wheat varieties was higher when sown on 25 September and after the soybean predecessor. The maximum yield (5.52 t/ha and 6.24 t/ha) for sowing on 25 September was produced by the variety MIP Yuvileina after sunflower and soybean predecessors, respectively, while for sowing on 5 October, MIP Fortuna achieved 5.46 t/ha after the soybean predecessor. The factors that had the most significant impact on the yield of winter wheat were the growing conditions of the year and the predecessor, contributing 67.8% and 20.9% respectively. The factors "variety" and "sowing date" had contributions of 3.5% and 3.0% respectively.

Therefore, to determine the level of adaptability of new-generation winter wheat varieties to adverse growing conditions and to approach their potential genetic yield, it is necessary to know the optimal and permissible sowing dates, which determine the formation of the highest grain yield of winter wheat in the spring-summer period. Sowing dates also have a certain influence on the physical characteristics of winter wheat grain. Research data shows that the hectolitre weight of most winter wheat varieties exceeded the 1st class standard of Ukraine (775 g/L) for soft winter wheat (Table 8).

Table 8. Hectolitre weight of winter wheat, g/L (average for 2021-2022)

Variety	Sowing date			Average
	25 September	5 October	15 October	
Zhytnytsia Odeska	781.0	783.0	772.0	778.6
Lira Odeska	778.0	787.0	785.0	783.3
Fortetsia	769.0	778.0	775.0	774.0
Palitra	779.0	785.0	789.0	784.3
Liha Odeska	775.0	778.0	770.0	774.3
Nasnaha	767.0	772.0	761.0	766.6
Veteran	771.0	776.0	761.0	769.3
Pokrovska	760.0	766.0	768.0	764.6
Storytsia	776.0	785.0	770.0	777.0
Dovira Odeska	785.0	792.0	781.0	786.0
Average	774.1	780.2	773.2	775.8

Source: *developed by the group of authors based on the conducted research*

According to the presented data, on average over two years, the highest hectolitre weight was observed in the following varieties: Dovira Odeska – 786.0 g/L; Liha Odeska – 784.3 g/L; and Lira Odeska – 783.3 g/L. On average, a higher hectolitre weight was recorded for the sowing date of 5 October (780.2 g/L), followed by the sowing date of 25 September (774.1 g/L). The lowest value of hectolitre weight was observed with the late sowing date of 15 October (773.2 g/L). Sowing dates in 2023 showed a variable influence on the hectolitre weight of winter wheat (Table 9).

Table 9. Winter wheat grain hectolitre weight depending on sowing dates, g/L (2023)

Variety	Sowing date			Average
	25 September	5 October	15 October	
Katrusia Odeska	760.9	766.4	769.2	765.2
Mudrist Odeska	795.0	808.2	788.0	797.1
Fortetsia	787.0	783.7	791.5	787.4
Udacha Odeska	775.2	764.7	770.7	770.2
Hospodarka Odeska	776.0	780.0	786.5	780.8
Oranta Odeska	808.0	783.3	782.2	791.2
Peremoha Odeska	790.7	804.2	797.0	797.3
Pokrovska	796.7	798.2	804.7	799.9
Vyhoda Odeska	779.0	783.0	784.2	782.1
Dovira Odeska	768.2	802.0	778.5	782.9
Average	783.7	787.4	785.3	785.5
%, before sowing on 25 September	100.0	100.5	100.2	-

Source: *developed by the group of authors based on the conducted research*

Research data indicates that the hectolitre weight of most winter wheat varieties exceeded the 1st class standard. Only a few varieties, such as Katrusia Odeska (765.2 g/L) and Udacha Odeska (770.2 g/L), had a hectolitre weight that corresponded to the 2nd class of the national standard. On average, a higher hectolitre weight was recorded for the sowing date of 5 October (787.4 g/L). The lowest hectolitre weight was observed with the first sowing date, namely 25 September (783.7 g/L). In terms of varieties, on average, the highest hectolitre weight was observed in the following varieties: Pokrovska – 799.9 g/L, Peremoha Odeska – 797.3 g/L, and Mudrist Odeska – 797.1 g/L.

Sowing dates had a varied impact on the hectoliter weight of different winter wheat varieties. For instance, varieties such as Mudrist Odeska (808.2 g/L), Peremoha Odeska (804.2 g/L), and Dovira Odeska (802.0 g/L) exhibited the highest hectolitre weight when sown on 5 October. Conversely, varieties such as Katrusia Odeska (769.2 g/L), Fortetsia (791.5 g/L), and Hospodarka Odeska (786.5 g/L), as well as Pokrovska (804.7 g/L), showed the highest hectolitre weight when sown on 15 October. The varieties Udacha Odeska (775.2 g/L) and Oranta Odeska (808.0 g/L) had the highest hectolitre weight with the first sowing date (25 September). A similar pattern was observed for the 1,000-grain weight (Table 10).

***Table 10**. 1,000-grain weight of winter wheat seeds, g (average for 2021-2022)*

Variety	Sowing date			Average
	25 September	5 October	15 October	
Zhytnytsia Odeska	41.0	42.7	41.1	41.6
Lira Odeska	40.6	41.9	40.8	41.1
Fortetsia	40.5	42.3	40.8	41.2
Palitra	39.2	39.7	39.4	39.4
Liha Odeska	36.0	36.8	34.4	35.7
Nasnaha	37.9	38.5	37.8	38.0
Veteran	39.9	39.8	38.1	39.2
Pokrovska	38.9	40.4	38.9	39.4
Storytsia	40.7	41.9	40.1	40.9
Dovira Odeska	42.0	42.9	41.1	42.0
Average	39.6	40.6	39.2	39.8
%, before sowing on 25 September	100.0	102.3	98.8	-

***Source:** developed by the group of authors based on the conducted research*

On average over two years across 10 winter wheat varieties, the highest 1,000-grain weight (40.6 g) was achieved with a sowing date of 5 October. This represented a 2.3% increase compared to the first sowing date (25 September) and a 3.6% increase compared to the third sowing date (15 October). With a sowing date of 5 October, the following varieties produced the highest average 1,000-grain weight over two years: Dovira Odeska (42.9 g), Zhytnytsia Odeska (42.7 g), and Fortetsia (42.3 g). For the sowing date of 25 September, the highest 1,000-grain weight was observed in the varieties Dovira Odeska (42.0 g) and Zhytnytsia Odeska (41.0 g). On average, the varieties Dovira Odeska (42.0 g), Zhytnytsia Odeska (41.6 g), and Lira Odeska (41.1 g) had the largest grain size. A similar pattern was observed for the 1,000-grain weight in 2023 (Table 11).

***Table 11**. 1,000-grain weight of winter wheat seeds depending on sowing dates, g (2023)*

Variety	Sowing date			Average
	25 September	5 October	15 October	
Katrusia Odeska	45.1	46.6	47.1	46.3
Mudrist Odeska	40.7	46.4	41.4	42.8
Fortetsia	45.0	45.8	45.1	45.3
Udacha Odeska	41.9	41.2	42.9	42.0
Hospodarka Odeska	42.3	43.3	40.8	42.1
Oranta Odeska	40.7	42.4	40.4	41.2
Peremoha Odeska	40.6	40.0	40.3	40.3
Pokrovska	41.9	41.9	41.5	41.8
Vyhoda Odeska	40.1	40.8	38.4	39.8
Dovira Odeska	41.3	42.0	40.7	41.3
Average	42.0	43.0	41.9	42.3
%, before sowing on 25 September	100.0	102.6	99.8	-

***Source:** developed by the group of authors based on the conducted research*

On average across 10 winter wheat varieties, the highest 1,000-grain weight (43.0 g) was achieved with a sowing date of October 5th. The 1,000-grain weight for both the first (25 September) and third (15 October) sowing dates was almost identical at 42.0 g and 41.9 g, respectively. With a sowing date of 5 October, the varieties Mudrist Odeska (46.4 g), Hospodarka Odeska (43.3 g), and Oranta Odeska (42.4 g) produced the highest 1,000-grain weight. The varieties Katrusia Odeska (47.1 g) and Udacha Odeska (42.9 g) achieved their highest 1,000-grain weight with a sowing date of 15 October. For nearly all sowing dates, the varieties Fortetsia (45.0 g, 45.8 g, and 45.1 g, respectively), Peremoha Odeska (40.6 g, 40.0 g, and 40.3 g, respectively), and Pokrovska (41.9 g, 41.9 g, and 41.5 g, respectively) had similar 1,000-grain weight values. On average, the varieties Katrusia Odeska (46.3 g) and Fortetsia (45.3 g) had the largest grain size. Results from the two-year agrochemical analysis (2021-2023) indicate that sowing dates have a significant impact on the quality of winter wheat grain (Table 12).

***Table 12**. Quality of winter wheat grain depending on sowing dates*

Sowing date	Protein content (% of dry matter) over years			Crude gluten content (%) over years		
	2021	2022	2023	2021	2022	2023
25 September	11.9	11.5	11.4	24.5	19.0	19.8
5 October	12.2	11.9	11.5	25.9	19.3	19.5
15 October	12.6	12.4	11.6	27.6	19.7	19.9
Average	12.2	11.9	11.6	26.0	19.3	19.7

***Source:** developed by the group of authors based on the conducted research*

Throughout all the years of study, a trend towards increased protein (12.6%, 12.4%, and 12.6%, respectively) and gluten content (27.6%, 19.7%, and 19.9%, respectively) was observed for the sowing date of 15 October. The lowest protein content (11.9%, 11.5%, and 11.4%, respectively) was recorded for the sowing date of 25 September. In terms of gluten, the lowest levels in 2021 and 2022 were noted for the sowing date of 25 September (24.5% and 19.0%), while in 2023, the lowest level was recorded for the sowing date of 5 October (19.8%). The highest protein content (12.6%) and gluten content (26.0%) were observed in 2021, whereas the lowest levels occurred in 2023 for protein content (11.6%) and in 2022 for gluten content (19.3%). It should be noted that over the three years of the study, with the first (25 September) and second (5 October) sowing dates, the winter wheat grain met the 3rd class of the national standard in terms of protein and gluten content. Only in 2021, with the third sowing date (15 October), did the winter wheat grain meet the 2nd class standard. The grain quality in 2022 and 2023 with a sowing date of 15 October met the 3rd class standard in terms of protein and gluten content. Regarding varieties, sowing dates had a varied impact on grain quality (Fig. 1).

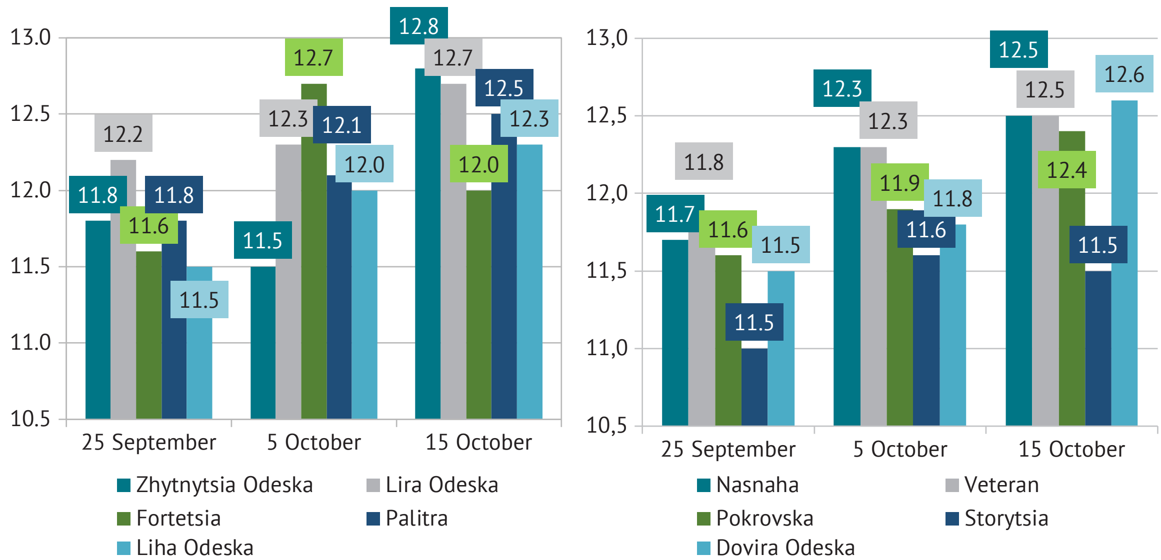

***Figure 1**. Protein content in winter wheat varieties depending on sowing dates (average for 2021-2022)*
***Source:** developed by the group of authors based on the conducted research*

Varieties such as Zhytnytsia Odeska (12.8%), Lira Odeska (12.7%), Palitra (12.5%), Nasnaha (12.5%), Veteran (12.5%), and Dovira Odeska (12.6%) exhibited the highest protein content when sown on 15 October, with their grain quality meeting the requirements of the 2nd class national standard. All varieties sown on 25 September and 5 October produced grain with protein content corresponding to the 3rd class. Varieties like Fortetsia (12.7%) and Storytsia (11.6%) produced the highest protein content when sown on 5 October. The results of the chemical analysis indicate that sowing dates in the 2022/2023 agricultural year had a negligible impact on the quality of winter wheat grain (Fig. 2). However, a general trend was observed: the higher the grain yield, the lower the protein content (assuming equal nutrient supplies). The variety Katrusia Odeska produced grain with a protein content of 10.4%, meeting the requirements of the 4th class according to the national standard when sown on the second date (5 October). For the remaining varieties, the protein content of the grain, regardless of the sowing date, corresponded to the 3rd class of the national standard.

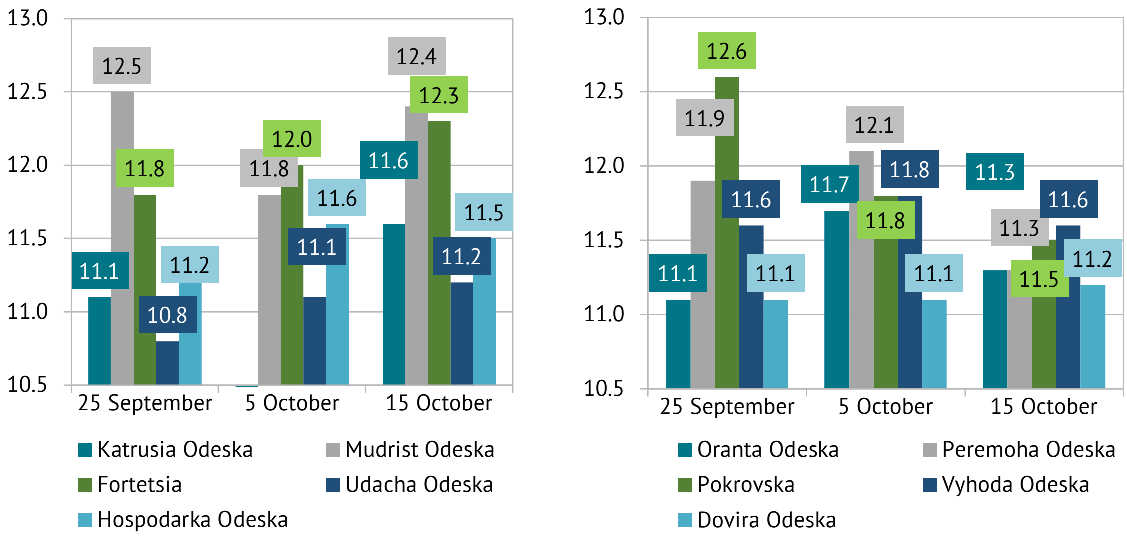

***Figure 2**. Protein content in winter wheat varieties depending on sowing dates (2023)*
***Source:** developed by the group of authors based on the conducted research*

On average across all sowing dates, the varieties Mudrist Odeska (12.2%) and Fortetsia (12.0%) produced the highest protein content. However, the pattern for gluten content is somewhat different (Fig. 3).

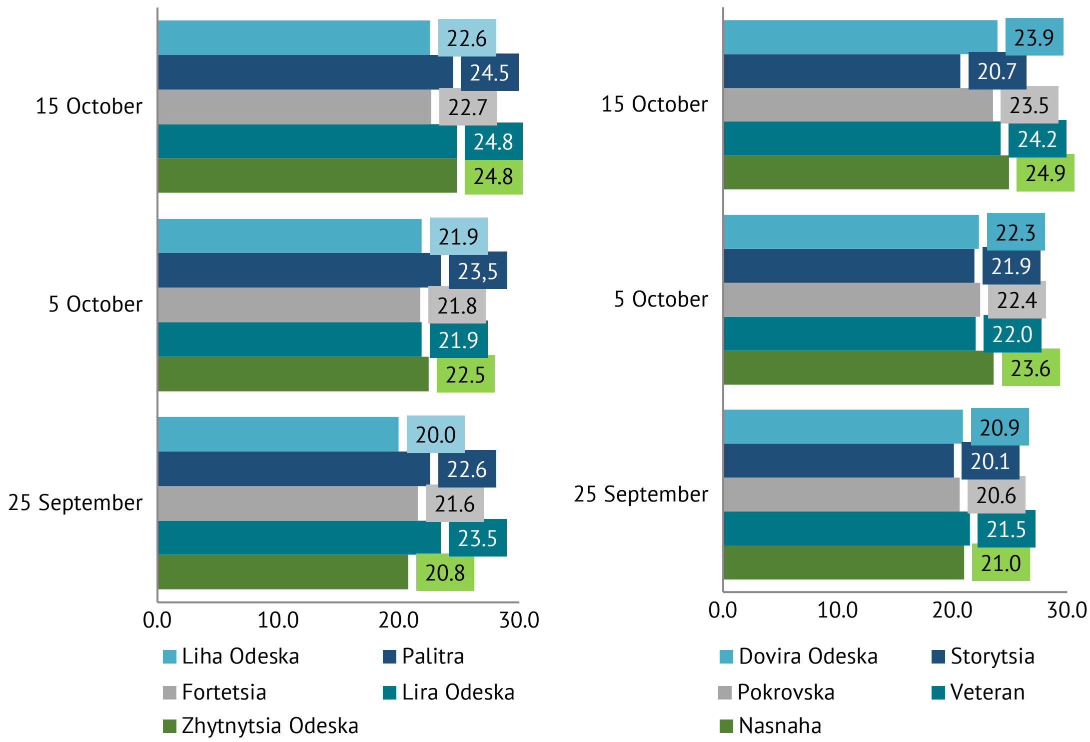

***Figure 3**. Gluten content in winter wheat varieties depending on sowing dates (average for 2021-2022)*
***Source:** developed by the group of authors based on the conducted research*

On average over 2021-2022, almost all winter wheat varieties, except Storytsia, accumulated the highest amount of gluten with a sowing date of 15 October, with the content in most varieties meeting the requirements of the 2nd class. With a sowing date of 5 October, all varieties showed a decrease in gluten content, with the amount corresponding to the 3rd class, except for the varieties Palitra and Nasnaha, where the gluten content of the grain met the 2nd class. With a sowing date of 25 September, the grain quality in terms of gluten content met the 3rd class for almost all varieties (except Lira Odeska, which met the 2nd class). In 2023, a decrease in gluten content was observed in the grain of all studied winter wheat varieties (Fig. 4).

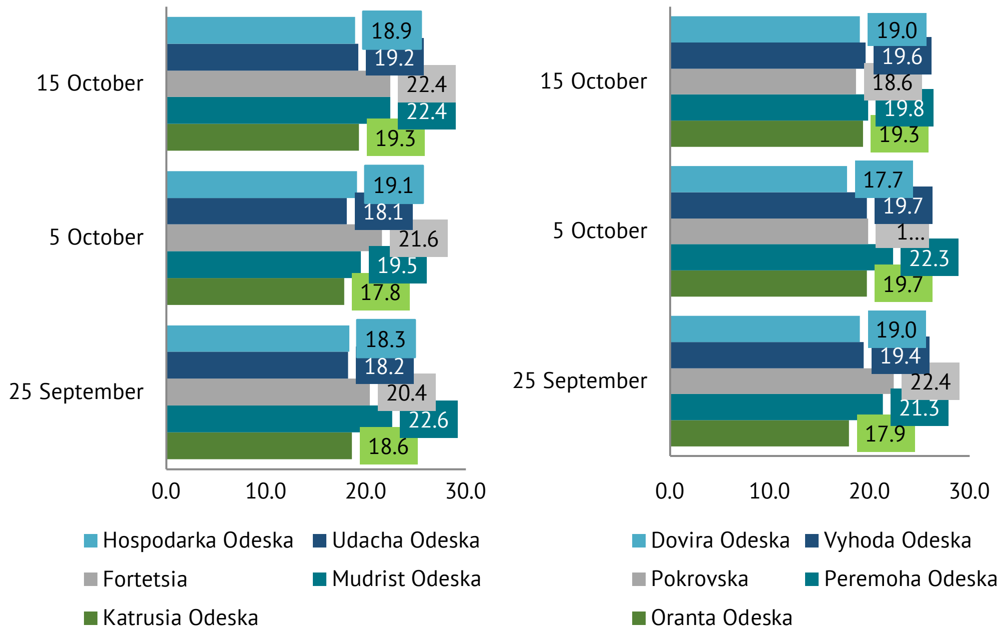

***Figure 4**. Gluten content in winter wheat varieties depending on sowing dates (2023)*
***Source:** developed by the group of authors based on the conducted research*

In terms of gluten content in the grain, all varieties, regardless of sowing date, met the requirements of the 3rd class. Only a few varieties (Katrusia Odeska and Dovira Odeska) when sown on 5 October, and Oranta odeska when sown on 25 September, produced grain with gluten content meeting the requirements of the 4th class. O. Vinyukov *et al.* (2021), based on their research, emphasised that, regardless of the variety, plants sown earliest exhibited the best biometric indicators. It was found that as the sowing dates were shifted towards later periods, the grain number per spike decreased, while the 1,000-grain weight increased.

K. Lachutta and R. Jankowski (2024) in their experiments conducted in Bałcyny (53°35′46.4˝ N, 19°51′19.5˝ E, NE Poland) determined the impact of different sowing dates on the technological quality of winter wheat grain (*Triticum aestivum* L.). The experimental variables were the sowing date (early: 6 September 2018, 5 September 2019, and 3 September 2020; delayed by 14 days: 1720 September; and delayed by 28 days: 1-4 October). A 14- and 28-day delay in sowing contributed to an increase in the total protein content of the grain (by 1% and 2%, respectively). Furthermore, a 28-day delay in sowing led to an increase in the moisture content of gluten in the grain (+0.5-0.6%) and an improvement in the quality of the protein complex in the Zeleny sedimentation test (+1.5%).

V. Spanic *et al.* (2023) concluded that, due to significant average differences for all winter wheat genotypes with the third sowing date (14 November 2022), the grain had a higher protein and crude gluten content. The lowest content of technological quality traits was observed with the second sowing date (4 November 2022). The second sowing date, compared to the first (13 October 2022), had advantages in terms of 1,000-grain weight (22.5%), protein content (1.2%), and crude gluten content (2.1%). However, it should be noted that the authors did not analyse the stability of these factors in the context of changing weather conditions, such as cultivation practices, weed infestation, and soil fertility levels, which is crucial for a comprehensive assessment of the impact of these factors on grain quality and yield. Nevertheless, from the perspective of developing agronomic science, both positive, negative, and complex results often have contradictory and debatable aspects, but despite this, they complement and deepen knowledge, contributing to the development of more substantiated elements of crop production technology, taking into account many factors such as climate change, varietal characteristics, and soil type and condition.

CONCLUSIONS

Multi-year experimental data on determining the optimal sowing dates for winter wheat indicate a trend towards later sowing, primarily due to changing weather conditions with warmer autumn temperatures and the use of varieties with a shorter vernalisation requirement. Over three years of research, the highest yields of winter wheat were obtained when sown on 5 October for most of the varieties studied: in 2021-2022, the average yield was 3.60 t/ha, which was 10.9% higher than the result of sowing on 25 September and 11.1% higher than the result of sowing on 15 October; in the 2022/2023 agricultural year, the yield was 3.90 t/ha, which is 10.2% more compared to sowing on 25 September and 8.3% compared to sowing on 15 October. The highest yields when sown on 5 October (on average for 2021-2022) were obtained by the following winter wheat varieties: Dovira Odeska (4.03 t/ha), Storytsia (3.90 t/ha), Palitra (3.80 t/ha), Katrusia Odeska (5.16 t/ha), Pokrovska (4.48 t/ha), Hospodarka Odeska (4.28 t/ha), and Udacha Odeska (4.08 t/ha) in the 2022/2023 agricultural year.

On average, the highest hectolitre weight of winter wheat was recorded with sowing on 5 October: in 2021-2022, the hectolitre weight averaged 792.0 g/L; in the 2022/2023 agricultural year, it was 787.4 g/L. The highest hectolitre weight on average was recorded for the following varieties: in 2021-2022: Dovira Odeska (786.0 g/L), Zhytnytsia Odeska (778.6 g/L), Storytsia (777.0 g/L); in the 2022/2023 agricultural year: Pokrovska (799.9 g/L), Peremoha Odeska (797.3 g/L), Mudrist Odeska (797.1 g/L). On average for 10 winter wheat varieties, the highest 1,000-kernel weight was obtained with sowing on October 5th: in 2021-2022, the 1,000-grain weight was 40.6 g; in the 2022/2023 agricultural year, it was 43.0 g. The highest 1,000-grain weight with sowing on 5 October was recorded for the following varieties: in 2021-2022: Dovira Odeska (42.9 g), Zhytnytsia Odeska (42.7 g), Fortetsia (42.3 g); in 2022/2023: Katrusia Odeska (46.6 g), Mudrist Odeska (46.4 g), Fortetsia (45.8 g).

Results of the agrochemical analysis of winter wheat grain show that in 2021-2022: most varieties produced 3rd class grain; the highest protein content (12.4%) and gluten content (23.7%) on average over two years was observed with the late sowing date (15 October). Zhytnytsia Odeska (12.8% protein and 24.8% gluten), Lira Odeska (12.7% protein and 24.8% gluten), Nasnaha (12.5% protein and 24.9% gluten), Veteran (12.5% protein and 24.2% gluten), Palitra (12.5% protein and 24.5% gluten), and Dovira Odeska (12.6% protein and 23.9% gluten) had the best indicators for protein and gluten content when sown on 15 October and met the requirements of the 2nd class standard. In the 2022/2023 agricultural year: the protein and gluten content in winter wheat grain was lower compared to previous years; most varieties produced 3rd class grain; the highest protein content (11.6%) and gluten content (19.9%) on average was observed with the late sowing date (15 October).

The data presented above confirms that there is no universal sowing date. This issue must be addressed on a case-by-case basis, taking into account a whole range of factors: weather conditions, the specific response of different varieties to sowing dates, predecessors, the availability of prepared areas for sowing, soil moisture reserves in the sowing layer, the availability of effective protection measures, the technical level of farms, and so on. Such an approach underlines the importance of a flexible and scientifically sound approach to agricultural practices, especially in the context of climate change.

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None.

CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Урожайність та якість пшениці озимої залежно від строків сівби в умовах південного степу України

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Анотація. Зміна кліматичних умов спонукають до продовження досліджень щодо уточнення строків посіву для нових сортів пшениці озимої та визначення рівня їх впливу на показники продуктивності з урахуванням особливостей погодних умов року. Тому, вивчення строків сівби, особливо сортів-інновацій з інтенсивним стартовим ростом, було і залишається актуальним питанням. Мета досліджень – визначення та адаптація до умов Південного Степу України оптимальних та допустимих строків сівби озимої пшениці нових сортів для досягнення максимально можливого рівня реалізації їх генетичного потенціалу з урожайності і якості зерна. Експериментальні досліди проводили у 2021–2023 роках в умовах Одеської області. Основний метод, який застосовувався у досліді – польовий, який доповнювався аналітичними дослідженнями, вимірами, підрахунками і спостереженнями. Облік врожаю проводився методом суцільного обмолоту за допомогою селекційного комбайну SAMPO. Встановлено, що найвищу урожайність зерна пшениці озимої за три роки досліджень одержано при сівбі 5 жовтня. У 2021-2022 роках – урожайність у середньому становила 3.60 т/га, а у 2022/2023 с.-г. році – 3.90 т/га. Виявлено, що найвищий врожай сформували (середнє за 2021-2022 рр.) при сівбі 5 жовтня такі сорти пшениці озимої: Довіра одеська – 4.03 т/га і Катруся одеська – 5.16 т/га; Покровська – 4.48 т/га, Господарка одеська – 4.28 т/га, Удача одеська – 4.08 т/га (2022/2023 с.-г. р.). Встановлено, що якість зерна озимих зернових значною мірою залежить від погодних умов року, сорту і строків сівби. Найкращі результати за цими показниками були отримані при сівбі 5 жовтня. Але, за всіма сортами пшениці озимої спостерігається тенденція до збільшення білка й клейковини в зерні при строку сівби 15 жовтня. Таким чином, практична цінність дослідження полягає у виявленні рівня адаптивності до несприятливих умов вирощування сортів нового покоління пшениці озимої та реалізації їх потенційно-генетичної урожайності а також визначення оптимальних та допустимих строків їх сівби в грунтово-кліматичних умовах Південного Степу України

Ключові слова: озимі зернові культури; погодні умови; терміни сівби; продуктивність; фізичні і хімічні показники якості

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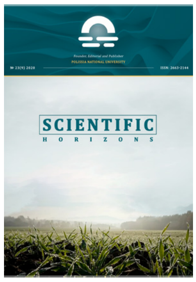

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Tillage influence on agrophysical soil properties and crop productivity in the irrigated conditions of the steppe zone of Ukraine

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Abstract. The main goal of the study was to establish the influence of various tillage systems on the agrophysical properties of dark-chestnut soil under short-grain crop rotation in the irrigated conditions of southern Ukraine. The research was carried out during 2021-2022 in a stationary experiment on a four-field crop rotation: grain maize – winter rapeseed – winter wheat – soybeans. The experimental field was in the

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Tomnytskyi, A., Hranovska, L., Lykhovyd, P., Reznichenko, N., & Kozyriev, V. (2024). Tillage influence on agrophysical soil properties and crop productivity in the irrigated conditions of the steppe zone of Ukraine. *Scientific Horizons*, 27(10), 70-78. doi: 10.48077/scihor10.2024.70.

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semi-arid steppe climate zone at the Institute of Climate-Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine. Three tillage systems were studied for their influence on soil bulk density, porosity, and water permeability, namely: mouldboard-differentiated ploughing tillage; differentiated chisel tillage; and differentiated ploughless tillage with soil slitting. Soil bulk density was determined using the core method. Soil porosity was calculated as the ratio of total bulk density to solid fraction bulk density. Water infiltration rates, established through the water absorption test method, were used to measure soil water permeability. The results of the study were statistically analysed using the common ANOVA procedure with Fisher's least significant difference test at P<0.05. In addition to the agrophysical parameters of the soil, the energy output of the crop rotation was assessed. It was established that mouldboard tillage did not provide significant benefits in terms of bulk density and soil porosity. However, ploughing showed the best results for soil water permeability across all crops in the rotation. The highest energy output of crop rotation (119.1 GJ/ha) was recorded for the ploughless-differentiated tillage system with soil slitting, whereas the mouldboard ploughing and chisel tillage systems produced somewhat lower energy yields of 112.0 and 108.6 GJ/ha, respectively. Therefore, ploughless-differentiated tillage with soil slitting is the most effective option for short-grain crop rotations in irrigated conditions of southern Ukraine, in terms of creating optimal soil agrophysical properties and achieving the highest crop productivity

Keywords: mouldboard ploughing; ploughless tillage; soil bulk density; soil porosity; water permeability; yield

INTRODUCTION

Tillage is a farming technique that uses mechanical manipulation to alter soil properties and improve conditions for crop growth and development. Tillage significantly affects the physical characteristics and functions of soil, which can influence its health and overall fertility. Enhancing soil properties and increasing crop yields through proper soil tillage is crucial, but ensuring sustainability requires careful soil tillage management. Tillage is vital for maintaining the sustainability and quality of soil as it modifies its physical, chemical, and biological properties.

The physical characteristics of soil and the viability of agriculture are greatly impacted by tillage, an essential soil management practice. Tillage affects the bulk density, porosity, and hydraulic properties of soil, which in turn influence water infiltration and retention (Steponavičienė *et al.*, 2023). Although tillage can improve soil conditions for crop emergence and yield, it also has potential drawbacks. Excessive tillage can lead to erosion and soil degradation, which harm long-term productivity (Nweke, 2018). Conservation tillage techniques have emerged as alternatives to address these challenges, balancing productivity, profitability, and environmental protection. By reducing degradation, promoting sustainable food production, and preserving soil health, these methods minimise negative environmental impacts. Selecting the appropriate tillage system is critical, as it affects not only the soil's physical health but also plant growth and the broader environment. Suitable tillage practices must be implemented to safeguard soil quality, environmental integrity, and food security (Ramzan *et al.,* 2019). Implementing scientifically proven optimal tillage practices is particularly important in regions with unstable natural humidification, such as southern Ukraine, where frequent and severe droughts, erosion, freshwater shortages, and increased aridity exacerbate soil degradation (Lykhovyd, 2021). Furthermore, tillage practices must account for the specifics of crop rotations, which to some extent determine the required parameters of agrophysical soil properties.

It was established that tillage systems in crop rotations significantly impact not only soil properties but also the overall productivity of crop rotations and the efficiency of water use. Different types of crop rotation in varying growing conditions require distinct approaches to tillage. For example, H. Sharifnasab *et al.* (2024) reported that tillage minimisation was found to be essential for improving soil water retention under wheat crops, as demonstrated by the results of a meta-analysis. No-tillage and minimal-tillage methods enhanced soil organic carbon content, total nitrogen levels, and beneficial earthworm populations when compared to standard mouldboard tillage. However, long-term studies revealed that no-till farming greatly increased weed populations and that population and that crop rotations had no effect on weed infestation. The study by A. Woźniak and M. Soroka (2018) demonstrated that in a semi-arid climate, reduced tillage (rototiller and chisel) could boost the economic profitability of crop production and decrease weed infestation compared to traditional mouldboard ploughing. M. Maliarchuk *et al.* (2021) concluded that the best agrophysical properties of dark-chestnut soil in the irrigated conditions of southern Ukraine were achieved under differentiated ploughless tillage. Moreover, this option also provided the best economic return for crop cultivation in a four-field grain crop rotation involving grain maize, sorghum, soybeans, and winter wheat. The differentiated tillage system with disc tillage at a depth of 8-10 cm was also found to be the most efficient in terms of water use (Vozhehova *et al.,* 2019). However, it is difficult to disagree with S. Khursheed *et al.* (2019) that adaptive tillage systems in Ukraine are currently insufficiently studied and, as a result, inefficient and irrational practices are still implemented, leading to the loss of organic matter, erosion, deterioration of agrophysical properties, and, consequently, a general decline in fertility. O. Menshov and O. Kruglov (2023) stressed that one of the most prominent reasons for soil degradation in Ukraine is irrational tillage, particularly, excessive ploughing. Therefore, current research must provide robust evidence in support of alternative tillage methods that are less harmful to soil health. It should also be emphasised that tillage systems should be studied in close relation to soil and climate conditions, which are changing nowadays rapidly and must take into account the water supply and the range of cultivated crops. Novel studies and approaches to soil tillage should be based on the principles of sustainability and climate-smart agricultural technologies.

Thus, the primary objective of this study was to determine the ideal tillage strategy under current climate change conditions in southern Ukraine, to optimise the agrophysical properties of dark-chestnut soil under irrigated short four-field grain crop rotation.

MATERIALS AND METHODS

The study was conducted during 2021-2022 as part of a stationary field experiment on the experimental plots of the Institute of Climate-Smart Agriculture of the National Academy of Agrarian Sciences, located in the village of Naddniprianske, Kherson Region. The location of the experiment was 46°44'34.9"N, 32°42'20.1"E. The scheme of the short grain crop rotation and the studied tillage options are presented in Table 1.

***Table 1**. Experimental scheme of the study on tillage effects on agrophysical soil properties and crop productivity in the irrigated conditions of southern Ukraine*

No.	Tillage system	Crop rotation and tillage depth, cm			
		Grain maize	Winter rapeseed	Winter wheat	Soybeans
1	Mouldboard-differentiated (standard control option)	28-30 (p)	14-16 (p)	20-22 (p)	23-25 (p)
2	Ploughless-differentiated	28-30 (c)	14-16 (c)	20-22 (c)	23-25 (c)
3	Ploughless-differentiated with slitting on 38-40 cm	10-12 (d) + 38-40 (s)	12-14 (d) + slitting aftereffect	14-16 (d) + slitting aftereffect	10-12 (d) + slitting aftereffect

***Note:** p – ploughing; c – chisel tillage; d – disc tillage; s – soil slitting*
***Source:** prepared based on the authors' research*

The study was carried out on dark-chestnut, middle loamy soil with a humus horizon depth of 40 cm, humus content of 2.3%, total nitrogen content of 0.17%, gross phosphorus content of 0.09%, and a pH of the soil ranging from 6.8 to 7.3. The climate of the zone is characterised as semi-arid steppe, and the region is considered risky for rainfed agriculture (Beck *et al.,* 2018). The study was conducted in four replications using a systematic plot distribution design. The sown area was 450 m^2, while the accounted area was 50 m^2.

Tillage was conducted using standard manufactured machines. The plant genotypes used in the study were: the variety Skadovskyi for grain maize, with a sowing rate of 80,000 pcs./ha; the variety Diona for soybeans, a sowing rate of 10 kg/ha; the variety Konka for winter wheat, with a sowing rate of 220 kg/ha (5 million pcs./ha); the variety Chornyi Veleten for winter rapeseed, with a sowing rate of 8 kg/ha (800,000 pcs./ha). Irrigation was conducted using a DDA-100-MA overhead sprinkler machine. Irrigation occurred when soil moisture dropped to 70% of the field water-holding capacity in the root zone. In general, the cultivation technology of the studied crops within the crop rotation was standard for southern Ukraine, except for the tillage options. The depth of tillage was controlled using a furrow meter, and at least 50 measurements were taken on each experimental plot. The bulk density of the soil was established using the core method in layers of 0-10, 10-20, 20-30, and 30-40 cm at the beginning of the growing season and after harvesting (Al-Shammary *et al.,* 2018). Soil porosity was determined by the equation (1):

$$P = (1 - d/dsf), \tag{1}$$

where P is porosity; d – soil bulk density, g/cm^3; dsf – bulk density of the solid fraction of the soil, g/cm^3.

Water permeability was measured using the water absorption test after three hours of exposure (Nagy *et al.,* 2013). Soil moisture was determined by the gravimetric method (Reynolds, 1970). The yield of the studied crops was established through direct harvesting using a combined harvester. The energy output of the crop rotation was calculated based on the standardised energy income from crop products (Konieczna *et al.,* 2020). Statistical data processing was performed at $P < 0.05$ using the common ANOVA procedure, with Fisher's least significant difference test (Williams & Abdi, 2010). The authors adhered to the standards of the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1979).

RESULTS AND DISCUSSION

The study's findings showed that, for the majority of the crops examined, traditional mouldboard tillage had no impact on the bulk density of the soil – with soybeans being the exception. Mouldboard ploughing resulted in noticeably improved soil loosening under soybeans at the start of the crop growing season, but this difference was negated after harvest (Table 2).

***Table 2**. Soil bulk density (g/cm$_3$) in the 0-40 cm layer under the studied crops depending on the tillage system*

No.	Tillage system	Method and depth (cm)	Crops			
			Grain maize	Winter rapeseed	Winter wheat	Soybeans
At the beginning of the growing season						
1	Mouldboard	28-30 (p)	1.28a	1.25a	1.27a	1.25a
2	Ploughless	28-30 (c)	1.29a	1.29b	1.28a	1.29b
3	Ploughless-differentiated	10-12 (c) + 38-40 (s)	1.25a	1.27ab	1.26a	1.27ab
After harvesting						
1	Mouldboard	28-30 (p)	1.29a	1.27a	1.28a	1.31a
2	Ploughless	28-30 (c)	1.31a	1.31a	1.30a	1.31a
3	Ploughless-differentiated	10-12 (c) + 38-40 (s)	1.27a	1.29a	1.28a	1.35a

***Note:** different letters indicate that the difference between the experimental variants is significant according to Fisher's least significant difference test at $P < 0.05$*
***Source:** prepared based on the authors' research*

This was also true for soil porosity, which did not significantly differ between the variants of the tillage system. Generally, soil porosity was somewhat higher under mouldboard ploughing, but this difference was insignificant and could not be attributed to the effect of the tillage system itself (Table 3).

***Table 3**. Soil porosity (%) in the 0-40 cm layer under the studied crops depending on the tillage system*

No.	Tillage system	Method and depth (cm)	Crops			
			Grain maize	Winter rapeseed	Winter wheat	Soybeans
At the beginning of the growing season						
1.	Mouldboard	28-30 (p)	51.1ab	52.0a	51.5a	52.0a
2.	Ploughless	28-30 (c)	50.6a	50.7a	50.9a	50.7a
3.	Ploughless-differentiated	10-12 (c) + 38-40 (s)	52.0c	51.3a	51.6a	51.2a
After harvesting						
1.	Mouldboard	28-30 (p)	50.7ab	51.2a	50.9a	51.3a
2.	Ploughless	28-30 (c)	49.9b	49.9a	50.4a	50.0a
3.	Ploughless-differentiated	10-12 (c) + 38-40 (s)	51.2a	50.6a	50.8a	50.3a

***Note:** different letters indicate that the difference between the experimental variants is significant according to Fisher's least significant difference test at $P < 0.05$*
***Source:** prepared based on the authors' research*

Another trend was observed regarding soil water permeability. It was established that mouldboard ploughing and the ploughless-differentiated tillage system with soil slitting provided significantly better water permeability compared to ploughless chisel tillage under grain maize. Under soybeans and winter rapeseed, mouldboard ploughing was significantly superior to both ploughless tillage systems, while ploughless-differentiated tillage with soil slitting performed better than chisel loosening. As for soil water permeability under winter wheat, mouldboard ploughing outperformed all other systems at the beginning of crop growth. However, after harvesting, it was no longer superior to the differentiated system of ploughless tillage with soil slitting. In general, it can be concluded that conventional tillage with mouldboard machines creates better conditions for water absorption in dark-chestnut soil in the semi-arid climate of southern Ukraine (Table 4).

***Table 4**. Soil water permeability (infiltration rates in mm/min) under the crops of the studied four-field crop rotation depending on tillage options*

No.	Tillage system	Method and depth (cm)	Time of measurement	
			Beginning of growing	After harvesting
		Under grain maize		
1	Mouldboard	28-30 (p)	4.4a	4.0a
2	Ploughless	28-30 (c)	3.9b	3.4b
3	Ploughless-differentiated	10-12 (d) + 38-40 (s)	4.6a	4.3a
		Under soybeans		
1	Mouldboard	28-30 (p)	4.9a	4.3a
2	Ploughless	28-30 (c)	3.7b	3.0b
3	Ploughless-differentiated	10-12 (d) + 38-40 (s)	4.2c	3.6c
		Under winter wheat		
1	Mouldboard	28-30 (p)	4.2a	3.6a
2	Ploughless	28-30 (c)	3.0b	2.3b
3	Ploughless-differentiated	10-12 (d) + 38-40 (s)	3.6c	3.0a
		Under winter rapeseed		
1	Mouldboard	28-30 (p)	3.8a	3.4a
2	Ploughless	28-30 (c)	2.6b	2.2b
3	Ploughless-differentiated	10-12 (d) + 38-40 (s)	3.2c	2.7c

***Note:** different letters indicate that the difference between the experimental variants is significant according to Fisher's least significant difference test at $P < 0.05$*
***Source:** prepared based on the authors' research*

In terms of the crop rotation's productivity and energy output, it was found that the ploughless-differentiated tillage with soil slitting produced the highest yields across all the crops studied. This also applies to the energy output of the crop rotation, which was the highest in this variant and reached 119.1 GJ/ha, while the lowest energy output was recorded under the chisel ploughless tillage – 108.6 GJ/ha. Conventional mouldboard ploughing tillage occupied an intermediate position in terms of both crop productivity and energy production (Table 5).

***Table 5**. Productivity of the crop rotation depending on tillage options*

No.	Tillage system	Indicators	Crops				
			Grain maize	Winter rapeseed	Winter wheat	Soybeans	Average for the crop rotation
1	Mouldboard-differentiated	yield, t/ha	12.69	2.62	6.51	3.56	-
		GJ/ha	209.4	52.4	116.9	69.3	112.0
2	Ploughless-differentiated	yield, t/ha	12.27	2.55	6.37	3.41	-
		GJ/ha	202.6	51.0	114.3	66.4	108.6
3	Ploughless-differentiated with slitting on 38-40 cm	yield, t/ha	13.70	2.70	6.76	3.85	-
		GJ/ha	226.2	53.9	121.3	74.9	119.1

***Source:** prepared based on the authors' research*

To summarise the results of the current study, it was established that the agrophysical properties of the soil, and soil water permeability in particular, are to some extent dependent on the tillage options, especially how the layers are mixed and turned. Tillage effects vary across different crops, which could be attributed to differences in their root systems, as it is a well-established fact that plant roots play a crucial role in the formation of physical traits in soil (Wang *et al.*, 2023). Additionally, tillage greatly affects crop productivity and energy production; therefore, it is essential to consider these impacts of soil cultivation on the final results of crop production.

Tillage is one of the major factors affecting soil agrophysical and hydraulic indicators, especially those such as soil bulk density, porosity, and water permeability. Its effects on the soil are strongly dependent not only on the machinery used but also on soil type, climate conditions, and the overall level of agricultural practice. Therefore, considerable variations in results are obtained in studies conducted under different agro-industrial conditions. Most studies claim that tillage systems significantly impact soil bulk density and related properties, such as porosity and hardness. Conventional tillage, usually involving mouldboard ploughing, generally decreases soil bulk density compared to minimal or no-till systems, particularly in the arable soil layer. For example, R. Hashimi *et al*. (2023) determined that although soil compaction occurs to some extent under zero tillage, it generally improves soil structure and increases the proportion of beneficial agrophysical aggregates in the soil. However, excessive soil compaction in some cases can pose significant hazards to normal crop growth and development, resulting in decreased yields and yield quality due to impediments to plant roots accessing water and nutrients. It was found that compacted soils reduced barley, wheat, maize, and soybean yields by 15.1%, 4.1%, 37.7%, and 27.7%, respectively, providing evidence that maize crops are the most susceptible to soil compaction (Zhang *et al.,* 2024).

Several studies have claimed that chisel ploughing can be even more effective than mouldboard ploughing in reducing soil bulk density. In this regard, disc ploughless tillage is inferior to both mouldboard and chisel ploughing, but the aspects of soil bulk density formation under the influence of tillage are also dependent on the speed of tillage machinery, which was not considered as a factor in this study (Isaak *et al.,* 2024). However, in this study, a very subtle difference was found between mouldboard and ploughless tillage options in terms of soil bulk density. Reports on the influence of tillage on crop yields are inconsistent and sometimes even contradictory. In this research, the most productive tillage option was differentiated disc ploughless tillage with soil slitting. However, D. Burger *et al*. (2023) reported different results, confirming improved yields under ploughing during dry years in Northern Germany. The difference in the study results could be due to the fact that although this research was conducted in a more arid climatic zone than Northern Germany, irrigation was applied to mitigate the effects of drought on the crops studied. The authors' hypothesis is supported by the study conducted by B. Zhang *et al*. (2023), who claimed that crop productivity under deficit irrigation could be significantly improved through deeper tillage, which provides better water-retention capacity. In this case, there was no need for the creation of water-saving storage in the soil; thus, the highest yield and energy output were obtained in the variants with conservation ploughless tillage.

As for soil water permeability, this study showed that tillage provided the best water penetration rates under all the studied crops except for maize. In general, it can be concluded that deeper tillage with soil layer rotation is the best practice to increase soil water filtration rates while minimising tillage and neglecting soil rotation results in slower infiltration rates. This theory is supported by the study conducted by Y. Qian *et al*. (2024), who compared soil permeability under conventional and no-till systems. However, no-till systems seem to be the better option for soil health in irrigated conditions, as zero tillage results in better water flux and a more beneficial soil structure in the long-term (Talukder *et al.,* 2023). As this study covered a relatively short period of two years, it is difficult to support or refute this statement.

Another important question is the effect of tillage options on overall crop productivity. A meta-analysis of European studies determined that tillage minimisation (through ridge and strip tillage methods) increased the yields of major crops by 5% compared to conventional mouldboard ploughing, while no-till systems reduced yields by 5.1% (Achankeng & Cornelis, 2023). In the US Corn Belt, satellite data analysis revealed a subtle (3.3%) increase in grain maize yields and a 0.74% increase in soybean yields under a long-term conservation (ploughless) tillage system (Deines *et al.,* 2019). These results align with the findings of the authors of this article, but it should be stressed that the ploughless tillage system with chisel ploughing was inferior to the differentiated system with disc cultivation and soil slitting.

In addition, it should be acknowledged that studying tillage must consider soil wind and water erosion hazards. Tillage-related erosion on slopes can reduce wheat and maize yields over time, and current climate change potentially exacerbates this effect (Quinton *et al.,* 2022). Conservation tillage practices, which mainly include ploughless and no-till options, provide benefits such as improved soil health, reduced erosion, and generally better soil structure, which can contribute to sustainable crop production (Kumar *et al.,* 2022). However, this statement is not universally applicable to all agro-industrial conditions, as this study demonstrated that a significantly better water infiltration capacity of the dark-chestnut soil under irrigated conditions is achieved with conventional mouldboard ploughing. However, no erosion effects were studied; therefore, it cannot be established whether ploughless tillage is superior or inferior to mouldboard ploughing.

At the same time, it must be acknowledged that in this study deep ploughing does not provide significant benefits in terms of soil agrophysical properties; therefore, excessive ploughing should be avoided. The future of agriculture lies in the rational combination of differentiated tillage systems within short- to moderate-length crop rotations, especially in the so-called bio-tillage. Consequently, experimental and theoretical research into the efficiency of tillage systems and their impact on soil agrophysical, agrochemical, agromeliorative, biological, and fertility properties should be conducted under different agroclimatic conditions in Ukraine to ensure a rational transition to innovative tillage systems in the context of the current food crisis and climate change (Lykhovyd, 2024; Pavlova & Litvinov, 2024).

To sum up, the current study demonstrates that, in general, the ploughless-differentiated tillage system with disc cultivation and soil slitting provides the best balance between the formation of optimal soil agrophysical properties and crop productivity of the irrigated steppe zone of southern Ukraine.

CONCLUSIONS

Soil bulk density was insignificantly influenced by tillage systems and fluctuated between 1.25-1.29 g/cm^3 at the beginning and 1.27-1.35 g/cm^3 at the end of the growing season for the studied grain crops. The same is true for soil porosity, which was approximately 50.0% across all the studied tillage variants. As for soil water permeability, it was established that mouldboard ploughing resulted in a significantly higher rate both at the beginning of the growing season and after harvesting under soybeans and winter rapeseed, averaging between 3.8-4.9 and 3.4-4.3 mm/min, respectively. However, in grain maize, mouldboard tillage did not statistically outperform the ploughless-differentiated tillage system, and in winter wheat crops, it performed significantly better only at the beginning of the growing season. Regarding the productivity of the crop rotation, it was established that mouldboard tillage performed better than the ploughless chisel tillage system, but was still inferior to disc tillage with soil slitting, as evidenced by the energy output of the crop rotation – 119.1 vs. 112.0 GJ/ha. Thus, there is no scientifically sound evidence supporting conventional mouldboard ploughing over the studied ploughless tillage systems on irrigated dark-chestnut soil in the short grain crop rotation. Therefore, a ploughless-differentiated tillage system with disc tillage to a depth of 10-12 cm, accompanied by soil slitting to a depth of 38-40 cm once per crop rotation, should be preferred over conventional mouldboard ploughing systems on the irrigated lands of the semi-arid zone of southern Ukraine to provide the best ratio between soil health and crop yields. Further investigations will focus on studying no-till systems in irrigated short grain crop rotations, regarding their influence on agrophysical and agrochemical soil properties and overall fertility, as well as the impact of no-till practices on crop productivity.

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CONFLICT OF INTEREST

None.

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Вплив обробітку на агрофізичні властивості ґрунту та продуктивність сільськогосподарських культур в зрошуваних умовах степу України

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Анотація. Основною метою дослідження було встановити вплив різних систем обробітку ґрунту на агрофізичні властивості темно-каштанового ґрунту в короткоротаційній зернопросапній сівозміні в зрошуваних умовах півдня України. Дослідження проводили протягом 2021-2022 рр. у стаціонарному досліді в чотирипільній сівозміні: кукурудза на зерно – ріпак озимий – пшениця озима – соя. Дослідне поле знаходилося в напівпосушливій степовій кліматичній зоні Інституту кліматично орієнтованого землеробства НААН. Досліджували три системи обробітку ґрунту з точки зору їх впливу на насипну щільність, пористість та водопроникність ґрунту, а саме: відвальний диференційований обробіток; диференційований чизельний обробіток; диференційований безполицевий обробіток з щілюванням ґрунту. Об'ємну масу ґрунту визначали за допомогою кернового методу. Пористість ґрунту розраховували як відношення загальної об'ємної маси до об'ємної маси суцільного зламу. Швидкість інфільтрації води, встановлена за допомогою методу водопоглинання, була використана як міра водопроникності ґрунту. Результати дослідження були статистично оброблені за допомогою загальноприйнятої процедури ANOVA з використанням критерію найменшої значущої різниці Фішера при $P < 0{,}05$. Крім агрофізичних параметрів ґрунту, оцінювали енергетичну продуктивність сівозміни. Встановлено, що відвальний обробіток ґрунту не має суттєвої переваги за показниками насипної щільності та пористості ґрунту. В той же час, оранка показала найкращі результати за водопроникністю ґрунту під всіма культурами сівозміни. Проте найвищий вихід енергії в сівозміні (119,1 ГДж/га) був зафіксований за безполицевої системи диференційованого обробітку ґрунту з щілюванням, тоді як відвальна оранка та чизельний обробіток забезпечили дещо нижчий вихід енергії – 112,0 та 108,6 ГДж/га відповідно. Таким чином, безполицевий диференційований обробіток ґрунту з щілюванням є найкращим варіантом для короткоротаційних зернових сівозмін в зрошуваних умовах півдня України з точки зору створення оптимальних агрофізичних властивостей ґрунту та отримання найвищої продуктивності сільськогосподарських культур

Ключові слова: полицева оранка; безполицевий обробіток; щільність складення ґрунту; пористість ґрунту; водопроникність; урожай

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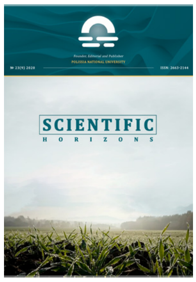

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Microbial inoculants as a means of improving soil and crop yields

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Abstract. The article presented the results of studies of the influence of microbial inoculants *Rhizobium japonicum* and *Azospirillum brasilense* on the growth and development of soybean (Glycine max) at the initial stages of its development. The study was conducted on three experimental plots: a control plot without treatment, a plot with *Rhizobium japonicum* inoculum and a plot with *Azospirillum brasilense* inoculum. The main indicators were evaluated: plant height, number of leaves, root system development and total biomass at different stages of plant growth, as well as laboratory analysis of nitrogen content in plant tissues. The results of the study showed that the inoculants had a significant impact on all measured parameters compared to

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the control plot. In particular, the plants in the plot with *Rhizobium japonicum* showed 50% higher growth and development of the root system, which was confirmed by the formation of root nodules responsible for nitrogen fixation. *Azospirillum brasilense* also improved plant growth and root system development, but its effect was less pronounced compared to *Rhizobium japonicum*. Nitrogen content analysis showed that plants in the Rhizobium japonicum plot had 45% higher tissue nitrogen content compared to the control plot, indicating effective nitrogen fixation. In plants treated with *Azospirillum brasilense*, the nitrogen content was also 25% higher, but without nodule formation, the effect was less pronounced. The aim of the study was to evaluate the effectiveness of microbial inoculants in improving plant growth and development in the early stages of vegetation, to investigate their effect on root system productivity, nitrogen uptake and total plant biomass, and to determine the optimal conditions for maximising the impact of inoculants in agricultural conditions. The results emphasised the importance of using microbial inoculants to increase soybean productivity and resistance in the early stages of its development. The application of *Rhizobium japonicum* provided a greater increase in biomass, root system and nitrogen assimilation, which makes this inoculant more effective than *Azospirillum brasilense*

Keywords: soybean; nitrogen fixation; atmosphere; climate; chemical fertilizers

INTRODUCTION

Soybeans (*Glycine max*) are one of the key crops that are actively used in the food, feed, and energy industries. Due to its high protein and oil content, it is an indispensable crop, particularly for the production of food, animal feed and biofuels. As demand for soybeans continues to grow globally, increasing its yields remains important for the agricultural sector, especially in countries where soybeans are the main crop, such as Ukraine. One of the most promising approaches to increasing soybean productivity is the use of microbial inoculants, which stimulate plant growth and improve nitrogen uptake from the atmosphere (Didur *et al.*, 2023).

The relevance of the research topic is driven by the need to increase soybean yields using environmentally friendly methods that reduce dependence on chemical fertilizers. Traditional methods often have environmental and economic limitations, so microbial inoculants such as *Rhizobium japonicum* and *Azospirillum brasilense* are a promising solution. For example, a study by A.M. Chibeba *et al*. (2020) showed that *Rhizobium japonicum* increased yields by 35-40% in the field, confirming its effectiveness. These findings confirm that ensuring optimal conditions for inoculant application can significantly affect the effectiveness of this microorganism, especially in conditions similar to those in Ukraine. J.Z. Barbosa *et al*. (2021) also confirmed that *Azospirillum brasilense* increased yields by 28%, demonstrating its versatility in different agroclimatic conditions.

The research is aimed at identifying the most effective microbial inoculants for the conditions of Ukraine, in particular the Sumy region, where the climate is temperate continental and soil types are diverse. J. Bais *et al*. (2023) demonstrated that the combined use of *Rhizobium* and *Azospirillum* can increase yields by up to 45%, indicating a possible synergy between these bacteria. Meanwhile, a study by L.B. Alessandro *et al*. (2023) showed that *Rhizobium* increased nitrogen content in plant tissues by 42%, confirming its significant effectiveness in temperate climates. F. Marcos Brignoli *et al*. (2023) investigated the effectiveness of *Azospirillum* in combination with fertilizers, showing that it increases yields by 30%, especially in conditions of limited access to chemical fertilizers. They note that this inoculant has particular potential in regions with limited access to fertilizers, making it a promising option for Ukrainian farmers.

D.M. Zeffa *et al*. (2020) focused on *Rhizobium japonicum*, confirming that it provided a 38% increase in yield. C.J. Michelon *et al*. (2020) showed that this inoculant works effectively even in dry conditions, providing a 33% increase in yield. The work of D.F. Ribeiro *et al*. (2020) confirmed that the combined use of *Rhizobium* and *Azospirillum* can reduce the use of chemical fertilizers by 30%, contributing to environmental sustainability. This is an approach that can be effectively integrated into sustainable agriculture in Ukraine, while maintaining the quality of the rationale. P.H. Da Silva Libório *et al*. (2020) proved that *Rhizobium* application increased yields by 32% and also increased soybean resilience to extreme weather conditions, which is important for regions with a changing climate. M.M. Picoli *et al*. (2022) studied the impact of *Rhizobium* and *Azospirillum* in subtropical climates, showing that they increased yields by 34-37%, confirming the effectiveness of the combination. Ongoing research shows the high potential of microbial inoculants to increase soybean yields and reduce fertilizer costs under different agro-climatic conditions. This indicates the prospects for their introduction into Ukrainian agriculture, which will allow the technology to be adapted to local conditions and contribute to the development of an environmentally sustainable agricultural sector.

The purpose of the study was to evaluate the effectiveness of microbial inoculants *Rhizobium japonicum* and *Azospirillum brasilense* in increasing soybean yield in Ukraine, in particular, in Sumy region.

MATERIALS AND METHODS

Three equal plots of 100 m^2 were selected for the study. One of the plots served as a control, where seeds were sown without pre-treatment with microbial inoculants, which allowed us to establish the main indicators of growth and yield. Only soybean seeds were treated. The second plot was sown with seeds treated with the nitrogen-fixing *bacterium Rhizobium japonicum*, which forms nodules on plant roots and helps to fix atmospheric nitrogen. The third plot was sown with seeds pre-treated with the inoculant *Azospirillum brasilense*, which stimulates the development of the root system and improves nutrient absorption, as noted by I.M. Didur (2023). Two types of microbial inoculants from different manufacturers were used. *Rhizobium japonicum* was provided by Badische Anilin- & Soda-Fabrik (BASF), Germany, a world leader in biotechnology and production of inoculants for crops. *Azospirillum brasilense* was supplied by Biotrop, Brazil, a company specializing in the development of biological products to improve crop growth and yield. This approach made it possible to compare the effectiveness of inoculants produced in different countries and assess their impact in different agroclimatic conditions, as shown in Table 1.

***Table 1**. Efficiency of microbial inoculants*

Site	Seed treatment scheme
Control plot	No seed treatment. Pure soybean seeds (Glycine max) were sown, without the use of inoculants.
Plot with *Rhizobium japonicum*	The seeds were treated with *Rhizobium japonicum* at a concentration of 10^8 CFU/g. The treatment was carried out by soaking the seeds in a solution with an inoculant concentration of 2 ml/kg of seeds for 1 hour.
Plot with *Azospirillum brasilense*	The seeds were treated with *Azospirillum brasilense* inoculum at a concentration of 10^9 CFU/ml. The treatment was carried out by wetting the seeds with the inoculant at a concentration of 3 ml/kg of seeds 30 minutes before sowing.

***Source:** created by the authors based on A.O. Rozhkov et al. (2024)*

The concentration of microbial inoculants is usually measured in colony forming units (CFU) per gram or millilitre of the product used for seed treatment. It indicates how many active bacteria are contained in one gram or millilitre of inoculant. The plot with *Rhizobium japonicum* demonstrated a significant impact of this nitrogen-fixing microorganism on soybean productivity. The application of the inoculant at a concentration of 10^8 CFU/g led to the active formation of root nodules, where atmospheric nitrogen is fixed, which provides plants with additional nutrients. The plot with *Azospirillum brasilense* showed a different mechanism of influence on plants. The high concentration of the inoculant (10^9 CFU/ml) stimulated the development of the root system, which ensured better absorption of nutrients from the soil. Prior to the experiment, the soil was analysed for fertility and acidity based on DSTU 4289:2004 (2005) and DSTU 7863:2015 (2016). For soil treatment, the complex fertilizer NPK 16-16-16 was used in all plots, which provided the same level of nutrients. The amount of fertilizer applied was 150 kg/ha, which was in line with the recommendations for growing soybeans under these conditions. The fertilizer was produced by Yara International, a leading manufacturer of fertilizers and agricultural products. The country of origin is Norway (Chudak, 2023).

All plots were irrigated using a drip system that ensured a uniform level of moisture throughout the growing season to avoid fluctuations due to uneven moisture (Iutynska *et al.*, 2022). This allowed us to focus on assessing the effect of inoculants and plant growth, minimizing the influence of extraneous factors. Water was supplied evenly, taking into account the needs of soybeans at each stage of their development, which helped maintain a stable level of soil moisture. The system controlled the amount of water supplied and the frequency of irrigation, which ensured the same conditions for each plot, regardless of weather changes (Vorobey *et al.*, 2023). This ensured optimal conditions for soybean growth and contributed to a more accurate assessment of the effectiveness of microbial inoculants (Romanko, 2021).

Data were collected at three key stages of soybean development: at the initial growth stage, during flowering and at harvest. Plant height, number of leaves, root development and total biomass were measured at each stage. After harvesting, the number of pods and seeds was counted, and the average weight of 1000 grains was determined to assess the quality of the crop. Representative samples of grains and leaves were taken for laboratory analysis of nitrogen content, which allowed us to assess the effectiveness of microbial inoculants in nitrogen fixation. The condition of root nodules was assessed only in the plot with *Rhizobium japonicum*, as this inoculant is responsible for the formation of nodules on plant roots, where atmospheric nitrogen is fixed. However, a detailed analysis of the root system was also carried out for two other plots – the control plot and the plot with *Azospirillum brasilense*.

In the control plot, where no inoculants were used, the analysis of root mass and root development allowed us to assess the standard level of nutrient and water uptake without additional biological effects. In the *Azospirillum brasilense* plot, since this inoculant does not form nodules but stimulates root growth, the assessment focused on the total weight of the roots and their ability to absorb water and nutrients. Accordingly, the analysis of root mass and development allowed assessing the effectiveness of root system stimulation by this inoculant and its impact on resource uptake (Saribekyan, 2022). In addition, retrospective tests such as the Tukey test were used to compare the results between the groups (control and plots with different inoculants) (Reinhart *et al.*, 2024). The data were processed using Statistica 3.0, which allowed us to analyse in detail the effect of each inoculant on soybean yield. This approach ensured the reliability of the results and made it possible to assess the effectiveness of microbial inoculants in specific research conditions (Jin *et al.*, 2024). The experimental studies of plants (both cultivated and wild), including the collection of plant material, were in accordance with institutional, national or international guidelines. The authors adhered to the standards of the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1979).

RESULTS

The initial growth stage of plants is an essential stage of research because it is at this stage that the foundations for future plant development, health, and productivity are laid. In agricultural practice, the initial stage of soybean growth is particularly important for the formation of a stable and productive plant capable of withstanding various stress factors during development. It is at this stage that the plant begins to actively use nutrients from the soil, including nitrogen, which is critical for the formation of protein structures and the maintenance of overall metabolism. The microbial inoculants used in this study play a key role at the initial stage of growth, as their action is aimed at activating physiological processes in plants. For example, the nitrogen-fixing *bacterium Rhizobium japonicum* begins to form a symbiotic relationship with the plant immediately after seed treatment, and this process affects the plant's ability to receive additional nitrogen from the atmosphere. In the early stages of growth, this mechanism allows plants to use the available soil resources more efficiently, which increases stability and the potential for further development.

The importance of the initial growth stage is also due to the fact that the basis for the development of the root system is laid at this stage. A strong and well-developed root system ensures that the plant will be able to absorb water and nutrients better throughout the growing season (Ismayilzada *et al.*, 2023). Microbial inoculants, such as *Azospirillum brasilense*, actively stimulate the growth of the root system, which increases the overall adaptability of the plant to stresses such as drought or lack of nutrients. Thus, the initial stage of growth determines the effectiveness of inoculants and lays the foundation for optimal plant development (Table 2).

***Table 2**. Initial growth stage*

Parameter	Control plot	*Rhizobium japonicum*	*Azospirillum brasilense*
Plant height (cm)	12	18	16
Number of plants	5	7	6
Root system development (d)	8	12	10
Total biomass (g)	20	30	28

***Source:** created by the authors based on N. Rehan et al. (2024)*

The analysis of the data showed that the inoculants had a positive effect on all measured parameters compared to the control plot. The height of plants in the plot treated with *Rhizobium japonicum* was 50% higher than in the control plot, and 33% higher in the *Azospirillum brasilense* plot. This indicates that both inoculants promoted more intensive plant growth at the initial stage of development. The number of leaves also increased in both plots with microbial inoculants, with 40% more leaves observed in the *Rhizobium japonicum* plot and 20% more in the *Azospirillum brasilense* plot compared to the control plot. This result suggests that the inoculants improved the photosynthetic activity of the plants, contributing to a greater accumulation of energy for growth.

The development of the root system in the plots treated with inoculants was significantly better. *Rhizobium japonicum* provided a 50% increase in root mass compared to the control, due to its ability to form root nodules for nitrogen fixation. *Azospirillum brasilense* showed a slightly lower effect, increasing root mass by 25%, but its effect was also significant because this inoculant stimulated root growth through the production of phytohormones. The total plant biomass was 50% higher in the *Rhizobium japonicum* plot and 40% higher in the *Azospirillum brasilense* plot compared to the control. This indicates a general improvement in the condition of the plants treated with inoculants, which makes it possible to expect an increase in yield at further stages of development.

A significant increase in nitrogen content was observed in the area with *Rhizobium japonicum*. The results showed that the nitrogen level in the plants of this zone was 35-40% higher than in the control group. This indicates that the bacteria effectively fixed nitrogen and provided it to the plants (Olougou *et al.*, 2024).

More developed root nodules could be visually observed, which confirmed the successful symbiotic interaction between *Rhizobium japonicum* and the plant. The high nitrogen content contributed to the active growth of plants and the formation of more fruits, which indicates the effectiveness of this inoculant in the study. The results obtained indicate that the use of inoculants significantly affects the initial stages of soybean development. *Rhizobium japonicum* provided a greater increase in plant growth and biomass compared to *Azospirillum brasilense*. This result can be explained by the ability of *Rhizobium japonicum* to form root nodules, which ensures effective nitrogen fixation from the atmosphere, while *Azospirillum brasilense* mainly focuses on stimulating the root system, which also has a positive effect on plant growth, but less pronounced.

The control plot, where the seeds were not treated with inoculants, showed significantly lower growth and development rates, which underlines the importance of using microbial inoculants to improve soybean productivity. The data from the initial growth stage confirms that the use of biological products provides significant benefits to plants at early stages of development, which can lead to higher yields at later stages. The flowering stage is a critical period in the development of soybean (*Glycine max*), as it directly affects future yields and seed quality (Kiurchev *et al.*, 2020). This is the period when the potential of the crop is laid, as the number of flowers and the success of pollination determine the number of pods and, consequently, the overall yield of the crop. The efficient development of plants at this stage indicates that they receive sufficient nutrients and water, which allows them to form a sufficient number of flowers and pods. In the study of the use of microbial inoculants, in particular *Rhizobium japonicum* and *Azospirillum brasilense*, it was important to assess the impact on plants during the flowering stage, as this period is crucial for the productivity of the entire plant. Nutrient or water deficiencies at this time can lead to a decrease in the number of flowers and their death, and thus a decrease in future yields. Accordingly, the effectiveness of inoculants can be assessed through the impact on the number and quality of flowers, pods, and seeds.

One of the key reasons for the importance of the flowering stage is that at this time, the processes of protein synthesis and the formation of organic compounds, which are necessary for the development of pods and seeds, are actively underway. Plants spend a lot of energy and resources to ensure flowering, so it is at this stage that there is a need for optimal nutritional conditions, including nitrogen and other crucial macro- and microelements. The use of inoculants helps to improve the nitrogen nutrition of plants, which is crucial for increasing the number of flowers and improving their condition. At the flowering stage, the same parameters were measured: plant height, number of leaves, root system development, and total biomass. In addition, the number of flowers was estimated, which is an important indicator of future yield. The data obtained are presented in Table 3.

***Table 3**. Stages of flowering*

Parameter	Control plot	*Rhizobium japonicum*	*Azospirillum brasilense*
Plant height (cm)	40	55	50
Number of leaves	20	28	25
Root system development (d)	25	35	30
Total biomass (g)	80	120	110
Number of flowers	15	25	22

***Source:** created by the authors based on Agriculture, forestry and fisheries (2024)*

Analysing the data obtained, it can be seen that at the flowering stage, inoculants continue to have a positive effect on plant growth and development. Compared to the control, the height of plants in the plot with *Rhizobium japonicum* increased by 37.5%, and in the plot with *Azospirillum brasilense* by 25%. The number of leaves was also higher in both inoculation sites: *Rhizobium japonicum* resulted in a 40% increase and *Azospirillum brasilense* in a 25% increase. This indicates that both inoculants improve the photosynthetic capacity of plants, which increases their overall productivity.

As for the root system, *Rhizobium japonicum* showed the best results with a 40% increase compared to the control. *Azospirillum brasilense* also increased root weight, but its effect was less pronounced, showing a 25% increase. The number of flowers in the plot with *Rhizobium japonicum* was 66% higher than in the control plot, and 46% higher than in the plot with *Azospirillum brasilense*. This is an important indicator, as the number of flowers directly affects the potential yield. The total plant biomass was also 50% higher in the plot with *Rhizobium japonicum* and 37.5% higher in the plot with *Azospirillum brasilense*. At the harvest stage, the number of pods and seeds was counted and the average weight of 1000 grains was determined for each plot. The data obtained are presented in Table 4.

***Table 4**. Harvesting stage*

Parameter	Control plot	*Rhizobium japonicum*	*Azospirillum brasilense*	Standard deviation (control)	% increase (*Rhizobium japonicum*)	% increase (*Azospirillum brasilense*)
Plant height (cm)	45	55	50	2	22.2	11.1
Number of leaves	15	20	18	1	33.3	20.0
Root system development (d)	25	35	30	3	40	20
Total biomass (g)	80	110	100	4	37.5	25

***Source:** created by the authors based on M.N.E. Olougou et al. (2024)*

The analysis of the data showed that at different stages of plant development, the use of microbial inoculants had a positive effect on the growth and development of soybeans. The height of plants in the *Rhizobium japonicum* plot increased by 22.2% compared to the control plot, while the *Azospirillum brasilense* treatment provided an increase of 11.1%. This indicates that both inoculants promoted plant growth, but *Rhizobium japonicum* was more effective in promoting height. The number of leaves also increased significantly in the treated plots. In particular, the *Rhizobium japonicum* plot showed a 33.3% increase in the number of leaves, while the *Azospirillum brasilense* plot showed an increase of more than 20%. This confirms the better effect of *Rhizobium japonicum* on the vegetative development of plants, which is important for increasing their productivity (Agriculture, forestry and fishery, 2024). The development of the root system also showed significant differences. In the plot with *Rhizobium japonicum*, the increase in root development was 40%, and in the plot with *Azospirillum brasilense* – 20%. This indicates that *Rhizobium japonicum* is more effective in promoting root formation and improves nitrogen fixation, which provides the plants with the necessary nutrients and water. However, both inoculants had a positive effect on the root system, improving the ability to absorb nutrients.

In terms of total biomass, the plot with *Rhizobium japonicum* showed an increase of 37.5%, while the plot with *Azospirillum brasilense* showed an increase of 25%. This indicates that both inoculants increased the overall plant productivity, but *Rhizobium japonicum* provided better results. The increase in total biomass is a critical indicator of the overall effectiveness of inoculants, which demonstrates their impact on plant growth and development. In order to assess the effectiveness of nitrogen fixation in different experimental plots, a laboratory analysis of nitrogen content in plant tissues was conducted. The results of the analysis showed that the nitrogen content was highest in the plot with *Rhizobium japonicum*, which confirms the high efficiency of this inoculant in nitrogen fixation. The formation of nitrogen-fixing nodules on soybean roots contributed to a significant increase in nitrogen uptake from the atmosphere, which was reflected in tissue parameters. Compared to the control plot, where no inoculants were applied, the plot with *Rhizobium japonicum* showed a 45% increase in nitrogen content.

An increase in nitrogen content was also recorded in the plot with *Azospirillum brasilense*, but to a lesser extent than in the plot with *Rhizobium japonicum*. This can be explained by the fact that *Azospirillum brasilense*, although it promotes the development of the root system, does not form root nodules and has a less pronounced nitrogen-fixing effect. The nitrogen content in this area was 25% higher compared to the control, which indicates a positive, but less pronounced effect of the inoculant. In the control plot, where the seeds were not treated with inoculants, the nitrogen content in plant tissues was the lowest, which is a natural result of the absence of microbial inoculants that promote nitrogen fixation. This highlights the need for inoculants to improve nitrogen nutrition in soybeans. Additional indicators of laboratory nitrogen analysis refer to the total amount of nitrogen absorbed per plant weight and the level of nitrogen uptake from the soil. These two indicators allow for a deeper assessment of the efficiency of nitrogen fixation and the impact of inoculants on overall plant nutrition. The data obtained for these parameters are shown in Table 5.

***Table 5**. Nitrogen content in grains and leaves*

Site	Nitrogen content (%)	Increase in nitrogen content compared to control (%)	Total amount of nitrogen absorbed (g/m^2)	Level of nitrogen uptake from the soil (%)
Control	1.8	–	10	100%
Rhizobium japonicum	2.6	+45%	14.5	80%
Azospirillum brasilense	2.25	+25%	12.5	90%

***Source:** created by the authors based on M. Cao et al. (2024)*

The analysis of the data in the table shows that the effect of microbial inoculants on the nitrogen content of soybean plants differs significantly depending on the preparation used. In the control plot, where the seeds were not treated with inoculants, the nitrogen content in plant tissues was the lowest at 1.8%. This is the baseline for comparison with other plots, as the plants in the control plot were only able to use soil resources without additional biological stimuli. The total amount of nitrogen absorbed in this plot was 10 g/m², indicating the limited ability of plants to use nitrogen efficiently without the help of inoculants. In the plot with *Rhizobium japonicum*, the results were much higher. The nitrogen content in plant tissues was 2.6%, which is 45% higher than in the control plot. This indicates the high efficiency of the inoculant in improving nitrogen nutrition of plants. The total amount of nitrogen absorbed reached 14.5 g/m², which is a significant increase. These data indicate active nitrogen fixation by *Rhizobium japonicum* bacteria, which allowed plants to obtain a significant part of nitrogen from the atmosphere. In addition, the reduction in soil nitrogen uptake to 80% indicates that some nitrogen was supplied through symbiosis with the bacteria, rather than from the soil alone.

The plot where *Azospirillum brasilense* inoculant was used also showed an improvement in plant nitrogen nutrition, although the results were less pronounced than in the plot with *Rhizobium japonicum*. The nitrogen content in plant tissues was 2.25%, which is 25% higher than in the control plot, but less than in the plot with *Rhizobium japonicum*. The amount of nitrogen absorbed reached 12.5 g/m², which indicates an average level between the control plot and the plot with *Rhizobium japonicum*. The level of nitrogen uptake from the soil remained high at 90%, indicating that *Azospirillum brasilense* has a greater impact on root development and improved nutrient uptake from the soil than on atmospheric nitrogen fixation. The analysis of root development and weight in the three experimental plots revealed significant differences depending on the inoculants used. In the control plot, where no microbial inoculants were used, the average weight of the root system was 12 g, and the length of the roots was 20 cm. These indicators are baseline and served as a comparative basis for evaluating the effectiveness of microbial inoculants in other plots. The lack of inoculants led to limited root development, which affected the ability of plants to absorb nutrients and water.

In the plot where *Rhizobium japonicum* was used, significant improvements in root development were observed. The average weight of the root system increased to 16 g, and the length of the roots reached 25 cm, indicating a positive effect of nitrogen-fixing bacteria on root growth. In addition, nodules were formed on the roots – an average of 30 nodules per plant. The average weight of the nodules was 0.8 g. These nodules perform a key function in fixing atmospheric nitrogen, converting it into a form available to plants. Thanks to this process, plants received an additional source of nitrogen, which ensured better growth and development. The 33% increase in root mass compared to the control plot also indicates an improvement in the plants' ability to absorb water and nutrients from the soil. In the plot where *Azospirillum brasilense* inoculant was applied, improvements in root development were also observed, although the effect was less pronounced compared to *Rhizobium japonicum*. The average weight of the root system in this plot was 14 g and the root length was 23 cm, which is 16% more than in the control plot. Although *Azospirillum brasilense* does not form nodules, this inoculant stimulates the growth of the root system, which allows plants to absorb nutrients and water more efficiently. The increase in root mass indicates an improved ability of plants to adapt to soil conditions, in particular to dry periods. The data obtained for the study are presented in Table 6.

***Table 6**. Analysis of the root system and its mass in three plots*

Site	Average weight of the root system (g)	Root length (cm)	Number of bulbs (pcs)	Average bulb weight (g)
Control	12	20	0	0
Rhizobium japonicum	16	25	30	0.8
Azospirillum brasilense	14	23	0	0

***Source:** created by the authors based on L. Koziol et al. (2024)*

The data analysis shows that the use of inoculants had a positive effect on the development of the root system. *Rhizobium japonicum* provided the best results, contributing not only to an increase in root mass and length, but also to the formation of nodules for nitrogen fixation, which is important for plant nutrition. *Azospirillum brasilense*, although it does not form nodules, improved root development, allowing plants to better absorb nutrients.

DISCUSSION

The use of microbial inoculants *Rhizobium japonicum* and *Azospirillum brasilense* demonstrated a positive effect on soybean productivity compared to the control plot where seeds were sown without treatment. In the area treated with *Rhizobium japonicum*, there was a significant increase in yield by 35-40%. This indicates the effectiveness of the inoculant in the moderate climatic conditions typical for the Sumy region. A significant increase in the number of root nodules indicates a successful symbiosis between bacteria and plants, which allowed plants to absorb nitrogen from the atmosphere more efficiently. The analysis of plant tissues showed an increased level of nitrogen, which confirms active nitrogen fixation.

J. Li *et al.* (2022) analysed the results of studies from 2010 to 2024 in a meta-analysis and confirmed that microbial inoculants contribute to a 30-40% increase in yield. These data are generally consistent with the results, where *Rhizobium japonicum* provided a 35-40% increase in yield. This indicates the stability and versatility of this inoculum in different agroclimatic conditions. M. O'Callaghan *et al.* (2022), however, identified certain limitations in the use of inoculants, especially in dry climates and insufficient soil moisture. They noted that the effectiveness of such biological products can be reduced by 15-20% in adverse conditions when the soil does not receive enough moisture. This is in line with the findings that optimal irrigation conditions are required for maximum efficiency. The plot with *Azospirillum brasilense* also showed positive results, but the effect was less pronounced compared to *Rhizobium japonicum*. The 25-30% increase in yield indicates that this inoculant improves root growth and overall plant development. Plants in this area had a more developed root system, which probably contributed to increased drought tolerance and better absorption of nutrients from the soil, as indicated by R. Sammauria *et al.* (2022).

L. Canfora *et al.* (2021) focused on scientific trends in the use of inoculants and noted that inoculants that are adapted to local plant strains are most effective. They also pointed out that commercial strains may not achieve the expected results if soil and climate conditions are not taken into account. In the present study, standardized inoculants were used, which confirm the overall effectiveness of *Rhizobium japonicum*, but point out the importance of adapting inoculants to the specific conditions of the Sumy region. A comparison of the experimental plots with the control plots confirmed that microbial inoculants significantly increase soybean yields. The positive impact of inoculants on yield is evidenced by an increase in the number of pods and grains per plant and an increase in the average weight of 1000 grains. It is important to note that *Rhizobium japonicum* was more effective as it not only stimulated plant development but also significantly increased the level of nitrogen in plant tissues. A.S.M. Elnahal *et al.* (2022) found that inoculants can have a significant impact on improving nitrogen fixation in the root system of plants, but their effectiveness is highly dependent on the soil type. They also noted that in soils with a high organic matter content, the effectiveness of inoculants can increase by 10-15%, which is consistent with the data obtained in areas with neutral acidity and rich organic matter content. However, the effectiveness of microbial inoculants may vary depending on climatic conditions and soil type (Mamchur & Studinska, 2024). Although the results of this study show that *Rhizobium japonicum* is very effective in temperate climates, it may be less effective in other conditions, such as tropical or subtropical climates. Therefore, it is important to adapt inoculant recommendations to regional conditions, taking into account soil type, climatic features and soybean varieties grown. M.M. Saad *et al.* (2020) conducted experiments with different strains of microorganisms and found that effectiveness depends on the combination with other factors such as phosphorus and potassium. They also recommend combining inoculants with mineral fertilizers for better results. The results of using inoculants in natural conditions without additional fertilizers showed that microbial preparations can increase yields on their own, but the use of fertilizers could enhance this effect.

From an economic point of view, the use of microbial inoculants has significant potential. Reducing the need for chemical nitrogen fertilizers helps to reduce costs for farmers and also has a positive impact on the environment (Gamayunova & Sydiakina, 2023; Shahini *et al.*, 2024). The use of such inoculants helps to preserve soil fertility and prevents the contamination of water sources, making it an important element of sustainable agriculture. Y. Chen *et al.* (2021) studied the use of inoculants on wheat in soils with high phosphorus content and found that nitrogen-fixing bacteria significantly increased the availability of nitrogen and phosphorus to plants. Although the study included a different crop, their findings confirm that microbial inoculants can effectively improve plant nitrogen nutrition under appropriate conditions, which is consistent with our results for soybeans.

Further research on the use of microbial inoculants to increase soybean yields has broad prospects and can significantly expand knowledge about effective agricultural technologies. Firstly, it is important to study the effectiveness of different strains of microorganisms in different agroclimatic conditions. As our experiments have shown, the results can vary significantly depending on the type of soil, level of irrigation and climatic features of the region. In this context, it is advisable to conduct similar experiments in areas with different soil conditions and moisture levels to determine the optimal conditions for the use of *Rhizobium japonicum* and *Azospirillum brasilense*. This will help to determine which strains are suitable for specific regions of Ukraine, including areas with arid climates or acidic soils. M.S. Santos *et al.* (2021) found that the use of inoculants in combination with pesticides can significantly reduce their effectiveness due to chemical incompatibility. This aspect is essential for further research in the Sumy region, as the impact of pesticides was not taken into account in our experiments. Further research should focus on studying the compatibility of inoculants with different chemicals to ensure crop stability.

Another perspective is to study combinations of inoculants with other biological agents, such as mycorrhizal fungi or phosphate-mobilising bacteria. Conducting experiments with different combinations of inoculants can help identify the most effective options for Ukrainian conditions and reduce dependence on chemical fertilizers. This approach will help preserve soil fertility and reduce environmental impact, which is an important aspect of sustainable agriculture. M. Shen *et al.* (2021) focused on the impact of inoculants on bacterial communities in the maize rhizosphere. They found that inoculants can improve soil microbial diversity, which contributes to soil fertility. Although the study did not include soybeans, the results show that microbial inoculants have the potential to improve the overall soil ecosystem, which is consistent with the findings of increased soil nitrogen.

Another perspective is to investigate the impact of inoculants on crops other than soybeans. The use of *Azospirillum* and *Rhizobium* in combination with other legumes or cereals could help determine whether these inoculants are versatile and suitable for a wide range of plants. This will open up opportunities to expand the use of biotechnology in agriculture, allowing farmers to optimize production processes and increase productivity. G. Bizos *et al.* (2020), in a study of olive trees, also showed that inoculants can improve plant growth and productivity, but only under conditions of sufficient moisture and nutrients. This aspect is consistent with the findings on the importance of irrigation and adequate nutrient levels for the effectiveness of inoculants in soybean cultivation. Thus, the analysis of other authors' studies confirms the overall effectiveness of microbial inoculants in increasing yields, but also highlights the importance of adapting to specific regional conditions and technological approaches, such as irrigation and fertilization.

CONCLUSIONS

Analysis of the root system and mass in different plots showed significant differences, reflecting the impact of the microbial inoculants used. In the control plot, where no inoculants were applied, the average weight of the root system was 12 g, and the length of the roots reached 20 cm. This is a basic indicator that reflects the standard development of plants in the absence of biological stimulation. The limited development of the root system in this area indicates a limited ability of the plants to effectively absorb nutrients and water, which can negatively affect overall development and yield. On the plot with the use of *Rhizobium japonicum* inoculant, a significant improvement in the development of the root system was observed. The average weight of root crops increased to 16 g, and the length reached 25 cm. This indicates a positive effect of the inoculant on the development of the root system, especially on the formation of root nodules, which play a key role in nitrogen fixation. The average number of nodules was 30 per plant, and their weight reached 0.8 g, indicating an effective symbiotic interaction between bacteria and plants. This allowed the plants to receive additional nitrogen, which contributed to their active growth and development. Another important result of this effect is the improved ability of plants to absorb water and nutrients.

In the plot with *Azospirillum brasilense* inoculum, an improvement in root development was also recorded, although the effect was less pronounced than in the plot with *Rhizobium japonicum*. The average weight of the root system was 14 g, and the length of the roots reached 23 cm, which is 16% more than in the control plot. Although *Azospirillum brasilense* does not form root nodules, its ability to stimulate root growth provides improved nutrient and water uptake. This is especially relevant in resource-limited environments where a developed root system gives plants an advantage. The obtained results clearly demonstrate that the use of *Rhizobium japonicum* and *Azospirillum brasilense* inoculants has a positive effect on the development of the root system and the general condition of soybean plants. Particularly important is the ability of *Rhizobium japonicum* to stimulate the formation of nodules that provide additional nitrogen nutrition, which is a significant advantage of this inoculant. *Azospirillum brasilense*, although it does not form nodules, helps to improve the root system by stimulating root growth.

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CONFLICT OF INTEREST

None.

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Мікробні інокулянти як засіб покращення ґрунту та врожайності сільськогосподарських культур

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Анотація. У статті наведено результати досліджень впливу мікробних інокулянтів *Rhizobium japonicum* та *Azospirillum brasilense* на ріст і розвиток сої (*Glycine max*) на початкових етапах її розвитку. Дослідження проводили на трьох дослідних ділянках: контрольний без обробки, ділянці з інокулянтом *Rhizobium japonicum* та ділянці з інокулянтом *Azospirillum brasilense*. Оцінювали основні показники: висоту рослин, кількість листків, розвиток кореневої системи та загальну біомасу на різних етапах росту рослин, а також лабораторний аналіз вмісту азоту в тканинах рослин. Результати дослідження показали, що інокулянти мали значний вплив на всі виміряні параметри порівняно з контрольною ділянкою. Зокрема, рослини на ділянці з *Rhizobium japonicum* показали на 50 % більший ріст і розвиток кореневої системи, що підтверджувалося утворенням кореневих бульбочок, відповідальних за фіксацію азоту. *Azospirillum brasilense* також покращував ріст рослин і розвиток кореневої системи, але його ефект був менш вираженим порівняно з *Rhizobium japonicum*. Аналіз вмісту азоту показав, що рослини на ділянці *Rhizobium japonicum* мали на 45 % вищий вміст азоту в тканинах порівняно з контрольною ділянкою, що свідчить про ефективну фіксацію азоту. У рослинах, оброблених *Azospirillum brasilense*, вміст азоту також був на 25 % вищим, але без утворення бульбочок ефект був менш вираженим. Метою дослідження було оцінити ефективність використання мікробних інокулянтів для покращення росту та розвитку рослин на початкових етапах вегетації, дослідити їхній вплив на продуктивність кореневої системи, поглинання азоту та загальну біомасу рослин, а також визначити оптимальні умови для максимального впливу інокулянтів у сільськогосподарських умовах. Отримані результати підкреслюють важливість використання мікробних інокулянтів для підвищення продуктивності та стійкості сої на початкових етапах її розвитку. Застосування *Rhizobium japonicum* забезпечило більший приріст біомаси, кореневої системи та засвоєння азоту, що робить цей інокулянт більш ефективним порівняно з *Azospirillum brasilense*

Ключові слова: соя; азотфіксація; атмосфера; клімат; хімічні добрива

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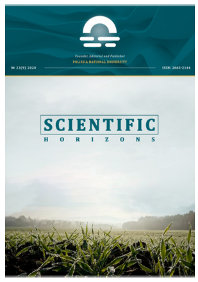

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Influence of biological products on the productivity of high oleic sunflower hybrids

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Abstract. The study's objective was to evaluate the efficacy of biological substances in enhancing the growth, development, and productivity of high-oleic sunflower hybrids, aiming to increase seed yields and improve oil quality. An investigation was carried out in the Mykolaiv region throughout 2021-2023 to assess the efficacy of seven different foliar feeding methods in producing five high-oleic sunflower hybrids. The findings indicated that the application of foliar feeding had a beneficial impact on the measured variables, such as plant height, leaf area, seed count per boll, seed yield, and oil and oleic acid concentration in seeds. In particular, the P64HE133 hybrid demonstrated the highest average yield of 3.89 t/ha, followed by Columbi with 3.69 t/ha, with no statistically significant difference between them. Foliar application of Organic-Balance helped to increase the average seed yield to 3.58 t/ha, exceeding the results of other types of spraying. The use of biological products contributed to an increase in yield by

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0.21-0.4 t/ha compared to control, as well as an increase in oil content by 0.6-2.1-5.3%. The overall impact of the combination of hybrid and foliar feeding methods on yield was the largest, amounting to 63.5%, where the impact of hybrid selection was 27.4%, foliar feeding – was 5.4%, and other factors had an impact of 3.7%. The findings suggest that the use of foliar feeding can significantly enhance productivity and enhance the quality attributes of sunflower seeds. The utilisation of these biological substances not only enhances seed production and oil content but also promotes more sustainable and robust plant development. Accordingly, the integration of such methods into agronomic practices can significantly improve crop productivity and increase the economic benefits of growing high-yielding sunflower hybrids

Keywords: fertilisation; yield; hybrids; oleic acid; biological products

INTRODUCTION

Modern agriculture is facing challenges related to climate change, preserving soil fertility, reducing the use of chemical fertilisers and pesticides, and ensuring sustainable agricultural production. The use of biopreparations containing natural microorganisms can improve plant and soil health, increase plant adaptability to stressful conditions and increase the content of oleic acid in seeds, which together will contribute to higher commercial yields of these crops.

Sunflower (*Helianthus annuus L.*) is among the four primary oilseeds of worldwide significance, alongside soybean, palm, and rapeseed (Kovalenko *et al.* 2021). The use of foliar fertilisers and growth regulators has significant potential for developing the biological capabilities of plants. As noted by Y. Domaratskiy *et al.* (2023), foliar fertilisation acts as a complementary method to root fertilisation, offering in some cases a more cost-effective and efficient solution. This method ensures that nutrients are instantly absorbed and distributed within the plants through the leaves, quickly eliminating nutritional deficiencies. The studies emphasise the importance of sunflower as one of the key oilseeds and the potential of biological products in its cultivation, some gaps have not yet been fully covered in the scientific literature. There has been limited investigation on the effects of foliar feeding on the soil microbiota and plant health. Furthermore, there is a dearth of evidence regarding the effects of these fertilisers on various sunflower cultivars, especially those that have a significant concentration of oleic acid or omega-6 carbohydrates.

High-oleic sunflower hybrids stand out among other oilseeds due to their higher oleic acid content, which exceeds 90% (Malik *et al.*, 2024). According to O. Kovalenko *et al.* (2024) and O. Yeremenko *et al.* (2023), these hybrids are characterised by increased resistance to oxidation, which reduces the risk of toxic compounds during processing, storage or use. From a nutritional point of view, the most valuable oils are those with a high content of oleic acid (up to 70%) and a low content of linoleic acid (up to 20%). Thus, modern sunflower breeding is focused on adjusting the ratio of fatty acids to create oils and fats that are highly stable and efficient. The sunflower breeding goals are focused on creating hybrids with yields of more than 5 t/ha and oil content of more than 50%, which underlines the growth of the planned development and quality requirements for this crop. Following Y.M. Hadzalo *et al.* (2023) and Y. Domaratskiy *et al.* (2022), even with the recognised potential of sunflower, environmental factors remain a significant constraint to achieving maximum yields, which currently range between 1.5 and 3 t/ha. They stressed the need for breeders to prioritise overcoming or reducing the impact of negative environmental conditions to ensure yields of 4 t/ha or higher.

Foliar nutrition is also recommended for integrated crop protection, as it not only increases yields and improves product quality, but is also an environmentally friendly way of feeding, reducing the environmental impact associated with fertiliser applied to the soil (Lopushnyak *et al.*, 2022). This is made possible by precise dosing of nutrients directly to the plant, limiting their overuse and minimising the environmental impact (Poltoretskyi *et al.*, 2020; Tsyliuryk *et al.*, 2021). Plant growth regulators (PGRs) are a heterogeneous collection of chemical substances capable of stimulating, inhibiting, or influencing physiological and morphological processes in plants. According to S. Vdovenko *et al.* (2024), the use of PGR for controlling sunflower development has already proven to be effective in counteracting the negative impact of stressful conditions on crop productivity, as noted in the study. Although their research indicates the effectiveness of PGR in increasing crop adaptation to stressful conditions, there is room for further development of this topic. Specifically, the long-term effects of the use of PGRs on the soil microbiome and environmental safety remain insufficiently investigated (Dehtiarova, 2023). It is also worth addressing the potential development of plant resistance to PGR over time, as well as considering the impact of excessive use of PGR on non-target species, which can lead to ecological imbalances.

Therefore, this study aimed to assess the influence of biological substances on the yield and characteristics of sunflower seeds with high oleic content in the Southern Steppe environment of Ukraine. This work aimed to assess the influence of different biological chemicals on the seed development of high-oleic sunflower hybrids and to examine how these chemicals contribute to the concentration of oleic acid in the liquid extracted from the seeds.

MATERIALS AND METHODS

This study was conducted experimentally at the Educational and Research Centre of Mykolaiv National Agrarian University between 2021 and 2023. The experimental plots consist of soil characterised by southern chernozem, low-power, slightly saline, and predominantly loamy deposits found in woodlands. The pH of the soil solution was within the range of 6.8-7.2, indicating a neutral reaction. Humus concentration in the 0-30 cm layer ranged from 123 to 125 gkg^{-1} (Drobitko *et al.*, 2023). The average composition of the topsoil included nitrates, which are created via the complexation of nitrates with disulfophenolic acid, resulting in the formation of trinitrophenol. Upon exposure to an alkaline environment, it produces potassium trinitrophenolate (or sodium trinitrophenolate, depending on the alkali employed) in a proportion that matches the nitrate concentration, resulting in a yellow hue. The extraction of mobile phosphorus and potassium compounds from the soil using a 1% ammonium carbonate solution with a pH of 9 at a temperature of 25 ± 20°C yields a mobile phosphorus concentration of 15-25 mg/kg^{-1}. 41-46 mg/kg^{-1}, exchangeable potassium (measured with a flame photometer). The range is 389-425 mg/kg^{-1} of soil. Located in the southern Steppe subzone of Ukraine, the experimental plot area is classified under the third agroclimatic region (Panfilova *et al.*, 2019; 2023). The experimental design comprised the subsequent alternatives (Table 1):

***Table 1**. Scheme of the experiment*

Factor A – hybrids	Factor B (foliar feeding with biological products)
1. NK Ferty	1. Control (water treatment)
2. Mas 86.OL	2. Sclerocide (1 l/ha)
3. Columbi	3. Azotophyt-p (0.5 l/ha)
4. LG 5452 HO KL	4. Organic balance (0.5 l/ha)
5. P64HE133	5. Sclerocide (1 l/ha) + Helprost-Bor (1 l/ha)
	6. Azotophyt-p (0.5 l/ha) + Helprost-Bor (1 l/ha)
	7. Organic-Balance (0.5 l/ha) + Helprost-Bor (1 l/ha)

***Source:** compiled by the authors*

The plants underwent two treatments with the specific biological compounds under investigation. The first treatment occurred during the stage of 5-6 pairs of leaves, while the second treatment took place during the budding phase. Seed sowing of the examined hybrids was conducted at a soil temperature ranging from 8 to 10°C. Harvesting took place during the third decade of September in the specified years of study. The origin of hybrids is as follows: "NK Ferty" (Syngenta, France); "Mas 86.OL" (MAS Seeds, France); "Columbi" (Limagrain, France); "LG 5452 HO KL" (Limagrain, France); "P64HE133" (Pioneer, USA). The study was organised in a randomised design with four replications in each plot of 25 m^2. The planting density is 50 thousand plants/ha, with a row spacing of 70 centimetres. Generally, the appropriate fertiliser rates and agronomic procedures for sunflower production were established.

To study the growth and development of terrestrial plants, measurements and analyses were conducted using the following techniques: the height of the plants and the dimensions of the seventh leaf (its breadth and length) were evaluated immediately before the onset of pollenation. For this purpose, five typical plants marked with labels were selected from the two central rows. The leaf area was determined according to the method described by S.O. Tkachyk *et al.* (2016). Harvesting was carried out manually at full ripeness. All measurements and observations were made following the field research methodology of S.O. Tkachyk *et al.* (2016). The oil and oleic acid content of the seeds was determined using magnetic resonance techniques from Spinlock Magnetic Resonance Solutions.

RESULTS

A three-year field trial evaluated the growth characteristics of five sunflower hybrids with a high oleic acid content. The analysis of plant height showed that the hybrid "P64HE133" had the highest average height, which was 178.93 cm. This result may indicate the high adaptability of this hybrid to growing conditions and its potential to form a productive leaf surface for efficient photosynthesis. A slightly lower average height was demonstrated by the Columbi hybrid, which is 176.79 cm, which also indicates its high phenotypic qualities and adaptability. At the same time, the NK Ferty hybrid with an average height of 175.88 cm was ranked third among the tested variants, emphasising its potential for creating optimal conditions for development. Next on the list are the hybrids "Mas 86.OL" and "LG 5452 HO KL" with average heights of 174.67 cm and 173.64 cm, respectively, demonstrating a variety of growth characteristics among high-oleic sunflower hybrids. The statistical study yielded a statistically significant difference ($P < 0.05$) between the hybrid "P64HE133" and all other variations, except for

"Columbi". Thus, the results emphasise the hierarchy of growth characteristics among the studied hybrids, indicating a significant heterogeneity in the response of plants to environmental conditions (Table 2).

Table 2. *The impact of biological products on sunflower growth: analysis of the average plant height for the period 2021-2023, cm*

Foliar feeding with biological products (factor B)	Hybrid (factor A)					
	LG 5452 HO KL	Mas 86.OL	NK Ferty	Columbi	P64HE133	Average value
Control (water treatment)	160.54	163.76	165.54	168.95	170.98	165.55
Sclerocide	168.4	168.74	172.95	170.98	172.39	170.29
Azotophyt-p	170.6	172.61	174.55	172.61	175.32	173.54
Sclerocide + Helprost-Bor	174.76	175.26	176.71	176.98	177.71	176.28
Organic balance	177.1	177.71	178.57	178.84	179.57	178.36
Azotophyt-p + Helprost-Bor	179.73	180.29	180.75	180.75	181.39	180.98
Organic-Balance + Helprost-Bor	182.32	182.29	183.07	183.04	184.73	183.49
Average value	173.64	174.67	175.88	176.79	178.93	-

Source: *compiled by the authors*

Within the realm of biological products, the application of the "Organic-Balance + Helprost-Bor" combination on two occasions throughout the growth season resulted in an average plant height of 183.49 cm, surpassing the achievements of other experimental variations. For instance, the combined use of "Azotophyt-p + Helprost-Bor" resulted in an average height of 180.98 cm, while the separate use of "Organic-Balance" resulted in an average height of 178.36 cm. The variants with "Sclerocide + Helprost-Bor" and "Azotophyt-p" showed 176.28 cm and 173.54 cm, respectively. The application of "Sclerocide" demonstrated a result of 170.29 cm, and the control variant without fertilisation showed the lowest average height – 165.55 cm, which demonstrates a significant ($P < 0.05$) increase in plant growth for all fertilisation options compared to the control.

Specifically, the application of "Sclerocide + Helprost-Bor" twice during the growing season resulted in a substantial rise in the average height of hybrids "NK Ferty" to 176.71 cm and "Mas 86.OL" to 175.26 cm. These results surpassed those achieved by applying the combination "Azotophyt-p" individually twice. For the "Mas 86.OL" hybrid, a similar effect was observed when the combination of "Azotophyt-p + Helprost-Bor" was used as isolated treatments twice, reaching an average height of 180.29 cm. However, when "Organic-Balance + Helprost-Bor" was applied separately twice, a slight increase in average height to 182.29 cm was found. At the same time, for all the hybrids studied, any foliar feeding contributed to an increase in plant height.

The most significant effect on plant height was the relationship between the choice of hybrids and foliar feeding regimes, which accounted for 76.5% of the total effect. In isolation, hybrid selection had a significant impact, accounting for 18.9%, while foliar fertilisation itself played a role with a 2.9% share. The other factors studied had a 1.7% effect on plant height. This highlights the importance of optimising the foliar application regime for specific sunflower hybrids to maximise their growth and development. During a detailed field study aimed at studying the morphological characteristics of high-oleic sunflower hybrids, the leaf area was assessed, which is a key factor in the process of photosynthesis and affects the overall productivity of plants. The analysis determined that hybrids "P64HE133" and "Columbi" showed the highest average leaf area among all the studied hybrids, each of which had an identical indicator (Table 3). This indicates the significant potential of these hybrids in forming an efficient photosynthetic system capable of optimising the use of sunlight.

Table 3. *Leaf area of sunflower hybrids in the flowering phase depending on biological products, m2 (average for 2021-2023)*

Foliar feeding with biological products (factor B)	Hybrid (factor A)					
	LG 5452 HO KL	Mas 86.OL	NK Ferty	Columbi	P64HE133	Average value
Control (water treatment)	0.585	0.592	0.6	0.609	0.617	0.601
Sclerocide	0.628	0.642	0.65	0.66	0.673	0.651
Azotophyt-p	0.661	0.665	0.675	0.687	0.7	0.678
Sclerocide + Helprost-Bor	0.7	0.71	0.715	0.72	0.725	0.714
Organic balance	0.725	0.73	0.735	0.74	0.745	0.735
Azotophyt-p + Helprost-Bor	0.75	0.755	0.76	0.765	0.77	0.76
Organic-Balance + Helprost-Bor	0.775	0.78	0.785	0.79	0.795	0.785
Average value	0.689	0.711	0.717	0.741	0.756	-

Source: *compiled by the authors*

Compared to “P64HE133” and “Columbi”, hybrids “NK Ferty” and “Mas 86.OL” showed slightly lower average leaf area for each of them. These results indicate that these hybrids also have good adaptability to external conditions and can effectively develop leaf areas to meet the plant’s photosynthetic needs. The lowest average leaf area was found in the hybrid “LG 5452 HO KL”. Even though this value is only slightly inferior to other hybrids, statistical analysis confirmed that all hybrids had a statistically significant advantage over “LG 5452 HO KL” in this parameter ($P < 0.05$). This finding emphasises the significance of genetic elements and the influence of agricultural methods on the formation of the leaf surface, which is directly linked to the plant’s ability to undertake photosynthesis and hence its production.

Thus, the analysis of leaf area identified the variability between the studied hybrids and emphasised the importance of this parameter in determining photosynthetic efficiency and overall adaptation of plants to growing conditions. The analysis of the impact of foliar feeding on the leaf surface development of sunflowers revealed that the combination of long-term “Organic-Balance” and “Helprost-Bor”, together with a separate two-time application of “Azotophyt-p” and “Helprost-Bor”, resulted in the attainment of the highest average leaf area. “Organic-Balance” and “Sclerocide + Helprost-Bor” fertilisers provided the average leaf area, indicating a decrease in efficiency with a decrease in fertiliser intensity. The application variant “Azotophyt-p” and the separate application “Sclerocide” showed an even smaller average area of 0.678 m^2 and 0.651 m^2 respectively. The control variant, without any fertilisation, showed the lowest average leaf area, emphasising the importance of foliar fertilisation for leaf surface development.

Among the analysed hybrids, “P64HE133” showed that a two-time application of “Azotophyt-p” resulted in an increase in the average leaf area to 0.678 m^2, which was higher than with long-term use of the biological product “Sclerocide”. A similar trend was observed with the two-time application of “Organic-Balance + Helprost-Bor” compared to the long-term use of “Azotophyt-p + Helprost-Bor”. Interestingly, for Mas 86.OL, the use of “Organic-Balance” alone resulted in a slightly higher average leaf area compared to the long-term use of “Sclerocide + Helprost-Bor”. Hybrids and the use of foliar fertilisers had the greatest impact on leaf area, accounting for 50.1%. The next most important factor was the use of foliar fertilisers, which contributed 33.2% to the increase, while hybrids themselves contributed 11.4%. Other factors accounted for 5.3% of the impact. The “P64HE133” hybrid showed the highest average number of kernels per basket, followed by “Columbi”, “NK Ferty”, “Mas 86.OL”, and “LG 5452 HO KL” with 1048.1 kernels. At the same time, “P64HE133” was distinguished by a significantly higher average number of grains compared to other hybrids, and all hybrids showed a statistically significant ($P < 0.05$) excess over “LG 5452 HO KL”.

The highest average number of grains per basket among the hybrids was recorded with the double use of “Organic-Balance + Helprost-Bor”, amounting to 1224.4 pieces. “Azotophyt-p + Helprost-Bor”, “Organic-Balance”, “Sclerocide + Helprost-Bor”, “Azotophyt-p” and “Sclerocide” were next, while the control group had the lowest number of 1128 units. The application of each foliar fertiliser led to a statistically significant ($P < 0.05$) rise in the mean grain count when compared to the control group. The difference between the highest result with “Organic-Balance + Helprost-Bor” and the results with “Azotophyt-p + Helprost-Bor” or “Organic-Balance” was not statistically significant, unlike the results for the other hybrids (Table 4).

Table 4*. Number of seeds in a basket of sunflower hybrids depending on biological products, pcs. (average for 2021-2023)*

Foliar feeding with biological products (factor B)	Hybrid (factor A)					
	LG 5452 HO KL	Mas 86.OL	NK Ferty	Columbi	P64HE133	Average value
Control (water treatment)	1,001	1,036	1,110	1,232	1,261	1,128
Sclerocide	1,041	1,050	1,121	1,242	1,289	1,148.6
Azotophyt-p	1,043	1,058	1,158	1,265	1,294	1,163.6
Sclerocide + Helprost-Bor	1,051	1,070	1,177	1,281	1,295	1,174.8
Organic balance	1,054	1,077	1,216	1,294	1,320	1,192.2
Azotophyt-p + Helprost-Bor	1,058	1,089	1,232	1,295	1,330	1,200.8
Organic-Balance + Helprost-Bor	1,089	1,121	1,242	1,324	1,346	1,224.4
Average value	1,048.1	1,066.6	1,182.3	1,276.1	1,305	-

Source: *compiled by the authors*

Essentially, the application of biological products twice led to a marginal rise in the seed count per basket in comparison to the extended treatment. However, for the “NK Ferty” hybrid, long-term use of fertiliser has proved particularly effective. When using the “Sclerocide” combination, the number of seeds per head slightly decreased compared to the two-time use of “Azotophyt-p”. A similar trend was observed for the “LG 5452 HO KL” hybrid, where “Sclerocide” yielded 1041 seeds, while the use of “Azotophyt-p” alone yielded 1043 seeds. Moreover, in the case of “LG 5452 HO KL”, the use of “Azotophyt-p + Helprost-Bor” resulted in a decrease in the number of seeds per head compared to the use of "Organic-Balance + Helprost-Bor". For the hybrid "Mas 86.OL", the long-term application of "Organic-Balance" also showed a slight decrease in the number of seeds compared to the two-time application of "Azotophyt-p + Helprost-Bor".

Despite this, each use of biological products showed better results on average on each hybrid than the control. The combined effect of hybrids and foliar treatments was most effective (56.4%) in increasing the number of seeds per head. Hybrids came in second with 39.5%, followed by foliar fertilisers (1.1%) and other factors (3%). Seed yield is considered a key indicator for assessing the economic benefits of using modern hybrids. In this context, the "P64HE133" hybrid had the highest average seed yield, reaching 3.82 t/ha. In the last season, agricultural research showed that among the various hybrids tested at the test site, such as "Columbi", "NK Ferty", "Mas 86.OL" and "LG 5452 HO KL" stand out in terms of seed yield. In particular, "Columbi" showed a result of 3.72 t/ha, "NK Ferty" – 3.56 t/ha, "Mas 86.OL" – 3.29 t/ha, and "LG 5452 HO KL" – 3.11 t/ha.

Interestingly, the increase in yields in these studies may be due not only to the genetic characteristics of the hybrids themselves but also to the use of modern biological products that contribute to better plant development, increased stress resistance and protection against pests and diseases. This underscores the importance of an integrated approach to agricultural production that combines high-quality seed with advanced agricultural technologies. Among the different methods of using biological products, the use of double fertilisation "Organic-Balance + Helprost-Bor" demonstrated significantly better average seed yields compared to other methods. Notably, the average yields between the different methods of foliar feeding and the control group differed significantly (Table 5).

***Table 5**. Influence of biological products on seed yield of sunflower hybrids, t/ha (average for 2021-2023)*

Foliar feeding with biological products (factor B)	Hybrid (factor A)					
	LG 5452 HO KL	Mas 86.OL	NK Ferty	Columbi	P64HE133	Average value
Control (water treatment)	2.98	3.01	3.17	3.4	3.56	3.23
Sclerocide	2.97	3.11	3.36	3.56	3.87	3.37
Azotophyt-p	3.14	3.31	3.35	3.74	3.82	3.48
Sclerocide + Helprost-Bor	3.37	3.47	3.5	3.68	3.69	3.54
Organic balance	3.06	3.22	3.49	3.81	3.83	3.48
Azotophyt-p + Helprost-Bor	3.08	3.5	3.54	3.91	3.95	3.8
Organic-Balance + Helprost-Bor	3.19	3.41	3.5	3.93	4.04	3.93
Average value	3.11	3.29	3.56	3.72	3.82	-

***Source:** compiled by the authors*

Usually, seed yields with three times foliar spraying exceeded the results of two times application of similar fertilisers and PGR. This demonstrates the effectiveness of more intensive nutrient application methods in increasing the productivity of most hybrids. However, some exceptions to this rule have been recorded, especially for specific hybrids that show differences in response to cultivation. Specifically, the hybrid "NK Ferty" showed a slightly reduced seed yield when sprayed twice with the "Sclerocide" combination, compared to a single application of "Azotophyt-p". This may indicate that for some hybrids, increasing the number of sprays does not always lead to better results, possibly due to the achievement of optimal nutrition levels or due to the specific needs of the hybrid itself. Moreover, the hybrid "Mas 86.OL" showed a lower seed yield when "Sclerocide + Helprost-Bor" was applied twice compared to the two-time application of "Organic-Balance". This confirms the idea that not all hybrids are more productive with more intensive fertilisation regimes, and in some cases, less intensive but well-targeted fertilisation can be more beneficial. As such, although the general trend shows the benefits of double foliar spraying to increase seed yields in most cases, some exceptions require an individualised approach to each hybrid. It is necessary to address the specific needs of each hybrid and the possibility of reaching yield requirements, which may make further investments in additional sprays less effective.

The application of any form of foliar treatment on each hybrid resulted in an enhancement in seed yield when compared to the control group that did not receive any further fertilisation. The combined effect of hybrid characteristics and foliar nutrition proved to be the most powerful factor affecting yield, accounting for 64.5% of the total impact. The hybrids themselves contributed 27.3% of the impact, while biological products had 6.9%. Other aspects accounted for only 1.3% of the impact on the final yield. The key indicator for assessing the quality of oilseeds is their oil content. In this respect, the "P64HE133" hybrid showed the highest average level of 51.3%. The oil content of other hybrids decreased in the following order: "Columbi", "NK Ferty", "Mas 86.OL", "LG 5452 HO KL". The use of biopreparations increased the oil content by 0.9-1.8% (Fig. 1).

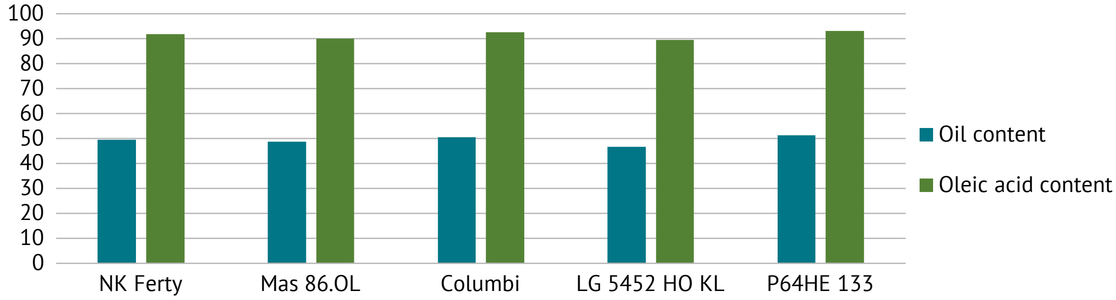

***Figure 1**. Oil and oleic acid content in sunflower hybrid seeds, %*
(average for 2021-2023 and by the factor of foliar feeding of crops with biological products)
Source: *compiled by the authors*

In conducting a thorough examination of the qualitative attributes of seeds from several sunflower hybrids, particular emphasis was given to the oleic acid concentration, a crucial constituent that governs the nutritional and industrial worth of oil. In particular, the hybrid "P64HE133" had the highest average oleic acid content among all the hybrids studied, reaching 93.1%. This high figure underlines the potential of the "P64HE133" hybrid not only in terms of yield but also in terms of the quality of the products obtained. Compared to "P64HE133", the other hybrids showed lower average values of oleic acid content, but they also showed significant variability in this component, depending on growing conditions and applied agricultural technologies. Notably, the use of foliar fertilisers can increase the content of oleic acid in seeds by 2.1-5.3%, which underlines the importance of agrochemical measures to improve the quality of agricultural products.

It is worth noting that the content of oleic acid in each of the studied hybrids exceeded the total oil content, which indicates a high level of saturation with this valuable component. This high oleic acid content makes these hybrids particularly attractive to produce high-quality oils suitable for use in the food industry and for other purposes, such as biofuel production.

DISCUSSION

The investigation's results indicated a significant correlation between the use of biological products and the yield of sunflowers. Furthermore, it was imperative to emphasise the contingent nature of the indications on the sunflower hybrid. Nevertheless, to enhance the credibility and pertinence of the analysis, it is essential to analyse these indicators about comparable studies conducted by other authors.

In the context of agronomic research, analysis of the impact of biological products on the physiological characteristics of plants is prioritised. Specifically, one of the areas of research is the effects of mycorrhizal preparations that facilitate symbiosis between plant roots and fungi, thereby increasing their ability to assimilate nutrients and resistance to stressful conditions. One of these drugs is MycoFriend, which was studied by G. Sousa *et al.* (2021), M. Gandariasbeitia *et al.* (2022), and S. Hafez *et al.* (2021), to determine its potential for increasing sunflower (*Helianthus annuus L.*) yield. The application of MycoFriend in the row before sowing resulted in a 1.9-2.5 cm increase in plant height compared to the control group that did not utilise the biological product. At full maturity, the plants in the treated plots had an average height of 170.3-174.5 cm. These results correlate with the original study, which also recorded an improvement in plant growth using mycorrhizal preparations, although, in the original study, plant height was slightly higher. This may indicate the variability of plant responses to mycorrhizal preparations depending on specific growing conditions, soil properties and climatic factors. Thus, research confirms the potential of biological products as a means of optimising crop growth and development, offering an effective tool for increasing productivity in conditions where traditional agrochemical intervention may be limited or undesirable.

In agronomic studies by S. Li *et al.* (2023) and J. Li *et al.* (2022), the impact of the BTU biocomplex, which includes natural nitrogen-fixing, fungicidal and other beneficial bacteria, as well as biologically active products of their vital activity, on different types of crops in different agroclimatic conditions, was studied. The results indicated that the use of this product enhances the efficacy of seed germination, enhances the regularity of seedlings, decreases plant infection with various diseases without causing addiction, and boosts yields by 10-30%. These results confirm the high efficiency of the BTU biocomplex and its prospects for application in agronomy, especially in Ukraine, seeking sustainable methods to increase the productivity of agricultural systems.

Contemporary academic research in the subject of agronomy is increasingly focussing on investigating the influence of biological products on the morphological and physiological traits of plants. One of the main areas is the analysis of the ability of these products to improve growth parameters, such as the number of leaves per plant, which can significantly affect the overall productivity of plants by increasing photosynthetic activity.

A study conducted by B. Han *et al.* (2021), A. Oliveira *et al.* (2022) and D. Nguyen *et al.* (2021) conducted the effect of Biopolin on sunflower plants, in particular, on changes in the number of leaves. An observed variation in the leaf count per plant, ranging from 17.8 to 27.1 pieces, was attributed to the administration of Biopolin. All hybrids examined showed a substantial rise in leaf count, with a difference ranging from 4.4 to 8.8 leaves compared to the control plots without the preparation. These data should be compared with the results of the original study, which focused on measuring leaf area rather than directly on the number of leaves. Initial observations revealed a rise in leaf area, suggesting a beneficial impact of mycorrhizal preparations on the growth of leaf mass. However, by assessing the number of leaves, a more detailed picture can be obtained of changes in the growth characteristics of the plant, indicating a potential improvement in not only the quantitative but also the qualitative parameters of photosynthesis.

Studies have shown moderate correlations between yield and oil quality, which demonstrates that changes in the growing season due to heat stress do not directly affect oil quality. Heat stress can affect the oil content by reducing the number of seeds and increasing the sterile zone on the sunflower heads. In particular, a drop in seed count can lead to a decrease in oil content, while an increase in sterile area can contribute to an increase in oil content (Shahini *et al.*, 2023). Under conditions of heat stress, variations in the concentration of oleic acid are observed, which becomes more sensitive to high temperatures. Under normal temperature conditions, oil content is positively correlated with an oleic acid content, which is in line with previous results of H. Amimy *et al.* (2023), but under heat stress this relationship becomes negative. Reducing the oil content at high temperatures does not necessarily increase the oleic acid concentration. Along with the increase in oleic acid caused by genetic and environmental factors, there may be a decrease in the concentration of palmitic acid, as confirmed by the study by P. Lemos *et al.* (2022). These discoveries are important for Ukraine, one of the world's leading sunflower producers. An assessment of the influence of heat stress on the quality and yields of oil can be utilised to formulate climate change adaptation measures that will guarantee the sustainability of production in the presence of global warming and climate fluctuations.

Considerable focus is given in agriculture to the influence of biological products on crop productivity (Tanchyk *et al.*, 2024). These are based on biological products that show great promise in increasing sunflower yields. P. Evon *et al.* (2023) and N. Alzamel *et al.* (2022), who studied the ES Balistic hybrid, noted that the use of Polymyxobacterin increased yields compared to control plots where the biological product was not used. Under control conditions, the average yield was 2.62 t/ha. However, after the application of the biological product, an increase in this indicator was recorded, which confirms its effectiveness. Also noted are the results of J. Anandappa *et al.* (2023), M. Al-Zaidy and I. Al-Hilfy (2023) and B. Adeleke *et al.* (2022), who analysed the "SY Experto" hybrid and acquired similar trends. In the control plots, the yield of this hybrid was slightly higher and amounted to 2.7 t/ha. The use of biological products also increased yields, but, as in the first case, slightly higher results were reported in the original study, which could be due to differences in weather conditions, soil, or other agronomic parameters between the studies. These results indicate the potential of biological products as an effective means of increasing sunflower productivity, providing a rationale for further research and the potential integration of this biological product into modern agronomic practices.

The growing season is a critical factor that affects harvest time and quality, and the ability of biological products to modulate this period can have significant agronomic implications. A. Ghendov-Moșanu *et al.* (2023), R. Puttha *et al.* (2023) and K. Asish *et al.* (2023) examined the length of the growth season for two sunflower hybrids, namely the medium-early hybrid "P64LE121" and the medium-ripening hybrid "P64LE99". Following the study results, the vegetation period of the medium-early hybrid ranged from 102 to 113 days, while that of the medium-ripening hybrid lasted from 108 to 116 days. Particular attention was devoted to the effect of the biological product FitBest applied to the row before sowing. This application of the product demonstrated the ability to extend the growing season of both hybrids to a maximum duration of 113-116 days. This increase may be due to improved growth conditions, such as increased nutrient availability or improved water nutrition. Significantly, the aforementioned study did not include the duration of the growing season as a crucial factor. Instead, the focus was directed towards other agronomic aspects such as the yield of crops and the overall quality of seeds. Failure to address the length of the growing season in previous studies may indicate an underestimation of its impact on the final result of crop production.

CONCLUSIONS

The study findings showed that the biological products proved efficacy in enhancing several important parameters of sunflower agriculture, such as plant height, leaf area, seed count in baskets, seed yield, and seed quality, including oil and oleic acid content. Particularly striking results were obtained for the "P64HE133" and "Columbi" hybrids, which showed the highest average yield with the highest oil content.

The application of "Organic-Balance + Helprost-Bor" twice, as well as the double use of "Azotophyt-p +

Helprost-Bor", proved to be effective, providing high average seed yields for all hybrids in general – 3.8 t/ha and 3.93 t/ha respectively, which is significantly higher than the control group. Importantly, the biological products enhanced seed production by 0.21-0.4 metric tonnes per hectare compared to the control group and increased oil content by 0.9-1.8%. This highlights the importance of foliar feeding as a strategy to optimise sunflower production performance. Additionally, the average oleic acid content in the seeds, which is critical for oil quality, was increased by 2.1-5.3% due to foliar nutrition. In general, the combined effect of hybrid and biological product selection had the largest share in improving seed yields (63.5%), followed by the contributions of the hybrid itself (27.4%) and biological products (15.3%). In conclusion, to achieve optimal yields (between 3.37 and 3.93 t/ha) and seed quality, it is recommended to use targeted biological products, in particular, the combination of "Organic-Balance + Helprost-Bor" or "Azotophyt-p + Helprost-Bor" twice on the hybrids "P64HE133" and "Columbi".

Further research should address the genetic interaction between high oleic sunflower hybrids and biological products, which may reveal which genetic markers influence the increased response to biological products. This could be used to develop targeted breeding strategies to improve plant response to biological fertilisation.

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None.

CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Вплив біопрепаратів на продуктивність високоолеїнових гібридів соняшнику

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Анотація. Дослідження було спрямоване на вивчення ефективності застосування біопрепаратів на ріст, розвиток та продуктивність високоолеїнових гібридів соняшнику з метою підвищення урожайності насіння та покращення якості олії. Протягом 2021-2023 років в Миколаївській області було проведено експеримент, спрямований на вивчення ефективності семи варіантів позакореневого підживлення на продуктивність п'яти високоолеїнових гібридів соняшнику. Результати показали, що позакореневе підживлення мало позитивний вплив на визначені параметри, включаючи висоту рослин, площу листка, кількість насінин у кошику, урожайність насіння, вміст олії та олеїнової кислоти в насінні. Зокрема, гібрид "Р64НЕ133" продемонстрував найвищу середню урожайність 3,89 т/га, наступним був "Коломбі" з показником 3,69 т/га, при цьому між ними не було виявлено статистично значущої різниці. Позакореневе підживлення "Органік-баланс" сприяло підвищенню середньої урожайності насіння до 3,58 т/га, перевищуючи результати від інших видів обприскувань. Застосування біопрепаратів сприяло збільшенню урожайності на 0,21-0,4 т/га порівняно з контролем, а також збільшенню вмісту олії на 0,6-2,1-5,3%. Загальний вплив комбінації гібриду та способів позакореневого підживлення на урожайність був найбільшим, склавши 63,5%, де вплив вибору гібрида становив 27,4%, позакореневого підживлення – 5,4%, а інші чинники мали вплив 3,7%. Результати вказують на те, що позакореневе підживлення може ефективно підвищити урожайність та покращити якісні характеристики насіння соняшнику. Застосування цих біопрепаратів не лише збільшує кількість насіння та вміст олії в них, але й сприяє більш стійкому та здоровому росту рослин. Відповідно, інтеграція таких методів у агротехнічні практики може значно покращити продуктивність сільськогосподарських культур і збільшити економічну вигоду від вирощування високоврожайних гібридів соняшнику

Ключові слова: підживлення; урожайність; гібриди; олеїнова кислота; біопрепарати

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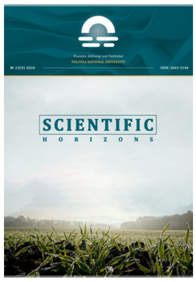

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Prospects and directions of diversification of oilseed group crops

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Abstract. The purpose of the study was to examine the influence of technology elements on the yield and quality of oil from various varieties of oilseeds, in particular, sunflower, rapeseed, safflower, camelina, and brown mustard. The effectiveness of various agricultural techniques (types of fertilisers, protection systems, and agrotechnical measures) to improve the quality and quantity of these crops was evaluated. The study was conducted for three years on the basis of the educational-scientific-practical centre of Mykolaiv National Agrarian University. The methodology included conducting field experiments with various agronomic technologies, laboratory analysis of the obtained plant samples, and determining the oil content. According to the results, the use of an optimised nutrition system and a combined protection system increases the yield of sunflower seeds by 15%, the quality of oil by 10%, and the use of innovative elements for growing flax increases the oil content of seeds by 8%. For rapeseed, it was determined that the use of adopted nutrition and

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protection systems increases the yield by 20%. The mustard examination showed a substantial improvement in the crop's resistance to diseases when using integrated plant protection. The findings confirmed the importance of choosing the optimal elements of the technology that contribute to improving the yield and quality of oilseeds. This issue is relevant since oilseeds occupy a substantial place in the agro-industrial complex. Increasing the efficiency of growing oilseeds not only provides stable profits for farmers but also has a positive impact on the food security of the country in general. Thus, the introduction of scientifically based agronomic measures is a critical factor for achieving sustainable development of the agricultural sector and diversification of oilseeds

Keywords: agro-industrial complex; oilseed plants; technology elements; varieties and hybrids; yield; seed quality

INTRODUCTION

Oilseeds play a vital role in the agro-industrial complex, providing important resources not only for the food industry but also for other applications, such as the production of biofuels, various types of oils, paints, and cosmetics. In the context of global climate change, economic fluctuations and growing requirements for food security, the issue of diversification of oilseeds is becoming particularly relevant. The main problem is to assess the possibilities of expanding the range of oilseeds in addition to sunflower, which will help reduce economic risks, increase productivity and reduce the negative impact on soil fertility due to the oversaturation of sunflower crop rotations. Crop diversification in the field of agronomy is associated with the selection of plant species of the oilseed group to improve disease resistance and increase their productivity (Kairbayeva *et al.*, 2022).

The subject of diversification of oilseeds is extremely relevant in the context of ensuring food security, economic stability and environmental sustainability of the agro-industrial complex (Shevchuk *et al.*, 2024). In particular, T. Zelt (2022) noted that diversification of oilseeds can substantially reduce the risks associated with monoculture cultivation, such as vulnerability to pests, diseases, and climate change. I. Nahvi *et al.* (2023) noted that the introduction of new varieties and hybrids of oilseeds, such as sunflower, rapeseed, soy, etc., can help increase yields and reduce production costs. A study conducted by L. Magno *et al.* (2022), focuses on the potential of oilseeds such as flax and hemp. These plants can provide additional sources of oil that not only meet consumer demand but also help reduce dependence on traditional oilseeds such as sunflower and rapeseed. Researchers C. Wen *et al.* (2023) determined that the diversification of oilseeds is an important factor that directly affects the development of the fat and oil industry engaged in processing these crops. D. Rudoy *et al.* (2023) established that the growing demand for oils with lower saturated fat content, such as flax or hemp seed oil, substantially encourages innovation in manufacturing processes. The paper of S. Jopony *et al.* (2023) analysed in detail how crop diversity can substantially change the structure of acreage and improve the efficiency of land use. The authors emphasise that optimisation of acreage and the introduction of adapted crops that can withstand climate change and diseases can not only increase the productivity of agricultural production but also ensure its sustainability in the long term.

Y. Jiang *et al.* (2024) examine in detail the environmental aspects of diversification, in particular, its positive impact on biodiversity, water management, and reducing the use of chemical fertilisers, which is critical for ecosystem conservation. I.V. Chekhova (2021) believes that the diversification of oilseeds improves the product range and also contributes to the development of more sustainable and innovative production practices in the industry. Despite the available research, there is a lack of work on niche oilseeds that can be commercially profitable, and there is also a lack of detailed data on the long-term economic benefits of diversification and the impact of these crops on the ecological aspects of agronomic systems. Therefore, it is necessary to assess the potential for diversification of oilseed group crops to improve agricultural production and economic benefits due to changes in climate and market conditions, including in the southern steppe zone of Ukraine. The main purpose of this study was to identify new approaches to the diversification of agricultural production and study the impact of various elements of technology on the yield and quality of oilseeds, such as sunflower, rapeseed, mustard, safflower, brown mustard, and camelina.

MATERIALS AND METHODS

An experiment was conducted on the basis of the educational-scientific-practical centre of Mykolaiv National Agrarian University during 2022-2024 to achieve the study goal. The selection of oilseed crops for the study was conducted at the educational and scientific-practical centre, where a decision was made to focus on six crop species (Table 1).

Sunflower is included with the greatest distribution, high yield and oil content, rapeseed – for its low content of saturated fats and the possibility of their use for the production of biodiesel, safflower – as a drought-resistant crop, with high nutritional value and fairly high oil content, mustard – due to its resistance to environmental conditions and additional value in medicine, oilseed flax – for its widespread use in medicine, industry, and camelina – for its prospects in the production of oil for cosmetics, food, jet fuel.

***Table 1.** Varieties and hybrids of crops used in the study*

Culture	Variety/hybrid	Year of registration	Originator	Country
Sunflower	Forward	2018	V.Ya. Yuriev Plant Production Institute	Ukraine
Sunflower	P64LE25	2015	Pioneer	Netherlands
Winter rapeseed	Phoenix CL	2023	DSV	Austria
Winter rapeseed	Abacus	2010	NPZ (LEMBKE)	Germany
Safflower	Dobrynya	2016	Institute of Oilseed Crops of NAAS	Ukraine
Safflower	Lahidny	2011	Institute of Oilseed Crops of NAAS	Ukraine
Brown mustard	Dzhyonka	2008	Institute of Oilseed Crops of NAAS	Ukraine
Brown mustard	Zabahanka	2020	Institute of Oilseed Crops of NAAS	Ukraine
Oilseed flax	Orpheus	2002	Institute of Oilseed Crops of NAAS	Ukraine
Oilseed flax	Dobrodar	2022	Institute of Oilseed Crops of NAAS	Ukraine
Camelina	Prestige	2006	Institute of Oilseed Crops of NAAS	Ukraine
Camelina	Euro 12	2015	Institute of Oilseed Crops of NAAS	Ukraine

***Source:** compared by the authors on the basis of data from the Ministry of Agrarian Policy and Food of Ukraine (2024)*

The experiment of the study was conducted on southern chernozem in the educational-scientific-practical centre of Mykolaiv National Agrarian University during 2022-2024. Weather conditions during the experiment were marked by the following trends: spring was characterised by gradual warming. The beginning of March was cool with periodic frosts, but in April and May the temperature increased substantially, precipitation decreased from March to May, but light precipitation fell. In summer, temperatures were high, with daily averages ranging from +20°C to +35°C, with temperature peaks above 40°C in July and August. The summer months were accompanied by short-term rains, alternating with prolonged droughts. In autumn, the temperature gradually decreased. September was warm, with temperatures ranging from +20°C to +30°C, in October the temperature gradually decreased, precipitation fluctuated, and it became more windy. Rain fell periodically and unevenly. In general, the weather during the entire period had changes characteristic of the steppe zone of the Mykolaiv region.

The study evaluated the impact of various technological elements on the yield and oil quality of sunflower, rapeseed, safflower, brown mustard, oilseed flax, and camelina crops, along with their resistance to major diseases and pests. The study aimed to determine the effects of crop variety or hybrid, fertilisers, and protection measures on the yield and quality of these oilseed crops. The experiment scheme included the following options:

1. Factor A – varieties and hybrids of oilseed crops: Sunflower (P64LE25, Forward), Rapeseed (Abacus, Phoenix CL), Safflower (Lahidny, Dobrynya), Brown Mustard (Dzhyonka, Zabahanka), Oilseed Flax (Orpheus, Dobrodar), Camelina (Prestige, Euro 12).
2. Factor B – Nutrition System:
   - without fertilisers (control);
   - recommended ($N_{60}P_{40}$);
   - optimised ($N_{30}P_{30}K_{30}$+ soil spray with the organic biopreparation Metawight at 10 L/ha + treatment of seeds and crops with biopreparations and microelements recommended for the studied crops).
3. Factor C – Protection System:
   - without protection (control);
   - recommended (application of recommended agents when the economic threshold of pest damage is exceeded);
   - optimised (agrotechnical measures and biologically derived agents).

The yield was determined from each plot. Seed quality assessment, oil content for oilseeds taken, laboratory analysis for water content, and chemical residues were determined according to (DSTU 7011:2009, 2010; Methods of examination..., 2016). The experimental study with oilseeds followed the standards of the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1979).

RESULTS

Oilseeds continue to occupy leading positions among agricultural plants in the agro-industrial complex, as they are characterised by high profitability and substantial efficiency in production. These crops not only provide stable profits for farmers but also play an important role in shaping food security in countries. There are about 350 species of oilseeds in the world, which are grown in different climatic conditions, and have different agronomic properties. Among them, the most popular are sunflower, soy, and rapeseed, which are used for the production of oil, feed, and other food products.

Elements of the technology have substantially affected the yield of oilseeds, which is an extremely important factor for ensuring diversification (Shahini *et al.*, 2023). The inclusion of new varieties and hybrids of oilseeds in the study provided higher yields. This is due to unique genetic characteristics that allow them to adapt more effectively to different growing conditions, such as climate change, soil types, and the presence of moisture. The use of organic fertilisers also had a positive effect on yield, although not as substantial as the selection of varieties and hybrids. Organic fertilisers improve the quality of the soil, contribute to its enrichment with nutrients, and improve water retention capacity and structure, which provides better conditions for the growth and development of plants, allowing them to receive the necessary nutrients, which is important for their healthy development (Tonkha *et al.*, 2024). Irrigation and the use of pesticides also play an important role in ensuring a stable crop. Irrigation systems allow maintaining an optimal level of humidity in the soil, which is especially important during periods of drought (Yeraliyeva *et al.*, 2017). The use of pesticides helped to reduce losses from pests and diseases, and this substantially affected the entire crop volume of oilseeds of the Asteraceae and Linaceae families (Table 2), as well as the Brassicaceae family (Table 3). In general, the use of an integrated approach, including the latest varieties and agronomic technologies, usually gives better results compared to traditional methods. This will allow agricultural producers to maximise the use of resources, increase agricultural productivity, and ensure the sustainability of agricultural systems in the long term (Table 3).

***Table 2**. Seed yield of oilseed crops in the Asteraceae and Linaceae families (average for 2022-2024), t/ha*

Nutrition system (Factor B)	Protection system (Factor C)	Crop, Hybrid/Variety (Factor A)					
		Sunflower		Safflower		Oilseed flax	
		P64LE25	Forward	Lahidny	Dobrynya	Orpheus	Dobrodar
Without fertilisers	Without protection	1.74	2.02	1.57	1.6	1.22	1.27
	Recommended	1.81	2.15	1.62	1.6	1.25	1.33
	Optimised	1.82	2.17	1.63	1.67	1.25	1.33
Recommended	Without protection	2.29	2.69	1.77	1.85	1.41	1.47
	Recommended	2.37	2.75	1.83	1.93	1.47	1.53
	Optimised	2.38	2.78	1.83	1.93	1.47	1.53
Optimised	Without protection	2.37	2.89	1.82	1.99	1.47	1.62
	Recommended	2.47	3.12	1.89	2.09	1.47	1.69
	Optimised	2.51	3.19	1.9	2.1	1.51	1.71

***Source:** compiled by the authors*

***Table 3**. Seed yield of oilseed crops in the Brassicaceae family (Average for 2022-2024), t/ha*

Nutrition system (Factor B)	Protection system (Factor C)	Crop, Hybrid/Variety(Factor A)					
		Winter rapeseed		Brown mustard		Camelina	
		Abacus	Phoenix CL	Dzhyonka	Zabahanka	Prestige	Euro 12
Without fertilisers	Without protection	1.61	2.02	1.21	1.43	1.12	1.19
	Recommended	1.83	2.08	1.29	1.5	1.15	1.23
	Optimised	1.79	2.1	1.29	1.51	1.15	1.24
Recommended	Without protection	1.71	2.12	1.32	1.56	1.29	1.37
	Recommended	1.85	2.34	1.37	1.63	1.26	1.41
	Optimised	1.9	2.33	1.38	1.64	1.25	1.41
Optimised	Without protection	1.77	2.4	1.39	1.69	1.38	1.47
	Recommended	1.89	2.47	1.44	1.77	1.45	1.54
	Optimised	1.93	2.56	1.45	1.8	1.47	1.57

***Source:** compared by the authors*

The quality indicators of plant seeds are significantly influenced by cultivation technology. The introduction of new hybrids and varieties has led to an increase in oil content and improvements in other quality parameters, such as a reduction in chemical residues. The use of an optimised fertilisation system positively impacted overall quality but resulted in a slight increase in moisture content and chemical residues. Applying an optimised nutrition system alongside a combined protection system achieved optimal oil content and reduced chemical residues, though the impact on moisture content was variable (Table 4). Overall, implementing individual technological elements contributed to improved results, but their effectiveness depends on specific conditions and crop types.

Table 4*. Oil content and seed moisture of studied crops under optimised nutrition and protection systems (average for 2022-2024)*

Culture	Variety/hybrid	Oil content (%)	Seed moisture (%)	Pesticide residues (mg/kg)
Sunflower	P64LE25	42	9	0.5
	Forward	47	8.5	0.3
Winter rapeseed	Abacus	44	8.8	0.4
	Phoenix CL	48.5	7.5	1
Safflower	Lahidny	40	9.5	0.2
	Dobrynya	42	9	1
Brown mustard	Dzhyonka	32	10.5	1.5
	Zabahanka	34	10	0.9
Oilseed flax	Orpheus	33	9.8	1.1
	Dobrodar	38	10	0.4
Camelina	Prestige	29	12.5	0.3
	Euro 12	31	11	0.8

Source: *compared by the authors*

The technological elements used in sunflower cultivation had a significant impact on oil quality. The application of irrigation and pesticides ensured stable and high oil content, contributing to reduced acid and peroxide values, which indicate improved oil stability. The Omega-6 content, essential for human health, remained stable and may even increase with these agronomic practices. The optimised nutrition system positively affected sunflower oil quality, enhancing oil content and reducing the acid value, indicative of lower oxidative processes in the oil. Omega-6 content remained high, thus preserving valuable nutritional properties. In the control treatment, oil quality was at a basic level. Oil content, acid and peroxide values, and omega-6 content were indicators that provide baseline oil quality (Table 5). Overall, the use of fertilisers, irrigation, and pesticides significantly improved sunflower oil quality, promoting stability, reducing oxidation, and maintaining essential nutritional components.

Table 5*. Quality indicators of sunflower oil from the hybrid forward*

Treatment	Oil content (%)	Quality indicators
Without fertilisers and protection	-	Acidity (°K): 0.3
		Peroxide value (meq/kg): 7
		Free fatty acid content (%): 0.1
		Iodine index (g iodine/100 g): 120
		Omega-6 content (%): 65
		Colour (Yellow scale): 2
Recommended nutrition and protection system	45.5	Acidity (°K): 0.2
		Peroxide value (meq/kg): 5
		Free fatty acid content (%): 0.1
		Iodine index (g iodine/100 g): 122
		Omega-6 content (%): 62
		Colour (Yellow scale): 1.8
Optimised nutrition and protection system	47	Acidity (°K): 0.4
		Peroxide value (meq/kg): 6.5
		Free fatty acid content (%): 0.2
		Iodine index (g iodine/100 g): 119
		Omega-6 content (%): 64
		Colour (Yellow scale): 2.5

Table 5. Continued

Treatment	Oil content (%)	Quality indicators
Combined protection+irrigation	48.5	Acidity (°K): 0.2
		Peroxide value (meq/kg): 4
		Free fatty acid content (%): 0.1
		Iodine index (g iodine/100 g): 123
		Omega-6 content (%): 65.5
		Colour (Yellow scale): 1.7

Source: *compared by the authors*

Agronomic practices, such as the introduction of new varieties and hybrids, optimised nutrition and protection systems, and irrigation, significantly impact plant disease resistance, helping either to improve or maintain disease resistance at control levels across different crops. Selecting new crop varieties or hybrids often leads to increased resistance to disease. Specifically, new sunflower and rapeseed hybrids have shown improved resistance to respective diseases compared to control variants. The application of an optimised nutrition system can positively influence plant disease resistance, though results may vary depending on the crop and specific conditions. For instance, in some cases, fertilisation enhances resistance to certain diseases, while in others, the effect may be less pronounced. Irrigation and pesticide application also positively impact plant resistance, potentially improving resilience against diseases such as Fusarium or fungal infections, though effectiveness varies based on the crop and methods applied (Table 6). Overall, implementing new agronomic practices is an effective method for enhancing plant disease resistance, though their efficacy may fluctuate depending on specific growing conditions and crop type.

Table 6*. Resistance of studied oilseed crops to diseases and pests depending on production intensification*

Culture	Variety/hybrid	Disease resistance
Sunflower	P64LE25	Fusarium resistance – 80%, powdery mildew resistance – 75%
	Forward	Fusarium resistance – 90%, powdery mildew resistance – 85%
	Forward	Fusarium resistance – 80%, powdery mildew resistance – 75%
Winter rapeseed	Abacus	Fusarium resistance – 80%, powdery mildew resistance – 75%
	Phoenix CL	Soybean moth resistance – 75%, Fusarium resistance – 70%
	Phoenix CL	Soybean moth resistance – 85%, Fusarium resistance – 80%
Safflower	Dobrynya	Blackleg resistance – 75%, fungal infection resistance – 70%
	Dobrynya	Fungal infection resistance – 70%
	Lahidny	Fungal infection resistance – 70%
Brown mustard	Dzhyonka	Blackleg resistance – 75%, fungal infection resistance – 70%
	Zabaganka	Fungal infection resistance – 70%
	Zabaganka	Fungal infection resistance – 70%
Oilseed flax	Orpheus	Whitefly resistance – 70%, fungal infection resistance – 65%
	Dobrodar	Fungal infection resistance – 70%
	Dobrodar	Fungal infection resistance – 70%
Camelina	Prestige	Fungal infection resistance – 70%
	Euro 12	Fungal infection resistance – 80%
	Euro 12	Fungal infection resistance – 70%

Source: *compared by the authors*

New varieties and hybrids have demonstrated superior results compared to standard cultivars. They have shown increased yields and enhanced seed adaptability, making them recommended for further use. The application of organic fertilisers is an effective measure for improving the yield and quality of crop seeds. It is advisable to use organic fertilisers for all oilseed crops. This not only boosts yields but also positively influences the overall condition of the plants. The integrated use of pesticides and irrigation has proven effective in increasing plant productivity and reducing crop losses. The use of Confidor and Fury is recommended for pest control, along with ensuring adequate irrigation levels for optimal growth conditions. To achieve the best results, it is crucial to conduct regular monitoring of plant growth processes. Laboratory analysis for oil content and other physicochemical properties will ensure high product quality. The results of the study indicate a positive impact of selected elements on plant resistance and economic benefits. Therefore, this study demonstrates that innovative agronomic practices and the selection of high-yielding varieties can significantly enhance the effectiveness of oilseed cultivation.

DISCUSSION

The results obtained suggest that the introduction of new varieties (hybrids) of oilseed crops and the application of innovative technological elements positively influence yield and product quality. The findings establish that new varieties (hybrids) and agronomic measures (such as organic fertilisers and irrigation) enhance yield, oil content, and resistance to diseases and pests. For instance, the new sunflower hybrid, Forward, exhibited a yield increase to 3.2 t/ha compared to the standard variety, P64LE25, which yielded 2.5 t/ha. This is important, as such improvements can substantially enhance the economic benefits for agricultural producers, stabilise profits, and contribute to food security.

Research has substantiated that innovative technological elements, such as new varieties (hybrids) and cultivation conditions, significantly affect the yield and seed quality of camelina. The findings align with the conclusions of F. Al Juhaimi *et al*. (2024), who noted that new camelina varieties can substantially enhance yields. However, their study focuses more on the genetic characteristics of the varieties. In contrast, another study places greater emphasis on the agronomic elements of these technologies. According to F. Chen *et al*. (2024), agronomic practices, particularly the use of organic fertilisers, positively influence yield and seed quality. This is consistent with data showing that the application of organic fertiliser increased flax yield to 1.8 t/ha compared to the control, along with a reduction in oil acidity with the addition of organic fertilisers.

In studies conducted by A. Wenda-Piesik and K. Ambroziak (2022), it was determined that the application of irrigation could significantly improve the oil quality of safflower. The results affirm the importance of optimising cultivation conditions to achieve high-quality raw material outputs. Specifically, in one of the trials where an irrigation system was implemented, a notable improvement in oil quality was observed. This suggests that proper water resource management can considerably influence the agronomic characteristics of safflower, which, in turn, may positively impact the expansion of its cultivated area (Bulgakov *et al.*, 2020).

K. Kuzmin *et al.* (2024) focused on a detailed examination of the impact of various fertiliser types on camelina productivity. Their study revealed that the application of a combination of organic and mineral fertilisers could significantly enhance both yield and seed quality. These findings are consistent with data from the current study, which demonstrated that the use of organic fertiliser increased mustard yield to 1.8 t/ha. This underscores the importance of using organic fertilisers as a technological element to achieve optimal results in the cultivation of various agricultural crops (Malik *et al.*, 2024).

S. Deekshitha *et al*. (2024) established that new varieties (hybrids) can improve oil content. Their research confirms that the camelina variety Euro 12 provided a higher oil content in seeds compared to the standard. F. Xiang *et al*. (2024) investigated the effect of irrigation on seed quality, noting significant improvements in oil quality. The results corroborate these findings, as irrigation in one of the trials raised the iodine value to 123 gI_2/100 g. A. Kaolaor *et al.* (2024) indicated that fertilisers and growing conditions can enhance resistance to pests and diseases. The data from this study support these findings, as all variants exhibited high resistance to diseases and pests. L. Nguyen *et al.* (2024) discovered that new varieties with enhanced agronomic traits significantly improve yield and product quality. This aligns with the results obtained in this study, where new varieties (hybrids) demonstrated higher yields and seed quality compared to control variants.

V.A. Vytoptova (2023) reported the advantages of modern hybrids, although specific comparative values were not provided. She also explored the state and challenges of Ukrainian agriculture during wartime, noting that performance may vary by region. A. Snetkova (2019) and A.A. Mazaraki and V.D. Lagutin (2016) focused their studies on new oilseed varieties, although their findings indicate lower oil content in seeds, dependent on growing conditions. These findings suggest that the Forward sunflower hybrid used in this study indeed holds competitive advantages under favourable conditions. In addition, K. Tkachenko (2022) and K. Vasylkivska (2021) examined oilseed production and export trends, closely linked to the new hybrids and varieties featured in this study.

F. Zanetti *et al.* (2019) engaged in mathematical modelling of crop processes. The results of this study confirm the researchers' conclusions on the effectiveness of irrigation technologies, offering valuable insights into optimising production processes, which could substantially enhance productivity in the sector. The researchers also explored new crops for biofuel, providing relevant context to the study's focus on new varieties and hybrids. Their findings open up new possibilities for alternative energy sources, reducing reliance on conventional fuels. L. Pari *et al.* (2019) examined the environmental impact and economic viability of oilseed crops. The findings of their studies directly correlate with the conclusions of this study, particularly in the context of using organic fertilisers and irrigation. This highlights the importance of integrating environmental aspects into oilseed crop production, which can not only improve yields but also conserve ecosystems. Such a holistic approach to agronomy helps to mitigate environmental impact and supports sustainable development within the agricultural sector.

J. Ji *et al.* (2024) provided a detailed analysis of the positive effects of organic fertilisers and modern irrigation methods on agricultural crop yields. The findings of this study not only corroborate prior conclusions but also demonstrate consistency in the data obtained, underscoring the importance of adopting organic farming methods that can enhance productivity and increase the resilience of agro-systems to climate change. S. Yaheliuk and M. Fomych (2024) highlighted significant achievements in the development of resilient crop varieties that demonstrate similar adaptation results under stress conditions. This enabled a comparison of the resilience levels of the sunflower, flax, rapeseed, mustard, camelina, and safflower varieties under study, aligning them with top-performing varieties in the field and confirming their competitiveness. L. Pokopceva *et al.* (2024) identified a positive impact on sunflower productivity through the application of various biopreparation modifications and growth regulators. The importance of such studies lies in the potential to create new varieties and hybrids capable of withstanding extreme conditions, thereby ensuring high yields and reducing risks for agricultural producers. The findings of this study not only corroborate numerous conclusions drawn in other studies but also expand the understanding of how agronomic practices and conditions affect the cultivation of key oilseed crops such as sunflower, flax, rapeseed, mustard, safflower, and camelina.

CONCLUSIONS

This study assessed the impact of new oilseed crop varieties, particularly camelina, on seed yield, quality, and the economic appeal for agricultural production. Results indicate the positive effects of innovative technologies, such as organic fertilisers and irrigation systems, on key agronomic factors. The average yield of camelina reached 1.6 t/ha, substantially higher than that of standard varieties. The influence of irrigation and pesticides on plant growth was positive, providing optimal conditions for plant development. High oil quality was observed in the seeds of new varieties and hybrids, with improved oil content and reduced chemical residues. The use of organic fertilisers also enhanced product quality, though moisture content was slightly higher. New varieties and hybrids demonstrated increased resistance to major diseases. Organic fertilisers positively affected overall plant health, although their impact on disease resistance varied. The combined use of irrigation and pesticides increased plant disease resistance, but the effect was inconsistent depending on the specific crop.

It is recommended to continue the selection and introduction of new crop varieties (hybrids) to enhance yield and product quality, with a particular focus on their resistance to diseases and stress conditions. For maximising outcomes, the optimisation of fertiliser types and dosages is advised, with an emphasis on organic fertilisers, which have shown promising results. The continued use of irrigation and pesticides is also encouraged, with ongoing evaluation under varying conditions to optimise costs and improve efficiency. Regular monitoring of product quality is essential, including analysis of oil content and chemical residues. However, this study has limitations; it was conducted within a single geographic area (Mykolaiv region), which may restrict the generalisability of the findings to other regions. Furthermore, it did not extensively explore different types of organic fertilisers or irrigation methods in the context of climate change impacts. Future research should encompass multiple geographic zones, include a broader range of crop varieties (hybrids), organic fertiliser types, irrigation methods, and schedules, and evaluate the effects of climate change and new technological elements. Long-term monitoring of soil fertility and crop productivity changes is also essential to obtain more accurate and sustainable results.

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CONFLICT OF INTEREST

None.

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Перспективи та напрямки диверсифікації культур олійної групиї

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Анотація. Метою роботи було дослідити вплив елементів технології на врожайність і якість олії з різних сортів олійних культур, зокрема соняшника, ріпаку, сафлору красильного, льону, рижію, гірчиці сизої. Оцінено ефективність різних методів агротехніки (види добрив, системи захисту та агротехнічні заходи) для покращення якості і кількості врожаю цих культур.Дослідження проводили впродовж трьох років на базі навчально-науково-практичного центру Миколаївського національного аграрного університету. Методологія включала проведення польових експериментів дослідів із різними агрономічними технологіями, лабораторний аналіз отриманих рослинних зразків, визначення вмісту олії. За результатами встановлено, що застосування оптимізованої системи живлення та комбінованої системи захисту підвищує врожайність насіння соняшника на 15 % та якість олії на 10 %, застосування інноваційних елементів за вирощування льону збільшує олійність насіння на 8 %. Для ріпака було встановлено, що використання прийнятих систем живлення та захисту підвищує врожайність на 20 %. Дослідження гірчиці продемонструвало значне поліпшення стійкості культури до хвороб при застосуванні інтегрованого захисту рослин. Висновки підтверджують важливість вибору оптимальних елементів технології, які сприяють підвищенню врожайності та якості олійних культур. Це питання є надзвичайно актуальним, оскільки олійні культури займають значне місце в агропромисловому комплексі. Підвищення ефективності вирощування олійних культур не лише забезпечує стабільний прибуток для аграріїв, але й позитивно впливає на продовольчу безпеку країни в цілому. Таким чином, впровадження науково обґрунтованих агрономічних заходів є ключовим фактором для досягнення сталого розвитку аграрного сектору та диверсифікації культур олійної групи

Ключові слова: агропромисловий комплекс; рослини олійної групи; елементи технології; сорти і гібриди; урожайність; якість насіння

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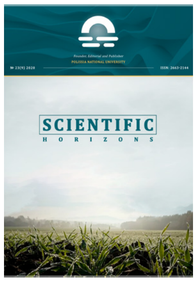

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Changes in the composition of grass and shrub layer plants in common oak stands since the closure of leaf canopy

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Abstract. Nowadays, there are threats of extinction of plant communities and individual plant species. Therefore, it is important to investigate the possibilities of restoring the composition of plants of different levels of forest phytocoenoses after clear-cutting in specific types of forest conditions. The purpose of the study was to establish the possibilities and features of restoring the species composition of plants of the grass and shrub layer, new growth and undergrowth after closing of canopies on common oak stands in fresh sugruds. Methods used: species accounting, comparison of species diversity at different sites, assessment of species abundance. It was established that the grass and shrub layer in closed forest stands consists of 54-57 species and it has a significant foliage cover – 60%. The base of the layer consists of 18-20 species. There

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is an increase with age in the value of the foliage cover of the main types of grass and shrub layer from 57-58% to 63-68%. It was found that the largest number of plants of the grass and shrub layer belongs to the forest ecological and coenotic group: in 9-10-year-old stands, 53.5% of species belong to the group, in 20-40-year-olds – 66.0%, and in 50-60-year-olds – 69.2%. It was found that the undergrowth consists of 4-6 species with a total closeness of 20-30% in crops of all age groups. The new growth consists of 6-8 species in forest stands of all age groups and their foliage cover ranges from 20-25%. The ratio of plants of different ecological and coenotic groups in oak stands of 50-60 years is quite close to overripe (130-140 years) natural stands. The practical significance of the study lies in the possibility of using the results in the practice of creating environmental protection facilities and justifying the systems of harvesting for the general use

Keywords: grass, shrubby and woody vegetation; foliage cover; Sorensen species similarity index; ecological and coenotic group

INTRODUCTION

Forestry in Ukraine has always been aimed at increasing the productivity of forest stands to obtain more wood. With this in mind, pure stands of certain tree species were created, introductions were made, agrotechnical measures were taken to increase the intensity of forest crop growth, etc. Only over time did the forestry doctrines include provisions on ensuring the sustainability of forest ecosystems, the need to preserve biodiversity and ensure social functions. But even to this day, the practice of continuous logging for the general use and subsequent artificial afforestation of cutting areas continues.

According to V. Khryk and I. Kimeichuk (2021), in the forest management system, the most destructive for forest ecosystems are continuous logging, which leads to the destruction of the existing forest phytocoenosis and drastic changes in environmental conditions on the logging site. On logging sites there is: a sharp increase in the illumination of the soil and plants of the lower tiers of vegetation; significant damage to the soil cover and changes in many indicators that characterise its condition; destruction of soil animals, microorganisms, and flora; changes in the temperature conditions of air and soil, and their humidification modes. All of the above leads, in turn, to the disappearance or reduction of the spread of particularly shade-loving and shade-tolerant plants. Thus, O. Ostapchuk and O. Sovakov (2024) after a survey of 1-6 year-old logging sites in the Right-Bank Forest-Steppe, a generalisation was made that there was a sharp change in the species diversity and species of forest plants in comparison with ripe oak stands. Further changes in environmental conditions and vegetation cover are facilitated by silvicultural operations, which in the practice of forestry in Ukraine are carried out within one year after logging. Preparation of the soil for planting forest crops often consists in creating furrows. V. Tkachuk *et al.* (2019) found significant damage to blueberry thickets during the period of soil preparation for forest crops.

Notably, the deforestation of the territory of the state in the first half of the 20th century (before, during, and after the Second World War) significantly disrupted the age structure of the state's forests. The Public report of the Head of the State Forest Resources Agency of Ukraine for 2023 (2024) notes that the age structure is dominated by middle-aged plantings, the share of ripe and overripe stands is 18.7%. The average age of forests is more than 60 years, and there is a gradual ageing of forests, which leads to a deterioration in their sanitary condition. Thus, most of the forests of Ukraine are artificial. However, the Cabinet of Ministers of Ukraine approved the state strategy forest management of Ukraine until 2035 (2021), which provides for a gradual reduction in the total area of continuous logging, and also proposes to ensure the transition to close-to-nature methods of forestry with the establishment of forests of natural composition and structure. The latter involves the use of natural renewal of the main forest-forming tree species. This, according to P. Yavorovskyi *et al.* (2019), will ensure the conservation of biological diversity and sustainability of forest stands. V. Tkach *et al.* (2024), after a long study of the natural renewal of common oak after continuous logging, also state that the use of natural renewal is an important element in close-to-nature forestry. However, information is needed on the state of plant diversity in forest crops of different ages and the possibility of its restoration after continuous logging for the general use on a forest-type basis. Thus, in the Volyn and Zhytomyr Polissia, I. Ivanyuk (2021) investigated the restoration of plants of living ground cover in common oak crops in moist sugruds. The researcher established a very significant dynamism of plant composition after logging and creation of forest crops: disappearance and reduction of the spread of sciophytes and sciogeliophytes and the appearance of a significant number of sciophytes in open forest crops; gradual restoration of the presence of sciophytes in closed forest crops. O. Andrushchenko *et al.* (2018), who studied the species composition of plants of living ground cover in oak stands of different ages in the forest-steppe of Ukraine, also came to similar conclusions. L. Borsukevych (2023), who investigated floodplain forests of various species composition, concluded that artificial and overgrown oak forests have a weak ability to self-heal and require measures to optimise their structure. Therefore, studies aimed at establishing the possible restoration of the composition of forest plants after continuous logging in various types of forest conditions are relevant.

One of the areas of research that would allow conducting a system of measures to implement the above-mentioned areas for the protection of biodiversity is objective information about its state. An important part of this is indicators on the impact of various anthropogenic factors on the distribution, productivity, and fruiting of plants in various natural and anthropogenic systems. Thus, studies aimed at identifying the dynamics of the composition of plants of living subsurface cover in stands of different ages after canopy closure, created after continuous logging, in different types of forest conditions are important. Their results, after comparing them with the materials of the plant species composition in sub-climactic forests, will help to make generalisations about the possibilities of restoration.

The purpose of the study was to establish the possibilities and features of restoring the species composition of plants of the grass and shrub layer, new growth and undergrowth after closing canopies of common oak crops in fresh sugruds.

MATERIALS AND METHODS

The research was conducted on trial plots (TP) established in the forest plantations of state forestry enterprises (now branches "Luhyn Forestry" and "Radomyshl Forestry and Hunting Range" of the State Enterprise "Forests of Ukraine") in Zhytomyr Oblast, Ukraine. Trial plots were selected using a relational database of the Forest Fund of Ukraine as of 2017, and according to the accounting books of forest crops as of 2020 of specific forestry enterprises/forest districts. Technologies for creating forest crops were representative of the study region for a specific period, and the taxational characteristics of forest crops of the noted age groups were quite close (Table 1). This allows making certain generalisations about the course of the processes under study.

***Table 1**. Taxational and forestry characteristics of forest stands on trial plots*

No.	Composition	Age, years	Height, m	Diameter, cm	Quality class	Stand density	Inventory, $m^3 \cdot ha^{-1}$	Forest type
13	10Co	10	3	4	II	0.85	18	C_2HOP
14	7Co1Ep1Wb1Ch+Ba	10	3	4	III	0.80	16	C_2HO
15	8Co2Nm	10	3	4	III	0.80	18	C_2HO
16	8Co2Wb	29	12	10	I	0.75	116	C_2HOP
17	10Co	40	13	16	III	0.75	124	C_2HOP
18	10Co	20	10	10	II	0.75	110	C_2HOP
19	10Co+Wb	62	20	24	I	0.70	235	C_2HOP
20	8Co2Sp	56	19	22	I	0.70	230	C_2HOP
21	10Co	48	18	22	I	0.70	196	C_2HOP
1	9Co1Sp+Wb	130	25	40	3	0.60	270	C_2HOP
2	8Co1Sp1Wb	131	27	40	2	0.70	340	C_2HOP
3	10Co+Wb	126	28	46	2	0.60	308	C_2HOP

***Source:** developed by the authors based on the relational database of the Forest Fund of Ukraine (2017)*

Trial plots measuring 100×100 m (1 ha) were established according to the description of O. Pakhomov and V. Petrushevskyi (2021). Within each TP, accounting areas (1×1 m) were established diagonally, where the foliage cover or presence of each plant species was determined according to the method described by I. Kuzmishina (2019). The study was conducted during April-October 2020. The study of the species composition of plant biodiversity in forest stands and overripe plantings was carried out according to identification guide by D. Dobrochayeva *et al.* (1999), and Latin names were specified in the Global Biodiversity Information Facility database (2024). In order to compare plant biodiversity in forest stands of different ages and compare it with data obtained in overripe plantings, the Sorensen similarity index adopted in geobotany described by A. Dzyba (2021) was applied according to the equation:

$$\mathrm{I}ss = \frac{2\mathrm{C}}{\mathrm{A}+\mathrm{B}}, \tag{1}$$

where I*ss* – index of species similarity; A and B – number of species in the trial plots that are compared; C – number of common species in the trial plots. In the course of the study, ecological and coenotic groups of plants described by I. Kovalenko (2024) were identified – forest, edge, meadow, swamp, and ruderal.

RESULTS AND DISCUSSION

The grass shrub layer in closed forest crops (9-10 years old) consists of 54-57 species and it has a significant foliage cover of 60% (Table 2). However, the basis of the layer is 18-20 species.

***Table 1**. Main species of the grass and shrub layer in forest crops of common oak of different ages and their foliage cover in fresh sugruds (Quercetum franguloso (alni)-convallarioso-variaherbosum)*

Name of shrub and grass species	Foliage cover of species (%) and their presence (+, -) in forest stands of different ages								
	9-10 years old			20-40 years old			50-60 years old		
	13	14	15	16	17	18	19	20	21
Convallaria majalis L.	8	10	8	8	13	10	20	20	20
Stellaria holostea L.	7	7	8	10	8	10	10	10	8
Pteridium aquilinum (L.) Kuhn	8	8	14	10	10	12	10	10	10
Clinopodium vulgare L.	+	+	1	+	+	+	+	+	+
Genista tinctoria L.	+	1	+	+	+	+	+	+	+
Veronica chamaedrys L.	+	+	+	+	+	1	+	+	+
Fragaria vesca L.	1	1	1	3	1	1	1	1	1
Anemone nemorosa L.	+	+	+	+	5	7	8	8	12
Clematis recta L.	3	5	3	5	3	3	3	3	3
Calamagrostis arundinacea (L.) Roth	-	-	-	3	1	3	1	1	+
Carex montana L.	+	+	-	3	5	3	5	10	10
Festuca rubra L.	8	5	5	5	5	3	3	3	1
Brachypodium sylvaticum (Huds.) P. Beauv.	+	+	-	+	1	+	-	+	-
Poa angustifolia L.	5	5	5	5	5	3	1	+	+
Betonica officinalis L.	1	1	+	1	1	+	+	1	1
Agrostis vinealis Schreb.	7	5	5	5	3	5	+	+	+
Potentilla alba L.	+	+	+	+	-	+	1	1	1
Erigeron canadensis L.	5	7	7	+	-	-	-	-	-
Erigeron annuus (L.) Pers.	3	3	+	-	-	-	-	-	-
Hypericum perforatum L.	1	+	+	+	+	+	+	+	+
Calamagrostis epigeios (L.) Roth	1	+	+	-	-	-	-	-	-
Foliage cover of the main species, %	***58***	***58***	***57***	***58***	***61***	***61***	***63***	***68***	***67***
Number of main species, units	***20***	***20***	***18***	***19***	***17***	***18***	***17***	***18***	***17***
Overall foliage cover of the layer, %	***60***	***60***	***60***	***60***	***65***	***63***	***65***	***70***	***70***
Total number of species, units	***55***	***57***	***54***	***51***	***60***	***57***	***51***	***65***	***54***

Source: *developed by the authors of this study*

The most widely distributed in terms of area are: *Convallaria majalis* L. with foliage cover – 8-10%, *Pteridium aquilinum* (L.) Kuhn – 8-14%, *Stellaria holostea* L. – 7-8%, *Festuca rubra* L. – 5-8%, *Agrostis vinealis* Schreb. – 5-7%, and *Poa angustifolia* L. – 5%. The first three species belong to the forest and their presence in older forest crops increases slightly – in *Convallaria majalis* – up to 8-13% in 20-40 year-old stands and 20% in 50-60 year-old stands, or stays at the same level. The following three species are those that prefer open areas and their presence decreases significantly with increasing age of forest stands. The latter also include *Erigeron canadensis* L., *Erigeron annuus* (L.) Pers., *Calamagrostis epigejos* (L.) Roth, *Hypericum perforatum* L. All of them probably became somewhat widespread before the closure of forest canopies, retained some presence after the closure of the latter, and disappeared in the following period. The dynamics of the spread of *Anemone nemorosa* L. in the common oak stands over time is noteworthy: absence in 9-10-year-old stands and reaching a foliage cover of 8-12% in 50-60-year-old stands. There is an increase in the foliage cover of the main species of the grass and shrub layer with age from 57-58% to 63-68% due to an increase in distribution of *Convallaria majalis*, *Carex montana* L., *Stellaria holostea* and *Anemone nemorosa* in older crops.

The number of sparsely distributed species in the grass and shrub layer is quite significant: in 9-10-year-old common oak stands – 23-24 species and their foliage cover is in the range of 2-3% (Table 3). The distribution of these species by area remains at the same level in older forest stands, and their number increases slightly. Thus, in 20-40-year-old oak crops, their number is 27-30 units, and for 50-60-year-olds – 23-34 units. A general trend can be noted: the increase in the number of species of the grass and shrub layer in older forest crops occurs due to the restoration of shade-tolerant and shade-loving species against the background of the disappearance of some light-loving ones. However, there is also the appearance of the latter in older forest stands. This may be conditioned by a number of reasons – the presence of a source of seed supply, the natural possibilities of seed entering a certain area (its volatility, displacement factors, etc.), and the presence of meadows, forest edges, sparse stands, the development of new growth and undergrowth. The necessary conditions appear both naturally (the death of trees, shrubs), and after logging and forest maintenance. It is known that during the latter, not only some trees are selected, but also, more often, a significant part of the new growth and undergrowth is cut down. It is possible that the presence of a significant number of light-loving species in common oak stands of different ages is associated precisely with the system of forest sanitation.

***Table 3**. Composition of sparsely distributed plants of the grass and shrub layer in common oak stands of different ages in fresh sugruds (Quercetum franguloso (alni)-convallarioso-variaherbosum)*

Name of shrub and grass species	Foliage cover of species (%) and their presence (+, -) in forest stands of different ages								
	9-10 years old			20-40 years old			50-60 years old		
	13	14	15	16	17	18	19	20	21
Viola montana L.	+	+	+	+	+	+	-	+	+
Geum urbanum L.	+	+	+	+	+	+	+	+	+
Vicia sepium L.	-	-	-	+	-	-	+	-	-
Pilosella onegensis Norrl.	-	-	-	+	+	+	-	+	+
Galium tinctorum L.	+	-	-	-	-	-	-	+	-
Trifolium alpestre L.	-	+	+	+	+	+	+	+	+
Trifolium medium L.	+	-	-	-	-	-	+	-	-
Astragalus glycyphyllos L.	+	+	+	+	+	+	-	+	+
Primula veris L.	+	+	+	+	+	+	+	+	+
Euphorbia angulata Jacq.	-	-	-	-	-	-	-	+	-
Ranunculus auricomus L.	-	-	-	+	+	+	-	-	-
Ranunculus polyanthemos L.	-	-	-	-	+	+	+	+	+
Cruciata glabra (L.) Opiz	+	+	+	+	+	-	+	+	+
Taraxacum officinale Weber ex FHWigg.	+	-	-	-	-	+	+	+	+
Campanula trachelium L.	-	-	-	-	+	-	-	-	-
Campanula persicifolia L.	+	+	+	+	+	+	+	+	+
Dactylis glomerata L.	-	-	+	-	+	+	-	-	-
Lathyrus niger (L.) Bernh.	-	+	+	+	+	+	+	+	-
Tanacetum corymbosum (L.) Sch.Bip.	-	-	-	+	+	+	-	+	+
Serratula tinctoria L.	-	-	-	+	+	+	+	+	+
Viola hirta L.	+	+	+	+	-	+	-	+	+
Anthericum ramosum L.	-	-	-	+	+	+	+	+	-
Pulmonaria obscura Dumort.	-	-	-	-	-	+	+	+	+
Pulmonaria angustifolia L.	-	-	-	+	+	+	-	+	+
Pulsatilla patens (L.) Mill.	-	-	-	+	+	+	+	+	-
Dryopteris filix-mas (L.) Schott	-	-	-	-	-	-	+	+	-
Dryopteris carthusiana (Vill.) H.P.Fuchs	+	+	+	+	+	+	+	+	+
Scrophularia nodosa L.	+	+	+	+	+	+	+	+	+
Silene nutans L.	-	+	+	+	+	+	-	+	+
Silene vulgaris (Moench) Garcke	-	-	-	-	-	-	+	+	+
Platanthera bifolia (L.) Rich.	-	-	-	+	+	+	-	+	-
Viola reichenbachiana Jord. ex Boreau	+	+	+	+	+	+	+	+	+
Moehringia trinervia (L.) Clairv.	-	+	-	-	+	-	+	+	+
Stachys sylvatica L.	+	+	+	+	+	+	+	+	-
Lilium martagon L.	-	-	-	-	-	-	-	+	+

Table 3. Continued

Name of shrub and grass species	Foliage cover of species (%) and their presence (+, -) in forest stands of different ages								
	9-10 years old			20-40 years old			50-60 years old		
	13	14	15	16	17	18	19	20	21
Potentilla argentea L.	+	+	+	-	-	-	-	-	-
Poa pratensis L.	-	-	+	-	-	-	+	-	+
Achillea millefolium L.	+	+	-	-	-	-	-	-	-
Hypochaeris radicata L.	-	-	-	+	+	-	-	+	+
Linaria vulgaris Mill.	+	-	+	-	-	-	-	-	-
Persicaria maculosa Gray	-	-	+	-	-	-	-	-	-
Pimpinella saxifraga L.	+	+	+	+	+	+	-	+	+
Lapsana communis L.	-	-	-	-	+	-	-	+	+
Mycelis muralis (L.) Dumort.	+	-	-	+	+	+	-	-	-
Leucanthemum vulgare Lam.	+	+	-	-	-	-	-	-	-
Saponaria officinalis L.	-	+	+	-	-	-	-	-	-
Daucus carota L.	+	+	-	-	-	-	-	-	-
Oreoselinum nigrum Delarbre	+	+	+	-	+	+	+	+	+
Carex leporina L.	-	-	+	-	-	-	-	-	-
Carex hirta L.	+	+	+	+	-	-	-	-	-
Foliage cover of species, %	***2***	***2***	***3***	***2***	***4***	***2***	***2***	***2***	***3***
Number of species, units	***23***	***23***	***24***	***27***	***30***	***28***	***23***	***34***	***27***

Source: *developed by the authors of this study*

Plants listed in the Red Book of Ukraine were found in some of the surveyed areas: in 20-40-year-old and in 50-60-year-old stands – *Platanthera bifolia* (L.) Rich. and for 50-60-year-old stands – *Lilium martagon* L. The presence of these Red Book species was established only in half of the surveyed areas. Notably, during similar studies in 130-140-year-old natural oak forests of fresh sugrud, *Platanthera bifolia* was found in all surveyed plots and *Lilium martagon* in half of them.

For the purpose of comparing samples of plants of the grass and shrub layer in oak stands of different ages, the Sorensen index was used between each other and those obtained by the authors. Thus, this indicator increases from 0.67 (9-10-year-old stands) to 0.86 (50-60-year-old stands) (Ivanyuk & Ivanyuk, 2019). The latter indicates a gradual restoration of the composition of plants of the grass and shrub lever after continuous logging. Similar results were obtained by M. Vedmid *et al.* (2008), who investigated the recovery of grass and shrub level plants after continuous logging of ripe pine forests in fresh subors. Comparison of the species composition of plants of the grass and shrub layer of forest stands of different ages among themselves (determination of the Sorensen index) indicates that this indicator practically does not change with increasing age of forest crops: from 0.72 (comparison of 9-10-year and 20-30-year crops) to 0.70 (comparison of 9-10-year and 50-60-year crops).

Distribution of the species composition of grass and shrub layer plants by ecological and coenotic groups (Fig. 1) established that the largest number of them belongs to the forest (in cultures of all age groups). Thus, in 9-10-year – old stands, this group includes 53.5% of species, in 20-40-year-olds – 66.0%, and in 50-60-year-olds – 69.2%. The most common forest species in all trial plots were: *Anemone nemorosa*, *Stellaria holostea*, *Convallaria majalis*, *Carex montana*, *Pteridium aquilinum*. There was a gradual increase in this indicator with increasing age of planting. This may indicate the restoration of forest plants that disappeared during the period and as a result of cutting down the edificatory layer of tree species. The number of species in all other ecological and coenotic groups decreases with increasing age of forest crops. In cultures of 50-60 years, the ratio of plants of different ecological and coenotic groups is quite close to the data obtained in overripe (130-140 years) natural stands of common oak. All test areas, regardless of the age of the stand, contained *Convallaria majalis, Stellaria holostea, Fragaria vesca*, *Veronica chamaedrys*, *Betonica officinalis*, *Clinopodium vulgare*, *Genista tinctoria*, *Clematis recta*, *Festuca rubra*, *Poa angustifolia*, *Agrostis vinyl*, and *Pteridium aquilinum*. Surely these species can withstand quite long-term lighting, competition with light-loving species, and have effective methods of recovery.

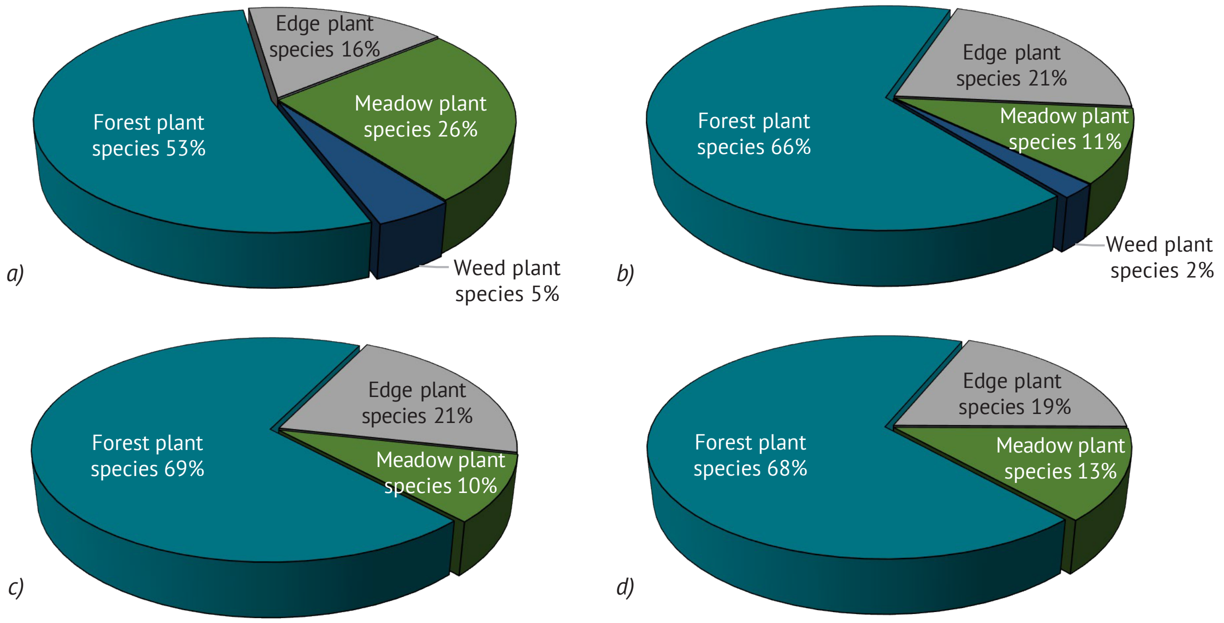

***Figure 1**. Distribution of grass and shrub layer plants by ecological and coenotic groups in common oak stands of different ages, in fresh sugruds*
***Note:** a – 9-10 years, b – 20-40 years, c – 50-60 years, and d – 130-140 years*
***Source:** obtained based on research*

The number of new growth and undergrowth species is also quite significant (Table 4). In the undergrowth, which consists of 4-6 species, the vast majority are two species: *Frangula alnus* Mill. and *Sorbus aucuparia* L. Their total foliage cover is 20-30% in forest crops of all age groups. Both species are shade-tolerant plants and recover well after deforestation and the creation of forest crops. The other 6 types of undergrowth are much less common in the area and are not found in all the surveyed areas. Attention is drawn to the distribution of the light-loving plant *Chamaecytisus ruthenicus* (Fisch. Ex Wol.) Klask in 9-10-year-old and 50-60-year-old crops. This may be due to its preservation after greater distribution in open forest crops and, possibly, the presence of small areas with some thinning of the stand (due to the reasons noted above) in older ones. Shade-tolerant *Corylus avellane* L. and *Euonymus verrucosus* Scop. are present in half of the surveyed areas and do not become widespread.

***Table 4**. Composition of new growth and undergrowth plants in common oak crops of different ages in fresh sugruds (Quercetum franguloso (alni)-convallarioso-variaherbosum)*

Name of shrubby and woody species	Foliage cover of species (%) and their presence (+, -) in forest stands of different ages								
	9-10 years old			20-40 years old			50-60 years old		
	13	14	15	16	17	18	19	20	21
Undergrowth									
Frangula alnus Mill.	20	20	20	20	20	20	20	20	20
Sorbus aucuparia L.	10	10	10	+	10	10	10	10	10
Rosa canina L.	+	+	+	+	+	+	-	+	-
Rosa villosa L.	-	+	-	+	-	+	-	-	+
Rosa tomentosa Sm.	-	-	-	-	+	+	-	-	-
Chamaecytisus ruthenicus (Fisch. ex Wol.) Klask.	+	+	-	-	-	-	1	+	+
Corylus avellana L.	+	+	+	+	-	-	+	+	-
Euonymus verrucosus Scop.	+	+	+	-	+	+	-	+	-
Closeness of the undergrowth, %	**30**	**30**	**30**	**20**	**30**	**30**	**30**	**20**	**30**
Number of undergrowth types, units	**5**	**6**	**4**	**5**	**5**	**6**	**4**	**6**	**4**
Undergrowth									
Quercus robur L.	8	7	8	7	10	5	10	10	10
Populus tremula L.	10	10	10	10	8	10	10	8	10
Betula pendula Roth.	7	3	1	3	2	5	+	2	5

Table 4. Continued

Name of shrubby and woody species	Foliage cover of species (%) and their presence (+, -) in forest stands of different ages								
	9-10 years old			20-40 years old			50-60 years old		
	13	14	15	16	17	18	19	20	21
Betula pubescens Ehrh.	-	-	1	-	+	5	-	-	+
Pyrus communis L.	+	+	1	+	+	+	+	+	+
Prunus cerasifera Ehrh.	-	+	-	+	-	-	+	-	-
Malus sylvestris Mill.	+	+	-	-	+	+	+	+	+
Pinus sylvestris L.	+	+	+	-	+	+	+	+	+
Carpinus betulus L.	-	+	+	+	-	-	+	+	-
Closing of the undergrowth, %	**25**	**20**	**21**	**20**	**20**	**25**	**20**	**20**	**25**
Number of undergrowth species, units	**6**	**8**	**7**	**6**	**7**	**7**	**8**	**7**	**7**

Source: *compiled by the authors*

The new growth consists of 6-8 species in forest stands of all age groups and their foliage cover ranges from 20-25%. Three species are the most widespread: *Populus tremula* L., *Quercus robur* L. and *Betula pendula* Roth. The materials obtained during the research period somewhat complement the existing ideas about the impact of continuous logging on plant biodiversity, and also indicate a high probability of its restoration in forest stands over time. It was established that the value of the Sorensen index, when comparing the number of species in sub-climatic oak forests and in common oak stands of different ages, increases with increasing age of the latter from 0.67 (9-10-year stands) to 0.86 (50-60-year stands). The success of this restoration will depend on many factors: technologies for clear-cutting and creating forest crops; specifics of the latter's care before closure and thinning afterwards; location (distance, location to prevailing winds) of specific areas in relation to settlements and agricultural land, etc. Similar conclusions were reached by O. Orlov *et al.* (2021), who studied vegetation on objects of the nature reserve fund in the territory of Zhytomyr Polissia. According to V. Agiy et *al.* (20-19), timely inspection logging aimed at preserving common oak in Transcarpathia will allow forming mixed stands for the dominance of this particular tree species, and the delay in carrying out such logging in forest stands under the age of 10 years may lead to the loss of this main forest-forming tree species and its replacement with common hornbeam. P. Spathelf *et al.* (2023), who investigated the reforestation potential of oak stands in the forest-steppe of Ukraine (Central Podillia and Roztochchia), found that it is quite high, but further research is needed on the relationship between plants of the ground cover and undergrowth.

The possibility and success of restoring plant biodiversity may have its own characteristics in certain types of forest-growing conditions, which requires further similar studies on a forest-typological basis. Based on materials from M. Bondaruk and O. Tselishchev (2021), who investigated the rare component of forest coenoses of the West and Central Polissia Forestry District of Ukraine on monitoring sites, forest vegetation is characterised by forest type diversity and is represented by 23 forest site types, and vegetation – by 222 species. Indicators for rare plants in fresh sugruds only partially coincide with the results of this research. In the course of the study, two plant species were identified that are listed in the Red Book of Ukraine – *Platanthera bifolia* (L.) Rich. and *Lilium martagon* L., and the authors of the study describe only the latter. It was established that restoration of *Lilium martagon* occurred on 66.7% of the surveyed areas, and *Platanthera bifolia* – only on a third. The researchers also state that the determination of the places of growth of rare plant species should be done on a typological basis and then conduct monitoring observations (once every 5 years) or survey of allocated areas during the FSC certification of forestry branches.

Studies aimed at investigating the dynamics of plant diversity in the development of successions of natural sub-climactic forests, again on a forest-typological basis, are also necessary. It is known that the further development of such forests in many cases leads to changes in the composition of tree species of the first tier. These circumstances may lead to some or significant changes in the composition of the lower layers. P. Ustymenko *et al.* (2019) in their research, came to the conclusion that the introduction of an absolute conservation regime in sub-climatic forests quite often leads to complete degradation of these natural objects that were created for their conservation. They explain this fact by the fact that in their development, plant groups do not reproduce their own kind (there is a change in the structure of phytocoenoses – the composition of plants, the structure of the coenosis itself), and the direction and speed of these changes depends on numerous abiotic and biotic factors. Comparison of the above areas of research will significantly expand the understanding of the protection, conservation, and restoration of plant diversity in the forests of a particular region of Ukraine. M. Bondaruk *et al.* (2019) support the above proposals and propose a revision of some provisions of the reserve case, which, in their opinion, are based on conservative plant protection with an absolute reserve regime.

Thus, the results of the conducted studies confirm the importance of a typological approach in the study and monitoring of forest ecosystems for the conservation and restoration of plant biodiversity. It was established that continuous logging and features of forest sanitation can both contribute to and hinder the natural restoration of species, depending on the technologies used and growing conditions.

CONCLUSIONS

As a result of the conducted studies, the species composition of grass and shrub layer plants in common oak stands of different ages was established: 9-10, 20-40, and 50-60 years. It was revealed that the most common plants include 18-20 species, among which forest plants have the greatest foliage cover – *Convallaria majalis* L. with foliage cover – 8-10%, *Pteridium aquilinum* (L.) Kuhn – 8-14%, *Stellaria holostea* L. – 7-8%, and types of open areas and forest edges – *Festuca rubra* L. – 5-8%, *Agrostis vinealis* Schreb. – 5-7%, and *Poa angustifolia* L. – 5%. Establishment of an increase in the foliage cover of the most common species of this layer with the age of forest crops. This is mainly conditioned by an increase in the areas of *Convallaria majalis*, *Carex montana* L., *Stellaria holostea*, and *Anemone nemorosa* L. The study also revealed a significant number of less common plants in stands of all age groups – 23-24 species in 9-10-year-old stands, 27-30 species in 20-40-year-old stands, and 23-34 species in 50-60-year-old stands. An increase in the number of species of the grass and shrub layer in older forest crops was observed due to the restoration of shade-tolerant and shade-loving species, which is also confirmed by calculations of the Sorensen index. When comparing the number of species in crops of different age groups with materials obtained in natural oak forests, its increase was found from 0.67 (9-10-year-old stands) to 0.86 (50-60-year-old stands). The distribution of the species composition of plants of the grass and shrub layer by ecological and coenotic groups was carried out, which revealed a significant number of plants at the forest edge, meadow, and weeds: in 9-10-year-old forest crops – 46.5%, in 20-40-year-old – 34.0%, and in 50-60-year-old – 30.8%. Their number was found to decrease with the age of common oak crops. The ratio of plants of different ecological and coenotic groups in cultures of 50-60 years is quite close to it in overripe (130-140 years) natural forests of common oak. The appearance of plants listed in the Red Book of Ukraine was also revealed – *Lilium martagon* L. and *Platanthera bifolia* (L.) Rich.) occurred on half of the surveyed areas of older forest stands. This coincides with the level of their presence in the surveyed areas of overripe natural forests.

The results of the study are important in terms of understanding the possibilities of restoring plants of the grass and shrub layer as a result of continuous logging for the general use in ripe forests with a predominance of common oak in fresh sugruds. Further research may focus on studying these processes in plantings of different species compositions on a forest-typological basis. Monitoring studies on the state of sub-climatic forests in protected areas are also promising.

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CONFLICT OF INTEREST

None.

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Зміна складу рослин трав'яно-чагарничкового ярусу у лісових культурах дуба звичайного з часу їх змикання

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Анотація. Внаслідок інтенсивної вирубки лісу у XX столітті в Україні порушена вікова структура лісових насаджень. Нині існують загрози зникнення рослинних угрупувань і окремих видів рослин. З огляду на це, актуальними є дослідження з вивчення можливостей відновлення складу рослин різних ярусів лісових фітоценозів після суцільної вирубки лісу в конкретних типах лісорослинних умов. Мета дослідження – встановлення можливостей і особливостей відновлення видового складу рослин трав'яно-чагарничкового ярусу, підросту та підліску після змикання культур дуба звичайного у свіжих сугрудах. Методи, які використовувались: облік видів, порівняння видового багатства на різних ділянках, оцінка рясності видів. Встановлено, що трав'яно-чагарничковий ярус у лісових культурах, що зімкнулися складається з 54-57 шт. і має значне проєктивне покриття – 60 %. Основу ярусу складають 18-20 видів. Спостерігається збільшення з віком величини проєктивного покриття основних видів трав'яно-чагарничкового ярусу від 57-58 до 63-68 %. З'ясовано, що найбільша кількість рослин трав'яно-чагарничкового ярусу відноситься до лісової еколого-ценотичної групи: у 9-10-річних культурах належить 53,5 % видів, у 20-40-річних – 66,0 % і у 50-60-річних – 69,2 %. Виявлено, що підлісок складається з 4-6 видів з сумарною зімкнутістю 20-30 % у культурах усіх вікових груп. До складу підросту входить 6-8 видів у лісових культурах всіх вікових груп і їх проєктивне покриття коливається у межах 20-25 %. Співвідношення рослин різних еколого-ценотичних груп у дубових культурах 50-60 років досить близьке до перестійних (130-140 років) природних деревостанів. Практична цінність досліджень полягає у можливості використання результатів у практиці створення природоохоронних об'єктів і обґрунтування систем рубок головного користування

Ключові слова: трав'яна, чагарникова та деревна рослинність; проєктивне покриття; індекс видової подібності Соренсена; еколого-ценотична група

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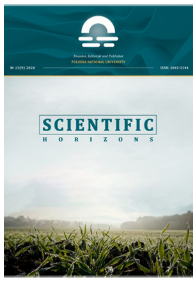

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Ecological typology of woody vegetation of the Starohut forest massif as a component of the restoration of disturbed ecotopes

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Abstract. The study aimed to determine the typological structure of the Starohut forest massif within the Desniansko-Starohutskyi National Nature Park based on the Ukrainian school of forest typology by Alekseev-Pogrebnyak. The massif covers an area of 6,778.8 hectares. The following trophotopes were identified in the study area: bors, subors, and sugruds. Bors, consisting of pine communities with some birch, occupy dry and fresh hygrotopes on flat terrain, covering 1.8 hectares or 0.02% of the total

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forest area. Subors, comprising pine-oak communities with birch and aspen, are found in fresh, moist, damp, and swamp hygrotopes, covering 4,510.1 hectares or 66.53% of the territory. Sugruds, consisting of oak-pine-linden-maple forests on plains and aspen-birch-linden-maple forests in depressions, cover 2,266.9 hectares or 33.44% of the Starohut forest massif. No gruds were identified. The study found that the primary forest-forming species in the national park is *Pinus sylvestris*, occupying 5,092.7 hectares. *Betula pendula* covers 1,207.1 hectares, *Alnus glutinosa* – 201.4 hectares, *Picea abies* – 111.5 hectares, and *Quercus robur* – 90.0 hectares. Other tree species are sparsely represented. In the protected area of the park, fresh subors and sugruds dominate, including a mixture of birch, black alder, and spruce over a total area of 1,181.1 hectares, along with moist pine-birch-alder-oak subors and sugruds covering 849.4 hectares. The recreational zone is dominated by fresh hygrotopes with subors and sugruds totalling 2,336.4 hectares. In the economic zone, subors and sugruds prevail on fresh sandy soils, totalling 430.7 hectares. The results support the restoration of natural forest types in areas affected by large-scale fires in 2023-2024 and other anthropogenic influences

Keywords: forest types; hygrotopes; trophotopes; biodiversity; rare species of flora

INTRODUCTION

Forests are essential biosphere components, providing numerous ecological functions such as oxygen production and regulating soil water balance. They also support vast areas of biodiversity, which are integrated into the ecological network. M. Pesendorfer *et al.* (2020) state that forest resources have significant economic value. The degradation of forest vegetation due to anthropogenic impact represents a global issue. Over the past 30 years, global forest cover has declined from 29% to 27%. L. Oitsius *et al.* (2020), through an analysis of forest phytocenoses in Ukrainian Polissia, concluded that their sustainability depends, to some extent, on biodiversity levels. S. Chadro *et al.* (2024) observed that significant changes in the vegetation cover of forest phytocenoses in recent years are attributed to climate change and human economic activities. D. Litvinov *et al.* (2020) assert that global climate change contributes to the reduction of species diversity in forest phytocenoses. M. Trifanova *et al.* (2024) emphasise that the EU's Biodiversity Strategy 2030 aims to strictly protect 10% of land to prevent the loss of rare flora and fauna species.

I. Koval and S. Sidorenko (2019) argue that intense anthropogenic impacts, such as deforestation, fires, and military operations, have significantly altered the structure of forest communities in Ukraine, particularly in Polissia, leading to a decline in biodiversity. A long-term consequence of the full-scale war in 2022-2024 is the destruction of forest ecosystems. The forests of the Desniansko-Starohutskyi NNP are suffering from constant shelling, forest fires, and extensive fortifications. Military actions have had a significant impact on the condition of the area's vegetation. Restoring and preserving forests for future generations is a critical task for foresters, ecologists, and environmental organisations, including the Desniansko-Starohutskyi NNP. This challenge can be addressed through the use of the Alekseev-Pogrebnyak forest typology, which is based on ecological and edaphic principles. Forest typology enables the restoration of disturbed forest phytocenoses, preserving the characteristic natural diversity of a given climate zone. According to E. Collado *et al.* (2023), close-to-nature forest management, also known as continuous cover forestry, is a sustainable forest management approach that maximises the conservation of species diversity in forest ecosystems while providing significant economic benefits.

According to R. Seidl *et al.* (2018), when restoring forest phytocenoses damaged by fires, hurricanes, or other negative factors, it is important to adhere to an environmentally appropriate forest crop typology to create a sustainable ecosystem; specifically, moisture-loving species should be planted on moist soils and mesotrophic species on moderately moist soils. As noted by V. Melnyk (2020), this is particularly relevant for spruce plantations, as spruce is highly sensitive to soil moisture levels and requires 30% to 80% soil moisture for optimal growth and development during the initial stages of ontogenesis. This ecological characteristic of the European spruce explains why the island spruce forests of Polissia are listed in the Green Book of Ukraine.

The restoration of disturbed ecotopes, including the forest ecosystem of the Starohut forest, requires a thorough understanding of the typology of the area across edaphic and hydrological gradients. An essential component of the restoration process is the development of an ecological and typological characterisation of this territory, which was the purpose of this article.

MATERIALS AND METHODS

The research was conducted within the Desniansko-Starohutskyi National Nature Park (Ukraine, Sumy region) from 2021 to 2023. According to the physical-geographical classification of Ukraine, the study area is located within the Pridesnyansky district of the Novgorod-Seversky physical-geographical region of Ukrainian Polissia. The coordinates of the Starohut massif as recorded by Landsat-8 are as follows: westernmost point – 52°18'39"N, 33°34'50"E; easternmost point – 52°19'55"N, 33°51'16"E; northernmost point – 52°22'06"N, 33°47'18"E; and southernmost point –

52°18'97"N, 33°39'47"E. The terrain of the natural park is a moraine low plain with a gentle slope westward towards the Desna River. The elevation above sea level in the park's western part, within the Desna floodplain, is 122 metres, while in the eastern part, it reaches 163 metres (Marynych *et al*., 2003). The soil cover of the Starohut forest includes soddy-podzolic gleyed fluvioglacial sandy soils in flat areas and peat, peat-bog soils in low-flow and drainage-deficient depressions. The study area has approximately 7% swampy terrain. The climate is temperate continental, with warm, humid summers and mild winters. The average annual air temperature is 6.5°C. In winter, the average temperature is -3.9°C, while in summer it is 18.7°C. Annual precipitation totals 608.7 mm and is unevenly distributed across the seasons: an average of 136 mm falls in winter, 148.5 mm in spring, 193.8 mm in summer, and 130.4 mm in autumn.

The data analysed included information from the Starohut forestry on forest management. The calculations were based on forest management tax descriptions provided by the SEBR of Ukraine (2022) in the Desniansko-Starohutskyi NNP. Route surveys and geobotanical descriptions by S. Panchenko (2013) were used to identify forest types. A total of 6,778.8 hectares of the Starohut forest was studied, focusing on the distribution of forest area by forest type, soil moisture conditions, and main tree species. The study of the typological structure of the park's forest vegetation was conducted using methods from the Ukrainian school of forest typology (Hensiruk, 2002; Ostapenko & Tkach, 2002; Andriienko, 2006). Pogrebnyak's edaphic table served as a methodological framework for determining forest conditions: horizontal lines represent five hygrotopes based on soil moisture, while vertical columns represent trophotopes based on soil fertility (Table 1).

Table 1*. Predominant tree species in trophotopes according to Alekseev-Pogrebnyak*

Hygrotopes	Trophotopes			
	A - Bor	**B - Subor**	**C - Sugrud**	**D - Grud**
0 – Xerophilic (Very dry)	Pine	Pine, oak	Oak, pine	Oak, maple
1 – Mesoxerophilic (Dry)	Pine	Pine, oak	Oak, linden, maple, pine,	Oak, linden, maple, ash
2 – Mesophilic (Fresh)	Pine, birch	Pine, oak, birch	Oak, birch, linden, maple, pine	Oak, linden, maple, ash
3 – Mesohygrophilic (Moist)	Pine, birch	Pine, oak, birch	Oak, birch, linden, maple, pine	Oak, linden, maple, ash
4 – Hygrophilic (Damp)	Pine, birch	Pine, oak, birch, alder	Alder, linden, maple, pine, oak	Oak, birch, maple, ash, alder
5 – Ultrahygrophilic (Swamp)	Pine, birch	Pine, alder	Alder, birch	Alder, ash

Source: *prepared based on research data by P.S. Pogrebnyak (1959)*

In various types of forests, according to Pogrebnyak's typology, different perennials dominate the herbaceous-shrub layer depending on moisture conditions: A_0 – *Stipa pennata*, *Helichrysum arenarium;* A_1 – *Arctostaphylos uva-ursi, Pulsatilla patens;* A_2 – *Vaccinium vitis-idaea;* A_3 – *Vaccinium myrtillus, Polytrichum commune;* A_4 – *Molinia caerulea, Vaccinium uliginosum;* A_5 – *Ledum palustre, Eriophorum angustifolium, Vaccinium oxycoccos;* B_0 – *Stipa pennata*; B_1 – *Pulsatilla patens;* B_2 – *Pulmonaria angustifolia;* B_3 – *Vaccinium myrtillus;* B_4 – *Sphagnum palustre;* B_5 – *Potentilla palustris;* C_0 – *Carex pilosa, Melica nutans;* C_1 – *Stellaria holostea;* C_2 – *Pulmonaria angustifolia, Rubia tinctorum;* C_3 – *Pulmonaria angustifolia;* C_4 – *Athyrium filix-femina, Filipendula ulmaria, Impatiens noli-tangere;* C_5 – *Thelypteris palustris, Caltha palustris;* D_0 – *Melica nutans, Carex hirta;* D_2 – *Rubia tinctorum;* D_3 – *Pulmonaria angustifolia;* D_4 – *Impatiens noli-tangere, Filipendula ulmaria;* D_5 – *Chrysosplenium alternifolium*. The plant community studies were conducted in line with national and international guidelines. The authors adhered to the standards of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1979) and the Convention on Biological Diversity (1992).

RESULTS AND DISCUSSION

According to forest management data, forests within the Desniansko-Starohutskyi NNP cover an area of 8,583.9 hectares, with 6,778.8 hectares located in the Starohut forest massif. The vascular plant flora of the Desniansko-Starohutskyi NNP includes 852 species, making it the richest among the national parks and reserves of Polissia. The park is home to 35 plant species listed in the Red Book of Ukraine and 49 regionally rare species. The main natural factors influencing plant community distribution are soil fertility and the proximity of groundwater to the surface. Anthropogenic impact on this area is significant – unauthorised logging and fires have nearly eradicated oak forests from the park.

The forest vegetation in the study area primarily comprises coniferous, moderately humid mixed, and broad-leaved forests of the *Vaccinio-Piceetea, Quercetea robori-petraeae*, and *Querco-Fagetea* classes. In the eastern and central areas of the park, mesotrophic forest swamps of the *Scheuchzerio-Caricetea nigrae* class are present. In the northern part of the park and the Chern River floodplain, there are oligotrophic and oligo-mesotrophic forest swamps of the Oxycocco-Sphagnetea class. The classification of forest vegetation types in the Starohut massif according to ecological and floristic criteria is shown in Table 2.

***Table 2**. Taxonomy of forest vegetation of the Starohut massif*

Ecological and floristic classification (after Brown-Blanque)			**Types of forest vegetation (after P. Pogrebnyak, 1959)**		**Growing conditions**
Order	**Union**	**Association**	**Forest type**	**Code**	
Menyantho trifoliatae-Betuletalia pubescentis Grygora *et al.* 2005	*Oxycocco palustri-Betulion pubescentis* Grygora *et al.* 2005	*Menyantho trifoliati-Betuletum pubescentis* Grygora *et al.* 2005 em.	Damp birch-pine subor; Moist oak-pine subor	B_4BP B_3OP	Mesotrophic bogs on peatlands and their periphery
Vaccinietalia uliginosi R. Tx. 1955	*Ledo palustris-Pinion sylvestris* R. Tx. 1955	*Eriophoro vaginati-Pinetum sylvestris* Hueck 1925	Damp birch-pine subor; Swamp birch-pine subor	B_4BP B_5BP	Peat-bog soils in closed low-flow depressions
Alnetalia glutinosae R.Tx. 1937	*Alnion glutinosae* Malcuit 1929	*Sphagno squarrosi-Alnetum glutinosae* Solińska-Górnicka (1975)	Damp birch-pine subor; Moist oak-pine subor	B_4BP B_3OP	Along the edges of swamps in closed depressions
		Carici elongatae-Alnetum Schwickerath 1933	Swamp black alder sugrud	C_5A	In swamps, along the edges of streams on swampy and peat-marsh soils
Salicetalia auritae Doig 1962	*Salicion cinereae* T. Müller et Gors 1958	*Salicetum pentandro-cinereae*	Damp black alder sugrud	C_4A	Peat bogs, peat-bog soils in depressions
		Betulo-Salicetum repentis	Damp black alder sugrud	C_4A	Peat meadows
Fagetalia sylvaticae Pawlowski 1928	*Querco roboris-Tilion cordatae* Bulokhov et Solomeshch 2003	*Mercurialo perennis-Quercetum roboris* Bulokhov et Solomeshch 2003	Fresh linden-oak-pine sugrud; Moist linden-oak-pine sugrud	C_2-LOP C_3-LOP	Moderately humid flat areas along the edges of swamps on soddy-podzolic gleyed sandy soils
		Corylo avellanae-Pinetum sylvestris Bulokhov et Solomeshch 2003	Fresh linden-oak-pine sugrud; Moist linden-oak-pine sugrud	C_2-LOP C_3-LOP	Plakors on soddy-slightly podzolic gleyed sandy soils
	Alnion incanae Pawlowski 1928	*Ficario-Ulmetum minoris* Knapp 1942 em. J. Matuszkiewicz 1976	Moist linden-oak-pine sugrud	C_3-LOP	Small narrow areas along the edges of eutrophic swamps
		Fraxino-Alnetum W. Matuszkiewicz 1952	Moist linden-oak-pine sugrud; Damp black alder sugrud	C_3-LOP C_4A	Outskirts of eutrophic swamps and alder forests
Quercetalia roboris R. Tx. 1931	*Pino-Quercion* Medw.-Korn. 1959	*Querco-Pinetum (sylvestris)* (W. Matuszkiewicz 1981) J. Matuszkiewicz 1988	Fresh oak-pine subor; Fresh linden-oak-pine sugrud; Moist linden-oak-pine sugrud	B_2OP C_2LOP C_3LOP	Flat relief on soddy-podzolic gleyed sandy soils

Table 2. Continued

Ecological and floristic classification (after Brown-Blanque)			Types of forest vegetation (after P. Pogrebnyak, 1959)		Growing conditions
Order	**Union**	**Association**	**Forest type**	**Code**	
Cladonio-Vaccinietalia Kielland-Lund 1967	*Dicrano-Pinion* Libbert 1933	*Cladonio-Pinetum* (sylvestris) Juraszek 1927	Dry pine bor; Fresh pine bor;	A_1P A_2P	River terrace
		Molinio caerulea-Pinetum sylvestris W. Matuszkiewicz et J. Matuszkiewicz 1973	Fresh oak-pine subor; Moist oak-pine subor; Damp oak-pine subor; Fresh linden-oak-pine sugrud	B_2OP B_3OP B_4OP C_2LOP	Levelled, moderately moist areas on soddy-podzolic sandy and sandy-clayey gleyed soils
		Peucedano-Pinetum (sylvestris) W. Matuszkiewicz (1962) 1973	Fresh pine bor; Fresh oak-pine subor; Moist oak-pine subor	A_2P B_2OP B_3OP	Dry flat areas and slopes of ridges on sod-podzolic sandy and sandy-clayey gleyed soils
		Veronico incanae-Pinetum sylvestris Bulokhov et Solomeshch 2003	Fresh oak-pine subor	B_2OP	Flat areas on sod-podzolic sandy and sandy-clayey gleyic soils
		Vaccinio uliginosi-Pinetum sylvestris Kleist 1929	Swamp birch-pine subor; Damp oak-pine subor	B_5BP B_4OP	Closed depressions, edges of swamps on peat-bog and sod-podzolic sandy gley soils
Vaccinio-Piceetalia Br.-Bl. 1939	*Melico nutanstis-Piceion abietis* (Killand-Lund 1981) Onyshchenko 2005 prov	*Querco-Piceetum* (W.Matuszkiewicz 1952) W.Matuszkiewicz et Polak 1955	Fresh linden-oak-pine sugrud; Moist linden-oak-pine sugrud	C_2LOP C_3LOP	Flat areas on soddy-podzolic sandy gleyic soils
Chelidonio-Robinietalia Hada... et Sofron 1980	Chelidonio-Robinion Hada... et Sofron 1980	*Chelidonio-Robinietum* Hada... et Sofron 1963	Fresh linden-oak-pine sugrud	C_2LOP	Sandy soddy-podzolic soils

Source: *prepared by the authors based on the research data of S. Panchenko (2013)*

According to the established classification, the flora of the natural park includes three types of vegetation, seven classes of formations, eleven formation groups, twenty-three formations, thirty-five subformations, 104 association groups, and 242 associations, encompassing forest, meadow, and aquatic vegetation. Research has shown that the forest vegetation of the area comprises five hygrotopes: dry, fresh, moist, damp, and swamp. Monospecific pine forests cover a small area of 1.8 hectares, occupying 0.02% of the total forest area. Subors occupy the largest forest area, spanning 4,510.1 hectares, or 66.5% of the total. Sugruds cover a significantly smaller area of 2,266.9 hectares or 33.4% of the forest area. The oak grove forest type is absent in the Starohut forest massif (Table 3).

Table 3*. Structure of woody vegetation by hygrotopes in the Starohut forest, ha*

Trophotopes	Hygrotopes						Total, ha
	0	**1**	**2**	**3**	**4**	**5**	
	Very dry	**Dry**	**Fresh**	**Moist**	**Damp**	**Swamp**	
Bor (A)	–	0.7	1.1	–	–	–	1.8
Subor (B)	–	–	2,594.5	1,430.5	425.7	59.4	4,510.1
Sugrud (C)	–	–	1,352.6	720.6	179.7	14.0	2,266.9
Grud (D)	–	–	–	–	–	–	-
Total, ha	–	0.7	3,948.2	2,151.1	605.4	73.4	6,778.8

Source: *compiled by the authors*

Fresh soils predominate among hygrotopes, covering 3,948.2 hectares, or 58.24% of the total forest area. A smaller area is occupied by moist soils, which account for 2,151.1 hectares, or 31.73%. Damp soils are located in depressions, covering 605.4 hectares, or 8.93%. Wet, swamp soils and lakes occupy 73.4 hectares, or 1.08%. Conditions of very dry soil are completely absent. *Pinus sylvestris* is the primary forest-forming species, encompassing 75.1% of the area (5,092.7 hectares). Significantly smaller areas are occupied by *Betula pendula* (1,207.1 hectares, or 17.8%), *Alnus glutinosa* (201.4 hectares, or 2.9%), *Quercus robur* (90.0 hectares), *Picea abies* (111.5 hectares), and *Populus tremula* (71.7 hectares). Species such as *Tilia cordata, Pinus strobus, Larix decidua*, and *Robinia pseudoacacia* collectively occupy an area of 4.4 hectares, which represents 0.2% of the forest area. *P. abies* can sustain its populations over extended periods only in sufficiently fresh soils with 30-80% of full moisture content. Spruce phytocenoses are localised in areas adjacent to swamp ecosystems, where the soil remains consistently moist regardless of the weather. Only such environmental conditions facilitate the continuous regeneration of *P. abies* populations. Most of the sugruds in the Starohut forest area are situated in the regulated recreation zone of the National Natural Park, with considerably fewer located in the protected area.

In the Starohut forest massif, ten forest types have been identified. The predominant type is the fresh oak-pine subor, covering 2,594.5 hectares or 38.3% of the total area. Almost half of the area – 1,430.5 hectares – is occupied by the moist oak-pine subor, which constitutes 21.1% of the total forest area, along with the fresh linden-oak-pine sugrud, which spans 1,352.6 hectares or 19.95%. Smaller areas are occupied by the moist birch-oak-pine sugrud (720.6 hectares or 10.6%), the damp oak-pine subor (425.7 hectares or 6.3%), and the damp alder-pine sugrud with black alder (179.7 hectares or 2.6%) (Table 4).

Table 4. *Forest types in the Starohut forest massif*

Forest type index	Forest types	Area, ha
A_1P	Dry pine bor	0.7
A_2P	Fresh pine bor	1.1
B_2OP	Fresh oak-pine subor	2,594.5
B_3-OP	Moist oak-pine subor	1,430.5
B_4-BP	Damp birch-pine subor	425.7
B_5-BP	Swamp birch-pine subor	59.4
C_2-LOP	Fresh linden-oak-pine sugrud	1,352.6
C_3- LOP	Moist birch-oak-pine sugrud	720.6
C_4-A	Damp alder sugrud	179.7
C_5-A	Swamp alder sugrud	14.0
Total area, ha		6,778.8

Source: *compiled by the authors*

The park is divided into several functional zones: the protected area, the zone of regulated recreation (which is subdivided into two subzones), and the economic zone (also divided into two subzones) (Fig. 1).

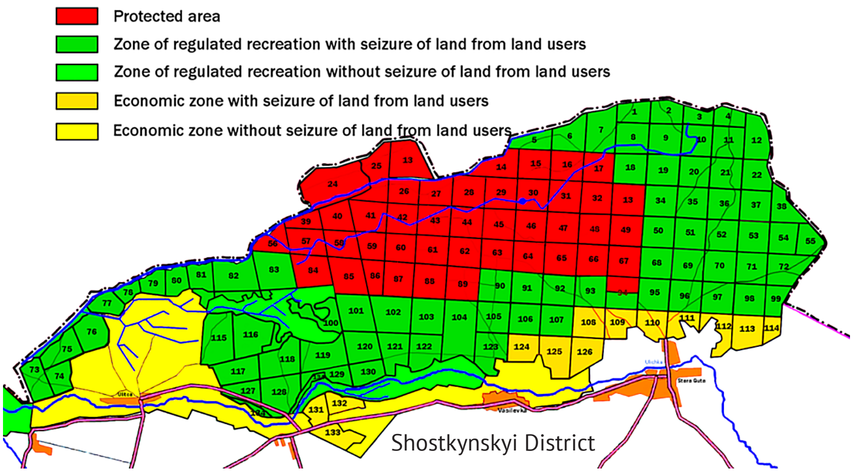

Figure 1. *Functional zones of the Starohut forest massif of the Desniansko-Starohutskyi NNP*
Source: *NNP Desniansko-Starohutskyi (2024)*

The protected area is located in the northern part of Starohut forest, covering 2,294.3 hectares. Trophotopes in the protected zone are divided into two categories: subors, which occupy 1,500.5 hectares, and sugruds, which cover 793.8 hectares. An analysis of the hygrotopes within the park's protected area shows that the largest areas are occupied by fresh soil – 1,181.1 hectares – and moist soil – 849.4 hectares. Smaller areas are occupied by damp soils – 238.0 hectares – and swamp areas – 25.8 hectares. There are no dry or very dry conditions within the reserve zone. The recreational zone occupies 57.55% of the study area, amounting to 3,901.3 hectares. This zone is dominated by subors – 2,746.3 hectares – with a significantly smaller area occupied by sugruds – 1,153.2 hectares. There are very few monoculture pine forests – 1.8 hectares – and no oak forests. Forest-covered hygrotopes in the recreational zone are distributed as follows: fresh – 2,336.4 hectares, moist – 1,164.2 hectares, damp – 352.4 hectares, swamp – 47.6 hectares, and dry – 0.7 hectares.

The economic functional zone of the Starohut part of the park occupies 583.2 hectares, constituting 8.6% of the total area. The trophotopes in this zone are primarily represented by sugruds, covering 319.9 hectares, while subors occupy 263.3 hectares. There are no monoculture pine forests or oak forests. Among the hygrotopes, fresh soil conditions predominate – 430.7 hectares, followed by moist soils – 137.5 hectares. Very small areas are occupied by damp soils in depressions – 15.0 hectares. There are no very dry, dry, or swamp conditions in the park's economic zone. The distribution of forest types by edaphic conditions in the Starohut forest can be seen in Table 5. The percentage of hygrotopes in various functional zones of the Starohut forest is presented in Figure 2.

***Table 5**. Typological structure of forest vegetation by functional zones of the Starohut part of the Desniansko-Starohutskyi NNP*

Functional areas of the park	Hygrotopes	Trophotopes				Total area, ha
		Bor	Subor	Sugrud	Grud	
Protected area	Very dry	–	–	–	–	–
	Dry	–	–	–	–	–
	Fresh	–	862.9	318.2	–	1,181.1
	Moist	–	470.3	379.1	–	849.4
	Damp	–	148.0	90.0	–	238.0
	Swamp	–	19.3	6.5	–	25.8
Recreation zone	Very dry	–	–	–	–	–
	Dry	0.7	–	–	–	0.7
	Fresh	1.1	1,543.0	792.3	–	2,336.4
	Moist	–	893.8	270.4	–	1,164.2
	Damp	–	269.4	83.0	–	352.4
	Swamp	–	40.1	7.5	–	47.6
Economic zone	Very dry	–	–	–	–	–
	Dry	–	–	–	–	–
	Fresh	–	188.6	242.1	–	430.7
	Moist	–	66.4	71.1	–	137.5
	Damp	–	8.3	6.7	–	15.0
	Swamp	–	–	–	–	–
Total area, ha		1.8	4,510.1	2,266.9	–	6,778.8

***Source:** compiled by the authors*

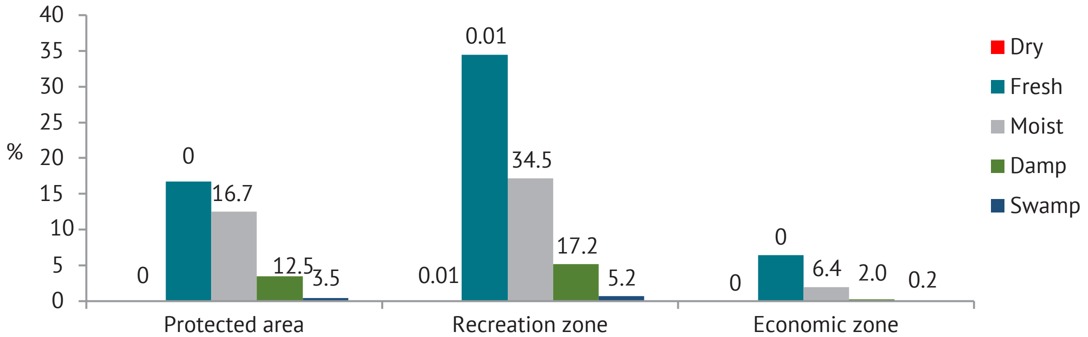

***Figure 2**. The ratio of hygrotopes in various functional zones of the Starohut part of the National Park, %*
***Source:** compiled by the authors*

The study of the typological structure of the Starohut forest massif, based on the main forest-forming species, revealed that the largest areas are occupied by *P. sylvestris* and *B. pendula* (Table 6).

***Table 6**. Typological structure of the Starohut forest massif according to the main forest-forming species*

Trophotopes	Species	Soil moisture conditions, ha						Total, ha
		Very dry	Dry	Fresh	Moist	Damp	Swamp	
Bor	*P. sylvestris*	–	0.7	1.1	–	–	–	1.8
Subor	*P. sylvestris*	–	–	2,486.1	1,076.7	171.4	15.6	3,749.8
	B. pendula	–	–	94.8	326.9	232.8	25.3	679.8
	A. glutinosa	–	–	–	1.3	20.7	18.5	40.5
	P. abies	–	–	10.1	13.2	–	–	23.3
	P. tremula	–	–	1.3	12.4	0.8	–	14.5
	Q. robur	–	–	2.1	–	–	–	2.1
Sugrud	*P. sylvestris*	–	–	1,039.0	299.2	2.9		1,341.1
	B. pendula	–	–	205.2	271.2	47.8		527.3
	A. glutinosa	–	–	–	21.0	129.0		160.9
	P. abies	–	–	54.3	33.9	–		88.2
	Q. robur	–	–	21.1	66.8	–		87.9
	P. tremula	–	–	30.8	26.4	–		57.2
	P. strobus	–	–	0.6	2.1	–		2.7
	R. pseudoacacia	–	–	1.0	–	–		1.0
	T. cordata	–	–	0.6	–	–		0.6
	L. decidua	–	–	0.1	–	–		0.1

***Source:** compiled by the authors*

P. sylvestris is an ecologically adaptable species, adapted to various moisture conditions; it is found in five hygrotopes and dominates in fresh, moist, and damp soil conditions. Of all ten forest-forming species, only *P. sylvestris* is present under dry conditions. The total area of *P. sylvestris* in the Starohut forest massif is 5,092.7 hectares. *B. pendula* is also a species with a wide ecological amplitude, growing in various types of hygrotopes but preferring fresh, moist, and damp conditions. In the Starohut forest, *B. pendula* occurs in subors – 679.8 hectares – and sugruds – 527.3 hectares. *A. glutinosa*, like birch, is found in two trophotopes – subors and sugruds – covering 40.5 and 160.9 hectares, respectively. This species prefers moist, damp, and swamp soil conditions. *P. abies* has a narrow ecological amplitude, growing in two trophotopes – subors and sugruds – and occupying very small areas – 23.3 and 88.2 hectares, respectively. This species prefers fresh and moist soils.

Q. robur is a plant that does not tolerate damp or swamp soil. In the study area, the species primarily grow on elevated terrain in sugruds, occupying 87.9 hectares. In some locations, *Q. robur* is found as an associated species in subors, covering an area of 2.1 hectares. The species thrives in two hygrotopes – fresh and moist. *P. tremula* is also present in two trophotopes and predominates in sugruds. The total area of presence of this species in the forest is 71.7 hectares and it prefers fresh and moist soil conditions. *T. cordata* is found only on fresh soil in drifts, covering an area of 0.6 hectares. Introduced forest-forming species in the Starohut forest massif – *Larix decidua, P.strobus,* and *Robinia pseudoacacia* – occupy a small area in sugruds on fresh hygrotopes.

During the fire of 2023, all types of forests growing on the eastern side of the massif were damaged, except swamp communities: dry pine bor – A_1P, fresh pine bor – A_2P, fresh oak-pine subor – B_2OP, fresh linden-oak-pine sugrud – C_2LOP, and moist birch-oak-pine sugrud – C_3LOP. This fire became the largest since the establishment of the Desniansko-Starohutskyi National Natural Park. According to Sentinel satellite images, the area of forest affected by the fire is approximately 500 hectares. In the northeastern part of the park, the fire raged for more than a week, with no possibility of extinguishing it due to the danger posed by proximity to the state border. As a result of the fire, 33 forest blocks were damaged to varying degrees. This represents a fifth of the land covered with forest vegetation and a third of the recreational zone. The area of forest phytocenoses damaged by the fire consists mainly of forest crops of *P. sylvestris* older than 60 years. According to the Global Fire Information Management System, 15.6% of the forest area in the park was affected by fire in May 2023 (Fig. 3).

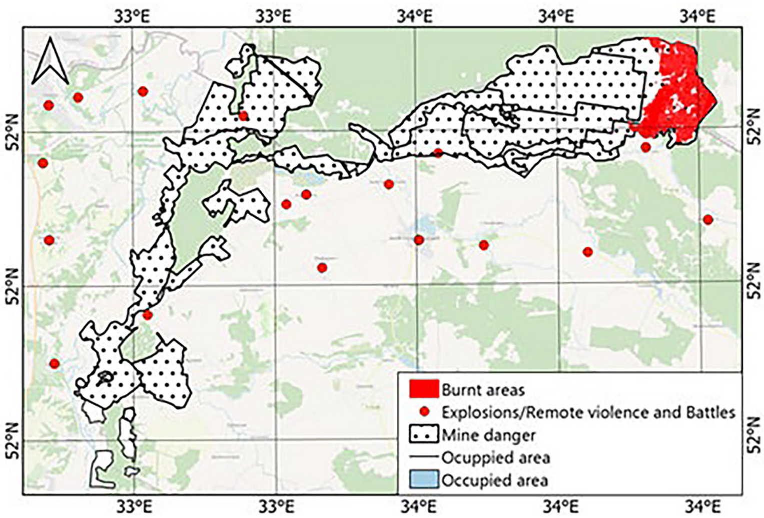

Figure 3*. Area of fire spread in the NPP Desniansko-Starohutskyi, May 2023*
Source: *compiled by the authors*

As a result of repeated shelling and fires, populations of many species in the eastern and northern parts of the Starohut forest were on the verge of extinction: *Primula veris* L., *Corydalis solida* (L.) Clairv., *Clinopodium vulgare* L., *Trifolium montanum* L., *Galium boreale* L., *Cardamine bulbifera* (L.) Crantz, *Pimpinella saxifrage* L., *Steris viscaria* (L.) Rafin., *Vaccinium myrtillus* L., *Vaccinium vitis-idaea* L., *Filipendula vulgaris* Moench, *Ulmus glabra* Huds, *Acer campestre* L., *Acer platanoides* L., and *Pinus sylvestris* L.. Plant species listed in the Red Book of Ukraine were affected, including *Lycopodium annotinum* L., *Huperzia selago* (L.) Bernh., *Diphasiastrum zeilleri* (Rouy) Damboldt, *Dactylorhiza incarnata* (L.) Soo, *Dactylorhiza fuchsia* (Druce) Soo, *Epipactis helleborine* (L.) Crantz, *Carex brunnescens* (Pers.) Poiret., *Platanthera bifolia* (L.) Rich., and *Salix starkeana* Willd.. Populations of regionally rare species protected in the Sumy region were also negatively affected by the fire, such as *Gymnocarpium dryopteris* (L.) Newman, *Viola riviniana* Rchb., *Helianthemum chamaecistus* Mill., *Salix rosmarinifolia* L., and *Salix myrsinifolia* Salisb.. Some of these are included in the list of the International Union for Conservation of Nature.

The problem of using forest typology in practice can be traced in the works of modern authors. According to I. Myskovets *et al.* (2024), forest typology is crucial for forest restoration. The authors assert that anthropogenic landscapes should be incorporated into forest typology, but priority attention should be given to natural forest phytocenoses to ensure the rational use of forest resources and the maximum preservation of the natural forest ecosystem. The idea of restoring damaged forest phytocenoses based on an ecological approach is present in the works of authors such as O. Prikhodko *et al.* (2019), H. Berglund and T. Kuuluvainen (2021), and A. Bugno-Pogoda and T. Durak (2021).

The problem of the restoration of damaged forest phytocenoses is also relevant for intrazonal territories. According to M. Dodan and S. Peric (2019), reforestation efforts in the coniferous forests of Croatia should be conducted with consideration for the ecology of spruce forests and their resistance to hurricane winds. A study by E. Collado *et al.* (2023) of mixed forests in the Mediterranean revealed a direct relationship between pine growth processes and soil moisture. The study assesses the impact of local moisture on the quality of forest cover formation. The authors advocate for forest restoration in conditions that closely resemble natural ones, taking into account the landscape diversity of the area to cultivate forests that are more adapted to climate change by increasing the biodiversity of the planted forest species. Ch. Haga *et al.* (2022) employed the LANDIS-II Net Ecosystem Carbon and Nitrogen Succession extension to optimally plan forest species composition for restoring damaged forest cover in the Oshima-Hiyama National Forest in southern Hokkaido, northern Japan. R. Seidl *et al.* (2018) are deeply convinced that, in the conditions of the Austrian Alps, mixed forests are better adapted to the impact of the bark beetle than monoculture coniferous stands. In analysing the spatial patterns of landscape disturbance, the authors identified an uneven distribution of risk for coniferous phytocenoses. Therefore, they propose planting broad-leaved species adapted to the changed climate alongside conifers. W. Mason *et al.* (2021) note that there is a growing interest in Europe in ecological forest management strategies that enhance species and structural diversity through the use of irregular silvicultural systems. The core principle of the European Continuous Forest Cover Strategy is that natural forest stands are established following the forest types of the original stands, using authentic seed sources.

Thus, researchers highlight the importance of using forest typology for the restoration of forest ecosystems, with priority given to natural phytocoenoses and the consideration of landscape diversity. The significance of an ecological approach in forest restoration is emphasised by numerous authors who note that utilising natural conditions is key to successfully adapting forests to climate change and enhancing their biodiversity. Ecological forest management, particularly based on the establishment of mixed forests and the use of irregular silvicultural systems, contributes to improving the resilience of forest stands to adverse factors such as pests and extreme weather conditions. In many regions of the world, emphasis is placed on restoring forests under conditions that closely resemble natural ones to preserve biodiversity and ensure their resilience to future challenges.

CONCLUSIONS

In the course of the study, it was established that the territory of the Starohut forest massif within the Desniansko-Starohutskyi NNP is represented by the following trophotopes: bors, subors, and sugruds. Bors occupy dry and fresh hygrotopes on flat terrain, covering an area of 1.8 hectares or 0.02% of the total forest area. Subors occupy fresh, moist, damp, and swamp hygrotopes, with a total area of 4,510.1 hectares, or 66.53%. In the same hygrotopes, sugruds grow on an area of 2,266.9 hectares, which is 33.44% of the study area; no gruds were found. *P. sylvestris* is the main forest-forming species, growing on an area of 5,092.7 hectares. *B. pendula* occupies a much smaller area – 1,207.1 hectares, *A. glutinosa* – 201.4 hectares, *P. abies* – 111.5 hectares, and *Q. robur* – 90.0 hectares. Other tree species are poorly represented. The protected area of the Starohut forest massif is dominated by fresh pine subors, with an admixture of birch, black alder, and spruce – 1,181.1 hectares, and moist pine-birch-alder-oak subors – 849.4 hectares. The recreation zone is dominated by fresh subors – 2,336.4 hectares, and moist sugruds – 1,164.2 hectares. The economic zone is dominated by subors – 430.7 hectares, and sugruds – 137.5 hectares. As a result of logging, military aggression, and forest fires in the eastern part of the park, the following forest types were damaged: dry pine forest, fresh pine forest, fresh oak-pine subor, fresh linden-oak-pine subor, and damp birch-oak-pine subor. After the end of military actions, it is necessary to study the condition of trophotopes in more detail to carry out forest restoration works that are close to natural conditions.

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CONFLICT OF INTEREST

None.

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Екологічна типологія деревної рослинності Старогутського лісового масиву як складова відновлення порушених екотопів

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Анотація. Метою дослідження було визначення типологічної структури Старогутського лісового масиву в межах Деснянсько-Старогутського національного природного парку на основі української школи лісової типології Алексєєва-Погребняка. Площа лісового масиву становить 6778,8 га. На досліджуваній території виділено такі трофотопи: бори, субори та сугруди. Бори, що складаються з соснових угруповань з домішкою берези, займають сухі та свіжі гігротопи на рівнинних місцевостях, займаючи 1,8 га або 0,02 % від загальної площі лісу. Субори, що складаються з сосново-дубових угруповань з участю берези та осики, зустрічаються у свіжих, вологих, мокрих та перезволожених гігротопах, займаючи 4510,1 га або 66,53 % території. Сугруди, що складаються з дубово-соснових липово-кленових лісів на рівнинах та осиково-березово-липово-кленових лісів у зниженнях, займають 2266,9 га або 33,44 % території Старогутського лісового масиву. Порушень не виявлено. Дослідженнями встановлено, що основною лісоутворюючою породою в національному парку є сосна звичайна (*Pinus sylvestris*), яка займає 5092,7 га. *Betula pendula* займає меншу площу – 1207,1 га, ще меншу – *Alnus glutinosa* (201,4 га), *Pice aabies* (111,5 га) та *Quercus robur* (90,0 га). Інші породи дерев представлені незначною мірою. У заповідній зоні парку переважають свіжі субори та сугрудки з домішкою берези, вільхи чорної та ялини загальною площею 1 181,1 га та вологі сосново-березово-вільхово-дубові субори та сугрудки – 849,4 га. У рекреаційній зоні переважають свіжі гідротопи з суборами та сугрудами – 2336,4 га. У господарській зоні переважають субори та сугруди на свіжих піщаних ґрунтах, загальною площею 430,7 га. Отримані результати дають змогу відновити природні типи лісів на територіях, що постраждали від масштабних пожеж 2023-2024 рр. та інших антропогенних впливів

Ключові слова: типи лісу; гідротопи; трофотопи; біорізноманіття; рідкісні види флори

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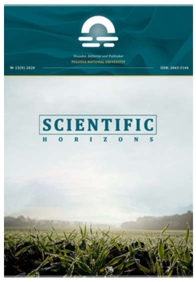

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Chorological analysis of species of the genus *Dianthus* in the South Caucasus region with an emphasis on the flora of Nakhchivan Autonomous Republic

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Abstract. The purpose of the study was to conduct a chorological analysis of six species of the genus *Dianthus* in the Nakhchivan Autonomous Republic, using field and molecular genetic methods. The main focus was on their geographical distribution, population status, and genetic diversity. As a result of the study of six species of the genus *Dianthus*, significant differences were found in their geographical distribution, ecological niches, and adaptation to local conditions. Molecular genetic analysis showed a high level of genetic diversity among populations of *Dianthus nahcivanensis* and *Dianthus nakhchivanicus*, which confirmed their uniqueness and endemic status. It has also been proven that the genus *Dianthus* has a high species diversity and shows significant variations in its distribution depending on the geographical and climatic conditions of the region. The study used geobotanic methods to determine the habitats of species and analyse factors affecting their distribution. In particular, features of the habitat were identified in connection with the local conditions of the Nakhchivan Autonomous Republic, which included a significant number of endemic species. The genetic analysis performed using the polymerase chain reaction method and sequencing helped to identify the level of genetic diversity and establish phylogenetic relationships between populations. It was found that *Dianthus raddeanus*, *Dianthus parviflorus, Dianthus nakhchivanicus* and *Dianthus nahcivanensis* species are more vulnerable due to the impact of anthropogenic activities, in particular agriculture and grazing, which has led to a decrease in the area of their habitat. Modelling using the maximum entropy method showed potential zones of expansion of species habitats under favourable ecological conditions. The results obtained were important for understanding the ecological patterns of distribution of the genus *Dianthus* in the region and the development of strategies for preserving biodiversity in the face of climate change and anthropogenic pressure. Based on the study, it was recommended

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to create or expand protected areas, limit anthropogenic impact, and conduct additional monitoring of the number and genetic structure of *Dianthus* populations

Keywords: endemics; distribution zone; genetic diversity; anthropogenic impact; biodiversity conservation

INTRODUCTION

Genus *Dianthus* (carnation) belongs to the family *Caryophyllaceae* and includes more than 300 species distributed mainly in temperate and subtropical zones of Eurasia. This genus is an important object of botanical research due to its considerable diversity and the presence of endemic and rare species in need of protection. In the South Caucasus region, in particular in the Nakhchivan Autonomous Republic, *Dianthus* is represented by a number of unique species that demonstrate significant ecological plasticity and genetic diversification. However, despite this, research on biogeography, ecology, and genetic diversity of representatives of the genus *Dianthus* in this region is limited.

In the scientific literature, it has been repeatedly noted that species of the genus *Dianthus* are of great evolutionary and ecological importance because of their ability to adapt to diverse ecosystems. Some species of this genus are widely distributed in Eurasia and North Africa, while others are narrowly endemic. In particular, studies have shown that endemic species such as *Dianthus nahcivanensis* and *Dianthus nakhchivanicus*, have limited geographical distribution and significant genetic isolation, which makes them vulnerable to environmental changes (Terlević *et al.*, 2022). In addition, studies of the chemical composition of extracts of these compounds can open up new opportunities for creating safer pharmaceutical products. Thus, M.Z. Mladenović *et al.*, (2023) study identified 275 components that were part of the flower extract, while 18 of them, as it was noted, were completely new compounds. In a more up-to-date 2024 paper, P.-J. Hou *et al.* (2024) discovered the medicinal properties of clove extract in the prevention of urolithiasis. A large study was also conducted by the A.K. Celik *et al.* (2024) research group, which revealed high anti-cancer and antioxidant activity of endemic species of *Dianthus*. In the Republic of Azerbaijan, researchers from the Institute of Plant Chemistry of the Academy of Sciences identified 6 compounds with *Dianthus helenae* which subsequently demonstrated anticonvulsant and nootropic effects (Yusupova *et al.*, 2021). All this may indicate a significant potential for further study of this genus not only in the framework of geobotanical research, but also in medical and pharmaceutical chemistry.

Previous studies of the Caucasus flora have shown that the South Caucasus region is an important centre of biodiversity with a large number of endemic species, including not only representatives of the genus *Dianthus*, but also many other families (Beker & Rączka, 2019; Kuljanishvili *et al.*, 2021). In particular, the Nakhchivan Autonomous Republic is a place of distribution of numerous endemic and rare plant species, which makes this region key for the protection of biodiversity. Given the climatic and geological features of the region, many species show high adaptation to harsh conditions such as lack of moisture, increased solar radiation, and stony soils (Elizbarashvili *et al.*, 2021). One of the key problems that arose in the study of the flora of the Caucasus is the influence of anthropogenic factors on natural ecosystems. A number of studies show that grazing, agriculture, and construction have a significant impact on the population status of many plant species, including members of the genus *Dianthus*.

However, since 2018, significant progress has been made in understanding the molecular genetics of species of the genus *Dianthus* (Lin *et al.*, 2022; Franzoni *et al.*, 2023). The use of modern methods of molecular genetic analysis allows researchers to determine the genetic structure of populations, the level of genetic diversification, and to identify relationships between similar species. In particular, studies using polymerase chain reaction (PCR) and DNA (deoxyribonucleic acid) sequencing methods have revealed unique genetic markers for rare and endemic species (Meng *et al.*, 2023). However, anthropogenic factors can lead to a significant decrease in the number and fragmentation of populations, which increases the risk of extinction of individual species. Vulnerable species growing in regions with significant anthropogenic impact showed low regenerative capacity and genetic diversity (Glibovytska *et al.*, 2024). Thus, the study of species of the genus *Dianthus* in Nakhchivan is an important contribution to understanding the biogeography and genetic structure of the plant world of the Caucasus. The results of this study can serve as a basis for developing strategies for the protection of rare and endemic plant species in the region, and contribute to further research on the genetic diversity of representatives of the genus *Dianthus*.

The purpose of this study was to carry out a comprehensive chorological analysis of six species of the genus *Dianthus* growing in the Nakhchivan Autonomous Republic, using both field and molecular genetic methods. Special attention was paid to the following species: *Dianthus raddeanus, Dianthus orientalis, Dianthus parviflorus, Dianthus elegans, Dianthus nahcivanensis,* and *Dianthus nakhchivanicus*, which are important components of the region's mountain ecosystems.

MATERIALS AND METHODS

In the course of the study, several aspects of the biology and ecology of six species of the genus *Dianthus*, which grew up in the Nakhchivan Autonomous Republic, were analysed. In addition to molecular genetic analysis, laboratory analysis and the use of modern methods of geographic information systems (GIS), a comprehensive field study was conducted, which included the collection of geographical data, soil characteristics, determination of microclimatic conditions, and assessment of the state of populations and anthropogenic impacts. The collected samples were identified at the species level using Caucasian flora determinants, after which they were dried and herbarised for further analysis in the laboratory. During the collection of samples, the main ecological parameters of habitats were recorded, in particular the type of soil, the level of illumination, air temperature, and relative humidity.

Field studies were conducted during two growing seasons (from May to October 2022 and 2023) in various environmental conditions of the Nakhchivan Autonomous Republic. Surveys were carried out by route expeditions, during which samples of plant material were collected from various types of habitats, such as highlands, foothills, and forest-steppe and steppe ecosystems. During field work, GPS devices were used to accurately determine the coordinates of species' growth sites. The following parameters were recorded in each growth site: geographical coordinates (latitude, longitude, altitude above sea level); soil type and its physical and chemical properties; microclimatic conditions (temperature, humidity, illumination); population status (density, number, degree of damage); presence of anthropogenic influences (degree of grazing, traces of land cultivation, development).

Herbarium collections from local and international botanical institutes and museums were also used to obtain more detailed data, which contained information about collection sites, morphological features, flowering periods, and other characteristics of species *Dianthus*. The herbarium of the National Institute of Azerbaijan provided access to important data on species collected in the country, including the Nakhchivan Autonomous Republic: herbarium samples of *Dianthus elegans* and *Dianthus parviflorus*, information on historical collection sites of species and their prevalence in mountainous areas, data on soil conditions and climatic characteristics of places where samples were collected. The herbarium of the Nakhchivan Institute of Botany was one of the key sources of information about endemic species of *Dianthus* from the region. The following data were used: herbarium samples of *Dianthus nahcivanensis* and *Dianthus nakhchivanicus*, sample collection sites, in particular exact coordinates and environmental conditions, morphological descriptions such as stem length, leaf shape, flower structure, information about flowering periods, which helped in determining the phenological phases of species.

Methods of chorological analysis were used to determine the distribution areas of species of the genus *Dianthus*. Habitat maps were created based on literature data, herbarium collections, and samples obtained during field surveys. For this purpose, geographic information systems (GIs) were used, which helped to combine various data sources and build maps of areas indicating high-altitude belts and habitat types. QGIS software was used for data analysis and visualisation. 1:50000 and 1:100000 scale topographic maps of the region were used to analyse the geographical distribution of species, and geological maps to determine the relationship between soil conditions, topography, and species distribution of *Dianthus*.

Data on the distribution of species were analysed using the maximum entropy model (MaxEnt), which helped to identify potential distribution zones of species considering environmental factors. Data on temperature, humidity, light, and other parameters that are critical for species survival in certain conditions were collected and processed. The model was calibrated based on 75% of the training data, and the remaining 25% was used to test the accuracy of the forecast. During field studies, soil samples were collected from different areas where the species grew. Each soil sample was collected at a depth of 10-15 cm, which is standard for analysing the surface layer of soil. Soil collection sites corresponded to the coordinates of species growth points of *Dianthus*.

The collected samples were dried at room temperature and then transported to a laboratory for further analysis. The laboratory analysis included several stages. First, the physical properties of the soil were determined by granulometric analysis to determine the content of sand, silt, and clay in the samples. The sieve analysis method and the hydrometric method were used to assess the mechanical composition of the soil. Soil pH values were also measured using an electronic pH meter. The Valkley-Black method (burning organic matter) was used to analyse the organic matter content. Nitrates, phosphates, and other mineral elements were determined by spectrophotometry. Atomic absorption spectrometry and colour testing methods were used to analyse the content of macro- and microelements such as nitrogen, potassium, and phosphorus. This helped to assess the potential of the soil to support plant growth. These data were used to establish correlations between soil types, their physical and chemical properties, and the state of populations of species in the genus *Dianthus*.

Molecular genetic analysis was performed to assess the genetic diversity of Dianthus species and their evolutionary relationships. For this purpose, the polymerase chain reaction (PCR) method with randomly amplified DNA (RAPD) and DNA sequencing analysis were used. DNA was isolated from the samples using the standard CTAB (cetyltrimethylammonium bromide method) method, which is effective for isolating DNA in plant samples with a high content of polyphenols and polysaccharides. The quantity and quality of the isolated DNA were checked by spectrophotometry and agarose gel electrophoresis. The optimal DNA concentration index for subsequent manipulations was at least 50 ng/μL. A total of 48 samples were taken from different populations of each species, followed by DNA extraction using the QIAamp DNA Mini Kit in accordance with the manufacturer's instructions. PCR primers were developed based on chloroplast genes and nuclear markers, which made it possible to assess both chloroplast and nuclear genetic diversity. The PCR procedure was performed using a temperature cycle consisting of denaturation at 95°C, annealing of primers at 50-60°C, and elongation at 72°C. The number of PCR cycles was optimised to 35-40 to achieve the maximum amount of amplified product without losing the specificity of the reaction.

The samples were then sequenced using the Sanger method, and the results were analysed using MEGA X software suite to build phylogenetic trees and determine the level of genetic diversity of each species. The obtained DNA sequences were compared with National Center for Biotechnology Information (NCBI) (2024) databases to determine phylogenetic relationships and potential evolutionary lineages. PCR products were analysed by electrophoresis in 2% agarose gel using molecular weight markers to compare amplicon sizes. The obtained data from sequencing and PCR analysis of amplicons were used to assess the level of genetic diversity among populations. Indicators such as the Nei's genetic diversity index, heterozygosity level, and the number of alleles per locus were used. Calculations were performed using the GenePop and Arlequin programmes. To assess the impact of anthropogenic factors on the number and distribution of species, local residents, agricultural workers and environmentalists were interviewed, and satellite images of the territory over the past 20 years were analysed. The survey was conducted using standardised questionnaires that contained the following blocks of questions: general information on land use, the impact of grazing, anthropogenic load and development, nature conservation, and species conservation.

A total of 150 respondents were interviewed, including: 75 people, mostly farmers and landowners, who had many years of economic experience in the region, 50 people who worked directly in fields and pastures, with an average work experience of more than 15 years, 25 experts who participated in field research and observations in the Nakhchivan Autonomous Republic. The work experience of environmentalists ranged from 10 to 25 years, which provided a high level of expert assessment of anthropogenic impacts. Areas with active anthropogenic activity were also identified, in particular, agricultural land, grazing and development areas. Based on the collected data, indices of the impact of anthropogenic factors for each habitat of species of the genus *Dianthus* were calculated. The data was integrated into GIS, which helped to create risk maps for each species and assess the degree of extinction of populations. All the data obtained were subjected to statistical analysis using software. Variance analysis (ANOVA) was used to compare environmental parameters between different species and populations. Correlation analyses were used to analyse the relationship between genetic diversity and anthropogenic factors.

RESULTS

During the study, field and laboratory studies of six species of the genus *Dianthus* were conducted in the Nakhchivan Autonomous Republic, covering the collection of geographical, environmental, and genetic data for a comprehensive assessment of their biological and environmental characteristics. The results of the study showed that the species of the genus *Dianthus* in the South Caucasus region has an uneven distribution associated with the diverse environmental conditions present in this region. It was found that the greatest abundance and diversity of species is observed at altitudes from 1500 to 2500 metres above sea level, where species such as *Dianthus raddeanus* and *Dianthus orientalis* grow. In the flat and foothill areas, in particular in the Nakhchivan Autonomous Republic, species *Dianthus parviflorus* and *Dianthus elegans* were mainly found. These species have shown resistance to harsher climatic conditions, such as high temperatures and low precipitation (Table 1).

***Table 1**. Distribution of the main species of Dianthus in the region by climatic conditions*

Species of *Dianthus*	Height belt (m)	Latitude	Longitude	Soil type	Average annual temperature (°C)	Average precipitation (mm)
Dianthus raddeanus	1,200-1,400	39.240° N	45.420° E	Stony, gravel	10-15	600-800
Dianthus orientalis	1,500-1,700	39.300° N	45.450° E	Sandy loam	8-12	500-700
Dianthus parviflorus	1,100-1,300	39.200° N	45.480° E	Sandy, clay	12-18	200-400
Dianthus elegans	1,700-1,900	39.380° N	45.400° E	Forest, loamy	14-20	300-500
Dianthus nahcivanensis	1,400-1,600	39.400° N	45.360° E	Stony soils	14-24	
Dianthus nakhchivanicus	1,600-1,800	39.290° N	45.390° E	Mountain chernozems	14-22	

***Source:** created by the author*

Species *Dianthus elegans* was found on the highest mountain areas (1700-1900 m), while *Dianthus parviflorus* grew at lower altitudes (1100-1300 m). *Dianthus nakhchivanicus* grows at altitudes of 1600-1800 m, while *Dianthus nahcivanensis* recorded at altitudes of 1400-1600 m. Species of *Dianthus raddeanus*, *Dianthus parviflorus*, and *Dianthus orientalis* were found at altitudes from 400 to 1500 m, indicating a wider ecological habitat. These data showed that the species adapted to different ecological niches in the study region.

Microclimatic conditions in the growing areas varied depending on the altitude zone and location. Temperatures ranged from 18°C to 28°C during the growing season. The highest temperatures were recorded in populations *Dianthus raddeanus*, *Dianthus orientalis*, and *Dianthus parviflorus*, which grew in low-lying areas where the average daily temperature reached 28°C. In high-altitude populations of *Dianthus nahcivanensis* and *Dianthus nakhchivanicus,* the temperature conditions were more moderate, with an average of about 20-22°C. In terms of humidity, populations located at high altitudes had higher humidity levels (up to 70% in the morning), while low-lying populations were characterised by lower humidity levels (40-50%). Illumination in all places of growth was high, which met the specific environmental requirements of the genus *Dianthus* to the light.

The population status showed different levels of density and abundance for each species. *Dianthus parviflorus* showed the highest abundance, while *Dianthus elegans* had the lowest density. *Dianthus nahcivanensis* and *Dianthus nakhchivanicus* showed moderate levels of density and abundance, which indicated stable populations in their habitats (Table 2). Population assessment also included analysis of plant damage. Significant damage was recorded in populations of *Dianthus raddeanus* and *Dianthus orientalis* through grazing and trampling, which led to a decrease in the population size and damage to root systems.

***Table 2**. Status of Dianthus species populations in the South Caucasus region*

Species	Density (plants/m²)	Number (individuals)	Degree of damage (%)
Dianthus raddeanus	30-40	150-200	10-15
Dianthus orientalis	25-35	100-150	20
Dianthus parviflorus	40-50	250-300	10
Dianthus elegans	10-20	50-80	15
Dianthus nahcivanensis	20-30	120-180	10-12
Dianthus nakhchivanicus	15-25	80-100	12

***Source:** created by the author*

Dianthus elegans, which was recorded at altitudes up to 1900 m, was characterised by the ability to grow on red soils in more severe climatic conditions. The soils on which the species grew ranged from stony to red clay soil. Species *Dianthus elegans* was characterised by the highest content of organic matter and nitrogen in the soil, which explains its distribution on red soils. *Dianthus nahcivanensis* and *Dianthus nakhchivanicus* had a slightly lower content of organic substances and grew on stony and mountainous chernozems (Table 3).

***Table 3**. Soil types and their physical and chemical properties*

Species	Soil type	pH	Organic content (%)	Nitrogen (%)
Dianthus raddeanus	Stony soils	7	3.2.	0.14
Dianthus orientalis	Mountain chernozems	6.8	3.5	0.15
Dianthus parviflorus	Loam	7.1	2.8	0.12
Dianthus elegans	Red soils	7	4	0.18
Dianthus nahcivanensis	Stony soils	6.7	2.9	0.13
Dianthus nakhchivanicus	Mountain chernozems	6.9	3.7	0.16

***Source:** created by the author*

Analysis of the soils in which the studied species grew showed significant variability in physical and chemical properties. The soils in the areas of *Dianthus nahcivanensis* growth had high stoniness and low humus content (about 2.5%), which indicated that this species was adapted to poor conditions. The soils on which *Dianthus nakhchivanicus* grew were characterised by moderate stoniness and a humus content of 3.1-3.5%, which created more favourable conditions for growth. In species populations of *Dianthus raddeanus*, *Dianthus orientalis*, and *Dianthus parviflorus*, the soils were moderately sandy with a low content of nitrogen and organic matter, which indicated their adaptability to harsh environmental conditions.

As a result of the conducted chemical analysis of the soil for the study of six species of the genus *Dianthus* in the Nakhchivan Autonomous Republic, the following results were obtained regarding the content of mineral elements and macronutrients in various soil samples. Soil samples were taken from growth sites of the species *Dianthus raddeanus*, *Dianthus orientalis*, *Dianthus parviflorus*, *Dianthus elegans*, *Dianthus nahcivanensis,* and *Dianthus nakhchivanicus*. Nitrogen content was relatively stable in all samples, with the highest rates found at growth sites of *Dianthus parviflorus* and *Dianthus nahcivanensis*. The potassium and phosphorus content were highest in the soils with *Dianthus parviflorus*, which may indicate more fertile soils in these areas (Table 4).

Table 4*. Content of trace elements in the soils at the growth sites of the species of Dianthus*

Species	Nitrogen (mg/kg)	Potassium (mg/kg)	Phosphorus (mg/kg)
Dianthus raddeanus	8	300	15
Dianthus orientalis	7	250	12
Dianthus parviflorus	9	320	18
Dianthus elegans	7.5	280	14
Dianthus nahcivanensis	8.2	310	16
Dianthus nakhchivanicus	6.5	230	10

Source: *created by the author*

The results obtained showed that the soils of the growth of species of the genus *Dianthus* have a neutral-alkaline pH, moderate organic matter content, and different levels of macro- and microelements. This indicated that the species of the genus *Dianthus* can adapt to different soil conditions, but there have been some advantages over more fertile and nutrient-rich areas. The conducted interview of local residents, agricultural workers and environmentalists helped to collect information on the impact of anthropogenic factors on species of the genus *Dianthus* in the Nakhchivan Autonomous Republic. The data obtained were presented in the form of tables for convenient evaluation of the results. According to the results of the interview, most agricultural workers used pesticides to treat their land, which contributed to a change in the flora and, possibly, a decrease in the number of some species of *Dianthus*. Environmentalists recorded a high proportion of changes in the flora of the region (Table 5).

Table 5*. General information on land use*

Category of respondents	Number of respondents	Average duration of activity (years)	Pesticide use (%)	Changes in flora and soil (%)
Local residents	75	20	60	55
Agricultural workers	50	15	80	70
Environmentalists	25	18	N/A	80

Source: *created by the author*

Grazing significantly affected vegetation, especially in regions with large livestock numbers. Environmentalists noted a significant decline in the populations of some species of *Dianthus* in such territories (Table 6).

Table 6*. Impact of grazing*

Category of respondents	Number of respondents	Average number of livestock per farm	Vegetation changes (%)	Changes in soils (%)
Local residents	75	50	65	50
Agricultural workers	50	75	70	65
Environmentalists	25	N/A	85	70

Source: *created by the author*

Anthropogenic activities, in particular development and changes in water resources, have caused a deterioration in the environmental situation. Environmentalists noted that new buildings have had a particularly negative impact on the growth areas of rare species of *Dianthus* (Table 7).

***Table 7**. Anthropogenic load and development*				
Category of respondents	**Number of respondents**	**New buildings nearby (%)**	**Changes in water resources (%)**	**Environmental degradation (%)**
Local residents	75	40	30	50
Agricultural workers	50	55	45	60
Environmentalists	25	75	65	80

Source: *created by the author*

Only a small proportion of local residents and agricultural workers were aware of the existence of rare and endemic species of *Dianthus* (Table 8), while environmentalists actively supported measures to protect them.

***Table 8**. Nature conservation and species conservation*			
Category of respondents	**Number of respondents**	**Knowledge of rare species (%)**	**Support for security measures (%)**
Local residents	75	30	20
Agricultural workers	50	25	35
Environmentalists	25	95	90

Source: *created by the author*

The survey showed that a small part of the local population showed knowledge of rare species, which indicates the need to raise awareness and implement conservation measures to preserve the local flora. Anthropogenic factors such as agricultural activity, grazing, and land-use changes were also found to have a significant impact on populations of rare and endemic species (Table 9). In areas with intensive grazing, significant vegetation damage has been observed, which may lead to a decrease in the number of species, such as *Dianthus nahcivanensis*, in the future.

***Table 9**. Influence of anthropogenic factors on population size*				
Species	**Type of anthropogenic impact**	**Impact level**	**Change in population (%)**	**Main threats**
Dianthus orientalis	Grazing of livestock	Moderate	-20	Soil degradation, trampling
Dianthus raddeanus	Agriculture	Moderate	-25	Reduction of natural habitats
Dianthus parviflorus	Development	High	-40	Loss of habitat
Dianthus elegans	Agriculture	Low	-15	Limited competition with other species
Dianthus nahcivanensis	Intensive grazing	High	-50	Trampling, environmental pollution
Dianthus nakhchivanicus	Agriculture	High	-45	Loss of natural habitats

Source: *created by the author*

Spectrophotometric analysis showed that the concentration of isolated DNA in all samples exceeded 50 ng/μL, which allowed the use of these samples for further PCR analysis. The quality of the isolated DNA was checked by agarose gel electrophoresis, where all samples showed clear bands, indicating no DNA degradation. For each of the six species of *Dianthus*, several genetic markers were amplified, in particular microsatellites or single sequence repeats (SSRs) and internally transcribed spacers (ITS). The optimal annealing temperature of the primers was 55°C for SSR markers and 52°C for its markers. After 35 PCR cycles, amplicons of the desired size were obtained, which was confirmed by the results of electrophoresis in 2% agarose gel. All the samples under study gave different amplification products, the dimensions of which coincided with those expected in previous studies.

The amplified DNA fragments were sequenced according to the Sanger method. The resulting DNA sequences were mapped to databases to identify species and verify the relationship between them. All six species were found to have characteristic genetic polymorphisms that clearly distinguished their genetic profiles. In general, the species *Dianthus nahcivanensis* and *Dianthus nakhchivanicus* they found a high level of genetic diversity compared to other species. This was due to the isolation of their populations, which adapted to specific growth conditions. Genetic diversity in these species was concentrated within populations, which indicated their long-term existence in localised regions.

On the contrary, species *Dianthus raddeanus*, *Dianthus parviflorus*, and *Dianthus orientalis* exhibited lower levels of genetic diversity, indicating greater genetic isolation and possible historical "bottle necks". This indicated a decrease in genetic exchange between populations, which may be the result of anthropogenic factors, such as habitat fragmentation. Molecular genetic analysis of *Dianthus raddeanus* showed significant genetic diversity, which indicates the potential for adaptation and survival in changing environmental conditions. However, the identified local genetic isolates confirmed the need for more research on the impact of population fragmentation on the long-term conservation of the species.

Phylogenetic analysis showed the evolutionary relationships between populations of different species of the genus *Dianthus*. It was found that *Dianthus nahcivanensis* and *Dianthus nakhchivanicus* form separate clusters, which confirmed their genetic isolation from other species. They showed genetic closeness to each other, indicating a common origin or a long history of coevolution in isolation. Other species, such as *Dianthus raddeanus* and *Dianthus orientalis*, formed separate phylogenetic groups that also indicated a long evolutionary history and specific adaptations to different environmental conditions.

Genetic analysis determined the level of genetic diversity within and between populations, established phylogenetic relationships between species, and identified possible mechanisms of adaptation to different environmental conditions. Most species, such as *Dianthus orientalis* and *Dianthus raddeanus,* demonstrated a high level of genetic diversity (heterozygosity up to 0.75), which indicates their adaptive potential and high resistance to changes in environmental conditions (Table 4). Endemic species of *Dianthus nahcivanensis* and *Dianthus nakhchivanicus* had significantly lower rates of genetic diversity (heterozygosity up to 0.42), which indicates limited genetic variability (Table 10). This may be due to their isolation and narrow distribution area, which increases the risk of extinction due to environmental changes or anthropogenic factors.

***Table 10**. Genetic diversity of species of the genus Dianthus*

Species	Heterozygosity	Level of genetic diversification	Number of allelic variants
Dianthus orientalis	0.75	High	12
Dianthus raddeanus	0.68	High	10
Dianthus parviflorus	0.55	Average	8
Dianthus elegans	0.60	Average	9
Dianthus nahcivanensis	0.42	Low	6
Dianthus nakhchivanicus	0.40	Low	5

***Source:** created by the author*

A study of six species of the genus *Dianthus* in the Nakhchivan Autonomous Republic revealed significant genetic and ecological diversity, but all species were vulnerable to anthropogenic factors and climate change. Analysis of species habitats of *Dianthus* in the South Caucasus established that most species of this genus demonstrate highly specialised ecological requirements and have a limited habitat. This makes them vulnerable to environmental changes and anthropogenic impacts. Endemic species, such as *Dianthus nahcivanensis* and *Dianthus nakhchivanicus*, require special attention to the protection and preservation of their places of growth. The conducted research investigated in detail the chorological features of the species of the genus *Dianthus* in the South Caucasus, identified key factors affecting their distribution and conservation, and developed specific measures to preserve these species. The results can serve as a basis for further research and strategies for managing the conservation of plant biodiversity in the region.

DISCUSSION

The study of the six species of the genus *Dianthus*, which are common in the territory of the Nakhchivan Autonomous Republic, used a comprehensive approach to investigate their ecology, geographical distribution, population status, and genetic structure. The selected methods helped to obtain a holistic view of the ecological features and threats to the species under study. The main research methods were field surveys, analysis of physical and chemical properties of soils, study of microclimatic conditions of growth sites, and molecular genetic surveys to investigate the genetic diversity of populations.

Field studies have made it possible to determine the geographical coordinates of the growth sites of each species, including altitude above sea level, soil type, and microclimatic conditions (temperature, humidity, and light). These data were important for understanding each species' ecological niches and their adaptive potential. The analysis of the physical and chemical properties of soils included the determination of acidity (pH), the concentration of basic minerals, and the content of organic substances. This approach revealed how soil conditions affect the growth and distribution of these species.

Studies of endemic plant species required special attention. The literature sources contain conflicting opinions about their adaptability to different climatic conditions. M. Behroozian's *et al.* (2020a) research team questioned the general assumption that endemic species have narrow ecological niches. Using environmental modelling and functional characteristics analysis, they found that some species of *Dianthus* demonstrate significant ecological variability and adaptation to different environmental conditions. This led to the assumption of their ability to occupy a variety of ecological niches, which goes against traditional ideas about endemics as highly specialised species (Behroozian *et al.*, 2020a).

In this study of *Dianthus* species, both specific and more general ecological traits characteristic of different populations were identified. The ability of endemic species to adapt and occupy wider ecological niches is partially substantiated, since some species, such as *Dianthus raddeanus* and *Dianthus nahcivanensis* indeed, demonstrated adaptation to various microclimatic conditions and changes in soil composition (Zubtsova *et al.*, 2019). These species can grow in different conditions of altitude and humidity, which confirms the idea of ecological plasticity. However, for some other species, such as *Dianthus parviflorus* and *Dianthus nakhchivanicus*, environmental specialisation remained prominent. They showed a significant dependence on specific environmental conditions, such as soil type and light levels, which is consistent with the traditional view of highly specialised endemics. Thus, the study partially confirmed Behroozian's thesis about the variability of ecological niches in some endemic species. However, it did not refute the opinion about the traditional highly specialised ecological strategy in certain populations. On the other hand, C.A.M. Marshall *et al.* (2022) study, which examined factors affecting plant endemism in two West African forest centres, focused on various environmental and climatic variables, and environmental characteristics such as altitude, forest type, climatic conditions, and anthropogenic impact. Climatic conditions, in particular precipitation, were found to be a significant predictor of endemism. The paper also highlighted the importance of protecting these forests due to their significant role in the conservation of rare and endemic species.

The South Caucasus region is characterised by the proliferation of zones with endemic plant crops (Nikolaishvili & Dvalashvili, 2015; Noroozi *et al.*, 2019). Although it is also possible to find among the literature sources of fauna research, in particular amphibians (Gasimova, 2021), with similar habitats, in the framework of this study, were rather auxiliary. A successful object of research can be called the species of the family *Caryophyllaceae,* not only in the study of endemics (Behroozian *et al.*, 2020b), and to better understand the overall impact of climatic conditions and human influence on the flora of regions with specific climatic conditions. In general, the high diversity of species in this family allows studying a wide range of morphological, ecological, and genetic variations. This helps to study the processes of evolution, adaptation, and speciation. In addition, species of this family are found in various ecosystems – from mountainous areas to steppes and deserts. This allows to study their adaptation to various climatic and environmental conditions and their interaction with the environment. Many representatives of *Caryophyllaceae* have relatively small genomes, which makes them convenient for molecular genetic research such as DNA sequencing and genetic mapping. Their genetic characteristics allow studying speciation processes, population genetics, and evolutionary history (Boxriker *et al.*, 2018). As indicated by G.A.W. Bauer *et al.* (2021), species of this family exhibit various morphological features, which allows studying phenotypic plasticity and the influence of various environmental factors on external features.

P. Sharma's *et al.* (2022) paper was considered an important literary source for analysis, as the molecular methods used, such as SSR markers, helped to investigate genetic diversity and phylogenetic relationships between species, which is relevant in the context of analysing the genetic variability of endemic species. Both studies used molecular genetic markers to assess the genetic diversity of species. The difference was that P. Sharma's *et al.* research focused on the cultural species of *Dianthus caryophyllus* and its mutant forms that emerged after radiation exposure. But the current study focused on natural populations of endemic species of the genus *Dianthus* in the natural environment, without the intervention of the mutation process. While improving breeding methods and species identification helps to prevent the extinction of entire plant and animal habitats, it is also important to improve conservation measures and monitor the overall situation of species (Honchar *et al.*, 2023). Great contribution to the study of adaptability and factors influencing distribution of *Dianthus* was made at the Latvian Institute of Biology using the example of *Dianthus superbus*, researchers have found that destructive human activity causes more damage to the population than the specifics of local soils (Osvalde *et al.*, 2021). Such results indicate the expediency of conducting lectures and activities to inform the population about the environmental situation in the region.

Heavy metals, which have become an integral part of environmental impact factors with the development of industrialisation, also have high risks for plant populations (Jalilova *et al.*, 2024). Thus, the K. Qiao *et al.* (2023) research group found that *Dianthus spiculifolius* had higher resistance to cadmium and lead compared to five other species of the genus *Dianthus*, and it was able to accumulate more heavy metal ions in its shoots. Future studies of the endemic species of the genus *Dianthus* on the ability to accumulate heavy metal ions are relevant, as they can form an idea of their resistance to polluted environments and potential for phytoremediation. To assess the level of genetic diversity both between different populations and within the same population, molecular genetic analysis performed for each of the species is widely used in modern scientific practice. Molecular markers such as microsatellites and DNA sequencing help to identify genetic relationships between populations and assess their status in terms of genetic metabolism (Burdina & Priss, 2016). Genetic data allowed studying how individual populations of species *Dianthus* adapted to different environmental conditions, such as climate change or specific soil characteristics. This was important for understanding the ecological boundaries of species and determining their optimal habitat zones.

The study of six species of the genus *Dianthus* in the Nakhchivan Autonomous Republic provided important data on their geographical distribution, population status, influence of anthropogenic factors, and genetic structure. The species *Dianthus raddeanus*, *Dianthus orientalis*, *Dianthus parviflorus*, *Dianthus elegans*, *Dianthus nahcivanensis*, and *Dianthus nakhchivanicus* are important components of the region's flora, and their study helps not only to better understand local biodiversity, but also to develop strategies for their conservation.

CONCLUSIONS

In the course of the study, a comprehensive chorological analysis of the species of the genus *Dianthus* in the South Caucasus was carried out, with a particular focus on the flora of the Nakhchivan Autonomous Republic. The results obtained established the specific distribution areas of the species under study, their ecological and genetic characteristics, and determined the influence of anthropogenic factors on the state of their populations. This study found that each species has a well-defined ecological niche, depending on factors such as temperature, humidity, soil type, and the degree of anthropogenic impact.

The relevance of this study is to expand knowledge about the biogeography of species of the genus *Dianthus* and their distribution in the South Caucasus region. The study provided a more accurate understanding of species distribution patterns, their environmental preferences, genetic diversity, and potential risks associated with anthropogenic factors. Such knowledge can be used to develop a conservation strategy for rare and endemic species, and to manage biodiversity in the region, which is particularly important given climate change and increasing anthropogenic pressures. However, the study was subject to certain limitations that affected the results obtained. First, field studies were conducted only during two growing seasons, which could lead to limited understanding of seasonal population dynamics. Long-term observations can provide more data on changes in the abundance and distribution of species over different years. Second, the analysis of molecular genetic diversity was performed only based on randomly selected samples, which could limit the completeness of the genetic profile of populations. A wider selection of samples and the use of additional genetic markers can improve the accuracy of the results obtained. In addition, potential species habitats were modelled using a specific set of ecological parameters, while other factors, such as competition with other species or interaction with local fauna, were not taken into consideration. The use of additional biotic and abiotic parameters can improve the accuracy of distribution models.

Thus, this study has made an important contribution to understanding the ecological and genetic characteristics of species in the genus *Dianthus* in the South Caucasus. Despite some limitations, the results obtained are valuable for further research and preservation of the floral diversity of the region. Further research that addresses the above omissions could greatly expand knowledge about these species and contribute to their more effective conservation.

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CONFLICT OF INTEREST

None.

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Хорологічний аналіз видів роду *Dianthus* у Південнокавказькому регіоні з акцентом на флорі Нахічеванської Автономної Республіки

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Анотація. Метою дослідження було проведення хорологічного аналізу шести видів роду *Dianthus* у Нахічеванській Автономній Республіці з використанням польових та молекулярно-генетичних методів. Основна увага була приділена їх географічному поширенню, популяційному статусу та генетичному різноманіттю. В результаті дослідження шести видів роду *Dianthus* були виявлені значні відмінності в їх географічному поширенні, екологічних нішах та адаптації до місцевих умов. Молекулярно-генетичний аналіз показав високий рівень генетичного різноманіття серед популяцій *Dianthus nahcivanensis* та *Dianthus nakhchivanicus*, що підтвердило їх унікальність та ендемічний статус. Також доведено, що рід *Dianthus* має високе видове різноманіття і демонструє значні варіації поширення залежно від географічних та кліматичних умов регіону. У дослідженні використано геоботанічні методи для визначення ареалів видів та аналізу факторів, що впливають на їх поширення. Зокрема, були виявлені особливості ареалу у зв›язку з локальними умовами Нахічеванської Автономної Республіки, до складу якого входить значна кількість ендемічних видів. Генетичний аналіз, проведений за допомогою методу полімеразної ланцюгової реакції та секвенування, дозволив виявити рівень генетичного різноманіття та встановити філогенетичні зв'язки між популяціями. Встановлено, що види *Dianthus raddeanus*, *Dianthus parviflorus*, *Dianthus nakhchivanicus* та *Dianthus nahcivanensis* є більш вразливими через вплив антропогенної діяльності, зокрема сільського господарства та випасання худоби, що призвело до скорочення площі їхніх ареалів. Моделювання з використанням методу максимальної ентропії показало потенційні зони розширення ареалів видів за сприятливих екологічних умов. Отримані результати є важливими для розуміння екологічних закономірностей поширення роду *Dianthus* в регіоні та розробки стратегій збереження біорізноманіття в умовах зміни клімату та антропогенного тиску. За результатами дослідження рекомендовано створити або розширити заповідні території, обмежити антропогенний вплив та проводити додатковий моніторинг чисельності та генетичної структури популяцій *Dianthus*

Ключові слова: ендеміки; ареал; генетичне різноманіття; антропогенний вплив; збереження біорізноманіття

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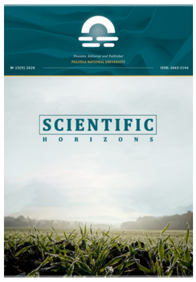

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Management of sustainable land use projects in accordance with EU requirements

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Abstract. The purpose of the study was to conduct a comparative analysis of the management of sustainable land use development projects in Ukraine and the EU countries and to develop recommendations for improving Ukrainian practices in accordance with the requirements of the European Union. The methodology was based on a systematic approach and included the analysis of statistical data, an expert survey, and the calculation of an integral index of compliance with EU requirements. The study focused on three key aspects: restoration of degraded land,

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introduction of organic farming, and water management. The results revealed that Ukraine is lagging behind in all the parameters under study. In the area of restoration of degraded land, it was found that the area of restored land in Ukraine (8.4% of the total area of degraded land) is almost twice less than the average of the EU countries studied (16.8%). Investments in land restoration in Ukraine (310 EUR/ha) are only 39% of the EU average (792 EUR/ha). The analysis of the development of organic farming showed that the share of organic land in Ukraine (1.1% of the total agricultural area) is almost 6 times lower than the average for the EU countries under study (6.7%). The number of certified organic producers in Ukraine (3.2 per 100,000 population) is 6.4 times lower than the average for EU countries. In the field of water management, it was found that the irrigation efficiency in Ukraine (60%) is significantly lower than the EU average (77.4%), and the share of land with drip irrigation in Ukraine (5.2%) is 3.5 times lower than the EU average (18%). The calculation of the integral index of compliance with EU requirements showed that Ukraine (56 points) significantly lags behind the average of the EU countries studied (82.4 points). Based on the identified differences and successful practices of EU countries, a set of recommendations has been developed to improve the management of sustainable land use projects in Ukraine. Key recommendations include the development of a comprehensive national strategy, the creation of specialised financing funds, the introduction of economic incentives, the modernisation of monitoring systems, the harmonisation of standards with the EU, and large-scale educational campaigns. The study emphasises the need to significantly improve approaches to managing sustainable land use projects in Ukraine to bring them closer to EU standards and ensure efficient use of land resources

Keywords: environmental efficiency; institutional capacity; integral index; economic incentives; adaptation of legislation; innovative technologies

INTRODUCTION

Sustainable development of land use is one of the priorities of the European Union (EU) policy aimed at ensuring the rational use of land resources, preserving ecosystems, and improving the quality of life of the population. In the context of Ukraine's European integration aspirations, the issue of adapting Ukrainian approaches to land management in accordance with EU requirements and standards is becoming particularly relevant. The problem of managing sustainable land use development projects attracts the attention of many researchers. Theoretical aspects of sustainable land use were considered by W.C. Adinugroho *et al.* (2024). The researchers emphasised the need to integrate environmental, economic, and social factors in land use planning. Project management issues in the context of sustainable development were investigated by R. Sweeney *et al.* (2023). The researchers emphasised the importance of considering the principles of sustainability at all stages of the project life cycle, emphasising the need to integrate economic, environmental, and social aspects into project management processes.

The specifics of implementing the principles of sustainable land use in the EU countries are the subject of active research. V. Maliene *et al.* (2024) analysed the positive impact of European directives and programmes on the development of sustainable land use practices. In particular, the study by P. Borrelli *et al.* (2020) was devoted to the analysis of soil erosion risks in EU countries and the development of strategies for adapting land use to climate change. These studies were of particular importance for Ukraine, given its significant agricultural potential and vulnerability to climate change, and can serve as a basis for developing its own sustainable land use strategies using the European experience.

An important aspect of the study was also the analysis of modern technological solutions used in the EU to manage sustainable land use projects. A. Vitale and C. Salvo (2024) considered the prospects for using Geographic Information Systems (GIS), remote sensing technologies, and artificial intelligence to monitor land resources and support management decision-making. L. Kong *et al.* (2020) focused on the potential of using big data technologies to optimise land use in EU urban agglomerations. Special attention should be paid to the issue of involving stakeholders in the management of sustainable land use projects. The EU experience demonstrates the importance of active participation of the public, business and scientific community in land policy planning and implementation processes. The study by J. Newig *et al.* (2023), S. Yudina *et al.* (2024) showed that effective interaction between different stakeholder groups contributes to improving the quality of management decisions and reducing land-use conflicts.

In the context of Ukraine, T.V. Lisova (2024) analysed the current state of land reform and challenges associated with the implementation of European land use standards. The researcher noted the need to improve the regulatory framework, develop institutional capacity and effectively control land resources. However, the specific mechanisms for adapting successful European practices to Ukrainian conditions remain understudied. To deepen our understanding of sustainable land use issues in the context of Ukraine's European integration, attention should be paid to the research by M.I. Semych (2019). The researchers considered the issue of harmonisation of the Ukrainian land management system with European standards. They stressed the need for an integrated approach to implementing changes that considers both legislative and institutional aspects.

The issues of digitalisation of land management and the introduction of innovative technologies in land use processes were considered by U. Sakthi *et al.* (2023). The researchers analysed the potential of using blockchain technologies and the Internet of Things to increase the transparency and efficiency of land transactions in the EU, which can be a useful experience for Ukraine in the context of land market development. The study by D. Stober *et al.* (2021) focused on spatial planning and the integration of sustainable development principles into territorial governance processes in EU countries. The researchers proposed innovative approaches to balancing economic, social and environmental interests in land use planning, which can be adapted to Ukrainian realities. An important aspect is also the study of economic mechanisms for promoting sustainable land use. In this context, the study by B. Bartkowski *et al.* (2020) was devoted to the analysis of the effectiveness of various instruments of economic regulation in the field of land use in the EU, including tax incentives, subsidies, and market mechanisms. These studies can be useful for developing economic incentives for sustainable land use in Ukraine.

Issues of land management in the context of rural development were considered by G. Pe'er *et al.* (2020). The researchers analysed the experience of EU countries in implementing integrated approaches to rural development that combine the tasks of landscape conservation, maintaining biodiversity and ensuring the economic viability of rural communities. The fundamental impact of sustainable land use policies on the socio-economic development of EU regions was investigated by L. Scheurer and A. Vranken (2024). It concerned the analysis of the impact of sustainable land use policies on the socio-economic development of EU regions. The researchers proposed a methodology for evaluating the effectiveness of sustainable land use projects, which can be adapted for monitoring and evaluating such initiatives in Ukraine.

The purpose of this study was to conduct a comparative analysis of the management of sustainable land use development projects in Ukraine and the EU countries and develop recommendations for improving Ukrainian practices in accordance with the EU requirements.

MATERIALS AND METHODS

A comprehensive approach was used to conduct the study, including an analysis of statistical data, the regulatory framework, and an expert survey. The study was conducted in the period from January to June 2024 and covered five member states of the European Union – Poland, Romania, Bulgaria, Greece, Spain, and Ukraine. Official sources Eurostat (2024) and State Service of Ukraine for Geodesy, were used for analysis. In particular, the following indicators were analysed:

- area of restored land (% of the total area of degraded land);
- land restoration investment (EUR/ha);
- share of organic land (% of total agricultural area);
- number of certified organic producers (per 100,000 population);
- organic products market (EUR per capita);
- irrigation efficiency;
- share of land with drip irrigation;
- water reuse (million m^3/year);
- water stress index.

The SPSS Statistics 28.0 software suite was used for processing statistical data. Descriptive statistics, correlation analysis (Pearson correlation coefficient), and regression analysis were used. The statistical significance of the results was evaluated using the Student's t-test at the significance level of $p < 0.05$. The analysis of the regulatory framework included the study of key EU documents on sustainable land use development, in particular: European Green Deal (2021), Common Agricultural Policy for 2023-2027 (2022), and EU Biodiversity Strategy for 2030 (2020), Directorate-General for Environment (2021), European Environment Agency (2024). The relevant Ukrainian regulations and strategic documents were also analysed to assess their compliance with EU requirements.

To obtain expert assessments, an anonymous online survey of 30 specialists in the field of land administration and sustainable development from the EU and Ukraine was conducted in the period from May 15 to June 30, 2023. The sample was formed by the snowball method with the initial selection of experts according to the following criteria: the presence of a specialised higher education (Master's or Doctor of Science in the field of land management, ecology, or sustainable development), at least 5 years of experience in the relevant field, the presence of at least three peer-reviewed publications on the research topic over the past 5 years. Distribution of respondents: 25 experts from EU countries (5 each from Poland, Romania, Bulgaria, Greece, and Spain) and 5 experts from Ukraine. The questionnaires were sent out by email with a link to the Google Form survey. Prior to the survey, all participants provided informed consent to the processing of their data in accordance with the requirements of the GDPR and national legislation. The survey was conducted in English, with the possibility of providing answers in Ukrainian for experts from Ukraine. The study was approved by the Ethics Committee of the National University "Kyiv-Mohyla Academy" (protocol No. 5 of 31 March 2016) and was conducted in compliance with ethical standards, including voluntary participation, data confidentiality, and the ability to withdraw at any stage of the survey. The questionnaire contained 20 closed-ended questions on a 5-point Likert scale and 5 open-ended questions. Questions related to assessment:

- effectiveness of existing mechanisms for managing sustainable land use development projects;
- compliance of national policies with EU requirements;
- major challenges and obstacles to implementing sustainable land use principles;
- promising areas for improving project management.

Methods of descriptive statistics, factor analysis, and content analysis of answers to open-ended questions were used to analyse the survey results. Based on the data obtained, an integral index of compliance of the management of sustainable land use development projects with EU requirements was developed. The index was calculated using the equation (1):

$$I = 0.3A + 0.3B + 0.2C + 0.2D, \qquad (1)$$

where I – integral index; A – indicator of regulatory compliance (0-100 points); B – indicator of institutional capacity (0-100 points); C – indicator of resource provision (0-100 points); D – indicator of project performance (0-100 points). The weight coefficients were determined based on expert assessments.

RESULTS

The study focused on three key aspects of managing sustainable land use projects in line with EU requirements: restoration of degraded land, introduction of organic farming and water management in the context of agricultural production. A comparative analysis of the situation in Ukraine and five EU countries (Poland, Romania, Bulgaria, Greece, and Spain) revealed significant differences in approaches and effectiveness of sustainable land use development projects. The results were based on the analysis of statistical data, an expert survey, and calculation of the integral index of compliance with EU requirements. The overall picture indicates a significant lag of Ukraine from the studied EU countries in all the aspects considered. Differences in investment volumes, the effectiveness of implemented measures, and the development of appropriate infrastructure are particularly noticeable. The analysis of successful practices of EU countries helped to identify key success factors and develop recommendations for bridging the existing gap.

Restoration of degraded land. The restoration of degraded land is a key aspect of sustainable land use development, which is an integrated approach to land management and use. This approach is aimed at ensuring long-term land productivity, maintaining its ecological integrity and promoting the socio-economic sustainability of regions. In the context of global challenges such as climate change, population growth and increased food security, the restoration of degraded land is of particular importance. It not only facilitates productive use of areas that have lost their fertility due to erosion, pollution, or overexploitation, but also contributes to the conservation of biodiversity, improving water and air quality, and mitigating the effects of climate change (Skydan *et al.*, 2021).

A comparative analysis of approaches to the restoration of degraded land in the countries of the European Union and Ukraine revealed significant differences in methodologies, investments, and the effectiveness of implemented measures. EU countries, guided by the principles of EU Soil Strategy for 2030 (2021), implement comprehensive strategies that combine innovative technologies with traditional land use methods. In particular, the following advanced approaches are widely used: phytoremediation – the use of specially selected plants to remove pollutants from the soil; bioremediation – the use of microorganisms to decompose toxic substances; agroforestry – the creation of protective forest belts to prevent soil erosion (Melnychenko, 2024; Skliar *et al.*, 2024). In addition, considerable attention is paid to the introduction of crop rotations and the cultivation of cover crops that contribute to the restoration of the structure and increase soil fertility. These methods not only effectively restore degraded land, but also ensure the long-term sustainability of agroecosystems.

In contrast, approaches to the restoration of degraded land in Ukraine are characterised by a certain limitation and predominance of traditional methods. In particular, simple reclamation is often used by filling with a fertile layer of soil, which does not always ensure sustainable restoration of the ecosystem. The use of modern remediation technologies and biological recovery methods remains limited, due to both insufficient funding and the lack of a comprehensive national strategy in this area (Fedoniuk *et al.*, 2024). The introduction of agroecological practices that would promote natural soil restoration is also slow, due to a lack of awareness among farmers and a lack of appropriate incentives. To quantify the effectiveness of measures to restore degraded land, a comparative analysis of key indicators was conducted between the EU countries and Ukraine (Table 1).

Table 1. *Comparison of indicators of restoration of degraded land (2024)*

Country	Area of restored land (% of the total area of degraded land)	Recovery investment (EUR/ha)	Recovery efficiency (points*)
Poland	18.5	850	7.2
Romania	15.3	720	6.8
Bulgaria	14.1	680	6.5

Table 1. Continued

Country	Area of restored land (% of the total area of degraded land)	Recovery investment (EUR/ha)	Recovery efficiency (points*)
Greece	16.7	790	7.0
Spain	19.2	920	7.5
Ukraine	8.4	310	4.3

Note: *recovery efficiency was evaluated by experts on a 10-point scale*
Source: *created by the authors based on D. Verhoeven et al. (2024) and the State Service of Ukraine for Geodesy*

An analysis of the data presented in Table 1 revealed significant differences between approaches to the restoration of degraded land in Ukraine and the EU countries. Comparison of Ukraine's indicators with the average values for the EU countries under study showed a significant lag in Ukraine in all key parameters. The difference in investment in land restoration is particularly noticeable, which directly affects the effectiveness of these measures. Such low indicators of Ukraine indicate the presence of serious systemic problems in the approach to the restoration of degraded land and the need for drastic changes in this area.

An expert survey conducted as part of the study revealed key success factors that contribute to the effective restoration of degraded land in the EU countries. Among them, the implementation of comprehensive national soil restoration programmes, significant investments in research and innovative restoration technologies, and the active involvement of local communities and the private sector in restoration projects stand out. Notable examples of such successful initiatives are the Land Restitution (2024) in Spain and the Land from Scratch (2024) in Poland. These programmes are distinguished by their integrated approach, which provides not only direct financing of land restoration work, but also a wide range of related activities. In particular, they include systematic training of farmers in modern methods of restoring and preserving soil fertility, the introduction of advanced soil monitoring systems using satellite technologies and drones, and the development and implementation of economic incentives to promote sustainable land use practices. The effectiveness of these programmes is confirmed by impressive results: over the past 5 years, significant areas of degraded land have been successfully restored. The LIFE programme, which aims to finance innovative environmental projects, provides significant support for initiatives to restore degraded land and preserve biodiversity. The Common Agricultural Policy for 2023-2027 (2022) also plays an important role, which encourages farmers to adopt sustainable land use practices through a system of subsidies and economic incentives. These initiatives create a strong institutional and financial framework for implementing effective measures to restore degraded land in EU countries. These achievements not only reflect the quantitative aspect of restoration, but also indicate qualitative changes in approaches to land management, the development of a new culture of land use, and an increase in environmental awareness among farmers and landowners.

In contrast to the successful experience of the EU countries, the situation in Ukraine is characterised by the presence of significant obstacles to the effective restoration of degraded land (Bragina *et al.*, 2018). According to experts, the main problems are chronic underfunding of restoration projects, the use of outdated technologies and methods, and imperfect legislative framework in the field of soil protection and restoration. The lack of a clear national strategy for the restoration of degraded land that sets out long-term goals, funding mechanisms and criteria for evaluating the effectiveness of rehabilitation measures is particularly acute. Limited budget resources allocated for this purpose lead to the fact that most restoration projects in Ukraine are fragmented, cover small areas and do not have a long-term effect. The experts also noted the serious problem of insufficient coordination between different departments and levels of government, which often leads to duplication of efforts, inefficient use of already limited resources, and a lack of a systematic approach to solving the problem of land degradation.

A diagram was created to visually represent the identified differences in the efficiency of restoring degraded land between the EU countries and Ukraine (Fig. 1). It shows a significant lag in Ukraine in terms of the effectiveness of restoring degraded land compared to European countries. The diagram not only illustrates the current state of affairs, but also highlights the scale of challenges facing Ukraine in the field of restoring degraded land, and the need for drastic changes in approaches to land management.

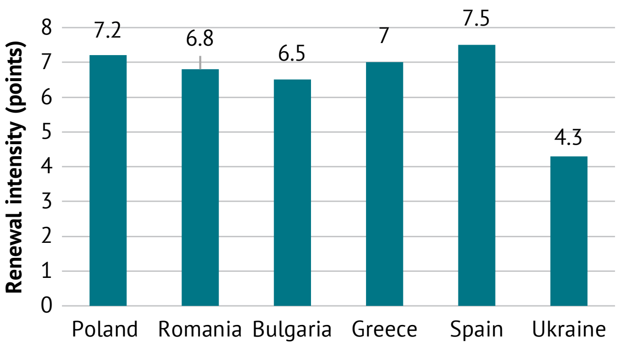

Figure 1. *Comparison of the efficiency of degraded land restoration*
Source: *created by the authors*

Considering the identified problems and the significant lag of Ukraine in the restoration of degraded land, experts have developed a number of recommendations to bridge this gap. The key recommendation was the development and implementation of a comprehensive national programme for the restoration of degraded land, which should be coordinated with the EU Soil Strategy for 2030 (2021). Such a programme should include clearly defined objectives and indicators of success, detailed funding mechanisms (with a particular focus on attracting private investment through public-private partnership mechanisms), and an effective monitoring and evaluation system. Experts emphasise the need for a significant increase in investment in land restoration, suggesting the use of various sources of funding, including the state budget, funds from international financial organisations, and the creation of a specialised fund for the restoration of degraded land. Special attention is paid to the importance of implementing a modern system for monitoring and evaluating the efficiency of recovery projects, similar to that used in the EU. Such a system will not only track progress in real time, but also provide the ability to quickly adjust the land restoration strategy based on the data obtained and analyse the effectiveness of implemented measures.

The implementation of these recommendations will require significant efforts and resources, but it is critical for ensuring the sustainable development of agriculture and the conservation of natural resources in Ukraine. Successful implementation of best practices in restoring degraded land will not only increase agricultural productivity and improve the environmental situation, but also bring Ukraine closer to European standards of land management, which is an important step towards European integration.

Introduction of organic farming. The introduction of organic farming is another key aspect of sustainable land use development, which plays a crucial role in ensuring long-term agricultural land productivity and maintaining ecological balance. Organic farming involves moving to crop cultivation methods that minimise the use of synthetic pesticides and fertilisers, thereby reducing the chemical load on the soil and water resources. This approach not only helps restore natural soil fertility and preserve biodiversity, but also ensures the production of safe and nutritious food. In the context of growing global concerns about food security, climate change and environmental degradation, organic farming is becoming increasingly important as a strategic area for the development of the agricultural sector. A comparative analysis of the development of organic farming in the European Union and Ukraine reveals significant differences in the approaches, scale of implementation, and efficiency of this sector. To quantify these differences, a detailed study of key indicators of organic sector development in selected countries was conducted (Table 2).

***Table 2**. Comparison of organic farming development indicators (2024)*

Country	Share of organic land (% of total agricultural area)	Number of certified organic producers (per 100,000 population)	Organic products market (EUR per capita)
Poland	5.8	18.3	62
Romania	4.2	12.7	41
Bulgaria	3.5	10.9	35
Greece	10.3	32.1	95
Spain	9.7	28.6	87
Ukraine	1.1	3.2.	8

***Source:** created by the authors based on Eurostat (2024)*

The analysis of the data presented in Table 2 revealed a significant lag in the development of the organic sector in comparison with the EU countries studied. Ukraine is significantly inferior in all key indicators. The share of organic land in Ukraine is almost 6 times less than the average indicator of the studied EU countries. The number of certified organic producers per 100,000 population in Ukraine is significantly lower than in the EU countries. The organic market in Ukraine also remains underdeveloped: the cost of organic products per capita in Ukraine is 7-12 times less than in the EU countries under study. These data clearly illustrate the significant potential for the development of the organic sector in Ukraine and the need to step up efforts in this line.

An expert survey conducted as part of the study revealed key success factors for the introduction of organic farming in the EU countries. Central to this process is the effective implementation of the EU's organic production action plan, which is part of the broader Farm to Fork Strategy (2020). This plan provides for a comprehensive approach to stimulating both demand and supply of organic products, including financial support for producers, infrastructure development, and raising consumer awareness. Significant financial support for farmers in the transition to organic production plays an important role. For example, Spain has a subsidy programme that covers up to 70% of certification costs and up to 50% of necessary equipment costs during the first three years of switching to organic production. This support allows farmers to overcome initial financial difficulties and achieve profitability faster. In addition, the EU has a well-developed system of certification and quality control of organic products, which ensures a high level of consumer confidence. Active consumer awareness is also an important element of success. A striking example of such activities is the Greek national programme Organic Food in Schools (Malissiova *et al.*, 2022), which not only provides students with organic products, but also conducts regular educational events for children and their parents about the benefits of organic food. This initiative contributes to the development of sustainable demand for organic products in the long term and educates a new generation of conscious consumers. As part of the EU's Green Course policy, innovative projects aimed at supporting organic farming are also being actively implemented. For example, the Organic Farm Knowledge Platform (2021) project provides farmers with access to the most up-to-date knowledge and practices in the field of organic production, and the European Innovation Partnership initiative "Agricultural Productivity and Sustainability" (2023) promotes collaboration between scientists, farmers, and agribusiness to develop innovative solutions in the field of sustainable agriculture.

In contrast to the successful experience of the EU countries, the situation with the development of organic farming in Ukraine is characterised by the presence of significant obstacles. The main problem, according to experts, is insufficient state support for the organic sector. Unlike the EU countries, Ukraine does not have systematic programmes to subsidise farmers who switch to organic production (Borko & Jammal, 2024). This leads to the fact that the cost of transition falls entirely on the shoulders of producers, which in conditions of limited financial resources becomes a significant barrier. Another important problem is the difficulty of certifying products according to EU standards. The cost of certification for an average farm can reach EUR 5,000-7,000, which is a significant financial burden for Ukrainian farmers. In addition, the certification procedure is often lengthy and bureaucratically complex, which further discourages potential producers of organic products. Experts also noted the problem of low purchasing power of the population and insufficient consumer awareness of the benefits of organic products. This leads to low domestic demand for organic products: about 70% of Ukrainian organic products are exported, while the domestic market remains underdeveloped. The lack of a well-developed infrastructure for storing, processing, and distributing organic products is also a significant constraint on the development of the sector. To visually represent the difference in the share of organic land between the EU countries and Ukraine, a comparative chart was created (Fig. 2).

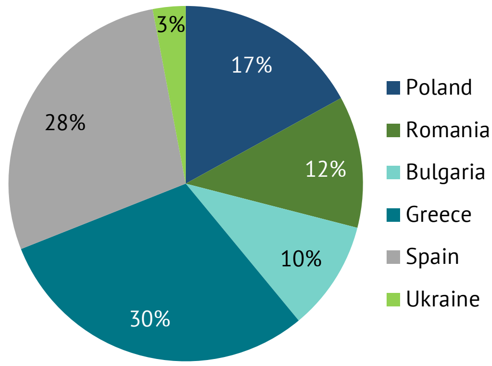

Figure 2. *Comparison of the share of organic land*
Source: *created by the authors*

This chart clearly demonstrates Ukraine's significant lag in the share of organic land from the leading EU countries, which underlines the need to step up efforts in this area and implement comprehensive measures to stimulate the development of the organic sector. Considering the identified problems and the significant lag of Ukraine in the field of organic farming, experts have developed a number of recommendations to bridge this gap. The key recommendation is the development and implementation of a comprehensive national strategy for the development of organic production, coordinated with the Action Plan for the Development of Organic Production in the EU (2022). This strategy should include a number of interrelated activities. First, it is necessary to create an effective system of financial support for farmers in the transition to organic production, which may include subsidies, soft loans, and tax preferences. This support should be sufficient to cover the additional costs associated with the transition to organic farming methods and ensure a stable income for farmers during the transition period. Second, it is important to simplify the certification procedure for organic products and harmonise Ukrainian standards with European ones. This will reduce producers' certification costs and facilitate access of Ukrainian organic products to EU markets. The creation of a national certification system that is internationally recognised can be an important step in this line. Third, it is necessary to stimulate the development of the domestic market for organic products. This can be achieved through the introduction of public procurement of organic products for schools, hospitals, and other government agencies, and through the support of local producers and the creation of specialised markets for organic products. An important aspect is also the development of infrastructure for the storage, processing, and distribution of organic products, which can be implemented through public-private partnerships.

Experts stressed the importance of conducting large-scale educational campaigns to raise consumer awareness of the benefits of organic products. These campaigns should reach different segments of the population and use a variety of communication channels, including social media, television, schools, and community organisations. In addition, it is recommended to introduce specialised educational programmes for farmers who want to switch to organic production, in order to improve their competencies in the field of organic technologies and management methods. Ultimately, an important aspect is the promotion of scientific research and innovation in organic farming. The creation of specialised research centres, support for cooperation between scientists and farmers, and the implementation of pilot projects on testing innovative organic technologies can be key factors for improving the efficiency and competitiveness of the Ukrainian organic sector.

The implementation of recommendations for the development of organic production in Ukraine will require significant efforts and resources, but it is necessary to ensure the sustainable development of agriculture, approach European standards, and obtain broad positive effects. Successful implementation of these measures will contribute to the development of the organic sector, have a positive impact on the economy, environment, and public health. The development of organic farming also opens up new opportunities for the export of Ukrainian products to the markets of the EU and other countries, which can become an important factor in economic growth and integration of Ukraine into the global value chains of sustainable agriculture.

Water resources management. Studies have shown significant differences between the approaches of Ukraine and EU countries to water resources management. In particular, it is important to implement the Water Framework Directive (2000), which introduces an integrated approach to water management at the river basin level. This directive promotes integrated water management by combining quantitative and qualitative aspects. Similar approaches are being implemented in Ukraine, but much more slowly than in the European Union countries. Data analysis showed that EU countries have made significant strides in implementing water-saving technologies in agriculture. For example, in Spain and Greece, drip irrigation systems are widely used, which can save up to 50% of water compared to conventional methods. In Ukraine, the share of land with drip irrigation remains low, due to insufficient funding and low awareness of farmers about the benefits of this technology. Experts note that the introduction of modern irrigation systems could significantly increase the efficiency of water resources use in Ukrainian agriculture.

Water reuse is also an important aspect of water management. In EU countries, especially in regions with limited water resources, technologies for wastewater treatment and reuse for irrigation are actively developing. For example, in Spain, more than 100 million m^3 of water is reused annually, which significantly reduces the load on natural water sources. In Ukraine, this practice has not yet become widespread, which is reflected in the following data (Table 3).

***Table 3**. Comparison of water management performance indicators (2024)*

Country	Irrigation efficiency (%)	Share of land with drip irrigation (%)	Water reuse (million m^3/year)	Water stress index*
Poland	78	15.3	42	1.8
Romania	72	12.7	35	2.1
Bulgaria	70	11.9	28	2.3
Greece	82	22.6	89	3.5
Spain	85	27.4	124	3.8
Ukraine	60	5.2	12	2.7

Note: *water stress index: ratio of total water intake to available renewable water resources*
Source: *created by the authors based on C. McLennan et al. (2024), Eurostat (2024) and State Service of Ukraine for Geodesy*

The expert survey revealed the key factors that determine the higher efficiency of water resources management in the EU countries: consistent implementation of the EU Water Framework Directive, significant investments in the introduction of innovative irrigation and water conservation technologies, a developed system for monitoring the state of water resources, active involvement of farmers in programmes to improve water use efficiency. But in Ukraine, experts noted a number of significant problems: the obsolescence of irrigation systems (up to 70% require modernisation or replacement), the slow introduction of water-saving technologies due to the lack of financial resources, an inefficient system for monitoring water resources, and a low level of awareness of farmers about modern methods of efficient water use.

Based on the analysis of the situation and recommendations of experts, a set of measures was developed to overcome the backlog of Ukraine in the field of water resources management: development and implementation of a national water resources management strategy, a large-scale programme for the modernisation of irrigation systems, the creation of a system of economic incentives for farmers, improving the system of monitoring and management of water resources. To assess the overall level of compliance of sustainable land use project management with EU requirements, an integral index was developed and calculated for each of the countries under study (Table 4). This index considers four key components: regulatory compliance, institutional capacity, resource support, and project performance. Each component was evaluated on a scale from 0 to 100 points based on statistical analysis and expert assessments.

Table 4*. Integrated EU compliance index (2024)*

Country	Regulatory compliance (A)	Institutional capacity (B)	Resource provision (C)	Project performance (D)	Integral index (I)
Poland	92	85	78	83	85.3
Romania	88	79	72	76	79.5
Bulgaria	85	76	68	72	76.1
Greece	90	82	75	80	82.4
Spain	95	88	82	87	88.7
Ukraine	65	58	45	52	56.0

Source: *created by the authors based on Schillaci et al. (2023), State Service of Ukraine for Geodesy*

The integral index was calculated by equation (1). The results showed that Ukraine lags significantly behind the EU countries in all components of the index. The greatest lag is observed in terms of resource provision and project performance. This indicates the need not only to harmonise legislation and develop institutional capacity, but also to significantly increase investment in sustainable land use development projects and improve the efficiency of their implementation in Ukraine.

Recommendations for improving project management. Based on the identified differences and successful practices of the EU countries, it is possible to develop a comprehensive set of recommendations for improving the management of sustainable land use development projects in Ukraine in accordance with EU requirements. The key element should be the development and implementation of a comprehensive national strategy for sustainable land use development, which would cover all three aspects under study: the restoration of degraded land, the development of organic farming, and water resources management. This strategy should be consistent with relevant EU directives and initiatives, in particular the EU Soil Strategy for 2030 (2021), Organic Action Plan, and Water Framework Directive (2000).

In the field of restoration of degraded land, it is recommended to create a specialised fund to finance restoration projects, which would combine funds from the state budget, international financial organisations, and private investors. It is important to introduce a system of economic incentives for landowners who are actively engaged in the restoration of degraded land, including tax incentives and subsidies. It is also necessary to develop and implement a modern soil monitoring system that would allow rapid identification of problem areas and assessment of the effectiveness of restoration measures. For the development of organic farming, the key recommendation is to create an effective system of financial support for farmers during the transition to organic production. This system may include subsidies to cover certification costs, soft loans to purchase necessary equipment, and compensation for possible temporary crop declines during the transition period. It is also important to simplify the certification procedure for organic products and harmonise Ukrainian standards with European ones. It is recommended to develop and implement a national infrastructure development programme for the storage, processing, and distribution of organic products, which can be implemented through public-private partnership mechanisms. It is equally important to conduct large-scale information and educational campaigns to raise consumer awareness of the benefits of organic products and stimulate domestic demand.

In the field of water resources management, the key recommendation is the development and implementation of a national programme for the modernisation of irrigation systems, with a focus on the introduction of water-saving technologies such as drip irrigation. It is important to create a system of economic incentives for farmers who implement efficient water use methods, including grants for the purchase of modern equipment and preferential tariffs for irrigation water. It is also recommended to develop technologies for wastewater treatment and reuse for irrigation, especially in regions with limited water resources. To enhance institutional capacity, it is important to establish an interagency coordinating body that ensures the coordinated implementation of sustainable land use policies at all levels. It is also necessary to develop and implement a comprehensive professional development programme for specialists in the field of land and water management, which would consider the best practices of EU countries.

An important aspect is to develop cooperation with research institutions and universities to encourage innovation and transfer knowledge to practical land use.

To improve monitoring and evaluation of the effectiveness of projects, it is recommended to implement a single information system that would allow real-time monitoring of progress in the implementation of sustainable land use development projects and assess their impact on environmental and socio-economic indicators. This system should be integrated with the relevant European databases to ensure comparability of indicators. The implementation of these recommendations will require significant efforts and resources, but it is critical for ensuring the sustainable development of the agricultural sector of Ukraine and approaching European standards of land management. The successful implementation of these measures will not only contribute to solving environmental problems and improving agricultural efficiency, but also create new opportunities for economic growth and integration of Ukraine into the European space.

DISCUSSION

The results of the study indicate a significant lag between Ukraine and the EU countries in the field of managing sustainable land use development projects. This lag is observed in all three key aspects: the restoration of degraded land, the introduction of organic farming, and water management. Such results are important for understanding the current state and prospects for the development of sustainable land use in Ukraine in the context of European integration processes.

In the field of restoration of degraded land, Ukraine shows significantly lower indicators compared to the EU countries under study. The share of restored land in Ukraine (8.4%) is almost half that of the EU countries (14.1-19.2%), and the volume of investment in restoration (310 EUR/ha) is 2-3 times lower. These findings are consistent with the study by P. Borrelli *et al.* (2020), which identified high risks of soil erosion in Eastern European countries, including Ukraine, and stressed the need to step up efforts to restore degraded land. The results also support the findings of T.V. Lisova (2024) on the need to improve the regulatory framework and increase the institutional capacity of Ukraine in the field of land management. It is important to note that the low efficiency of restoring degraded land in Ukraine (4.3 points out of 10) compared to the EU countries (6.5-7.5 points) can be associated not only with insufficient funding, but also with the use of outdated technologies and methods. This confirms the arguments of A. Vitale and C. Salvo (2024) on the importance of implementing modern geoinformation systems and remote sensing technologies for effective land management.

In the field of organic farming, the results of the study show an even more significant lag in Ukraine. The share of organic land in Ukraine (1.1%) is significantly lower than in the EU countries (3.5-10.3%), and the number of certified organic producers per 100,000 population in Ukraine (3.2) is 3-10 times less than in the EU countries under study. These data are consistent with conclusions of I. Soares *et al.* (2024) on the importance of integrating sustainability principles into all aspects of project management in the agricultural sector. Especially important is the significant lag of Ukraine in terms of Organic Market Development (EUR 8 per capita compared to EUR 35-95 in the EU countries). This confirms the arguments of E. Butenko and Y. Loshakova (2019) on the need for an integrated approach to the development of the organic sector, which would include not only supporting producers, but also stimulating demand for organic products.

In the field of water resources management, the results of the study also indicate a significant lag in Ukraine. Low irrigation efficiency (60% compared to 70-85% in the EU) and a small share of land with drip irrigation (5.2% compared to 11.9-27.4% in the EU) indicate the need to modernise irrigation systems in Ukraine. These results support the conclusions of P.A. Schulte *et al.* (2022) on the importance of introducing innovative technologies to improve the efficiency of water resources use in agriculture. It is important to note that the low level of water reuse in Ukraine (12 million m^3/year compared to 28-124 million m^3/year in the EU countries) indicates the need to develop technologies for wastewater treatment and reuse for irrigation. This is consistent with recommendation of D. Stober *et al.* (2021) on the integration of sustainable development principles into water management processes. The analysis of the integral index of compliance with EU requirements shows that Ukraine significantly lags behind the countries under study in all components: regulatory compliance, institutional capacity, resource support, and project performance. Particularly low indicators of Ukraine in terms of resource provision components (45 points compared to 68-82 in the EU countries) and project performance (52 points compared to 72-87 in the EU countries) confirm the conclusions of I. Fazey *et al.* (2020) on the need for an integrated approach to implementing sustainable development principles in land use.

It is important to note that the identified problems in the field of managing sustainable land use development projects in Ukraine are systemic in nature and require a comprehensive solution. This is consistent with the conclusions of J. Newig *et al.* (2023) on the importance of effective interaction between different groups of stakeholders to improve the quality of management decisions in the field of land use. The results of the study also support arguments of F. Terribile *et al.* (2024) on the positive impact of European directives and programmes on the development of sustainable land use practices. Significantly higher indicators of EU countries in all the studied aspects indicate the effectiveness of the European policy in the field of sustainable land use development. An important aspect identified in the course of the study is the significant lag of

Ukraine in the implementation of innovative technologies for land and water resources management. This the findings of L. Kong *et al.* (2020) on the importance of using big data and artificial intelligence technologies to optimise land use. The results of the study also indicate the need to develop economic mechanisms for stimulating sustainable land use in Ukraine. Low investment rates in the restoration of degraded land and the development of organic farming support arguments of B. Bartkowski *et al.* (2020) on the importance of effective economic regulatory instruments in the field of land use.

Special attention should be paid to the significant lag of Ukraine in the development of organic farming. This confirms the conclusions of R.M. Petrescu-Mag *et al.* (2019) on the need to implement integrated approaches to rural development that would simultaneously address the challenges of landscape conservation, maintaining biodiversity, and ensuring the economic viability of rural communities. It is important to continue studying the impact of sustainable land use policies on the socio-economic development of regions. The results of the study are also consistent with the findings of L. Scheurer and A. Vranken (2024) on the need to develop a methodology for evaluating the effectiveness of sustainable land use projects that would consider both environmental and socio-economic aspects. The analysis of the results of the study also highlights the need to develop a system for monitoring and evaluating the effectiveness of sustainable land use projects in Ukraine. This is in line with recommendations of U. Sakthi *et al.* (2023) on the use of blockchain technologies and the Internet of Things to improve transparency and efficiency of land management. Special attention should be paid to problems in the field of water resources management in Ukraine, considering global climate changes, which is confirmed by the conclusions of P. Borrelli *et al.* (2020), B. Rexha *et al.* (2024) on the need to develop adaptive land-use strategies for climate change.

The results of the study highlight the need for significant changes in approaches to managing sustainable land use development projects in Ukraine. A significant lag behind the EU countries in all the aspects under study indicates the need for a comprehensive solution to problems, which would include improving the regulatory framework, strengthening institutional capacity, increasing investment, and introducing innovative technologies. Overcoming this gap is critical not only for ensuring the sustainable development of the agricultural sector of Ukraine, but also for the successful European integration of the country. The implementation of European standards and practices in the field of land and water resources management will not only increase the efficiency of agriculture and solve environmental problems, but also open up new opportunities for economic growth and integration of Ukraine into the European space.

The results of the study also indicate the need for further scientific research in the field of evaluating the effectiveness of sustainable land use development projects, developing innovative technologies for land and water resources management, and studying the impact of sustainable land use policies on the socio-economic development of the regions of Ukraine. These areas of research should become a priority for the Ukrainian scientific community in the context of the country's European integration aspirations and global challenges to sustainable development.

CONCLUSIONS

The study comprehensively analysed the management of sustainable land development projects in Ukraine and the EU countries, focusing on three key aspects: restoration of degraded land, introduction of organic farming, and water resources management. The results of the study revealed a significant lag of Ukraine from the studied EU countries in all the parameters considered. In the field of restoration of degraded land, it was found that the area of restored land in Ukraine (8.4% of the total area of degraded land) is almost twice less than the average for the EU countries under study (16.8%). Investment in land restoration in Ukraine (310 EUR/ha) accounts for only 39% of the EU average (792 EUR/ha). The recovery efficiency estimated by experts in Ukraine (4.3 points) is significantly lower than the average of the EU countries (7 points). Analysis of the development of organic farming showed that the share of organic land in Ukraine (1.1% of the total agricultural area) is almost 6 times less than the average indicator of the studied EU countries (6.7%). The number of certified organic producers in Ukraine (3.2 per 100,000 population) is 6.4 times less than the EU average (20.5). The market of organic products in Ukraine (EUR 8 per capita) is 8 times less than the average of the EU countries (EUR 64). In the field of water resources management, it was found that irrigation efficiency in Ukraine (60%) is significantly lower than the EU average (77.4%). The share of land with drip irrigation in Ukraine (5.2%) is 3.5 times less than the average of EU countries (18%). The volume of water reuse in Ukraine (12 million m^3/year) is 5.3 times less than the average of the EU countries (63.6 million m^3/year). The calculation of the integral index of compliance with EU requirements showed that Ukraine (56 points) significantly lags behind the average of the EU countries surveyed (82.4 points). The greatest lag is observed in terms of resource provision and project performance. Based on the identified differences and successful practices of the EU countries, a set of recommendations has been developed to improve the management of sustainable land use development projects in Ukraine. The key recommendations are: development of a comprehensive national strategy; creation of specialised funding funds; introduction of economic incentives; modernisation of monitoring systems; harmonisation of standards with the EU and large-scale educational campaigns.

Among the limitations of the study, it is worth noting the insufficient consideration of regional features of implementing the principles of sustainable land use in Ukraine and the limited number of experts involved. In addition, the impact of land reform on the possibilities of implementing European land use standards was not considered in detail. Promising areas for further research are the analysis of the impact of land reform on the implementation of the principles of sustainable land use, the development of differentiated approaches to project management in different regions of Ukraine, the study of the impact of climate change on land use practices and the improvement of methods for evaluating the effectiveness of sustainable development projects. Special attention should be paid to the study of mechanisms for involving various groups of stakeholders in the management of sustainable land use development projects and the development of economic instruments to encourage sustainable practices.

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CONFLICT OF INTEREST

None.

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Управління проєктами сталого розвитку землекористування відповідно до вимог ЄС

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Анотація. Метою дослідження було проведення порівняльного аналізу управління проєктами сталого розвитку землекористування в Україні та країнах ЄС та розробка рекомендації щодо вдосконалення українських практик відповідно до вимог Європейського Союзу. Методологія базувалася на системному підході та включала аналіз статистичних даних, проведення експертного опитування та розрахунок інтегрального індексу відповідності вимогам ЄС. Дослідження зосереджувалося на трьох ключових аспектах: відновленні деградованих земель, впровадженні органічного землеробства та управлінні водними ресурсами. Результати виявили значне відставання України за всіма досліджуваними параметрами. У сфері відновлення деградованих земель встановлено, що площа відновлених земель в Україні (8,4 % від загальної площі деградованих земель) майже вдвічі менша за середній показник досліджуваних країн ЄС (16,8 %). Інвестиції у відновлення земель в Україні (310 €/га) становлять лише 39 % від середнього показника країн ЄС (792 €/га). Аналіз розвитку органічного землеробства показав, що частка органічних земель в Україні (1.1 % від загальної сільськогосподарської площі) майже в 6 разів менша за середній показник досліджуваних країн ЄС (6,7 %). Кількість сертифікованих органічних виробників в Україні (3,2 на 100 000 населення) у 6,4 рази менша за середній показник країн ЄС. У сфері управління водними ресурсами виявлено, що ефективність зрошення в Україні (60 %) значно нижча за середній показник країн ЄС (77,4 %), а частка земель з крапельним зрошенням в Україні (5,2 %) у 3,5 рази менша за середній показник країн ЄС (18 %). Розрахунок інтегрального індексу відповідності вимогам ЄС показав, що Україна (56 балів) суттєво відстає від середнього показника досліджуваних країн ЄС (82,4 бали). На основі виявлених відмінностей та успішних практик країн ЄС розроблено комплекс рекомендацій для вдосконалення управління проєктами сталого розвитку землекористування в Україні. Ключові рекомендації включають розробку комплексної національної стратегії, створення спеціалізованих фондів фінансування, впровадження економічних стимулів, модернізацію систем моніторингу, гармонізацію стандартів з ЄС та проведення масштабних освітніх кампаній. Дослідження підкреслює необхідність суттєвого вдосконалення підходів до управління проєктами сталого розвитку землекористування в Україні для наближення до стандартів ЄС та забезпечення ефективного використання земельних ресурсів

Ключові слова: екологічна ефективність; інституційна спроможність; інтегральний індекс; економічні стимули; адаптація законодавства; інноваційні технології

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