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Morphometric analysis of nuts in the hybrid population of purple-leaved hazelnut (*Corylus avellana* L.)

Abstract. Hazelnut (*Corylus avellana* L.) is a strategically important nut crop with high nutritional value and significant economic potential. While industrial cultivation is primarily based on green-leaved genotypes, interest in purple-leaved forms has been steadily increasing due to the ornamental appeal and elevated antioxidant potential associated with anthocyanin pigmentation. However, information on nut morphometric traits and the variability in hybrid populations of purple-leaved hazelnut remains limited. This study aimed to analyse the morphometric characteristics of nuts in a hybrid population of purple-leaved hazelnut developed at the National University of Life and Environmental Sciences of Ukraine. The experimental material included nuts from 23 mature own-rooted hybrid shrubs derived from a cross between 'Akademik Yablokov' and 'Moskovskij Rubin'. Nut and kernel weight, linear dimensions, shell thickness, kernel percentage, and nut shape index were evaluated using standard morphometric and statistical methods. The results revealed substantial phenotypic variability in nut morphological traits, with average nut weight ranging from 1.4 g to 3.4 g. Based on the aggregate of economic traits, the 'Profesorskyi' genotype was identified as the most promising overall, producing the heaviest nuts (3.4 g) and kernels (1.6 g). Correlation analysis revealed a strong negative relationship between kernel percentage and shell thickness (r = -0.670), indicating shell thickness as the primary limiting factor for kernel yield. The studied hybrid

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population represents a valuable genetic resource for hazelnut breeding, enabling the selection of genotypes suitable for both commercial nut production and ornamental use. The combination of high nut quality with pronounced decorative traits associated with stable anthocyanin pigmentation highlights the potential of purple-leaved hazelnut for diversified agricultural production and landscape applications

Keywords: anthocyanin pigmentation; phenotypic variability; nut weight; kernel percentage; shell thickness; breeding

INTRODUCTION

The study of hazelnut genetic diversity, particularly focusing on purple-leaved genotypes, is of paramount importance due to the shifting priorities in global horticulture. As agricultural systems face increasing pressure from climate change and evolving market demands, the reliance on a narrow range of green-leaved cultivars poses a risk to long-term crop resilience and economic stability. Investigating the morphometric characteristics of hybrid populations allows for the identification of genotypes that harmoniously combine high productivity with enhanced biological activity and ornamental appeal. This dual-purpose potential is crucial for the diversification of nut production, providing raw materials for the food industry while simultaneously offering aesthetic value for sustainable urban landscaping. Establishing a robust morphometric database for these hybrids is therefore essential for accelerating the selection process and ensuring the development of superior cultivars that meet modern quality standards.

The global significance of hazelnut as a primary nut crop is underscored by its massive production volumes. According to FAOSTAT (n.d.), the world production of in-shell hazelnuts exceeds 1.1 million tonnes, reflecting its vital role in the global agricultural economy. Researchers A. Campa Negrillo *et al.* (2021) and M. Rondanelli *et al.* (2023) emphasised that hazelnuts are indispensable in the confectionery industry due to the high nutritional density, whether consumed fresh or processed. Within the Ukrainian market, a marked domestic production deficit exists. Consequently, V. Mezhenskyj (2022) identifies a significant opportunity for the industrial scaling of hazelnut cultivation. The aesthetic and biological advantages of anthocyanin-rich genotypes have also gained scientific attention. The genotypes with purple pigmentation in leaves and husks provide significant ornamental value, which led O. Johnson & R. Moore (n.d.) to advocate for the integration of such pigmented mutants into breeding programmes in Europe and North America. I. Kosenko *et al.* (2023) have demonstrated that these genotypes can be effectively utilised to improve specific nut characteristics.

In Eastern Europe, the latest advancements in variety registration and testing have been highlighted by the official entry of the ‘Barbakan BSI’ and ‘Bahrianyi’ cultivars into the State Register of Plant Varieties of Ukraine (Vishtak *et al.*, 2021; Vishtak *et al.*, 2022; Ministry of Economy, Environment, and Agriculture of Ukraine, 2025). Meanwhile, G. Hodun & S. Pluta (2024, pers. commun.) have noted the increasing inclusion of such forms in Polish breeding efforts. Despite the promising results, at the National University of Life and Environmental Sciences of Ukraine, a critical challenge remains. Breeding programmes in hazelnut are exceptionally time-consuming, often requiring 15-20 years from the initial cross to the release of a new cultivar. This long duration necessitates the use of advanced morphometric analysis at early stages to identify the most promising material. This is particularly relevant when evaluating offspring from crosses of highly productive cultivars such as ‘Akademik Yablokov’ and ‘Moskovskij Rubin’, which serve as the genetic foundation for many purple-leaved hybrids in the region.

Furthermore, the introduction of new crops and cultivars better adapted to climate change is relevant for many countries, including Ukraine, as established by R. Kızılkaya *et al.* (2022) and

M. Nazarenko & O. Simchenko (2023). Hybrid populations derived from purple-leaved forms represent a valuable source of phenotypic variability, particularly in nut size, shape, and shell characteristics. Understanding the correlation between shell thickness and kernel yield is vital for selecting genotypes that meet the industrial requirement for high-efficiency processing. Modern standards demand thin-shelled nuts with a kernel percentage exceeding 45%, a threshold that requires rigorous selection within hybrid populations.

The aim of this study was to analyse nut morphological traits in hybrid population genotypes of purple-leaved hazelnut developed at the National University of Life and Environmental Sciences of Ukraine (NULES).

MATERIALS AND METHODS

Purple-leaved hazelnut cultivars 'Akademik Yablokov' and 'Moskovskij Rubin' were cultivated at a separate site. Nuts from 'Akademik Yablokov' were used to generate a hybrid seedling population, from which purple-leaved genotypes were selected and planted in a breeding orchard at the Educational, Research and Production Laboratory of Genetic Resources, Introduction, and Breeding of Rare Fruit and Ornamental Plants (NULES of Ukraine), located at the Agronomic Research Station in Pshenychne village, Bila Tserkva district, Kyiv region (50°05'20.1" N 30°12'54.9" E). The experimental material comprised nuts collected from 23 mature shrubs of the hybrid population. Seedlings were planted in 2014 at a spacing of 5 × 4 m and maintained under rainfed conditions using standard horticultural practices. Nuts were harvested at full physiological maturity in mid-August 2025. All procedures complied with the Convention on Biological Diversity (1992) and Convention on International Trade in Endangered Species of Wild Fauna and Flora (1973).

Nut length, width, thickness, and shell thickness (all in mm) were measured using a mechanical calliper with a resolution of 0.1 mm. Nut and kernel weights were determined with an Adventurer™ electronic laboratory balance (China) with a precision of 0.01 g. Kernel percentage was calculated as the ratio of kernel weight to nut weight. Nut shape index was expressed as the length-to-width ratio. Nut and kernel traits were evaluated according to the International Board for Plant Genetic Resources Descriptors (Bioversity, FAO & CIHEAM, 2008). Statistical analysis included descriptive statistics (mean, standard deviation (SD), and coefficient of variation (CV%)) and correlation analysis. All calculations were performed using Microsoft Excel Office 2019. One-way analysis of variance (ANOVA) was used to compare genotypes for each morphometric trait, and differences were considered significant at the 95% confidence level ($p \leq 0.05$) using R. Fisher's least significant difference (LSD) test. Skewness and kurtosis were calculated using standard Excel statistical functions. K. Pearson correlation coefficients were calculated, and the statistical significance was assessed using Student's t-test at $p \leq 0.05$.

RESULTS AND DISCUSSION

In 2003, nuts of the purple-leaved hazelnut cultivars 'Moskovskij Rubin' and 'Akademik Yablokov' from the collection of the Bakhmut Experimental Station of Nursery Cultivation were evaluated. 'Moskovskij Rubin' had a higher nut weight (4.4 g) but a lower kernel percentage (38.0%) compared with 'Akademik Yablokov', which produced smaller nuts (2.8 g) with a higher kernel percentage (49.7%). The relatively low kernel content of 'Moskovskij Rubin' was associated with poor kernel filling, presumably due to cultivation under rainfed conditions; in addition, approximately half of the nuts were damaged by the hazelnut weevil (*Curculio nucum* L.).

These two cultivars, grafted onto Turkish hazel (*Corylus colurna* L.), were established at a separate site. Given that hazelnut is a self-incompatible species, it is highly probable that 'Akademik Yablokov' was cross-pollinated by 'Moskovskij Rubin'. Nuts collected from 'Akademik Yablokov' trees were characterised by an average nut weight of 2.6 g and a kernel percentage of 48.6%. An analysis of the hybrid population of purple-leaved seedlings derived from 'Akademik Yablokov' nuts revealed a variation in average nut weight ranging from 1.4 g to 3.4 g (Table 1).

Table 1. Morphometric indicators of nuts ($\bar{x}$ ± SD, *n* = 20), 2025

Genotype	Nut weight, g	Nut length, mm	Nut width, mm	Nut thickness, mm	Kernel weight, g	Kernel percentage, %	Shell thickness, mm
‘Profesorskyi’	3.4 ± 0.29	25.1 ±1.29	19.6 ± 0.70	18.8 ± 1.32	1.6 ± 0.02	47.4 ± 3.46	1.1 ± 0.08
‘Aspirantskyi’	2.9 ± 0.29	22.6 ± 0.97	17.9 ± 0.88	16.7 ± 0.95	1.1 ± 0.02	37.1 ± 6.23	1.4 ± 0.18
#58	2.7 ± 0.39	27.5 ± 1.27	18.8 ± 1.14	16.7 ± 0.67	1.3 ± 0.03	48.1 ± 3.79	1.0 ± 0.11
#34	2.6 ± 0.29	25.7 ± 1.42	17.2 ± 0.92	15.8 ± 0.92	0.7 ± 0.02	27.8 ± 3.57	1.3 ± 0.07
#66	2.5 ± 0.25	19.5 ± 0.85	18.6 ± 0.84	15.4 ± 0.70	1.1 ± 0.01	46.5 ± 3.24	1.2 ± 0.08
#61	2.5 ± 0.23	26.2 ± 0.63	19.9 ± 0.32	18.7 ± 0.67	0.6 ± 0.03	25.3 ± 5.95	1.1 ± 0.12
#60	2.5 ± 0.40	29.4 ± 1.26	17.8 ± 1.39	16.8 ± 0.92	1.2 ± 0.05	47.9 ± 3.92	1.1 ± 0.07
‘Akademichnyi’	2.5 ± 0.16	21.8 ± 0.42	17.5 ± 0.53	16.6 ± 0.52	1.2 ± 0.01	48.1 ± 2.85	1.1 ± 0.06
#68	2.4 ± 0.19	24.8 ± 0.42	16.7 ± 0.48	15.4 ± 0.70	1.1 ± 0.02	44.7 ± 3.56	1.1 ± 0.08
#65	2.4 ± 0.19	22.8 ± 0.63	16.7 ± 0.67	15.7 ± 0.48	0.9 ± 0.01	38.0 ± 2.17	1.2 ± 0.05
#57	2.3 ± 0.22	21.1 ± 0.74	16.5 ± 0.71	15.9 ± 0.74	1.1 ± 0.02	46.6 ± 2.60	1.2 ± 0.09
#36	2.3 ± 0.18	26.7 ± 0.95	15.6 ± 0.52	14.5 ± 0.85	0.9 ± 0.01	39.0 ± 3.32	1.2 ± 0.04
#54	2.3 ± 0.15	24.4 ± 0.84	16.0 ± 0.82	15.1 ± 0.57	0.8 ± 0.03	37.3 ± 8.23	1.1 ± 0.08
#64	2.1 ± 0.14	22.4 ± 0.70	16.9 ± 0.74	13.5 ± 0.53	1.1 ± 0.00	54.3 ± 2.14	0.8 ± 0.28
#56	1.9 ± 0.17	19.8 ± 0.42	16.6 ± 0.84	13.4 ± 0.70	0.9 ± 0.07	46.2 ± 9.45	1.0 ± 0.07
#38	1.8 ± 0.17	20.3 ± 0.67	15.9 ± 0.74	12.6 ± 0.52	0.8 ± 0.00	43.7 ± 2.64	1.1 ± 0.09
#42	1.7 ± 0.21	22.5 ± 0.53	15.1 ± 0.32	14.8 ± 0.42	0.8 ± 0.02	44.5 ± 3.71	0.8 ± 0.13
#45	1.7 ± 0.20	22.0 ± 0.47	15.6 ± 0.52	14.3 ± 0.67	0.6 ± 0.03	34.4 ± 6.83	1.1 ± 0.13
#52	1.7 ± 0.14	19.6 ± 0.70	15.2 ± 0.42	13.2 ± 0.42	0.8 ± 0.01	44.0 ± 2.59	1.1 ± 0.11
#62	1.6 ± 0.10	18.7 ± 0.67	15.4 ± 0.70	13.3 ± 0.67	0.9 ±0.01	54.6 ± 2.52	0.8 ± 0.13
#49	1.5 ± 0.12	21.7 ± 0.67	13.1 ± 0.57	12.1 ± 0.57	0.8 ± 0.01	55.3 ± 1.77	0.9 ± 0.14
#44	1.5 ± 0.11	20.1 ± 0.32	14.1 ± 0.74	12.3 ± 0.48	0.6 ± 0.00	41.7 ± 1.58	1.1 ± 0.05
#37	1.4 ± 0.14	20.2 ± 0.63	12.6 ± 0.52	11.4 ± 0.52	0.7 ± 0.01	47.9 ± 2.83	1.0 ± 0.03
$LS_{D0.0}5$	*0.19*	*0.72*	*0.64*	*0.62*	*0.12*	*3.85*	*0.10*
Lim	1.4-3.4	18.7-27.5	12.6-19.6	11.4-18.8	0.6-1.6	25.3-55.3	0.8-1.4
CV, %	23.6	12.7	11.3	13.4	29.9	17.7	13.9

Note: $\bar{x}$ – mean; SD – standard deviation; n – number of replications; LSD0.05 – least significant difference ($p \leq 0.05$); lim – range of variation (min–max); CV – coefficient of variation
Source: compiled by the authors

In the course of evaluating the hybrid population, the distribution of genotypes by nut weight was found to be fairly symmetrical and close to normal. The observed slight positive skewness (0.305) indicates a predominance of individuals with nut weights below the arithmetic mean of 2.2 g, while a few variants are characterised by significantly higher nut weights. This is further supported by the negative kurtosis value (-0.195), suggesting a platykurtic distribution where values are spread relatively evenly across the range (1.4-3.4 g) without a sharp concentration around the centre. These distributional patterns are analogous to the findings of N. Valentini *et al.* (2025), who reported skewness values of 0.355-0.512 and kurtosis of -0.086 to 0.018 in F_1 populations from crosses between two European cultivars. In the research, the average nut weight of the parental forms ranged from 2.3-2.4 g and 3.1-3.5 g, respectively, while the nut weight range in the F_1 population fluctuated between 1.3 g and 3.9 g across different years.

The phenotypic superiority of specific genotypes within the studied material is noteworthy. Significantly, the heaviest nuts were produced by the ‘Profesorskyi’ genotype (3.4 ± 0.29 g), which surpasses its maternal cultivar, ‘Akademik Yablokov’. ‘Aspirantskyi’ genotype also significantly outperforms others in kernel weight (2.9 ± 0.29 g). Both ‘Profesorskyi’ and ‘Aspirantskyi’) produce “very large” nuts, placing these genotypes among the top tier of the population, where 43.5% of genotypes are categorised as “large” or “very large”. When comparing these results to international commercial standards, the hybrid population demonstrates high competitive potential. In a review by K. Król & M. Gantner (2020), commercial hazelnut cultivars typically exhibit nut weights between 1.4 g and 3.8 g, kernel weights between 0.5 g and 2.0 g, and kernel content from 33.2 to 49.5%. Genotypes with nut weights between 1.2 g and 5.4 g, kernel weights between 0.5 g and 2.0 g, and kernel content from 39 to 57% were involved in the American breeding programmes (Yao & Mehlenbacher, 2000). As noted by Q. Yao & S. Mehlenbacher (2001), nut weight is a function of shell thickness and kernel weight. Most crosses in the investigations exhibited transgressive segregation toward lighter nuts.

The analysis of shell thickness revealed it to be one of the most stable traits in the hybrid population, with a coefficient of variation of 13.9% and an average value of 1.1 mm. The distribution of this trait is characterised by a moderate negative skewness (-0.64) and a positive kurtosis (0.39), indicating a slight predominance of genotypes with a thicker shell. The biological significance of this parameter is directly confirmed by its correlation with kernel yield. In particular, the genotypes that demonstrated the highest kernel content (exceeding the 54.0% threshold), namely #49, #62, and #64, were found to possess the thinnest shells (0.8 mm) in the entire population. This aligns with the breeding objectives for commercial hazelnut cultivars, where a thin shell is a primary indicator of high technological quality. In comparison, the 'Profesorskyi' genotype, while having the largest nut weight, possesses a shell of medium thickness, which results in an average kernel content of 47.4%. These observations highlight the importance of shell thickness as a limiting factor for kernel productivity in the studied hybrid material. It should be noted that in the current study, the genotype 'Aspirantskyi' exhibited the thickest shell (1.4 mm), which harmed its kernel percentage. According to previous observations over two seasons, nuts of 'Aspirantskyi' had an average shell thickness of 1.1 mm and a mean kernel percentage of 51.0% (Mezhenskyj *et al.*, 2025). In contrast, for top-performing genotypes such as 'Profesorskyi' and 'Akademichnyi', shell thickness and kernel percentage remained stable without significant fluctuations.

Finally, the observed higher variability in weight indicators (CV = 23.6-29.9%) compared to linear parameters of nuts (CV = 11.3-13.4%) aligns with the biological patterns established by D. Falconer (1995), confirming that weight-based traits offer the greatest scope for further clonal selection. The distribution of width was found to be the most symmetrical and closest to the theoretical normal curve (skewness = -0.17; kurtosis = 0.00), while height and thickness showed slight deviations. Specifically, the height of the nuts exhibited a moderate positive skewness (0.61), indicating a predominance of genotypes with below-average height (22.8 mm) alongside individual tall specimens. The correlation analysis revealed significant relationships between the morphological and weight-based traits of the studied hybrids (Table 2).

Table 2. Pearson correlation coefficients between traits (*r*)

Trait	Nut weight	Nut length	Nut width	Nut thickness	Kernel weight	Kernel percentage	Shell thickness
Nut weight	1						
Nut length	**0.611**	1					
Nut width	**0.858**	**0.498**	1				
Nut thickness	**0.890**	**0.644**	0.891	1			
Kernel weight	**0.743**	0.334	**0.599**	**0.560**	1		
Kernel percentage	-0.250	-0.333	-0.290	-0.386	**0.450**	1	
Shell thickness	**0.481**	0.178	0.319	0.385	-0.011	**-0.670**	1

Note: significant coefficients ($p \leq 0.05$) are shown in bold ($n = 23$)
Source: compiled by the authors

A very strong positive correlation was observed between nut weight and its transverse dimensions: thickness (r = 0.890) and width (r = 0.858). Nut weight also showed a strong correlation with nut length (r = 0.611), although to a lesser extent than with width and thickness. The nearly perfect correlation between nut width and nut thickness (r = 0.891) suggests that these two dimensions develop proportionally in the studied population. Kernel weight is strongly and positively correlated with total nut weight (r = 0.743), confirming that selection for heavier nuts will likely lead to heavier kernels. Among linear parameters, nut width (r = 0.599) and thickness (r = 0.560) have a greater influence on kernel weight than nut length (r = 0.334). Interestingly, there is virtually no correlation between kernel weight and shell thickness (r = -0.011), indicating that these two traits vary independently. The most critical findings for breeding concern the kernel percentage. A strong negative correlation was found between kernel percentage and shell thickness (r = -0.670), identifying shell thickness as the primary limiting factor for productivity. Furthermore, kernel percentage showed negative correlations with all nut dimensions, most notably with nut thickness (r = -0.386) and nut length (r = -0.333), as well as with total nut weight (r = -0.250). This suggests a potential "trade-off" in selection: while selecting for larger or heavier nuts, there is a risk of decreasing the relative kernel yield if the shell mass increases disproportionately. These results confirm that for the evaluated *Corylus avellana* purple-leaved hybrids, the most effective breeding strategy for high productivity should prioritise genotypes that combine a large nut volume (width and thickness) with a thin shell. The positive correlation between kernel weight and kernel percentage (r = 0.450) further supports the possibility of selecting for both high absolute weight and high relative yield. As noted by Q. Yao & S. Mehlenbacher (2001), nut dimensions represent a critical parameter for both the in-shell and kernel sectors of the industry. This is due to the inherent relationship where nut volume serves as a reliable proxy for kernel size, provided that significant kernel shrinkage is absent. Correlation analysis further supports this.

The parental cultivars 'Akademik Yablokov' and 'Moskovskij Rubin' produce elongated nuts, with shape indices of 1.6 and 1.3, respectively, according to measurements from 2003. Significant variation in nut shape was observed among the progeny. Specifically, the shape index ranges from 1.1 to 1.7, with 39.1% of the genotypes producing elongated fruits with a shape index of 1.5 or higher. This range is wider than that reported by A. Ferrão *et al*. (2021), who studied industrial cultivars and reported shape index fluctuations ranging from 0.9 to 1.5. Genotype #66 is characterised by oblate nuts with globular kernels. Four other genotypes: #61 (with ovate nuts), as well as #62, #57, and #38 (all with obovate nuts), also possess globular kernels (Figs. 1-2, Table 3).

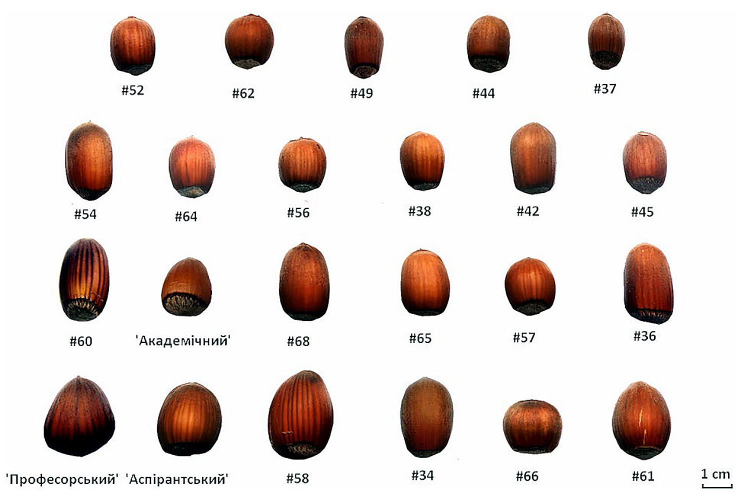

Figure 1. Nuts of genotypes tested

Source: compiled by the authors

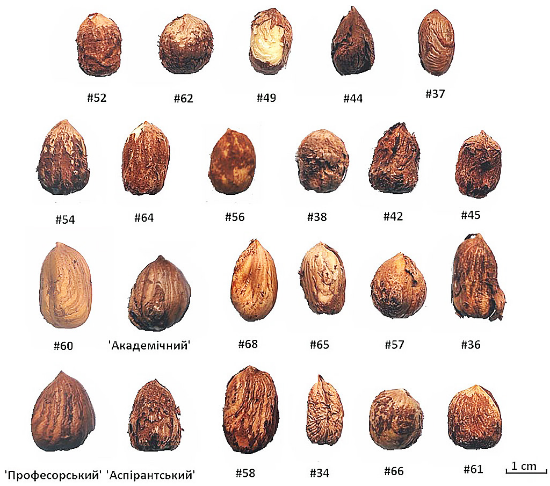

Figure 2. Kernels of genotypes tested

Source: compiled by the authors

Table 3. The characteristics of the nuts and kernels

Genotype	Shape index	Nut shape	Kernel shape	Kernel: Presence of fibre
#66	1.1	oblate	globular	weak
'Akademichnyi'	1.2	conical	ovoid	medium
#56	1.2	obovoid	ovoid	medium
#62	1.2	obovoid	globular	strong
'Profesorskyi'	1.3	ovoid	ovoid	absent
'Aspirantskyi'	1.3	ovoid	ovoid	medium
#61	1.3	ovoid	globular	weak
#57	1.3	obovoid	globular	medium
#64	1.3	obovoid	obovoid	medium
#52	1.3	obovoid	obovoid	very strong
#38	1.3	obovoid	globular	very strong
#65	1.4	ovoid	obovoid	strong
#45	1.4	obovoid	obovoid	very strong
#44	1.4	obovoid	ovoid	very strong
#58	1.5	ovoid	subcylindrical	strong
#34	1.5	subcylindrical	obovoid-subcylindrical	weak
#68	1.5	subcylindrical	obovoid-subcylindrical	weak
#54	1.5	subcylindrical	ovoid	strong
#42	1.5	obovoid-subcylindrical	ovoid	very strong
#60	1.6	subcylindrical	obovoid-subcylindrical	absent
#37	1.6	obovoid-subcylindrical	subcylindrical	medium
#36	1.7	ovoid-subcylindrical	subcylindrical	strong
#49	1.7	obovoid-subcylindrical	obovoid	very strong

Source: compiled by the authors

The observed inheritance patterns in the study are consistent with the findings of Q. Yao & S. Mehlenbacher (2001), who noted that when parents with long nuts are crossed, long nuts predominate in the offspring, and oblate nuts are seldom observed. In this context, the appearance of genotype #66 in the progeny of the 'Akademik Yablokov' × 'Moskovskij Rubin' cross represents a rare exception to this trend. This occurrence highlights the presence of transgressive segregation for fruit shape within this specific hybrid combination, suggesting that even with elongated parental forms, the genetic potential for diverse morphotypes remains accessible. According to Q. Yao & S. Mehlenbacher (2000), the world kernel market needs globular kernels. The amount of fibre on the kernel is a critical trait for both the unblanched kernel and in-shell markets. As noted by Q. Yao & S. Mehlenbacher (2001), transgressive segregation for this trait was observed in most crosses, with a high overall frequency of very fibrous kernels. Many progenies exhibited mean values exceeding those of the higher parent, while only two progenies showed means lower than the lower parent.

In the studied hybrid population, genotypes with "strong" and "very strong" presence of fibre account for 52.2% of all seedlings. In contrast, only two genotypes, 'Profesorskyi' and #60, produce kernels that are virtually free of fibre. While fibre can be removed during industrial processing through high-temperature blanching, kernels intended for the fresh market (direct consumption) must be naturally free of fibre to ensure high consumer attractiveness. It should be noted that breeding requirements vary significantly depending on whether a cultivar is intended primarily for direct consumption (the in-shell market) or for dual-purpose use (both processing and the fresh market) (Valentini *et al.*, 2006). Within the hybrid population, the segregation of traits allows for selection in both directions. Genotypes with large nuts (e.g., 'Profesorskyi') align with the requirements for direct consumption, while those with globular kernels are more suited for the processing sector. This diversity highlights the potential of the 'Akademik Yablokov' × 'Moskovskij Rubin' cross to produce cultivars tailored to specific industrial and retail needs. Furthermore, all purple-leaved hazelnuts in the studied population are distinguished by the exceptional ornamental value. The intense anthocyanin pigmentation of both leaves and husks makes these genotypes highly attractive for landscape architecture and ornamental gardening. Combining high-quality nut production with aesthetic appeal significantly enhances the economic attractiveness of the selected hybrids, as these hybrids can be utilised both in commercial orchards and as decorative elements in urban environments or private gardens.

CONCLUSIONS

The studied hybrid population of purple-leaved hazelnut exhibits substantial phenotypic variability in nut morphological traits, providing a robust and scientifically sound basis for targeted breeding. Average nut weight ranged from 1.4 to 3.4 g, while kernel percentage varied widely from 25.3 to 55.3%, indicating a broad segregation of economically valuable traits within the population. Among the evaluated genotypes, 'Profesorskyi' was identified as the most promising overall, while 'Aspirantskyi' was distinguished by the production of "very large" nuts, exceeding the corresponding parameters of the maternal cultivar 'Akademik Yablokov' and confirming the high genetic potential of the applied hybrid combination. The results demonstrate that selection for high productivity should prioritise genotypes combining large nut volume with reduced shell thickness, as shell thickness was identified as the primary factor limiting kernel yield. This conclusion is supported by the strong negative correlation between kernel percentage and shell thickness (r = -0.670). Transgressive segregation for nut shape was observed within the hybrid population, most notably in genotype #66, which produced oblate nuts despite the elongated nut shape of both parental cultivars. This finding indicates a complex inheritance of nut morphology in purple-leaved hazelnut hybrids and highlights the breeding value of segregating populations for generating novel phenotypes. The identification of genotypes characterised by large nuts, high kernel quality, and low fibre content, particularly 'Profesorskyi' and genotype #60, demonstrates the suitability for direct consumption and industrial processing. At the same time, all evaluated genotypes possess pronounced ornamental value due to stable anthocyanin pigmentation of leaves and husks, which significantly enhances the attractiveness for landscape use. Overall, the best-performing genotypes identified in this study represent a valuable genetic resource for hazelnut breeding. The future multi-year evaluation, official registration, and regionalisation will provide a reliable foundation for the development of sustainable nut production systems and multifunctional ornamental plantings in Ukraine and comparable temperate regions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Морфометричний аналіз горіхів гібридної популяції пурпуроволисткового фундука (*Corylus avellana* L.)

Анотація. Фундук (*Corylus avellana* L.) є стратегічно важливою горіховою культурою з високою харчовою цінністю та значним економічним потенціалом. Хоча промислове вирощування переважно базується на зеленолисткових генотипах, інтерес до пурпуроволисткових форм неухильно зростає завдяки їхній декоративній привабливості та підвищеному антиоксидантному потенціалу, пов'язаному з антоціановою пігментацією. Однак інформація про морфометричні ознаки горіхів та їхню мінливість у гібридних популяціях пурпуроволисткового фундука залишається обмеженою. Метою цього дослідження було проаналізувати морфометричні характеристики горіхів у гібридній популяції пурпуроволисткового фундука, створеної в Національному університеті біоресурсів і природокористування України. Експериментальний матеріал включав горіхи з 23 дорослих кореневласних гібридних кущів, отриманих у результаті схрещування сортів 'Академик Яблоков' та 'Московский Рубин'. Масу горіха та ядра, лінійні розміри, товщину шкаралупи, відсоток ядер та індекс форми горіха оцінювали за допомогою стандартних морфометричних і статистичних методів. Результати виявили значну фенотипову мінливість морфологічних ознак горіхів, із середньою масою горіха від 1,4 г до 3,4 г. За сукупністю господарських ознак генотип ‘Професорський’ було визначено найперспективнішим, який дав найважчі горіхи (3,4 г) та ядра (1,6 г). Кореляційний аналіз виявив сильний негативний зв’язок між відсотком ядра та товщиною шкаралупи (r = -0,670), ідентифікуючи останню як основний лімітуючий фактор для врожайності ядра. Досліджувана гібридна популяція являє собою цінний генетичний ресурс для селекції фундука, що дозволяє добирати генотипи, придатні як для комерційного виробництва горіхів, так і для декоративного використання. Поєднання високої якості горіхів з вираженими декоративними ознаками, пов’язаними зі стабільною антоціановою пігментацією, підкреслює потенціал пурпуроволисткового фундука для диверсифікованого сільськогосподарського виробництва та ландшафтного застосування

Ключові слова: антоціанова пігментація; фенотипова мінливість; маса горіха; вихід ядра; товщина шкаралупи; селекція

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Patterns of formation of morphometric traits and productivity of daikon (*Raphanus sativus* L. convar. *acanthiformis* Sazon.) under different sowing dates

Abstract. The adaptation of agrotechnologies to climate challenges and the sustainable production of functional food products determined the relevance of searching for optimal cultivation parameters for underutilised vegetable crops. The aim of the study was a comprehensive analysis of the formation of biometric and marketable characteristics of daikon radish as influenced by sowing dates to adapt production technology to the conditions of the Right-Bank Forest-Steppe of Ukraine. Field experiments (2022-2024) were conducted to study the cultivars 'Gulliver' and 'Minowase' across four sowing dates from early July to early August. For the 'Minowase' cultivar, sowing in the third decade of July resulted in the highest leaf count (20.8), rosette height (45.4 cm), and photosynthetic

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surface area (4,886 cm²), exceeding the indicators of other dates by 7.6-13.8%. The 'Gulliver' cultivar demonstrated a similar trend with maximum morphological parameters. Sowing in the first decade of July led to the lowest leaf weight in the studied cultivars, with a decrease of 7.4% and 29.9% compared to the optimal date. Root crop development correlated with the leaf rosette ($r = 0.31 - 0.90$): under the optimal sowing date, 'Minowase' formed root crops with a length of 35.4 cm and a diameter of 7.2 cm, ensuring the highest average weight of 426 g. In the 'Gulliver' cultivar, the average root crop weight across the studied sowing dates was 0.9-4.4% lower than that of 'Minowase'. Variability in root crop weight was determined by the sowing date at 44.9% ($p < .001$), while the influence of annual weather conditions (23.5%) and genotype (5.1%) was significantly lower. A moderate correlation was found between sowing dates and root crop weight ($r = 0.79 - 0.89$), and regression analysis confirmed a weight change of 8.82 g ('Gulliver') and 11.92 g ('Minowase') for every 10-day deviation. Sowing daikon in the third decade of July was optimal for yield stabilisation as it ensured a favourable hydrothermal regime; meanwhile, early sowing dates cause a significant reduction in the linear dimensions of root crops. The improved cultivation technology for daikon radish is recommended for industrial and small-scale producers to expand the assortment of vegetable crops and provide the market with fresh, valuable vegetable produce

Keywords: morphometric parameters; leaf area index; correlation analysis; climate change adaptation; varietal characteristics

INTRODUCTION

Global food security under the pressure of climatic and geopolitical factors requires the implementation of technologies for stabilising agricultural production. In this context, it is relevant to expand the range of vegetable crops due to daikon, which is characterised by high adaptive potential and biological value. Establishing the patterns of daikon morphogenesis at different sowing dates is the basis for the development of adaptive technologies, since optimising the growing dates allows coordinating the needs of organogenesis with hydrothermal resources, preventing premature bolting and yield quality deterioration. According to the forecasts given by A. Farah *et al.* (2025), the intensification of climate shocks could lead to a decrease in global food production by 14% by 2050. Researchers emphasise that traditional planning of the sowing campaign is losing its effectiveness, since anomalous warming destroys the usual biological rhythms of crops, requiring flexible decisions in choosing the timing of cultivation. For Ukraine, these challenges are especially acute, because, according to V. Vyshnevskyi (2025), the growth rate of the average annual temperature within the country significantly exceeds the world average. The researcher showed that the transformation of the thermal regime, which is manifested in an increase in the number of extremely hot days (over 30°C) by 3-5 times with a relatively stable level of precipitation, has led to increased aridisation of territories and the actual displacement of the boundaries of natural zones by hundreds of kilometres. In conditions of such instability, the agricultural economy, as noted by I. Fedosiy *et al.* (2026), requires a significant increase in the stability of yields of niche crops, in particular daikon, in conditions of resource scarcity. One of the most effective tools for adapting to environmental changes remains the optimisation of elements of cultivation technology. Q. Xu *et al.* (2024) argue that the choice of sowing dates is the most important among these elements. A scientifically sound sowing date allows synchronising the biological needs of the crop with the ecological resources of the territory. Also, S. Sajid & G. Hu (2022), in the study, proved that ignoring a systematic approach to planning sowing dates leads to crop losses of 30-50% and logistical failures.

The temperature regime is especially critical for daikon. Compliance with optimal sowing dates ensures the accumulation of the necessary amount of active temperatures, which is critical for the realisation of genetic potential.

J. Li *et al.* (2025) found that the temperature effect on the soil (over 30.7°C) in the early phases of development inhibits the division of xylem cells, which leads to the formation of a hollow core. Also, the deterioration of quality is often manifested in the form of internal browning, which is difficult to identify visually until the moment of consumption. Along with the temperature factor, the light regime is of critical importance. In particular, P. Chutimanukul *et al.* (2024) indicate that the regulation of the spectral composition and intensity of lighting, which directly correlate with the timing of sowing, allows for the intensification of photosynthesis and stimulation of the synthesis of secondary metabolites. This approach modulates the accumulation of ascorbic acid, soluble solids and anthocyanins, which ultimately determines the antioxidant potential of the product. At the same time, due to accelerated ontogenesis, radish reacts sharply to any moisture imbalance. In particular, M. Sultana *et al.* (2025) note that excess precipitation leads to cracking of root crops, while moisture deficiency at any stage critically inhibits growth, which determines the importance of optimising sowing dates.

A crucial additional argument in favour of optimising the calendar sowing dates is the possibility of biological control of the number of phytophagous organisms, as pointed out by I. Fedosiy *et al.* (2025). In conformity with the conclusions of S. Rai *et al.* (2023), this agrotechnical measure allows disrupting the synchrony between vulnerable phases of plant development and peaks of pest activity, minimising the entomological load on the agrocenosis without excessive pesticide pressure on the environment. The generalisation of the results of scientific research shows that most of the works on the morphogenesis of radish and daikon are devoted either to isolated physiological reactions to temperature, or to the influence of individual elements of agricultural technology without taking into account the complex action. The issue of stabilising daikon productivity in conditions of dynamic climate change remains insufficiently studied, which causes the low efficiency of existing recommendations on sowing dates. This necessitates an urgent need for a detailed study of adaptive reactions of plants to a combination of abiotic stressors in specific soil and climatic conditions.

In this regard, the purpose of the study was to establish the patterns of the formation of the assimilation apparatus and morphometric parameters of daikon root crops depending on the hydrothermal conditions of vegetation to determine the optimal calendar period of cultivation in the conditions of the Right-Bank Forest-Steppe of Ukraine.

MATERIALS AND METHODS

The experimental study was conducted during 2022-2024 at the National University of Life and Environmental Sciences of Ukraine (NULES of Ukraine). The field experiment was conducted on the experimental plots of the NL "Fruit and Vegetable Garden" (Ukraine). The coordinates of the experimental site: 50° 22' 42.21" N, 30° 30' 05.17" E, altitude 158 m above sea level. The soils of the experimental plots were sod-podzolic on loess. The granulometric composition of the soil was light loam. The quality indicators were as follows: pH of the salt extract 6.74, organic matter (humus) 2.71%, nitrate nitrogen 6.42 mg/kg, ammonium nitrogen 11.72 mg/kg, mobile phosphorus 568.83 mg/kg, mobile potassium 152.93 mg/kg.

During 2022-2024, the average monthly air temperature in January ranged from -0.3 to -2.7°C (Fig. 1). On average, these temperatures were 1.4°C higher than the multi-year data. February turned out to be quite warm, from -0.2 to 3.0°C, exceeding the long-term data by 3.8°C. For March, the temperature regime was characterised by a distribution from 2.6 to 4.7°C, which is 1.5°C higher than the long-term data. In April, the air temperature was close to the multi-year data and exceeded its value by only 0.1°C. May was marked by a significant increase in air temperature to 14.6-16.2°C, but the average value was 0.2°C lower than the multi-year data. In June, the air temperature (19.7-21.6°C) was slightly lower than the multi-year data by 0.2°C. In July, the air temperature was almost the same as the multi-year data, with a deviation of only 0.9°C. The hottest month was August (22.3-23.7°C) and the air temperature increased relative to the multi-year data by 2.6°C. September had a rather unstable temperature regime over the years of research in the range from 12.7 to 20.5°C, but its average monthly temperature exceeded the multi-year data by 2.4°C. The average monthly temperature in October was 10.6-11.4°C, exceeding the long-term data by 2.4°C. November was marked by a decrease in air temperature to 2.7-4.1°C, which is at the level of long-term data (the deviation was 0.7°C). In December, the air temperature ranged from 0.0 to -0.7°C and was 1.8°C higher than the long-term data.

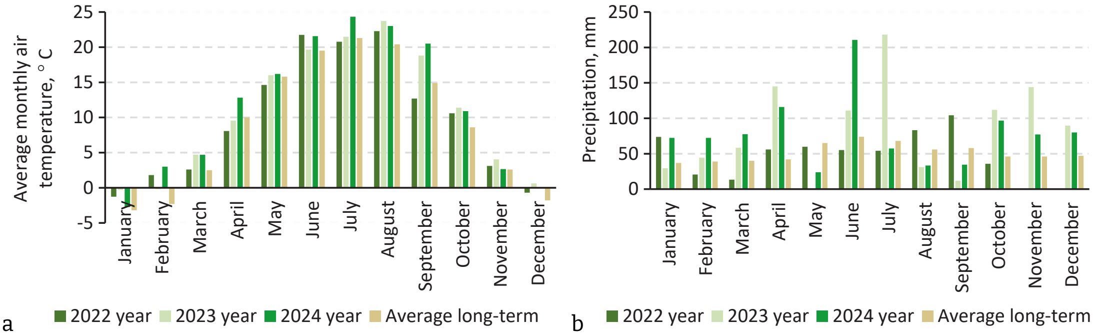

Figure 1. Monitoring of meteorological indicators: Comparative analysis of temperature (a) and precipitation (b) in 2022-2024

Source: compiled by the authors based on the conducted study

Precipitation in the winter period (December-February) was quite stable over the years, ranging from 45.8 to 58.5 mm monthly, with an average deviation from multi-year data of 12.6 mm. In contrast, the spring period (March-May) was characterised by an uneven distribution of precipitation with an average monthly indicator from 28.2 to 105.7 mm, both dry decades and periods with intense precipitation were observed. At the same time, multi-year indicators were lower in March (by 9.7 mm) and April (by 63.7 mm), and higher in May (by 36.8 mm). The autumn period (September-October) was also marked by an uneven distribution of precipitation by month, from 50.2 to 81.5 mm. Compared with long-term indicators, in October and November there was more precipitation (by 35.5 mm and 27.7 mm), and in September – less (by 7.8 mm).

Field research was conducted at the Department of Vegetable Growing and Indoor Soils of the NULES of Ukraine during 2022-2024. The scientific work programme encompassed the study of two factors influencing daikon plants: varietal characteristics (factor A) and sowing dates (factor B). The objects of the study were the varieties 'Gulliver' (control, Ukraine) and 'Minowase' (Netherlands). Sowing was carried out in four stages: in the first, second (control) and third decades of July, as well as in the first decade of August. The experiment was set up using the randomised plot method in three replications. The size of the plot for one variant of the experiment in each replication was 5 m². Sowing was carried out manually to a depth of 4-5 cm according to the 45×10 cm scheme. Taking into account the biological characteristics of the studied varieties, harvesting was carried out in a differentiated manner: after 50 days for 'Gulliver' and 60 days for 'Minowase'. Harvesting was carried out at the BBCH 49 stage, which allowed obtaining maximum marketable indicators and preventing physiological overripeness of root crops.

For morphological descriptions, a randomised selection of 10 plants from each plot was used, which had average biometric indicators for the variant. The number of leaves and the total length of the largest leaf (including the petiole) were determined. The area of the assimilation surface was determined by contour scanning using Petiole Pro Basic software, followed by calculation of the area of leaves per 1 ha. In parallel, the morphometric parameters of root crops were recorded: the length and maximum diameter. The crop structure was assessed by separately accounting for marketable and non-marketable fractions. The average weight of marketable root crops was determined with an accuracy of 1 g. The non-marketable part of the product was subjected to a comprehensive analysis, classifying it by the reasons for rejection: disease damage, damage by pests, the presence of cracks, the tendency to bolt (premature flowering), deformation (ugliness) and undergrowth (diameter <3 cm).

Statistical processing of the results was carried out using the programmes Statistica 13.1 (StatSoft, Inc., Tulsa, OK, USA) and JASP (Version 0.95.4, JASP Team, 2025). The data distribution was checked for normality using the Shapiro-Wilk test and visual analysis of the probability plots of the residuals. Since the residuals corresponded to the law of normal distribution, a three-factor analysis of variance (Factorial ANOVA) was used to assess the influence of sowing date, variety, year of research and the interactions on morphometric indicators and average root weight. Tukey post-hoc test (HSD) was used to compare the average values and identify significant differences between the options. The strength of the influence of the studied factors was determined by the "eta-square" indicator (η^2). The relationships between the biometric parameters of the rosette and the weight of the root crop were established by calculating the Pearson correlation coefficients. Statistical significance was assessed at $p < 0.05$. Graphical interpretation of data (Box-plots and graphs) was performed in Statistica 13.1 and Excel 2024 (Microsoft Corp., Redmond, WA, USA). The study was conducted in accordance with the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1976).

RESULTS AND DISCUSSION

The results of the study of morphological characteristics of the leaf rosette showed that the variety 'Minowase' was distinguished by the maximum indicators, namely: leaf length (36.9-45.4 cm), number of leaves per plant (18.5-20.8 pcs.), leaf weight (249-267 g) and leaf plate area (3,904-4,886 cm^2). These values were greater than the control variety 'Gulliver' by 1.4-3.7 cm (3.9-9.4%), 2.4-3.4 pcs. (13.2-21.0%), 25-63 g (10-34%) and 560-1,133 cm^2 (13-41%) (Table 1).

Table 1. Morphometric indicators of daikon varieties depending on sowing dates (2022-2024)

Factor A, variety	Factor B, sowing date	Number of leaves, pcs.	Leaf rosette height, cm	Leaf weight, g	Leaf plate area, cm²	Root crop length, cm	Root crop diameter, cm	Average weight of root crop, g
'Gulliver' (c*)	First decade of July	15.4 ± 0.83 a	35.5 ± 1.43 a	186 ± 3.09 c	2,771 ± 86.0 d	25.7 ± 0.81 a	4.8 ± 0.15 a	371 ± 5.78 a
	Second decade of July (c*)	16.0 ± 0.98 ab	37.4 ± 1.46 abc	224 ± 9.69 a	3,387 ± 152.7 cd	27.2 ± 0.79 ab	5.1 ± 0.08 ab	382 ± 5.88 a
	Third decade of July	18.4 ± 1.05 abcd	42.3 ± 1.35 bcd	242 ± 13.83 ab	4,326 ± 250.4 ab	30.5 ± 1.01 bc	5.6 ± 0.13 bcd	414 ± 7.56 bc
	First decade of August	16.5 ± 0.84 abc	39.5 ± 1.26 abcd	232 ± 13.88 ab	3,526 ± 214.8 c	28.4 ± 0.84 ab	5.4 ± 0.15 abc	396 ± 8.70 ab
'Minowase'	First decade of July	18.5 ± 0.33 abcd	36.9 ± 1.39 ab	249 ± 5.42 ab	3,904 ± 83.7 a	28.0 ± 0.65 ab	5.9 ± 0.17 cd	374 ± 5.84 a
	Second decade of July (c*)	19.4 ± 0.30 bcd	40.1 ± 1.54 abcd	253 ± 5.59 ab	4,295 ± 89.3 ab	28.7 ± 0.68 ab	6.2 ± 0.17 de	399 ± 5.34 abc
	Third decade of July	20.8 ± 0.66 d	45.4 ± 1.46 d	267 ± 3.29 ab	4,886 ± 63.3 b	35.4 ± 0.62 d	7.2 ± 0.19 f	426 ± 6.09 c
	First decade of August	19.7 ± 1.03 cd	43.2 ± 1.00 cd	260 ± 3.60 b	4,492 ± 73.8 ab	33.2 ± 0.71 cd	6.8 ± 0.17 ef	412 ± 5.66 bc

Note: (c*) – control; the values given are the averages of 3 repetitions in the territory and 3 repetitions in time (2022, 2023, 2024); the error of the mean is displayed as Std.Err (±); the results of multiple comparisons of means were performed using the Tukey criterion (HSD); differences that are insignificant at p ≤ .05 are indicated by identical lowercase letters

Source: compiled by the authors based on research conducted

Significant differences were observed in the growth parameters of the leaf rosette of the varieties depending on the sowing date. Earlier sowing (the first decade of July) gave the lowest indicators of morphological characteristics in the studied varieties, namely: leaf length (35.5 cm; 36.9 cm), number of leaves per plant (15.4 pcs.; 18.5 pcs.), leaf weight (186 g; 249 g) and leaf plate area (2,771 cm^2; 3,904 cm^2) for 'Gulliver' and 'Minowase', respectively. The dynamics of changes in the height of the leaf rosette indicates that sowing in the second decade of July (control) provided significantly higher indicators (37.4 cm; 40.1 cm), which were 5.1-7.8% (or 1.9-3.1 cm) higher than the values of the previous period. The maximum height of the rosette was recorded when sowing in the third decade of July (42.3 cm; 45.4 cm), which was 4.9-5.3 cm (13.1-13.2%) higher compared to the control. The latest sowing date gave slightly lower figures (39 cm and 43.2 cm), but these figures were still 2.0-3.1 cm (5.4-7.7%) higher than the control values. In addition, an increase in the number of leaves per plant was observed compared to the control (second decade of July): by 0.3-0.5 pcs. (1.5-3.1%) for the first decade of August and by 1.5-2.4 pcs. (7.6-15.0%) for the third decade of July.

The combination of various environmental factors, primarily temperature, contributed to plant growth. Among the sowing dates, the third decade of July provided a significantly higher leaf weight (242 g; 267 g) (respectively, 'Gulliver' and 'Minowase'), which exceeded the control (second decade of July) by 14-17 g (5.7-7.7%). This indicator was followed by the first decade of August (332 g; 260 g), where the excess of the control was 7-8 g (2.8-3.4%). The increase in leaf area relative to the earliest sowing date was recorded in the second decade of July (control), where the indicators were 3,387 cm^2 and 4,295 cm^2, as well as in the third decade of July (4,326 cm^2; 4,886 cm^2) (respectively, 'Gulliver' and 'Minowase'). At the same time, in the first decade of August, a gradual decrease in this indicator was observed, which reached 3,526 cm^2 and 4,492 cm^2.

Both environmental and genetic factors are associated with the development of root crops. An increase in vegetative growth indicators (plant height, number, mass, and area of leaves) contributed to an increase in the size of root crops. The variety 'Minowase' demonstrated a higher length (29-35 cm) and diameter (5.9-7.2 cm) of the root crop compared to the variety 'Gulliver' (control) at all sowing dates, exceeding it by 2-5 cm (5.5-16.9%) and 1.1-1.6 cm (21.6-28.6%), respectively. Among the sowing dates, the maximum length (30.5; 35.4 cm) and diameter (5.6; 7.2 cm) of the root crop (respectively, 'Gulliver' and 'Minowase') were recorded during sowing in the third decade of July. This was followed by the first decade of August (length: 28.4; 33.2 cm; diameter: 5.4; 6.8 cm) and the second decade of July (control) (length: 27.2; 28.7 cm; diameter: 5.1; 6.8 cm). While during sowing in the earliest term (the first decade of July), the smallest values of the length and diameter of the root crop were noted. The average weight of daikon roots was significantly influenced by the sowing dates, varieties, and the interaction. Regardless of the sowing dates, the 'Minowase' variety had a higher average root weight (403 g), while the 'Gulliver' (control) variety had a lower average weight by 12 g or 3.1%. When the 'Minowase' variety was sown in the first decade of July, the minimum deviation in the weight of the root crops was recorded in the experimental plots (3 g or 0.8%) compared to the control ('Gulliver' variety), but the weight of the root crops (399-426 g) differed significantly in the sowing options from the second decade of July to the first decade of August – by 12-17 g or 2.9-4.5%.

Among the sowing dates of the 'Gulliver' (control) variety, the sowing option in the third decade of July was noted, which had a significantly higher weight of the root crop (414 g) compared to the control (second decade of July). Next came the variant of the first decade of August – 396 g, while the first decade of July had the lowest root weight (371 g). The deviation of the indicator relative to the control for these variants was as follows: 32 g (8.4%), 14 g (3.7%) and – 11 g (-2.9%), respectively. Also, among the variants of sowing dates, the variety 'Minowase' in the third decade of July showed the maximum indicator of root weight (426 g), which was significantly greater (by 27 g or 6.8%) than in the control variant (second decade of July). An increase in root weight (up to 412 g) relative to the control (second decade of July) (by 13 g or 3.3%) was also observed when sowing in the first decade of August. The lowest root weight (374 g) was recorded in the first decade of July, which is 25 g or 6.3% less than the control (second decade of July). Statistical analysis confirmed that the average weight of the root crop is significantly influenced by all three main factors: sowing date, year of study and variety (Table 2). The most important among these factors was sowing date, which determines 44.9% of all changes in root crop weight ($p < .001$). The influence of weather conditions of the year is 23.5% ($p < .001$), while the characteristics of the variety have a much smaller weight – only 5.1% ($p < .001$).

Table 2. Indicators of significance and influence of factors on the formation of root crop mass (2022-2024)

Cases	Sum of Squares	df	Mean Square	F	p	η^2	95% CI for η^2	
							Lower	Upper
Sowing period	21,894.6	3	7,298.19	38.984	<.001	0.449	0.220	0.596
Veriate	2,476.9	1	2,476.91	13.231	<.001	0.051	0.000	0.211
Year	11,461.3	2	5,730.63	30.610	<.001	0.235	0.045	0.414
Sowing period ∗ Veriate	474.2	3	158.06	0.844	.476	0.010	0.000	0.048
Sowing period ∗ Year	2,318.2	6	386.37	2.064	.075	0.048	0.000	0.086
Veriate ∗ Year	735.4	2	367.70	1.964	.151	0.015	0.000	0.111
Residuals	8,986.2	48	187.21					
Sowing period ∗ Veriate ∗ Year	429.9	6	71.65	0.383	.886	0.009	0.000	0.000

Note: type III sum of squares
Source: compiled by the authors based on the conducted study

A critical feature is that no interaction between the factors produced a statistically confirmed result. The interaction of sowing date with the year of the study explains 4.8% of the variations ($p = .075$), and the effect of variety in combination with year is 1.5% ($p = .151$). The combination of variety with sowing date has an effect of 1% ($p = .476$), and the simultaneous interaction of all three factors is only 0.9% ($p = .886$). Since in all these cases the significance indicator exceeds the .05 threshold, this means that the effect of sowing date and variety is stable and does not significantly depend on the year of the study or the chosen combination of conditions.

The high reliability of these data is confirmed by the calculations of confidence intervals. In particular, for the factors of sowing date and year of the study, the limits of probable error are far from zero (the lower limit is 22% for sowing and 4.5% for year). This indicates that the influence of these factors is guaranteed. For the variety, the lower limit of the interval is 0.0%, which indicates a less stable nature of this factor. The variety 'Minowase' in the variants of the first-third decades of July demonstrates a distribution of the number of leaves that mimics a close to normal one, since its mean and median are very close, and there are no outliers (Fig. 2). In contrast, the distribution of the variety 'Gulliver' (control) in the variants of the third decade of July and the first decade of August has a similar characteristic. In the sowing terms of the first-second decades of July for the variety 'Gulliver' (control) and the first decade of August for the variety 'Minowase', the measures of central tendencies are in different places, so the distribution is not normal, and a right-sided asymmetry is observed (the mean value is noticeably higher than the median).

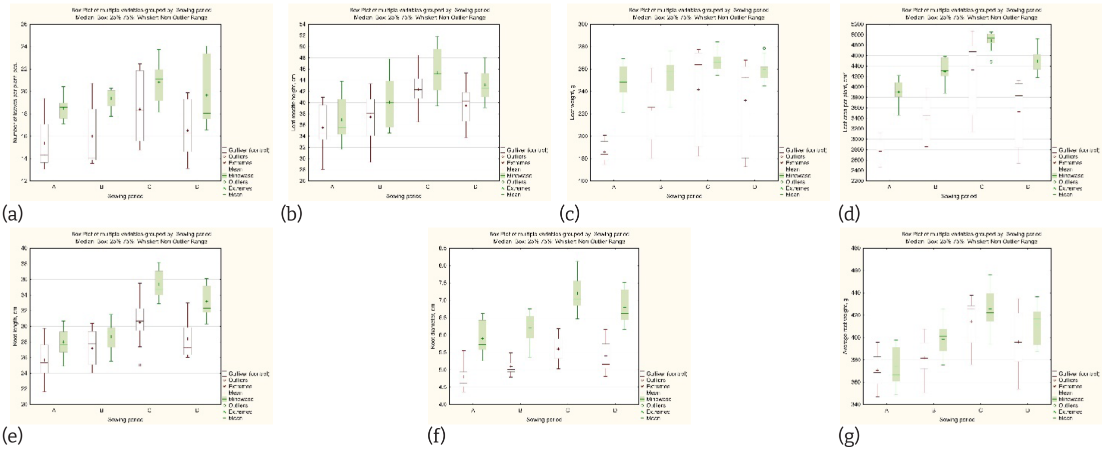

Figure 2. The influence of sowing dates on the variability of morphometric and productive indicators of daikon varieties (Box-plot)

Note: sowing dates: A – 1st decade of July; B – 2nd decade of July (control); C – 3rd decade of July; D – 1st decade of August. The studied indicators: (a) – number of leaves, pcs.; (b) – height of the leaf rosette, cm; (c) – leaf weight, g; (d) – leaf plate area, cm²; (e) – root length, cm; (f) – root diameter, cm; (g) – average root weight, g

Source: compiled by the authors based on the conducted research

The height of the leaf rosette for the variety 'Minowase' in the second decade of July and for 'Gulliver' in the third decade of July demonstrates a normal distribution. Also, a distribution close to normal was noted in the variant 'Minowase' in the third decade of July. The median (the line inside the rectangle) is located closer to the lower (Q1) boundary of the box (the first quartile), which indicates a right-sided asymmetry for the variety 'Gulliver' (control) in the variants of the first decade of July and the first decade of August. In contrast, a left-sided asymmetry was noted for the variety 'Gulliver' (control) in the second decade of July and the first decade of August. In all cases, there are no outliers. In the variant of the variety 'Minowase' in the first decade of July, the distribution of leaf weight indicates normality. The mean and median for the variety 'Gulliver' (control) of the first-second decades of July and the variety 'Minowase' of the third decade of July are almost at the same point, but do not pass the normality test. In the case of the variety 'Minowase' of the third decade of July, an outlier was recorded beyond the upper limit (upper whisker). Left-sided asymmetry can be clearly observed in the variants of the variety 'Minowase' of the second decade of July and the variety 'Gulliver' (control) of the third decade of July and the first decade of August, and right-sided asymmetry in 'Minowase' of the third decade of July. The mean and median of the leaf area index for sowing the variety 'Gulliver' (control) of the first-second decades of July and the variety 'Minowase' of the first-second decades of July and the first decade of August are almost at the same point, but not quite, while in other variants, the mean and median are located at different points of the distribution. One case of deviation (beyond the lower limit of the diagram) was detected for the variety 'Minowase' sown in the third decade of July.

The root length indicator demonstrates a non-normal distribution; one case of outlier beyond the lower limit of the diagram was recorded ('Gulliver' (control), sown in the third decade of July). The median shift to the upper part of the rectangle is observed only for the variety 'Gulliver' (control) sown in the second-third decades of July and for 'Minowase' sown in the second decade of July. The diagram demonstrates a distribution of root diameter that is not normal, although no outliers (or anomalous values) were detected. In most cases, a right-sided asymmetry is observed; only in the variants of the second decade of July ('Minowase') and the third decade of July ('Gulliver' (control)) was a left-sided asymmetry recorded. This is confirmed by the fact that the mean value is less than the median. The initial data of the average weight of the root crop do not contain any outliers. The diagram indicates the normality of the distribution only for the variants of the second decade of July ('Gulliver' (control)) and the first decade of August ('Minowase'), since the mean and median are at the same point (or are almost equal). Right-sided asymmetry is predominant, which is confirmed by the fact that the mean is greater than the median. Left-sided asymmetry was established exclusively in the variants of the second decade of July, the first decade of August ('Minowase') and the third decade of July ('Gulliver' (control)).

A positive correlation was found between the morphological indicators of daikon (Table 3). In the Gulliver variety (control), strong correlations (r = 0.67-0.89) were observed between leaf area and leaf weight (r = 0.89), between rosette height and root length (r = 0.80), number of leaves and rosette height (r = 0.80), leaf area and average root weight (r = 0.79), leaf weight and rosette height (r = 0.74), leaf weight and average root weight (r = 0.73), number of leaves and leaf weight (r = 0.70), as well as between number of leaves and leaf area (r = 0.68) and number of leaves and root length (r = 0.67). Average correlations (r = 0.36-0.63) were recorded between the sowing period and most morphological traits: root diameter (r = 0.52), average root weight (r = 0.41), leaf weight (r = 0.41), leaf area (r = 0.41), root length (r = 0.40) and rosette height (r = 0.36). The average also correlations include the relationships between root length and diameter (r = 0.63), number of leaves and average root weight (r = 0.61), rosette height and average root weight (r = 0.59), number of leaves and root diameter (r = 0.56) and rosette height and root diameter (r = 0.50).

Table 3. Correlation matrix of morphological indicators in daikon varieties (2022-2024)

Varieties	Variable	SD	LW	LA	NL	LRH	RL	RD	ARW
'Gulliver' (control)	SD	–							
	LW	0.407*	–						
	LA	0.412*	0.892***	–					
	NL	0.183	0.704***	0.680***	–				
	LRH	0.364*	0.736***	0.741***	0.798***	–			
	RL	0.399*	0.437**	0.523**	0.667***	0.802***	–		
	RD	0.518**	0.311	0.408*	0.557***	0.496**	0.631***	–	
	ARW	0.409*	0.734***	0.791***	0.614***	0.592***	0.487**	0.396*	–
'Minowase'	SD	–							
	LW	0.340*	–						
	LA	0.601***	0.750***	–					
	NL	0.256	0.594***	0.633***	–				
	LRH	0.512**	0.785***	0.807***	0.747***	–			
	RL	0.668***	0.580***	0.783***	0.367*	0.729***	–		
	RD	0.560***	0.581***	0.738***	0.400*	0.781***	0.897***	–	
	ARW	0.576***	0.413*	0.658***	0.365*	0.501**	0.652***	0.604***	–

Note: SD – sowing date; LW – leaf weight; LA – leaf area per plant; NL – number of leaves per plant; LRH – leaf rosette height; RL – root length; RD – root diameter; ARW – average root weight. Correlation is significant at the * p<.05, ** p<.01, *** p<.001 level; n=36

Source: compiled by the authors based on the conducted study

The 'Minowase' variety is characterised by even higher consistency of morphological traits, as most of the relationships are strong (r=0.66-0.90): between root length and diameter (r = 0.90), leaf area and rosette height (r = 0.81), rosette height and root diameter (r = 0.78), leaf weight and rosette height (r = 0.79), leaf area and length (r = 0.78), leaf area and root diameter (r = 0.74), rosette height and number of leaves (r = 0.75), leaf area and leaf weight (r = 0.75), leaf area and average root weight (r = 0.66), as well as between the sowing period and root length (r = 0.67). Moderate relationships were noted between length and average root weight (r = 0.65), number of leaves and leaf area (r = 0.63), leaf weight and number of leaves (r = 0.59), between diameter and average root weight (r = 0.60), leaf weight and root length (r = 0.58), leaf weight and diameter (r = 0.58), as well as between the sowing period and the remaining morphological indicators – leaf area (r = 0.60), rosette height (r = 0.51), diameter (r = 0.56) and average root weight (r = 0.58). Thus, the presence of a correlation between the indicators of the vegetative part of the plant (leaf area, leaf weight, rosette height) and the parameters of the root crop (diameter, length, weight) confirms the key role of leaves in ensuring the productivity of daikon, since the intensive development of the assimilation apparatus directly affects the accumulation of biomass and the formation of the marketable part of the plant. Statistical processing of experimental data and the graphic representation in Figure 3 allowed establishing that an increase in the duration of the growing season by 10 days for the variety 'Gulliver' (control) contributed to an increase in the following indicators: the number of leaves – by 0.44 pcs.; the height of the leaf rosette – by 1.41 cm; the weight of the leaves – by 12.79 g; the area of the leaf plate – by 262.44 cm²; the length of the root crop – by 1.00 cm; root diameter by 0.21 cm, and average root weight by 8.82 g. In turn, for the variety 'Minowase', shifting the sowing dates by 10 days increased the number of leaves by 0.42 pcs.; the height of the leaf rosette by 2.13 cm; the weight of the leaves by 4.18 g; the area of the leaf plate by 209.07 cm²; the length of the root by 2.01 cm; the diameter of the root by 0.33 cm, and the average weight of the root by 11.92 g.

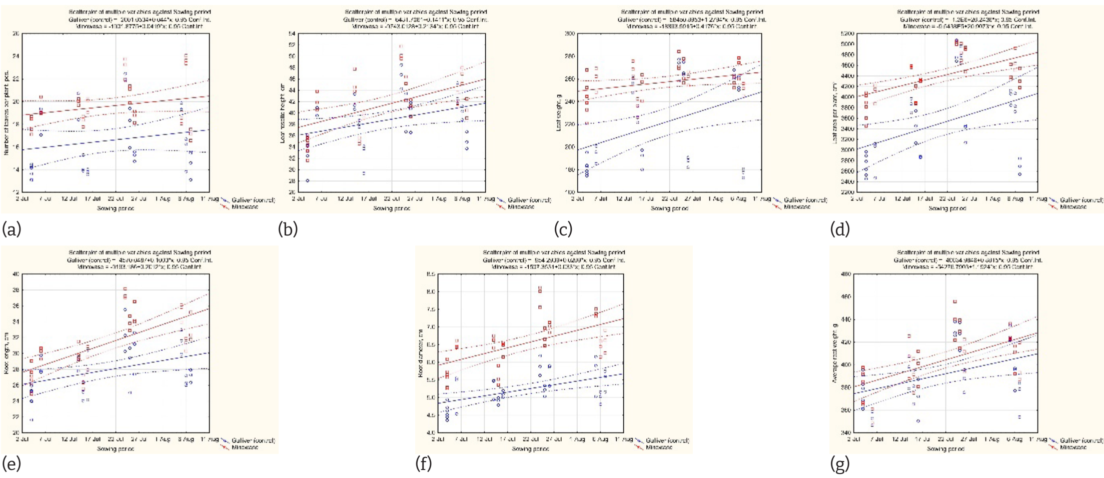

Figure 3. Dependence of morphometric and productive indicators of daikon varieties on sowing dates, approximated by linear regression

Note: sowing dates: A – 1st decade of July; B – 2nd decade of July (control); C – 3rd decade of July; D – 1st decade of August. Investigated indicators: (a) – number of leaves, pcs.; (b) – height of leaf rosette, cm; (c) – leaf weight, g; (d) – leaf plate area, cm²; (e) – root length, cm; (f) – root diameter, cm; (g) – average root weight, g

Source: compiled by the authors based on research conducted

This confirms that a developed assimilation apparatus is a key resource for biomass accumulation, and sowing in the third decade of July and the first decade of August stimulate its intensive formation. It has been statistically confirmed that the weight of the root crop directly depends on its length and diameter: the larger these parameters, the higher the total mass of the plant. It has been established that the yield of daikon depends on hydrothermal conditions during critical phases of vegetation. Sowing in the third decade of July is a defining term for the studied area, as it allows for the maximum realisation of the biological potential of the crop.

This correlates with international trends in the search for the most profitable periods of production. In particular, A. Sharma *et al.* (2023) using the example of India proved that growing radish in the off-season is more economically profitable due to the higher market value than in the main period. This statement is based on a comprehensive analysis of morphometry, correlations and statistical distribution of traits, which is consistent with and significantly complements the results of world scientific practice. During the experiment, a significant amplitude of variability in leaf rosette growth depending on the sowing date was recorded. The lowest indicators were observed with early sowing (first decade of July), while a shift to the third decade of July led to an increase in rosette height to 42.3-45.4 cm (+13.1-13.2% compared to the control). This phenomenon finds a deep physiological justification in the works of S. Oh *et al.* (2022), where it was established that temperature is a limiting factor in radish morphogenesis: at indicators above 29/23°C (day/night), inhibition of root growth and abnormal elongation of the hypocotyl are observed. The low productivity of crops in the first decade of July (root weight 371-374 g) in this study is explained precisely by the coincidence of the initial phases of development with the July temperature maximum, which exceeds the physiological optimum (19-24°C).

The revealed pattern between sowing dates and productivity contrasts with the data published by S. Khan *et al.* (2022) and S. Parajuli & P. Dhital (2023). If in the subtropical zones of Pakistan and Nepal, "early" crops (October-November) demonstrate better results (leaf length 22.61 cm) than late ones, due to the coincidence with the beginning of the cool season, then in temperate climates, "early" crops (early July) fall into the peak of heat, which creates stressful conditions. This is consistent with the data of N. Khusanov *et al.* (2024), which indicate a temperature threshold for germination of 30°C. In addition to temperature, the light regime is an important factor. The results of M. Hudu *et al.* (2025) in Nigeria showed that in the wet season (longer daylight hours, better moisture) a more powerful rosette is formed. This explains the slight decrease in indicators in the sowing option in the first decade of August (leaf weight decreased by 7-11 g compared to the third decade of July), since the reduction in daylight hours in August limits the photosynthetic potential of plants. This effect is even more pronounced in the study of P. Cottney *et al.* (2022) in Ireland, where late sowing (September) led to a critical drop in biomass (from 1,200 to <100 kg/ha) due to a deficiency of solar radiation. Also, the decrease in yield with early sowing correlates with biotic pressure, since S. Patra *et al.* (2024) established a direct relationship between the increase in temperature and the number of pests, which in the first decade of July led to damage to the assimilation apparatus.

It was found that the variety 'Minowase' demonstrated not only higher productivity (root weight up to 426 g, length up to 35 cm), but also higher ecological plasticity compared to the variety 'Gulliver'. This is confirmed by the analysis of the data distribution, where the variety 'Minowase' in the variants of the first-third decades of July demonstrated a distribution close to normal (mean ≈ median), without significant outliers, which indicates genetic stability. In contrast, the variety 'Gulliver' more often demonstrated distribution asymmetry, responding to stress by deviating from the mean values. This confirms the classification of S. Kumar *et al.* (2023) and A. Dhand & N. Garg (2023), who characterise Asian variety types (to which 'Minowase' belongs) as more tolerant to summer temperatures. In contrast to the data of S. Ali *et al.* (2023), where the plant height reached only 25.84 cm, the variety 'Minowase' under experimental conditions formed a rosette 45.4 cm high, which indicates better adaptation of this genotype to the long-day conditions of the Forest-Steppe.

Extremely close correlations were also found between the mass of the root crop and the indicators of the leaf apparatus, which are stronger than those described in the modern literature. In particular, the correlation between the length and diameter of the root crop in the variety 'Minowase' was $r = 0.90$ (versus $r = 0.60$), and the relationship between the mass of the leaves and the mass of the root crop in the variety 'Gulliver' was r = 0.89 (versus r = 0.41) (Esho & Jasim, 2022; Luitel *et al.*, 2024). Such high determination allows stating that in the conditions of the research region, the limiting factor in yield is precisely the development of the leaf area. This is confirmed by regression analysis, according to which the extension of the vegetation period by 10 days provides an increase in leaf area by 209-262 cm^2, which directly transforms into an increase in root weight by 8.8-11.9 g. These calculations are consistent with the data of N. Pokharel *et al.* (2023), who proved that maximising the weight of the root crop is impossible without achieving optimal parameters of the photosynthetic surface. Thus, the obtained data confirm that sowing the 'Minowase' variety in the third decade of July is a strategically important condition for its adaptation to specific soil and climatic conditions and the realisation of yield potential, since it is this term that ensures balanced growth and development, high genetic stability and the most efficient use of available agroclimatic resources.

CONCLUSIONS

Comprehensive studies of the growth and development of daikon in the conditions of the Right-Bank Forest-Steppe of Ukraine have allowed establishing a clear relationship between the sowing dates and the realisation of the biological potential of varieties. It has been confirmed that the meteorological conditions of the second half of summer are decisive for the formation of both the assimilation apparatus and the productive part of the plant. The most intensive growth of vegetative mass occurs when sowing in the third decade of July. It is this period that provides the optimal combination of temperature regime and daylight hours, which allows the 'Minowase' variety to form a powerful rosette with a leaf area of 4,886 cm^2, which is 13.8% higher than the control sowing date. Early July sowing causes premature ageing of leaves and inhibition of photosynthetic processes due to excessive solar insolation. The sowing date is a determining factor in the productivity of root crops, as evidenced by the obtained correlation coefficients at the level of 0.79-0.89. The highest average root weight (414-426 g) in the studied varieties is provided by sowing in the third decade of July. When shifting the sowing dates to August, there is a tendency to a slight decrease in the mass of root crops. The variety 'Minowase' demonstrated better adaptive ability to the conditions of the region, forming root crops with higher linear parameters (by 5.5-16.9% length; by 21.6-28.6% diameter) compared to the control variety. Analysis of morphometric indicators confirmed the stable superiority of the variety 'Minowase' in all the studied parameters.

The results of the analysis of variance indicate that the determining factor in the formation of the mass of root crops is the sowing date, the share of influence of which is 44.9% ($p < .001$). Weather conditions of the year have a significant, but half as much influence (23.5%). Varietal characteristics, although significant, play a secondary role (5.1%). The absence of significant interaction between factors ($p > .05$) confirms the independence of the action of agrotechnical techniques from the genotype and climatic fluctuations of the year. Further scientific work in this direction should be focused on studying the influence of mineral nutrition and irrigation systems in combination with optimised sowing dates. An important aspect is also the study of keeping quality and biochemical composition of root crops depending on the time of harvest, which will allow developing comprehensive recommendations for extending the shelf life of fresh daikon products in the autumn-winter period.

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CONFLICT OF INTEREST

None.

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Закономірності формування морфометричних ознак та продуктивності дайкону (*Raphanus sativus* L. *convar*. *acanthiformis* Sazon.) за різних термінів сівби

Анотація. Адаптація агротехнологій до кліматичних викликів та стале виробництво функціональних продуктів визначили актуальність пошуку оптимальних параметрів вирощування малопоширених овочевих культур. Мета дослідження полягала у комплексному аналізі формування біометричних і товарних характеристик дайкону під впливом термінів сівби для адаптації технології виробництва в умовах Правобережного Лісостепу України. Польовий експеримент (2022-2024 роки) проведено із вивченням сортів ‘Гулівер’ і ‘Міновасі’ за чотирьох термінів сівби від початку липня до початку серпня. У сорту ‘Міновасі’ за сівби у третій декаді липня зафіксовано найбільшу кількість листків (20,8 шт.), висоту розетки (45,4 см) та площу фотосинтетичної поверхні (4886 см^2), що на 7,6-13,8 % перевищило показники інших термінів. Сорт ‘Гулівер’ продемонстрував аналогічну тенденцію з максимальними морфологічними показниками. Сівба у першій декаді липня призвела до формування найменшої ваги листків у досліджуваних сортах із зниженням на 7,4 % та 29,9 % порівняно з оптимальним терміном. Розвиток коренеплодів корелював із розеткою листків ($r = 0,31 - 0,90$): сорт ‘Міновасі’ за сівби в оптимальний термін формував коренеплоди довжиною 35,4 см та діаметром 7,2 см, що забезпечило найбільшу середню масу 426 г. У сорту ‘Гулівер’ середня маса коренеплоду за досліджуваних термінів сівби була на 0,9-4,4 % меншою ніж у ‘Міновасі’. Варіабельність маси коренеплоду на 44,9 % ($p < 0,001$) зумовлювалась саме терміном сівби, тоді як вплив погодних умов року (23,5 %) та генотипу (5,1 %) був значно меншим. Виявлено середній кореляційний зв'язок між термінами сівби та масою коренеплоду (r = 0,79 - 0,89), а регресійний аналіз підтвердив зміну маси на 8,82 г (сорт 'Гулівер') та 11,92 г ('Міновасі') на кожні 10 діб відхилення. Сівба дайкону в третій декаді липня була оптимальною для стабілізації врожайності, оскільки забезпечила сприятливий гідротермічний режим; водночас ранні терміни сівби зумовили суттєве зменшення лінійних розмірів коренеплодів. Удосконалена технологія вирощування редьки дайкону рекомендується для товаровиробників промислового і присадибного сектору для розширення асортименту овочевих культур і забезпечення ринку свіжою цінною овочевою продукцією

Ключові слова: морфометричні параметри; індекс листкової поверхні; кореляційний аналіз; адаптація до змін клімату; сортові особливості

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Sowing parameters as a factor in emergence synchrony and productivity of sugar beet crops

Abstract. Modern sugar beet production is characterised by rising climate instability and the increasing impact of the early stages of organogenesis on crop productivity. In the conditions of a cool and wet spring, the problem of optimising the manageable sowing parameters, which can ensure the uniformity of seed germination and the synchrony of plant growth, acquires particular relevance. This study aimed to provide a scientific justification for the influence of coulter downforce and seeder travel speed on emergence synchrony and root mass formation in the sugar beet hybrid KWS 'Riorita' in the conditions of the Western Forest-Steppe of Ukraine. The research was carried out in 2025 on a productive research field on podzolised medium loam chernozem in the contrasting hydrothermal conditions of the sowing period. The experimental scheme included varying the coulter downforce and the seeder travel speed. The dynamics of seedling emergence, emergence synchrony, the spatial uniformity of the stand and root mass formation were measured. Statistical analysis was processed using analysis of variance and regression methods. It was found that an increase in coulter downforce to 40-60 kg significantly reduced the number of late-emerging seedlings and provided emergence synchrony at 96%, while with minimal pressure, it did not exceed 84%. A decrease in the proportion of seedlings emerging on days 9-10 after sowing was accompanied by an improvement in the uniformity of the stand and a stabilisation of the average root mass. A biological optimum was determined for the seeder travel speed at 7 km·h^{-1}, at which

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the best combination of spatial uniformity of sowing, high emergence synchrony and maximum root mass was achieved. A decrease and excessive increase in speed negatively affected productivity. The quantitative analysis showed that a delay in emergence of 1 to 2 days led to a sharp decrease in root mass; the late-emerging plants formed roots with 24% to 39% lower mass compared to the early emerging ones. The practical significance of the study is to substantiate the sowing parameters aimed at minimising emergence asynchrony as a key prerequisite for the stable formation of sugar beet yield under production conditions

Keywords: coulter downforce; seeder speed; early plant development; root mass; sowing technology

INTRODUCTION

In the last few years, sugar beet cultivation has faced technological and agroecological problems concentrated in the phase of crop establishment. In the conditions of spring sowing, a significant variability in the temperature and humidity of the soil, as well as technological parameters of the sowing unit, is observed, which can cause significant differences in the uniformity and synchrony of the seedlings' emergence, which in turn determine the structure of the yield. The synchrony of the emergence is a critical element of the productive potential of sugar beet, since the first few days after the emergence of the seedlings determine the competitive advantages of the plants, fix the spatial distribution of the biomass and lay the foundation for the subsequent heterogeneity of the root formation. That is why the research on the influence of the controlled technological parameters of the sowing (mainly the downforce of the coulter and the operating speed of the sowing unit) is important for the scientific substantiation of technological solutions for the uniformity of the sowing and the stability of the yield, in the conditions of variable technologies of the modern sugar beet cultivation.

The sensitivity of the establishment phase of the sugar beet crop to the risk of weather factors was consistently studied by A. Tataridas *et al.* (2022), who, by modelling methods, showed that the combination of low temperatures and excessive humidity of the soil significantly increases the risk of asynchronous emergence and uneven density of plant stand. This indicates that technological parameters, which affect the microconditions of germination, have a disproportionately strong effect in these conditions. A similar conclusion, but in a broader agroclimatic aspect, was made by S. Bastaubayeva *et al.* (2022), who noted that the productivity of sugar beet is largely determined by weather conditions in the initial growth period, and the stabilisation of the initial development processes is one of the most effective ways to reduce the inter-annual variability of the yield. The deepening of the research into the issue of the emergence synchrony is connected with the research in the physics of the seedbed and the seed-soil contact. K. Tuğrul (2022) experimentally showed that at the same agrochemical conditions, the determining factor of the uniformity of germination is the quality of the mechanical seed-soil contact, which regulates the local water regime, the rate of soaking and initial gas exchange. In applied terms, this means that the factors influencing the downforce and the stability of the coulter operation can be decisive in ensuring the uniform emergence of the crop in the conditions of a cool spring. The biological aspect of the problem is confirmed by the results of the research of M. Kaya & E. Kulan (2020), who showed that sugar beet seeds in conditions of low-temperature stress are particularly sensitive to the prolonged phase of germination; in these conditions, any heterogeneity of the microenvironment of the sowing layer increases the dispersity of the timing of emergence. Therefore, the management of the sowing parameters should be considered not only as a "mechanical" control, but also as a means of physiological stabilisation of the initial development phases of the plants.

A separate group of contemporary studies indicates that non-uniform emergence manifests in the harvest yield and quality. R. Nowicki *et al.* (2025), analysing technological operation timing in sugar beet production, draw attention to the fact that the initial growth rate discrepancy remains in the yield structure until harvest, resulting in a shift in root fractions, and, as a consequence, in the economic efficiency of the production system. A similar line of research was carried out by H. Rimaz *et al.* (2020), who found that the change in sowing and harvesting time leads to changes in the stability of white sugar yield, and the initial development stage constitutes an essential part of the variance in productivity between treatments. This indicates that under modern intensive production conditions, the initial development stage remains a bottleneck that depends on technological accuracy. Generalisations at the system level on the sustainability of sugar beet production technologies are contained in the research of Z. Gazdík *et al.* (2025), who, in a review of the concept of sustainable production, emphasised the importance of field operation accuracy and the controllability of the processes determining crop uniformity. It is also important that modern technological solutions are evaluated not only for the final yield level but also for their ability to reduce the risk and losses due to heterogeneity of the crop. In this sense, D. Grunwald *et al.* (2025), considering integration within crop protection systems, effectively emphasise the need for integration of technological factors: even the strongest elements of the crop management systems realise their full potential more effectively against the background of a uniform and well-established crop stand. In this context, the authors' results on the role of agronomic factors in sugar beet production systems indicate that technological details of individual operations influence the efficiency of resource use in crop rotation rather than at the single-season level.

A practical scheme for interpreting controllable agronomic factors is given by S. Paul (2022), who systematised the key elements of sugar beet management and noted that optimising technological parameters is one of the most cost-effective ways to increase yield stability in production conditions. In this respect, the sowing parameters are of primary importance as they predetermine the initial conditions that cannot be fully compensated for by other agronomic measures. The reviewed body of contemporary research indicates that the problem of emergence synchrony is located at the intersection of seedbed physics, germination physiology, and technological controllability of the sowing process and therefore requires applied quantitative assessment directly in production-research field conditions.

This study aimed to establish quantitative relationships between coulter downforce and seeder travel speed and their effects on emergence synchrony and productivity indicators of sugar beet crops under the conditions of the Western Forest-Steppe of Ukraine. The novelty of the research is the quantitative evaluation of the synchrony of emergence as a comprehensive index of the agrotechnical quality of sowing conditions under conditions of current production technologies.

MATERIALS AND METHODS

The field experiments were carried out in 2025 on the production research field of the Shymkivtsi unit of PE "Zakhidnyi Buh" (Zolochiv District, Lviv Region; 49°97′ N, 25°15′ E). The soil of the experimental site was podzolised medium loam chernozem, with a humus content of 3.4%, soil solution reaction pH (KCl) 6.7, available phosphorus content of 58 mg $P_2O_5 \cdot kg^{-1}$ of soil, and potassium content of 112 mg $K_2O \cdot kg^{-1}$ of soil. The long-term average annual precipitation in the experimental zone was 640 mm, and the mean daily air temperature during the growing season (April-October) was 17.1°C. In the spring of 2025, the first decade of April was noted for its increased moisture (up to 28 mm of precipitation) and low soil temperatures during the sowing period (+8°C to +10°C), which contributed to the formation of contrasting agroecological conditions for germination and activation of the agrotechnical sensitivity of the crop to sowing parameters, first of all mechanical (Lamichhane *et al.*, 2019; Bastaubayeva *et al.*, 2022).

The object of the study was sugar beet (*Beta vulgaris* L.), hybrid KWS 'Riorita', which is widely used in commercial crops in the region and is characterised by a high productivity potential. Soil tillage was carried out according to a conventional combined system, which included primary tillage by Horsch Tiger AS unit

(Germany) to a depth of 30-32 cm, spring harrowing by Degelman StrawMaster unit (Canada) and pre-sowing cultivation by FARMET Kompaktomat unit (Czech Republic) to a depth of 2.5-3.5 cm. The pre-sowing moisture content of the arable layer was 22.8%, the bulk density was 1.25 g·cm^{-3}, and the temperature of the soil at a depth of 5 cm was 10.2°C.

Sugar beet was sown on 17 April 2025 by Kverneland Monopil 24SE seeder (Norway) with a row spacing of 45 cm and a seeding rate of 120 thousand seeds per ha^{-1}. Within the framework of the experiment, two technological factors were changed: factor A is coulter downforce: 20 kg (minimum pressure), 40 kg (moderate, conventionally optimal pressure), and 60 kg (increased pressure); factor B is seeder travel speed: 4 km·h^{-1}, 7 km·h^{-1}, and 10 km·h^{-1}. Counting of seedlings was carried out on days 6, 7, 8, 9, and 10 after sowing. Field germination (%) was calculated by formula (1):

$$P = \frac{N_f}{N_s}, \tag{1}$$

where Nf is the number of emerged seedlings within the sampling area, Ns is the number of sown seeds.

After row closure (BBCH 16-18), the number of plants was counted in four rows, each with a length of 22.2 m. The uniformity of the plant stand was evaluated by the coefficient of variation (CV, %) of the distance between plants, which is a commonly used indicator of sowing quality (Varga *et al*., 2021). Yield evaluation was carried out on a sampling area of 10 m², from which all roots were dug out, after which the average root weight, the number of marketable and non-marketable roots, as well as the proportion of small (< 300 g) and large (> 1.2 kg) roots were determined. Sugar content, dry matter and technological impurities were determined in the root composite samples by the ICUMSA polarimetric method, which has been widely used in the international sugar beet research. P. Stevanato *et al*. (2019) stressed the importance of using a standardised polarimetric method to ensure the comparability of data among different research institutions.

Air and soil temperature and precipitation were measured using the automatic Davis Vantage Pro2 weather station (USA) installed on the experimental field. Data processing was carried out using Statistica 13.3 and Microsoft Excel 365 software packages. The data were analysed by analysis of variance (ANOVA), followed by Duncan's test at a significance level of $p < 0.05$. The relationships between the emergence synchrony, the uniformity of plant distribution and the root mass were estimated using the Pearson correlation and multiple regression analysis, where the explanatory variables included coulter downforce, sowing unit travel speed, soil temperature and soil moisture (Anar *et al*., 2019; Varga *et al*., 2021).

The field experiments were carried out in compliance with the ethical standards of the Convention on Biological Diversity (1992) and the Convention on the Trade in Endangered Species of Wild Fauna and Flora (1973). The study did not involve the use of genetically modified organisms, it did not imply interference into the natural ecosystems beyond the experimental plot, it did not create a risk for human or animal health, it did not include human subjects as the object of study, it did not involve any biomedical or socio-humanitarian manipulations and, therefore, it did not require a permission from the bioethics commission. Although the research was funded by the enterprise at whose facilities it was carried out, its results were independent, objective and served no private interests.

RESULTS AND DISCUSSION

The data of the 2025 field experiments showed that the coulter downforce and the sowing unit travel speed were the determining technological factors of the emergence synchrony of the sugar beet hybrid KWS 'Riorita' and, therefore, of the uniformity of the productivity elements. In the conditions of the cool and moist weather during the sowing period (soil temperature +8°C to +10°C), the mechanically caused differences in the seedbed structure and the seed-soil contact were manifested mainly through the changes in the proportion of late-emerged seedlings. These results agree with the data of S. Bastaubayeva *et al*. (2022), who found that the fluctuations in the soil temperature within the range of 5-10°C prolonged the seed imbibition period and increased the variation in the emergence terms. The high sensitivity of the sugar beet seeds to the low temperatures was also noted by M. Kaya & E. Kulan (2020). R. Esmaeili *et al.* (2022) pointed out the decisive importance of the uniform seed-soil contact for synchronous germination. Similar response of crop establishment processes to seedbed physical parameters was reported by S. Blunk *et al.* (2018), who emphasised the role of microsite stability for germination and J. Lamichhane *et al.* (2019), who pointed out that a higher risk of asynchronous emergence can occur under fluctuating temperature and moisture conditions in early spring. The change in coulter downforce within the studied range (20-60 kg) caused significant differences in emergence synchrony and in the distribution of emergence timing. The highest proportion of seedlings emerged in the optimal period (6-8 days) was found at an increased downforce (60 kg), while at the minimum pressure (20 kg), a higher proportion of late-emerged seedlings (9-10 days) was observed, which is undesirable for the formation of a uniform crop structure. In addition, M. Licht & M. Al-Kaisi (2005) found that even a small delay in emergence leads to permanent biomass differentiation during the growing season.

The differences in the average root mass between the downforce levels were less pronounced; however, the direction of influence generally repeated the pattern: the reduction in the proportion of lateemerged plants was accompanied by greater stability in root mass formation. Thus, the influence of coulter downforce was realised indirectly, through the regulation of the emergence timing distribution rather than through direct stimulation of growth processes. The main quantitative indices of the influence of coulter downforce on emergence synchrony and average root mass are given in Table 1.

Table 1. Effect of coulter downforce on emergence synchrony and average root mass (observations on 04 August 2025)

Coulter downforce, kg	Emergence on days 6-8, %	Emergence on days 9-10, %	Average root mass, g
20	84.0 ± 2.1 b	16.0 ± 1.8 a	526 ± 18 b
40	85.0 ± 1.9 b	15.0 ± 1.5 a	552 ± 17 a
60	96.0 ± 1.2 a	4.0 ± 0.9 b	545 ± 16 a

Note: letters within a column indicate significant differences according to Duncan's test ($p < 0.05$)
Source: original research data

The ANOVA results showed that the effect of the "coulter downforce" factor was significant ($p < 0.01$) for the proportion of seedlings emerged in the periods of 6-8 and 9-10 days, and ($p < 0.05$) for the average root mass. This indicates a cause-and-effect relationship between seedbed parameters and the distribution of emergence timing. According to H.-J. Koch *et al.* (2018), emergence synchrony determines the level of intraspecific crop stand uniformity and is a premise for the further realisation of the yield potential, as an asynchronous start results in sustained morphological plant-to-plant differentiation. The analysis of the effect of seeder travel speed revealed the biological optimum at which the combination of technical evenness of plant spacing and physiological evenness of emergence provides the most favourable conditions for root mass formation. At a speed of 7 km·h^{-1}, moderate values of the coefficient of variation (CV) of interplant spacing coincided with a high proportion of seedlings emerging on days 6-8 and the maximum average root mass. A reduction in speed to 4 km·h^{-1} was associated with increased CV and reduced biological efficiency of the sowing. An increase in speed up to 10 km·h^{-1}, despite the reduction in CV and the increase in the proportion of early-emerged seedlings, was accompanied by a decrease in the average root mass.

The obtained results are in agreement with the data of R. Afshar *et al.* (2019), who found that the influence of tillage technologies and seedbed formation conditions on the emergence rate depends on the actual weather conditions. D. Górski *et al.* (2022) pointed out that water retention is usually associated with an increase in the heterogeneity of seedbed structure, while N. Mioduszewska *et al.* (2018) reported that an increase in the scatter of seed placement depth contributes to the asynchrony of emergence. A similar difference between geometric uniformity and biological effect is explained by S. Blunk *et al.* (2018), who showed that the disturbance of the stability of seed-soil contact at high operating speed of the seeder leads to latent stress of seedlings, which is not reflected in the indicators of spatial variability, but has a negative effect on further growth. The quantitative parameters of the effect of seeder operating speed on sowing uniformity, emergence synchrony and root mass are given in Table 2.

Table 2. Effect of seeder operating speed on sowing uniformity, emergence synchrony and root mass (observations on 04 August 2025)

Speed, $km \cdot h^{-1}$	CV of interplant spacing, %	Emergence on days 6-8, %	Average root mass, g
4	12.0 ± 0.8 a	80.0 ± 2.4 c	387 ± 21 b
7	10.0 ± 0.6 b	89.0 ± 1.7 b	464 ± 19 a
10	9.0 ± 0.5 c	94.0 ± 1.4 a	358 ± 23 b

Note: letters within a column indicate significant differences according to Duncan's test ($p < 0.05$)
Source: original research data

It was found by ANOVA that the effect of the "seeder operating speed" factor was significant ($p < 0.01$) for the coefficient of variation of the distance between plants, the share of seedlings emerged during days 6-8 and average root mass, which testifies to the direct influence of the kinematic parameters of the seeder operation on the biological effect of the sowing process. The biological sensitivity of the germination process to the physical properties of the seedbed, in particular to the stability of seed-soil contact and to the micro-irregularities of compaction, is in agreement with the results of S. Blunk *et al.* (2018), who found that even minor violations of the seedbed structure lead to asynchronous emergence. The main result of this research is the quantitative substantiation of the fact that the timing of seedling emergence determines the subsequent differentiation of plant productivity, while technological factors influence indirectly through the regulation of the share of late-emerged seedlings. A consistent decrease in the average root mass with a shift of seedling emergence to later dates was observed, which indicates the formation of a stable hierarchical structure of the crop stand. This dependence corresponds to the concept of the "priority effect", which consists of the fact that earlyemerged plants receive a competitive advantage in terms of light, nutrients and root space at the initial stages of organogenesis.

Similar dependences were obtained by I. Varga *et al.* (2021), who found a decrease in both the proportion of commercial roots and the sucrose content in plants that emerged later. R. Esmaeili *et al.* (2022) also pointed out the long-term differentiation of biomass due to asynchronous initial development. In the growth models of M. Anar *et al.* (2019), the day of emergence appeared as one of the most sensitive parameters for predicting yield. Similar conclusions were drawn by H.J. Koch *et al.* (2018), who showed the limited compensatory potential of late-emerging plants even under favourable agrotechnical conditions. D. Grunwald *et al.* (2025) also pointed out that the hierarchy formed in the early stages remains until harvest and is responsible for the variation in root mass. Consequently, sowing conditions should be viewed not only as a means of controlling the technical uniformity of seed placement but also as a biological synchroniser of plant development, which is a necessary condition for creating a uniform and productive sugar beet crop.

A quantitative study of the relationship between root mass and day of emergence revealed a clearly defined gradient (Table 3). The highest average root mass was formed by plants that emerged on the 6th day after sowing, and with each subsequent delay in emergence, this indicator statistically significantly decreased. According to the ANOVA, the effect of the "day of emergence" factor on root mass was highly significant ($p < 0.001$), which indicates the determining role of temporal synchrony at the early stages of development in the formation of productivity.

Table 3. Dependence of root mass on the day of emergence (observations on 04 August 2025)

Day of emergence	Average root mass, g	Deviation from maximum, %
6	573 ± 19 a	0
7	541 ± 18 a	-6
8	506 ± 17 b	-12
9	436 ± 21 c	-24
10	352 ± 24 d	-39

Note: letters within a column indicate significant differences according to Duncan's test ($p < 0.05$)
Source: original research data

As can be seen from Table 3, a one-day delay in germination reduced the average root mass by 6%, two days by 12%, while the potential losses from seedlings that emerged on the 9th-10th days reached 24-39% of the maximum. Different letter indices in the columns indicate significant differences between most variants according to the Duncan test ($p < 0.05$). The graphic representation of this dependence is presented in Figure 1, where a practically linear decrease in root mass with a delay in emergence is observed.

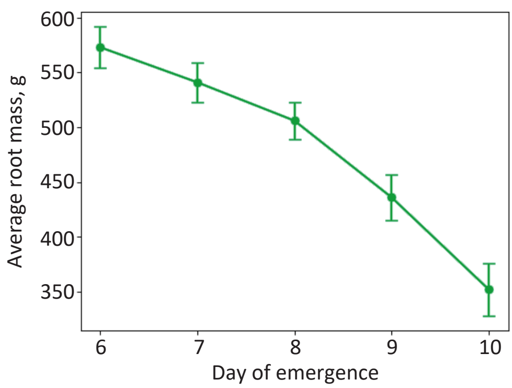

Figure 1. Dependence of average root mass on the day of sugar beet emergence (observations on 04 August 2025)
Source: original research data

The biological interpretation of this regularity is in line with the research of C. Chomontowski *et al.* (2020), M. Mirzaei & S. Hemayati (2022), and R. Nowicki *et al.* (2025), which emphasise the irreversibility of the early developmental superiority of seedlings in relation to root mass. A similar position is expressed by W. Samociuk *et al.* (2025), who note that even against the background of a uniform agrotechnical complex, late-emerging plants remain functionally dependent in the structure of the crop. Therefore, the synchrony of early emergence is an essential factor in the formation of the morphofunctional uniformity of the crop stand. The obtained data allow a practically significant conclusion to be drawn: control of the technological parameters of sowing should be directed, first of all, not at minimising the values of the indicators of geometric uniformity, but at minimising the share of late sprouts. Increasing the coulter downforce to the value ensuring the stability of the seed-soil contact and choosing the speed of the seeder, which does not worsen the biological indicators of the quality of sowing, are the necessary conditions for the creation of a uniform productivity structure. This conclusion is consistent with the modern paradigm of sustainable production of sugar beet, according to which the early development stage is considered a critical control point for the formation of the yield and the efficiency of its production, as was pointed out by P. Stevanato *et al.* (2019) and S. Paul (2022).

However, according to the results of J. Lamichhane *et al.* (2019), climate change will increase the risks of asynchrony of emergence, which will further increase the importance of technological stabilisation of the seedling emergence in the future sugar beet production systems. In general, it can be concluded that the synchrony of seedling emergence is an integrated indicator of the quality of sowing, which unites the mechanical, physical, and biological components of the creation of the productivity structure and is the main channel through which the influence of the studied technological parameters is realised.

CONCLUSIONS

The results of this study have confirmed the determining role of the sowing parameters in the formation of the seedling emergence synchrony and productivity of sugar beet (*Beta vulgaris* L.) crops of the KWS 'Riorita' hybrid under the conditions of the Western Forest-Steppe of Ukraine. It has been established that the coulter downforce and the speed of the seeder are the key manageable technological elements that control the uniformity of the early plant development and, as a consequence, have a significant effect on the root mass formation. Under the cool and wet weather conditions of the spring of 2025, the reduction of the asynchrony of the seedling emergence has proved to be especially important, as the late-emerged seedlings were not able to compensate for the delay in their growth and development during the growing season. An increase in the coulter downforce up to 40-60 kg has significantly reduced the proportion of the late-emerged seedlings and contributed to the creation of a more uniform stand structure. An increase in the seedling emergence synchrony from 84% to 96% has been accompanied by the stabilisation of the average root mass and a reduction in the in-stand variability. It has been demonstrated that the effect of the coulter downforce is realised not through the direct stimulation of the growth processes, but through the improvement of the physical conditions of the seed germination and the alignment of the rates of the early organogenesis.

A biological optimum of seeder travel speed was found: at 7 km·h^{-1}, the best spatial uniformity of seed placement, emergence synchrony and highest average root mass were combined. Both decreasing (4 km·h^{-1}) and increasing (10 km·h^{-1}) the seeder travel speed resulted in a productivity decline. This implies the impossibility of determining sowing quality based on geometric criteria only without accounting for the biological reaction of the crop. It is concluded that a delay in the emergence of even one or two days results in a loss in potential root mass of 24-39% for late-emerging seedlings. Therefore, the optimisation of the sowing regime should be aimed at ensuring maximum emergence synchrony as the main prerequisite for the formation of a uniform crop structure and stable realisation of the productive potential of sugar beet. Further studies should be aimed at the multi-year and multi-local verification of the revealed patterns, taking into account contrasting soil and climatic conditions, different genotypes of sugar beet, and a varied agrotechnical background. Of particular interest is the study of the interaction of coulter downforce and seeder travel speed with the physical properties of the seedbed (density, moisture, aggregate structure), and the integration of field experiments with modelling of the emergence process and yield formation. The application of digital monitoring systems for crop establishment, precise definition of the temporal dynamics of emergence and spatial analysis of the hierarchisation of plants within the stand seems promising. This will allow the development of adaptive recommendations for the calibration of the seeder for different soil types and weather conditions, which will increase the stability of sugar beet productivity in the context of increasing climate change.

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CONFLICT OF INTEREST

None.

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Параметри сівби як фактор синхронності сходів і продуктивності посівів цукрових буряків

Анотація. Сучасні умови вирощування цукрових буряків характеризуються підвищеною кліматичною мінливістю та зростанням ролі стартових етапів органогенезу у формуванні продуктивності культури. За прохолодних і зволожених умов весняного періоду особливої актуальності набуває оптимізація керованих параметрів сівби, здатних забезпечити вирівняне проростання насіння та синхронний розвиток рослин. Метою дослідження було наукове обґрунтування впливу притискного зусилля сошників і швидкості руху сівалки на синхронність сходів і формування маси коренеплоду цукрових буряків гібриду КВС ‘Ріоріта’ в умовах Західного Лісостепу України. Дослідження проводили у 2025 році на виробничо-дослідному полі на чорноземі опідзоленому середньосуглинковому за контрастних гідротермічних умов посівного періоду. Схема досліду передбачала варіювання притискного зусилля сошників та швидкості руху сівалки. Обліки включали динаміку появи сходів, визначення синхронності сходів, оцінку просторової рівномірності посіву та формування маси коренеплоду. Статистичну обробку результатів здійснювали з використанням дисперсійного аналізу та регресійних методів. Установлено, що підвищення притискного зусилля сошників до 40-60 кг істотно зменшувало частку пізніх сходів і підвищувало синхронність сходів до 96 %, тоді як за мінімального тиску вона не перевищувала 84 %. Зменшення частки сходів, що з’являлися на 9-10-ту добу після посіву, супроводжувалося вирівнюванням агроценозу та стабілізацією середньої маси коренеплоду. Виявлено біологічний оптимум швидкості руху сівалки на рівні 7 км·год$^{-1}$, за якої забезпечувалося найкраще поєднання просторової рівномірності посіву, високої синхронності сходів і максимальної маси коренеплоду. Як зниження, так і надмірне підвищення швидкості негативно впливали на продуктивність. Кількісно доведено, що затримка появи сходів на одну-дві доби призводила до істотного зменшення маси коренеплоду, а пізні сходи формували коренеплоди на 24-39 % меншої маси порівняно з ранніми. Практична цінність роботи полягає в обґрунтуванні параметрів сівби, спрямованих на мінімізацію асинхронності сходів як ключової передумови стабільного формування врожаю цукрових буряків у виробничих умовах

Ключові слова: притискне зусилля сошників; швидкість сівалки; стартовий розвиток рослин; маса коренеплоду; технологія сівби

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Study of the influence of phosphite fertilisers on the quality of apple fruits in plantings of the 'Fuji' variety

Abstract. The purpose of the study was to establish the effectiveness of foliar fertilisation with phosphite fertilisers by the company LLC "Ecoorganic" in apple plantations of the 'Fuji' cultivar and to determine their influence on the dynamics of accumulation of dry soluble substances, sugars, and organic acids in fruits. The research was conducted in 2022 on the experimental orchards of Uman National University of Horticulture (Ukraine). In the experiment, a control without fertiliser application and variants treated with three types of fertilisers (Poliaktiv Twinfos, Poliaktiv Twinfos Amino, Ecoline Phosphite K-Amino) at a dose of 1.5 L/ha during the fruit ripening phase were compared. Refractometric, spectrophotometric, and titrometric methods were used to assess the biochemical composition of the fruits. The findings showed that foliar fertilisation significantly affected the biochemical indicators of apple fruit quality. During the fruit ripening period, an increase in the content of dry soluble substances was observed in all experimental variants; however, the highest values were recorded when using the fertiliser Poliaktiv Twinfos, where this indicator significantly exceeded the control. A similar pattern was established for sugar accumulation: their maximum content was noted in fruits treated with the fertiliser Poliaktiv Twinfos, while the use of fertilisers Poliaktiv Twinfos Amino and Ecoline Phosphite K-Amino also ensured an increase in this indicator compared to the control. Furthermore, in all variants with foliar fertilisation, a decrease in

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the content of organic acids in fruits was observed, which contributed to the development of a more balanced taste. The most pronounced effect of acidity reduction was established when using the fertiliser Poliaktiv Twinfos. Practical significance of the study consists in the scientific substantiation of the feasibility of foliar fertilisation of the 'Fuji' apple cultivar during the fruit ripening phase to increase sugar content, reduce acidity, and improve the commercial and consumer qualities of fruits under conditions of increasing climate stress

Keywords: foliar fertilisation; biochemical composition of fruits; dry soluble substances; sugar content; organic acids

INTRODUCTION

In contemporary horticulture, the stability of production and the competitiveness of products are largely determined by the ability of plantations to withstand high temperatures and moisture deficiency during the summer period. It is precisely in the phase of fruit filling and ripening that intensive processes of carbohydrate synthesis and accumulation, transformation of organic acids, development of aroma, coloration, and flesh density occur. Disturbances in water balance and heat load during this period negatively affect the accumulation of soluble solids, reduce sugar content, and worsen taste characteristics and the market appearance of fruits. In this regard, the search for effective foliar fertilisation techniques capable of correcting physiological and biochemical processes in plants and increasing their adaptive potential under stressful growing conditions becomes especially relevant.

Analysis of contemporary scientific studies indicates a growing interest in the problem of optimising fruit crop nutrition during periods of climate change. V. Do *et al.* (2024) in their study established that the combination of high temperatures and uneven watering throughout the growing season leads to disturbances in physiological processes in apple trees, in particular, to a decrease in the intensity of photosynthesis, suppression of growth processes, and a reduction in average fruit weight. The researchers concluded that moisture deficiency in critical developmental phases negatively affects the accumulation of soluble solids and worsens the market quality of products, which requires the implementation of adaptive agrotechnical measures to increase plant resistance to climate stresses. Developing this area, S. Świerczyński *et al.* (2021) showed that foliar application of microelements, fertilisers, and biostimulants during the phase of intensive fruit growth activates enzymatic processes, stabilises metabolism, and contributes to increased apple yield under conditions of abiotic stress. The researchers emphasised that such operational correction of mineral nutrition through the leaf apparatus allows plants to quickly respond to changes in the external environment and reduce the negative impact of stressful growing factors.

Similar results were obtained by some Ukrainian researchers who investigated the influence of humic and fulvic acids on plants. In particular, O. Rylskyi *et al.* (2023) noted that the application of humic compounds in plant cultivation contributes to increased plant resistance to abiotic stresses, activation of enzymatic processes, and improvement of product quality indicators, which confirms the appropriateness of using such substances as an element of adaptive agrotechnologies. Contemporary studies emphasise the importance of improving apple foliar feeding systems to increase productivity and quality characteristics of fruits. In particular, V. Leus *et al.* (2023) showed that the application of biostimulant micronutrients within the framework of foliar feeding contributes to increased fruit size, their marketability, and the economic efficiency of cultivation. The application of such preparations stimulates physiological processes in the plant, which ensures more uniform fruit growth and improves their consumer properties.

The literature analysis conducted by R. Yakovenko *et al.* (2025) confirmed that the combination of soil and foliar fertilisation is optimal for ensuring high yields of apples and pears. They emphasised that balanced plant nutrition not only improves quantitative yield indicators but also contributes to increasing the biochemical quality of fruits, including increasing soluble sugar content and reducing titratable acidity, which directly affects the market and consumer properties of the products. These results were also confirmed by recent studies on other types of foliar fertilisation. Y. Lu *et al.* (2025) showed that foliar application of zerovalent iron nanoparticles significantly improves photosynthetic activity, enzymatic activity of leaves, and fruit quality of 'Yanfu No.6' apples. In the experiment, an increase in fruit weight, vitamin C content, soluble proteins, and overall quality indicators was observed, indicating the effectiveness of foliar microelement application in enhancing the physiological condition of the plants.

In Ukrainian studies, I. Trushev & R. Yakovenko (2023) investigated methods of rational use of fertilisers and foliar feeding in intensive apple orchards, confirming similar results. It was shown that the application of such approaches contributes to improved tree growth, accumulation of nutrients in leaves, and increased overall yield. Additionally, O. Tkachuk & A. Mizeriy (2024) demonstrated that the use of biofertilisers and foliar applications in apple orchards improves the physiological condition of the plants, increases yield, and enhances fruit quality characteristics, including flesh firmness, content of vitamin C and soluble solids. Their results confirm that integrating biofertilisers and foliar feeding is an effective method for improving apple resilience to stress factors and fruit quality.

Phosphite fertilisers attract particular interest in contemporary research as a promising element of the foliar feeding system. X. Han *et al.* (2021) substantiated that phosphite compounds are characterised by systemic mobility and the ability to induce plant defence responses in response to abiotic stresses. The researchers noted that phosphites influence the regulation of metabolic processes associated with plant adaptation to adverse conditions, improving physiological and biochemical reactions, which can contribute to increased yield and product quality under stressful conditions. Further studies show that phosphite compounds can enhance antioxidant protection of plants, improve their water status, and increase resistance to temperature and water stresses. In particular, M. Nyoni *et al.* (2021) demonstrated in apple trees that foliar application of phosphonates contributes to the accumulation of phosphite residues in plant tissues and increases their resistance to abiotic factors. Thus, Z. Li *et al.* (2025) argued that the application of phosphite fertilisers as foliar feeding not only provides effective plant nutrition but also acts as a powerful stimulator of physiological processes that increase plant resistance to abiotic stress. Researchers noted that foliar application of phosphites promotes the regulation of processes of soluble sugar accumulation and reduction of titratable acidity of fruits, which directly affects their market and consumer properties.

The purpose of the study was to evaluate the effect of foliar application of phosphite fertilisers on the development of biochemical indicators of apple fruit quality during their ripening period.

MATERIALS AND METHODS

The study on the effectiveness of foliar application of fertilisers by LLC "Ecoorganic" in apple plantations was conducted in 2022 at the experimental orchards of Uman National University of Horticulture, located in the Mankivka natural-agricultural region of the Middle Dnipro-Bug district of the Right-Bank Forest-Steppe of Ukraine. The object of the study was the 'Fuji' apple cultivar, included in the "Register of Plant Varieties Suitable for Distribution in Ukraine" (UIPVE, n.d.). 'Fuji' apple (*Fuji*) is an autumn-winter cultivar, selected by Japanese breeders in the Fujisaki region (from which its name originates) by crossing the 'Rolls Janet' and 'Red Delicious' cultivars (Fig. 1). The 'Fuji' apple tree is of medium size, 3.0-3.5 m tall, with a compact crown. It begins fruiting in the 3rd year. Flowering occurs in mid-late periods, which reduces the risk of fruit set damage by spring frosts. Fruiting is periodic, and yield is high. The fruits are round, large, with an average weight of approximately 200 g, red-pink in colour with yellow spots beneath the skin. The

Figure 1. Fruits of the 'Fuji' apple cultivar
Source: authors' photo

flesh is creamy, crisp, with a sweet-sour taste and high sugar content. Harvesting is carried out in mid to late October, and the fruits reach consumer ripeness 3-4 weeks after picking. The cultivar is characterised by good winter hardiness, high transportability, and a long storage period. Resistance to diseases is average. The fruits have universal use – suitable for fresh consumption and processing. The experimental design of the study included a control (treated with water) and three variants in which phosphite fertilisers were applied: Poliaktiv Twinfos, Poliaktiv Twinfos Amino, and Ecoline Phosphite K-Amino at a rate of 1.5 L/ha (Table 1).

Table 1. Experimental design

Application phase	Control	Variant 1 – Polyactive Twinphos	Variant 2 – Polyactive Twinphos Amino	Variant 3 – Ecoline Phosphite K-Amino
Fruit ripening period	Water treatment	1.5 L/ha	1.5 L/ha	1.5 L/ha

Source: the research scheme was approved by Ecoorganic (n.d.)

Fertilisers were applied on August 6, 15, and 25, 2022, during the fruit ripening period of the 'Fuji' cultivar (Fig. 2).

Figure 2. Images of trees of the experimental variants
Note: 1 – Polyactive Twinphos, 2 – Polyactive Twinphos Amino, 3 – Ecoline Phosphite K-Amino
Source: authors' photo

During the development of the experimental design and methodology, the main methodological recommendations of Ukrainian researchers G. Karpenchuk & A. Melnyk (1987) and V. Moiseychenko & V. Eshchenko (1994), and the authors' original methods, were used. For all experimental variants, the control was foliar treatment of the trees with water. During the fruit formation period on the tree, the content of the main organic substances was determined: dry soluble substances – by refractometric method according to DSTU ISO 2173-2007 (2009); sugars – by spectrophotometric method according to DSTU 4954-2008 (2009), organic titratable acids – by titration with 0.1N NaOH solution. The study was conducted in accordance with ethical standards of the Convention on Biological Diversity (1992) and the Convention on the Trade in Endangered Species of Wild Fauna and Flora (1973).

RESULTS AND DISCUSSION

The investigation of the effect of foliar fertilisation of 'Fuji' apple trees on the development of biochemical indicators of fruits showed that the accumulation of main soluble dry substances (SDS) largely depended on the type of fertilisers and the time of application. A. Hernández-Rodríguez *et al.* (2021) emphasised that foliar nutrition significantly affects fruit quality, including the content of soluble dry substances and sugars, which confirms the relevance of the study. The results indicate that during fruit ripening there was a gradual accumulation of SDS. The maximum SDS values were recorded in the variants treated with phosphite fertilisers, particularly Poliactiv Twinfos, which indicates the effectiveness of this preparation in increasing the biochemical value of fruits. Notably, direct studies on the effect of phosphite fertilisers on the accumulation of SDS and sugars in 'Fuji' apples are currently absent, therefore, the current findings are the first to demonstrate the positive effect of phosphite foliar applications on the biochemical composition of fruits of this cultivar.

Similar tendencies were observed in studies of foliar fertilisation with microelements and complex fertilisers. Thus, F. Mirzavandi *et al.* (2025) noted that foliar application of microelements, including phosphorus, contributes to the increase in the content of soluble dry substances and sugars in apple fruits. D. Ljavić *et al.* (2023) showed that foliar fertilisation changes the concentration of nutrients in leaves and affects the development of the biochemical composition of fruits. Thus, the increase of SDS in the variants with Poliactiv Twinfos corresponds to the general tendency of improving fruit quality due to foliar nutrition. At the beginning of September, SDS ranged from 11.0% in the control to 12.4% in the variant with Poliactiv Twinfos. At the end of the second ten days of September, the SDS level slightly decreased due to about 100 mm of precipitation, which led to the dilution of soluble substances in fruits. During the entire observation period, the lowest SDS content (11.2%) was recorded in the control, and the highest (12.8%) – with the treatment of Poliactiv Twinfos (Fig. 3).

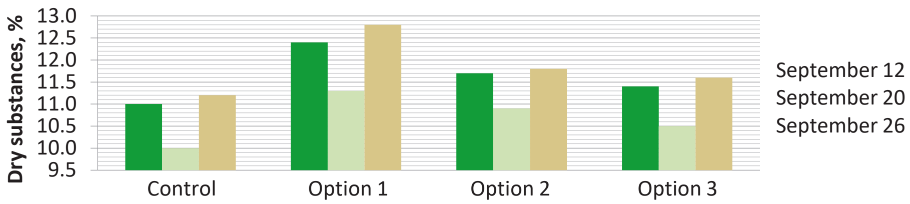

Figure 3. Dynamics of dry soluble substances (DSS) accumulation in 'Fuji' apple fruits during ripening depending on foliar treatment of plantations
Source: developed by the authors

Thus, the results indicate the effectiveness of foliar application of phosphite fertilisers in increasing the biochemical value of fruits of the 'Fuji' apple and confirm the importance of foliar nutrition for optimising fruit quality under conditions of summer heat and water stress. The same tendencies were observed in the assessment of the soluble sugar content, which was also the highest in the variant with Poliactiv Twinfos (9.6%), while the application of Poliactiv Twinfos Amino and Ecoline Phosphite K-Amino provided 8.85% and 8.7% of sugars, respectively (Fig. 4).

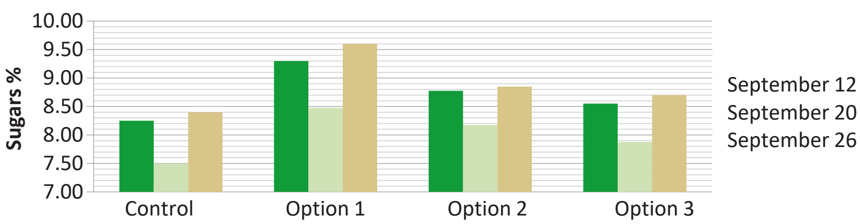

Figure 4. Dynamics of sugar accumulation in 'Fuji' apple fruits during ripening depending on foliar treatment of plantations
Source: developed by the authors

H.-F. Xu *et al.* (2025) in their paper noted that the application of amino acid and mineral foliar fertiliser contributes to an increase in soluble sugars in the 'Snick' apple cultivar, simultaneously improving Brix values and the accumulation of soluble dry substances. In addition, they noted that amino acid fertilisation stimulates fruit metabolism and contributes to increased sweetness while maintaining optimal acidity. Similar results were described by S. Mousavi *et al.* (2024), who showed that the use of seaweed extract in 'Golden Delicious' not only increased the content of soluble sugars by 1.2-1.5% compared to the control, but also improved the sugar-to-acid ratio, which positively affects the taste quality of fruits. The researchers also noted that foliar treatments increased the activity of enzymes involved in sugar synthesis, which fully corresponds to observations regarding the effectiveness of Poliactiv Twinfos in the current study.

Regarding organic acids, the present data showed their decrease with the application of phosphite preparations. In September, the acid content in fruits ranged from 0.304% in the control variant (water treatment) to 0.272% in the variant with Poliactiv Twinfos, 0.278% – in the variant with Poliactiv Twinfos Amino, and 0.288% – with the treatment of Ecoline Phosphite K-Amino (Fig. 5). The use of Poliactiv Twinfos during the fruit ripening period led to a reduction in organic acid content by 0.033% compared to the control.

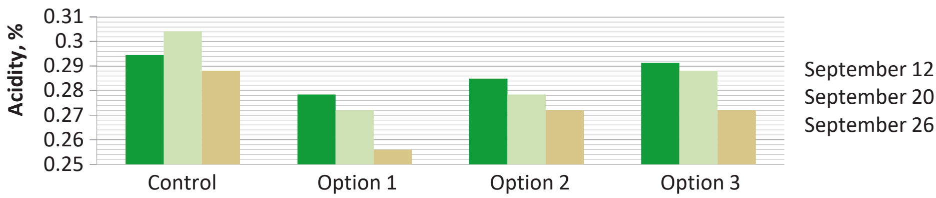

Figure 5. Dynamics of organic acid accumulation in 'Fuji' apple fruits during ripening depending on foliar treatment of plantations

Source: developed by the authors

Similar tendencies are observed in other studies. S. Yang *et al.* (2023) noted that foliar fertiliser based on seaweed extract contributes to a moderate decrease in acidity and a simultaneous increase in the sugar content of 'Fuji' fruits. F. Mirzavandi *et al.* (2025) showed that the combined application of phosphorus with nitrogen and iron increases the concentration of soluble sugars in fruits and moderately reduces acidity. In addition, S. Banyal (2021) noted that foliar application of mineral fertilisers during the fruit filling period increases sweetness and SDS, while acidity decreases within 0.02-0.04%, which corresponds well to the observations regarding the effect of Poliactiv Twinfos. Thus, the results indicate that phosphite fertilisation not only stimulates the accumulation of soluble dry substances and sugars in fruits, but also provides a moderate reduction in organic acidity, which contributes to the optimal combination of sweetness and taste properties of 'Fuji' apples.

CONCLUSIONS

As a result of the conducted studies, it was established that foliar feeding of 'Fuji' apple trees with the phosphite fertilisers Polyactive Twinphos, Polyactive Twinphos Amino, and Ecoline Phosphite K-Amino has a significant positive effect on the development of fruit quality indicators. In particular, an increase in soluble sugar content and a simultaneous decrease in titratable acidity were noted compared to the control variant, which directly affects the taste properties of the fruits and their consumer attractiveness. The improvement of the chemical composition of fruits is conditioned by the activation of key biochemical processes during the ripening period, in particular, the optimisation of carbohydrate metabolism, transformation of organic acids, increased activity of enzymes responsible for the synthesis of biologically active compounds, and improvement of the antioxidant potential of the fruits. This indicates a systemic effect of phosphite fertilisers on the physiological and biochemical processes of the plant, ensuring more uniform fruit development and increasing their market value.

Based on the obtained results, for commercial plantings of 'Fuji' apple trees, it is advisable to carry out foliar feeding during the fruit ripening phase using the Polyactive Twinphos fertiliser at a rate of 1.5 L/ha, which will contribute to an increase in the consumer quality of the fruits and improvement of their taste characteristics. The use of other phosphite preparations, such as Polyactive Twinphos Amino and Ecoline Phosphite K-Amino, is also effective; however, it is advisable to carry out comparative testing to determine the optimal rate and frequency of application. Prospects for further research lie in a comprehensive study of the effect of phosphite fertilisers on morpho-physiological and biochemical indicators of apple trees of different varieties at various phases of vegetation, and in combination with other adaptive agrotechnical measures (irrigation, mulching, biostimulant application). Particularly relevant is the study of the mechanisms of phosphite action under conditions of summer heat and water stress, and their impact on the storage duration of fruits and the development of resistance to physiological disorders. In addition, it is advisable to investigate the economic efficiency of their use in commercial plantings by evaluating the ratio of fertiliser costs to product quality improvement.

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CONFLICT OF INTEREST

The publication of the research data has been agreed upon between the authors and the company "Ecoorganic".

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Дослідження впливу дії фосфітних добрив на якість плодів яблуні в насадженнях сорту 'Фуджі'

Анотація. Метою роботи було встановлення ефективності застосування позакореневого підживлення фосфітними добривами компанії ТОВ «Екоорганік» в насадженнях яблуні сорту 'Фуджі' та визначення їх впливу на динаміку накопичення сухих розчинних речовин, цукрів і органічних кислот у плодах. Дослідження проводили у 2022 році в умовах дослідних насаджень Уманського національного університету садівництва (Україна). У досліді порівнювали контрольний варіант без внесення добрив і варіанти з обробкою трьома видами добрив («Поліактив Твінфос», «Поліактив Твінфос Аміно», «Еколайн Фосфітний К-Аміно»), у дозі 1,5 л/га у фазу дозрівання плодів. Для оцінки біохімічного складу плодів використовували рефрактометричні, спектрофотометричні та титрометричні методи аналізу. Результати досліджень засвідчили, що позакореневе підживлення істотно впливало на біохімічні показники якості плодів яблуні. Упродовж періоду дозрівання плодів у всіх дослідних варіантах спостерігалося зростання вмісту сухих розчинних речовин, однак найвищі їх значення зафіксовано при застосуванні добрива «Поліактив Твінфос», де цей показник достовірно перевищував контроль. Аналогічну закономірність установлено щодо накопичення цукрів: максимальний їх вміст відзначено у плодах за обробки добривом «Поліактив Твінфос», тоді як застосування добрив «Поліактив Твінфос Аміно» та «Еколайн Фосфітний К-Аміно» також забезпечувало підвищення цього показника порівняно з контролем. Водночас у всіх варіантах із позакореневим підживленням спостерігалося зниження вмісту органічних кислот у плодах, що сприяло формуванню більш збалансованого смаку. Найбільш виражений ефект зменшення кислотності встановлено при використанні добрива «Поліактив Твінфос». Практична цінність роботи полягає в науковому обґрунтуванні доцільності застосування позакореневого підживлення яблуні сорту 'Фуджі' у фазу дозрівання плодів з метою підвищення цукристості, зниження кислотності та покращення товарних і споживчих якостей плодів в умовах зростання кліматичних стресів

Ключові слова: позакореневе підживлення; біохімічний склад плодів; сухі розчинні речовини; вміст цукрів; органічні кислоти

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Effect of foliar nutrition on the content of organic compounds in apple fruits (*Malus domestica* Borkh.)

Abstract. The article presents the results of a study on the effect of foliar application of calcium-containing products HelpRost Ca, Brexil Ca and Avangard Ca on the accumulation of soluble solids, sugars, titratable acids and total phenolics in the fruits of apple cultivars 'Ligol', 'Jonagold Early Queen' and 'Fuji'. The cultivar 'Fuji' showed the highest responsiveness to treatments, where the application of Brexil Ca (2.0 kg/ha) and Avangard (6.0 L/ha) increased the soluble solids content to 16.5-16.7% (compared with 14.6% in the control) and sugars to 13.6-13.7%. In the 'Ligol' cultivar, the highest sugar content (9.7-9.8%) was recorded following the application of HelpRost Ca (3.0 L/ha) and Brexil Ca (2.0 kg/ha). The 'Jonagold' cultivar responded positively to HelpRost Ca (3.0 L/ha), with an increase in sugar content of 0.4%. A cultivar-specific dynamics of titratable acids was identified. In 'Ligol', all treatments increased the content of titratable acids, whereas in 'Fuji' a decrease to 0.42% was observed. The highest sugar-acid ratio (32) was recorded in 'Fuji' under the maximum rates of Brexil Ca and Avangard. The highest antioxidant activity was also observed in 'Fuji', where the maximum total phenolic content reached 227 mg/100 g following treatment with Avangard (6.0 L/ha). For 'Ligol', Brexil Ca proved to be the most effective (155 mg/100 g), whereas in 'Jonagold' no statistically significant difference from the control was found for this parameter. The practical value of the results lies in enabling a scientifically grounded selection of calcium foliar treatments and application rates for different apple cultivars

Keywords: calcium-containing preparations; sugars; titratable acidity; carbohydrates; polyphenols

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INTRODUCTION

Apple (*Malus domestica* Borkh.) occupies a leading position in global fruit production, owing to the outstanding biochemical composition and excellent storage potential of its fruits. As of 2025, world apple production stood at 81.7 million tonnes, with plantations covering 4.7 million ha (OECD & FAO, 2025). According to O. Shubravska *et al*. (2025), apple orchards in Ukraine occupy 76,000 ha, total production amounts to 1.1 million tonnes, and yields on commercial farms reach 45-65 t/ha. S. Yang *et al*. (2021) emphasised that sugars account for 85-90% of the soluble solids content in apple fruits, with fructose being the dominant sugar, making apples an important component of dietary nutrition. As noted by H. Nybom *et al*. (2020), the sugar-acid ratio – a key determinant of dessert quality and flavour – depends not only on sugar content but also on the concentration of titratable acids. The accumulation of sugars and other biochemical constituents in apple fruits is governed by a combination of factors, among which cultivar characteristics, climatic conditions, ripening stage, as well as the mineral nutrition system and orchard management practices play an important role. In particular, according to I. Ahmad & M. Sajid (2025), agronomic practices, including balanced application of macro- and micronutrients, substantially influence the intensity of synthesis and accumulation of sugars, organic acids, and biologically active compounds, which is directly reflected in fruit quality and nutritional value.

I. Ali *et al*. (2021) demonstrated that foliar application of boron and zinc to trees of the 'Royal Gala' cultivar facilitated improved sugar transport from leaves to fruits. Specifically, the soluble solids content in apples from treatments with foliar feeding was 10-15% higher than in the control. O. Sürücü & Z. Küçükyumuk (2023) noted that treatment of apple orchards with manganese and zinc compounds contributes to the stability of titratable acids even under drought conditions, preventing their excessive degradation. X. Yang *et al*. (2024) demonstrated that the contents of fructose and glucose in apples increase following treatment with amino acid-chelated iron; the researchers attributed this to enhanced photosynthetic activity of leaves and accelerated transport of sugars to fruits via the phloem, whilst the quantity of organic acids remained unchanged. According to S. Soppelsa *et al*. (2020), treatment of apple trees with seaweed as a biostimulant promotes an increase in titratable acids in the fruits, which, as the authors note, is beneficial for cultivars intended for prolonged storage, given that titratable acids serve as a natural preservative and respiratory substrate in apples. Foliar application of macro- and micronutrient complexes to apple trees promotes improvement of the biochemical composition of fruits, in particular an increase in soluble sugars and soluble solids, and also exerts a positive influence on fruit quality and storability (Świerczyński *et al*., 2022; Xu *et al*., 2025). A. Hassan *et al*. (2024) reported that the application of nano-potassium and phenylalanine 30 days before harvest increased the concentration of pigment-forming compounds (anthocyanins) and sugars, whilst not causing excessive reduction in organic acid levels, thereby preserving their optimal ratio with sugars – a key indicator of harmonious flavour and high fruit quality.

Given the data obtained by various researchers across different regions and for different apple cultivars, studying the effect of calcium-containing products is necessary for developing a nutritional programme that will ensure not only high contents of organic and biologically active substances, but also optimal storability of fruits during the post-harvest period. The objective of the study was to determine the effect of foliar treatments with calcium-containing products (HelpRost Ca, Brexil Ca, and Avangard) applied at different rates on the contents of soluble solids, sugars, titratable acids, and total phenolics in the fruits of apple cultivars 'Ligol', 'Jonagold Early Queen' ('Jonagold'), and 'Fuji' at harvest maturity.

MATERIALS AND METHODS

Fruits for the study were sampled in the educational and research laboratory orchard "Plidoovochevyi Sad" (Fruit and Vegetable Garden) of the National University of Life and Environmental Sciences of Ukraine (Kyiv, latitude 50.3838° N, longitude 30.5042° E; altitude 188 m a.s.l.), located in the Polissia natural climatic zone of Ukraine. The regional climate is moderately continental, with mild winters and warm summers. According to long-term average data (2015-2025), the mean annual temperature in the region was 10.4°C, the absolute minimum was -20.2°C, the maximum +32.2°C, and mean annual precipitation was 598 mm. The experimental apple orchards of cultivars 'Ligol', 'Jonagold', and 'Fuji' were established in 2019; the planting scheme for the first two cultivars was 4 × 1 m (rootstock M.9), and for 'Fuji' – 4 × 2.5 m (rootstock 54-118). The orchards were managed under rainfed conditions. The soils of the experimental plot are sod-medium podzolised soils on fluvioglacial deposits, suitable for horticultural production. Humus content was 1.8%, pH – 6.5, phosphorus supply – 8 mg/100 g, and potassium – 10 mg/100 g. To raise nutrient levels to their optimum (phosphorus – 10 mg/100 g and potassium – 12 mg/100 g), double superphosphate (60 g/tree) and potassium chloride (125 g/tree) were applied in autumn during 2022-2024. Nitrogen fertilisation was carried out annually in three stages: the first at bud burst, the second two weeks after flowering, and the third after the second wave of fruit drop. Fertilisers were applied to the root zone at a rate of 200 g ammonium nitrate per tree.

Pest and disease management in the apple orchards was conducted in accordance with the standard spray programme for apple orchards developed by Syngenta. In addition, the orchards of the abovementioned cultivars were treated with calcium-containing products throughout the growing season: the first application was carried out at the end of June after fruit drop, subsequent applications at 20-day intervals, and the final application 14 days before harvest. The study was conducted in three separate experiments, with cultivar as the factor; each experiment comprised six treatment variants (including a control) in three replicates of five trees each; the duration of the study was two years. The products used, their application rates, and compositions are presented in Table 1.

Table 1. Products for foliar fertilisation of apple cultivars 'Ligol', 'Jonagold', and 'Fuji', application rates, and composition

Product (variant)	Application rate	Composition
Control	600 L/ha	**H_2O – 100%**
HelpRost Ca	1.0 L/ha	CaO – 150 g/L; N – 40-60 g/L; amino acids (more than 16 types) – 50 g/L; vitamins; polysaccharides
HelpRost Ca	3.0 L/ha	
Brexil Ca	1.8 kg/ha	Ca – 20%; B – 0.5%
Brexil Ca	2.0 kg/ha	
Avangard Calcium+M/E (Avangard)	3.0 L/ha	N – 100 g/L; CaO – 180 g/L; MgO – 10 g/L; B – 0.25 g/L; Fe – 0.25 g/L; Mn – 0.1 g/L; Cu – 0.4 g/L; Zn – 0.4 g/L; Mo – 0.02 g/L; Co – 0.01 g/L
Avangard Calcium+M/E (Avangard)	6.0 L/ha	

Source: developed by the authors

Analytical examination of apple fruits grown under the different foliar fertilisation variants was carried out at the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine. Fruits were sampled at harvest maturity, determined by the degree of starch degradation. For the studied cultivars, this indicator corresponded to a score of 6. Soluble solids content was determined using a portable refractometer ATAGO PAL-1 (Japan). Fruit quality parameters were assessed using the standard methods described by P.V. Kondratenko *et al.* (2008). For the preparation of analytical samples, fruits (5-7 specimens) were homogenised, after which a drop of juice was expressed through muslin onto the refractometer prism; temperature correction was applied when recording readings. Data were expressed as a percentage on a fresh weight basis. Sugar content was determined by a spectrophotometric method. For this purpose, a ground sample of apples was washed with hot distilled water. The resulting extract was purified from proteins and pigments by precipitation with lead acetate. Sucrose was hydrolysed to glucose and fructose by heating in the presence of 10% hydrochloric acid. The hydrolysis products were oxidised with Fehling's solution. The optical density of the resulting solutions was measured on a ULAB 102UV spectrophotometer at a wavelength of 640 nm. The sugar content in the sample was calculated using the calibration curve equation. To construct the calibration curve relating optical density (absorbance units) to glucose concentration (mg/mL), standard glucose solutions of varying concentrations were used. Data were expressed as a percentage on a fresh weight basis. Titratable acid content was determined by alkalimetric titration to a colour endpoint. For acid extraction, a 25 g ground sample was transferred without losses – by rinsing with hot distilled water in a volume not exceeding 150 mL – into a 250 mL volumetric flask. The flask was held in a water bath at 80°C for 30 min and then cooled. The contents were brought to volume with distilled water and filtered through a filter paper into a 250 mL conical flask. A 20 mL aliquot of the extract was transferred by pipette into a 250 mL conical flask, 3-4 drops of phenolphthalein were added, and the mixture was titrated with 0.1 N sodium hydroxide solution until the appearance of a pale pink colour corresponding to pH 7.0. The titratable acid content in the sample was calculated from the formula, taking into account the titre of the 0.1 N sodium hydroxide solution and the conversion factor for citric acid. Data were expressed as a percentage on a fresh weight basis. The sugar-acid ratio was calculated as the ratio of total sugar content to titratable acid content in apple fruits.

For polyphenol extraction, a weighed sample was ground in a porcelain mortar with the addition of a small quantity of ethanol and filtered under vacuum through a Büchner funnel using a blue-ribbon filter paper into a Büchner flask. The residue on the filter was washed with small portions of ethanol until complete decolorisation of the sample. The volume of ethanol used (mL) was recorded. Into a test tube, 7.9 mL of distilled water, 0.1 mL of the extract, and 1 mL of Folin-Denis reagent were introduced, mixed, and after 3 minutes 1 mL of saturated sodium carbonate solution was added and mixed again. After one hour, the optical density of the tube contents was measured on a ULAB 102UV spectrophotometer at a wavelength of 640 nm. The blank was prepared as follows: 8 mL of distilled water and 1 mL of Folin-Denis reagent were placed in a test tube, mixed, after 3 minutes 1 mL of saturated sodium carbonate solution was added and mixed again. No fewer than three replicate measurements were taken and the mean optical density value was calculated. The polyphenol content in the sample was calculated using the calibration curve equation. To construct the calibration curve relating optical density (absorbance units) to chlorogenic acid concentration (μg/mL), standard chlorogenic acid solutions of varying concentrations were used. Data were expressed as mg/100 g fresh weight. All research involving plant material was conducted in compliance with international ethical requirements for the use of biological resources in accordance with the provisions of the Convention on Biological Diversity (1992). Meteorological data for the study years were obtained from a Vantage Pro2 Plus weather station located at the educational and research orchard laboratory of the National University of Life and Environmental Sciences of Ukraine. Statistical assessment of the effect of foliar treatments on the biochemical parameters of apple fruits was performed using one-way analysis of variance (ANOVA). The analysis was conducted separately for each cultivar, with 'Treatment variant' (including the untreated control) as the independent factor. Prior to analysis, data were tested for normality of distribution (Shapiro-Wilk test) and homogeneity of group variances (Levene's test). Assessment of the significance of differences between means within each cultivar was carried out using Tukey's test.

RESULTS AND DISCUSSION

The soluble solids content in fruits of the 'Ligol' cultivar ranged from 11.7% (control and the Avangard variant at 3.0 L/ha) to 12.4% in the variant with Brexil Ca (1.8 kg/ha). A significantly higher soluble solids content compared with the control was recorded, in addition to the above-mentioned variant, in fruits grown with the application of HelpRost Ca (3.0 L/ha). In the variants with Brexil Ca (2.0 kg/ha) and both Avangard variants, the soluble solids content was within the margin of statistical error (Table 2).

Table 2. Soluble solids and sugar contents in apple fruits depending on foliar treatment with calcium-containing products, % per 100 g fresh weight

Variant	Soluble solids			Sugars		
	'Ligol'	'Jonagold'	'Fuji'	'Ligol'	'Jonagold'	'Fuji'
Control	11.7±0.1	13.5±0.1	14.6±0.2	8.7±0.2	9.4±0.1	11.7±0.2
HelpRost Ca (1.0 L/ha)	11.8±0.2	13.8±0.3	14.5±0.2	8.6±0.3	9.6±0.3	12.9±0.2[a]
HelpRost Ca (3.0 L/ha)	12.1±0.2[a]	14.1±0.2[a]	14.6±0.2	9.7±0.1[a]	9.8±0.2[a]	13.5±0.2[a]
Brexil Ca (1.8 kg/ha)	12.4±0.1[a]	13.8±0.2	16.0±0.2[a]	9.8±0.1[a]	9.4±0.2	13.0±0.2[a]
Brexil Ca (2.0 kg/ha)	11.8±0.2	13.6±0.2	16.5±0.2[a]	9.0±0.2	9.4±0.2	13.6±0.2[a]
Avangard (3.0 L/ha)	11.7±0.3	14.2±0.1[a]	16.6±0.2[a]	9.0±0.2	9.8±0.1[a]	12.6±0.3[a]
Avangard (6.0 L/ha)	11.8±0.2	14.2±0.1[a]	16.7±0.2[a]	9.2±0.2[a]	9.4±0.1	13.7±0.2[a]

Note: [a] indicates values that differ significantly from the control variant
Source: developed by the authors

In the 'Jonagold' cultivar, the soluble solids content was significantly higher than in the control (13.5%) in the variants where HelpRost Ca (3.0 L/ha) and Avangard (at both rates) were applied foliarly. When HelpRost Ca (1.0 L/ha) and Brexil Ca at rates of 1.8 and 2.0 kg/ha were used for foliar feeding, no significant difference from the control was established. Fruits of the 'Fuji' cultivar grown in orchards treated with HelpRost Ca at rates of 1.0 and 3.0 L/ha were characterised by a soluble solids content at the level of the control, namely 14.5 and 14.6% respectively, compared with 14.6% in the control. However, 'Fuji' fruits grown with the foliar application of Brexil Ca (1.8 and 2.0 kg/ha) and Avangard (3.0 and 6.0 L/ha) accumulated significantly more soluble solids than those grown in the control. The sugar content in 'Ligol' fruits in the control was 8.7%. Values at the level of the control (within the margin of statistical error) were recorded in the variants with HelpRost Ca (1.0 L/ha), Brexil Ca (2.0 kg/ha), and Avangard (3.0 L/ha), namely 8.6 and 9.0% in the latter two variants respectively. A higher sugar content than in the control was found in apples from trees treated with HelpRost Ca at 3.0 L/ha (9.7%), Brexil Ca at 2.0 kg/ha (9.8%), and Avangard at 6.0 L/ha (9.2%). 'Jonagold' apples harvested from the variants with HelpRost Ca (3.0 L/ha) and Avangard (3.0 L/ha) contained 0.4% more sugar than the untreated control. No significant increase in sugar content was established in the other 'Jonagold' variants; in particular, apples from trees treated with Brexil Ca at both studied rates and with Avangard (6.0 L/ha) had a sugar content at the level of the control – 9.4%. In the 'Fuji' cultivar, the sugar content in all treatment variants with calcium-containing products was significantly higher than in the control, where it stood at 11.7%. A sugar content exceeding 13.5% was recorded in apples grown with Avangard (6.0 L/ha) and Brexil Ca (2.0 kg/ha), at 13.7 and 13.6% respectively (Table 2).

In the variants with calcium-containing product treatments of 'Ligol' apple orchards, the titratable acid content was higher than in the water control. The greatest difference from the control (by 0.20%) was observed with Avangard at both rates (3.0 and 6.0 L/ha), where the titratable acid content was 0.80%. The smallest difference from the control was recorded with Brexil Ca (2.0 kg/ha), at 0.95% (Table 3).

Table 3. Titratable acid content in apple fruits and sugar-acid ratio depending on foliar treatment with calcium-containing products

Variant	Titratable acids, % per 100 g fresh weight			Sugar-acid ratio		
	'Ligol'	'Jonagold'	'Fuji'	'Ligol'	'Jonagold'	'Fuji'
Control	0.60±0.03	0.66±0.01	0.50±0.2	15	14	24
HelpRost Ca (1.0 L/ha)	0.76±0.01[a]	0.62±0.01[b]	0.52±0.2	11	16	25
HelpRost Ca (3.0 L/ha)	0.80±0.07[a]	0.65±0.02	0.48±0.2	12	15	28
Brexil Ca (1.8 kg/ha)	0.75±0.05[a]	0.64±0.02	0.42±0.3[b]	13	15	31
Brexil Ca (2.0 kg/ha)	0.69±0.07[a]	0.74±0.01[a]	0.42±0.3[b]	13	13	32

Table 3, Continued

Variant	Titratable acids, % per 100 g fresh weight			Sugar-acid ratio		
	'Ligol'	'Jonagold'	'Fuji'	'Ligol'	'Jonagold'	'Fuji'
Avangard (3.0 L/ha)	0.80 ± 0.05[a]	0.57 ± 0.01[b]	0.43 ± 0.3[b]	11	17	29
Avangard (6.0 L/ha)	0.80 ± 0.05[a]	0.73 ± 0.02[a]	0.42 ± 0.3[b]	12	13	32

Note: [b] indicates values that differ significantly from the control variant
Source: developed by the authors

In the 'Jonagold' cultivar, the titratable acid content in the variants with HelpRost Ca (3.0 L/ha) and Brexil Ca (2.0 kg/ha) was within the margin of the control error, at 0.65 and 0.64% respectively. The most significantly elevated acidity was recorded in 'Jonagold' fruits grown in the variants with Brexil Ca at 2.0 kg/ha (0.74%) and Avangard at 6.0 L/ha (0.73%). The titratable acid content in 'Fuji' fruits in the variants with HelpRost Ca (1.0 and 3.0 L/ha) was within the margin of the control error, at 0.52 and 0.48% respectively. In the remaining treatment variants, the titratable acid content in 'Fuji' fruits was significantly lower than in the untreated control and ranged from 0.42% in the variants with Brexil Ca at both rates and Avangard (3.0 L/ha) to 0.43% in the Avangard (6.0 L/ha) variant. The sugar-acid ratio in 'Ligol' fruits ranged from 15 (control) to 11 in the Avangard variant at 3.0 L/ha. In the 'Jonagold' cultivar, the sugar-acid ratio was higher than in the control (14) in the HelpRost Ca variants at both rates – 16 and 15 respectively – and also in the variant with Brexil Ca (1.8 kg/ha). In the 'Fuji' cultivar, the sugar-acid ratio reached its maximum value (32) in two treatment variants: Brexil Ca (2.0 kg/ha) and Avangard (6.0 L/ha). Values below 30 for this flavour indicator were recorded in the control and in the variant with HelpRost Ca at 1.0 L/ha (Table 3).

The total phenolic content in 'Ligol' apple fruits in the control was 123 mg/100 g; significantly higher values were recorded in the variant with Brexil Ca at 2.0 kg/ha (155 mg/100 g), as well as with HelpRost Ca at 1.0 L/ha (149 mg/100 g) and Avangard at 3.0 L/ha (141 mg/100 g). In the remaining variants, the total phenolic content in 'Ligol' apple fruits was within the margin of the control error (Table 4).

Table 4. Total phenolic content in apple fruits depending on foliar treatment with calcium-containing products, mg/100 g fresh weight

Variant	'Ligol'	'Jonagold'	'Fuji'
Control	123 ± 7	148 ± 17	190 ± 6
HelpRost Ca (1.0 L/ha)	149 ± 12[a]	152 ± 12	213 ± 7[a]
HelpRost Ca (3.0 L/ha)	139 ± 7[a]	150 ± 16	212 ± 8[a]
Brexil Ca (1.8 kg/ha)	119 ± 8	162 ± 11	209 ± 5[a]
Brexil Ca (2.0 kg/ha)	155 ± 11[a]	144 ± 5	218 ± 8[a]
Avangard (3.0 L/ha)	141 ± 8[a]	138 ± 14	215 ± 7[a]
Avangard (6.0 L/ha)	140 ± 12	134 ± 15	227 ± 7[a]

Source: developed by the authors

The total phenolic content in 'Jonagold' apple fruits ranged from 134 to 162 mg/100 g. The maximum value was recorded in the variant with Brexil Ca (1.8 kg/ha), whilst the minimum was observed with Avangard (6.0 L/ha). It should be noted that no statistically significant difference from the control was found in any of the experimental variants for total phenolic content. In 'Fuji' fruits, the maximum total phenolic content was recorded in the Avangard variant at 6.0 L/ha, amounting to 227 mg/100 g, which significantly exceeded the control by 37 mg/100 g. Application of the same product at a lower rate also yielded a value above the control, at 215 mg/100 g. The use of HelpRost Ca at 1.0 and 3.0 L/ha increased total phenolic content by 23 and 22 mg/100 g respectively compared with the control. In the Brexil Ca variants, the control values were exceeded by 19 mg/100 g (at 1.8 kg/ha) and by 28 mg/100 g (at 2.0 kg/ha).

The study established that foliar treatment of 'Ligol', 'Jonagold', and 'Fuji' apple orchards with calcium-containing products affects the soluble solids content in fruits differently, which can be explained by the genetic response of each cultivar to the products applied. In particular, a weak response to most products was established for 'Ligol'. Researchers J. Lanauskas *et al*. (2012) noted that 'Ligol' possesses a genetically determined threshold for sugar accumulation that is difficult to alter through exogenous intervention. The increase in sugar content in fruits of 'Ligol' (up to 9.8%) and 'Fuji' (up to 13.7%) under the influence of Brexil Ca and Avangard is consistent with the findings of S. Murtić *et al*. (2017), who established that foliar treatments with calcium-containing products promote an increase in soluble solids content in fruits. This is associated with the role of calcium as a signalling element that activates carbohydrate metabolism enzymes, in particular sucrose phosphate synthase, which is responsible for sucrose synthesis. The most effective variants for 'Jonagold' were those with Avangard (3.0 and 6.0 L/ha) and HelpRost Ca (3.0 L/ha), where an increase in soluble solids content compared with the control of 0.7 and 0.6% respectively was observed.

The absence of a significant sugar increment in 'Jonagold' with certain rates of Brexil Ca (9.4%, at the level of the control) is consistent with the data of L.C. Argenta *et al*. (2022), who established that excessive or untimely calcium application may sometimes lead to antagonism with other elements – namely potassium: when the Ca/K balance shifts too strongly towards calcium, this can slow the rate of carbohydrate accumulation. A. Asgharzade *et al*. (2012) demonstrated that the effect of calcium fertilisers on the biochemical composition of apples is substantially dependent on the weather conditions of the growing year; in particular, in dry years calcium may retain moisture, thereby reducing sugar content. Of all the cultivars studied, 'Fuji' showed the best soluble solids response to foliar feeding. The soluble solids content of 16.0-16.7% achieved with Brexil Ca and Avangard is high, which is consistent with the results of A.G. Shewa *et al*. (2022), who noted that 'Fuji' has a tendency towards high accumulation of sorbitol and sugars, and that additional feeding with calcium and micronutrient products enhances this potential by improving the osmotic balance of cells. The significant increase in sugar content in 'Fuji' across all treatment variants may be related to the genetic characteristic of this cultivar for late fruit ripening. As noted by I. Ahmad & M. Sajid (2025), late-maturing cultivars have a longer period of carbohydrate complex formation, and calcium protection of cell membranes during this period prevents premature starch degradation, allowing sugars to accumulate gradually and consistently.

For the 'Ligol' cultivar, application of Avangard and Brexil Ca contributed to the maintenance of a higher acid content compared with the control. The increase in acidity by 0.20% with Avangard indicates effective penetration of Ca^{2+} ions into the fruit mesocarp. This is consistent with the data of M. Serrano *et al*. (2004), who noted that exogenous calcium inhibits the activity of enzymes responsible for the degradation of malic acid, which is the principal substrate for respiratory processes during ripening. Similar data were obtained by P. Wójcik (2023), who established that calcium contributes to the maintenance of a higher level of acidity, which is critical for cultivars with moderate acidity, of which 'Ligol' is one. In the 'Jonagold' cultivar, the greatest increase in acidity was observed in the Brexil Ca variants (0.74%). As stated by M. Serrano *et al*. (2004), this allows for the maintenance of the metabolic status of the cell at a higher level. In the 'Fuji' cultivar, in contrast to 'Ligol' and 'Jonagold', a tendency towards a reduction in acidity under the influence of the studied calcium-containing products was established (to 0.42-0.43%). According to the findings of P.R. Ernani *et al*. (2008), in certain cultivars – particularly those with late ripening – an excess of calcium may stimulate specific enzymatic pathways that accelerate the conversion of organic acids to sugars, especially under conditions of high insolation characteristic of the ripening period of 'Fuji'. The highest sugar-acid ratio values (up to 32) were recorded in 'Fuji', which indicates the genetic predisposition of this cultivar towards intensive sugar accumulation against a background of low acidity. The 'Fuji' cultivar is characterised by intensive carbohydrate accumulation, and the application of exogenous calcium (in particular in the form of foliar treatments) promotes an increase in soluble solids content through the activation of sugar metabolism enzymes, notably sucrose phosphate synthase (Murtić *et al*., 2017).

The increase in total phenolic content in 'Ligol' and 'Fuji' in the treatment variants is consistent with the findings of N. Ahmed *et al*. (2024), who noted the important role of micronutrients in the regulation of secondary metabolite biosynthesis, particularly phenolic compounds. Moreover, phenylalanine ammonia-lyase is the key enzyme of the phenylpropanoid pathway, and calcium may indirectly influence its activity through signalling mechanisms, thereby promoting phenolic accumulation (Lang *et al*., 2025). Exogenous calcium is capable of enhancing the activity of antioxidant enzymes and promoting the accumulation of phenolic compounds and flavonoids in plant tissues, as confirmed by a number of studies, as noted by Y. Gao *et al*. (2024). Similar patterns were established by Y. Xi *et al*. (2016) for apple, where treatment with bioactive compounds stimulates the antioxidant system and accumulation of phenolic compounds. The effectiveness of Brexil Ca in promoting phenolic accumulation is attributed to the presence of lignosulphonate in its composition, which facilitates better penetration of calcium through the leaf cuticle, thereby activating phenylpropanoid metabolism (Val *et al*., 2018). The results obtained confirmed that the optimisation of calcium nutrition should be based on the genetic characteristics of the cultivar and growing conditions in order to achieve the maximum quality effect in fruits.

CONCLUSIONS

The 'Fuji' cultivar proved to be the most responsive to foliar treatments with calcium-containing products. The application of Brexil Ca and Avangard contributed to a significant increase in soluble solids content, to 16.0-16.7% compared with 14.6% in the control. For 'Ligol', the best soluble solids result was obtained in the Brexil Ca (1.8 kg/ha) variant; for 'Jonagold' – with HelpRost Ca (3.0 L/ha) and Avangard at both rates. In the 'Fuji' cultivar, all treatment variants ensured a significant increase in sugar content (reaching a maximum of 13.7%), whilst in 'Ligol' and 'Jonagold' the positive effect was selective and dependent on the specific product rate. Foliar feeding affected acidity differently depending on the cultivar. In 'Ligol', treatments contributed to acid accumulation (up to 0.80-0.95%), which is important for prolonged storage. In contrast, in 'Fuji' a decrease in acid content was observed (to 0.42%). The highest flavour quality indices (sugar-acid ratio – 32) were recorded in 'Fuji' with the application of high rates of Brexil Ca (2.0 kg/ha) and Avangard (6.0 L/ha). For 'Jonagold', the best variant was HelpRost Ca. The 'Fuji' cultivar demonstrated the greatest capacity for phenolic compound accumulation under the influence of calcium fertilisers, reaching a maximum of 227 mg/100 g (Avangard, 6.0 L/ha), which is 19.5% above the control. The most universally positive effect on the biochemical composition of fruits (particularly in 'Fuji') was demonstrated by Avangard at 6.0 L/ha and Brexil Ca at 2.0 kg/ha. It was established that foliar calcium application is an effective tool not only for improving storability but also for enhancing the nutritional and flavour characteristics of apples. Further research should focus on optimising the timing and rates of foliar calcium application, taking into account cultivar characteristics, weather conditions, and interactions with other nutritional elements, in order to improve fruit quality and storability.

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Вплив позакореневого живлення на вміст органічних речовин у плодах яблуні (*Malus domestica* Borkh.)

Анотація. У статті представлено результати досліджень впливу позакореневого підживлення препаратами HelpRost Ca, Brexil Ca та Авангард Ca на накопичення сухих розчинних речовин, цукрів, титрованих кислот та загальних фенолів у плодах яблуні сортів ‘Лігол’, ‘Джонаголд Ерлі Квін’ та ‘Фуджі’. Найбільшу чутливість до обробок виявив сорт ‘Фуджі’, де застосування Brexil Ca (2,0 кг/га) та Авангарду (6,0 л/га) забезпечило зростання вмісту сухих розчинних речовин до 16,5-16,7 % (проти 14,6 % у контролі) та цукрів до 13,6-13,7 %. У сорту ‘Лігол’ максимальний вміст цукрів (9,7-9,8 %) зафіксовано при застосуванні HelpRost Ca (3,0 л/га) та Brexil Ca (2,0 кг/га). Сорт ‘Джонаголд’ позитивно зреагував на HelpRost Ca (3,0 л/га), збільшивши цукристість на 0,4 %. Встановлено сортову специфіку динаміки титрованих кислот. У сорту ‘Лігол’ всі варіанти обробок підвищували вміст титрованих кислот, тоді як у сорту ‘Фуджі’ спостерігалося їх зниження до 0,42 %. Найвищий цукрово-кислотний індекс (32) зафіксовано у сорту ‘Фуджі’ при застосуванні максимальної норми Brexil Ca та Авангарду. Найвищу антиоксидантну активність продемонстрував сорт ‘Фуджі’, де зафіксовано максимальний вміст загальних фенолів – 227 мг/100 г (при обробці Авангардом 6,0 л/га). Для сорту ‘Лігол’ ефективним виявився Brexil Ca (155 мг/100 г), тоді як у сорту ‘Джонаголд’ статистично значущої різниці з контролем за цим показником не виявлено. Практична цінність отриманих результатів полягає в можливості науково обґрунтованого добору препаратів і норм позакореневого підживлення кальцієм для різних сортів яблуні

Ключові слова: кальцієвмісні препарати; цукри; титровані кислоти; вуглеводи; поліфеноли

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Features of embryonic development in sugar beet under apospory and cytoplasmic male sterility

Abstract. The aim of the study was to evaluate the theoretical foundations of the phenomenon of high seed self-reproduction in substituted lines and to develop a methodology for studying embryonic development in sugar beet breeding materials with cytoplasmic male sterility (CMS) of different genetic origins. Experimental investigations included field methods, pollen-free seed production in CMS breeding materials, laboratory methods, cytological analysis of embryo

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development during apozygoty over 34 days after flowering fixation, and a cytophotometric method for assessing genome-level ploidy variability in materials with different terms of apozygotic seed reproduction. The methodology for studying embryonic development during apozygoty in materials of different CMS source origins was improved, incorporating flowering marking, shoot isolation, and embryo fixation from 5 to 34 days until complete embryo development. It was established that apozygoty in sugar beet is characterised by delayed genetically determined development of 4, 8, or 10 days within the 28-day developmental cycle, depending on the origin of the material, as well as by polyembryony and a high percentage of degenerated embryos. Adventitious embryogenesis, apospory, and simultaneous embryo development from integuments and nucellus in both micropylar and chalazal regions were characteristic of male-sterile lines of different genetic origins. It was found that substituted lines based on new CMS sources derived from wild species of the genus *Beta* L. exhibit the phenomenon of high seed self-reproduction under pollen-free conditions. The methodology for studying embryo development synchrony was developed to investigate the nature of apozygoty (generative and somatic embryogenesis) in substituted lines with introduced sterile cytoplasms, compared with monoecious male-sterile lines carrying the F. Owen S-vulgaris cytoplasm. The type of apozygoty manifested through gametophytic and sporophytic embryogenesis and determined the genetic heterogeneity (variability in quality) of apozygotic seeds. Apomixis using substituted lines and new sterile cytoplasms derived from wild species of the genus *Beta* L., combined with a high percentage of apomictic seed reproduction, may provide significant advantages for the production of new crop hybrids, particularly through the fixation of heterosis and preservation of the maternal plant genotype

Keywords: adventitious embryogenesis; apospory; new sources of cytoplasmic male sterility; *Beta maritima*; *Beta patula*; cytoplasmic male sterility phenotypes

INTRODUCTION

Apomixis is a process of asexual plant reproduction that yields seeds in the absence of meiosis and fertilisation. Since apomictic plants can produce clonal offspring, preserving the genotype of the maternal plant through seeds, this process offers numerous agronomic advantages for crop production, namely the fixation of heterosis, the introduction of new germplasm from wild species into the breeding process, the simplification of hybrid seed production, and positive variation in vegetatively propagated varieties. In sugar beet breeding, the application of apomixis using substituted lines with new sources of sterile cytoplasms from wild species of the genus *Beta* L., *Beta maritima* and *Beta patula*, consolidates the positive characteristics of the maternal component controlled by the cytoplasmic genome, such as disease resistance, early carbohydrate accumulation, high adaptive potential for the development of stubble-free seed production, the creation of energy hybrids with a high monosaccharide content, and the simplification of sterility maintainer breeding.

F. Krahulec *et al*. (2020) indicated the existence of various modes of seed reproduction as intra-population polymorphism of reproductive traits. Y. Xu *et al*. (2022) note that apomixis can duplicate genotypes corresponding to the maternal plant, and that it has important applications for genotype fixation and acceleration of the breeding process. D. Li *et al*. (2021) concluded that apomixis occurs in more than 400 plant species but remains insufficiently studied among major agricultural crops. However, A. Schmidt (2020) indicates that achieving practical application of apomixis in breeding requires in-depth theoretical investigation of the epigenetic regulation of genes using methods for analysing the genetic basis of their expression. P. van Dijk *et al*. (2020) investigated the dominance of the parthenogenesis allele in dandelions and the complex nature of autonomous perisperm formation. Based on experimental results, K. Wang *et al*. (2021) concluded that the developmental characteristics of embryos during apozygoty remain insufficiently studied, as in some cases they may clone the genotype, or have a recombinant basis and develop from embryo sac cells. Based on the research of M. Roik *et al.* (2024), high economic interest and prospects for profit are already determining competition among the world's leading agricultural corporations (Syngenta, Pioneer Hi-Bred International, Rijk Zwaan Zaadteelt B.V., Partec GmbH, and others). Embryological investigations play a primary role in establishing the nature of embryos – specifically gametophytic reduced and unreduced parthenogenesis – but require the refinement of methodologies for studying the synchrony of apomictic and hybrid embryo development using genetic selective markers. Using embryological investigations, the authors established that, depending on the mode of embryo sac formation, the nature of apozygotic embryo development is determined by the type of apomixis: diplospory, apospory, adventitious embryogenesis, and parthenogenesis. In diplospory, the embryo sac develops from an unreduced megasporocyte. In some cases, meiosis may be replaced by mitosis or the second division may be absent. Cytoembryological investigations showed that in aposporous embryo sacs of this type, parthenogenetic embryos form and develop to the globular stage, after which further development does not occur due to the absence of endosperm.

D. Li *et al.* (2021) and K. Wang *et al.* (2021) demonstrated that haploid parthenogenesis occurs in reduced embryo sacs whose cells were formed as a result of meiotic division. They showed that haploid embryos, if diploidisation does not occur, are not viable; their proportion amounts to only 1-2%, and this phenomenon has been described in a range of species (*Datura*, *Nicotiana*, *Triticum*, *Oryza*, *Hordeum*, *Brassica*, *Crepis*, *Solanum*, *Zea*, *Gossypium*, and others). The proportion of haploid parthenogenesis and haploid embryos in certain species, such as the alloplasmic line of *Triticum aestivum*, can reach 90%. It has been established that seeds of sugar beet breeding materials formed without pollination, by virtue of polyembryony, are characterised by genetic heterogeneity and the presence of two or three seedlings in certain schizocarpic fruits. B. Heidemann *et al.* (2025) concluded that low seed productivity is characteristic of many other cultivated plant species, including sugar beet, regardless of the type of apozygoty, and represents one of the unresolved challenges for the broad application of this new mode of seed reproduction in breeding. Y. Xu *et al.* (2022) note that in most species, nucellar and integumental embryogenesis is combined with generative parthenogenesis and with embryos that penetrate the embryo sac from outside. The simultaneous presence of a haploid embryo derived from reduced cells and an adventitious embryo derived from somatic cells is entirely possible. Among their principal differences are: differing ploidy levels of embryo sac cells (aposporous and meiotic); differences in embryo genome structure (with the maternal genome, as in adventitious embryogenesis, or recombinant, derived from reduced and unreduced embryo sac cells). The majority of studies on apozygoty in sugar beet are ambiguous and contradictory, and require additional experimental data using methods of genetics, embryology, and cytogenetics.

The aim of this article was to investigate the characteristics of embryonic development of embryos under pollen-free conditions in sugar beets with different sources of sterility derived from wild species of the genus *Beta* L., *Beta maritima*, and *Beta patula*, and to refine the methodology for analysing the synchrony of embryogenesis stages in maternal components with sterile cytoplasms of different genetic origins, with a view to identifying breeding accessions with a high rate of apozygotic seed set.

MATERIALS AND METHODS

The investigations were conducted during the period 2016-2023. Experimental material for embryological studies was selected on the basis of phenotypic traits: monoecism, sterility under apozygoty, and genome ploidy level. The following source material was used: the best apozygotic progenies A_4 of sugar beet for embryogenesis analysis and characterisation of apozygoty types – 12-136MS, 12-157MS, 12-222MS, 12-138MS, 12-153MS; substituted sugar beet lines based on sterile cytoplasms of wild species *Beta maritima* and *Beta patula* with apozygotic seed reproduction and high seed productivity, comprising the following breeding accessions: No. 15/1BC$_2$S (Greece), No. 15/2BC$_2$S (Greece), No. 15/3BC$_2$S (Greece), No. 16/1BC$_2$S (Greece), No. 16/2BC$_2$S (Greece), No. 16/3BC$_2$S (Greece), No. 3/1 dihaploid, F$_1$S clones (Turkey) *in vitro*, No. 3/2 dihaploid, F$_1$S clones (Turkey) *in vitro*, No. 1/17BC$_3$S *patula*, No. 2/7BC$_3$S *patula*; substituted lines of the third, fourth, fifth, and sixth backcross cycles were studied under pollen-free conditions in comparison with the development of hybrid embryos of *Beta vulgaris* cmS 0-type; breeding accessions: BC$_6$S maritima (Turkey) – c.2/1, c.4/1, c.5/1; BC$_6$S *patula* (Portugal, Madeira Island) – c.1/4, c.3/4, c.4/4, c.3/7; BC$_6$S *maritima* (Turkey) – c.5/1, c.7/2, c.6/3, c.7/5, for studying the seed productivity of new plasmotypes.

The characteristics of apozygotic seed reproduction in substituted lines were determined using a modified methodology for evaluating embryogenesis in sugar beet, adjusted with respect to flowering timing (Kovalchuk *et al.*, 2019). Selection based on sterility and monoecism traits was carried out in group isolators and in the plant-breeding glasshouse complex at Yaltushkivska Research Breeding Station, in accordance with the classification of F. Owen (1945). Monoecism was determined by characterising fruits on central shoots. Microscopic examination of preparations was performed using Carl Zeiss (Germany) and Polam (Poland) microscopes at a magnification of 5 × 12.5, and MBS-11 stereomicroscopes. The ploidy level of experimental apomictic materials was stabilised using the flow cytophotometry method and a Partec ploidy analyser.

The efficacy of embryo staining on embryological preparations was assessed on days 16 and 28 after flowering marking. Stains applied included: 0.03% methylene blue, iodine solution, and bromophenol orange. Seed set under pollen-free conditions in substituted lines was studied over a 10 cm segment in 5 replications per seed-bearing plant. Breeding accessions were evaluated in relation to one another by origin. Data were processed according to statistical analysis methods (Prysiazhniuk *et al.*, 2016). For each seed-bearing plant, the mean value of embryo development per 10 cm segment was determined using formula (1):

$$X = \frac{\sum \bar{M} - x}{n}, \tag{1}$$

where X is the mean number of developed seeds per 10 cm segment under apozygoty; x is the number of seeds per 10 cm segment for each breeding accession; $\bar{M}$ is the mean number of developed seeds for each seed-bearing plant across 5 replications per 10 cm segment; n is the total number of seeds from each seed-bearing plant across all 5 replications.

The standard error of the arithmetic mean is determined by formula (2):

$$m = \frac{\eth}{\sqrt{n}}, \tag{2}$$

where ð is the standard deviation of the arithmetic mean for each measurement variant, namely the number of fruits per 10 cm segment; *n* is the total number of fruits for each breeding accession (seed-bearing plant).

The investigations were conducted in accordance with the ethical standards of the Convention on Biological Diversity (1992) and the Convention on the Trade in Endangered Species of Wild Fauna and Flora (1973).

RESULTS AND DISCUSSION

To refine the methodology for differentiating early stages of embryogenesis during apozygoty, fixation of ovules in Carnoy's solution (3:1) was applied, along with various maceration and staining techniques for studying embryo sacs. Embryo development in sugar beet is genetically determined (Kovalchuk *et al.*, 2019). The characteristics of embryonic development of apomictic embryos in male-sterile lines of sugar beet at Yaltushkivska Research Breeding Station were studied. Thus, in apomictic embryos of the second cycle of apomictic reproduction (A$_2$), degeneration on day 28 after fixation occurred in 16.3% of cases. By day 28, a normal hybrid embryo should have been fully developed. Such development was observed in only 3.3% of ovules. Instead, ovules with embryo development at the 1/2 ES stage accounted for 23.3%, and at the 1/4 ES stage for 24.3%. This indicates a developmental delay of 8-10 days. The phenomenon of polyembryony – the development of two embryos – was also observed; the proportion of such plants was high and amounted to 32.7% (Fig. 1).

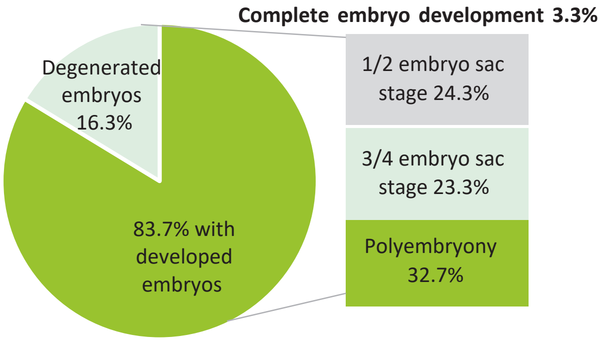

Figure 1. Embryogenesis of embryos from apozygotic progenies, day 28, A_2
Note: A_2 – apomictic pollen-free development of the second reproduction cycle
Source: compiled by the authors

The results of apomictic embryo development in the best A_4 progenies of sugar beet obtained under pollen-free conditions are presented in Table 1.

Table 1. Analysis of embryogenesis and characterisation of apozygoty types in the best apozygotic progenies A_4 of sugar beet

Breeding accession	Embryos examined, n	Unfertilised, %	Number of embryos with developmental delay, %	Anomalous embryo position, %		Type of apozygoty
				From nucellus	From integuments	
12-136MS	67	4.5	55.2	40.3	–	adventitious embryogenesis
12-157MS	75	6.7	66.7	26.6	–	adventitious embryogenesis
12-222MS	80	12.5	43.8	–	43.7	apospory
12-4652-12-12-2-4MS c.52	69	11.6	40.4	–	48.0	apospory
12-138MS	70	8.6	48.4	43.0	–	adventitious embryogenesis
12-153MS	72	12.5	45.8	41.7	–	adventitious embryogenesis

Note: A_4 – apozygotic progeny of the fourth reproduction cycle
Source: compiled by the authors

According to Table 1, the materials under study were characterised by a high proportion of embryos with anomalous positioning (up to 48.0%). The anomalies observed were represented by embryos of integumental (26.6% to 43.0%) and nucellar (up to 48.0%) origin. The number of underdeveloped embryos varied between accessions in this group from 40.4% to 66.7%. Unfertilised embryos ranged from 4.5% to 12.5%. Embryos with polyembryony were not observed in A_4, which may be associated with the stabilisation of monoecism over four cycles of reproduction. Optimisation of the embryogenesis methodology under apozygoty was carried out using substituted lines with a high apozygotic seed set rate, with new cmS sources derived from the wild species *Beta maritima* and *Beta patula* L., propagated as clones under an in vitro system. Ovules were fixed on day 10. Using the modified methodology, embryo sacs were studied from day 10 to day 16, and cytological investigations of embryo development in the micropylar, chalazal, and central regions, as well as perisperm development, were conducted from day 16 to day 38 after flowering marking. Data are presented in Table 2.

Table 2. Indicators of embryonic development of apomictic embryos under pollen-free conditions on day 16 after flowering marking in backcross progenies with new sterile cytoplasms from wild species *Beta maritima* and *Beta patula*

No.	Breeding accession	Developmental stages, fixation date, %										Number with polyembryony	
		Degenerated		club stage		sphere stage		heart stage		heart = globular			
		n	%	n	%	n	%	n	%	n	%	n	%
1	2	3	4	5	6	7	8	9	10	11	12	15	16
1	No. 15/1B$_2$CS (Greece)	5	16.7	4	13.2	10	33.4	16	20	5	16.7	5	16.7
2	No. 15/2B$_2$CS (Greece)	7	23.3	2	6.7	7	23.3	11	36.7	3	10.0	4	13.2
3	No. 15/3B$_2$CS (Greece)	10	33.4	1	3.2	9	30.0	10	33.4	–	–	3	10.0
4	No. 16/1B$_2$CS (Greece)	6	20.0	–	–	10	33.4	10	33.4	4	13.2	5	16.7
5	No. 16/2B$_2$CS (Greece)	9	30.0	2	6.6	9	30.0	5	16.7	5	16.7	4	13.2
6	No. 16/3B$_2$CS (Greece)	5	16.7	5	16.7	10	33.4	10	33.4	–	–	3	10.0
7	No. 3/1 dihaploid, F$_1$S clones (Turkey) *in vitro*	4	13.2	–	–	2	6.8	24	80.0	–	–	–	–
8	No. 3/2 dihaploid, F$_1$S clones (Turkey) *in vitro*	4	13.2	–	–	–	–	26	86.8	–	–	6	20.0
9	No. 1/17B$_3$CS *patula*	4	13.2	2	6.7	14	46.7	10	33.4	–	–	12	40.0
10	No. 2/7B$_3$CS *patula*	2	6.7	4	13.2	19	53.4	5	16.7	–	–	10	33.4

Note: degenerated embryos as a proportion of 30 analysed per breeding accession
Source: compiled by the authors

Male-sterile seed-bearing clones obtained by in vitro deposition and rooted under pot conditions differed from one another in various anomalies of apomictic embryo development compared with hybrid embryos. Thus, for breeding accessions No. 15/1, No. 15/3, and No. 16/3 on the *Beta maritima* L. cytoplasmic background, originating from Greece, on day 16 the development of embryos at the "sphere" and "heart" stages was predominant, reaching a combined value of 53.4-60%, compared with 16.7-33.4% of degenerated embryos. For some breeding accessions, developmental delay on day 16 was considerable: embryo development at the "club" stage was observed in 16.7% of cases, and at the "sphere" stage in 33.4%. For dihaploid line No. 3 (Turkey), embryos were delayed in development by 8 days and were characterised by relatively stable values of 80% to 86.8% at the "heart" stage. The proportion of degenerated embryos differed between breeding accessions and substantially exceeded the values recorded for hybrid embryos. In certain backcross progenies on the sterile *Beta patula* cytoplasmic background, a particularly high proportion of embryos with polyembryony and developmental delay in the micropylar region compared with the chalazal and central regions were observed.

On days 10-12 from the onset of flowering, following maceration, washing, and staining, embryo sacs were obtained showing embryo development from synergids at the "club" stage, degeneration of the egg cell, and two unfused polar nuclei. Only by means of a methodology that involves shifting the embryo fixation date by 8-10 days under apozygoty is it possible to assess polyembryony and its progression throughout embryogenesis. The efficacy of embryo staining on days 16 and 28 after flowering marking is illustrated in Figure 2 (a, b, c). Based on embryological investigation results, polyembryony in the apomictic line No. 2/7 BC$_3$S *patula* A$_1$ was observed in the experimental material at a proportion of 33.4%.

Simultaneous embryo development in both the micropylar and chalazal regions is shown in Figure 2 (d). During the course of investigations, degeneration of one of the embryos was frequently observed – more often that of the adventitious one (Fig. 2 (e)). Within the embryo sac, alongside the development of an apomictic embryo from the egg cell, an embryo from the synergids develops, lagging behind the hybrid by 8 days (Fig. 2 (f)). In line 1-16 BC_2S (Greece) A_1, alongside normal embryo development corresponding to the hybrid pattern, ovules were present with embryo development in both the micropylar and chalazal regions (Fig. 2 (g)). A developmental delay of embryos from generative cells of 8-10 days relative to the zygotic embryo is shown in Figure 2 (h). Complete development of the apomictic embryo in lines 1/16 BC_2S (Greece), corresponding to the zygotic pattern, lags by 10 days (Fig. 2 (i)).

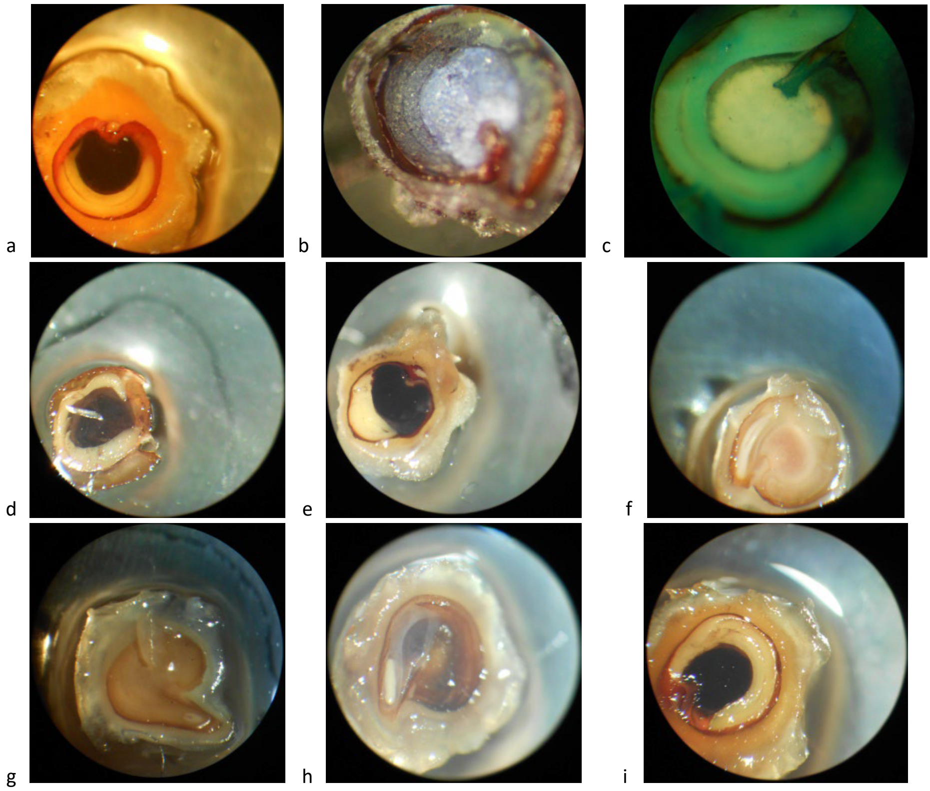

Figure 2. Study of embryonic development of apozygotic embryos using lines with high apozygotic seed self-reproduction. Magnification: 5×12.5

Note: a) embryo staining with 0.3% iodine solution; b) staining of apomictic embryos with bromophenol orange; c) response of apomictic embryos to methylene blue staining; d) development of apomictic embryos with polyembryony in line BC_3S *patula*; e) degeneration of one of the embryos in line BC_3S *patula*; f) developmental delay of embryos on days 16-20 after flowering marking; g) development of haploid embryos at the "club" stage from synergids; h) developmental delay of an embryo in line 1/15 BC_2S (Greece) A_1; i) normal embryo development in line 1/16 BC_2S (Greece) A_1, corresponding to the zygotic pattern on day 32 after flowering fixation

Source: photo by the authors

The characteristics of embryonic development during apozygoty were studied in substituted lines BC_3, BC_4, BC_5, and BC_6 under pollen-free conditions, in comparison with the development of hybrid embryos of *Beta vulgaris* cmS 0-type (Table 3). The analysis of embryo development presented in Table 3 was conducted taking into account the previously established developmental delay in sugar beet, with the fixation period extended to 10 days. Based on the results of cytological analysis, polyembryony in the majority of the lines under study was observed both on day 24 after flowering marking and on day 38, depending on the genetic origin of the breeding material.

Table 3. Embryonic development of embryos in breeding materials with apomixis of different genetic structure in substituted lines

No.	Breeding accessions	Fixation period, days	Number of seeds	Developmental stages							Number of cases with polyembryony, %
				Degenerated embryos, %	Polyembryony, %	Heart stage, %	"heart = globular" 1/4 embryo sac stage, %	1/2 embryo sac stage, %	3/4 embryo sac stage, %	Complete embryo development, %	
1	2	3	4	5	6	8	9	10	11	12	13
2	*Beta vulgaris* CMS-0302	16	30	–	–	–	–	30	–	–	–
		20	30	–	–	–	–	–	30	–	–
		24	30	–	–	–	–	–	–	30	–
		28	30	–	–	–	–	–	–	30	–
3	B_3CS No. 3 (Greece)	16	44	2.27	22.73	–	56.81	18.19	–	–	36.4
4	B_3CS No. 5 (Greece)	38	58	12.07	20.69	–	–	8.62	58.62	–	22.4
5	B_6CS No. 7 (Greece)	38	43	4.65	–	–	–	30.23	–	65.12	69.7
6	B_4CS No. 4 *patula*	20	40	10	–	–	60	–	30	–	40
7	B_4S No. 5 *patula*	28	41	26.83	–	–	43.90	29.27	–	–	48.7
8	B_5CS No. 11 (Turkey)	24	45	20	–	–	17.78	40	22.22	–	51.1
9	B_6CS No. 13 (Turkey)	28	50	28	–	–	32	–	40	–	40
10	B_4CS No. 1 (Turkey)	38	29	41.38	–	–	–	24.14	13.79	20.69	–
11	B_4CS No. 2 (Turkey)	38	30	56.67	–	16.67	–	–	10	16.66	–
12	B_3CS No. 5 (Turkey)	38	32	31.26	9.37	–	–	9.37	–	50	9.4

Note: BC_6S No. 7 (Greece) – polyembryony was observed in all studied ovules with complete embryo development in the chalazal region and a 1/2 embryo sac developmental delay in the micropylar region on day 38 after the onset of flowering

Source: compiled by the authors

A distinctive characteristic of breeding material with apozygoty, in comparison with hybrid embryos, is the high proportion of degeneration, amounting to 41.38% and 56.65%, at a fixation period of 38 days across all breeding accessions studied. However, low degeneration values of 4.65% and 12.07% were observed in backcross progenies at different embryo developmental stages in material on the *Beta maritima* (Greece) cytoplasmic background. The percentage values for complete embryo development at the adjusted fixation period of 38 days ranged from 16.60% to 65.12%, depending on the genetic origin of the material. The seed productivity of breeding accessions in substituted lines under apozygoty is presented in Table 4.

Table 4. Seed productivity of new plasmotypes under apozygoty A_1, based on introduced sterile cytoplasm of *Beta patula* and *Beta maritima* under pollen-free conditions

Breeding accession	Origin	Number of fruits per 10 cm segment, X ± m	Of these, %	
			Developed	Degenerated
1	2	4	5	6
BC_6S** *maritima* (Turkey), c.4/1	Amb. No. 17224	36 ± 0.22	92.5	7.5
BC_6S *maritima* (Turkey), c.5/1	Amb. No. 17224	32 ± 0.1	46.2	53.8
B_6CS *maritima* (Turkey), c.2/1	Amb. No. 17224	21 ± 0.2	39.1	60.9
BC_6S *patula* (Portugal, Madeira Island), c.1/4	Amb. No. 17222	32 ± 0.3	45.7	54.3
BC_6S *patula* (Portugal, Madeira Island), c.3/4	Amb. No. 17222	38 ± 0.42	51.3	48.7
BC_6S *patula* (Portugal, Madeira Island), c.3/4	Amb. No. 17226	37 ± 0.21	50.5	50.0
BC_6S *patula* (Portugal, Madeira Island), c.3/7	Amb. No. 17226	37 ± 0.29	68.8	31.2
BC_6S *maritima* (Turkey), c.5/1	Amb. No. 17224	28 ± 0	79.3	20.7

Table 4, Continued

Breeding accession	Origin	Number of fruits per 10 cm segment, X ± m	Of these, %	
			Developed	Degenerated
BC_6S *maritima* (Turkey), c.7/2	Amb. No. 17224	27 ± 0.32	96.4	3.6
BC_6S *maritima* (Turkey), c.6/3	Amb. No. 17224	24 ± 0.27	95.7	4.3
BC_6S *maritima* (Turkey), c.7/5	Amb. No. 17224	24 ± 0.29	88.5	11.5

Note: pollen-free conditions ensured by spatial isolation and the use of parchment isolators; BC_6S^{**} – substituted lines of the sixth backcross cycle
Source: compiled by the authors

According to Table 4, on day 28 from the onset of flowering, the highest and most stable seed set values under spatial isolation conditions were characteristic of seed-bearing plants of substituted lines with breeding accessions BC_6S *maritima* (Turkey) c.7/2, c.6/3. Seed set values in substituted lines per 10 cm segment ranged from 39.1% to 96.4%. Fruit set values varied from 21 ± 0.2 to 38 ± 0.42, depending on the origin of the breeding accessions. In the BC_6S *patula* Line, the number of apozygotic seeds set was characterised by stable values of 32 ± 0.3 to 38 ± 0.42, whilst the proportion of degenerated embryos ranged from 31.2% to 54.3%. For the BC_6S *patula* L. line, the number of fruits per 10 cm segment substantially exceeded the values recorded for seed-bearing plants on the sterile *Beta maritima* L. (Turkey) cytoplasmic background. Seed-bearing plants with high seed self-reproduction were identified, notably BC_6S maritima (Turkey) c.4/1 and c.5/1, as well as those on the *Beta patula* L. (Madeira Island) cytoplasmic background under breeding accessions BC_6S *patula* c.3/4 and c.3/7.

Embryo sacs formed from the archespore belong to the *Polygonum* type. The sugar beet seed reproduction system encompasses all types of reproduction. The frequency of apozygoty in free-pollinating sugar beet populations is unknown. According to D. Hojsgaard *et al.* (2014), the development of diploid plants under pollen-free conditions proceeds through adventitious embryogenesis or apospory. T. Szkutnik (2010) established that the appearance of tetraploid cells in apomictic progenies is conditioned by the formation of an embryo from an egg cell with an unreduced chromosome set and by tissue differentiation during ontogenesis through the phenomenon of endopolyploidisation. Based on embryological investigations by L. Seilova & A. Sedlovskiy (2000), apomictic embryos differ from sexual ones in their mode of formation and position within the embryo sac. Additional embryos arise from synergid or antipodal cells – apogamy. M. Roik *et al.* (2024) indicate that embryo formation via adventitious embryogenesis proceeds from cells of the integuments and nucellus. It was noted that some apomictic embryos perish during embryogenesis. Selection for ploidy level and apozygotic seed set contributes to enhanced apozygotic tendency and the identification of apomixis donors.

The influence of polyploidy on trait variation in *Amelanchier* (*Rosaceae*) was described by M. Burgess *et al.* (2014). M. Hand *et al.* (2015) investigated the evolution of apomixis loci in *Pilosella* and *Hieracium* (*Asteraceae*) using marker analysis in natural and experimental populations. Research by D. Rodriguez-Leal & J.-P. Vielle-Calzada (2012) emphasises that apomixis may be considered a spatiotemporal deregulation of the sexual process rather than a wholly independent mechanism. The authors analyse new evidence that the regulation of apomixis – particularly female gametogenesis and seed formation – is governed by the same epigenetic mechanisms as sexual reproduction. Similar approaches to interpreting the molecular regulation of apomixis are presented in the work of T. Niccolò *et al.* (2023). G. Gerashchenkov *et al.* (2015) identified fragments of genes controlling endosperm development without fertilisation in *Arabidopsis* using molecular genetics methods.

On cytological preparations, the genetic heterogeneity of seeds under apozygoty is determined by the development of embryos from embryo sac cells or from somatic cells of the ovule. In addition to cytoembryological characterisation, morphological marker traits – such as recessive hypocotyl colouration – are capable of identifying embryos developed via generative reduced parthenogenesis from heterozygous apomictic plants. Using the embryo culture method, N. Kovalchuk *et al.* (2019) isolated haploid and dihaploid lines from apomictic progenies of male-sterile sugar beet lines. Embryological investigations of apozygoty across cmS sources of different origins highlighted several problematic issues: variability in phenotypic traits of monoecism and sterility in apozygotic progenies does not conform to Mendel's laws of genetics, but is determined by the genetic heterogeneity of seeds, which is linked to the differing nature of embryos developed from both somatic and generative cells (Matzk *et al*., 2000). It was established that apozygotic lines from different genetic sources of sterile cytoplasms differed from one another in their embryonic development characteristics. Breeding materials based on *Beta patula* germplasm were characterised by greater capacity for polyembryony. The use of apomictic lines with a high apozygotic seed set requires the identification of source material using genetic markers for the differentiation of generative and somatic embryogenesis, which in one case leads to new genetic variability, and in another to the cloning of breeding material and the preservation of its principal traits in subsequent generations.

CONCLUSIONS

Experimental investigations of embryological development based on different cmS sources revealed several characteristics of uniparental seed reproduction in sugar beet. It was established that the embryonic development of apomictic progenies is characterised by various types of anomalies, a developmental delay of 8-10 days, simultaneous embryo development in both the micropylar and chalazal regions, adventitious embryogenesis, and apospory. Embryonic development under pollen-free conditions was studied in substituted lines BC_3, BC_4, BC_5, and BC_6, in comparison with the development of hybrid embryos of Beta vulgaris cmS 0-type. Polyembryony was observed both on day 24 after flowering marking and on day 38, and was dependent on the genetic origin of the breeding material. In the course of the cytological experiment, the synchrony of developmental stages of apomixis and sexual plant reproduction was compared, and the possibility of differentiating apozygotic progenies by apomixis type was demonstrated. In the BC_6S *patula* Line, the number of apozygotic seeds set was characterised by stable values of 32 ± 0.3 to 38 ± 0.42, whilst the proportion of degenerated embryos ranged from 31.2% to 54.3%. For substituted lines based on the *Beta patula* sterility source, the number of fruits per 10 cm segment substantially exceeded the values of seed-bearing plants on the sterile *Beta maritima* L. (Turkey) cytoplasmic background. Breeding accessions with high apozygotic seed reproduction values were identified, notably BC_6S maritima (Turkey) c.4/1 and c.5/1, as well as those on the *Beta patula* L. (Madeira Island) cytoplasmic background under breeding accessions BC_6S *patula* c.3/4 and c.3/7. Notwithstanding that polyembryony frequently determines the genetic heterogeneity of apozygotic seeds, generative reduced parthenogenesis in sugar beet may be identified in future investigations using morphological marker traits and ploidy stabilisation techniques. The cytoembryological method, employed for the first time for the detection of adventitious embryogenesis, remains to this day one of the most reliable approaches for studying both the type of apomixis and the characteristics of embryonic structure development. Prospects for further research lie in the investigation of the molecular-genetic mechanisms of apomixis, the identification of genetic markers of generative and somatic embryogenesis, and the development of methods for stabilising apozygotic progenies for practical application in the breeding of high-yielding sugar beet hybrids.

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CONFLICT OF INTEREST

None.

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Особливості ембріонального розвитку зародків заміщених ліній буряків цукрових при апозиготії і цитоплазматичній чоловічій стерильності

Анотація. Метою дослідження було оцінити теоретичні основи феномену високої саморепродукції насіння у заміщених ліній та розробити методику ембріонального розвитку у селекційних матеріалів буряків цукрових з цитоплазматичною чоловічою стерильністю (ЦЧС) різного генетичного походження. Для експериментальних досліджень були використані методи польові, безпилкове отримання насіння у селекційних матеріалів у ЦЧС, лабораторні, цитологічний аналіз розвитку зародків при апозиготії впродовж 34 діб після фіксації цвітіння, цитофотометричний метод мінливості рівня геному за плоїдністю у матеріалів з різним терміном репродукції апозиготичного насіння. Було вдосконалено методику дослідження ембріонального розвитку при апозиготії у матеріалів різного походження джерел ЦЧС, а саме відмітка цвітіння, ізоляція пагонів, фіксація зародків за терміном від 5 до 34 діб, до повного розвитку зародка. Було проаналізовано, що при апозиготії у цукрових буряків спостерігається відставання генетично-детермінованого розвитку 28 діб на 4, 8, 10 діб залежно від походження матеріалу, поліембріонією, високим відсотком дегенерованих зародків. Адвентивна ембріонія, апоспорія та розвиток зародків з інтигументів і нуцелусу в мікропілярній і халазальній частині одночасно були характерними для пилкостерильних ліній різного генетичного походження. Було встановлено, що у заміщених ліній на основі нових джерел ЦЧС від диких видів роду *Beta* L. спостерігається феномен високої саморепродукції насіння у безпилкового режиму. Методика вивчення синхронності розвитку зародків розроблена для дослідження природи апозиготії (генеративний, соматичний ембріогенез) у заміщених ліній з інтродукційними стерильними цитоплазмами, в зрівнянні з роздільноквітковими пилкостерильними лініями з S-vulgaris цитоплазмою Ф. Оуена. Тип апозиготії проявлявся в гаметофітному та спорофітному ембріогенезі і визначав генетичну неоднорідність (різноякісність) апозиготичного насіння. Апоміксис з використанням заміщених ліній і новими стерильними цитоплазмами від диких видів роду *Beta* L. та високим відсотком апоміктичної репродукції насіння, може забезпечити багато переваг для виробництва нових гібридів сільськогосподарських культур, а саме фіксацію гетерозису і збереження генотипу материнської рослини

Ключові слова: адвентивна ембріонія; апоспорія; нові джерела цитоплазматичної чоловічої стерильності; *Beta maritima*; *Beta patula*; фенотипи цитоплазматичної чоловічої стерильності

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The effect of agronomic practices on belowground biomass formation in *Miscanthus × giganteus*

Abstract. The aim of this study was to experimentally determine the effect of different systems of mineral and organic fertilisation on the dynamics of belowground biomass accumulation in giant miscanthus (*Miscanthus × giganteus*), the intensity of soil respiration, and the carbon sequestration potential under the conditions of the Forest-Steppe of Ukraine. The study was conducted during 2021-2025 in a two-factor field experiment, in which Factor A comprised a control treatment, leonardite application (100 kg/ha), and complete mineral fertiliser $N_{60}P_{16}K_{80}$, whilst Factor B comprised foliar feeding with potassium humate at the tillering stage, at the stem elongation stage, and at both stages combined. Belowground biomass was determined by the monolith method in the 0-30 cm soil layer, and the biological activity of the rhizosphere was assessed using the closed static chamber method. It was established that the root system mass of miscanthus increased consistently throughout the first five years of vegetation, reaching 10.76 t/ha in 2025 under the optimal combination of $N_{60}P_{16}K_{80}$ with double foliar feeding with potassium humate, compared with 6.14 t/ha in the control treatment. Carbon input into the soil via root mass in this treatment amounted to 4.52 t C/ha, and the maximum soil respiration intensity (432 mg CO_2/m²·h) was recorded in the leonardite + double potassium humate application treatment. A strong positive correlation was found between root mass and soil respiration intensity (r = 0.74), and the constructed second-degree polynomial regression model (r = 0.96) adequately describes the dynamics of belowground biomass accumulation and enables projection of its further increase. The practical significance of this study lies in the substantiation of the combined use of mineral fertilisers and humic preparations to enhance the productivity of miscanthus plantations, strengthen the carbon sequestration function of the agrocoenosis, and stabilise the biological activity of the soil

Keywords: carbon sequestration; soil respiration; leonardite; potassium humate; regression model

INTRODUCTION

The cultivation of perennial energy crops is currently considered not only as a means of obtaining a stable biofeedstock supply, but also as a tool for enhancing the ecological resilience of agricultural landscapes. For Ukraine, where soil degradation, organic matter deficit, climatic

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variability, and the need for energy diversification are all intensifying simultaneously, crops capable of combining high productivity with long-term positive effects on the soil are of particular significance. In this context, *Miscanthus × giganteus* represents a promising research subject, as it forms substantial belowground biomass, supports long-term plantation functioning without annual tillage, and potentially enhances the accumulation of soil organic carbon. At the same time, for the Forest-Steppe conditions of Ukraine, the precise effects of combining mineral fertilisation, humic preparations, and organic amendments on the development of the miscanthus root system and the associated biological soil processes have yet to be fully elucidated.

S. Li *et al*. (2022) demonstrated that the productivity of *Miscanthus × giganteus* is closely linked to the morphophysiological characteristics of the leaf apparatus: tetraploid forms develop thicker leaves, higher leaf mass per unit area, and greater photosynthetic productivity than triploid genotypes. This finding is important for understanding that biomass formation in this crop is determined not only by nutritional supply but also by intrinsic genotypic characteristics, which may alter the plant's response to agronomic practices. C. Zheng *et al*. (2022) note that miscanthus is a promising lignocellulosic feedstock crop for bioenergy production, as it can be cultivated on low-productivity lands, thereby avoiding competition with food crops. V. Ivanina *et al*. (2021) note that a defining characteristic of miscanthus physiology is the seasonal redistribution of nutrients: at the end of the growing season, 50-60% of nitrogen, phosphorus, and potassium in the shoots is remobilised to the belowground rhizomes. These accumulated reserves are mobilised in spring to support new shoot growth, making the crop partially independent of the immediate availability of soil nutrients. H. Burdová *et al*. (2023), studying the resilience of *Miscanthus × giganteus* on petroleum-contaminated soils, concluded that the crop exhibits high tolerance to stress conditions, but its growth is substantially limited by nutrient deficiency. According to these authors, nutrient availability is one of the critical factors determining the adaptability and productivity of miscanthus on problem soils. Thus, the optimisation of crop nutrition is of not only productive but also ecological importance. L. Wechtler *et al*. (2024), investigating miscanthus on technosols contaminated with polycyclic aromatic hydrocarbons, found that combining *Miscanthus × giganteus* with a biologically active companion component enhanced microbial activity, microbial biomass, and the abundance of contaminant-degrading organisms. Their findings have broader implications: they confirm that the miscanthus rhizosphere responds actively to changes in the nutritional system and is capable of modifying the intensity of biological soil processes.

G. Aslan-Sungur *et al*. (2025) demonstrated that advances in stand establishment techniques and increased planting density can substantially increase carbon uptake in the establishment year. The authors attribute this to more intensive vegetation cover formation and a faster transition of the stand into active functioning. Thus, agronomic practices in the early stages of crop development have a direct impact on its carbon potential. N. Namoi *et al*. (2026), in a study of ageing *Miscanthus × giganteus* stands, demonstrated that the effect of nitrogen fertilisation manifests primarily through increased stem mass and maintenance of stand productivity, which tends to decline with age. The authors emphasised that soil fertility management remains a key condition for the stable functioning of even perennial plantations, which are traditionally considered low-input with respect to fertilisation. J. Nelson *et al*. (2025) established that after three years of cultivation, miscanthus increased the maximum water-holding capacity of the soil by an average of 14.7% compared to maize, and this effect was associated primarily with changes in soil structure. The study confirms that the development of miscanthus belowground biomass has not only biometric but also soil-ameliorating significance. S. Yıldız *et al*. (2025) demonstrated that the application of humic acid reduced growth losses in miscanthus under drought conditions and improved the morphometric parameters of plants compared with treatments without humic substances. The authors recorded that at different water supply levels, humic acid mitigated the negative effects of stress. V. Pidlisnyuk *et al*. (2026) investigated the feasibility of using ash from miscanthus biomass as a secondary resource for improving soil properties, and noted that low doses of this material promote the restoration of plant growth and increase soil phosphorus and potassium availability, whilst excessive doses may induce salt stress. This finding is important from the perspective of organic and organo-mineral nutritional systems, as it highlights the necessity of precise dosing of soil amendments and biostimulants.

Thus, contemporary research convincingly confirms the high bioenergetic and ecological value of *Miscanthus × giganteus*; however, the question of how, under the specific soil-climatic conditions of the Forest-Steppe of Ukraine, the combination of mineral fertilisation, leonardite, and foliar application of potassium humate affects belowground biomass formation and soil respiration intensity as an indicator of rhizosphere functioning remains open. The aim of this study was to establish the patterns of belowground biomass formation in giant miscanthus and the associated biological soil processes depending on the base fertilisation system and the timing of foliar potassium humate application under the conditions of the Forest-Steppe of Ukraine. The objective of this paper was to experimentally determine the effect of different systems of mineral and organic fertilisation on the dynamics of root system mass accumulation and soil respiration processes, and to develop mathematical models of the key factors influencing plant formation in miscanthus (*Miscanthus × giganteus*) cultivar 'Osinnii Zoretsvit'.

MATERIALS AND METHODS

Field studies were conducted during 2021-2025 at the Educational and Production Unit of the National University of Life and Environmental Sciences of Ukraine, "Velykosnytynske named after O.V. Muzychenko", Fastiv district, Kyiv region. The soil of the experimental site is strongly eroded, light loamy podzolised chernozem on loess-like loam. The agrochemical characteristics of the plough layer (0-30 cm) at the time of experiment establishment were as follows: humus content – 1.43%; bulk density – 1.17 g/cm^3; pH_{KCl} – 5.76; available phosphorus – 105 mg/kg; exchangeable potassium – 78 mg/kg. The climatic conditions of the region are characterised by a mean annual temperature of +7.8°C and an annual precipitation total of 540-620 mm. The experiment was two-factorial with three replications; treatments were arranged in a randomised complete block design. The area of the sown plot was 50 m^2, and of the harvest plot – 35 m^2. The study crop was giant miscanthus (*Miscanthus × giganteus*), cultivar 'Osinnii Zoretsvit'. Planting was carried out in April 2021 using rhizomes weighing 25-35 g at a density of 10,000 plants/ha (1.0 × 1.0 m spacing). Factor A (fertilisation system): A1 – control (no fertiliser); A2 – leonardite (fraction 0-2 mm, humic acid content ≥75%), 100 kg/ha (applied once under spring tillage in the plantation establishment year); A3 – mineral fertilisation $N_{60}P_{16}K_{80}$ (ammonium nitrate + ammonium phosphate + potassium chloride, banded application, annually). Factor B (foliar feeding with potassium humate at 2 L/ha, solution concentration 1%): B1 – no foliar feeding (control); B2 – at the tillering stage; B3 – at the stem elongation stage; B4 – at both stages (double application). The experimental design comprised 12 treatments × 3 replications = 36 plots in total. In this paper, the analysis is conducted on the extreme variants of Factor B (B1 – no foliar feeding; B4 – double foliar feeding), since this contrasting comparison enables the most clearly defined characterisation of the influence of potassium humate foliar feeding on carbon sequestration indicators within each fertilisation system of Factor A.

Root system mass was determined by the monolith method (0-30 cm layer) annually in autumn following the completion of the growing season. Carbon accumulation was calculated on the basis of dry root biomass mass, with a stable carbon content of 42%. Soil respiration was measured using the closed chamber method in July (active growth phase). Analysis of variance (ANOVA) was used to determine the proportion of the effect of each factor on the response variables. Regression analysis was conducted by constructing second-degree polynomial equations of the form:

$$y = ax^2 + bx + c, \quad (1)$$

where *y* is the root system mass (t/ha) and x is the year of vegetation.

This approach allowed for the biological deceleration of root growth rates in mature plantations to be accounted for. Calculations were performed using the Statistica 13.0 software package and the Python NumPy library. The generative AI tool Gemini was used as an auxiliary tool for language editing and preparation of technical descriptions. All results were verified and manually edited by the author. The study was conducted in accordance with the ethical standards of the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1973).

RESULTS AND DISCUSSION

The formation of belowground biomass in miscanthus is an integral indicator of the crop's response to the nutritional system, as it is the rhizomes and roots that determine the longevity of the plantation, its resilience under stress conditions, and its carbon sequestration potential. The results obtained indicate that already during the first five years of vegetation, fertilisation and foliar feeding significantly altered the rates of root mass accumulation and the biological activity of the rhizosphere. S. Fosu & S. Davis (2024) demonstrated that over a decade of miscanthus cultivation on abandoned agricultural land, soil carbon and nitrogen stocks changed slowly, and nitrogen deficiency was not always the limiting factor. At the same time, the authors emphasised that the crop's response to fertilisation and changes in the carbon pool depend on stand age and the initial state of the soil. This underscores the necessity of region-specific research, as results obtained from mature plantations in the United States cannot be directly extrapolated to young stands under the conditions of the Forest-Steppe of Ukraine.

The highest rates of belowground phytomass accumulation were recorded in the treatment with $N_{60}P_{16}K_{80}$ application combined with double foliar feeding with potassium humate: the value increased from 1.50 t/ha in 2021 to 10.76 t/ha in 2025. As noted in the study by F. Dohleman & S. Long (2009), under conditions in the midwestern United States this crop can surpass maize in productivity, whilst the work of M. Roik *et al*. (2019) draws attention to the prospects of using miscanthus as a perennial energy crop, which is particularly relevant in the context of Ukraine's biofeedstock deficit for energy purposes. Certain aspects of the introduction of bioenergy crops, including miscanthus, in Ukraine, also highlighting the exceptional prospects of this crop under Ukrainian conditions, were examined in the study by O. Melnychuk *et al*. (2019). The combination of mineral fertilisers and humates produced a synergistic effect through improved nutrient supply to plants, stimulation of root formation, and enhanced accumulation of reserve substances in the rhizomes. I. Lewandowski *et al*. (2000) established that perennial miscanthus plantations in Europe are capable of accumulating 1.1-2.4 t C/ha/year in the soil.

These results are consistent with the findings of other researchers. In particular, O. Prysiazhniuk *et al*. (2026), analysing the nutrition of *Miscanthus × giganteus* on chernozems in Ukraine, concluded that complete NPK fertilisation ensures higher productivity and better meets the nutritional requirements of the crop than nitrogen application alone. The authors also demonstrated that assessment of the nutritional status of stands is an effective tool for adjusting cultivation technology. For Ukrainian conditions, this is particularly valuable, as it confirms the expediency of combining agrochemical monitoring with technological decisions regarding fertilisation. J. Finnan & B. Burke (2014) established that in mature miscanthus stands, nitrogen fertilisation increased nitrogen concentration in the rhizome network in proportion to the application rate. Soil respiration intensity under miscanthus stands increased in parallel with the development of the root system. The maximum value – 432 mg $CO_2/m^2 \cdot h$ – was recorded in 2025 in the leonardite + potassium humate (both stages) treatment, confirming the pronounced effect of humic substances on the activation of the rhizosphere microbiocoenosis. Based on the experimental data for 2021-2025, a consistently positive growth dynamic of the *Miscanthus × giganteus* root system was established across all fertilisation and foliar feeding treatments (Table 1).

Table 1. Root system mass of giant miscanthus in the 0-30 cm soil layer, t/ha (2021-2025)

No.	Factor A	Factor B	2021	2022	2023	2024	2025	Mean, t/ha	Increment relative to unfertilised control (Factor A)	Increment relative to no-feeding treatment (Factor B)
1	Control (no fertiliser)	No foliar feeding	0.85	2.62	4.23	5.35	6.14	3.84	–	–
2		Potassium humate, tillering stage	0.94	2.91	4.69	5.93	6.81	4.26	–	0.42
3		Potassium humate, stem elongation stage	0.92	2.84	4.57	5.79	6.64	4.15	–	0.31
4		Potassium humate, both stages	1.02	3.14	5.06	6.40	7.35	4.59	–	0.75
5	Leonardite, 100 kg/ha	No foliar feeding	1.07	3.25	5.26	6.66	7.65	4.78	0.94	–
6		Potassium humate, tillering stage	1.18	3.60	5.83	7.38	8.48	5.29	1.03	0.51
7		Potassium humate, stem elongation stage	1.15	3.52	5.70	7.23	8.30	5.18	1.03	0.40
8		Potassium humate, both stages	1.28	3.85	6.29	7.96	9.15	5.71	1.12	0.93
9	$N_{60}P_{16}K_{80}$	No foliar feeding	1.25	3.80	6.18	7.82	8.98	5.61	1.77	–
10		Potassium humate, tillering stage	1.38	4.21	6.84	8.67	9.94	6.21	1.95	0.60
11		Potassium humate, stem elongation stage	1.35	4.12	6.71	8.48	9.74	6.08	1.93	0.47
12		Potassium humate, both stages	1.50	4.51	7.40	9.37	10.76	6.71	2.12	1.10

Note: $N_{60}P_{16}K_{80}$ – the subscript indicates the dose of the active nutrient element in kg per hectare
Source: compiled by the author

At the same time, the degree to which the biological potential of the crop was realised depended substantially on the intensity of nutrition and the nature of the agrochemical practices applied. In the control treatment, the mean root mass over 2021-2025 was 3.84-4.59 t/ha, whilst under leonardite application it was 4.78-5.71 t/ha, and under mineral fertilisation 5.61-6.71 t/ha. Thus, the base fertilisation treatment established the baseline level of belowground phytomass development, whilst potassium humate functioned as an amplifier of the nutritional background. The greatest effect within all nutritional backgrounds was achieved with double foliar feeding: the increment relative to the no-feeding treatment was 0.75 t/ha in the control, 0.93 t/ha under leonardite application, and 1.10 t/ha against the $N_{60}P_{16}K_{80}$ background. Root system mass demonstrates a strong positive correlation with the intensity of nutrition. The greatest stimulating effect is achieved when complete mineral fertiliser is combined with double foliar feeding with potassium humate. The studies of M. Rose *et al.* (2014) and F. Agostini *et al.* (2015) confirm that humic substances positively affect the root system development of perennial crops. Specifically, according to D. Pribyl (2010), the application of humic substances can increase the dry mass of shoots and roots by approximately 20%, improving nutrient uptake. This explains the higher increment in belowground biomass (0.75-1.10 t/ha) in the double potassium humate application treatments compared with the no-feeding background, observed across all three fertilisation systems. The development of the root system has a direct effect on the ecological indicators of the soil. In the treatment with maximum root mass (NPK + potassium humate at both stages), an average of 2.82 t C/ha is input into the soil (Table 2).

Table 2. Carbon input into the soil via the root mass of giant miscanthus, t C/ha (2021-2025)

No.	Factor A	Factor B	2021	2022	2023	2024	2025	Mean, t C/ha
1	Control (no fertiliser)	No foliar feeding	0.36	1.10	1.78	2.25	2.58	1.61
2		Potassium humate, tillering stage	0.39	1.22	1.97	2.49	2.86	1.79
3		Potassium humate, stem elongation stage	0.39	1.19	1.92	2.43	2.79	1.74
4		Potassium humate, both stages	0.43	1.32	2.13	2.69	3.09	1.93
5	Leonardite, 100 kg/ha	No foliar feeding	0.45	1.36	2.21	2.80	3.21	2.01
6		Potassium humate, tillering stage	0.50	1.51	2.45	3.10	3.56	2.22
7		Potassium humate, stem elongation stage	0.48	1.48	2.39	3.04	3.49	2.18
8		Potassium humate, both stages	0.54	1.62	2.64	3.34	3.84	2.40
9	$N_{60}P_{16}K_{80}$	No foliar feeding	0.53	1.60	2.60	3.28	3.77	2.36
10		Potassium humate, tillering stage	0.58	1.77	2.87	3.64	4.17	2.61
11		Potassium humate, stem elongation stage	0.57	1.73	2.82	3.56	4.09	2.55
12		Potassium humate, both stages	0.63	1.89	3.11	3.94	4.52	2.82

Note: $N_{60}P_{16}K_{80}$ – the subscript indicates the dose of the active nutrient element in kg per hectare; carbon content in root mass – 42%
Source: compiled by the author

Soil respiration indicators increased synchronously with root system development, indicating enhanced biological activity of the rhizosphere as the plantation matured (Table 3). Physiological studies by H. Zub & M. Brancourt-Hulmel (2010) demonstrate that *Miscantus × giganteus* can attain peak CO_2 assimilation rates of approximately 39-40 µmol m^{-2} s^{-1}, indicating high photosynthetic capacity even after a decade of growth. At the same time, different mechanisms of influence were identified according to fertilisation type: mineral nutrition ensured the highest root system mass, whilst leonardite more actively stimulated soil microbiological processes, resulting in mean respiration values in the A_2B_1-A_2B_4 treatments of 368-387 mg $CO_2/m^2 \cdot h$, which exceeded the corresponding values under the $N_{60}P_{16}K_{80}$ background (346-363 mg $CO_2/m^2 \cdot h$). This provides grounds for considering leonardite as an effective agent for biological activation of the rhizosphere, even where the parameters of belowground mass accumulation are somewhat lower.

Table 3. Soil respiration intensity under giant miscanthus, mg $CO_2/m^2 \cdot h$ (2021-2025)

No.	Factor A	Factor B	2021	2022	2023	2024	2025	Mean, mg $CO_2/m^2 \cdot h$
1	Control (no fertiliser)	No foliar feeding	285	312	338	356	368	332
2		Potassium humate, tillering stage	292	320	347	365	377	340
3		Potassium humate, stem elongation stage	289	317	343	362	374	337
4		Potassium humate, both stages	298	327	354	373	386	348
5	Leonardite, 100 kg/ha	No foliar feeding	310	345	378	398	411	368
6		Potassium humate, tillering stage	318	354	388	409	422	378
7		Potassium humate, stem elongation stage	315	350	384	405	418	374
8		Potassium humate, both stages	325	362	397	418	432	387
9	$N_{60}P_{16}K_{80}$	No foliar feeding	295	325	354	373	385	346
10		Potassium humate, tillering stage	303	334	363	383	395	356
11		Potassium humate, stem elongation stage	300	330	359	379	391	352
12		Potassium humate, both stages	309	341	371	391	404	363

Note: determined by the closed chamber method during the active growth phase (July); $N_{60}P_{16}K_{80}$ – the subscript indicates the dose of the active nutrient element in kg per hectare
Source: compiled by the author

Factor analysis of the data presented in Tables 1-3 enables the grouping of variables and the identification of which factors most strongly influence the variability of the results obtained. The first component combines the year of vegetation and the natural capacity of perennial miscanthus plants for growth (proportion of effect – 65%). This explains why even in the unfertilised control, root mass increased from 0.85 to 6.14 t/ha over the five years of the study (Table 1). The second component comprises mineral fertilisers, leonardite, and humates (proportion of effect – 25%); it is owing to this component that the difference between 6.14 t/ha (control) and 10.76 t/ha (NPK + humate) in 2025 was obtained (Table 1). The third factor is metabolic activity (proportion of effect – 10%), which integrates the indicators of soil respiration and carbon accumulation (Tables 2-3). It should be noted that leonardite carries the greatest weight in this factor, as it activates the microbiota more intensively than mineral fertilisation.

Pearson correlation coefficients (r) were calculated using mean values for the five-year period. The relationship between the agronomic treatment variant and mean root system mass was evaluated. It was established that the correlation coefficient between Factor A (base fertiliser) and mean root system mass was r = +0.82, indicating a strong positive relationship (according to the Chaddock scale); the correlation coefficient between Factor B (foliar feeding) and mean root system mass was r = +0.41 (moderate positive relationship); and the correlation between root mass and soil respiration was r = +0.74 (strong positive relationship). Thus, mineral fertilisation and leonardite serve as the principal 'architects' of root mass, whilst humates exert a moderate influence as an independent factor; however, their role lies not so much in generating mass 'from scratch' as in synergistically amplifying the effect of fertilisers. To project the further development of *Miscanthus × giganteus* in the experiment, second-degree polynomial regression equations were calculated on the basis of the experimental data for 2021-2025, which best describe the biological deceleration of growth rates in subsequent years of vegetation. For the fertilisation and foliar feeding treatment ($N_{60}P_{16}K_{80}$ + potassium humate at both stages), the equation takes the following form:

$$y = 0.30x^2 + 4.12x - 2.39.$$

According to the calculations, the projected root system mass in 2026 will be 11.64 t/ha. For the mean value across all treatments, the equation takes the following form:

$$y = -0.23x^2 + 3.10x - 1.78.$$

The projected value for 2026 is 8.73 t/ha. This enables agricultural enterprises to undertake long-term carbon balance planning and to assess the efficiency of miscanthus stands in terms of prospective biofeedstock provision and its marketing. Thus, on the basis of the results of the study conducted, it can be concluded that belowground phytomass formation and rhizosphere biological activity in miscanthus (*Miscanthus × giganteus*) under the conditions of the Forest-Steppe of Ukraine are determined by the combination of the crop's natural growth potential and the agrochemical practices applied. In this context, complete mineral fertilisation $N_{60}P_{16}K_{80}$ combined with double foliar feeding with potassium humate ensures maximum root system mass accumulation and intensification of carbon sequestration. At the same time, leonardite application demonstrates a specific mechanism of action, more actively stimulating the soil microbiocoenosis and soil respiration indicators even where the parameters of belowground organ development are somewhat lower. The constructed polynomial regression models enable projection of the further dynamics of plant productivity, which is of strategic significance for long-term carbon balance planning and assessment of biofeedstock yield.

CONCLUSIONS

The results of the study established that during 2021-2025, the root system mass of miscanthus in the 0-30 cm soil layer increased consistently across all experimental treatments, with the highest value of 10.76 t/ha recorded in the fifth year of vegetation under the combination of $N_{60}P_{16}K_{80}$ with double foliar feeding with potassium humate. The base fertilisation treatment proved to be the determining factor in belowground biomass formation: leonardite provided a mean increment of 0.94-1.12 t/ha, and mineral fertilisation of 1.77-2.12 t/ha relative to the control, depending on the foliar feeding regime. The maximum carbon input into the soil via root mass was 4.52 t C/ha in the $N_{60}P_{16}K_{80}$ + potassium humate (both stages) treatment, indicating the high carbon sequestration potential of the optimised miscanthus nutritional system. The highest soil respiration intensity (432 mg $CO_2/m^2 \cdot h$) was recorded under leonardite application combined with double foliar feeding with potassium humate, indicating pronounced stimulation of rhizosphere biological activity by the organic factor. The second-degree polynomial regression model with a high coefficient of approximation (r = 0.96) adequately describes the dynamics of belowground biomass accumulation and projects its further increase to 11.64 t/ha in 2026 under the optimal nutritional system. The combined use of mineral fertilisers with humic preparations is practically advisable for enhancing the productivity of miscanthus plantations, stabilising the biological activity of the soil, and increasing the carbon sequestration potential; for biological activation of the rhizosphere, the inclusion of leonardite in the fertilisation system is recommended. Thus, the optimisation of the miscanthus fertilisation system contributes not only to increased belowground biomass productivity, but also creates the prerequisites for the ecologically balanced use of agricultural landscapes through the activation of biological soil processes and the enhancement of the soil's carbon accumulation function. Among the priorities for further research, the continued validation of the developed predictive models directly under field conditions during subsequent years of miscanthus vegetation is of particular importance. Another significant direction is the in-depth study of qualitative changes in the structure of the rhizosphere microbiocoenosis under the influence of leonardite, and the assessment of the long-term carbon sequestration potential.

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Вплив агротехнічних заходів на формування підземної біомаси рослинами *Miscanthus × giganteus*

Анотація. Метою роботи було експериментально визначити вплив різних систем мінерального та органічного удобрення на динаміку накопичення підземної біомаси міскантусу гігантського (*Miscanthus × giganteus*), інтенсивність ґрунтового дихання та потенціал депонування вуглецю в умовах лісостепу України. Дослідження проводили впродовж 2021-2025 років у двофакторному польовому досліді, де фактор А включав контроль, внесення леонардиту (100 кг/га) та повне мінеральне добриво $N_{60}P_{16}K_{80}$, а фактор В – позакореневе підживлення гуматом калію у фазу кущіння, виходу в трубку та в обидві фази. Підземну біомасу визначали методом монолітів у шарі ґрунту 0-30 см, а біологічну активність ризосфери – методом закритих статичних камер. Встановлено, що маса кореневої системи міскантусу стабільно зростала протягом перших п'яти років вегетації і за оптимального поєднання $N_{60}P_{16}K_{80}$ з дворазовим підживленням гуматом калію досягла 10,76 т/га у 2025 році, тоді як у контролі – 6,14 т/га. Надходження вуглецю в ґрунт з кореневою масою у цьому варіанті становило 4,52 т С/га, а максимальна інтенсивність ґрунтового дихання (432 мг CO_2/м²·год) зафіксована у варіанті з леонардитом і дворазовим застосуванням гумату калію. Виявлено високий прямий зв'язок між масою коренів та інтенсивністю ґрунтового дихання ($r = 0,74$), а побудована поліноміальна регресійна модель другого ступеня ($r = 0,96$) адекватно описує динаміку накопичення підземної біомаси та дає змогу прогнозувати її подальше зростання. Практичне значення роботи полягає в обґрунтуванні поєднаного використання мінерального удобрення та гумінових препаратів для підвищення продуктивності плантацій міскантусу, посилення вуглецедепонувальної функції агроценозу та стабілізації біологічної активності ґрунту

Ключові слова: депонування вуглецю; ґрунтове дихання; леонардит; гумат калію; регресійна модель

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