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Impact of new triazole-based growth regulators on winter ontogenesis of new winter wheat varieties

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Triazole-derived compounds are increasingly regarded as promising tools for improving the adaptability and productivity of winter wheat under unstable environmental conditions. Their physiological activity is associated with regulation of growth processes, enhancement of stress tolerance, and support of metabolic functions linked with overwintering. In the present study, we evaluated the effects of three new triazole-type compounds, CA-64, CA-79 and CA-67, on winter period performance of five winter wheat varieties Khvylia Dnipra, MIP Roksolana, Vezha, LG Magirus, and LG Litopys. Seeds (1,000 per treatment) were soaked for 24 h in aqueous solutions of the tested compounds at concentrations of 0.01%, 0.02%, and 0.04%; water-treated seeds served as the control. The analysis focused on seed germination, plant survival before and after winter, and soluble sugar content in the tillering node on selected winter dates. The results demonstrated a clear concentration-dependent response. Among the tested agents, CA-79 showed the strongest and most stable stimulating effect, especially at 0.02%, which provided the highest values of germination, survival of plants during winter, and carbohydrate accumulation in the tillering node across most genotypes. CA-64 also produced a positive effect, particularly at 0.02%, while 0.01% was beneficial in several cases and may be regarded as a safer conservative option. By contrast, CA-67 did not provide reliable stimulation, 0.01% was mostly neutral, whereas 0.02% and especially 0.04% reduced sugar reserves and worsened the survival-related traits. All three compounds were uniformly depressive at a concentration of 0.04%, causing declines in germination, pre-winter plant survival, post-winter survival, and winter carbohydrate status, thus indicating a toxicity threshold. The response was genotype-specific. The Ukrainian varieties Khvylia Dnipra, MIP Roksolana, and Vezha generally exhibited higher baseline winter-resistance parameters and responded more positively to CA-79 and CA-64, whereas the French varieties LG Magirus and LG Litopys had lower baseline sugar levels and greater sensitivity to excessive concentration. In practical terms, CA-79 at 0.02% should be considered the most promising option for enhancing winter tolerance, while CA-64 at 0.02% may serve as an effective alternative. CA-67 cannot be recommended as a reliable stimulator under the studied conditions. Further studies should integrate these results with grain yield and grain quality traits to develop variety-specific protocols for the use of novel triazole-based growth regulators in winter wheat.

Keywords: *Triticum aestivum* L.; winter tolerance; triazole-based growth regulators; small grains; abiotic stress tolerance.

Introduction

Triazole derivatives are playing an increasingly important role in modern systems for optimizing agricultural technologies, as they are capable of influencing a wide range of biochemical and physiological processes in plants. In wheat, these compounds are particularly valuable because they promote a balanced vegetative and generative development, enhance resistance to abiotic and biotic factors, and create conditions for a fuller realization of the genetically determined yield potential. Their practical significance is due to a dual effect: A considerable number of triazoles combine the properties of growth regulators and broad-spectrum fungicides active against major diseases. For this reason, such compounds make it possible to more effectively integrate growth stimulation with crop protection within resource-saving cultivation technologies (Nazarenko & Khoroshun, 2025; Zhao et al., 2025).

The mechanism of action of triazole compounds is multicomponent. As fungicides, they primarily inhibit sterol biosynthesis in pathogenic fungi, which leads to disruption of the cell membrane structure. At the same time, some representatives of this group, particularly paclobutrazol and uniconazole, are also capable of interfering with the hormonal regulation of the plant itself, mainly through modification of gibberellin biosynthesis. As a result, more compact and stronger plants are formed, with a more efficient distribution of assimilates (Gao et al., 2025). In addition to their hormonal effects, triazoles can activate antioxidant enzyme systems, enhance osmotic regulation under drought or salinity stress, stabilize the chlorophyll complex and improve the control of respiration processes. Collectively, these mechanisms are expressed under field conditions through better crop establishment, development of a stronger root system, increased lodging resistance, and more efficient grain set and grain filling under stress conditions (Hu et al., 2025).

The practical effects of triazoles are often manifested already at the early stages of plant development. They can increase germination energy and ensure more uniform emergence, especially under reduced temperatures or unstable moisture conditions. During tillering and stem elongation, such regulators are capable of forming a more productive crop stand with a more optimal arrangement of the leaf surface, which improves light-use efficiency while simultaneously reducing the risk of lodging, particularly under intensive nitrogen nutrition. At the heading and flowering stages, triazole-based preparations can restrict the development of foliar and spike infections, maintain the functioning of the green leaf area, and thereby contribute to better crop formation. As a result, this may ensure a higher harvest index, more stable yields, and improvement in certain grain-quality parameters, particularly protein content or test weight, depending on the genotype and growing conditions (Zhang et al., 2025).

An important advantage of triazoles is their ability to improve plant water relations by regulating transpiration and maintaining photosynthetic activity during periods of moisture deficit. Improvement of root system architecture promotes more intensive uptake of mineral nutrients, thereby enhancing fertilizer efficiency and potentially increasing nitrogen-use efficiency. Owing to their compatibility with seed dressings, micronutrient formulations, and many foliar plant-protection products, triazoles can be readily integrated into conservation and precision agriculture systems. When applied at clearly defined doses and timings, they can improve production efficiency while reducing resource inputs, which is particularly important for modern resource-saving agricultural systems (Zhang et al., 2022; Rowshani et al., 2024).

In the context of global warming, the importance of such compounds is increasing even further, as wheat is more and more frequently exposed to short-term heat waves, unstable moisture conditions, and overwintering problems. In steppe and forest-steppe environments, where winter wheat often undergoes freeze–thaw cycles and suffers from spring drought, triazole-based programs can help stabilize yield by simultaneously enhancing plant stress tolerance and reducing disease development, which often intensifies during warmer and more humid periods. Their positive effect lies not so much in guaranteeing maximum yield every season as in lowering the risk of losses and increasing the probability of achieving the target level of productivity under variable weather conditions. The combination of fungicidal activity with growth-regulating effects strengthens the integrated management of productivity formation processes, and healthier and better-developed crops become less vulnerable to abiotic and biotic stresses, while timely regulatory action reduces the remaining risk (Du et al., 2024; Ahmad et al., 2025).

Despite their beneficial properties, triazole use is associated with potential environmental risks, which should also be taken into account. Some representatives of this group are characterized by high chemical stability in the environment and may reach groundwater and subsequently surface water bodies through runoff or spray drift. Their ability to bind with soil components and their rate of degradation depend both on the chemical nature of the compound and on soil type; therefore, repeated application without regard to soil and climatic conditions, as well as the timing of use, may increase local anthropogenic pressure. Although the toxicity of many triazoles to bees remains relatively low, sublethal and indirect effects are possible, especially in interaction with other crop-protection products, nutritional imbalances or disease pressure. For this reason, caution and toxicological planning are required when determining application timing and the composition of tank mixtures. In addition, long-term reliance on triazoles alone may accelerate the development of resistance in pathogen populations, thereby reducing their overall effectiveness and leading to the need for higher application rates (Zhang et al., 2024).

The objective of this study was to evaluate the potential of three new compounds, CA-64 (potassium [1,2,4]triazolo[1,5-c]quinazoline-2-thiolate), CA-79 (potassium tetrazolo[1,5-c]quinazoline-5-thiolate), and CA-67 (5-(2-aminophenyl)-1H-1,2,4-triazole-3-thiol), as plant growth stimulators. Particular attention was focused on their ability to induce winter hardiness and to enhance germination and survival, as well as on the specific features of the interaction of these compounds with different modern winter wheat varieties. An important objective of the study was also to assess the prospects for applying these compounds in winter wheat cultivation based on their predictive value for activating tolerance to the winter period and stimulating sugar accumulation.

Materials and methods

Field and laboratory studies were performed in 2025–2026 at the experimental facilities of the Department of Breeding and Seed Production, Dnipro State Agrarian and Economic University. The trials were established in the steppe zone under subhumid environmental conditions. Pre-sowing seed treatment was used as the main experimental factor. For this purpose, bread wheat seeds were exposed to aqueous solutions of three triazole-related compounds: CA-64 (potassium [1,2,4]triazolo[1,5-c]quinazoline-2-thiolate), CA-79 (potassium tetrazolo[1,5-c]quinazoline-5-thiolate), and CA-67 (5-(2-aminophenyl)-1H-1,2,4-triazole-3-thiol). Each compound was tested in three concentrations, namely 0.01, 0.02, and 0.04%. For every treatment option, as well as for the untreated control, 1,000 seeds were used. The soaking period was 24 h, which corresponds to standard approaches employed in experiments with physiologically active seed-applied compounds. In the control treatment, seeds were immersed in water only. The selected concentration range was based on previously obtained results for chemically related stimulatory compounds tested on cereal species (Nazarenko et al., 2022).

The experimental layout consisted of 60 treatment combinations generated from the interaction between varietal material and seed-treatment regimes. The varietal component included winter bread wheat cultivars MIP Roksolana (Ukraine), Vezha (Ukraine), Khvylia Dnipra (Ukraine), LG Magirus (France), and LG Litopys (France). The treatment component comprised nine combinations formed by three active compounds and three concentration levels, together with the water-treated control. Each experimental unit was represented by a ten-row plot; row spacing was maintained at 0.15 m and row length at 1.5 m. Agronomic maintenance and crop protection measures were implemented according to regional recommendations for the subhumid steppe zone, thereby reducing the risk that uncontrolled pest or disease pressure could distort the response to seed priming treatments (Brygadyrenko & Reshetniak, 2014; Ali & Baloch, 2020).

Plant development was monitored continuously throughout the vegetation period. Particular attention was paid to the evaluation of overwintering performance, which was assessed both by visual scoring and by determining the concentration of soluble sugars in the tillering node during the most critical phase of winter. In addition, field emergence, survival of plants in the stand, and uniformity of crop establishment were recorded in order to characterize the effect of pre-sowing treatment on the earliest stages of growth. To obtain a broader interpretation of trait relationships and treatment differentiation, multivariate statistical procedures were also employed where the dataset was suitable. In particular, linear discriminant analysis was used to identify the indicators that contributed most strongly to the separation of treatment groups and to estimate the practical reliability of classification. Such an approach made it possible to integrate information from seed response, winter hardiness physiology, and in-season plant functioning into a single analytical framework. This design provided a comprehensive basis for evaluating the agronomic potential of triazole-derived priming agents in winter wheat under local environmental conditions.

All numerical data were processed using Statistica 10.0 software (TIBCO, Palo Alto, USA). The influence of variety, chemical nature of the active compound, concentration, and their combined effects was examined by factorial analysis of variance. Statistical significance was accepted at the 5% probability level. Prior to formal testing, data distribution characteristics were checked with the Shapiro–Wilk test in order to assess compliance with assumptions of parametric analysis. Where necessary, the dataset was additionally adapted by transformation or analyzed using procedures appropriate for the observed distribution pattern. For each trait, descriptive indicators including arithmetic means and standard deviations were calculated, and graphical visualization was used to facilitate recognition of general tendencies and treatment-specific responses. When ANOVA revealed significant sources of variation, differences among means were further resolved with Tukey's honestly significant difference test.

Results

The first clear manifestation of the triazole-derived compounds was observed in the germination and survival of winter wheat plants. The effects of CA-64, CA-79, and CA-67 at different concentrations on seed germination, pre-winter survival, and post-winter survival are summarized in Tables 1–3.

For CA-64 (Table 1), a clear dose-dependent response was observed. At low and medium concentrations, the compound acted positively, whereas at the high concentration it suppressed all three parameters. When averaged across all varieties, the control values were approximately 91.31% for germination, 91.09% survival before winter, and 90.47% survival after winter. Against the background of 0.01% CA-64, these indicators increased on average to 93.54%, 93.22%, and 92.30%, respectively, indicating a stable improvement in early plant development and overwintering performance. An even stronger effect was provided by a 0.02% concentration, with the mean values reaching 94.58%, 94.25%, and 93.33%. By contrast, 0.04% sharply reduced the parameters to 84.98%, 83.56%, and 80.54%, respectively, indicating not stimulation but rather a toxic or stress-related effect.

Table 1
Effects of CA-64 on germination and survival (x ± SD, n = 10)

Variant	Germination	Pre-winter	Post-winter
Khvylia Dnipra	91.07 ± 0.12[a]	90.55 ± 0.14[a]	90.03 ± 0.13[a]
Khvylia Dnipra, CA-64 0.01%	93.60 ± 0.33[b]	93.58 ± 0.25[b]	93.06 ± 0.24[b]
Khvylia Dnipra, CA-64 0.02%	94.14 ± 0.34[b]	94.11 ± 0.35[b]	93.59 ± 0.32[b]
Khvylia Dnipra, CA-64 0.04%	86.24 ± 0.37[c]	85.22 ± 0.33[c]	80.70 ± 0.42[c]
MIP Roksolana	90.59 ± 0.24[a]	90.57 ± 0.24[a]	90.05 ± 0.23[a]
MIP Roksolana, CA-64 0.01%	93.12 ± 0.27[b]	92.60 ± 0.24[b]	92.08 ± 0.27[b]
MIP Roksolana, CA-64 0.02%	93.66 ± 0.26[b]	93.13 ± 0.27[b]	92.61 ± 0.28[b]
MIP Roksolana, CA-64 0.04%	85.76 ± 0.36[c]	84.24 ± 0.36[c]	81.22 ± 0.41[c]
Vezha	92.11 ± 0.22[a]	92.09 ± 0.23[a]	92.07 ± 0.22[a]
Vezha, CA-64 0.01%	93.14 ± 0.23[b]	92.62 ± 0.24[a]	92.10 ± 0.27[a]
Vezha, CA-64 0.02%	94.18 ± 0.23[c]	94.15 ± 0.27[b]	93.63 ± 0.27[b]
Vezha, CA-64 0.04%	84.78 ± 0.45[d]	82.76 ± 0.46[c]	80.74 ± 0.44[c]
LG Magirus	92.13 ± 0.22[a]	92.11 ± 0.23[a]	91.09 ± 0.27[a]
LG Magirus, CA-64 0.01%	93.16 ± 0.23[b]	93.14 ± 0.23[b]	92.12 ± 0.29[b]
LG Magirus, CA-64 0.02%	94.20 ± 0.35[c]	93.67 ± 0.35[b]	92.65 ± 0.32[b]
LG Magirus, CA-64 0.04%	85.30 ± 0.46[d]	84.28 ± 0.42[c]	80.26 ± 0.47[c]
LG Litopys	90.65 ± 0.22[a]	90.13 ± 0.24[a]	89.11 ± 0.23[a]
LG Litopys, CA-64 0.01%	94.68 ± 0.26[b]	94.16 ± 0.24[b]	92.14 ± 0.29[b]
LG Litopys, CA-64 0.02%	96.72 ± 0.26[c]	96.19 ± 0.26[c]	94.17 ± 0.27[c]
LG Litopys, CA-64 0.04%	82.82 ± 0.43[d]	81.30 ± 0.43[d]	79.78 ± 0.45[d]

Note. Within each variety, values in the same row marked with different letters indicate significant differences at P < 0.05 according to Tukey's HSD test; comparisons were made among the CA-64 concentration treatments only.

Thus, among the three concentrations, 0.02% was the most effective, since it ensured the highest values for all three parameters. The concentration 0.01% was also positive, although its effect was weaker. The concentration 0.04% was unequivocally negative and its depressive effect was most clearly expressed in survival, that is, at the final stage of plant overwintering. This means that the high concentration not only reduced initial germination, but also intensified plant losses during winter establishment and after the winter period.

It is also important that, when moving from one parameter to the next, the decline at the 0.01% and 0.02% treatments was generally small, indicating that the plants retained the advantage gained at earlier stages. At the 0.04% treatment, however, the decline between stages was much more pronounced, which suggests plant weakening already after emergence. Therefore, the general trend for CA-64 can be summarized as follows: Moderate doses improve germination, winter establishment, and survival, whereas the high concentration sharply worsens all parameters.

Regarding varietal variability, Khvylia Dnipra responded well to CA-64 at moderate doses. The control values were 91.07–90.55–90.03% respectevly, whereas at 0.01% they increased to 93.60–93.58–93.06%, and at 0.02% they rose further to 94.14–94.11–93.59%. Thus, for this variety both lower concentrations were beneficial, but 0.02% produced the best effect. At the same time, 0.04% reduced the parameters to 86.24–85.22–80.70%, with survival being affected most strongly. This indicates a high sensitivity of the variety to exceeding the optimal dose.

The variety MIP Roksolana showed a similar pattern of response. In the control, the parameters were 90.59–90.57–90.05%, and at 0.01% they increased to 93.12–92.60–92.08%. The best values were again observed at 0.02%, namely 93.66–93.13–92.61%. At the same time, the difference between 0.01% and 0.02% was not as large as in some other varieties, indicating that the variety responded positively but without an excessively strong increase. The concentration 0.04% caused a decline to 85.76–84.24–81.22%, which confirms the negative effect of the high dose.

The variety Vezha is distinguished by a very high control level, especially for the pre- and post-winter survival: 92.11–92.09–92.07%. This represents one of the best baseline levels among the studied varieties. However, at 0.01%, the increase was weak: 93.14–92.62–92.10%. In fact, a substantial improvement was observed only for germination, whereas pre- and post-winter survival differed only slightly from the control. By contrast, 0.02% already ensured a pronounced increase to 94.18–94.15–93.63%. At 0.04%, the variety responded very negatively 84.78–82.76–80.74%. Thus, Vezha is characterized by a relatively restrained response to 0.01%, but a good response to 0.02%.

Variety LG Magirus demonstrated high initial values of the studied parameters. Under the effect of 0.01%, a moderate improvement was observed, while 0.02% resulted in a further increase. Thus, the variety responded positively to CA-64, although the amplitude of increase was smaller than, for example, in LG Litopys. LG Litopys proved to be the most contrasting and, at the same time, the most sensitive variety to the action of CA-64. It showed the strongest realization of the stimulatory potential of CA-64. At the same time, this variety reacted very sharply to excessive concentration: 0.04% reduced the parameters to the lowest or nearly the lowest values. Accordingly, LG Litopys may be regarded as the variety with the greatest positive response to the optimal dose, but also with a very pronounced negative effect under overdosing.

If the varieties are evaluated by their control values, the best initial state was observed in Vezha and LG Magirus, whereas LG Litopys and, to some extent, MIP Roksolana started from somewhat lower levels. However, it was precisely the lower control background in LG Litopys that was accompanied by the strongest positive response to the optimal concentrations of CA-64. Thus, the varieties differed not only in their baseline level of the studied parameters, but also in the degree of responsiveness to treatment.

ANOVA confirmed that all studied parameters varied significantly across concentration treatments ($F = 21.77$; $F_{0.05} = 3.25$; $P = 2.12 \times 10^{-4}$). Significant varietal differences were likewise observed ($F = 7.12$; $F_{0.05} = 3.49$; $P = 0.01$). In addition, the genotype × mutagen interaction was statistically significant ($F = 6.79$; $F_{0.05} = 5.10$; $P = 0.02$), indicating that the response to treatment depended on the variety.

The obtained data clearly demonstrate that the effect of CA-64 on germination, winter establishment, and survival of winter wheat has a pronounced concentration-dependent character. All varieties responded positively to 0.01% and especially to 0.02%, with 0.02% being regarded as the optimal concentration because it ensured the maximum values of all three parameters. In all cases, the concentration 0.04% produced a negative effect, reducing both germination and the other traits, with the most pronounced decline observed in survival. Among the varieties, LG Litopys showed the best response, whereas Vezha and LG Magirus were characterized by a high baseline level but a more moderate increase after treatment. From a practical point of view, this means that CA-64 should be used only at low and medium concentrations, while increasing the dose to 0.04% is undesirable because of the clearly expressed suppression of all studied parameters.

For CA-79 (Table 2), a clear concentration-dependent response was also observed, although its effect in some varieties was somewhat more contrasting than that of CA-64. On average across all varieties, the control values were approximately 91.38% for germination, 91.12% for pre-winter, and 90.48% post-winter survival. Against the background of 0.01% CA-79, these indicators increased to 93.67%, 93.15%, and 92.03%, showing that the low concentration generally had a positive effect on early development and plant survival. The highest mean values were obtained at 0.02%, where they reached 95.91%, 95.28%, and 93.56%. By contrast, 0.04% caused a sharp decline to 83.81%, 82.19%, and 77.97%, indicating a pronounced depressive effect of the high dose.

Overall, 0.02% was the most effective concentration of CA-79, since it provided the highest average values for all three parameters. The concentration 0.01% also had a stimulatory effect, but it was weaker and not always equally expressed for all traits in all varieties. By contrast, 0.04% was clearly negative: It reduced not only laboratory and field germination, but also particularly strongly suppressed surviving, that is, the post-winter survival of plants. This parameter turned out to be the most sensitive to overdosing.

Khvylia Dnipra showed a very good response to CA-79. The best values were recorded at 0.02%, indicating a pronounced stimulatory effect on all three parameters simultaneously. At 0.04%, a sharp decline was observed. Thus, for this variety, CA-79 at 0.02% was clearly optimal, whereas 0.04% was harmful.

Table 2
Effects of CA-79 on germination and survival (x ± SD, n = 10)

Variant	Germination	Pre-winter	Post-winter
Khvylia Dnipra	91.10 ± 0.13[a]	91.08 ± 0.12[a]	90.56 ± 0.13[a]
Khvylia Dnipra, CA-79 0.01%	94.13 ± 0.32[b]	93.61 ± 0.34[b]	93.09 ± 0.23[b]
Khvylia Dnipra, CA-79 0.02%	96.17 ± 0.34[c]	95.64 ± 0.34[c]	95.12 ± 0.34[c]
Khvylia Dnipra, CA-79 0.04%	84.27 ± 0.35[d]	83.25 ± 0.34[d]	80.23 ± 0.35[d]
MIP Roksolana	90.62 ± 0.25[a]	90.10 ± 0.25[a]	90.00 ± 0.26[a]
MIP Roksolana, CA-79 0.01%	92.65 ± 0.24[b]	92.13 ± 0.25[b]	91.11 ± 0.24[b]
MIP Roksolana, CA-79 0.02%	96.19 ± 0.34[c]	95.66 ± 0.34[c]	94.64 ± 0.34[c]
MIP Roksolana, CA-79 0.04%	84.79 ± 0.35[d]	84.77 ± 0.36[d]	79.75 ± 0.38[d]
Vezha	92.34 ± 0.23[a]	92.10 ± 0.23[a]	91.60 ± 0.24[a]
Vezha, CA-79 0.01%	93.67 ± 0.22[b]	93.65 ± 0.25[b]	93.13 ± 0.24[b]
Vezha, CA-79 0.02%	96.21 ± 0.34[c]	95.68 ± 0.35[c]	95.16 ± 0.36[c]
Vezha, CA-79 0.04%	84.31 ± 0.34[d]	82.79 ± 0.38[d]	79.77 ± 0.37[d]
LG Magirus	92.16 ± 0.23[a]	92.14 ± 0.22[a]	91.12 ± 0.25[a]
LG Magirus, CA-79 0.01%	92.69 ± 0.22[a]	92.17 ± 0.23[a]	90.65 ± 0.23[a]
LG Magirus, CA-79 0.02%	94.73 ± 0.26[b]	94.20 ± 0.23[b]	90.68 ± 0.24[a]
LG Magirus, CA-79 0.04%	83.83 ± 0.43[c]	80.81 ± 0.37[c]	74.79 ± 0.38[b]
LG Litopys	90.68 ± 0.23[a]	90.16 ± 0.22[a]	89.14 ± 0.23[a]
LG Litopys, CA-79 0.01%	95.21 ± 0.32[b]	94.19 ± 0.29[b]	92.17 ± 0.30[b]
LG Litopys, CA-79 0.02%	96.25 ± 0.25[c]	95.22 ± 0.28[c]	92.20 ± 0.31[b]
LG Litopys, CA-79 0.04%	81.85 ± 0.42[d]	79.33 ± 0.41[d]	75.31 ± 0.41[c]

Note: see Table 1.

MIP Roksolana also responded positively to CA-79, but its response showed a more pronounced gradient between 0.01% and 0.02%. The concentration 0.02% produced a very strong increase, making this treatment the most effective for the variety. By contrast, 0.04% sharply worsened the plant performance. Thus, MIP Roksolana responded best to the medium dose, whereas the low concentration was less effective and the high one was clearly suppressive. Vezha had one of the best control backgrounds. A noticeable improvement was already observed at 0.01%, indicating that even the low concentration acted effectively. Once again, the best treatment was 0.02%. At 0.04%, a strong decline was recorded.

LG Litopys was characterized by a very low control background compared with Vezha and LG Magirus, but at the same time it responded very well to the low and medium concentrations in terms of the first two parameters. This indicates that, for LG Litopys, the concentration 0.02% mainly improved the early phases of development, but did not provide a substantial additional advantage in final survival compared with 0.01%. The high concentration 0.04% was sharply negative.

ANOVA revealed significant variation in all studied parameters across concentration treatments ($F = 21.34$; $F_{0.05} = 3.25$; $P = 1.17 \times 10^{-4}$). Significant differences among varieties were also identified ($F = 7.73$; $F_{0.05} = 3.49$; $P = 0.007$). In addition, the genotype × mutagen interaction was statistically significant ($F = 7.10$; $F_{0.05} = 5.10$; $P = 0.01$), indicating that varietal responses depended on the treatment applied.

The response to CA-79 depended not only on the initial state of the plants, but also on the genotypic sensitivity of each variety to the compound. The most complete and well-balanced positive response to 0.02% CA-79 was shown by Khvylia Dnipra, MIP Roksolana, and Vezha, since in these varieties all three parameters increased simultaneously, including survival. LG Litopys also responded well to the treatment, but mainly in terms of germination and pre-winter survival, whereas for post-winter survival the additional effect of 0.02% compared with 0.01% was minimal. The least pronounced positive response was observed in LG Magirus, in which CA-79 almost did not improve survival even at stimulatory concentrations. In response to 0.04%, all varieties consistently showed suppression; however, LG Magirus and LG Litopys were the most sensitive to the high dose, especially in terms of survival, which declined most strongly in these varieties.

For CA-67 (Table 3), a completely different pattern of action was revealed compared with CA-64 and CA-79. Whereas the previous compounds showed a stimulatory effect at low or medium doses, CA-67 generally did not demonstrate a distinct positive effect and with increasing concentration its influence became clearly negative. On average across all varieties, the control values were approximately 91.37% for germination, 91.05% for pre-winter, and 90.23% for post-winter survival. At the concentration of 0.01%, these parameters changed only slightly, reaching about 91.80%, 91.47%, and 90.36%, respectively. Thus, the observed increase was only at the level of tenths of a percent and, and, therefore, from a biological point of view, should rather be regarded as a neutral effect than as true stimulation.

Table 3
Effects of CA-67 on germination and survival (x ± SD, n = 10)

Variant	Germination	Pre-winter	Post-winter
Khvylia Dnipra	91.13 ± 0.12[a]	91.11 ± 0.15[a]	90.59 ± 0.14[a]
Khvylia Dnipra, CA-67 0.01%	91.16 ± 0.33[a]	91.14 ± 0.27[a]	90.62 ± 0.28[a]
Khvylia Dnipra, CA-67 0.02%	89.20 ± 0.34[b]	88.67 ± 0.34[b]	86.15 ± 0.36[b]
Khvylia Dnipra, CA-67 0.04%	86.30 ± 0.37[c]	83.78 ± 0.34[c]	80.76 ± 0.42[c]
MIP Roksolana	90.65 ± 0.24[a]	90.13 ± 0.24[a]	90.11 ± 0.24[a]
MIP Roksolana, CA-67 0.01%	91.18 ± 0.27[a]	91.10 ± 0.25[b]	90.14 ± 0.25[a]
MIP Roksolana, CA-67 0.02%	88.72 ± 0.26[b]	87.69 ± 0.28[c]	83.67 ± 0.29[b]
MIP Roksolana, CA-67 0.04%	84.82 ± 0.36[c]	83.80 ± 0.36[d]	79.78 ± 0.42[c]
Vezha	92.17 ± 0.22[a]	91.65 ± 0.25[a]	91.13 ± 0.26[a]
Vezha, CA-67 0.01%	92.70 ± 0.23[a]	92.18 ± 0.25[a]	91.16 ± 0.27[a]
Vezha, CA-67 0.02%	91.24 ± 0.23[b]	90.21 ± 0.25[b]	86.19 ± 0.36[b]
Vezha, CA-67 0.04%	83.84 ± 0.45[c]	82.82 ± 0.45[c]	77.80 ± 0.48[c]
LG Magirus	92.19 ± 0.22[a]	92.17 ± 0.24[a]	90.15 ± 0.23[a]
LG Magirus, CA-67 0.01%	92.72 ± 0.23[a]	92.20 ± 0.25[a]	90.18 ± 0.24[a]
LG Magirus, CA-67 0.02%	91.26 ± 0.35[b]	90.23 ± 0.35[b]	84.21 ± 0.32[b]
LG Magirus, CA-67 0.04%	83.86 ± 0.46[c]	82.84 ± 0.37[c]	74.82 ± 0.41[c]
LG Litopys	90.71 ± 0.22[a]	90.19 ± 0.24[a]	89.17 ± 0.27[a]
LG Litopys, CA-67 0.01%	91.24 ± 0.26[a]	90.72 ± 0.24[a]	89.70 ± 0.27[a]
LG Litopys, CA-67 0.02%	90.28 ± 0.26[ab]	89.25 ± 0.29[b]	83.23 ± 0.33[b]
LG Litopys, CA-67 0.04%	85.38 ± 0.43[c]	83.86 ± 0.33[c]	74.34 ± 0.41[c]

Note: see Table 1.

Khvylia Dnipra was one of the varieties that reacted least sharply to CA-67, although the general pattern of increasingly negative effects with rising concentration was still maintained. In the control, the values were 91.13–91.11–90.59%, and at 0.01% they remained almost unchanged. This indicates an actual absence of stimulation. At 0.02%, a noticeable decline was observed. MIP Roksolana reacted in a similar way, although somewhat more sharply. The control values were 90.65–90.13–90.11%. At 0.01%, no substantial improvement was recorded. At 0.02%, the values decreased. Thus, MIP Roksolana was fairly sensitive to the medium and high concentrations, especially in terms of survival. Vezha had a high control background. However, even for this variety, 0.01% produced virtually no beneficial effect, since the parameters changed only minimally. At 0.02%, the deterioration became distinct. LG Magirus in the control was also characterized by high values. At 0.01%, the compound had virtually no effect on the parameters. However, already at 0.02% a noticeable decline occurred, especially in survival. At 0.04%, the negative effect became one of the strongest among all varieties. Therefore, LG Magirus can be regarded as a genotype with high sensitivity to the toxic action of CA-67, particularly at the final stage of overwintering. The same is true for LG Litopys.

ANOVA showed that all studied parameters varied significantly with changes in concentration ($F = 24.11$; $F_{0.05} = 3.25$; $P = 2.14 \times 10^{-4}$). Differences among varieties were not statistically significant ($F = 2.76$; $F_{0.05} = 3.49$; $P = 0.08$). At the same time, the genotype × mutagen interaction reached the significance threshold ($F = 5.68$; $F_{0.05} = 5.10$; $P = 0.04$), indicating that varietal responses to the treatments were not uniform.

According to the control values, the best initial level was observed in Vezha and LG Magirus, whereas MIP Roksolana and LG Litopys were characterized by a somewhat lower baseline. None of the varieties demonstrated a truly positive stimulation from CA-67. At best, CA-67 0.01% produced a neutral effect or promoted only very slight changes that cannot be regarded as a pronounced improvement. In terms of sensitivity to 0.02% and 0.04%, LG Magirus and LG Litopys appeared to be the most problematic, as their survival declined especially strongly. MIP Roksolana also showed a marked deterioration. Khvylia Dnipra may be considered relatively less sensitive, since even under stress treatments its values remained somewhat higher than those of a number of other varieties. Vezha occupied an intermediate position, its control status was good, but losses also became substantial as concentration increased.

In general, the comparison of the three compounds showed that CA-64 and CA-79 were the most effective in improving germination, pre-winter, and post-winter survival, whereas CA-67 proved to be the least promising. For CA-64 and CA-79, a clear positive effect was observed at low and medium concentrations, with 0.02% providing the best results in most cases, as it produced the highest values for all three parameters. The concentration 0.01% also generally had a stimulatory effect, although weaker. At the same time, 0.04% for both compounds showed a pronounced depressive effect, reducing not only germination but also subsequent plant pre- and post-winter survival.

Against this background, CA-79 may be regarded as the compound with the strongest stimulatory potential, since it produced the highest absolute values in a number of varieties, especially at 0.02%. CA-64 also acted positively and rather consistently, although in some cases its stimulatory effect was somewhat weaker than that of CA-79. Meanwhile, CA-67 did not produce a distinct improvement even at the lowest concentration of 0.01%, and when the dose was increased to 0.02%, and especially 0.04%, it caused a consistent deterioration of all studied parameters. Thus, from a practical point of view, CA-79 is the most promising option for pre-sowing seed treatment, CA-64 is somewhat inferior to it, whereas CA-67 cannot be considered an effective stimulator because of its predominantly neutral or negative effect.

For CA-64 (Table 4), a clear dose-dependent pattern was observed in sugar accumulation. On average across all varieties, the control values of sugar content were about 29.4% at time point 11 (10 November, cessation of vegetation), 25.8% at time point 02 (20 February), and 21.8% at time point 03 (10 March, effectively just before the resumption of active vegetation). This reflects the natural decline in sugar reserves over the winter period. Against the background of 0.01% CA-64, the mean values increased to 31.1%, 27.3%, and 23.2%, respectively, indicating that the low concentration ensured a noticeable improvement in carbohydrate accumulation and pre-winter survival. An even stronger effect was produced by 0.02%, with the mean values reaching 32.6%, 28.4%, and 25.0%. By contrast, 0.04% reduced sugar content to 26.8%, 23.4%, and 19.4%, which already represents a clearly negative effect.

Thus, among all concentrations, 0.02% was the most effective, since it ensured the highest level of sugars in the tillering node at all observation dates. The concentration 0.01% also had a positive effect, although weaker. The high concentration 0.04%, by contrast, caused a reduction in carbohydrate reserves not only compared with the stimulatory treatments, but also in comparison with the control. This indicates that for CA-64 there is a relatively narrow beneficial range within which the compound stimulates the physiological mechanisms of winter hardiness, whereas exceeding this range is already accompanied by suppression.

A general trend across all treatments was a gradual decrease in sugar content from time point 11 to 2 and then to 3, which is a natural pattern during the winter period. However, under 0.01% and especially 0.02%, this decline occurred at a higher absolute level, meaning that the plants entered the winter period and passed through it with larger reserves of nutrients. Under 0.04%, the decline started from an already lower initial level; therefore, this treatment can be regarded as physiologically unfavorable.

The variety Khvylia Dnipra responded well to CA-64. In the control, sugar content was 33.2%, 29.7%, and 24.3% at the respective observation dates; under 0.01%, it increased to 35.3–32.7–28.5% and under 0.02% to 37.1–33.8–31.1%. Thus, 0.02% ensured the greatest accumulation of sugars at all observation dates. The concentration 0.04% reduced the values to 31.2–27.5–22.7%, that is, even below the control. This indicates a pronounced stimulatory effect of the low and medium concentrations and a negative effect of the increased dose. MIP Roksolana was characterized by one of the highest control levels of sugars, measuring 33.9–32.6–29.4%. For MIP Roksolana, 0.02% was also the best treatment, whereas 0.01% acted only moderately. Vezha had a lower baseline level of sugars than the two previous varieties: 30.2%, 26.0%, and 22.0% at the respective dates. Nevertheless, the positive response to CA-64 was very clear. The medium concentration again proved to be optimal. At 0.04%, a decrease to 28.8–24.0–19.9% was observed, indicating suppression of reserve formation during the winter period.

Table 4

Parameters of winter wheat CA-64 varieties during the winter period of the 2025/2026 growing season (x ± SD, n = 5)

Variety	Content of sugars in tillering nod, %		
	11	2	3
Khvylia Dnipra	33.2 ± 0.5[a]	29.7 ± 0.5[a]	24.3 ± 0.4[a]
Khvylia Dnipra, CA-64 0.01%	35.3 ± 0.5[b]	32.7 ± 0.4[b]	28.5 ± 0.5[b]
Khvylia Dnipra, CA-64 0.02%	37.1 ± 0.4[c]	33.8 ± 0.5[c]	31.1 ± 0.4[c]
Khvylia Dnipra, CA-64 0.04%	31.2 ± 0.5[d]	27.5 ± 0.4[d]	22.7 ± 0.5[d]
MIP Roksolana	33.9 ± 0.5[a]	32.6 ± 0.5[a]	29.4 ± 0.4[a]
MIP Roksolana, CA-64 0.01%	35.3 ± 0.5[b]	32.9 ± 0.4[a]	29.1 ± 0.5[a]
MIP Roksolana, CA-64 0.02%	36.8 ± 0.5[c]	33.7 ± 0.5[b]	30.3 ± 0.5[b]
MIP Roksolana, CA-64 0.04%	30.8 ± 0.5[d]	28.6 ± 0.4[c]	23.8 ± 0.5[c]
Vezha	30.2 ± 0.4[a]	26.0 ± 0.5[a]	22.0 ± 0.4[a]
Vezha, CA-64 0.01%	31.6 ± 0.5[b]	27.2 ± 0.5[b]	23.0 ± 0.5[b]
Vezha, CA-64 0.02%	33.1 ± 0.4[c]	28.2 ± 0.5[c]	25.0 ± 0.5[c]
Vezha, CA-64 0.04%	28.8 ± 0.4[d]	24.0 ± 0.5[d]	19.9 ± 0.5[d]
LG Magirus	25.3 ± 0.5[a]	21.0 ± 0.5[a]	16.7 ± 0.6[a]
LG Magirus, CA-64 0.01%	27.0 ± 0.5[b]	21.9 ± 0.4[b]	18.2 ± 0.6[b]
LG Magirus, CA-64 0.02%	28.5 ± 0.5[c]	23.1 ± 0.5[c]	19.8 ± 0.5[c]
LG Magirus, CA-64 0.04%	22.1 ± 0.5[d]	18.8 ± 0.6[d]	15.4 ± 0.4[d]
LG Litopys	24.4 ± 0.5[a]	19.8 ± 0.5[a]	16.4 ± 0.4[a]
LG Litopys, CA-64 0.01%	26.3 ± 0.5[b]	21.9 ± 0.4[b]	17.4 ± 0.5[b]
LG Litopys, CA-64 0.02%	27.6 ± 0.5[c]	23.0 ± 0.5[c]	18.9 ± 0.5[c]
LG Litopys, CA-64 0.04%	21.2 ± 0.5[d]	18.0 ± 0.6[d]	15.0 ± 0.4[d]

Note: see Table 1.

LG Magirus was characterized by a much lower initial level of carbohydrates: 25.3%, 21.0%, and 16.7% at the respective observation dates. At the same time, the compound had a positive effect on this variety at low concentrations. The concentration 0.04% reduced the parameters below the control level. LG Magirus also responded better to 0.02%, although its overall sugar level remained lower than that of the local varieties. LG Litopys had the lowest, or one of the lowest, control values. Nevertheless, the response to CA-64 was positive and quite pronounced. Even in this variety, 0.02% produced the best result. The high concentration 0.04% was negative.

The effect of genotype was statistically significant ($F = 21.17$; $F_{0.05} = 2.40$; $P = 1.54 \times 10^{-5}$), and the effect of concentration was likewise significant ($F = 32.16$; $F_{0.05} = 2.90$; $P = 3.37 \times 10^{-6}$). In addition, the genotype × concentration interaction was also significant ($F = 9.89$; $F_{0.05} = 5.11$; $P = 0.01$), indicating that the response to concentration varied depending on the genotype.

Regarding the control values, the highest natural level of sugar accumulation was observed in MIP Roksolana and Khvylia Dnipra, followed by Vezha, whereas LG Magirus and LG Litopys were characterized by a lower carbohydrate background. This may indicate certain genotypic peculiarities in the formation of winter reserves. At the same time, all varieties responded to CA-64 in a generally similar way: 0.01% and especially 0.02% increased sugar content, whereas 0.04% reduced it. In terms of the magnitude of the positive response to 0.02%, Khvylia Dnipra and Vezha performed particularly well, showing a clear increase at all observation dates. MIP Roksolana also exhibited high values, although this was partly due to its already high initial background. LG Magirus and LG Litopys also responded positively to the compound; however, their absolute sugar levels remained lower, which may indicate a lower potential for carbohydrate accumulation during the winter period. The obtained results indicate that the effect of CA-64 on sugar content in the tillering node of winter wheat has a clearly expressed concentration-dependent character.

For CA-79 (Table 5), a clear concentration-dependent response was also observed. On average across all varieties, the control values were about 29.5% at time point 11, 25.9% at time point 2 and 21.9% at time point 3. This reflects the natural decline in sugar reserves during the winter period. Against the background of 0.01% CA-79, the mean values increased to approximately 31.5%, 28.4%, and 24.3%, indicating that even the low concentration already ensured noticeable accumulation of carbohydrate reserves. The highest values were observed at 0.02%, where the mean levels reached about 33.4%, 30.4%, and 26.7%. By contrast, 0.04% reduced the indicators to 27.4%, 24.3%, and 20.2%, which indicates a pronounced depressive effect of the increased dose.

Table 5
Parameters of winter wheat CA-79 varieties during the winter period of the 2025/2026 growing season (x ± SD, n = 5)

Variety	Content of sugars in tillering nod, %		
	11	2	3
Khvylia Dnipra	33.4 ± 0.5^{a}	29.8 ± 0.5^{a}	24.5 ± 0.4^{a}
Khvylia Dnipra, CA-79 0.01%	35.8 ± 0.4^{b}	33.5 ± 0.5^{b}	29.1 ± 0.4^{b}
Khvylia Dnipra, CA-79 0.02%	37.6 ± 0.5^{c}	35.0 ± 0.4^{c}	32.1 ± 0.5^{c}
Khvylia Dnipra, CA-79 0.04%	31.1 ± 0.4^{d}	28.6 ± 0.5^{d}	23.8 ± 0.4^{d}
MIP Roksolana	33.9 ± 0.5^{a}	32.8 ± 0.5^{a}	29.4 ± 0.4^{a}
MIP Roksolana, CA-79 0.01%	35.0 ± 0.5^{b}	33.9 ± 0.5^{b}	30.0 ± 0.5^{a}
MIP Roksolana, CA-79 0.02%	36.7 ± 0.4^{c}	35.6 ± 0.4^{c}	32.4 ± 0.4^{b}
MIP Roksolana, CA-79 0.04%	31.6 ± 0.5^{d}	30.7 ± 0.5^{d}	26.9 ± 0.5^{c}
Vezha	30.3 ± 0.4^{a}	26.0 ± 0.5^{a}	22.1 ± 0.5^{a}
Vezha, CA-79 0.01%	32.6 ± 0.5^{b}	27.4 ± 0.4^{b}	23.9 ± 0.5^{b}
Vezha, CA-79 0.02%	34.2 ± 0.4^{c}	29.1 ± 0.4^{c}	26.1 ± 0.4^{c}
Vezha, CA-79 0.04%	28.9 ± 0.4^{d}	23.9 ± 0.5^{d}	19.5 ± 0.4^{d}
LG Magirus	25.4 ± 0.5^{a}	21.1 ± 0.5^{a}	16.8 ± 0.6^{a}
LG Magirus, CA-79 0.01%	27.5 ± 0.4^{b}	23.0 ± 0.4^{b}	19.0 ± 0.4^{b}
LG Magirus, CA-79 0.02%	29.6 ± 0.4^{c}	26.2 ± 0.4^{c}	21.8 ± 0.4^{c}
LG Magirus, CA-79 0.04%	23.2 ± 0.5^{d}	20.0 ± 0.5^{d}	15.3 ± 0.5^{d}
LG Litopys	24.5 ± 0.5^{a}	19.9 ± 0.5^{a}	16.5 ± 0.5^{a}
LG Litopys, CA-79 0.01%	26.8 ± 0.4^{b}	24.0 ± 0.4^{b}	19.4 ± 0.4^{b}
LG Litopys, CA-79 0.02%	28.7 ± 0.4^{c}	26.1 ± 0.4^{c}	21.0 ± 0.4^{c}
LG Litopys, CA-79 0.04%	22.4 ± 0.4^{d}	18.5 ± 0.5^{d}	15.3 ± 0.4^{d}

Note: see Table 1.

Thus, 0.02% was the best concentration of CA-79, since it ensured the highest sugar content in the tillering node at all observation dates. The concentration 0.01% also acted positively, but weaker. The 0.04% treatment was inferior in all cases not only to the stimulatory doses, but also, as a rule, to the control. This means that CA-79 has a clearly beneficial dose window within 0.01–0.02%, whereas exceeding it leads to depletion of carbohydrate reserves.

Khvylia Dnipra responded very well to CA-79. The variety showed a clearly positive response to 0.01% and especially to 0.02%, but a negative one to 0.04%. MIP Roksolana was also characterized by a high baseline level of sugar content. Even 0.04% in this variety gave relatively higher values than in most other varieties; however, these values were still lower than those of the optimal treatments and were inferior to the control at some observation dates. This indicates that MIP Roksolana has a high natural level of carbohydrate reserves and at the same time responds well to 0.02% CA-79. Vezha had a lower control level of sugars compared with Khvylia Dnipra and MIP Roksolana. However, its response to the compound was very clear. The medium concentration was the most effective. The concentration 0.04% sharply worsened the status of carbohydrate reserves. LG Magirus was characterized by a noticeably lower initial level of carbohydrates. Nevertheless, CA-79 clearly improved these values. At 0.04%, the indicators declined. Therefore, for LG Magirus as well, 0.02% was the best treatment, although the absolute level of sugars remained lower than in the local varieties. LG Litopys had the lowest, or one of the lowest, control levels and responded well to 0.01–0.02%, especially to 0.02%.

For the remaining varieties, particularly the western ecotypes, CA-67 should generally be avoided during winter establishment. The effect of genotype was statistically significant (F = 7.99; $F_{0.05}$ = 2.40; P = 0.007), and the effect of concentration was also significant (F = 10.90; $F_{0.05}$ = 2.90; P = 0.001). However, the genotype × concentration interaction was not statistically significant (F = 4.69; $F_{0.05}$ = 5.11; P = 0.07), indicating that varietal differences in response to concentration were not pronounced.

According to the control values, the highest natural level of sugar content was observed in MIP Roksolana and Khvylia Dnipra, somewhat lower in Vezha and the lowest in LG Magirus and LG Litopys. This indicates clear genotypic differences in the formation of carbohydrate reserves during the winter period. At the same time, all varieties responded to CA-79 in the same direction. Thus, 0.01% and especially 0.02% increased sugar content, whereas 0.04% reduced it. CA-79 at 0.02% appears to be the most promising treatment for strengthening the physiological basis of winter hardiness in winter wheat, whereas the use of 0.04% is inadvisable because of its clearly expressed depressive effect.

For CA-67 (Table 6), a different type of action was observed than for CA-64 and CA-79. While the previous two compounds showed a stimulatory range within 0.01–0.02%, no such clearly positive interval was found for CA-67. On average across all varieties, the control values were about 29.6% at time point 11, 26.0% at time point 02 and 21.9% at time point 03. At 0.01% CA-67, these indicators remained almost the same or were even slightly lower, approximately 29.5%, 25.9%, and 21.4%. Thus, the low concentration generally did not provide a true stimulatory effect and, at best, was neutral.

Table 6
Parameters of winter wheat CA-67 varieties during the winter period of the 2025/2026 growing season (x ± SD, n = 5)

Variety	Content of sugars in tillering nod, %		
	11	2	3
Khvylia Dnipra	33.4 ± 0.5^{a}	30.0 ± 0.4^{a}	24.5 ± 0.4^{a}
Khvylia Dnipra, CA-67 0.01%	33.6 ± 0.5^{a}	30.9 ± 0.4^{b}	24.8 ± 0.5^{a}
Khvylia Dnipra, CA-67 0.02%	32.2 ± 0.4^{b}	28.0 ± 0.5^{c}	23.7 ± 0.4^{b}
Khvylia Dnipra, CA-67 0.04%	29.4 ± 0.5^{c}	25.3 ± 0.4^{d}	19.9 ± 0.4^{c}
MIP Roksolana	34.0 ± 0.5^{a}	32.8 ± 0.5^{a}	29.5 ± 0.4^{a}
MIP Roksolana, CA-67 0.01%	33.7 ± 0.5^{a}	32.7 ± 0.4^{a}	29.1 ± 0.5^{a}
MIP Roksolana, CA-67 0.02%	32.0 ± 0.5^{b}	30.8 ± 0.5^{b}	25.5 ± 0.5^{b}
MIP Roksolana, CA-67 0.04%	29.0 ± 0.5^{c}	27.1 ± 0.4^{c}	20.0 ± 0.5^{c}
Vezha	30.4 ± 0.4^{a}	26.1 ± 0.5^{a}	22.2 ± 0.5^{a}
Vezha, CA-67 0.01%	30.7 ± 0.5^{a}	24.4 ± 0.5^{b}	20.4 ± 0.5^{b}
Vezha, CA-67 0.02%	29.3 ± 0.4^{b}	23.4 ± 0.5^{c}	19.5 ± 0.5^{c}
Vezha, CA-67 0.04%	27.0 ± 0.4^{c}	22.1 ± 0.5^{d}	17.9 ± 0.5^{d}
LG Magirus	25.5 ± 0.5^{a}	21.2 ± 0.5^{a}	16.9 ± 0.6^{a}
LG Magirus, CA-67 0.01%	25.2 ± 0.5^{a}	21.1 ± 0.4^{a}	16.4 ± 0.5^{a}
LG Magirus, CA-67 0.02%	23.7 ± 0.5^{b}	19.9 ± 0.5^{b}	15.3 ± 0.4^{b}
LG Magirus, CA-67 0.04%	21.3 ± 0.5^{c}	18.4 ± 0.6^{c}	13.7 ± 0.4^{c}
LG Litopys	24.6 ± 0.5^{a}	20.0 ± 0.5^{a}	16.6 ± 0.5^{a}
LG Litopys, CA-67 0.01%	24.4 ± 0.5^{a}	20.2 ± 0.4^{a}	16.4 ± 0.5^{a}
LG Litopys, CA-67 0.02%	23.6 ± 0.5^{b}	18.6 ± 0.5^{b}	15.3 ± 0.5^{b}
LG Litopys, CA-67 0.04%	21.4 ± 0.5^{c}	17.2 ± 0.6^{c}	13.2 ± 0.4^{c}

Note: see Table 1.

The concentration 0.02% already caused a clear decrease in sugar content. This indicates that the medium dose of CA-67 did not improve the physiological condition of plants during the winter period, but, on the contrary, weakened the accumulation of reserve carbohydrates. The negative effect was most strongly expressed at the later assessment date, that is, during the period when maintaining a sufficient level of sugars is especially important for the successful completion of overwintering. The most unfavorable treatment was 0.04%.

Khvylia Dnipra was one of the few varieties in which 0.01% CA-67 had not yet acquired a clearly negative character. However, even in this variety, no true positive stimulation was observed, and with increasing concentration the depressive effect became clearly expressed. MIP Roksolana had one of the highest control levels of carbohydrates. At the same time, even 0.01% did not improve these values, but only maintained them at approximately the control level or slightly below it; thus, for MIP Roksolana, the low concentration was nearly neutral, whereas the medium and high concentrations were clearly negative. Vezha proved to be more sensitive to CA-67, showing a distinctly negative response already beginning from the minimum dose. For LG Magirus and LG Litopys, the action of CA-67 did not exert any stimulatory effect at any concentration.

The effect of genotype was statistically significant (F = 17.16; $F_{0.05}$ = 2.40; P = 3.04 × 10^{-4}), and the effect of concentration was likewise significant (F = 19.00; $F_{0.05}$ = 2.90; P = 5.17 × 10^{-4}). In addition, the genotype × concentration interaction was also significant (F = 9.89; $F_{0.05}$ = 5.11; P = 0.01), indicating that the response to concentration varied depending on the genotype.

The highest natural sugar content was observed in MIP Roksolana and Khvylia Dnipra, somewhat lower in Vezha, and the lowest in LG Magirus and LG Litopys. This generally repeats the pattern identified for CA-64 and CA-79. However, in the case of CA-67, the main difference was that none of the varieties showed a clearly expressed stimulatory effect. The variety closest to a neutral-to-positive response was Khvylia Dnipra, in which 0.01% did not yet worsen the parameters. In MIP Roksolana, the low concentration was also close to neutral, but did not produce any increase. For Vezha, even 0.01% was already accompanied by deterioration at the later stages of the winter period. LG Magirus and LG Litopys were characterized mainly by a neutral or slightly negative response to 0.01% and a clearly negative response to 0.02% and 0.04%. The obtained results indicate that the effect of CA-67 on sugar content in the tillering node of winter wheat is predominantly neutral or negative.

The results of the factor analysis (Table 7) showed that compound concentration was the principal source of variation for such traits as seed germination, plant survival, and sugar accumulation in the tillering node before, during, and after the winter period, that is, for the set of characteristics collectively reflecting winter hardiness. Thus, the concentration factor accounted for most of the variability observed in the experiment. By contrast, the varietal factor had a more selective influence, being expressed mainly through differences in germination and the number of viable plants before wintering. The remaining traits, in turn, more fully reflected the contribution of genotype to winter tolerance. Overall, the number of variables included in the model was sufficient for reliable estimation of variability and for subsequent discriminant analysis.

Although fewer measured parameters were directly determined by genotype, the classification capacity of the model for identifying winter-tolerant plants remained high. In other words, even with a limited set of strongly genotype-dependent traits, the multivariate approach provided substantial discriminatory power. Classification accuracy for winter tolerance ranged from about 80% in Vezha to 90% in local varieties and 92–94% in western ecotype genotypes (LG varieties). This indicates that the model adequately captured biologically meaningful differences among the studied groups.

Table 7
Resaults of component analyze

Parameter	Dose	Genotype	Wilks' - Lambda	F_{remove} (5.01)	P-value
Germination	-0.873*	0.658	0.025	18.67	< 0.01
Befor winter period	-0.734*	0.462	0.019	13.28	< 0.01
Surviving	0.928*	0.754*	0.027	23.61	< 0.01
Sugar content 11	-0.741*	0.782*	0.019	17.21	< 0.01
Sugar content 2	0.776*	0.759*	0.018	16.58	< 0.01
Sugar content 3	0.831*	0.748*	0.021	18.03	< 0.01
Explenation variants	5.238	3.742	–	–	–
Non-explanation	0.327	0.248	–	–	–

The factor-space distribution also revealed clear differentiation among genotypes. According to Figure 1, the positions of the tested CA agents in multidimensional space were not identical. CA-64 and CA-79 did not merge into a single cluster, which indicates statistically significant differences in their effects on seed material, although the relative arrangement of objects suggests that their mechanisms of action may be at least partly similar.

At the same time, the varieties themselves formed two distinct groups: Western ecotype genotypes were concentrated in one cluster, whereas locally adapted varieties occupied another. Only Vezha showed slight ambiguity in classification. The main factor underlying this separation appears to be the degree of adaptation of each genotype to its specific growing environment. Importantly, the distribution in factor space was generally well structured, with no major overlap among genotype groups, confirming that the model described the observed patterns consistently and with high adequacy.

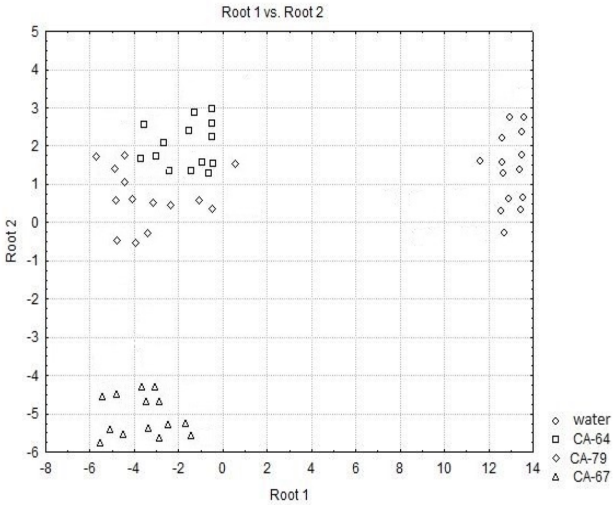

Fig. 1. Factor space for CA substances action

Discussion

Winter wheat is one of the key crops forming the foundation of food security. Its yield level is largely determined by the ability of plants to adapt to specific environmental conditions and by the efficiency of the cultivation technology applied. Over recent decades, scientific and practical interest in new-generation growth stimulators has increased markedly (Cohen et al., 2021; Nazarenko et al., 2022). Such preparations are regarded not only as means of increasing potential yield, but also as an important factor in strengthening plant resistance to adverse influences and improving the technological and nutritional properties of grain (Nazarenko et al., 2023).

The group of growth stimulators includes numerous synthetic and natural compounds capable of influencing metabolic processes, cell division, and tissue formation. Their effects are realized through the activation of enzymatic systems, enhancement of chlorophyll biosynthesis, and regulation of plant hormonal status, particularly auxins, cytokinins, and gibberellins (Xu et al., 2024; Zhou et al., 2025), as well as through increased efficiency of the photosynthetic apparatus. Modern regulators, among which triazole derivatives, amino acid complexes, humic substances, and epigenetically active compounds occupy an important place, are characterized by multifunctional activity (Mohsin et al., 2023; Li et al., 2025). They stimulate seed germination and root system development, promote leaf area expansion and productive tillering, contribute to stem strengthening and reduction of lodging risk, and improve grain filling, often accompanied by an increase in protein content. Under conditions of climatic instability, when agricultural production is increasingly confronted with droughts, sharp temperature fluctuations, and moisture deficit, such properties become especially important for yield stabilization (Wang et al., 2024).

One of the most important consequences of growth stimulator application is the stabilization, and in many cases the increase, of yield through the optimization of fundamental physiological processes. Pre-sowing seed treatment or the application of stimulators during vegetation is usually accompanied by higher germination energy and more uniform emergence, the formation of a more developed and efficient photosynthetic surface, an increase in the coefficient of productive tillering, as well as an improved balance between the number of grains per spike and thousand-kernel weight (Xu et al., 2024).

Results from numerous studies conducted under different environmental conditions and on diverse genotypes indicate that the use of growth stimulaors may provide a yield increase of approximately 1.0–1.5 t/ha, although the actual effect depends on varietal characteristics and weather conditions. For the steppe zone, where winter wheat productivity is often limited by water stress and insufficient mineral nutrition, such yield gains are of not only economic but also strategic importance (Janda et al., 2020; Ejaz et al., 2023).

Equally important is the role of new growth stimulators in shaping the adaptive potential of winter wheat. Under conditions of aridization and high temperatures, they are capable of reducing transpiration intensity through the regulation of stomatal conductance, increasing the water-holding capacity of the tissues, and stimulating the synthesis of compatible osmolytes, particularly proline and soluble sugars, which ensure stabilization of cell membranes (Lamlom et al., 2025; Li et al., 2025). At the same time, they weaken the accumulation of reactive oxygen species and strengthen antioxidant defense mechanisms, enabling plants to maintain cellular integrity more effectively under stress conditions (Rani et al., 2018; Xu et al., 2024).

The combined action of these mechanisms leads to increased crop resilience during periods of moisture deficit, late frosts, or heat waves. As a result, the risk of sharp yield decline is significantly reduced, and production itself becomes more stable and predictable even in seasons when plants are simultaneously exposed to several stress factors. From a practical point of view, growth stimulators contribute to the preservation of those yield components that are established at the early stages of organogenesis, and they also support the transport of assimilates to the grain during the critical phases of stem elongation and grain filling (Zhang et al., 2025).

The quality of winter wheat grain is shaped by a complex of traits, among which protein content, the quantity and properties of gluten, micronutrient composition, and the presence of biologically valuable compounds are of particular importance (Kosová et al., 2021; Nazarenko et al., 2023). Acting at the level of biochemical processes, growth stimulators can substantially improve these characteristics. They activate enzyme systems associated with protein synthesis, promote the translocation of nitrogen from vegetative organs to developing kernels, and often increase both the content and elasticity of gluten, which are critical determinants of baking quality (Xu et al., 2024). In addition, their application may enhance the accumulation of important micronutrients, which is significant both for the nutritional value of the product and for the functional properties of flour. The alleviation of oxidative stress in the plant also contributes to reducing the content of undesirable oxidation products in the grain, thereby indirectly improving its safety and quality (Lamlom et al., 2025; Parizi et al., 2025).

Under production conditions, growth stimulators can be effective at different stages of winter wheat ontogenesis. Pre-sowing seed treatment ensures faster and more uniform germination and promotes strong initial development of the root system. The use of such preparations at the tillering stage makes it possible to enhance the formation of productive shoots, improve their preservation and increase leaf area. During the period from stem elongation to heading, foliar applications can support photosynthetic intensity, promote carbohydrate redistribution, and positively affect grain filling and thousand-kernel weight (Jha et al., 2017; Korsukova et al., 2025).

The greatest effect from the use of growth stimulators is achieved when they are integrated into a comprehensive crop management system that includes balanced mineral nutrition, rational irrigation where available, and timely protection against weeds, pests, and diseases. Within such integrated technologies, each new stimulator may increase the overall efficiency of the production system by approximately 15–20%, although the specific outcome is largely determined by the interaction between genotype and environmental conditions, as well as by the nature of stresses during the growing season (Cohen et al., 2021; Ejaz et al., 2023).

Conclusion

Among the three studied compounds, CA-79 and CA-64 were the most effective, whereas CA-67 proved to be the least promising. A common pattern was observed for germination, pre-winter and post-winter survival, and sugar content in the tillering node. In most cases, the best results were obtained with the low and medium concentrations, while 0.04% showed a depressive effect in all cases. The most stable and pronounced stimulatory effect was produced by CA-79, especially at 0.02%. This treatment most often ensured the highest values of germination and survival, as well as the greatest sugar content throughout the winter period. CA-64 also acted positively, with 0.02% being optimal and 0.01% remaining effective in most cases. Therefore, both compounds may be regarded as promising agents for strengthening the physiological basis of winter hardiness and improving the early development of plants. Against this background, CA-67 did not produce a distinct stimulatory effect: 0.01% was mostly neutral, whereas 0.02% and especially 0.04% reduced most of the studied parameters. Among the varieties, MIP Roksolana and Khvylia Dnipra typically showed the highest baseline level of winter reserves and generally better control values, while Vezha also performed relatively well, though at a somewhat lower level. By contrast, LG Magirus and LG Litopys were characterized by a lower initial sugar content and greater sensitivity to unfavorable concentrations. At the same time, all varieties, although to different extents, responded positively to CA-79 at 0.02% and CA-64 at 0.02%. The strongest negative response to overdosing was observed at 0.04%, where both carbohydrate reserves and plant preservation parameters declined. Thus, compound concentration was the decisive factor, and the optimal range for practical application was 0.01–0.02%, primarily for CA-79 and CA-64. Under the studied conditions, CA-67 cannot be considered an effective stimulator. Further research should focus on evaluating the selected genotypes in terms of grain yield, improvement of technological grain quality, and enhancement of beneficial micronutrient content.

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