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Influence of new growth regulators based on triazoles on winter period ontogenesis of modern wheat varieities

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Abstract. Triazole derivatives are valuable tools in modern agriculture, regulating diverse plant processes. In wheat, they balance growth and reproduction, bolster tolerance to abiotic and biotic stress, and unlock stable, high yields. Their dual role, as growth regulators and broad-spectrum fungicides, enables integrated, efficient programs that couple crop protection with physiological stimulation strategies. Five winter wheat varieties in grains (1.000) were treated in water solutions of three triazole-type compounds CA-64, CA-79, and CA-67. Each compound was tested at 0.01%, 0.02%, and 0.04%. Soaking lasted 24 hours, following widely accepted protocols for plant growth stimulants; the control seeds were soaked in water. The results demonstrated that CA-79, especially 0.02%, most reliably increased carbohydrate reserves in the tillering node at all winter monitoring points while preserving post-winter stand scores. CA-64 similarly enhanced reserves and final survival at 0.02% (often 0.01%), making it a safe default for locally adapted material. CA-67 offered no dependable stimulation: 0.01% was at best neutral and 0.02% reduced reserves and post-winter scores. At 0.04%, all agents sharply lowered germination, pre-winter stands, and winter survival (winter losses 4–12%), marking a toxicity threshold to avoid. Responses were genotype-conditioned. Local varieties (Pozytsiia Odeska, MIP Feieriia, Tenor) showed predictable gains with CA-79 and CA-64 at 0.02%, maintaining stable stands (germination-survival drop 1.0–1.5%). Western ecotypes (MV Nador, MV Menrot) started with lower sugar baselines and were more sensitive; they still benefited from CA-79 0.02% (and modestly CA-64 0.02%), but losses at 0.04% were greatest, so operational range should remain 0.01–0.02%. Practical recommendation is to prioritize CA-79 (0.02%) for reserve building and overwintering, use CA-64 (0.02%) where validated, keeping 0.01% as a conservative option,; avoid 0.04% for all agents. Tailor protocols by variety, anchoring decisions on late-winter sugar status and the germination - survival gap (target 1.0–1.5%). In summary, CA-79 or, sometimes, CA-64, but not CA-67 can be used for winter-tolerance stimulation; 0.02% is the operative concentration, 0.01% for risk-averse or sensitive material (western ecotype). Future research will integrate these observations with analyses of variability in grain quality and yield traits, enhancing effect of new triazole-based agents and improving plant production of traditional cereals crops.

Keywords:cereals; winter tolerance; triazole; plant regulator; winter wheat; sugar concentration; abiotic stres.

Introduction

Triazole derivatives are becoming increasingly important tools for optimizing modern agricultural technologies because they regulate a wide spectrum of biochemical and physiological processes in plants. In wheat, they can promote balanced vegetative and reproductive development, enhance tolerance to abiotic and biotic stress, and help express the genetic potential for high, stable yields. Their appeal lies in a dual value proposition: Many triazoles function both as growth regulators and as fungicides with broad activity against key pathogens. This combination can streamline crop protection and stimulation strategies within integrated, resource-efficient production systems (Roostaei et al., 2018; Nazarenko et al, 2019).

Despite widespread practical use, the response of different crops to specific triazole molecules and doses can vary appreciably. Establishing the limits of response variability in promising crops is therefore essential to justify practical adoption and to tailor recommendations. Such work should quantify how germination, seedling vigour, survival, photosynthetic activity, yield components and quality respond across molecules and concentration ranges. Understanding this variability enables the development of robust, variety-specific protocols that deliver benefits while minimizing agronomic and ecological risks (Roostaei et al, 2018; Wang et al., 2020; Khalil et al., 2021).

Triazole fungicides primarily inhibit sterol biosynthesis in fungi, disrupting membrane integrity. Certain triazoles (e.g., paclobutrazol, uniconazole) also modulate plant hormone pathways—most notably gibberellin biosynthesis—leading to shorter, sturdier plants with improved partitioning of assimilates. Beyond direct hormonal effects, triazoles can enhance antioxidant enzyme systems, support osmotic adjustment under drought or salinity, stabilize chlorophyll, and improve stomatal regulation. These mechanisms underpin observed field outcomes such as improved stand establishment, increased root density, better lodging resistance, and more efficient grain set and filling under stress (Janda et al., 2020).

Triazoles often increase germination energy and promote uniform emergence, particularly under suboptimal temperatures or episodic moisture deficits. During tillering and stem elongation, growth-regulating triazoles can produce compact canopies with favourable leaf angles, improving light interception and reducing lodging risk – important for intensive systems and high-nitrogen regimes. Near booting and flowering, disease-oriented triazole applications can suppress foliar and spike pathogens, preserving green leaf area and protecting grain formation. Cumulatively, these effects may translate to higher harvest index and improved yield stability, with quality gains such as more consistent protein or test weight depending on variety and environment (Zhang et al., 2020).

Triazoles can contribute to higher water-use efficiency by moderating canopy transpiration and sustaining photosynthetic capacity during dry spells. Improved root architecture may enhance nutrient acquisition, complementing fertilizer programs and potentially allowing more efficient nitrogen use. Compatibility with seed dressings, micronutrient primers, and many foliar agrochemicals facilitates integration into conservation tillage and precision agriculture systems. When applied according to well-defined dose-timing windows, triazoles help producers generate higher yields with lower resource expenditure – an imperative in modern resource-saving agriculture (Zhang et al., 2022; Sumei et al., 2022).

Under global warming, wheat increasingly faces heat spikes, erratic precipitation, and overwintering challenges. In steppe and forest-steppe environments, where winter wheat is exposed to freeze–thaw cycles and spring drought, triazole-based programs can stabilize yields by strengthening stress physiology and curbing disease pressure that often accelerates under warmer, wetter intervals. The net effect is not necessarily maximal yield in every season, but a reduction in downside risk and a higher probability of reaching target productivity under variable weather. Their pronounced fungicidal activity helps control a wide range of pathogens, while their growth-modulating properties bolster the plant's inherent resilience. This synergy supports integrated disease management: healthier, better-structured canopies experience less microclimatic favourability for pathogens, and timely fungicidal action protects from the remaining risks (Shaki et al., 2022; Sumei et al., 2022).

Some triazoles exhibit moderate persistence and can be transported via runoff or drift, creating exposure pathways for aquatic systems. Soil binding and degradation rates vary by molecule and soil type; repeated use without attention to timing and weather can raise local environmental loads. While acute toxicity to bees is often low for many triazoles, sublethal or indirect effects (e.g., interactions with other chemistries, nutritional stress, or disease) are possible and should be considered in spray scheduling and tank mixes. Because many triazoles act on sterol or hormone pathways, careful toxicological evaluation and adherence to regulatory limits are essential to avoid unintended effects on non-target organisms. Repeated, sole reliance on triazole fungicides can accelerate resistance evolution in pathogen populations, leading to diminished field performance and the need for higher inputs (Zhang et al., 2020).

The study aimed 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 plantgrowth regulators, focusing on their ability to induce winter resistance and photosintetic activity, their interactions with different winter wheat genotypes and the specific characteristics of genotype–substance interactions. A key objective was to assess the feasibility of applying three new compounds in future plant production programs by examining their predictive value by the winter period tolerance activation and photosintetic activity stimulation.

Materials and methods

The study was carried out on the experimental fields of the Department of Breeding and Seed Farming, Dnipro State Agrarian and Economic University, during the 2024–2025 seasons under steppe, sub-humid conditions. Bread wheat grains (1,000 seeds per treatment and per control) were primed in aqueous solutions of three triazole-type 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 at 0.01%, 0.02%, and 0.04%. Soaking lasted 24 hours, following widely accepted protocols for plant growth stimulants; the control seeds were soaked in water. Tested concentrations were selected based on prior research with analogous compounds in cereals (Nazarenko et al, 2022).

In total, 60 variants were established: six wheat varieties × ten seed-treatment options (nine active-compound × concentration combinations plus water control). The varietal set comprised Pozytsiia Odeska, MIP Feieriia, Tenor (domestic breeding) and MV Nador, MV Menrot (Martonwashar). Each variant was sown in a plot of ten rows, with 0.15 m interrow spacing and 1.5 m row length. Crop protection followed best practices for the subhumid steppe zone to avoid interference from unmanaged pests and diseases (Ali & Baloch, 2020).

Phenological observations were recorded throughout the season. Overwintering success was evaluated both visually and by quantifying soluble sugar content in the tillering node during the critical winter period. Field emergence, plot-level plant survival, and stand uniformity were assessed to capture early establishment effects of seed priming. To probe multivariate structure and the predictive value of main traits (where collected), linear discriminant analysis was applied using standard modules. This allowed identification of variables with high discriminatory power among the treatment groups and estimation of classification accuracy for practical decision-making (e.g., which compound–dose combinations most reliably shift target traits). Collectively, the design links laboratory-scale seed responses, winter survival physiology, in-season photosynthetic performance providing a coherent basis for optimizing triazole-based seed priming in winter wheat under regional conditions.

All statistical analyses were performed in Statistica 10.0 (TIBCO, Palo Alto, USA). A factorial analysis of variance (ANOVA) was used to evaluate main effects and interactions of genotype (variety), active compoundidentity, and concentration. Significance was declared at $P < 0.05$. Assumptions of normality were checked with the Shapiro–Wilk W-test; where necessary, data were transformed or analyzed with distribution-appropriate options. Descriptive statistics (means, standard deviations) were compiled for all the traits, and the results were graphed to aid interpretation and highlight treatment patterns. Post-hoc pairwise comparisons were conducted with Tukey's HSD to separate means when ANOVA results were significant.

Results

First of all, the effect of triazole-type compounds manifested in the germination and survival of winter wheat plants. The data concerning germination, survival at the beginning and end of winter, and concentrations are provided in Tables 1–3 (for CA-64, CA-79, and CA-67, respectively).

As for CA-64 (Table 1), 0.01% concentration in general demonstrated small but significant improvements compared with water; 0.02% produced similar or slightly higher values than 0.01%. At 0.04% concentration, marked depression was observed for all three parameters (this was always the worst variant, most noticeable for the foreign genotypes MV Menrot and Nador).

Table 1
Germination and survival under action of CA-64 (x ± SD, n = 10)

Variant	Germination	Before winter	Surviving
Pozytsiia Odeska	91.00 ± 0.10^{a}	90.50 ± 0.11^{a}	90.00 ± 0.11^{a}
Pozytsiia Odeska, CA-64 0.01%	93.50 ± 0.30^{b}	93.50 ± 0.23^{b}	93.00 ± 0.21^{b}
Pozytsiia Odeska, CA-64 0.02%	94.00 ± 0.32^{b}	94.00 ± 0.32^{b}	93.50 ± 0.30^{b}
Pozytsiia Odeska, CA-64 0.04%	86.50 ± 0.34^{c}	85.50 ± 0.31^{c}	81.00 ± 0.39^{c}
MIP Feieriia	90.50 ± 0.22^{a}	90.50 ± 0.21^{a}	90.00 ± 0.21^{a}
MIP Feieriia, CA-64 0.01%	93.00 ± 0.24^{b}	92.50 ± 0.22^{b}	92.00 ± 0.24^{b}
MIP Feieriia, CA-64 0.02%	93.50 ± 0.24^{b}	93.00 ± 0.24^{b}	92.50 ± 0.26^{b}
MIP Feieriia, CA-64 0.04%	86.00 ± 0.33^{c}	84.50 ± 0.34^{c}	81.50 ± 0.38^{c}
Tenor	92.00 ± 0.20^{a}	92.00 ± 0.20^{a}	92.00 ± 0.20^{a}
Tenor, CA-64 0.01%	93.00 ± 0.20^{a}	92.50 ± 0.22^{a}	92.00 ± 0.24^{a}
Tenor, CA-64 0.02%	94.00 ± 0.21^{ab}	94.00 ± 0.24^{ab}	93.50 ± 0.25^{ab}
Tenor, CA-64 0.04%	85.00 ± 0.42^{c}	83.00 ± 0.44^{c}	81.00 ± 0.41^{c}
MV Nador	92.00 ± 0.20^{a}	92.00 ± 0.20^{a}	91.00 ± 0.25^{a}
MV Nador,CA-64 0.01%	93.00 ± 0.20^{a}	93.00 ± 0.21^{a}	92.00 ± 0.26^{b}
MV Nador,CA-64 0.02%	94.00 ± 0.33^{ab}	93.50 ± 0.32^{ab}	92.50 ± 0.30^{b}
MV Nador,CA-64 0.04%	85.50 ± 0.43^{c}	84.50 ± 0.40^{c}	80.50 ± 0.44^{c}
MV Menrot	90.50 ± 0.20^{a}	90.00 ± 0.21^{a}	89.00 ± 0.21^{a}
MV Menrot, CA-64 0.01%	94.50 ± 0.23^{b}	94.00 ± 0.22^{b}	92.00 ± 0.26^{b}
MV Menrot, CA-64 0.02%	96.50 ± 0.24^{c}	96.00 ± 0.23^{c}	94.00 ± 0.25^{c}
MV Menrot, CA-64 0.04%	83.00 ± 0.40^{d}	81.50 ± 0.41^{d}	80.00 ± 0.42^{d}

Note: values within a row followed by different letters differ significantly ($P < 0.05$), as determined using Tukey's HSD test with Bonferroni correction; comparisons were performed separately for each variety across CA-64 treatment concentrations.

We recommend avoiding CA-64 at 0.04% concentrations for all the genotypes due to significant depression. For working range, 0.01% was safe, providing a reliable enhancement of most genotypes and minimal overwinter loss; the concentration was often the best or second best option, but requires local verification, as some varieties showed only modest gains (e.g. Tenor).

The general opinion is to choose concentrations at which the difference between germination and survival is ≤1% (0.01–0.02% or 0.02% for MV Menrot). These doses should be used as working concentrations for winter wheat resistance improvement; a narrower range of 0.01–0.02% should be used for highly responsive varieties such as MV Menrot or MV Nador to elucidate the optimum. The ANOVA analysis confirmed a significant variability in all the parameters depending on concentration ($F = 22.15$; $F_{0.05} = 3.25$; $P = 2.17 \times 10^{-4}$). Also, significant differences among the varieties were detected ($F = 6.72$; $F_{0.05} = 3.49$; $P = 0.01$). In addition, a statistically significant genotype×mutagen interaction was observed ($F = 6.78$; $F_{0.05} = 5.10$; $P = 0.02$).

As for CA-79 (Table 2), the 0.01% concentration in general produced a small but significant improvement compared with water across all varieties; the 0.02% concentration provided higher parameters than 0.01%, often being the best for the crop populations; and the 0.04% concentration decreased all three parameters (germination, pre-winter stand, final surviving), always being the worst, with the effect especially pronounced for the foreign genotypes MV Nador and MV Menrot.

Table 2
Germination and survival under action of CA-79 (x ± SD, n = 10)

Variant	Germination	Before winter	Surviving
Pozytsiia Odeska	91.00± 0.10 [a]	91.00± 0.10 [a]	90.50± 0.10 [a]
Pozytsiia Odeska, CA-79 0.01%	94.00 ±0.30 [b]	93.50 ±0.31 [b]	93.00 ±0.21 [b]
Pozytsiia Odeska, CA-79 0.02%	96.00 ±0.31 [c]	95.50 ±0.32 [c]	95.00 ±0.31 [c]
Pozytsiia Odeska, CA-79 0.04%	84.50 ± 0.33 [d]	83.50 ± 0.31 [d]	80.50 ± 0.33 [d]
MIP Feieriia	90.50 ±0.22 [a]	90.00 ±0.23 [a]	90.00 ±0.23 [a]
MIP Feieriia, CA-79 0.01%	92.50 ±0.22 [b]	92.00 ±0.22 [b]	91.00 ±0.22 [b]
MIP Feieriia, CA-79 0.02%	96.00 ±0.31 [c]	95.50 ±0.32 [c]	94.50 ±0.31 [c]
MIP Feieriia, CA-79 0.04%	85.00 ± 0.33 [d]	85.00 ± 0.33 [d]	80.00 ± 0.36 [d]
Tenor	92.00± 0.20 [a]	92.00± 0.21 [a]	91.50± 0.21 [a]
Tenor, CA-79 0.01%	93.50± 0.20 [b]	93.50± 0.22 [b]	93.00± 0.22 [b]
Tenor, CA-79 0.02%	96.00 ± 0.31 [c]	95.50 ± 0.33 [c]	95.00 ± 0.33 [c]
Tenor, CA-79 0.04%	84.50 ± 0.32 [d]	83.00 ± 0.35 [d]	80.00 ± 0.35 [d]
MV Nador	92.00 ± 0.20 [a]	92.00 ± 0.20 [a]	91.00 ± 0.22 [a]
MV Nador,CA-79 0.01%	92.50 ± 0.20 [a]	92.00 ± 0.20 [a]	90.50 ± 0.21 [a]
MV Nador,CA-79 0.02%	94.50 ± 0.23 [b]	94.00 ± 0.21 [b]	90.50 ± 0.21 [a]
MV Nador,CA-79 0.04%	84.00 ± 0.41 [c]	81.00 ± 0.34 [c]	75.00 ± 0.36 [b]
MV Menrot	90.50 ± 0.20 [a]	90.00 ± 0.20 [a]	89.00 ± 0.20 [a]
MV Menrot, CA-79 0.01%	95.00 ± 0.30 [b]	94.00 ± 0.26 [b]	92.00 ± 0.28 [b]
MV Menrot, CA-79 0.02%	96.00 ± 0.22 [c]	95.00 ± 0.26 [c]	92.00 ± 0.28 [b]
MV Menrot, CA-79 0.04%	82.00 ± 0.40 [d]	79.50 ± 0.38 [d]	75.50 ± 0.39 [c]

Note: see Table 1.

The following gains (compared with the control) were observed: +4.5% (from 90.5% to 95.0%) in Pozytsiia Odeska (at CA-79 0.02%); +4.5 % (from 90.0% to 94.5%) in MIP Feieriia (the same variant); +3.5% (from 91.5% to 95.0%) in Tenor (CA-79 0.02%); +3.0% (from 89.0% to 92.0%) in MV Menrot (CA-79 0.01–0.02%). At the same time, no survival improvement was observed for MV Nador.

We recommend avoiding using CA-79 at a 0.04% concentration for any of the tested genotypes. For Pozytsiia Odeska, MIP Feieriia, and Tenor, CA-79 0.02% produced the best survival gains. The 0.01% concentration was safer, but the improvements were lower. For MV Menrot, both concentrations (0.01 and 0.02%) were similarly effective. For MV Nador, it is best to avoid higher doses. In general, the preferable concentrations was 0.02% for the most genotypes, producing a germination - surviving gap of ≤1–1.5%.

The ANOVA analysis confirmed a significant variability in all parameters with changing concentrations ($F = 20.17$; $F_{0.05} = 3.25$; $P = 1.34 \times 10^{-4}$). Significant differences across the varieties were also detected ($F = 7.78$; $F_{0.05} = 3.49$; $P = 0.006$). Also, a statistically significant genotype×mutagen interaction developed ($F = 7.11$; $F_{0.05} = 5.10$; $P = 0.01$).

As for CA-67 (Table 3), the concentration 0.01% had a neutral effect on all the varieties, producing neither improvements (compared with the control) not declines. The use of the 0.02% concentration was followed by significant declines for every variety. Under the action of the 0.04% concentration, the winter losses were the highest.

Table 3
Germination and survival under action of CA-67 (x ± SD, n = 10)

Variant	Germination	Befor winter	Surviving
Pozytsiia Odeska	91.00± 0.10 [a]	91.00± 0.12 [a]	90.50± 0.12 [a]
Pozytsiia Odeska, CA-67 0.01%	91.00 ±0.30 [a]	91.00 ±0.25 [a]	90.50 ±0.25 [a]
Pozytsiia Odeska, CA-67 0.02%	89.00 ±0.32 [b]	88.50 ±0.31 [b]	86.00 ±0.34 [b]
Pozytsiia Odeska, CA-67 0.04%	86.50 ± 0.34 [c]	84.00 ± 0.32 [c]	81.00 ± 0.39 [c]
MIP Feieriia	90.50 ±0.22 [a]	90.00 ±0.21 [a]	90.00 ±0.22 [a]
MIP Feieriia, CA-67 0.01%	91.00 ±0.24 [a]	91.00 ±0.23 [a]	90.00 ±0.22 [a]
MIP Feieriia, CA-67 0.02%	88.50 ±0.24 [b]	87.50 ±0.25 [b]	83.50 ±0.27 [b]
MIP Feieriia, CA-67 0.04%	85.00 ± 0.33 [c]	84.00 ± 0.34 [c]	80.00 ± 0.39 [c]
Tenor	92.00± 0.20 [a]	91.50± 0.22 [a]	91.00± 0.24 [a]
Tenor, CA-67 0.01%	92.50± 0.20 [a]	92.00± 0.23 [a]	91.00± 0.24 [a]
Tenor, CA-67 0.02%	91.00 ± 0.21 [b]	90.00 ± 0.22 [b]	86.00 ± 0.34 [b]
Tenor, CA-67 0.04%	84.00 ± 0.42 [c]	83.00 ± 0.43 [c]	78.00 ± 0.45 [c]
MV Nador	92.00 ± 0.20 [a]	92.00 ± 0.21 [a]	90.00 ± 0.21 [a]
MV Nador,CA-67 0.01%	92.50 ± 0.20 [a]	92.00 ± 0.23 [a]	90.00 ± 0.21 [a]
MV Nador,CA-67 0.02%	91.00 ± 0.33 [b]	90.00 ± 0.32 [b]	84.00 ± 0.30 [b]
MV Nador,CA-67 0.04%	84.00 ± 0.43 [c]	83.00 ± 0.35 [c]	75.00 ± 0.38 [c]
MV Menrot	90.50 ± 0.20 [a]	90.00 ± 0.21 [a]	89.00 ± 0.25 [a]
MV Menrot, CA-67 0.01%	91.00 ± 0.23 [a]	90.50 ± 0.22 [a]	89.50 ± 0.24 [a]
MV Menrot, CA-67 0.02%	90.00 ± 0.24 [ab]	89.00 ± 0.26 [b]	83.00 ± 0.31 [b]
MV Menrot, CA-67 0.04%	85.50 ± 0.40 [c]	84.00 ± 0.31 [c]	74.50 ± 0.38 [c]

Note: see Table 1.

For agricultural practice, we recommend avoiding CA-67 ≥0.02%, as this concentration consistently increased winter losses spike. At 0.01%, it was neutral, with winter loss ≤1.0–1.5%). Among the genotypes, Pozytsiia Odeska was the most tolerant to rising concentrations, whereas the varieties of western ecotype, MV Menrot and MV Nador, were the most sensitive. Therefore if CA-67 is used for these varieties, it should only be used at a 0.01% concentration.

The ANOVA analysis confirmed a significant variability in all the parameters with changing concentrations ($F = 34.14$; $F_{0.05} = 3.25$; $P = 3.44 \times 10^{-5}$). Non-significant differences across the varieties were also detected ($F = 2.78$; $F_{0.05} = 3.49$; $P = 0.08$), and a statistically significant genotype×mutagen interaction developed ($F = 5.15$; $F_{0.05} = 5.10$; $P = 0.05$).

Two compounds CA-64 and CA-79 showed good results in low doses. However, CA-67 was largely neutral at the lowest dose and uniformly harmful once increased. The concentrations of 0.01 and 0.02% exerted stimulating effects. In general. CA-64 and CA-79 demonstrated small but significant gains in germination and final survival.

One of the principal methods for accurately assessing overwintering status in conventional winter cereals of the region is measuring sugar concentrations in the plants' tillering node and tracking their depletion dynamics over the unfavorable period. The data from these laboratory analyses are presented in Tables 4–6 of the article. These results demonstrate that, initially, two genotypes of the western European ecotype are less adapted to winter conditions. Measurements were taken at three key dates, selected according to the temperature dynamics. Visual assessments of overwintering were also made, and these generally correlated with the laboratory findings.

As for the specific effects of individual compounds, Table 4 shows that CA-64 produced an overall trend of a gradual decline in sugar content across the varieties. However, this led to negative consequences only for MV Nador and MV Menrot, while treatments with 0.01% and 0.02% concentrations displayed a clearly positive effect for all the varieties.

Table 4
Winter wheat varieties parameters during winter period (2024/2025periods of vegetation dates) ($x \pm SD$, $n = 5$)

Variety	BW	Content of sugars in tillering nod, %			AW
		11	02	03	
Pozytsiia Odeska	5	33.3± 0.4[a]	29.9± 0.4[a]	24.4± 0.3[a]	5
Pozytsiia Odeska, CA-64 0.01%	5	35.5 ± 0.4[b]	32.8 ± 0.3[b]	28.7 ± 0.4[b]	5
Pozytsiia Odeska, CA-64 0.02%	5	37.0 ± 0.3[c]	33.9 ± 0.4[c]	31.0 ± 0.3[c]	5
Pozytsiia Odeska, CA-64 0.04%	4	31.7 ± 0.4[d]	27.9 ± 0.3[d]	23.2 ± 0.4[d]	4
MIP Feieriia	5	33.8± 0.4[a]	32.7± 0.4[a]	29.3± 0.3[a]	5
MIP Feieriia, CA-64 0.01%	5	35.4 ± 0.4[b]	32.8 ± 0.3[b]	29.2 ± 0.4[a]	5
MIP Feieriia, CA-64 0.02%	5	36.7 ± 0.4[c]	33.6± 0.4[c]	30.2 ± 0.4[b]	5
MIP Feieriia, CA-64 0.04%	4	31.2 ± 0.4[d]	28.9 ± 0.3[d]	24.2 ± 0.4[c]	4
Tenor	5	30.1± 0.3[a]	25.9± 0.4[a]	21.9± 0.4[a]	5
Tenor, CA-64 0.01%	5	31.5± 0.4[b]	27.1 ± 0.4[b]	22.9± 0.4[b]	5
Tenor, CA-64 0.02%	5	32.9 ± 0.3[c]	28.1 ± 0.4[c]	24.8 ± 0.4[c]	5
Tenor, CA-64 0.04%	4	29.1 ± 0.3[d]	24.2± 0.4[d]	20.2 ± 0.4[d]	4
MV Nador	4.5	25.1 ± 0.4[a]	20.9 ± 0.4[a]	16.5 ± 0.5[a]	4.5
MV Nador,CA-64 0.01%	4.5	26.9 ± 0.4[b]	21.7 ± 0.3[b]	18.1 ± 0.5[b]	4.5
MV Nador,CA-64 0.02%	4.5	28.2 ± 0.4[c]	22.9 ± 0.4[c]	19.5 ± 0.4[c]	4.5
MV Nador,CA-64 0.04%	4	22.3 ± 0.4[d]	18.9 ± 0.5[d]	15.6 ± 0.3[d]	3.5
MV Menrot	4.5	24.1 ± 0.4[a]	19.6 ±0.4[a]	16.1 ± 0.4[a]	4.5
MV Menrot, CA-64 0.01%	4.5	26.1 ± 0.4[b]	21.6 ± 0.3[b]	17.1 ± 0.5[b]	4.5
MV Menrot, CA-64 0.02%	4.5	27.2 ± 0.4[c]	22.7 ± 0.4[c]	18.5 ± 0.4[c]	4.5
MV Menrot, CA-64 0.04%	4	21.3 ± 0.4[d]	17.9 ± 0.5[d]	15.1 ± 0.3[d]	3.5

Note: BW – evaluation before winter period [points]; AW – evaluation after winter period [balls]; significant differences at P < 0.05 by by Tukey's HSD test with Bonferroni correction;comparison in terms of one variety.

Following the general recommendations, the working range was 0.02% > 0.01% for the most varieties, both being agronomically safe, while 0.04% concentration caused consistent sugar decrease, especially in the western ecotype. The high-responding varieties were Pozytsiia Odeska and MIP Feieriia(CA-64 0.02%); a moderate response was observed in Tenor (0.02%); and the western ecotypes MV Nador and MV Menrot were the most sensitive (CA-64 should be applied at 0.02% concentration). At 0.02%, CA-64 consistently boosted the carbohydrate reserves through winter without compromising stand parameters, while 0.04% caused a toxic effect. Statistically significant factors were genotype ($F = 28.11$; $F_{0.05} = 2.40$; $P = 4.14 \times 10^{-6}$), concentration ($F = 45.17$; $F_{0.05} = 2.90$; $P = 7.87 \times 10^{-8}$), and genotype × concentration interaction ($F = 9.14$; $F_{0.05} = 5.11$; $P = 0.008$).

As for the specific effects of CA-79, Table 5 shows the same overall trend of a gradual decline in sugar content during winter period. At a concentration of 0.01%, CA-79 increased sugars compared with the control, and 0.02% provided the highest sugar content without harming the stands (according to visual estimation). A concentration of 0.04% reduced sugars and lowered AW in each variety (-0.5% in the local varieties, from 0.5% to 1.0% in the western ecotypes). At CA-790.02%, the late-winter sugars were consistently high in each genotype, i.e. best reserve status by spring.

The factors such as genotype ($F = 23.94$; $F_{0.05} = 2.40$; $P = 1.33 \times 10^{-5}$), concentration ($F = 40.32$; $F_{0.05} = 2.90$; $P = 5.51 \times 10^{-7}$), and genotype × concentration ($F = 7.77$; $F_{0.05} = 5.11$; $P = 0.02$) interaction were statistically significant.

No stimulating effect was demonstrated by CA-67 (Table 6): a 0.01% concentration was at best neutral (and sometimes slightly negative toward sugar content), while ≥0.02% consistently reduced the reserves. In practice, CA-67 should be avoided for winter crops.

The local, better-adapted varieties (Pozytsiia Odeska, MIP Feieriia, Tenor) responded predictably to 0.02% CA-79 and CA-64, with higher late-winter sugars and stable preserved plants (except for 0.04% concentration). The western ecotypes (MV Nador and MV Menrot) started with lower baseline sugars and were more sensitive. They still gained from CA-79 0.02% (and to a lesser extent from CA-64 0.02%), but the absolute sugar levels remained modest. For these varieties, 0.01 and 0.02% should be the ceiling levels and 0.04% must be avoided.

For reserve planting and stable overwintering, CA-79 (0.02%) should be prioritized and CA-64 (0.02%) should be used where locally validated. A 0.01% concentration should remain as a conservative option. Application of CA agents at 0.04% to winter crops must be avoided, as it reduces sugars and is detrimental to plants. For MV Nador and MV Menrot, the doses should be within a range of 0.01–0.02%.

As a result of the factor analysis (Table 7), we found that the CA concentrations had effects on germination, survival, concentration of sugars in the tillering node before, during, and after the winter. At the same time, the factor of variety quite differed in germination and number of vital plants before the winter. The other traits in this case provided all information about the influence of genotype on winter tolerance. The number of parameters in the model was sufficient for estimation of variability using discriminant analysis.

Despite fewer parameters in our study being genotype-dependent, the model's classification power to identify winter-tolerant plants remained high. Even though relatively few measured traits depended on the source genotype, our framework still showed strong discriminatory ability for detecting winter tolerance. Although genotype effects were limited across parameters, the overall classification accuracy for winter tolerance was high (ranging 80% for Tenor to 90% for the local varieties and 92% - 94% for the western ecotypes).

Table 5
Winter wheat varieties parameters during winter period (2024/2025periods of vegetation dates) (x ± SD, n = 5)

Variety	BW	Content of sugars in tillering nod, %			AW
		11	02	03	
Pozytsiia Odeska	5	33.3 ± 0.4[a]	29.9 ± 0.4[a]	24.4 ± 0.3[a]	5
Pozytsiia Odeska, CA-79 0.01%	5	35.9 ± 0.3[b]	33.4 ± 0.4[b]	29.2 ± 0.3[b]	5
Pozytsiia Odeska, CA-790.02%	5	37.5 ± 0.4[c]	34.9 ± 0.3[c]	32.0 ± 0.4[c]	5
Pozytsiia Odeska, CA-79 0.04%	4.5	31.5 ± 0.3[d]	28.9 ± 0.4[d]	24.2 ± 0.3[d]	4.5
MIP Feieriia	5	33.8 ± 0.4[a]	32.7 ± 0.4[a]	29.3 ± 0.3[a]	5
MIP Feieriia, CA-79 0.01%	5	34.9 ± 0.4[b]	33.8 ± 0.4[b]	29.9 ± 0.4[a]	5
MIP Feieriia, CA-79 0.02%	5	36.5 ± 0.3[c]	35.5 ± 0.3[c]	32.2 ± 0.3[b]	5
MIP Feieriia, CA-79 0.04%	4.5	31.9 ± 0.4[d]	30.9 ± 0.4[d]	27.2 ± 0.4[c]	4.5
Tenor	5	30.1 ± 0.3[a]	25.9 ± 0.4[a]	21.9± 0.4[a]	5
Tenor, CA-79 0.01%	5	32.5 ± 0.4[b]	27.2 ± 0.3[b]	23.8 ± 0.4[b]	5
Tenor, CA-79 0.02%	5	33.9 ± 0.3[c]	28.9 ± 0.3[c]	25.8 ± 0.3[c]	5
Tenor, CA-79 0.04%	4.5	29.1 ± 0.3[d]	24.0 ± 0.4[d]	19.7 ± 0.3[d]	4.5
MV Nador	4.5	25.1 ± 0.4[a]	20.9 ± 0.4[a]	16.5 ± 0.5[a]	4.5
MV Nador,CA-79 0.01%	4.5	27.3 ± 0.3[b]	22.7 ± 0.3[b]	18.8 ± 0.3[b]	4.5
MV Nador,CA-79 0.02%	4.5	29.2 ± 0.3[c]	25.9 ± 0.3[c]	21.4 ± 0.3[c]	4.5
MV Nador,CA-79 0.04%	4	23.3 ± 0.4[d]	19.9 ± 0.4[d]	15.4 ± 0.4[d]	4
MV Menrot	4.5	24.1 ± 0.4[a]	19.6 ± 0.4[a]	16.1 ± 0.4[a]	4.5
MV Menrot, CA-79 0.01%	4.5	26.5 ± 0.3[b]	23.6 ± 0.3[b]	19.1 ± 0.3[b]	4.5
MV Menrot, CA-79 0.02%	4.5	28.2 ± 0.3[c]	25.7 ± 0.3[c]	20.5 ± 0.3[c]	4.5
MV Menrot, CA-79 0.04%	4	22.3 ± 0.3[d]	18.4 ± 0.4[d]	15.2 ± 0.3[d]	4

Note: see Table 4.

Table 6
Winter wheat varieties parameters during winter period (2024/2025periods of vegetation dates) (x ± SD, n = 5)

Variety	BW	Content of sugars in tillering nod, %			Visual estimation
		11	02	03	
Pozytsiia Odeska	5	33.3 ± 0.4[a]	29.9 ± 0.4[a]	24.4 ± 0.3[a]	5
Pozytsiia Odeska, CA-67 0.01%	5	33.5 ± 0.4[a]	30.8 ± 0.3[b]	24.7 ± 0.4[a]	5
Pozytsiia Odeska, CA-67 0.02%	5	32.0 ± 0.3[b]	27.9 ± 0.3[c]	23.5 ± 0.3[b]	4.5
Pozytsiia Odeska, CA-67 0.04%	4	29.7 ± 0.4[c]	25.5 ± 0.3[d]	20.2 ± 0.3[c]	4
MIP Feieriia	5	33.8 ± 0.4[a]	32.7 ± 0.4[a]	29.3 ± 0.3[a]	5
MIP Feieriia, CA-67 0.01%	5	33.6 ± 0.4[a]	32.5 ± 0.3[a]	29.0 ± 0.4[a]	5
MIP Feieriia, CA-67 0.02%	5	31.7 ± 0.4[b]	30.6 ± 0.4[b]	25.2 ± 0.4[b]	5
MIP Feieriia, CA-67 0.04%	4	29.2 ± 0.4[c]	27.2 ± 0.3[c]	20.2 ± 0.4[c]	4
Tenor	5	30.1 ± 0.3[a]	25.9 ± 0.4[a]	21.9 ± 0.4[a]	5
Tenor, CA-67 0.01%	5	30.5 ± 0.4[a]	24.1 ± 0.4[b]	20.2 ± 0.4[b]	5
Tenor, CA-67 0.02%	5	28.9 ± 0.3[b]	23.1 ± 0.4[c]	19.1 ± 0.4[c]	5
Tenor, CA-67 0.04%	4	27.1 ± 0.3[c]	22.0 ± 0.4[d]	18.0 ± 0.4[d]	4
MV Nador	4	25.1 ± 0.4[a]	20.9 ± 0.4[a]	16.5 ± 0.5[a]	4
MV Nador,CA-67 0.01%	4	24.9 ± 0.4[a]	20.7 ± 0.3[a]	16.1 ± 0.5[a]	4
MV Nador,CA-67 0.02%	4	23.2 ± 0.4[b]	19.5 ± 0.4[b]	15.1 ± 0.4[b]	4
MV Nador,CA-67 0.04%	3.5	21.2 ± 0.4[c]	18.3 ± 0.5[c]	13.6 ± 0.3[c]	3.5
MV Menrot	4.5	24.1 ± 0.4[a]	19.6 ± 0.4[a]	16.1 ± 0.4[a]	4.5
MV Menrot, CA-67 0.01%	4	24.0 ± 0.4[a]	19.7 ± 0.3[a]	16.0 ± 0.5[a]	4
MV Menrot, CA-67 0.02%	4	23.0 ± 0.4[b]	18.1 ± 0.4[b]	15.0 ± 0.4[b]	4
MV Menrot, CA-67 0.04%	3.5	21.3 ± 0.4[c]	17.0 ± 0.5[c]	13.1 ± 0.3[c]	3

Note: BW – evaluation before winter period (balls); visual estimation – evaluation after winter period (balls); significant differences at P < 0.05 by by Tukey's HSD test with Bonferroni correction;comparison in terms of one variety.

Table 7
Factor loadings (unrotated) and discriminant function

Parameter	Concentration	Genotype	Wilks'-lambda	F_{remove} (5,01)	P-value
Germination	–0.899*	0.640	0.022	18.22	< 0.01
Befor winter	–0.711*	0.449	0.016	12.93	< 0.01
Surviving	0.945*	0.771*	0.024	23.14	< 0.01
Sugar content 11	–0.757*	0.797*	0.016	17.64	< 0.01
Sugar content 02	0.798*	0.774*	0.015	16.23	< 0.01
Sugar content 03	0.848*	0.761*	0.018	17.64	< 0.01
Explenation variants	5.416	3.868	–	–	–
Non-explanation	0.311	0.234	–	–	–

The multivariate analysis revealed that Tenor and the western-ecotype varieties occupy a less favorable region of the factor space compared with the other genotypes, although all classes are still captured by a highly relevant, well-fitting model. Figure 1 displays the factor-space positions of all CA agents. Notably, CA-64 and CA-79 do not cluster into a single group; although differences in their effects on seed material are statistically significant, the spatial arrangement of individual objects suggests partially similar action mechanisms.

A clear bifurcation of the varieties emerges, with the western-ecotype genotypes forming one cluster and the locally adapted varieties forming another, with Tenor showing minor classification ambiguity. The primary discriminant appears to be each variety's adaptation to its target environment, which drives the separation. Importantly, there is no substantial mixing of genotypes in factor space; all groups remain well delineated, yielding a coherent, separable set of observations.

Discussion

Winter wheat is one of the cornerstone crops for global food security, including in Ukraine. Its productivity is largely determined by how well plants adapt to environmental conditions and by the technology used in cultivation. Over the past decades, particular attention has been paid to growth stimulators of a new generation (Cohen et al, 2021; Nazarenko et al., 2022). These products not only increase yield potential, but also enhance plant tolerance to adverse factors and improve the technological and nutritional quality of the grain (Nazarenko et al., 2023).

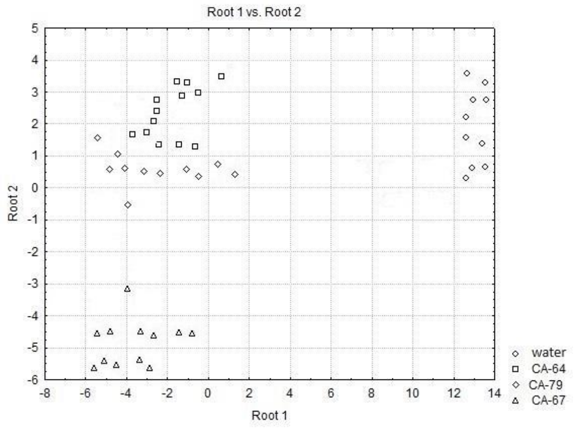

Fig. 1. Model for action on CA agents in factors space

Growth stimulators are a diverse group of synthetic and natural compounds that influence metabolism, cell division, and tissue differentiation. Their effects are mediated through activation of key enzyme systems, intensification of chlorophyll synthesis, modulation of the hormonal balance (auxins, cytokinins, gibberellins) (Feng et al., 2018; Zhao et al., 2023), and considerable increase in photosynthetic efficiency. Modern regulators, including triazole-based derivatives, amino acid complexes, humic compounds, and epigenetically active compounds, exert a broad spectrum of action (Li et al., 2023;Mohsin et al., 2023). They accelerate seed germination and the establishment of a strong root system; stimulate leaf area expansion and productive tillering; improve stem strength and reduce lodging risk; and support more complete grain filling with a tendency toward higher protein accumulation. In the era of climate variability, characterized by frequent droughts, sharp temperature swings, and moisture deficits, these properties become particularly valuable for stabilizing production (Rani et al., 2018; Zhao et al., 2023).

One of the most important outcomes of using growth stimulators is the stabilization (and often the increase) of yield through optimization of core physiological processes. Treating seeds or plants with new stimulators typically results in higher germination energy and more uniform emergence; a larger and more efficient photosynthetic surface; a greater coefficient of productive tillering; and an improved balance between grain number per spike and thousand-kernel weight (Rani et al., 2018; Kim et al., 2024).

Experimental results from a range of environments and genotypes indicate that stimulators can add roughly 0.5 to 1.5 t/he of grain, depending on the variety and weather conditions. For the steppe zone, where winter wheat yields are often constrained by drought stress and nutrient limitations, these gains are economically and strategically significant (Murphy et al., 2020; Ejaz et al., 2023).

New growth stimulants are equally important for building the adaptive capacity of winter wheat. Under arid conditions and high temperatures, they can reduce the intensity of transpiration by regulating stomatal conductance, improve tissue water storage capacity, and activate the synthesis of compatible osmolytes (such as proline and soluble sugars) that stabilize cell membranes (Wang et al., 2020; Li et al., 2023). Moreover, they decrease the accumulation of reactive oxygen species and reinforce the antioxidant defense system, helping the plants maintain cellular integrity during stress episodes (Iqbal et al., 2015; Ahmad et al., 2020).

These combined effects translate into greater resilience during periods of moisture deficit, late frosts, or heat spells. As a result, the likelihood of catastrophic yield losses is reduced, and production becomes more predictable – even when the season brings multiple stress events. In practical terms, stimulators help preserve the yield components set early in development and maintain assimilate flow toward the grain during the critical phases of stem elongation and filling (Zhang et al., 2022).

The quality of winter wheat grain is determined by protein content, gluten quantity and strength, micronutrient composition, and the presence of beneficial bioactive compounds (Nazarenko et al, 2019; Kosová et al., 2021). Growth stimulators, operating at the biochemical level, can significantly enhance these indicators. They increase the activity of enzymes involved in protein synthesis, promote nitrogen remobilization from vegetative tissues to the developing kernels, and often raise both the content and elasticity of gluten – key parameters for baking performance (Peng et al., 2014). Additionally, stimulators tend to support the accumulation of important micronutrients (Fe, Zn, Cu, Mn), which has implications for human nutrition and for the functional properties of flour. By mitigating oxidative stress in plants, they can also contribute to lower levels of undesirable oxidative products in the harvested grain, indirectly supporting food safety and nutritional value (Ahmad et al., 2020; Mohsin et al., 2021; Parizi et al., 2025).

In field practice, growth stimulators can be strategically applied at several stages of winter wheat ontogenesis pre-sowing seed treatment to secure fast, uniform germination and robust early root development. Also, tillering stage applications can enhance the formation and survival of productive shoots and expand leaf area. During stem elongation and heading, foliar sprays are applied to sustain photosynthesis, support carbohydrate partitioning, and improve grain filling and thousand-kernel weight (Jha et al., 2017; Korsukova et al., 2025).

The best results are obtained when stimulators are integrated into a broader crop management system that includes balanced mineral nutrition, precise irrigation (where available), and well-timed protection against weeds, pests, and diseases. In such integrated programs, each newly introduced stimulator can raise the efficiency of the production technology by roughly 10–20%, depending on genotype × environment interactions and the season's stress profile (Cohen et al., 2021; Ejaz et al., 2023).

Conclusion

Across three triazole-type agents (CA-64, CA-79, CA-67), concentration was the dominant driver of field outcomes, with a narrow, repeatable stimulatory window at 0.01–0.02% and uniform reduction at 0.04%. The most reliable stimulator was CA-79, especially at 0.02%, consistently elevating carbohydrate reserves in the tillering node at all winter checkpoints, while preserving post-winter stand scores. CA-64 improved reserves and final survival at 0.02% (often also at 0.01%), making it a safe default for locally adapted varieties. By contrast, CA-67 provided no dependable stimulation: 0.01% was at best neutral, whereas 0.02% decreased the reserves and post-winter scores. At 0.04%, all agents caused marked losses in germination, pre-winter stand, and winter survival (winter losses increasing by 4–12%), indicating a clear toxicity threshold that should be avoided for all genotypes. The responses were genotype-conditioned. The local varieties (Pozytsiia Odeska, MIP Feieriia, Tenor) showed predictable gains with CA-79/CA-64 at 0.02%, maintaining stable stands. The western ecotypes (MV Nador, MV Menrot) began with lower sugar baselines and were more sensitive; they still benefited from CA-79 0.02% (and modestly from CA-64 0.02%), but harm from a 0.04% concentration was the greatest, and operational ceilings should account for 0.01–0.02%. Following research will therefore prioritize evaluating selected genotypes for grain yield and improved technological grain qualities, as well as enhancements in beneficial microelement content. These studies will provide essential insights to harnessing the full potential of new triazole-based agents and their stimulating effects in sustainable crop improvement strategies.

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