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PECULIARITIES OF THE NEW EPIMUTAGEN ACTION ON VARIABILITY OF WINTER WHEAT

SUMMARY

The study of new agents for enhancing biodiversity in local varieties of winter wheat is a promising approach for developing both new commercial varieties and components for recombination selection. The objective of this study was to explore the potential of Triton-305X (TX-305) as a epimutagen, focusing on optimizing the yield of epimutant forms derived from local varietal resources. Winter wheat seeds from four varieties were treated with water (control) and TX-305 at concentrations of 0.01%, 0.05%, 0.1%, and 0.5%. The most successful results for all varieties were achieved with TX-305 concentrations of 0.1% and 0.5%. Lower concentrations of TX-305 were not optimal for producing valuable forms. All varieties exhibited significant genotype-mutagenic interactions, leading to notable positive changes in variability parameters. This agent shows promise for generating short-stemmed, early-ripening forms, long-spiked lines, and high-yielding forms. The findings highlight the potential of epimutagens as a valuable tool in winter wheat breeding. By regulating chromatin modifications, epimutagens provide a controlled approach to inducing variability, offering new possibilities for crop improvement in response to changing environmental conditions. Further research will focus on the long-term stability of epimutagen-induced traits and their integration into commercial breeding programs.

Keywords: winter wheat, epimutagen, Triton-305X, mutation, plant improvement.

INTRODUCTION

Bread winter wheat remains a highly valuable grain crop, particularly in regions with risky farming conditions, such as the entire territory of Ukraine (Nazarenko, 2016). Global warming and climate change present both opportunities and challenges for wheat production. On one hand, rising temperatures enable the expansion of heat-loving crops further north and contribute to milder overwintering conditions, which is especially beneficial for winter crops. On the other hand, climate change also increases the frequency and

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severity of droughts during critical growth stages, potentially reducing yields and threatening food security (Yuan *et al*., 2021; Turaeva *et al*., 2024).

The study explores the effects of a new epimutagen on the variability of winter wheat, focusing on its ability to induce heritable changes without directly altering DNA sequences. Unlike traditional mutagens, epimutagens influence chromatin structure and gene expression, leading to phenotypic modifications that can enhance crop adaptability and productivity (Cann *et al*., 2022). The research examines the extent of variability in treated wheat lines, assesses the stability of inherited traits across generations, and evaluates the practical implications for breeding programs (Hassine*, et al.,* 2023).

Chemical mutagenesis is particularly valuable due to its higher specificity in interacting with particular genotypes and its site-directed effects on native DNA, a characteristic less common in physical mutagens (Yali & Mitiku, 2022). This approach offers several promising opportunities for the genetic improvement of agricultural crops, enabling more targeted and controlled induction of variability compared to other mutagenic factors (Abdel-Hamed *et al*., 2021). These advantages make chemical mutagenesis a powerful tool for enhancing desirable traits such as yield potential, stress tolerance, and disease resistance in modern breeding programs.

The application of new factors to local genetic material, such as novel epimutagens, presents significant potential for expanding the spectrum and frequency of induced changes (Cann *et al*., 2022). This approach is particularly promising when working with highly susceptible genotypes, as it can lead to a predominance of rare but valuable mutations (Spencer-Lopes *et al*., 2018).

Furthermore, chemical mutagens tend to induce subtle yet complex biochemical mutations without introducing additional negative traits. These changes can enhance the nutritional properties of staple crops, addressing deficiencies in biologically active compounds and essential microelements that do not always align with consumer needs (Zulfiqar *et al*., 2024).

Additionally, using specific chemicals at moderate concentrations can optimize the source material-mutagen system, significantly increasing mutation yield. Under favorable conditions, the frequency of valuable mutations may reach 60–80%, and these values may not represent an upper limit, particularly for underutilized chemical classes (Abdel-Hamed *et al*., 2021). Notably, sodium azide has shown promising effects on certain cereal crops, further highlighting the potential of chemical mutagenesis in breeding programs (Hassine *et al*., 2023).

Many chemical mutagens, particularly those with strong damaging effects, can exhibit variability within a single concentration, influencing trait inhibition by as much as 10–12% of its absolute value. This highlights the critical importance of studying genotype-mutagen interactions to optimize mutation breeding strategies (Mangi *et al.*, 2021).

Typically, as the genetic activity of a mutagenic factor increases, the intensity of genotype-mutagen interactions tends to decrease. However, this is not always the case, as certain genotypes may exhibit a significantly lower rate of interaction reduction or even maintain stable interaction levels despite increased mutagenic activity. Identifying such genotypes is essential for refining mutation breeding approaches and enhancing the effectiveness of mutagenic treatments (Abdel-Hamed *et al*., 2021; Mahanish & Kin, 2025).

Epimutagens represent a novel approach in plant breeding, enabling controlled variability by affecting epigenetic markers rather than causing genetic mutations (Chakraborty *et al.,* 2023). This study investigates how a newly tested epimutagen influences key agronomic traits of winter wheat, including yield potential, stress tolerance, and disease resistance. The ability to induce beneficial traits without genetic damage offers promising applications for sustainable agriculture and breeding advancements (Mahanish & Kin, 2025).

The study aimed to analyze hereditary variability by conducting visual and biometric assessments of phenotypic changes in the second generation of mutant families. It sought to identify new lines with inherited altered traits in the third and subsequent generations, determine the boundaries of practically valuable variability in comparison with individual varieties, and assess the effects of established depressive impacts observed in the first generation. Additionally, the research aimed to identify optimal combinations within the system of source material and mutagen concentration to enhance the effectiveness of mutation breeding.

MATERIAL AND METHODS

The experiments were carried out under the conditions of the research fields station of the Science-Education Center of the Dnipro State Agrarian Economic University during 2022–2024 (48°51′10″ n. l. 35°25′31″ e. l.).

Winter wheat seeds (1000 grains for each concentration and water) were acted with a TX-305 (Triton-305X) 0.01%, 0.05%, 0.1%, 0.5% (Sigma-Aldrich, Germany) at water solution. Seed material was acted with an exposition of 24 hours by the generally recommended protocols for chemical mutagens action. Concentrations were trivial for this type of mutagens. The control was soaked in water (Spencer-Lopes *et al*., 2018).

Seeds material has been sown by 40 variants (in total) (2-rows plots for second generation, 5-rows for third generations and 10-rows plots for next generations, initial variety as control, interrows were 0.15 m, 1.5 m length of row). For varieties with difference at ecotype were used (in brackets FS – forest-steppe ecotype, all for all zones, S – steppe by state examination classification) Perspektyva Odeska (S), Sonata Poltavska (FS), Shpalivka (all), MIP Lada (all). The genotypes were identified by general national breeding classification as for Steppe conditions semi-intensive Perspektyva Odeska, MIP Lada; intensive Sonata Poltavska, Shpalivka.

Crop cultivation is a standard practice in the Steppe zone. As part of the study, untreated initial varieties served as controls and were grown alongside mutant families for comparison in the second generation. Each variant was planted in ten plots, with control rows included for accurate assessment.

Sowing was conducted manually at the end of September, ensuring a depth of 4–5 cm and a seeding rate of 100 viable seeds per row. Each sample consisted of two rows, with a control row of the initial variety for direct comparison. Mutant lines were planted in three replications, with control rows of the parent variety included for each twenty-row plot to ensure reliable evaluation of phenotypic variability. Phenotypic and biometric analyses were conducted across multiple generations to determine trait variability, stability and potential depressive effects. Comparisons were made between treated and control populations to evaluate the effectiveness of the epimutagenic intervention. (Mangi *et al.,* 2021).

At M_2–M_3 generations mutations were identified though visual evaluation and biometrical analyze of yield structure. At second generation preliminary evolution by visible changes, at second identification as epimutations by traits heredity. Estimation was conducted during 2022 – 2023 years for second-third generations and during for 2023 –2024 years for next generation in collection of genetic-value samples and lines grain production exam. The assessment of plant resistance to diseases was carried out for the following diseases powdery mildew, brown leaf rust and septoria. Significant presence of these diseases was observed: in 2022-2023 powdery mildew, 2022, 2024 brown leaf rust, 2022, 2024 septoriosis. The assessment was carried out on a trivial 9-points scale.

Level of changeability was calculated as $P\nu = \alpha * \gamma$, where $P\nu$ – level of changeability of variant; α – number of mutations for general number of families at variant; γ – number of types changed traits at variant.

Statistic analyze of data was performed by ANOVA-analysis, grouping and estimation of data was provided by discriminant and cluster analysis (Euclidian distance, single linkage) (Statistic 10.0, multivariant module, TIBCO, Palo Alto, USA). The normality of the data distribution was examined using the Shapiro–Wilk W-test. Differences between samples were assessed by Tukey HSD test.

RESULTS AND DISCUSSION

A total of 9,950 families in the second generation (M_2) and 272 mutant lines in the third generation (M_3) were analyzed, including both control and mutagen-treated materials. The mutagens were applied at concentrations recommended for cereal breeding. On average, each variant contained about 500 M_2 families, except for the extreme concentration TX-305 (0.5%) applied to one variety (Perspektyva Odeska).

Table 1 presents data on the general mutation rates observed in the second and third generations for four winter wheat genotypes, categorized by mutagen concentration. Notably, the level of mutagenic depression in the first generation was lower than that observed for chemical supermutagens in previous studies. Prior research has indicated a strong correlation between mutagenic depression in the first generation and mutation variability in subsequent generations. However, data on epimutagen activity remain unavailable, which underscores the importance of further investigations in early generations to assess its effects.As we can see from the table, the number of changes is at the same level for the more sensitive variety Perspektyva Odeska as for the more tolerant varieties to the action of moderate mutagens, such as alkyl compounds in previous studies (Shpalivka). The table presents mutation rates in the second and third generations of four winter wheat varieties treated with different concentrations of the mutagen TX-305.

Table 1. General rate of hereditary changes for winter wheat samples at second – third generations (x ± SD, n = 450-500).

Variety	Number of selecting families	Number of mutant families	Rate of mutations, %
Perspektyva Odeska	500	2	0.40 ± 0.09[a]
Perspektyva Odeska, TX-305 0.01%	500	9	1.80 ± 0.14[b]
Perspektyva Odeska, TX-305 0.05%	500	15	3.00± 0.19[c]
Perspektyva Odeska, TX-305 0.1%	500	19	3.80±0.22[d]
Perspektyva Odeska, TX-305 0.5%	450	27	6.00±0.24[e]
Sonata Poltavska	500	3	0.60 ± 0.10[a]
Sonata Poltavska, TX-305 0.01%	500	11	2.20± 0.15[b]
Sonata Poltavska, TX-305 0.05%	500	14	2.80±0.20[b]
Sonata Poltavska, TX-305 0.1%	500	19	3.80±0.22[c]
Sonata Poltavska, TX-305 0.5%	500	25	5.00±0.23[d]
Shpalivka	500	2	0.40 ± 0.08[a]
Shpalivka, TX-305 0.01%	500	9	1.80±0.13[b]
Shpalivka, TX-305 0.05%	500	14	2.80±0.20[c]
Shpalivka, TX-305 0.1%	500	17	3.40±0.21[c]
Shpalivka, TX-305 0.5%	500	23	4.60±0.22[d]
MIP Lada	500	3	0.60 ± 0.19[a]
MIP Lada, TX-305 0.01%	500	9	1.80±0.14[b]
MIP Lada, TX-305 0.05%	500	12	2.40±0.18[b]
MIP Lada, TX-305 0.1%	500	17	3.40±0.21[c]
MIP Lada, TX-305 0.5%	500	22	4.40±0.22[d]

Note: indicate significant differences at P < 0.05 by ANOVA-analyze with Bonferroni amendment. Comparison in terms of one variety.

Mutation rate increases with concentration, across all varieties, the mutation rate increased proportionally with higher TX-305 concentrations. The lowest mutation rate (0.40–0.60%) was observed in control groups, while the highest rates (4.40–6.00%) occurred at 0.5% concentration.

Perspektyva Odeska shows the highest mutability at 0.5% TX-305, this variety exhibited the highest mutation rate (6.00%), suggesting greater sensitivity to the mutagen ($F = 7.23$; $F_{0.05} = 4.10$; $P = 0.004$ regarding Tukey HSD test).

Note some similar trends across varieties, while absolute mutation rates varied slightly, the overall trend of increasing mutation rates with concentration remained consistent.

Sonata Poltavska, Shpalivka, and MIP Lada have moderate response. Both varieties demonstrated a gradual mutation rate increase, with Sonata Poltavska reaching 5.00% and MIP Lada 4.40% at the highest concentration. Changeability on the same level. Shpalivka exhibits intermediate sensitivity. This variety showed a maximum mutation rate of 4.60%, slightly lower than Perspektyva Odeska but higher than MIP Lada.

The findings suggest that Perspektyva Odeska is the most responsive genotype to TX-305, while MIP Lada has the lowest sensitivity. These results emphasize the importance of genotype-mutagen interactions in optimizing mutation breeding strategies. For all cases, the differences were statistically significant for all concentrations of mutagens in all varieties with tree exeptions. There are no statistic difference between Sonata Poltavska, TX-305 0.01% and Sonata Poltavska, TX-305 0.05% ($F = 3.03$; $F_{0.05} = 4.10$; $P = 0.08$), Shpalivka, TX-305 0.05% and Shpalivka ($F = 3.23$; $F_{0.05} = 4.10$; $P = 0.07$), TX-305 0.1%, MIP Lada, TX-305 0.01% and MIP Lada, TX-305 0.05% ($F = 3.25$; $F_{0.05} = 4.10$; $P = 0.07$), regarding Tukey HSD test, which show sometimes similar action for TX-305 0.01% and TX-305 0.05% ($F = 4.03$; $F_{0.05} = 4.10$; $P = 0.06$).

For this group of varieties epimutagen action was statistically significant for the variance in the change in mutagen concentration ($F = 112.14$; $F_{0.05} = 3.48$; $P = 1.15*10^{-7}$) and for genotype-mutagen interaction ($F = 5.55$; $F_{0.05} = 2.72$; $P = 0.02$), but not for genotypes ($F = 3.23$; $F_{0.05} = 3.86$; $P = 0.07$).

To further establish the differentiating ability of overall mutation frequency as an indicator, a cluster analysis was performed (Figure 1).

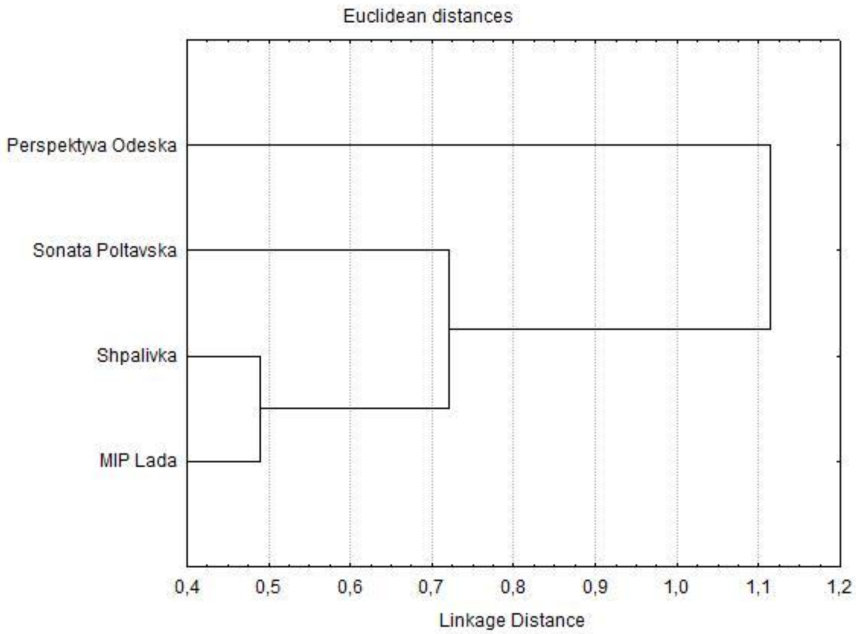

Figure 1. Results of cluster analysis by general mutation rate.

The analysis revealed that all the varieties are generally divided into three main groups when exposed to TX-305. The first group consists of the variety

Perspektyva Odeska, characterized by higher variability and a higher rate of general changes. The second group includes the varieties Sonata Poltavska, Shpalivka, and MIP Lada, which exhibit similar variability at an average level within the group. Notably, TX-305's action on individual varieties differs significantly from previously studied substances with lower damaging abilities.

It can also be concluded that the frequency of epimutations increases progressively with higher concentrations, with the highest frequency observed at the highest concentration.

Equally, important to overall variability is the number of traits that have undergone changes. An increase in overall mutation frequency does not always correlate with increased diversity in mutant material for selection, and in some cases, it may even lead to a significant reduction in this diversity. Therefore, the level of variability is calculated as the ratio between the number of mutant cases and the number of traits that have changed (Table 2; cluster analysis data for this parameter are presented in Figure 2).

Varieties showed the next level of changeability Perspektyva Odeska (level up to 1.20), Sonata Poltavska (up to 0.95), Shpalivka (up to 0.78), MIP Lada (up to 0.70). Herewith, a significant decrease in the number of traits for which mutations have passed with an increase in concentration to the maximum is observed for variety Perspektyva Odeska. Perspektyva Odeska ($F = 7.15$; $F_{0.05} = 4.10$; $P = 0.007$) and Sonata Poltavska ($F = 5.14$; $F_{0.05} = 4.10$; $P = 0.02$) differ in a higher level from two other varieties and among themselves, in which the dynamics in the number of traits, however, remains the same.

Although, for all genotypes in these two groups TX-305 action was statistically significant in all variants for the changeability with the change in epimutagen concentration ($F = 88.17$; $F_{0.05} = 3.10$; $P = 2.23*10^{-6}$), by genotypes ($F = 4.67$; $F_{0.05} = 3.59$; $P = 0.03$) and for genotype-mutagen interaction ($F = 8.17$; $F_{0.05} = 2.54$; $P = 0.009$). For all cases, the differences were statistically significant for all concentrations of epimutagen in all varieties. While cluster analysis of other chemical mutagens showed some differences in the classification of genotypes and a more systematic nature of the second indicator, the results with TX-305 did not differ significantly (Fig. 2). Again, all the varieties exposed to TX-305 were grouped into three categories. The first group includes the variety Perspektyva Odeska, which exhibited higher variability across all concentrations of the epimutagen (highest level). The second group consists of the variety Sonata Poltavska, which showed lower variability. The third group includes the varieties Shpalivka and MIP Lada, which demonstrated the lowest variability under the action of TX-305.

Cluster analysis for the level of changeability revealed a different division into three groups compared to the previous parameter (Fig. 2). Unlike previous cases with other mutagens, it can be mathematically justified that the estimate based on the level of changeability is more accurate than the general rate of changes.

Table 2. Level of changeability, caused by mutation variability (x ± SD, n = 450-500).

Variant	Level of changeability	Changed traits
Perspektyva Odeska	0.01±0.01[a]	2
Perspektyva Odeska, TX-305 0.01%	0.18±0.02[b]	10
Perspektyva Odeska, TX-305 0.05%	0.42±0.05[c]	14
Perspektyva Odeska, TX-305 0.1%	0.68±0.06[d]	18
Perspektyva Odeska, TX-305 0.5%	1.20±0.09[e]	20
Sonata Poltavska	0.02±0.01[a]	3
Sonata Poltavska, TX-305 0.01%	0.22±0.02[b]	10
Sonata Poltavska, TX-305 0.05%	0.36±0.03[c]	13
Sonata Poltavska, TX-305 0.1%	0.61±0.05[d]	16
Sonata Poltavska, TX-305 0.5%	0.95±0.07[e]	19
Shpalivka	0.01±0.01[a]	2
Shpalivka, TX-305 0.01%	0.16±0.02[b]	9
Shpalivka, TX-305 0.05%	0.31±0.03[c]	11
Shpalivka, TX-305 0.1%	0.51±0.05[d]	15
Shpalivka, TX-305 0.5%	0.78±0.07[e]	17
MIP Lada	0.02±0.01[a]	3
MIP Lada, TX-305 0.01%	0.14±0.02[b]	8
MIP Lada, TX-305 0.05%	0.24±0.03[c]	10
MIP Lada, TX-305 0.1%	0.51±0.04[d]	15
MIP Lada, TX-305 0.5%	0.70±0.05[e]	16

Note: indicate significant differences at P < 0.05 by ANOVA-analyze with Bonferroni amendment. Comparison in terms of one variety.

Mutational changes were categorized into six groups based on standard mutation breeding classification. The first group pertains to plant structure traits, including stem thickness, plant height variations, and waxy bloom presence.

TX-305 did not significantly induce mutations for stem thickness, dwarf, or semi-dwarf forms at lower concentrations. Only at 0.5% TX-305 was there any induction, with one case each in Perspektyva Odeska and Sonata Poltavska.TX-305 as an epimutagen effective in induction three types of changes by first group for forms with weak waxy bloom (up to 0.60 for all varieties, regular), short stem forms (up to 0.60, irregular, dependent on variety, less for Shpalivka) and the highest level for high-stem forms (up to 0.60%, regular for all varieties). TX-305 demonstrates a distinct epimutagenic effect on waxy bloom presence, short and high-stem variations, while being ineffective in inducing rare structural mutations like stem thickness and dwarfism (except at extreme concentration).

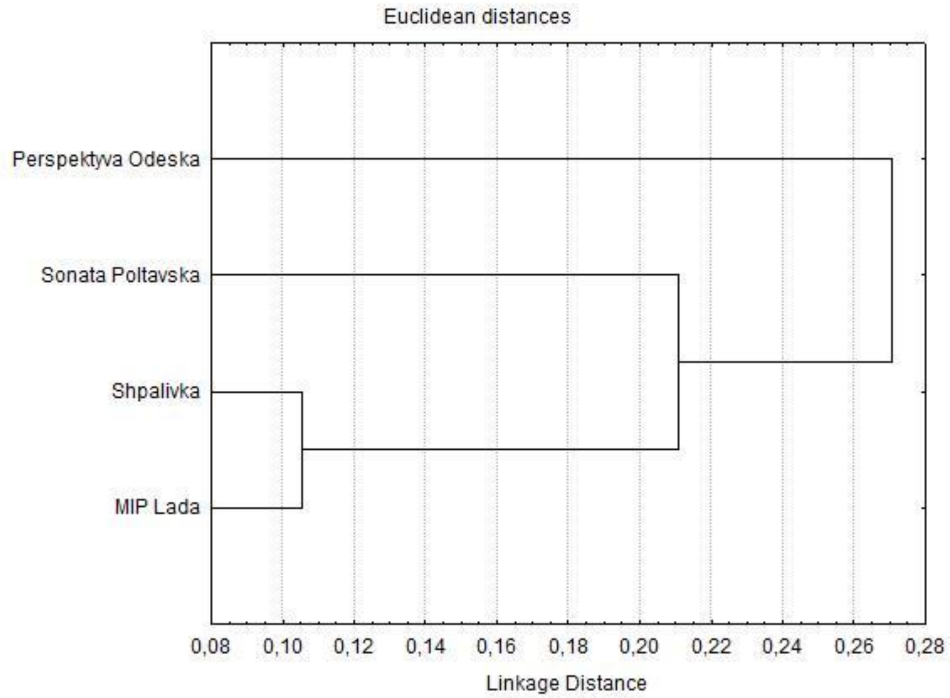

Figure 2. Results of cluster analysis by level of variability.

The second group of mutants includes grain size and shape variations, such as barrel-shaped grain, small and large grain. Small grain mutations occur at a frequency of up to 0.20%, with higher concentrations of TX-305 increasing the probability for all varieties. The effect is statistically significant ($F = 8.17$; $F_{0.05} = 4.32$; $P = 0.01$). Barrel-shaped and large grain were observed infrequently, indicating that TX-305 does not strongly induce them. Epimutants with small grain appeared more frequently at TX-305 concentrations of 0.1% and 0.5%, suggesting a dose-dependent response. TX-305 predominantly induces small grain mutations, especially at higher concentrations, while other grain shape variations remain rare.

The third group of mutants consists of spike structure variations, which includes 15 different types. As TX-305 concentration increases, the frequency of most spike-related mutations also increases. Exception: large spike and other rare mutations occur less frequently and do not follow this pattern. Long spike and semi-awn spike mutations were the most frequently observed. These occurred regularly at a rate of up to 0.20% for all tested varieties (regular). TX-305 shows strong epimutagenic effects in inducing these two specific spike variations.

The fourth group of mutations, which includes physiological changes in plant growth and development, exhibits high variability despite involving only four traits. Early maturity (up to 0.40%, regular) was more frequent in third and fourth TX-305 concentrations across all varieties. Late maturity (up to 0.67%, regular) was more common in Perspektyva Odeska, Shpalivka, and MIP Lada, especially in third and fourth TX-305 concentrations. Disease tolerance was a significant mutation trait in the mutation model, frequently observed. Sterility was extremely rare; no cases at first, second, or third concentrations, with only two occurrences at the fourth concentration. TX-305 effectively induces early and late maturity mutations, with late maturity being more frequent at higher concentrations. However, sterility is not typical, making TX-305 a potentially useful epimutagen for controlled breeding without major fertility loss.

The fifth group consists of systemic mutations, characterized by extreme changes in spike structure. These mutations go beyond the typical cultivated form, leading to a phenotype resembling wild wheat relatives. Such mutations are rare but significant in their deviation from the cultivated phenotype.Such traits are not probable under the action of TX-305. Only sometimes speltoids at fourth (in one case third) concertation (not for Perspektyva Odeska). This type of mutations isn't interesting for wheat genetic improvement.

The sixth group includes agriculture-value forms with high grain yield or tillering ability. It occurs in most varieties, preferably for TX-305 0.1 % and TX-305 0.5% concentrations. It's not occurrence decreases with increasing concentration for all varieties. This type of mutations is common for high grain yield.

In addition to establishing the variability of individual parameters and their groups, it is quite important to demonstrate the model variability (especially for common parameters and a group of valuable mutations), which was done through the discriminant analysis of individual variables (Table 3, Fig. 3). The model was the general rate of mutations, the level of changeability, mutations in the first, third, fourth and sixth groups.

Table 3. Results of discriminant analyze

Variables at model	Wilks Lambda λ	Partial Lambda	F_{remove} (4,14)	p-level
Changes rate	0.08	0.07	42.68	0.01
Level of changeability	0.04	0.15	18.99	0.01
First group	0.04	0.16	16.11	0.01
Second group	0.51	0.71	2.31	0.09
Third group	0.07	0,08	26,19	0,01
Fourth group	0.07	0.08	31.44	0.01
Fifth group	0.52	0.73	2.14	0.09
Sixth group	0.04	0.17	7.01	0.01

The data obtained confirm that the applied epimutagen is highly effective in both inducing general variability and influencing specific traits. The range of variable features is extensive, covering nearly all potential mutation types. TX-305 effective in inducing low-growing forms, essential for intensive ecotypes. A frequent and significant change, beneficial for adapting to shorter growing seasons. Disease resistance enhanced tolerance was observed, making it valuable for breeding resistant varieties. Sixth mutation group inclusion ensures the agent's practical significance, broadening its breeding applications. The TX-305 supermutagen is efficient for targeted breeding, particularly in dwarfism, early maturity, and disease resistance, making it a valuable tool in modern mutation breeding programs.

The discriminant analysis (Fig. 3) confirmed distinct effects of different TX-305 concentrations, emphasizing the concentration-dependent nature of mutagenic variability. First and second concentrations show mixed effects, but their data cloud remains far from the water-treated control. Second and third concentrations exhibit partial association, while the first concentration stands apart. The fourth concentration (highest TX-305 level) demonstrates a clear separation, indicating its strong mutagenic effect.

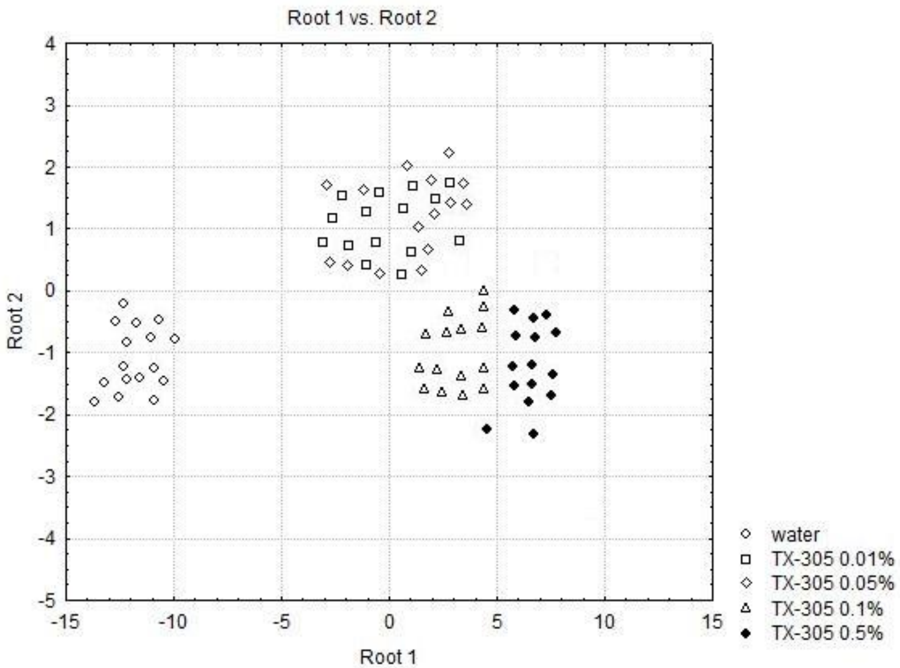

Figure 3. Results of discriminant analysis for model parameters

Perspektyva Odeska exhibited the highest genotype-mutagenic interaction, making it most responsive to TX-305. The three other varieties also showed effective mutation induction at higher concentrations (Table 4).

The findings suggest that third and fourth TX-305 concentrations are the most effective, though genotype-dependent. Perspektyva Odeska emerges as a promising candidate for mutagenic breeding due to its higher responsiveness to

TX-305. The data suggest that this substance should primarily be used as an epimutagenic factor to develop components for improving existing varieties through recombinant breeding (Ahumada-Flores *et al.*, 2021; Bora *et al.*, 2024). It is less likely to produce lines suitable for direct use as commercial varieties (Ergün *et al.*, 2023).

Table 4. Classification matrics - canonical roots.

Genotype	Percent of classification
Perspektyva Odeska	100.0
Sonata Poltavska	91.0
Shpalivka	91.0
MIP Lada	91.0
Total	93.5

This epimutagen is optimally used for inducing short-stemmed forms with long ears and early ripening lines, which can help avoid the typical May-June drought in our region (Nazarenko *et al.*, 2016). While the use of TX-305 in breeding for disease resistance shows promise, a wide range of substances have demonstrated effectiveness for this type of improvement (Anter, 2021)

In contrast to previous studies (Jankowicz-Cieslak *et al.*, 2022; Bora *et al.,* 2024), the concentrations tested, though approaching semi-lethal levels in the first generation, did not result in a significant decrease in activity (OlaOlorun *et al.*, 2020; Cann *et al.*, 2022). However, even at higher concentrations, TX-305 induced a significant increase in biodiversity (OlaOlorun *et al.*, 2021). Therefore, the applied dose range is generally suitable for obtaining genetically valuable forms when needed (Mahanish & Kin, 2025).

The data suggest that higher concentrations of the mutagen (0.1% - 0.5%) lead to a greater spectrum of mutations, including both beneficial and adverse changes (Ahumada-Flores *et al.*, 2021). Higher concentrations (0.1% - 0.5%) are more effective, these concentrations induce a broader range of mutations. The third (0.1%) and fourth (0.5%) concentrations show the strongest effects and can be used regardless of genotype. The fourth concentration (0.5%) is recommended only for varieties with low initial variability.

Genotype-mutagenic interaction is high and shows significantly higher interaction with genotype than previous agents show. The interaction is mainly positive, indicating effective induction of desired traits (Yali & Mitiku, 2022).

The findings support targeted mutagenesis strategies, where different genotypes can be exposed to specific concentrations for optimal breeding outcomes. The use of higher concentrations should be carefully controlled, especially in varieties with greater genetic stability, to avoid excessive adverse changes (Horshchar & Nazarenko, 2024).

The epimutagen induced significant phenotypic variability in key traits, with certain lines exhibiting improved environmental adaptability (Lal *et al.*, 2020; Anter, 2021). Unlike conventional mutagens, epimutagenic treatment did not result in high mutation loads or undesirable traits, ensuring greater stability in subsequent generations (Nazarenko *et al.*, 2017; Horshchar & Nazarenko, 2024). The study identified optimal epimutagen concentrations that maximize beneficial variability while minimizing negative impacts.

CONCLUSIONS

The studied TX-305demonstrated a fairly high overall level of variability and demonstrated extremely high activity in obtaining new forms for all essential features of winter wheat plants. The activity of this agent is predominantly focused on the induction of such types of mutations as changes in plant height, changes in the length of the spike and grain, positive in first and negative in second case, changes in ripeness, before the entire production of a significant number of early-ripening forms, the production of forms with high grain yield. Particular attention should be paid to the possibility of obtaining short-stem early-maturing forms, with a long spike. The findings highlight the potential of epimutagens as a valuable tool in winter wheat breeding. By regulating chromatin modifications, epimutagens provide a controlled approach to inducing variability, offering new possibilities for crop improvement in response to changing environmental conditions. Further research will focus on the long-term stability of epimutagen-induced traits and their integration into commercial breeding programs.

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