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Research Article

Peculiarities of the Action of a Novel Epimutagen on Variability in Winter Wheat

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Abstract: This research aimed to evaluate the potential of Triton-305X (TX-305) as an epimutagen, with a focus on optimizing general rate of epimutant forms from regional genetic resources. Seeds from four wheat varieties were treated with water (control) and TX-305 at concentrations of 0.01%, 0.05%, 0.1%, and 0.5%. The most number of perspective changes were observed at concentrations of 0.1% and 0.5%, particularly in combination with the varieties Farrell and NE 12443. Lower concentrations were less effective in generating valuable forms. All varieties demonstrated significant genotype-mutagen interactions, resulting in notable positive changes in variability parameters. TX-305 shows considerable promise for inducing short-stemmed, early-ripening forms, as well as long-spiked, high-yielding lines with large seeds. These findings underscore the potential of epimutagens as powerful tools in winter wheat breeding. By modulating chromatin structure, epimutagens offer a controlled and targeted method for inducing genetic variability, opening new opportunities for crop improvement under changing environmental conditions.

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1. Introduction

Bread winter wheat remains a highly valuable grain crop, particularly in regions with challenging farming conditions (Nazarenko et al., 2021). Global changes in climate present both opportunities and challenges for wheat production. On one hand, rising temperatures allow heat-loving crops to expand further north and create milder overwintering conditions, benefiting winter crops. On the other hand, climate change increases the frequency and severity of droughts during critical growth stages, which may reduce yields and threaten food security (Yuan et al., 2021; Turaeva et al., 2024).

This study investigates the effects of a novel epimutagen on the variability of winter wheat, emphasizing its ability to induce heritable changes without directly altering DNA sequences. Unlike traditional mutagens, epimutagens modify chromatin structure and gene expression, resulting in phenotypic changes that can enhance crop adaptability and productivity (Bayhan et al., 2024). The research evaluates the extent of variability in treated wheat lines, the stability of inherited traits across generations, and the practical implications for breeding programs (Kumar et al., 2024).

Chemical mutagenesis is particularly valuable due to its higher specificity in interacting with particular genotypes and its site-directed effects on native DNA, a trait less common in physical mutagens (Yanting et al., 2024). This approach presents promising opportunities for genetic crop improvement by enabling more targeted and controlled induction of variability compared to other mutagenic factors (Harb et al., 2025). 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 (Nazarenko et al., 2019).

The application of new factors to local genetic material, such as novel epimutagens, holds significant potential for expanding the general rate and number of types of novel mutations (Bayhan et al., 2024). This approach is especially promising when applied to highly susceptible genotypes, as it can lead to the emergence of rare but valuable mutations (Spencer-Lopes et al., 2018).

Furthermore, chemical mutagens often induce subtle yet complex biochemical mutations without introducing additional negative traits. These changes can improve the nutritional properties of staple crops, helping to address deficiencies in biologically active compounds and essential microelements that may not always meet consumer needs (Wanga et al., 2024).

Moreover, the use of specific chemicals at moderate concentrations can optimize the stability of variety-epimutagen system, significantly enhancing changeability of plant matherial. Under these conditions, the number of types of agriculture-valuable forms may reach 60–80%, and this may not represent the upper limit, particularly for underutilized chemical classes (Harb et al., 2025). Notably, sodium azide has demonstrated promising effects on certain cereal crops, further emphasizing the potential of chemical mutagenesis in breeding programs (Kumar et al., 2024).

The most part of agents, especially substances with higher damaging influence, may exhibit variability even within some concentrations, influencing trait inhibition by up to 11–13% from normal value. This underscores key importance of studying variety-epimutagen interactions to optimize mutation breeding strategies (Manjusha et al., 2024).

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 lower rate of interaction reduction or even maintain stable interaction levels despite increased mutagenic activity. Identifying such genotypes is crucial for refining mutation breeding strategies and improving the effectiveness of mutagenic treatments (Harb et al., 2025; Mahanish and Kin, 2025).

Epimutagens are chemical or biological agents that induce heritable changes in gene expression without altering the DNA sequence (Khare et al., 2025). These modifications primarily affect DNA methylation, histone modifications, and non-coding RNA interactions, leading to phenotypic variations. Traditional breeding methods often rely on genetic mutations, but epimutagenesis offers an alternative means to enhance crop variability and resilience (Mahanish and Kin, 2025).

The aim of the research was to analyze epimutagen changebility by conducting visual and morphometric assessments of visual changes at the second-third generations of epimutant plants. This study investigates the effects of a novel epimutagen on winter wheat by evaluating its influence on plant growth, productivity, and adaptability. We aim to determine how this agent modifies epigenetic markers and whether these changes persist across generations.

2. Materials and Methods

The experiments were conducted at the research field station of the Science and Education Center, Dnipro State Agrarian and Economic University, during 2022–2024 (48°51′11″ N.L. 35°25′30″ E.L.), ordinary low-humus black soil, humus content 3.1%, after fallow land, dose of fertilizations was $N_{110}P_{50}K_{30}$, without irrigation.

Seeds of winter wheat (1000 grains for every variant) were treated with a TX-305 (Triton-305X) 0.01%, 0.05%, 0.1%, 0.5% in water solution. The seed material was exposed for 24 hours in accordance with the generally recommended protocols for chemical mutagen treatments. The control seeds were soaked in water. From first generation about 500 well-formed spikes were selected for investigation in next generations (Spencer-Lopes et al., 2018).

Samples of treated seeds were sown (each spike separately) in two-rows plots for M_2, five-rows for next generation and ten-rows reseach plots for fourth-five, initial genotype as control, interrows were 0.15 m, 1.5 m length of row, 0.45, 1.05 and 2.10 m^2. 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 performed manually at the end of September, with a depth of 4–5 cm and a seeding rate of 100 viable seeds per row. Each plot accompanied by a control plot of the initial genotype for comparison. Mutant lines were planted in three replications and within each twenty plots, control plot of the parent variety were included to ensure reliable assessment of phenotypic variability. Phenotypic and biometric analyses (plant and yield structure, 10 typical plants) were conducted across multiple generations to determine trait variability, and stability. Comparisons were made between treated and control populations to evaluate the effectiveness of the epimutagenic intervention. The genotypes were identified by general national breeding classification as for Steppe conditions semi-intensive NE 12443; intensive Farrell, Ronin, Seilor.

In the second and third generations, epimutations were developed by visual estimation and morphometric analysis of yields structure. At the M_2, preliminary assessment was based on visible changes, while in the M_3 epimutations were confirmed by the heritability of traits. Evaluations were conducted in 2022–2023 for the second and third and in 2023–2024 for subsequent generations within the collection of genetically valuable samples and in grain production trials of selected lines. For the selected promising lines, the nature of the changes was confirmed using MSAP (methylation-sensitive AFLP; HpaII/MspI isoschizomers), performed in the laboratory of the Department of Plant Physiology and Introduction, Dnipro National University.

The level of variability was calculated using the formula $P\nu = \alpha \times \gamma$, where $P\nu$ represents the variability level of a given variant; α denotes the proportion of mutated families relative to the total number of families in the variant; γ indicates the number of trait types that were altered in the variant.

Data were analyzed with ANOVA, while multivariate structure was explored via discriminant analysis and hierarchical clustering (Euclidean metric, single-linkage) using Statistica 10.0 (multivariate module; TIBCO, Palo Alto, USA). Normality was checked with the Shapiro–Wilk W-test and pairwise group differences were evaluated using Tukey's HSD.

3. Results

In general of 9.950 families in the second generation (M_2) and 287 mutant lines in the third-forth generation ($M_{3\text{-}4}$) were analyzed, including both control and mutagen-treated materials. The mutagens were applied at concentrations recommended for cereal breeding. On average, each variant included approximately 500 M_2 families, except for the highest concentration of TX-305 (0.5%), which was applied to a single variety (Farrell).

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 reported for chemical supermutagens in previous studies. Prior research has indicated a strong correlation between mutagenic depression in the first generation and epimutation variability in subsequent generations. However, data on epimutagen activity remain unavailable, underscoring the need for further investigations in early generations to assess its effects.

As shown in the table, the number of changes in the more sensitive variety Farrell is comparable to that in more tolerant varieties exposed to moderate mutagens, such as alkyl compounds in previous studies. However, the difference between Farrell and NE 12443 was not statistically significant ($F = 3.23$; $F_{0.05} = 4.10$; $P = 0.07$, Tukey HSD test). The table presents mutation rates in the second and third generations for winter wheat varieties treated with different concentrations of the mutagen TX-305.

Mutation rate increases with concentration (but not with significant differences for all cases), 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 (4.40–5.11 %) occurred at 0.5% concentration. Although absolute mutation rates varied slightly among the varieties, all genotypes demonstrated a consistent trend of increased mutation rates with rising mutagen concentrations.

Farrell shows the highest mutability at 0.5% TX-305, this variety exhibited the highest mutation rate (5.11%), suggesting greater sensitivity to the mutagen ($F = 5.17$; $F_{0.05} = 4.10$; $P = 0.03$). There was not differences between TX-305 0.05% and TX-305 0.1% ($F = 2.03$; $F_{0.05} = 3.95$; $P = 0.11$).

While absolute mutation rates varied slightly among the varieties, a consistent trend of increasing mutation rates with higher concentrations was observed across all genotypes.

Table 1. Parameters of epimutation variability at $M_2 – M_3$ ($x \pm SD$, $n = 450\text{-}500$)

Variant	Number of families at M_1	Epimutant families	Rate of epimutations, %
Farrell	500	3	0.60 ± 0.08^{a}
Farrell, TX-305 0.01%	500	8	1.60 ± 0.11^{b}
Farrell, TX-305 0.05%	500	16	3.20 ± 0.18^{c}
Farrell, TX-305 0.1%	500	18	3.60 ± 0.21^{c}
Farrell, TX-305 0.5%	450	23	5.11 ± 0.25^{d}
NE 12443	500	2	0.40 ± 0.07^{a}
NE 12443, TX-305 0.01%	500	10	2.00 ± 0.13^{b}
NE 12443, TX-305 0.05%	500	15	3.00 ± 0.20^{c}
NE 12443, TX-305 0.1%	500	19	3.80 ± 0.21^{d}
NE 12443, TX-305 0.5%	500	24	4.80 ± 0.24^{e}
Ronin	500	2	0.40 ± 0.07^{a}
Ronin, TX-305 0.01%	500	14	2.80 ± 0.14^{b}
Ronin, TX-305 0.05%	500	16	3.20 ± 0.17^{b}
Ronin, TX-305 0.1%	500	21	4.20 ± 0.20^{c}
Ronin, TX-305 0.5%	500	22	4.40 ± 0.22^{c}
Seilor	500	2	0.40 ± 0.11^{a}
Seilor, TX-305 0.01%	500	12	2.40 ± 0.17^{b}
Seilor, TX-305 0.05%	500	15	3.00 ± 0.19^{b}
Seilor, TX-305 0.1%	500	22	4.40 ± 0.22^{c}
Seilor, TX-305 0.5%	500	23	4.60 ± 0.21^{c}

Note: statistically significant differences at $P < 0.05$ by resaults of ANOVA-analyze with Bonferroni amendment. Comparison in terms of each genotype.

The varieties NE 12443, Ronin, and Seilor demonstrated moderate responsiveness to TX-305 treatment. All three exhibited a gradual increase in mutation rates with rising concentrations. At the highest concentration, Ronin reached a mutation rate of 4.40%, NE 12443 4.80%, and Seilor 4.60% placing Seilor in an intermediate position between the other two in terms of sensitivity. The level of changeability remained consistent across these genotypes.

Farrell emerged as the most responsive genotype to TX-305, while Ronin showed the lowest sensitivity. These findings highlight the critical role of genotype–mutagen interactions in optimizing mutation breeding strategies. In nearly all cases, the differences in mutation rates across concentrations and genotypes were statistically significant, with only three exceptions.There are no statistic difference between Farrell, TX-305 0.05% and Farrell, TX-305 0.1% ($F = 3.12$; $F_{0.05} = 4.10$; $P = 0.08$), Ronin, TX-305 0.1% and Ronin, TX-305 0.5% ($F = 3.28$; $F_{0.05} = 4.10$; $P = 0.07$), Seilor , TX-305 0.1% and Seilor , TX-305 0.5% ($F = 3.29$; $F_{0.05} = 4.10$; $P = 0.07$), regarding Tukey HSD test, which show sometimes similar action for TX-305 0.1% and TX-305 0.5% ($F = 4.09$; $F_{0.05} = 4.10$; $P = 0.05$).

For this group of varieties epimutagen action was statistically significant for the variance in the change in mutagen concentration ($F = 78.17$; $F_{0.05} = 3.48$; $P = 2.34*10^{-5}$) and for genotype-mutagen interaction ($F = 7.17$ $F_{0.05} = 2.72$; $P = 0.02$), not for varieties ($F = 3.07$; $F_{0.05} = 3.86$; $P = 0.07$).

To further confirm the discriminating potential of overall epimutation rate as an indicator, a cluster analysis was conducted (Figure 1). The analysis indicated that the tested winter wheat varieties could be broadly classified into two distinct groups according to their response to TX-305. The first group, consisting of Farrell and NE 12443, is characterized by higher variability and a greater rate of general mutational changes. The second group includes Ronin and Seilor, which display moderate and relatively uniform variability within the group. Notably, the action of TX-305 on individual genotypes differs markedly from that of previously studied substances with lower mutagenic activity.

Furthermore, the frequency of epimutations was shown to increase progressively with rising concentrations of TX-305, reaching its peak for the TX-305 0.5%.

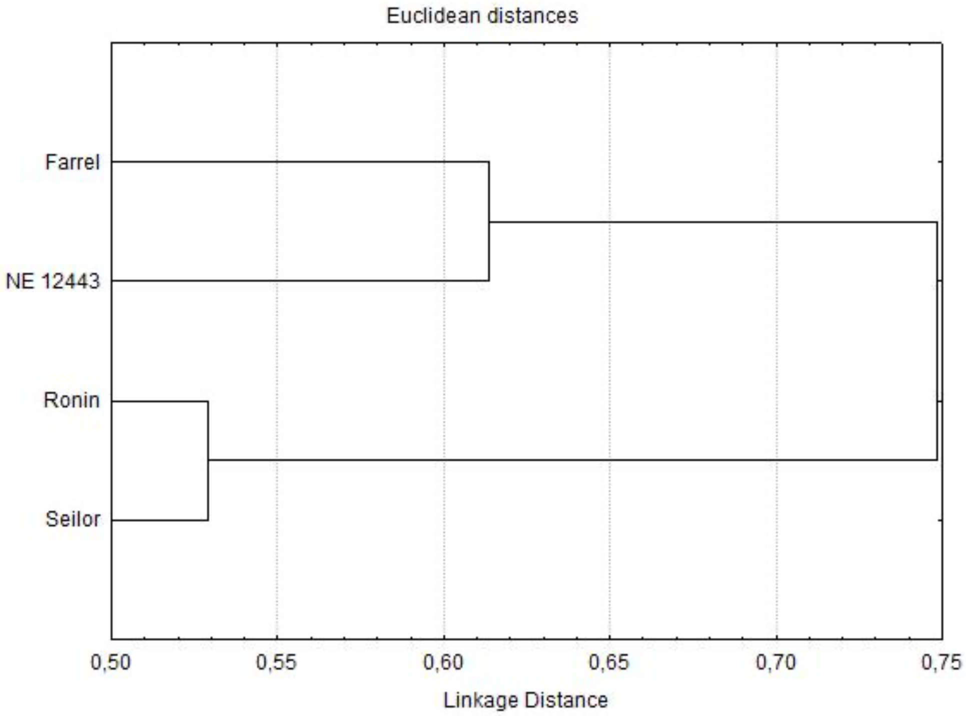

Figure 1. Groups after cluster analysis by epimutation rate.

In addition to overall variability, the number of traits affected by mutagenic changes is an equally important indicator. An increase in mutation frequency does not necessarily translate into a proportional expansion of the diversity of mutant material available for selection; in some cases, it may even narrow the range of useful variation. To address this, the level of variability is determined as the ratio between the number of mutant cases and the number of traits that have undergone modification (Table 2).

Table 2. Parameters of variability level at M2 – M3 (x ± SD, n = 450-500)

Variety	Level of changeability	Changed traits
Farrell	0.02±0.01^a	3
Farrell, TX-305 0.01%	0.11±0.02^b	7
Farrell, TX-305 0.05%	0.45±0.04^c	14
Farrell, TX-305 0.1%	0.54±0.05^c	15
Farrell, TX-305 0.5%	0.82±0.08^d	16
NE 12443	0.01±0.01^a	2
NE 12443, TX-305 0.01%	0.20±0.02^b	10
NE 12443, TX-305 0.05%	0.33±0.03^c	11
NE 12443, TX-305 0.1%	0.61±0.05^d	16
NE 12443, TX-305 0.5%	0.72±0.06^d	15
Ronin	0.01±0.01^a	2
Ronin, TX-305 0.01%	0.36±0.03^b	13
Ronin, TX-305 0.05%	0.38±0.03^b	12
Ronin, TX-305 0.1%	0.63±0.05^c	15
Ronin, TX-305 0.5%	0.66±0.05^c	15
Seilor	0.01±0.01^a	2
Seilor , TX-305 0.01%	0.26±0.03^b	11
Seilor , TX-305 0.05%	0.36±0.04^c	12
Seilor , TX-305 0.1%	0.79±0.05^d	18
Seilor , TX-305 0.5%	0.78±0.05^d	17

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

The genotypes demonstrated the following maximum levels of changeability: Farrell (up to 0.82), NE 12443 (up to 0.72), Ronin (up to 0.66), and Seilor (up to 0.78). Notably, less number of changed traits exhibiting epimutations at the highest concentration was observed for Farrell. Statistical analysis confirmed this trend, with Farrell showing a significant effect ($F = 5.95$; $F_{0.05} = 4.10$; $P = 0.003$), and Seilor also reaching significance ($F = 4.14$; $F_{0.05} = 4.10$; $P = 0.05$).

Farrell and Seilor differ both from each other and from the other two varieties in terms of variability patterns. In Farrell, the high overall mutation rate contributed to its elevated level of changeability, despite the reduced number of traits affected at higher concentrations. In contrast, Seilor exhibited its high changeability primarily due to a broader spectrum of traits undergoing mutation, rather than the overall frequency of changes. Meanwhile, NE 12443 and Ronin displayed similar dynamics, with consistent but lower levels of changeability

Across all genotypes in both groups, the effect of TX-305 on changeability was statistically significant at all concentration levels. Analysis of variance confirmed a highly significant influence of epimutagen concentration ($F = 86.19$; $F_{0.05} = 3.10$; $P = 3.17 \times 10^{-6}$), genotype ($F = 3.67$; $F_{0.05} = 3.59$; $P = 0.05$) and variety–mutagen ($F = 4.43$; $F_{0.05} = 2.54$; $P = 0.01$). These results indicate that, for all cases, differences in changeability across TX-305 concentrations and genotypes were statistically significant.

The results with TX-305 did not vary significantly (Figure 2). Once again, all varieties treated with TX-305 were grouped into three distinct categories. The first group comprised the variety Farrell, which exhibited the highest overall variability across all concentrations of the epimutagen. The second group included the variety Seilor, which showed a lower general mutation rate but higher variability across individual traits. The third group consisted of Ronin and NE 12443, both of which demonstrated the lowest levels of variability under TX-305 treatment.

Cluster analysis based on the level of changeability revealed a different grouping pattern compared to the previous parameter (Figure 2). Unlike earlier findings with other mutagens, the results here suggest that the estimate based on changeability provides a more accurate reflection of mutagenic effects than the general mutation rate, as supported by statistical justification.

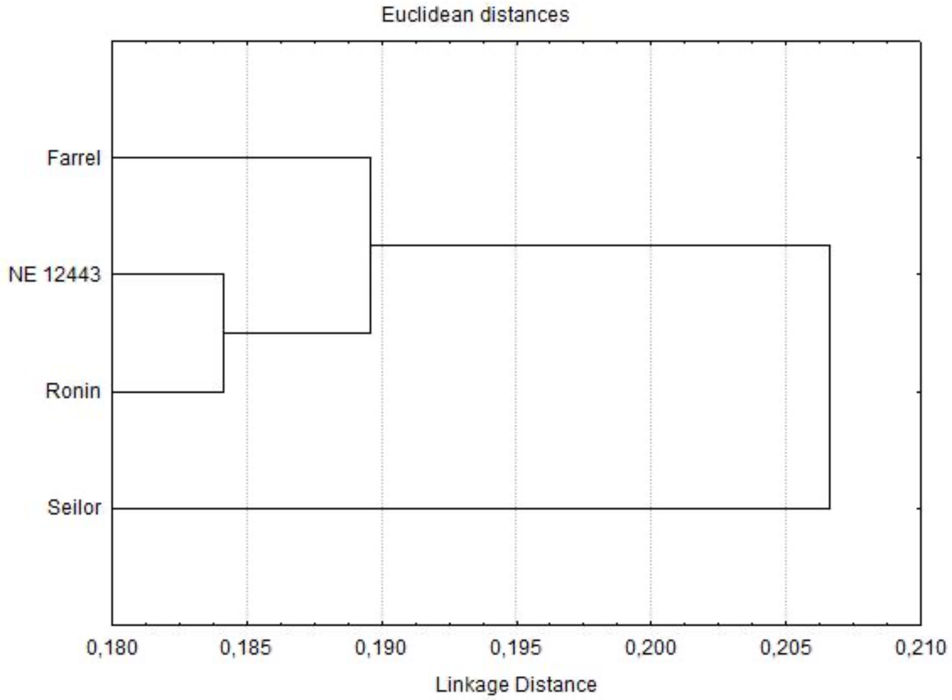

Figure 2. Groups after cluster analysis by level of changeability.

Mutational changes were categorized into six groups based on standard mutation breeding classifications. The first group includes traits related to plant structure, such as stem thickness, plant height variations, and the presence of a waxy bloom.

TX-305 did not significantly induce mutations affecting stem thickness or dwarf/semi-dwarf forms at lower concentrations. Only at the highest concentration (0.5%) was a single semi-dwarf case observed in the variety NE 12443. As an epimutagen, TX-305 was effective in inducing three types of changes within this group: forms with weak waxy bloom (up to 0.60 across all varieties), forms without waxy bloom (up to 0.60, genotype-dependent, less frequent in Farrell, absent in Seilor), and short-stemmed forms (up to 0.44). The highest induction was recorded for high-stem forms (up to 0.80%, consistently across all varieties).

TX-305 thus demonstrates a distinct epimutagenic effect on traits such as waxy bloom expression and plant height (short and tall forms), while showing limited effectiveness in inducing rarer structural mutations like increased stem thickness or dwarfism, except for a single thick-stem mutation at the highest concentration across all varieties.

The next epimutations group includes variations in sizes and shapes of grains, such as drum-shaped, small and big grains. Large grain mutations were observed at frequencies of up to 0.20%, with higher concentrations of TX-305 (0.1% and 0.5%) significantly increasing their occurrence across all varieties, except for Farrell, where lower concentrations were less effective. Barrel-shaped and small grain mutations appeared infrequently, indicating that TX-305 does not strongly induce these forms. The increased frequency of large grain mutants at higher concentrations suggests a dose-dependent response, highlighting TX-305’s selective epimutagenic effect on grain size.

The third group comprises spike structure variations, encompassing 15 distinct mutation types. A general trend was observed where mutation frequency increased with TX-305 concentration. However, exceptions included rare types such as large spikes and other anomalies, which did not follow this pattern. Long spike mutations were the most common, regularly occurring in all tested varieties at frequencies up to 0.20%, reinforcing TX-305’s efficacy in modifying key reproductive structures.

The fourth group involves physiological changes in plant growth and development, despite including only four traits, it showed high variability. Early maturity mutations occurred regularly (up to 0.67%) and were most frequent at the 0.1% and 0.5% TX-305 concentrations across all varieties. Late maturity mutations were less frequent, particularly in Ronin and Seilor, and primarily appeared at lower concentrations. Disease tolerance was not a dominant trait within this group and was observed only at 0.05% and 0.1% concentrations. Sterility was extremely rare, with no cases at the first three concentrations and only isolated cases at the highest (0.5%). These findings indicate that TX-305 is effective in inducing early and late maturity mutations with minimal impact on fertility, making it a favorable candidate for controlled breeding.

The fifth group comprises systemic mutations that cause extreme spike structure changes, often resembling wild wheat relatives. These mutations represent significant deviations from the cultivated phenotype and were exceedingly rare under TX-305 treatment. Occurrences were limited to occasional speltoid forms at the 0.5% concentration (and in one case, at 0.1%), excluding Ronin. Given their rarity and limited breeding value, such mutations are not considered relevant for genetic improvement in cultivated wheat.

The sixth group includes agronomical valuable mutations, primarily associated with high grain yield and enhanced tillering. These mutations were observed across most varieties, predominantly at TX-305 concentrations of 0.1% and 0.5%. Their frequency did not decline with increasing concentration, suggesting stability across treatment levels. These epimutations represent a common and desirable outcome of TX-305 treatment, supporting its utility in generating high-yielding lines for breeding programs.

It is essential to evaluate overall model variability, particularly for general indicators and the group of valuable mutations. This was achieved through discriminant analysis of selected variables (Table 3, Fig. 3). The model included the general mutation rate, the level of changeability, and mutation frequencies in the first, fourth, and sixth groups.

The data confirm that the applied epimutagen, TX-305, is highly effective in inducing both general variability and specific trait modifications. The range of affected traits is broad, encompassing nearly all standard mutation categories. TX-305 is particularly effective in inducing low-growing forms, which are essential for the development of intensive ecotypes and are especially beneficial for adaptation to shorter growing seasons.

Table 3. Classification by discriminants functions

Traits for model	Wilks Lambda λ	Partial λ	F_{remove} (4,14)	p-level
Changes rate	0.06	0.17	16.27	0.01
Level of changeability	0.06	0.17	16.72	0.01
First group	0.05	0.18	12.11	0.01
Second group	0.35	0.54	3.31	0.07
Third group	0.25	0.49	4.09	0.06
Fourth group	0.08	0.19	11.42	0.01
Fifth group	0.61	0.87	1.28	0.09
Sixth group	0.17	0.31	5.01	0.03

The inclusion of traits from the sixth mutation group further underscores the practical value of this agent by broadening its potential applications in breeding programs. Overall, TX-305 proves to be a highly efficient tool for targeted mutation breeding, particularly for the development of short-stemmed, early-maturing, large-grained, and high-yielding lines, making it a valuable asset in modern crop improvement strategies.

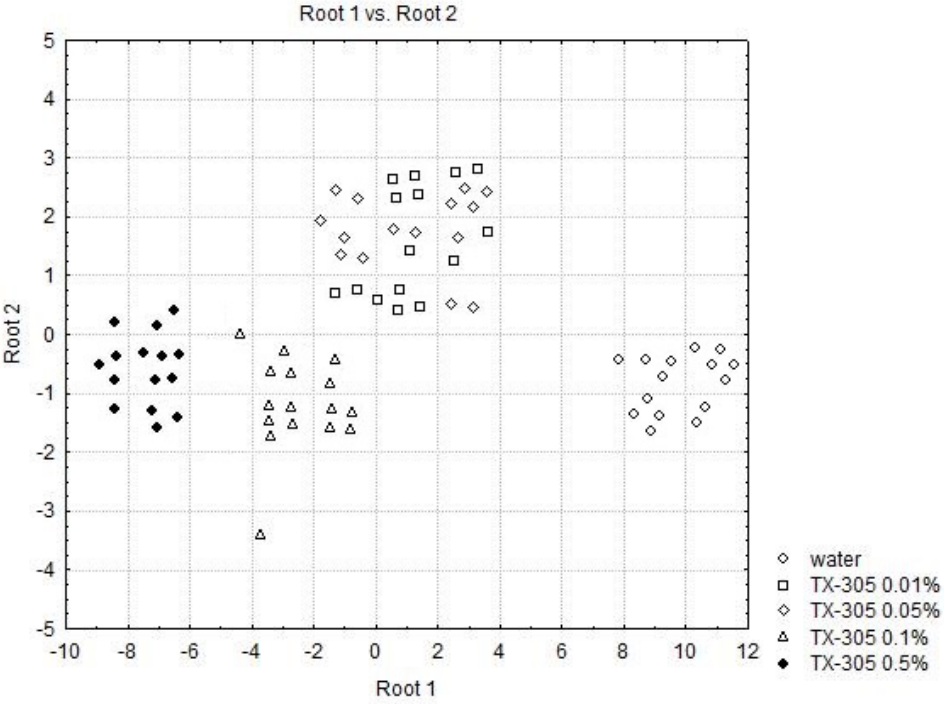

Figure 3. Results of discriminant analysis for model parameters.

The discriminant analysis (Figure 3) confirmed the distinct effects of different TX-305 concentrations, highlighting the concentration-dependent nature of mutagenic variability. The first and second concentrations showed mixed effects; however, their data clusters remained clearly separated from the water-treated control. The second and third concentrations exhibited partial overlap, whereas the first concentration stood distinctly apart. The fourth concentration (the highest TX-305 level) demonstrated a pronounced separation, indicating its strong mutagenic impact.

Among the genotypes (Table 4), Farrell exhibited the highest level of genotype-mutagenic interaction, making it the most responsive to TX-305 treatment. NE 12443 also showed effective mutation induction, particularly at higher concentrations.

Table 4. Classification matrics by canonical roots

Genotype	% of classification
Farrell	100.0
NE 12443	87.0
Ronin	62.5
Seilor	62.5
Total	74.5

In contrast, the other two varieties demonstrated comparatively lower responsiveness. These findings suggest that the third and fourth concentrations of TX-305 are the most effective overall, with Farrell emerging as a promising candidate for mutagenic breeding due to its heightened sensitivity to the epimutagen.

4. Discussion

The data suggest that this substance is best utilized as an epimutagenic factor for developing components to enhance existing varieties through recombinant breeding (Abdelhameed et al., 2024; Bora et al., 2024). It is less likely to generate lines suitable for direct commercialization as new varieties (Ergün et al., 2023).

This epimutagen is best suited for inducing reduced-height lines with elongated spikes and earliness forms, which are particularly valuable for avoiding the typical spring drought conditions (Afram et al., 2024). Although TX-305 shows promise for breeding disease-resistant forms, several other substances have likewise proven effective for this type of improvement (Nazarenko et al., 2016; Nazarenko et al., 2022)

In contrast to earlier reports (Animasaun and Oguntoye, 2024; Bora et al., 2024), the tested concentrations although approaching LD_{50} levels in the first generation did not cause a significant decline in activity (Bayhan et al., 2024; Jasmin et al., 2024). Furthermore, even at the higher concentrations, TX-305 markedly enhanced biodiversity (Rana et al., 2024). Therefore, the applied dose range can be considered generally suitable for generating genetically valuable forms when required (Mahanish and Kin, 2025).

The data suggest that higher concentrations of the mutagen (0.1%–0.5%) induce a broader spectrum of mutations, including both beneficial and adverse changes (Abdelhameed et al., 2024). These concentrations are more effective, with the 0.1% and 0.5% treatments showing the strongest effects, making them suitable for use regardless of genotype. The 0.5% concentration is particularly recommended for varieties with low initial variability.

The genotype-mutagenic interaction is strong, exhibiting significantly higher interaction with genotype than previous agents. This interaction is predominantly positive, suggesting effective induction of desired traits (Yanting et al., 2024).

The findings support targeted mutagenesis strategies, allowing genotype-specific exposure to optimized concentrations for improved breeding outcomes. However, the application of higher concentrations should be carefully regulated, particularly in genetically stable varieties, to prevent excessive undesirable mutations (Horshchar and Nazarenko, 2023).

Treated plants exhibited increased variation in plant height, tillering ability, and spikelet number. Enhanced drought and frost tolerance were observed in epimutagen-treated populations, suggesting a shift in gene expression linked to abiotic stress responses (Afram et al., 2024; Makebe et al., 2024). DNA methylation assays revealed specific loci with altered methylation patterns, indicating targeted epigenetic modifications induced by the epimutagen. Despite increased variability, some treated lines demonstrated higher yield stability, suggesting potential for breeding programs (Nazarenko et al., 2017; Horshchar and Nazarenko, 2024). The findings highlight the potential of epimutagens as tools for accelerating wheat adaptation to climate change. However, further studies are needed to evaluate the long-term heritability of the induced epigenetic modifications.

Conclusion

The epimutagen TX-305 exhibited a pronounced overall variability and proved highly effective in inducing novel forms with significant modifications in key agronomic traits of winter wheat. The varieties Farrell and NE 12443 exhibited particularly strong potential for epimutation induction when treated with TX-305 at concentrations of 0.1% and 0.5%. The agent's activity was primarily directed toward inducing changes in plant height, grain size, spike length, and maturity time, with a notable increase in early-ripening and high-yielding forms. Of particular interest is the emergence of short-stemmed, early-maturing lines with long spikes, traits highly desirable for breeding in drought-prone regions. This research provides new insights into the application of epimutagens in crop improvement. By inducing epigenetic variability, TX-305 offers a promising strategy for expanding the genetic potential of winter wheat without resorting to direct genetic modification. Future research should prioritize multi-generational studies to evaluate the stability and agronomic viability of the induced traits.

Ethical Statement

Ethical approval is not required for this study because the methods of the study does not require review by an ethics committee.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Artificial Intelligence Declaration

The authors declare that no generative artificial intelligence tools were used at any stage of the preparation of this manuscript, including the writing, editing, or refinement of the text, or the creation of any images, figures, graphics, tables, or related titles.

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Author Contributions

Mykola Nazarenko contributed to data collection, data analysis and interpretation, experimental execution and initial manuscript drafting. Oleh Okselenko assisted in data analysis and interpretation, participated in experiment. All authors read and approved the final version of the manuscript.

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