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Impact of ecogenetic factors on cytogenetic variability of winter wheat

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Abstract. The study focused on the mutagenic potentials of ethylmethanesulfonate (EMS) and sodium azide (SA), their abilities to induce chromosomal aberrations, interactions with various winter wheat genotypes, and the nature of genotype-mutagenic interactions. Additionally, their feasibility for future applications was examined, particularly regarding their predictive value at the cellular level in determining mutation-inducing capacity at the plant level. The cytogenetic effects of ethylmethanesulfonate (EMS) and sodium azide (SA) were tested on two winter wheat varieties, Vezha and Ihrysta. The seeds were treated with EMS at concentrations of 0.025%, 0.05%, and 0.1%, and SA at 0.01%, 0.025%, 0.05% and 0.1%. The cytogenetic activity was assessed through pollen sterility and the frequency and spectrum of chromosomal abnormalities in mid-phase cell mitosis. The results provided significant insights into the genotype-mutagenic interactions in these wheat varieties. The study found that the winter wheat variety Ihrysta exhibited a higher genotype-mutagenic specificity, making it a strong candidate for inducing genetic variability and producing mutant forms. The most effective mutagenic response was observed following the use of EMS and SA concentrations ranging 0.025% to 0.05%, optimizing mutation induction while minimizing the adverse effects. The study identified the variety Vezha as particularly responsive and promising for breeding programs targeting mutagenic variability. Moderate concentrations of EMS and SA were found to be the most effective, striking a balance between inducing beneficial genetic changes and minimizing adverse effects. The key indicators of genetic susceptibility to mutagens included pollen fertility, the overall frequency of chromosomal aberrations, and the number of induced fragments, while rare chromosomal rearrangements had limited analytical value. The induction patterns of EMS and SA were consistent with other chemical supermutagens, although the responses varied depending on the plant's genetic background. The findings will be integrated with further studies on hereditary changes in biochemical and physiological traits, providing a basis for refining mutagenic strategies and optimizing breeding programs.

Keywords: winter wheat; chromosomal abnormalities; ethylmethansulfonate; sodium azide; variety; cell; site-specific action; chemical mutagens.

Introduction

The development of stable, highly productive agrocenoses for grain crops relies on the integration of ecological and genetic variability. The ecological component addresses the adaptability of derived forms and communities to specific environments. It encompasses the manifestation of novel traits and properties within varying ecological conditions. The ecological-geographical approach is vital for selecting source material tailored to generate new forms suitable for specific ecological niches (Nazarenko et al., 2022). The genetic component defines the scope of variability, setting the limits for changes at both the level of individual genes and their associations. It plays a pivotal role in how new traits are expressed and stabilized from the pre- and post-mutation states. Genetic variability also involves the complex interaction between modified genes and the unaltered wild-type components of the original genotype (Nazarenko et al., 2019). The successful stabilization of newly introduced properties is critical, ensuring their consistency and functionality across generations (Fu et al., 2015). The interplay between ecological adaptability and genetic potential drives the creation of grain crop varieties that thrive under diverse environmental conditions while achieving high productivity. Balancing these components ensures sustainable agricultural systems, capable of responding to changing environmental pressures and advancing crop improvement. This integrated understanding underscores the necessity of combining ecological insights with genetic advancements to enhance agrocenosis productivity and resilience (Nazarenko et al., 2023).

The instability of modern wheat varieties stems from the superselectivity of their germplasm, which often contributes to their low adaptability. This issue is particularly significant in addressing the balance between adaptability and performance in different ecological and agricultural contexts. The highly selective breeding processes used to create modern varieties result in germplasm that excels in specific, controlled environments but lacks broad adaptability (Yuan et al., 2021).

These varieties often perform poorly outside of optimal conditions, making them vulnerable to environmental variability and stressors. Many local forms are characterized by their semi-intensive growth habits, tailored to thrive in specific regional conditions with high adaptability (Nazarenko, 2020). These forms are better suited to local ecological conditions, displaying resilience where modern varieties may falter. Despite their adaptability, local forms are often unsuitable for high-intensity agricultural technologies due to their genetically constrained potential for economically valuable traits (Ergün et al., 2023). Combining the adaptability of local forms with the high-performance potential of modern varieties could provide a solution. Utilizing genetic tools to expand the trait boundaries of local forms may enable their integration into more intensive agricultural systems without sacrificing adaptability.

Intensive ecotypes can be adjusted to thrive in specific local environments, using low doses and concentrations of mutagens to ensure a gradual transformation without disrupting the balance of traits significantly (Abdullah et al., 2018; Chernysky & Gumentyk, 2020). For landraces and local varieties, the focus shifts to intensification – improving specific economically or agronomically valuable traits while retaining the core genetic identity (Horshchar & Nazarenko, 2022a). The genetic changes induced by mutagenesis are inherently complex, often resulting in non-neutral shifts. While positive traits may emerge, there is a risk of deleterious or unwanted effects (Shabani et al., 2022). Utilizing a broad genetic base increases the likelihood of identifying favorable outcomes while minimizing risks. Testing on larger samples allows researchers to better identify and select for neutral-positive changes, ensuring success in experimental mutagenesis (Ergün et al., 2023). A carefully calibrated appro-

ach, considering both dose and genotype variability, remains critical. This method is validated by studies demonstrating its utility in developing new, resilient plant forms (Álvarez-Holguín et al., 2019; Yuan et al., 2021).

The second critical point in understanding the application of ecogenetic factors is the role of genetically determined resistance mechanisms to both individual mutagens and overall mutational pressure. These mechanisms are pivotal in determining the outcomes of mutagenic treatments. The use of chemical supermutagens has been shown to bypass initial resistance barriers effectively. These agents can override the intrinsic genetic mechanisms that typically shield DNA from mutations, allowing for higher mutation rates. Studies of Nazarenko et al. (2019) and Yuan et al. (2021) confirm that such agents are particularly effective in disrupting the primary defense mechanisms, enabling the induction of novel genetic variations. The site-specificity of chemical supermutagens introduces a unique set of challenges (Oprica et al., 2023). Precise interaction with DNA - unlike broader mutagens, site-specific agents target particular regions of DNA, which can lead to unpredictable outcomes due to the intricate interactions between the mutagen and the DNA sequence, localized mutations, which may not produce the desired variability across the genome. Different genotypes may exhibit widely varying responses to site-specific mutagens due to their distinct DNA architectures and repair mechanisms (Nazarenko, 2020).

The study highlights the importance of identifying specific genotypes within local genetic diversity that exhibit higher responsiveness to the site-specific action of individual chemical agents, particularly at moderate concentrations. Forms with enhanced sensitivity to site-specific actions of chemical mutagens, such as DMS, have been reliably identified in previous research (Oprica et al., 2023). These forms demonstrate a unique genomic architecture that responds effectively to correctly calibrated mutagen concentrations, enhancing the potential for desirable outcomes. Utilizing responsive genotypes in combination with optimal mutagen concentrations can result in significant improvements, increasing the yield of valuable forms by up to 80%. The effectiveness is attributed to the interplay between genome architecture and tailored mutagenic conditions (Nazarenko et al., 2023). Moderate concentrations of mutagens are particularly effective in inducing site-specific actions without excessive damage to the genome, making them suitable for breeding programs. The selected material should belong to high-yield, intensive varieties to maximize the potential benefits of mutagenic treatments. In less intensive local material, induced changes (micromutations) may not be sufficient to bridge the performance gap compared with Western European ecotypes (Jankowicz-Cieslak et al., 2022).

The main objective of this study was examining the variability and induced biodiversity in modern winter wheat genotypes under the influence of a mutagen with a high damaging capacity. The study focused on the following goals: characterizing the variability and biodiversity, genotypic effects, analyzing the impact of mutagen type and dosage on total rate of chromosomal abnormalities and levels of variability across general and specific traits; evaluating the variability induced by mutagens in the winter wheat genotypes and assessing the ability of mutagens to generate new forms exhibiting diverse traits across different trait groups, examining the influence of genotype on mutagenic exposure outcomes; identifying the extent to which genetic factors mediate responses to mutagenic agents. Also, we focused on determining the threshold concentrations of mutagen exposure, exploring potential plateaus in mutagenic effects, where increased dosage does not proportionally enhance variability, and exploring the relationship between genotype and mutagenic factors, specifically how source material responds to varying mutagenic conditions. This research provides insights into optimizing mutagenic treatments to enhance biodiversity and improve breeding outcomes for winter wheat. It emphasizes the role of genotype-mutagen interactions and aims to identify ideal conditions for generating promising mutant lines.

Materials and methods

The study on pollen viability and chromosomal rearrangements was conducted by the Laboratory of the Department of Breeding and Seed Production at the Dnipro State Agrarian Economic University. The research involved first-generation mutant populations grown at the experimental field station of the Science-Education Center, where pollen samples were systematically collected and analyzed.

The grain sample size was 1,000 grains per concentration. The mutagens used were ethylmethanesulfonate (EMS) in concentrations of 0.025%, 0.05%, and 0.1%; sodium azide (SA) in concentrations of 0.01%, 0.025%, 0.05%, and 0.1%. Both chemicals were sourced from Sigma-Aldrich, Germany. The control group was grains soaked in water without mutagenic treatment. The grains were exposed to the selected mutagen concentrations for 24 hours, following the established standard protocols specific to the cultivars (Spencer-Lopes et al., 2018). The mutagen concentrations were selected based on prior experimental data regarding the effectiveness of mutagenesis in cereals. These levels were chosen to optimize the induction of genetic variability while minimizing potential detrimental effects on the plant viability.

The experiment involved 18 treatment variants using two winter wheat varieties, Vezha and Ihrysta. Standard regional agricultural practices were applied to cultivate the first-generation mutant populations. The pollen viability was assessed during flowering by collecting an average of 25 samples per treatment from the ears without abnormalities and with well-developed yellow anthers. The pollen grains were stained with acetocarmine, and the fertility was determined based on staining intensity under light microscopy.

The cytogenetic analysis of the chemical mutagen activity was performed using light microscopy with a Micromed XS-3330 device (Micromed, Poltava, Ukraine) at 600x magnification. The microscope, equipped with a 5MP camera, facilitated enhanced observation and documentation. Mitotic cells from the primary root system of winter wheat seedlings were examined during late metaphase to anaphase stages, recording chromosomal abnormalities such as fragments, double fragments, chromatid and chromosomal bridges, micronuclei, and lagging chromosomes. Cells with complex rearrangements (two or more abnormalities) were counted separately.

Swollen seeds were germinated at 20–22 °C until the primary roots reached a maximum length of 1.1 cm. The root tips were excised and fixed for 24 hours in Clarke's fixative (1 part glacial acetic acid to 3 parts 96% medical alcohol). The fixed samples were stored in 70% ethanol under refrigeration until further analysis. At least 25 temporary pressure preparations were prepared for each treatment. Observations were conducted on up to 1,000 cells per concentration and control, with fewer cells analyzed at higher concentrations due to cytotoxic effects. The preparations were stained with acetocarmine for improved clarity during light microscopy. If the root tips were excessively rigid, they were softened by soaking in a 45% glacial acetic acid solution before staining. The protocols and methods were based on Spencer-Lopes et al. (2018), ensuring standardization and reproducibility. This method ensured robust and reliable preparation of samples for cytogenetic analysis, allowing the assessment of chromosomal rearrangements and other cytological parameters across treatments.

The statistical analyses were performed using Statistica 10.0 software (TIBCO, Palo Alto, USA). The ANOVA module was employed to evaluate the influence of experimental factors, particularly for genotype-mutagenic interactions. The significance level was $P < 0.05$. The results were visualized graphically to enhance interpretability. The Tukey HSD test was applied for post hoc pairwise comparisons, ensuring robust evaluation of significant differences among treatments. The key descriptive metrics included arithmetic mean (x) and standard deviation (SD), reported as $x \pm SD$. Data normality was assessed using the Shapiro–Wilk test (W-test). Standard program modules of discriminant analysis were utilized to identify model characteristics of the observed cytogenetic features and evaluate their statistical significance in differentiating treatments and genotypes. This comprehensive approach ensured accurate, statistically valid conclusions regarding genotype-mutagenic interactions and the effects of EMS and SA treatments on cytogenetic activity.

Results

The analysis of fertility in two mutant populations exposed to varying EMS concentrations revealed a linear relationship between increasing EMS concentrations and rising sterility levels, as indicated by a strong positive correlation ($r = 0.82$) (Table 1). The fertility decreased significantly with increasing EMS concentrations ($F = 125.14$; $F_{0.05} = 2.02$; $P = 7.40*10^{-6}$). The highest EMS concentration (0.1%) did not result in an LD50 level (lethal dose for 50% of the material), with the viability maintained at 60–70%. Lower concentrations (0.025% and 0.05%) preserved higher viability levels (90–70%), aligning with the international classification of optimal mutagen concentrations. The varieties did not significantly differ in sterility levels ($F = 1.01$; $F_{0.05} = 2.34$; $P = 0.08$). However, the variability due to EMS concentration was highly significant, as confirmed by a post-hoc Tukey test. The factor caused a significant decrease in

viability, although the highest EMS concentration (0.1%) did not reach LD_{50} levels and remained relatively acceptable at 60–70%. This concentration is borderline optimal according to international standards (up to 70%). The mutagen is highly harmful, with the first concentration already causing a statistically significant drop in fertility. However, the first and second concentrations are considered low, maintaining 90–70% of the trait value. The first concentration of EMS caused a statistically significant reduction in fertility but remained within the acceptable viability levels. Concentrations of 0.025% and 0.05% provided sufficient plant material for further studies on hereditary variability in the second and third generations. The highest concentration (0.1%) approached the semi-lethal levels for most wheat genotypes, underscoring its potentially harmful nature. A significant interaction was observed between genotype and mutagen ($F = 4.95$; $F_{0.05} = 4.60$; $P = 0.04$), highlighting the variability in survival rates among genotypes under EMS treatment.

Table 1
Influence of EMS action on pollen sterility trait of first-generation spikes ($x \pm SD$, $n = 25$)

Variety	Control	EMS 0.025%	EMS 0.05%	EMS 0.10%
Vezha	98.17 ± 0.82^{a}	88.92 ± 1.00^{b}	78.97 ± 1.01^{c}	68.90 ± 1.11^{d}
Ihrysta	97.92 ± 0.80^{a}	88.71 ± 0.99^{b}	77.90 ± 0.97^{c}	68.17 ± 1.24^{d}

Note: $^{a, b, c, d}$ indicate significant differences at $P < 0.05$ according to the Tukey HSD test with Bonferroni correction, analyzing each variety separately across different mutagenic treatments.

The analysis of fertility in the two mutant populations under different sodium azide concentrations showed a linear increase in the sterility with concentration ($r = 0.90$). Both varieties exhibited similar trait expression ($F = 2.00$; $F_{0.05} = 2.21$; $P = 0.07$), while the concentration variability was statistically significant ($F = 117.11$; $F_{0.05} = 2.00$; $P = 3.56*10^{-6}$). The Tukey's test confirmed the differences between 0.05% and 0.1% SA. The 0.1% concentration approached the LD_{50} (63%) and is classified as high to semi-lethal (50–70%). No significant genotype-mutagen interaction was found ($F = 3.23$; $F_{0.05} = 3.90$; $P = 0.07$). The mutagen is highly harmful, as even the lowest concentration reduced fertility by approximately 10%, although the first two concentrations still maintained relatively high viability (90–70%). The third concentration bordered low and optimal viability, providing sufficient plant material for further studies on hereditary variability in subsequent generations.

Table 2
Influence of SA action on pollen sterility trait of first-generation spikes ($x \pm SD$, $n = 25$)

Variety	Control	SA 0.01%	SA 0.025%	SA 0.05%	SA 0.10%
Vezha	98.17 ± 0.82^{a}	88.45 ± 1.01^{b}	80.89 ± 0.91^{c}	71.76 ± 1.19^{d}	64.77 ± 1.37^{e}
Ihrysta	97.92 ± 0.80^{a}	88.37 ± 1.01^{b}	80.49 ± 0.97^{c}	71.10 ± 1.18^{d}	63.65 ± 1.33^{e}

Note: see Table 1.

Within the same concentration range, SA exhibited relatively stronger mutagenic effects compared with EMS. Despite its higher mutagenicity, SA did not cause critical consequences, making it a viable mutagenic agent for controlled studies. The studied wheat varieties exhibited a similar level of tolerance to SA-induced mutagenesis. This suggests that the genetic background plays a role in response to SA but does not create drastic variations in tolerance among genotypes. In the case of EMS, the genotype-mutagen interaction was statistically significant, meaning that different wheat varieties responded differently to exposure to EMS. However, for SA, this interaction was not significant, implying that SA's effects are more uniform across different genotypes.

The study of chromosomal rearrangement frequency under EMS exposure (Table 3) revealed distinct effects of individual factors and their concentrations, establishing clear thresholds for chromosomal abnormalities. The factor analysis confirmed a significant effect of genotype, with reliable statistical differences observed between varieties ($F = 6.17$; $F_{0.05} = 2.35$; $P = 0.01$). An increase in EMS concentration consistently correlated with higher frequencies of chromosomal rearrangements ($F = 123.12$; $F_{0.05} = 2.71$; $P = 3.17 \times 10^{-11}$), demonstrating a concentration-dependent impact. The interaction was not statistically significant ($F = 2.79$; $F_{0.05} = 3.15$; $P = 0.07$), suggesting that the mutagen's effects were relatively uniform across genotypes. The control samples exhibited a 0.4% level of chromosomal rearrangements. The rearrangement frequency in Vezha and Ihrysta accounted for 6.47% and 7.86% at 0.025% EMS and 12.40% and 13.25%, respectively, at 0.05% EMS, highlighting a significant difference between the varieties. At 0.1% EMS, the frequencies rose to 17.08% in Vezha and 18.60% in Ihrysta, nearing a harmfulness threshold, as indicated by increased cell mortality. Treatment with 0.1% EMS led to significant cell death, suggesting the concentration approaches a toxicity threshold. The variety Ihrysta was slightly more vulnerable, but the difference was not substantial enough to significantly impact the plant viability in future generations. Despite moderate differences, both varieties remained viable for further mutagenesis experiments, with careful selection of concentration thresholds to balance genetic variability and plant survival.

Table 3
General rates of chromosomal abnormalities in properly dividing mitotic cells of the root tips under EMS action ($x \pm SD$, $n = 800–1000$)

Variety	Variant	Mitosis, number	Chromosomal aberrations	
			number	%
Vezha	water	1,004	4	0.40 ± 0.10^{a}
Vezha	EMS 0.025%	1,005	65	6.47 ± 0.20^{b}
Vezha	EMS 0.05%	1,008	125	12.40 ± 0.24^{c}
Vezha	EMS 0.10%	972	166	17.08 ± 0.32^{d}
Ihrysta	water	1,001	4	0.40 ± 0.11^{a}
Ihrysta	EMS 0.025%	1,005	79	7.86 ± 0.21^{b}
Ihrysta	EMS 0.05%	1,004	133	13.25 ± 0.29^{c}
Ihrysta	EMS 0.10%	903	168	18.60 ± 0.37^{d}

Note: see Table 1.

The study of chromosomal rearrangement frequency under SA exposure (Table 4) revealed distinct effects of individual factors and concentrations, as well as thresholds for chromosomal abnormalities. The factor analysis confirmed that the genotype factor had a significant impact, with statistically reliable differences between the varieties ($F = 3.17$; $F_{0.05} = 2.35$; $P = 0.04$). The SA 0.10% concentration led to significant cell death, suggesting that it approached a harmfulness threshold. In Vezha, this concentration was associated with a decrease in chromosomal abnormalities. An increase in SA concentration correlated with a higher frequency of chromosomal rearrangements, with statistically significant differences within each variety ($F = 148.99$; $F_{0.05} = 2.71$; $P = 2.15\times10^{-15}$). This interaction was found to be statistically significant ($F = 4.09$; $F_{0.05} = 3.15$; $P = 0.03$), emphasizing the varying responses of different genotypes to SA treatment.

Table 4
General rates of chromosomal abnormalities in properly dividing mitotic cells of root tips under SA action ($x \pm SD$, $n = 800 – 1000$)

Variety	Variant	Mitosis, number	Chromosomal aberrations	
			number	%
Vezha	water	1004	4	0.40 ± 0.10^{a}
Vezha	SA 0.01%	1009	52	5.15 ± 0.11^{b}
Vezha	SA 0.025%	1006	88	8.75 ± 0.22^{c}
Vezha	SA 0.05%	1001	135	13.49 ± 0.30^{d}
Vezha	SA 0.10%	946	119	12.58 ± 0.24^{e}
Ihrysta	water	1001	4	0.40 ± 0.12^{a}
Ihrysta	SA 0.01%	1003	73	7.28 ± 0.15^{b}
Ihrysta	SA 0.025%	1007	109	10.82 ± 0.20^{c}
Ihrysta	SA 0.05%	1002	150	14.97 ± 0.32^{d}
Ihrysta	SA 0.10%	921	150	16.29 ± 0.26^{e}

Note: see Table 1.

The control samples displayed a 0.4% level of chromosomal rearrangements. The frequency of chromosomal rearrangements in Vezha and Ihrysta measured 5.15% and 7.28% at 0.001%; 8.75% and 10.82% at 0.025%; and 13.49% and 14.97%, respectively, at 0.05% concentration. Thus, significant differences were observed between the varieties. Following the treatment with 0.10% concentration, the parameter equaled 12.58% in Vezha and 16.29% in Ihrysta, indicating a higher level of cytogenetic activity, but also approaching a harmfulness threshold, as evidenced by increased cell mortality. These findings indicate that higher SA concentrations lead to increased cytogenetic activity, though 0.10% SA approaches a harmful threshold. The variety Ihrysta showed higher vulnerability, suggesting a more substantial impact on its viability in subsequent generations.

The observed differences in the effects of sodium azide (SA) and ethylmethanesulfonate (EMS) were primarily reflected in the varied responses of the two winter wheat varieties. For Vezha, SA exhibited a

stronger damaging effect than EMS, indicating a higher susceptibility of this variety to SA. This suggests that genotype-mutagen interactions may amplify the impact of SA on Vezha. On Ihrysta, both mutagens (SA and EMS) demonstrated similar levels of impact, with no statistically significant differences in their cytogenetic effects or observed damage. Sodium azide exerted a stronger overall effect on both varieties, reinforcing its potency as a mutagen.

The study of chromosomal changes induced by the mutagens provided detailed insights into the types and frequencies of rearrangements observed in mitotic cells. The following chromosomal aberrations were identified: fragments and double fragments – the most common and quantifiable markers of chromosomal damage; bridges, dbserved in various forms, indicating improper chromosomal segregation during mitosis; micronuclei were indicated lagging chromosomal material that failed to integrate into daughter nuclei; lagging chromosomes, which highlighted errors in chromosomal movement during cell division. Complex changes were observed in cells exhibiting two or more chromosomal rearrangements, which were categorized separately due to their higher degree of cytogenetic instability (Tables 5 and 6). To further understand the mutagenic mechanisms, the ratio of fragments to bridges was calculated, providing insights into how different mutagens influence chromosomal integrity.

The number of fragments and double fragments in the control group was minimal but consistently present, serving as a reference point for assessing mutagenic effects. Even the lowest EMS concentration (0.025%) caused a clear and significant rise in chromosomal fragments, suggesting early mutagenic effect. Following the treatment with EMS 0.025%, the fragment count increased in both the varieties, measuring 40 in Vezha and 50 in Ihrysta. The number of fragments rose further after exposure to EMS 0.05%, accounting for 70 in Vezha and 87 in Ihrysta, demonstrating a stronger mutagenic effect. At EMS 0.10%, the fragment numbers plateaued, equaling 84 in Vezha and 90 in Ihrysta. This suggests a threshold effect, where further increases in concentration do not proportionally increase fragment induction. The Ihrysta variety consistently exhibited a higher susceptibility to EMS-induced fragmentation. At lower concentrations, the differences between varieties were substantial. At 0.10% EMS, the varietal differences were negligible, likely due to reaching a mutagenic saturation point. Ethylmethanesulfonate induced chromosomal fragmentation in a dose-dependent manner, with significant varietal differences at lower concentrations. At 0.10% EMS, a threshold is reached, where further increases in concentration do not significantly increase fragment numbers. Ihrysta was observed to be more susceptible than Vezha at lower EMS doses, but this difference disappeared at higher concentrations.

Table 5

Spectra of chromosomal abnormalities in properly dividing mitotic cells of the root tips, EMS action (x ± SD, n = 900–1000)

Variety	Variant	fragments (single + double)		bridges (chromosomal + chromatid)		fragments / bridges	other (micronucleus, lagging chromosomes)		double and more	
		n	%	n	%		n	%	n	%
Vezha	water	3.0 ± 0.2^{a}	75.0	1.0 ± 0.2^{a}	25.0	3.0	0.0 ± 0.0^{a}	0.0	0.0 ± 0.0^{a}	0.0
Vezha	EMS 0.025%	40.0 ± 0.6^{b}	61.5	17.0 ± 1.1^{b}	26.2	2.4	8.0 ± 1.0^{b}	12.3	8.0 ± 1.0^{b}	12.3
Vezha	EMS 0.05%	70.0 ± 1.2^{c}	56.0	42.0 ± 2.2^{c}	33.6	1.7	13.0 ± 1.2^{c}	10.4	20.0 ± 1.1^{c}	16.0
Vezha	EMS 0.10%	84.0 ± 1.4^{d}	50.6	65.0 ± 2.5^{d}	39.2	1.3	17.0 ± 1.4^{e}	10.2	32.0 ± 1.5^{d}	19.3
Ihrysta	water	3.0 ± 0.2^{a}	75.0	1.0 ± 0.3^{a}	25.0	3.0	0.0 ± 0.0^{a}	0.0	0.0 ± 0.0^{a}	0.0
Ihrysta	EMS 0.025%	50.0 ± 1.3^{b}	63.3	22.0 ± 0.9^{b}	27.9	2.3	7.0 ± 1.0^{b}	8.9	11.0 ± 0.8^{b}	13.9
Ihrysta	EMS 0.05%	87.0 ± 1.9^{c}	65.4	34.0 ± 1.1^{c}	25.6	2.6	12.0 ± 1.1^{c}	9.0	14.0 ± 1.2^{b}	10.5
Ihrysta	EMS 0.10%	90.0 ± 1.9^{c}	53.6	56.0 ± 1.3^{d}	33.3	1.6	22.0 ± 1.6^{d}	13.1	26.0 ± 1.8^{c}	15.5

Note: see Table 1.

Each EMS concentration showed a significant increase in fragment numbers compared with the control group. Successive concentrations also exhibited significant differences, except for 0.10% EMS, where a plateau effect was observed. Ihrysta exhibited significantly higher fragment induction at lower EMS concentrations, indicating greater sensitivity to mutagenic stress ($F = 5.34$; $F_{0.05} = 2.48$; $P = 0.01$). At 0.10% EMS, the differences between varieties diminished, suggesting a saturation point in mutagenic impact. Lower concentrations (0.025% and 0.05%) induced substantial chromosomal damage. Ethylmethanesulfonate in 0.1% concentration did not significantly increase fragment numbers, indicating that the mutagenic effect reached a threshold. The significant increase in fragments and double fragments reinforces the strong mutagenic potential of EMS.

Upon treatment with SA, the number of fragments increased in both Vezha and Ihrysta, measuring 28 and 41 at 0.01% (notable increase compared with the control group); 44 and 59 (a sharp increase) at 0.025%; and 71 and 75, respectively, at 0.05% concentration. At 0.10% concentration, SA caused the variability of 62 in Vezha and 82 in Ihrysta (a slight decline compared with the previous concentration in Vezha). Fragment numbers were significantly higher at each concentration compared with the control. The differences between successive concentrations were also statistically significant, except at 0.1% SA, when a decline was observed in Vezha. Ihrysta exhibited the highest fragment induction across all the concentrations, suggesting greater susceptibility to SA. Sodium azide induced chromosomal fragmentation in a dose-dependent manner, with a peak effect at 0.05% SA. A slight decline in fragments after exposure to 0.1% SA suggests a threshold beyond which cytotoxic effects may overshadow mutagenic induction. Ihrysta was the most susceptible variety, highlighting the role of genetic background in mutagenic response.

Sodium azide exerted high effectiveness in inducing chromosomal fragments, with concentrations up to 0.05% yielding the most pronounced effects. The decline in fragment induction caused by 0.1% SA suggests a potential threshold where cytotoxic effects begin to outweigh mutagenic induction, leading to increased cell mortality. The variation in response between Vezha and Ihrysta highlights the importance of genetic background in determining mutagenic efficiency. This suggests that different genotypes respond differently to the same concentration of SA. The consistent statistical significance of the observed differences confirms that fragment induction is a reliable metric for evaluating mutagenic activity. Sodium azide exhibited a strong mutagenic effect, with 0.05% being the optimal concentration for fragment induction in the varieties. The differences observed across genotypes and concentrations emphasize the need for customized mutagenesis strategies to maximize genetic variability while minimizing cytotoxicity.

The analysis of fragment induction provides valuable insights into the effects of genotype, mutagen concentration, and their interaction. The effect of genotype on fragment induction was statistically significant ($F = 8.17$; $F_{0.05} = 2.35$; $P = 0.007$). This indicates that the inherent genetic makeup of the varieties (Vezha and Ihrysta) significantly influences chromosomal fragmentation under mutagenic stress. Increasing mutagen concentration had a highly significant effect on the total number of fragments observed ($F = 112.01$; $F_{0.05} = 2.71$; $P = 1.35 \times 10^{-12}$). This highlights a clear dose-dependent relationship, with higher concentrations leading to greater chromosomal fragmentation across all genotypes. The interaction between genotype and mutagen concentration was also highly significant ($F = 5.54$; $F_{0.05} = 4.87$; $P = 0.03$). This demonstrates that the response to increasing concentrations varied significantly between the two genotypes, underscoring the importance of genotype-specific reactions in mutagenesis studies. The genetic background plays a critical role in determining the sensitivity and response to mutagenic factors, as seen in the differences between Vezha and Ihrysta. The consistent increase in fragments with higher concentrations reaffirms the dose-dependent mutagenic effects of both mutagens (excluding last critical concentrations). The interaction term highlights that fragment induction is not uniform across genotypes, pointing to complex interactions that depend on both the genetic material and mutagen concentration.

Chromatid and chromosomal bridge formation increases with EMS concentration. Both genotypes exhibited a minimal but consistent number of chromatid and chromosomal bridges. This establishes a baseline for evaluating EMS-induced changes. Following treatment with EMS, the bridge numbers significantly increased in both Vezha and Ihrysta, measuring 17 and 22 at 0.025%, 34 and 42 at 0.05%, and 56 and 65, respectively, at 0.10% concentration. Ihrysta exhibited greater bridge induction

following treatment with lower EMS doses, while Vezha responded to higher concentrations. Ethylmethanesulfonate in 0.10% concentration led to the most pronounced increase, suggesting a threshold for mutagenic stress.

These findings indicate a clear dose-dependent increase in chromatid and chromosomal bridges caused by EMS treatment, and also highlight differences in genotypic responses – demonstrating that while both varieties showed increased chromosomal damage with higher EMS concentrations, the extent and pattern of response between Vezha and Ihrysta varied ($F = 5.44$; $F_{0.05} = 2.48$; $P = 0.02$). At lower EMS concentrations (0.025%), Ihrysta exhibited higher bridge induction than Vezha. At higher EMS concentrations (0.10%), Vezha showed a stronger response, indicating differential sensitivity to mutagenic stress.

The higher susceptibility of Vezha to bridge formation suggests that it is more prone to chromosomal missegregation and structural damage under EMS treatment. This increased bridge induction may indicate that Vezha's chromosomal architecture is more vulnerable to improper segregation events, potentially leading to greater genomic instability. By contrast, the relatively lower bridge induction in Ihrysta may point to differences in chromosomal structure, stability, or DNA repair mechanisms between the two varieties. Ihrysta's ability to maintain a lower level of chromosomal bridges despite EMS exposure suggests it may possess more efficient pathways of response to DNA damage or structural resistance to bridge formation. These differences reinforce the importance of genotype-specific evaluations when assessing mutagenic effects and highlight potential variations in genomic resilience and repair efficiency between different wheat varieties.

The analysis of chromatid and chromosomal bridges induction under sodium azide (SA) revealed the key dose-dependent trends and genotypic variability in response to mutagenic exposure. The number of bridges in Vezha and Ihrysta measured 17 and 22 at 0.01% (significant increase compared with the control), 30 and 50 at 0.025% (sharp rise), and 46 and 50, respectively, at 0.05% concentration (the highest induction rate for Vezha). Sodium azide in 0.10% concentration caused a decline in bridge induction, with values accounting for 40 in Vezha and 46 in Ihrysta, suggesting a possible cytotoxic threshold. The plateau and subsequent decline at SA 0.10% suggest that beyond this concentration, SA's mutagenic efficiency is reduced, potentially due to increased cytotoxic effects or cell death limiting further chromosomal rearrangements. Ihrysta consistently exhibited higher bridge numbers across all concentrations, indicating a greater susceptibility to chromosomal missegregation. Vezha displayed lower bridge induction at each concentration and exhibited a milder decline at SA 0.10%, suggesting better tolerance at higher concentrations.

Vezha appears to be better suited for experiments requiring high mutagenic variability due to its stability and lower susceptibility to lethal chromosomal damage. Ihrysta, with its higher bridge induction, may be more advantageous for maintaining plant viability while still inducing genetic variability, making it a viable candidate for targeted mutagenesis approaches. These findings highlight the importance of genotype-specific responses in mutagenic studies and reinforce the need for tailored concentration protocols when utilizing SA-induced mutagenesis in plant breeding programs. The percentage of total aberrations and bridge induction analysis reveals consistent trends and highlights the interplay between mutagen concentration and genotype variability in winter wheat. Genotype factor associated with bridge induction statistically significant ($F = 4.91$; $F_{0.05} = 2.35$; $P = 0.02$), indicating some variability in the genotypes, though less pronounced than other types of chromosomal aberrations. The effect of mutagen concentration was also highly significant ($F = 143.19$; $F_{0.05} = 2.71$; $P = 4.17*10^{-10}$), confirming a strong dose-dependent response in bridge induction. We observed notable genotype × mutagen interaction ($F = 4.99$; $F_{0.05} = 1.87$; $P = 0.008$), suggesting that while genotypic differences in bridge formation are smaller, specific genotype-mutagen concentration combinations significantly influence the results. Supermutagens (SA and EMS) were most effective at moderate concentrations, subject to which the percentage of total aberrations increased consistently up to a threshold.

Higher mutagen concentrations reduced the site specificity beyond this threshold, mutagens may lose specificity, affecting broader genomic regions and leading to an overall increase in total aberrations. The genetic background plays a key role in determining the response to mutagenic factors, with notable differences between Vezha and Ihrysta in bridge induction levels. The bridge induction levels were lower compared with other aberration types. This suggests that bridge formation is a less frequent but still critical marker of chromosomal instability. Moderate mutagen concentrations were optimal for inducing targeted genetic variability without excessive genomic damage. Genotypic selection is crucial when designing experiments related to mutagenesis, as some varieties exhibit lower chromosomal stability under mutagenic stress. Further research on mutagen specificity at different concentrations may help optimize efficiency of mutation breeding while minimizing unwanted cytotoxic effects.

The wheat varieties exhibited an initial increase in chromosomal fragments, followed by a slight decline when subject to higher mutagen concentrations. This pattern suggests that certain genomic loci are more susceptible to mutagenic effects, but their response varies depending on the concentration. In Vezha, the fragment-to-bridge ratio remained stable across all SA concentrations. This suggests that Vezha's chromosomal architecture is less prone to site-specific variability under mutagenic stress. Vezha's lower susceptibility to SA may indicate a need for higher mutagenic doses or alternative mutagenic strategies to induce similar levels of genetic variability. The dominance of fragments over bridges in most varieties aligns with the mechanism of SA and EMS, which primarily induce single-strand breaks or localized chromosomal disruptions. The observed site-specific variability in most varieties can be strategically utilized for targeted genetic modifications, enhancing breeding efficiency.

We observed minimal or zero occurrences in the control group, establishing a reliable baseline. Dose-dependent increase in aberrations following treatment with EMS in both Ihrysta and Vezha, measuring 7 and 8 at 0.025% and 12 and 13, respectively at 0.05% concentration. The highest numbers of micronuclei and lagging chromosomes were observed after exposure to 0.1% concentration, specifically 17 in Vezha and 22 in Ihrysta. Higher EMS concentrations increased chromosomal abnormalities. Ihrysta exhibited a greater increase than Vezha, indicating higher sensitivity. The presence of micronuclei and lagging chromosomes confirms EMS mutagenicity. Optimal concentration EMS (0.05%) provided significant genetic variability while avoiding excessive cytotoxicity. Ethylmethanesulfonate induced a predictable increase in micronuclei and lagging chromosomes. Moderate EMS concentrations (0.05%) struck the best balance between mutagenic efficiency and cytotoxicity. The genotypic differences suggest that sensitivity to EMS between Vezha and Ihrysta varied.

Sodium azide exerted notable increases in abnormalities in Vezha and Ihrysta, measuring 7 and 10 at 0.01% (notable increase compared with the control), 14 and 15 at 0.025% (rise in induction – as compared with 0.01%), and 18 and 25, respectively, at 0.05% concentration. Following treatment with SA 0.1%, the number of abnormalities declined (compared with 0.05%), measuring 17 in Vezha and 22 in Ihrysta. This suggests a threshold effect where further increases in concentration lead to reduced effectiveness due to potential cytotoxicity. The variety Ihrysta had higher susceptibility. Ihrysta exhibited higher levels of chromosomal abnormalities at each SA concentration, suggesting greater mutagenic susceptibility than Vezha. The greatest number of abnormalities was seen at SA 0.05%. Vezha was observed to have lower tolerance subject to high concentration. The decline in abnormalities at SA 0.1% was more pronounced in Vezha than in Ihrysta. This suggests that Vezha has a lower mutagenic tolerance, possibly indicating genotype-specific thresholds for mutagenic response. Compared with SA 0.05%, SA 0.1% exhibited a decline in the number of abnormalities which may be attributed to cytotoxic effects or saturation of the mutagenic process at this concentration.

Genotype factor was not significant ($F = 2.33$; $F_{0.05} = 2.35$; $P = 0.06$), indicating that the induction of rare aberrations was relatively consistent in both the varieties. The effect of concentration was highly significant ($F = 52.17$; $F_{0.05} = 7.62$; $P = 3.12\times10^{-7}$), confirming a strong dose-dependent relationship. The genotype and mutagen interaction was not significant ($F = 1.19$; $F_{0.05} = 1.87$; $P = 0.09$), suggesting that genotypic responses to EMS were generally uniform. The proportion of rare aberrations increased consistently with higher EMS concentrations, peaking at a critical concentration, beyond which cytotoxic effects began to limit further induction. While not a primary factor, the interaction with mutagen concentration suggests subtle genotype-specific differences. A slight decline in rare aberrations at the highest concentration suggests that excessive cytotoxicity may counteract mutagenic efficiency. Ethylmethanesulfonate-induced rare aberrations followed a notable dose-dependent trend, with the highest efficiency observed before reaching cytotoxic limits. The genotypic differences were minor, but concentration-dependent variations may still exist. Optimal EMS concentration selection is crucial, balancing mutagenic efficiency and cytotoxicity.

Table 6

Spectra of chromosomal abnormalities in properly dividing mitotic cells of the root tips, SA action (x ± SD, n = 900 – 1000)

Variety	Variant	fragments (single + double)		bridges (chromosomal + chromatid)		fragments / bridges	other (micronucleus, lagging chromosomes)		double and more	
		n	%	n	%		n	%	n	%
Vezha	water	3.0 ± 0.2[a]	75.0	1.0 ± 0.5[a]	25.0	3.0	0.0 ± 0.0[a]	0.0	0.0 ± 0.0[a]	0.0
Vezha	SA 0.01%	28.0 ± 1.1[b]	30.4	17.0 ± 1.3[b]	18.5	1.7	7.0 ± 0.8[b]	7.6	5.0 ± 1.0[b]	5.4
Vezha	SA 0.025%	44.0 ± 1.8[c]	27.3	30.0 ± 1.6[c]	18.6	1.5	14.0 ± 1.1[c]	8.7	14.0 ± 1.7[c]	8.7
Vezha	SA 0.05%	71.0 ± 2.1[d]	33.0	46.0 ± 2.0[d]	21.4	1.5	18.0 ± 1.2[d]	8.4	28.0 ± 2.0[d]	13.0
Vezha	SA 0.10%	62.0 ± 2.0[e]	38.0	40.0 ± 2.1[d]	24.5	1.6	17.0 ± 1.2[d]	10.4	32.0 ± 2.2[d]	19.6
Ihrysta	water	3.0 ± 0.2[a]	75.0	1.0 ± 0.3[a]	25.0	3.0	0.0 ± 0.0[a]	0.0	0.0 ± 0.0[a]	0.0
Ihrysta	SA 0.01%	41.0 ± 1.1[b]	42.3	22.0 ± 1.1[b]	22.7	1.9	10.0 ± 1.2[b]	10.3	11.0 ± 1.7[b]	11.3
Ihrysta	SA 0.025%	59.0 ± 2.0[c]	35.1	35.0 ± 2.0[c]	20.8	1.7	15.0 ± 1.4[b]	8.9	18.0 ± 2.0[c]	10.7
Ihrysta	SA 0.05%	75.0 ± 2.2[d]	34.4	50.0 ± 2.2[d]	22.9	1.5	25.0 ± 2.1[c]	11.5	34.0 ± 2.2[d]	15.6
Ihrysta	SA 0.10%	82.0 ± 2.4[e]	46.6	46.0 ± 2.0[d]	26.1	1. 8	22.0 ± 2.0[c]	12.5	29.0 ± 2.1[d]	16.5

Note: see Table 1.

No complex chromosomal aberrations were observed in the control group, confirming a stable reference for comparison. Ethylmethanesulfonate increased chromosomal aberrations in both Ihrysta and Vezha, measuring 11 and 8 at 0.025%, 14 and 20 at 0.05%, and 26 and 32, respectively, at 0.1% concentration. The differences between the varieties were relatively minor, suggesting that complex chromosomal aberrations were primarily driven by EMS concentration rather than genotype-specific sensitivity. We observed strong dose-dependent effect, with higher EMS concentrations leading to greater chromosomal instability. The optimal EMS concentration was 0.05%, balancing mutagenic efficiency and cytotoxicity, making it a suitable concentration for mutagenesis programs. The genotypic effects were minimal, reinforcing the idea that EMS-induced complex aberrations are largely concentration-dependent rather than variety-specific.

Significant increase compared with the control was observed for cells with two or more aberrations. The parameter increased in both Vezha and Ihrysta, accounting for 5 and 11 at 0.01%, 14 and 18 at 0.025%, and 28 and 34, respectively, at 0.05% concentration. Following treatment with SA 0.1%, the parameter measured 32 in Vezha and 29 in Ihrysta. Ihrysta demonstrated peak at 0.05% SA, followed by a decline at 0.10% SA, suggesting cytotoxic effects at higher concentrations, as well as higher tolerance, maintaining elevated induction levels even at 0.1% SA, suggesting resilience to mutagenic stress. Vezha exhibited higher susceptibility, showing a reduction in aberration induction at higher SA levels. The most effective concentration for inducing multiple aberrations in the varieties was 0.05% SA. At the same time, 0.10% concentration may reach a cytotoxic threshold, leading to reduced mutagenic efficiency, as observed in Vezha. Genotypic variation plays a critical role in SA-induced aberration responses, with Ihrysta demonstrating higher tolerance and Vezha showing greater sensitivity. Future mutagenesis protocols should consider genotype-specific responses to optimize SA concentrations while minimizing cytotoxic effects. This study reinforces the dose-dependent effect of SA on chromosomal instability, while emphasizing the importance of genotype in determining mutagenic efficiency.

Genotype factor was not significant ($F = 2.00$; $F_{0.05} = 2.35$; $P = 0.07$), indicating that genotypic differences do not strongly influence the frequency of complex aberrations. The effect of mutagen concentration was highly significant ($F = 227.18$; $F_{0.05} = 4.67$; $P = 5.65*10^{-15}$), confirming that mutagen concentration is the dominant factor affecting complex aberration induction. Genotype × mutagen concentration interaction was not significant ($F = 1.76$; $F_{0.05} = 1.87$; $P = 0.06$), suggesting a uniform response to increasing mutagen concentration in the genotypes. The differences between the varieties and at the level of groups were statistically insignificant. The primary factor influencing the number of cells with complex aberrations was the mutagen concentration, not genotype. The data highlights a strong correlation between higher mutagen doses and increased chromosomal complexity, corroborating the dose-dependent mutagenic effect.

The discriminant analysis (Tables 7 and 8) conducted for traits linked to mutagenic effects under EMS and SA treatments highlights several significant findings. For most traits, such as pollen sterility, total rate of chromosomal rearrangements, number of fragments, and double fragments, a consistent relationship was observed with increasing mutagen concentration. This indicates that these traits follow predictable trends under the influence of both EMS and SA, supporting their reliability as indicators of mutagenic activity. While the presence of bridges displayed a discernible trend, it deviated from the model-consistent behavior exhibited by other traits. This suggests that the mechanisms or factors influencing bridge formation may differ or be less closely linked to mutagen concentration. The genotype (variety) did not have a significant impact on the majority of traits analyzed, indicating that the mutagen-induced effects are largely consistent across different wheat varieties. A significant interaction was observed for complex changes (cells with two or more types of chromosomal aberrations). This suggests that complex chromosomal changes are influenced by both the intensity of the mutagen and the genetic predisposition of the variety, highlighting a unique genotype-mutagen interplay for this trait.

The comparative analysis of EMS and SA highlights several key points regarding their mutagenic actions. Despite some differences in their specific effects (Fig. 1 and 2), such as on pollen sterility and the presence of bridges, both mutagens demonstrate broadly similar mutagenic profiles. The model parameters confirm that the effects of EMS and SA are predictable, reinforcing their reliability for inducing genetic changes under controlled experimental conditions. As the concentration increased, the effects became increasingly predictable: both mutagens exhibited consistent trends with higher concentrations, thereby validating their effectiveness in inducing mutations. This consistency makes them valuable tools for targeted genetic modification and controlled experimentation. Discriminant analysis affirmed the utility of these mutagens for controlled studies by identifying traits that exhibit predictable responses to concentration changes. Traits like pollen sterility, chromosomal rearrangements, and complex changes were particularly responsive, while the behavior of bridges deviated from the broader trends. The study underscores the importance of genotype selection in mutagenic research, as genotype-mutagen interactions significantly influence outcomes. In this case, the variety Vezha demonstrated responsiveness that makes it a suitable candidate for further mutagenic studies. Investigating mechanisms of such tolerance could enhance understanding of site-specific mutagenic actions.

Table 7

Trait in model after discriminant analysis of EMS

Parameter	Genotype			Concentration		
	Wilks' - Lambda	F_{remove} (12.45)	P-level	Wilks' - Lambda	F_{remove} (3.17)	P-level
Pollen fertility	0.015	5.11	0.08	0.040	26.17	0.01
General rates	0.011	4.44	0.10	0.038	10.11	0.01
Fragments	0.002	2.14	0.14	0.032	8.17	0.01
Bridges	0.002	2.11	0.14	0.022	2.33	0.08
Other	0.002	2.01	0.14	0.012	1.10	0.10
Double and more	0.032	12.92	0.05	0.041	27.38	0.01

The analysis derived from Figure 1 reveals key insights into the effects of EMS concentrations on the studied traits. The 0.025% and 0.05% EMS concentrations exhibited significant contrasting effects, striking an optimal balance between inducing genetic mutations and maintaining plant material viability. These concentrations are particularly effective in promoting variability while avoiding excessive cellular or physiological damage, making them highly suitable for generating viable mutant populations. The 0.10% EMS concentration displayed significantly higher mutagenic activity compared with the lower concentrations. However, this came at the cost of increased cellular damage, reduced viability, and compromised survival and regeneration of the plant material. While the concentration induces substantial mutational changes, its detrimental

impact on plant health diminishes its utility for creating viable mutant populations. Concentrations of 0.025% and 0.05% EMS are optimal for mutagenesis as they maximize genetic variability and mutational changes without compromising plant health. These levels provide a sustainable approach for creating diverse mutant populations, maintaining a balance between mutagenic efficacy and plant material survivability.

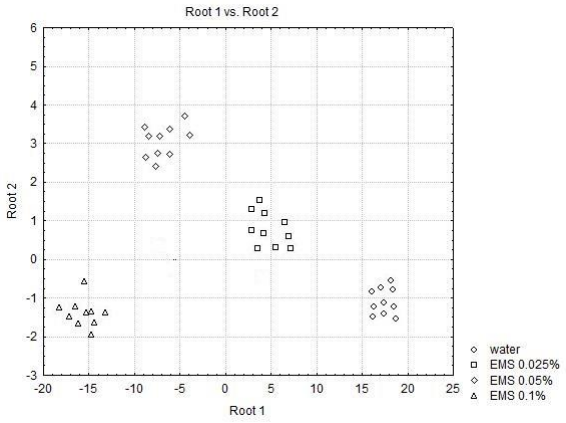

Fig. 1. Classification by canonical functions (discriminant analysis of EMS as a factor, by Mahalanobis distances)

Table 8
Trait in model after discriminant analyzis of SA

Parameter	Genotype			Concentration		
	Wilks' - Lambda	F_{remove} (12.45)	P-level	Wilks' - Lambda	F_{remove} (3.17)	P-level
Pollen fertility	0.012	5.90	0.09	0.050	31.11	0.01
General rates	0.011	4.00	0.10	0.014	3.44	0.05
Fragments	0.001	2.24	0.14	0.014	3.92	0.04
Bridges	0.001	2.30	0.14	0.012	2.59	0.07
Other	0.001	2.36	0.14	0.011	2.21	0.08
Double and more	0.001	2.12	0.15	0.054	37.11	0.01

The analysis of Figure 2 reveals notable trends in the mutagenic activity induced by sodium azide (SA) at varying concentrations, first of all distinct effects at lower concentrations. Concentrations of 0.01% and 0.025% SA exhibited clear, contrasting responses. These concentrations seem to provide more targeted mutagenic activity, producing distinguishable effects that may facilitate selection for specific traits. At 0.05% and 0.1% SA, the effects appeared to overlap or merge, displaying mixed group characteristics. This suggests that higher concentrations might lose specificity, likely due to excessive mutagenic pressure leading to extreme genomic instability, increased cell death or the emergence of aberrant forms that are less useful for breeding purposes. Higher concentrations may reduce the efficiency of trait selection, as the mutations induced might become more random or deleterious. Lower concentrations (0.01%–0.025%) are likely more effective for controlled mutagenesis, allowing for the induction of beneficial changes while minimizing collateral damage.

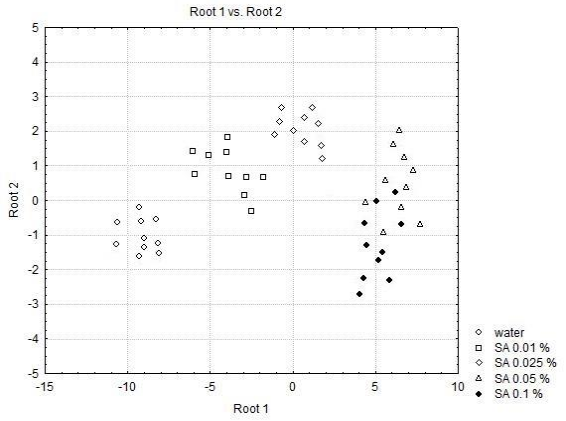

Fig. 2. Classification by canonical functions (discriminant analysis of SA as a factor, by Mahalanobis distances)

The classification results based on the influence of mutagen concentrations and varietal characteristics demonstrate a high degree of precision and reliability in distinguishing these effects. The accuracy of classification for different mutagen concentrations is robust, even for the third and fourth concentrations of SA. Minimal classification errors indicate the reliability of the chosen parameters in differentiating the effects of varying mutagen concentrations. The varietal response to mutagen exposure is effectively classified, confirming the ability of the selected indicators to capture the interaction between genotype and mutagenic factors. The first three studied parameters—pollen fertility, total frequency of rearrangements, and the number of fragments – exhibit high discriminative power. These parameters account for the majority of variability and are critical in assessing mutagenic effects and varietal differences. On average, 85% or more of the objects were accurately classified, underscoring the resolution and reliability of the selected parameters in capturing mutagenic effects and varietal distinctions.

The model developed in this study provides a robust framework for predicting the efficacy of sodium azide (SA) as a mutagen to induce high levels of genetic variability. The model incorporates the variability of individual factors effectively, providing a reliable means of forecasting SA-induced mutagenesis outcomes. The predictive capability highlights the robustness of the experimental parameters and design used. Vezha demonstrated a higher suitability for SA exposure, showing significant genetic variability and strong potential for mutant form development. While better suited for SA than EMS, it remains a reliable candidate for generating diverse mutant forms. Ihrysta exhibited comparatively lower variability under SA treatment than Vezha, indicating less responsiveness to this mutagen. The study affirms the utility of SA as a powerful mutagen in plant breeding, particularly for enhancing genetic diversity. Varieties like Vezha have shown significant promise, making them key targets for future SA-based mutagenesis studies. While Ihrysta displayed lower variability, its inclusion in breeding programs may still yield valuable results when combined with other mutagenic agents or techniques. The model offers a predictive tool for optimizing SA use in generating diverse and desirable genetic forms.

Discussion

The yielded results allow for assessing the potential of chemical mutagenesis and highly active substances in inducing genetic changes to develop new forms (Muhammad et al., 2021; Von Well et al., 2022) or refining the initial material (variety or line). This approach aligns with modern requirements but enables improvement in specific parameters (Andrew et al., 2021).

Those mutagens are widely applied in practice to develop new genetically and breeding-valuable forms (Hong et al., 2022), primarily due to their ability to induce high variability in plant structure (plant architecture) (Cabahug et al., 2020; Živković et al., 2024). This practice facilitates the creation of plants better suited to modern requirements for intensive varieties (Pathirana, 2021) by transforming locally adapted semi-intensive forms, such as national breeding varieties under specific conditions (Horshchar and Nazarenko, 2022a).

The examined mutagen exhibited a somewhat different range of changes in this material compared with foreign scientific programs (Ghasemi-Soloklui et al., 2023). While the overall mutation rate was significantly higher, particularly in genotypes identified as less resistant to this type of factor, the proportion of beneficial changes was lower than anticipated (Kiani et al., 2022). It is important to highlight the strong relationship between the rate and frequency of cytogenetic aberrations observed in these varieties and the general rate and spectrum of visually identified changes. This correlation suggests that cytogenetic data may serve as a reliable indicator of broader mutagenic effects, providing valuable insights into the mutagen's influence on the genome (Pathirana, 2021; Nazarenko et al., 2022).

The frequency of chromosomal aberrations closely aligns with the rate of visually identifiable mutations. This relationship strengthens the case for using cytogenetic markers as predictors of mutagenic efficiency and effectiveness. While this correlation has been identified in prior research, it is not consistent across all mutagens (Horshchar & Nazarenko, 2022b). The observed patterns emphasize the unique specificity of the tested mutagens in driving changes that are both cytogenetically detectable and visually apparent (Lethin et al., 2022).

The use of integrative indicators has been highlighted as a more promising approach, because they not only account for the total number

of mutant cases but also encompass the spectrum of changes based on the number of individual traits affected by the genetic activity of the factor, (Pathirana, 2021; Pathirana et al., 2023; Von Well et al., 2023). This was particularly confirmed for this mutagen, with stronger validation through mathematical and statistical analyses compared with previous studies on gamma rays (Muhammad et al., 2021), nitrosoalkylureas, or other mutagenic factors (Horshchar & Nazarenko, 2024).

Sodium azide as a mutagenic factor demonstrates a significant dependence on the genotype-mutagen interaction, influenced by the specific characteristics of the initial genotype and its genetically determined reactivity to the mutagenic factor (Murthy et al., 2024). Observations across cellular to whole-plant levels reveal a consistent relationship in the severity of the mutagenic effects, suggesting a direct impact on DNA structure rather than an indirect effect on the hereditary apparatus (Hong et al., 2022). A comprehensive assessment of the mutation rate and spectrum is crucial for evaluating the mutagen's success in inducing mutations (Pathirana et al., 2023). Focusing on only one component may result in incomplete data and hinder accurate classification analysis (Mahanish & Kin, 2025).

The findings emphasize the mutagen's significant value in inducing a diverse range of genetic changes, which, although not immediately yielding economically valuable forms or lines, provide critical insights for future breeding efforts. This positions the mutagen as an essential tool for exploring genetic control mechanisms and developing targeted improvements in existing varieties (Yan et al., 2021; Živković et al., 2024). The mutagens demonstrate a high capacity for inducing a wide spectrum of genetic alterations (Cabahug et al., 2020). These changes are instrumental for studying mechanisms of trait control and understanding complex genetic interactions (Bora et al., 2024). Ethylmethanesulfonate is more suited for generating pre-breeding material rather than immediate commercial varieties. Sodium azide offers a potential for use in gene pyramiding to combine favorable traits into elite varietie (Abdullah et al., 2018; Shabani et al., 2022).

The findings suggest that in at least two cases, there is a clear potential for obtaining a higher frequency of valuable forms, aligning with results observed in other wheat varieties in previous studies. While they do not drastically alter the overall trends in the frequency and spectrum of forms, microchanges define the understanding of mutagenic effects and offer supplementary data that can improve the predictability of outcomes (Bilgın et al., 2022). The study corroborates previous findings that microchanges, although informative, do not significantly adjust the broader patterns of mutagen-induced variability. The general frequency and spectrum of forms obtained remain consistent, even when microchanges are factored in (Von Well et al., 2023). By linking phenotypic changes to genetical and physiological markers, the study aims to enhance the predictability of identifying new valuable lines. Further research will focus on establishing correlations between observed changes and their inheritance and highlighting unique traits that could make certain lines more advantageous for specific breeding objectives.

Conclusion

The studied mutagens, despite high damaging ability, exhibited sufficient site-specificity to identify the most responsive genotypes, even in a relatively limited set. The analysis reinforces the potential of both EMS and SA as effective mutagens for mutation breeding and cytogenetic research. Their broad similarities, coupled with the importance of genotype-specific responses, provide a foundation for leveraging these agents in targeted genetic improvements. One genotype, Vezha, stood out as particularly responsive and more promising variety for breeding programs focused on mutagenic variability. The study supports the use of moderate concentrations as these concentrations often yield the best balance of positive changes. Lower concentrations (SA 0.01%–0.025%; EMS 0.025% – 0.05%) are likely more effective for controlled mutagenesis, allowing for the induction of beneficial changes while minimizing collateral damage. Higher concentrations tend to reduce the quality and quantity of obtained material, introducing undesirable traits or excessive sterility. Moderate concentrations maximize the mutagenic efficiency while minimizing negative effects. The interaction of mutagen concentration and genotype was not as significant as in the previous study. The mutagens demonstrated the potential to induce several desirable traits. The study also noted challenges, such as high sterility rates, especially at higher concentrations, and potential correlation of desirable traits with undesirable one. Further studies will evaluate the selected genotypes for tolerance to adverse environmental factors, including winter and drought resistance, technological qualities of grain, and positive changes in the content of valuable microelements in grain.

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