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Cytogenetic effect of highly active ecogenetic factors on winter wheat

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Abstract. The study was aimed at exploring the potential of ethylmethanesulfonate (EMS) as a mutagen by examining its ability to induce chromosomal aberrations, its interaction with different genotypes, and the characteristics of genotype-mutagenic interactions. It also focused on evaluating the feasibility of using EMS in future applications, including its predictive value when tested at the cellular level for determining its mutation-inducing capacity at the plant level. Seeds of winter wheat two varieties (Spivanka and Altigo) were treated with ethylmethansulfonate (EMS) at concentrations of 0.025%, 0.05%, and 0.1%, and sodium azide (SA) at concentrations of 0.01%, 0.025%, 0.05%, and 0.1%. The study of cytogenetic activity, evaluated through pollen sterility and the frequency and spectrum of chromosomal abnormalities in mid-phase cell mitosis, revealed significant findings regarding genotype-mutagenic interactions in the wheat varieties. Genotype-mutagenic interactions are crucial in determining variability in chromosomal aberrations. The wheat variety Altigo demonstrated significantly higher genotype-mutagenic specificity, making it a promising candidate for inducing variability and obtaining mutant forms. Altigo showed particularly effective responses when treated with EMS and SA concentrations ranging 0.025% to 0.05%, which optimized the mutagenic effects without excessive adverse impacts. The study underscores critical findings of the parameters that define the genetically determined susceptibility of wheat varieties to ecogenetic factors, particularly focusing on the mutagens EMS and SA. The pollen fertility, overall frequency of chromosomal aberrations, and number of induced fragments were observed to be the most reliable indicators of genetic susceptibility to mutagens. Other parameters, particularly rare chromosomal rearrangements, only partially reflected the trends or failed to provide meaningful data, indicating limited utility in such analyses. The agents under study exhibited induction patterns consistent with those observed for other chemical supermutagens, although variations occured based on the initial genetic material of the plant. The data will be integrated with studies on the frequency and quality of resulting hereditary changes, particularly in complex biochemical and physiological traits. These results provide a foundation for refining mutagenic strategies and identifying optimal conditions and materials for future breeding programs.

Keywords: bread wheat; chromosomal rearrangements; ethylmethansulfonate; sodium azide; variety; mitosis; site-specific; chemical supermutagens.

Introduction

The use of chemical factors as agents of ecogenetic improvement in cultivated plants plays a crucial role in enhancing and stabilizing food production to meet the needs of the Earth's growing population. Advances in ecological genetics significantly influence scientific research, species conservation, ecological management, and the development of adaptation strategies to address climate change. Understanding the genetic aspects of ecological interactions provides the foundation for more effective biodiversity conservation and sustainable resource management practices. Genetic regulation of organismal responses to environmental challenges is key to their adaptive potential, and studying these mechanisms offers valuable insights into how populations adjust to changing environmental conditions (Álvarez-Holguín et al., 2019; Pathirana & Carimi, 2023).

The study of chromosomal damage frequency provides insights into the general genetic and epigenetic activity of specific factors, while analyzing the spectrum of subsequent changes helps to associate the nature of future mutations with the induction of rare types of changes (Bilgın et al., 2022). Although no test system directly links changes at the organismal level with DNA damage, certain patterns can be observed (Álvarez-Holguín et al., 2019). Chemical agents, such as ethylmethansulfonate, which is increasingly used as a mutagen, exhibit heightened site specificity. This results in a higher frequency of specific types of changes (Horshchar & Nazarenko, 2022a), as demonstrated in plants by relatively increased occurrences of rare mutations, as well as specific types of dwarfism and sterility (Jalal et al., 2021; Bilgın et al., 2022).

Test systems are frequently employed for environmental monitoring to assess pollution levels in a specific area (Gupta et al., 2019). These systems provide data not only on the extent of pollution but also on its nature, underlying causes, and potential consequences (Nazarenko et al., 2023). Methods based on testing chromosomal changes are well-established, standardized, and internationally recognized for their reliability and effectiveness (Ghasemi-Soloklui et al., 2023).

Testing new mutagens at the cellular level often involves observing the elimination of cells at high concentrations or doses of the factor (Pathirana & Carimi, 2023). Typically, the frequency of chromosomal changes peaks at a certain dose or concentration, after which it declines, marking a practical limit for the mutagen's application (Horshchar & Nazarenko, 2022b). In many cases, a plateau is observed in the mutagenic effects over a range of doses or concentrations, making it logical to stop experimentation upon reaching this plateau.

However, maximum damage to the nuclear apparatus does not always correlate with a critical reduction in survival rates (LD_{50}) or morphometric parameters (RD_{50}) (Bora et al., 2024). In certain situations, doses at or even beyond the plateau may be utilized to induce rare genetic changes, aiding in the investigation of genetic control mechanisms for specific traits (Hong et al., 2022). This approach is frequently applied in modern genetic research, particularly with chemical supermutagens (Álvarez-Holguín et al., 2019).

The subjects of mutagenic effects, particularly for chemical agents, are of paramount importance, as genotype-specific features influence DNA damage patterns, shifts in mutable site locations, and the transition of sites from mutability to stability (Von Well et al., 2023). The selection of optimal genotype compositions and concentrations of ethyl methanesulfonate facilitates the induction of the desired types of changes with high frequency, while minimizing associated negative alterations. This approach maximizes the potential for enhancing specific traits and properties (Nazarenko et al., 2023).

Fluctuations in the response of individual subjects to mutagen treatment, particularly in terms of the overall frequency and specific types of chromosomal rearrangements, are critical for identifying genetically determined characteristics of DNA structure (Nazarenko et al., 2019). Equally important are individual differences in susceptibility or, more rarely, resistance to the mutagenic effects of a given factor (Ergün et al., 2023). These variations may also depend on the presence of specific tolerance mechanisms to the genetic activity of the mutagenic substance (Von Well et al., 2023).

In chemical mutagenesis, studies on genotype-mutagenic interactions are particularly crucial due to the significantly greater variability in the effects of even similar mutagenic agents (Nazarenko et al., 2019). Identifying changes at the chromosomal level provides more reliable and faster results compared with classical experimental wheat mutagenesis, which typically requires analyzing second or third generations. Notably, sodium azide remains underexplored in this context, with the genotypes of domestic winter wheat varieties being virtually unstudied, especially in comparative analyses. Importantly, ethylmethanesulfonate's mechanism of action differs significantly from previously studied chemical mutagens (Bora et al., 2024).

The study aimed to explore the potential of ethylmethanesulfonate (EMS) as a mutagen by examining its ability to induce chromosomal aberrations, its interaction with different genotypes, and the characteristics of genotype-mutagenic interactions. It also focused on evaluating the feasibility of using EMS in future applications, including its predictive value when tested at the cellular level for determining its mutation-inducing capacity at the plant level. Additionally, we examined the effect of EMS on the fertility of the first-generation material, identifying this as a significant limitation for its practical use in generating desired plant samples.

Materials and methods

The study on pollen viability and the frequency and spectrum of chromosomal rearrangements was conducted at the Laboratory of the Department of Breeding and Seed Production at the Dnipro State Agrarian Economic University. The pollen samples were collected from the first-generation mutant populations grown at the experimental field station of the Science-Education Center.

A thousand grains per concentration were treated with ethylmethanesulfonate (EMS) at concentrations of 0.025%, 0.05%, and 0.10%, and sodium azide (SA) at concentrations of 0.01%, 0.025%, 0.05%, and 0.10% (both from Sigma-Aldrich, Germany). The grains soaked in water served as the control. The selected concentrations were based on prior experimental mutagenesis data for cereals. The grain exposure lasted 24 hours following standard protocols for the cultivars (Spencer-Lopes et al., 2018).

The experiment included 18 treatment variants using two winter wheat varieties, Spivanka and Altigo. The agricultural practices for cultivating the first-generation mutant populations adhered to the standard methods for this region. The pollen viability was assessed at the flowering stage by collecting an average of 25 samples per treatment from ears without morphoses and with well-developed yellow anthers in the middle part of the ear. This ensured the collection of maximum material for analysis. The pollen grains were stained with acetocarmine, and the fertility was evaluated based on staining intensity, observed through light microscopy.

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

To prepare the samples, the swollen seeds were germinated at 20–22 °C until the primary roots reached a maximum length of 1.1 cm. The root tips were then excised and fixed for 24 hours in the Clarke's fixative, a mixture of 1 part glacial acetic acid and 3 parts 96% medical alcohol. The fixed samples were stored in 70% ethanol in a refrigerator. At least 25 temporary pressure preparations were prepared, observing up to 1,000 cells per concentration and control (with fewer cells analyzed at higher concentrations). The preparations were stained with acetocarmine for clarity. If root tips were excessively rigid, they were soaked in a 45% glacial acetic acid solution to facilitate observation (Spencer-Lopes et al., 2018).

The statistical analysis was conducted using Statistica 10.0 software (TIBCO, Palo Alto, USA). The ANOVA module (with a significance level of $P < 0.05$) was employed to evaluate the influence of factors and to determine genotype-mutagenic interactions, with graphical visualization of the results. The pairwise comparisons were carried out using the Tukey HSD test. The descriptive statistics included the calculation of the arithmetic mean and standard deviation ($x \pm SD$), while the normality of the data was assessed using the Shapiro–Wilk test (W-test). The discriminant analysis (standard program modules) was utilized to identify the model characteristics of the observed features and evaluate their significance.

Results

The analysis of the fertility of samples of two mutant populations under the action of different concentrations of EMS showed a linear dependence of the increase in sterility with increasing concentration ($r = 0.88$, Table 1), and for both varieties, although the varieties differed in the degree of manifestation of this trait ($F = 16.20$; $F_{0.05} = 2.34$; $P = 2.74*10^{-5}$), the variability in concentrations was ($F = 317.71$; $F_{0.05} = 2.02$; $P = 2.81*10^{-8}$). This comparison was also confirmed by the post-hoc Tukey test comparisons between different variants. We found that the effect of the factor led to a significant decrease in viability according to this parameter. However, the highest concentration of EMS 0.1% did not reach the LD_{50} level and resulted in viability levels of approximately 65–70%. Therefore, this concentration can be considered on the verge of optimal, as it is up to 70%, according to international classification. The mutagen is highly harmful, and the lowest concentration resulted in a statistically significant decrease in fertility. However, both the first and second concentrations were relatively low, maintaining 70–90% of the original trait value. Thus, under the action of EMS in the studied concentrations, it is possible to obtain a sufficient amount of plant material for further study of hereditary variability in the second-third generation. In general, according to many years of research involving a vast number of wheat genotypes, the last concentration still tends to be semi-lethal. The genotype-mutagen interaction manifested in different survival of the material and was statistically significant ($F = 7.10$; $F_{0.05} = 4.60$; $P = 0.008$).

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%
Spivanka	97.74 ± 0.84^a	89.32 ± 1.02^b	79.17 ± 1.03^c	69.93 ± 1.41^d
Altigo	95.82 ± 0.81^a	86.71 ± 0.92^b	77.33 ± 0.94^c	65.12 ± 1.44^d

Note: indicate significant differences at $P < 0.05$ according to the Tukey HSD test with the Bonferroni correction; comparison in terms of one variety within line.

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%
Spivanka	97.74 ± 0.84^a	88.64 ± 1.07^b	81.17 ± 1.01^c	72.51 ± 1.17^d	65.74 ± 1.17^e
Altigo	95.82 ± 0.81^a	87.07 ± 1.02^b	79.48 ± 0.87^c	67.10 ± 1.13^d	53.29 ± 1.28^e

Note: see Table 1.

The analysis of the fertility of samples of two mutant populations under the action of different concentrations of sodium azide showed a linear dependence of the increase in sterility with increasing concentration ($r = 0.92$, Table 2) for both the varieties. The varieties did not differ from each other in the degree of manifestation of this trait ($F = 2.10$; $F_{0.05} = 2.21$; $P = 0.06$), but at high concentrations, the difference was significant ($F = 8.01$; $F_{0.05} = 3.20$; $P = 0.009$). However, when examining the variability across different concentrations of the trait, the results indicated a significant effect ($F = 46.50$; $F_{0.05} = 2.00$; $P = 3.11*10^{-5}$), suggesting that concentration levels greatly influence the trait's expression. This comparison was also confirmed by the post-hoc Tukey test for pairwise comparisons between different variants. The variants SA 0.05% and SA 0.10% differed from each other in both parameters. We found that the effect of the factor led to a significant decrease in viability in this parameter, but the

concentration of SA 0.10% in the case of the Altigo variety was almost up to the LD_{50} level and was 53.3%, while for the variety Spivanka it was at the level of optimal concentrations from the point of view of experimental mutagenesis, i.e. this concentration in this indicator can be considered on the border of optimal and semi-lethal depending on the genotype (up to 50–70%, according to the international classification). The genotype-mutagen interaction manifested in different sterility in the material, showing statistical significance at the specified concentrations ($F = 6.23$; $F_{0.05} = 3.90$; $P = 0.01$). However, at low first and second concentrations, the results were not significant ($F = 3.43$; $F_{0.05} = 3.90$; $P = 0.07$). The mutagen is highly harmful and even the first concentration caused a significant statistically significant decrease in fertility (almost 10%), but in general the first and second concentrations are low (90–70% of the value of the trait), and the third is on the border between low and optimal in terms of viability. Thus, under the action of sodium azide in the studied concentrations, it is possible to obtain a sufficient amount of plant material for further study of hereditary variability in the second-third generation.

In general, within a similar concentration range determined by genetic factors, sodium azide exhibited relatively stronger mutagenic activity, although it did not lead to severe consequences. Additionally, the variety Altigo was less tolerant to such mutagenic effects, highlighting the significance of genotype-mutagenic interaction. However, for sodium azide, unlike ethyl methanesulfonate (EMS), this interaction became evident only at higher concentrations of the mutagenic factor.

The study of the frequency of chromosomal rearrangements under EMS exposure (Table 3) revealed clear distinctions in the effects of individual factors and their concentrations, as well as thresholds for the total number of chromosomal abnormalities. The factor analysis confirmed that the genotype factor had a significant influence, with statistically reliable differences observed between the varieties ($F = 8.17$; $F_{0.05} = 2.35$; $P = 0.008$). This effect was particularly notable in the EMS 0.10% variant, where significant cell death occurred, suggesting that this concentration approached a harmfulness threshold. An increase in EMS concentration was consistently associated with higher frequencies of chromosomal rearrangements, with statistically significant differences observed within each variety ($F = 118.01$; $F_{0.05} = 2.71$; $P = 2.27\times10^{-11}$). Furthermore, the genotype-mutagen interaction was significant ($F = 7.79$; $F_{0.05} = 3.15$; $P = 0.01$), highlighting the differential responses of genotypes to mutagenic treatment. The baseline cytogenetic activity in the control samples ranged 0.4% to 0.7%. Under the influence of 0.025% EMS concentrations, the frequency of chromosomal rearrangements ranged 8.5% (Spivanka) to 9.7% (Altigo); at 0.05% EMS, the range was 16.1% (Spivanka) to 16.3% (Altigo), showing no significant difference between the two varieties. At 0.10% EMS the range was 22.5% (Spivanka) to 23.4% (Altigo), indicating a higher level of cytogenetic activity, but also approaching a harmfulness threshold, as evidenced by increased cell mortality. Although the variety Altigo exhibited slightly higher vulnerability, the difference was not substantial enough to predict a significant impact on the viability of plants of this variety in subsequent generations. These findings suggest that both varieties exhibited moderate cytogenetic activity in response to EMS treatment and remain viable for further mutagenesis experiments, with appropriate consideration of concentration thresholds.

Table 3

General rates of chromosomal abnormalities in properly dividing mitotic cells of root tips under EMS action (x ± SD, n = 800–1000)

Variety	Variant	Mitosis, number	Chromosomal aberrations	
			number	%
Spivanka	water	1004	4	0.40 ± 0.10^{a}
Spivanka	EMS 0.025%	1009	86	8.52 ± 0.30^{b}
Spivanka	EMS 0.05%	1011	163	16.12 ± 0.22^{c}
Spivanka	EMS 0.10%	889	200	22.50 ± 0.31^{d}
Altigo	water	1001	7	0.70 ± 0.14^{a}
Altigo	EMS 0.025%	1002	97	9.68 ± 0.25^{b}
Altigo	EMS 0.05%	1003	163	16.25 ± 0.31^{c}
Altigo	EMS 0.10%	801	187	23.35 ± 0.36^{d}

Note: see Table 1.

The study of the frequency of chromosomal rearrangements under SA exposure (Table 4) revealed clear distinctions in the effects of individual factors and their concentrations, as well as thresholds for the total number of chromosomal abnormalities. The factor analysis confirmed that the genotype factor had a significant influence, with statistically reliable differences observed between the varieties ($F = 11.17$; $F_{0.05} = 2.35$; $P = 0.001$). This effect was particularly notable in the SA 0.10% variant, where significant cell death occurred, suggesting that this concentration approached a harmfulness threshold (related to theLD_{50} concentration for Spivanka concerning the decrease of chromosomal abnormalities). An increase in SA concentration was consistently associated with higher frequencies of chromosomal rearrangements, with statistically significant differences observed within each variety ($F = 145.14$; $F_{0.05} = 2.71$; $P = 3.11\times10^{-15}$). Furthermore, the genotype-mutagen interaction was significant ($F = 8.09$; $F_{0.05} = 3.15$; $P = 0.009$), highlighting the differential responses of genotypes to mutagenic treatment. The baseline cytogenetic activity in the control samples ranged 0.4% to 0.7%. Under the influence of SA concentrations at 0.001%, the frequency of chromosomal rearrangements accounted for 7.7% in Spivanka and 9.7% in Altigo; at 0.025%, it equaled 13.6% in Spivanka and 16.4% in Altigo; at 0.05%, it measured 20.3% in Spivanka and 25.3% in Altigo, showing significant difference between the two varieties. At 0.10%, the frequency varied between 16.2% in Spivanka and 23.1% in Altigo, indicating a higher level of cytogenetic activity, but also approaching a harmfulness threshold, as evidenced by increased cell mortality. Although the variety Altigo exhibited higher vulnerability, the difference was substantial enough to predict a significant effect on the viability of plants of this variety in subsequent generations.

The observed difference in the effects of sodium azide (SA) compared with ethylmethanesulfonate (EMS) was primarily seen in the varied responses of the two winter wheat varieties. On Spivanka, SA caused a more damaging effect than EMS. This indicates a higher susceptibility of Spivanka to the mutagenic activity of SA, possibly due to specific genotype-mutagen interactions that amplify the impact of SA on this variety. As with Altigo, both mutagens (SA and EMS) exerted similar levels of impact. There were no statistically significant differences in the cytogenetic effects or observed damage between the two mutagens for this genotype. Action of SA was stronger for both varieties.

Table 4

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

Variety	Variant	Mitosis, number	Chromosomal aberrations	
			number	%
Spivanka	water	1004	4	0.40 ± 0.10^{a}
Spivanka	SA 0.01%	1003	77	7.68 ± 0.10^{b}
Spivanka	SA 0.025%	1007	137	13.60 ± 0.21^{c}
Spivanka	SA 0.05%	932	189	20.28 ± 0.32^{d}
Spivanka	SA 0.10%	844	137	16.23 ± 0.25^{e}
Altigo	water	1001	7	0.70 ± 0.12^{a}
Altigo	SA 0.01%	1002	97	9.68 ± 0.17^{b}
Altigo	SA 0.025%	1010	166	16.44 ± 0.22^{c}
Altigo	SA 0.05%	817	207	25.34 ± 0.31^{d}
Altigo	SA 0.10%	753	174	23.11 ± 0.27^{e}

Note: see Table 1.

The study of the spectrum of chromosomal changes under the influence of mutagens provided detailed insights into the types and frequencies of rearrangements observed in mitotic cells. These included the following types of chromosomal aberrations: fragments and double fragments, which represent the most common and quantifiable markers of chromosomal damage; bridges, observed in various forms, indicating improper chromosomal segregation; and micronuclei, which indicate lagging chromosomal material that fails to be incorporated into daughter nuclei. Additionally, lagging chromosomes highlight errors in chromosomal movement during cell division, as shown in other aberrations in the tabulated data. Complex changes were also noted, with cells exhibiting two or more chromosomal rearrangements categorized separately, reflecting a higher degree of cytogenetic instability (Tables 5 and 6). The ratio of fragments to bridges was calculated, providing insights into the mutagenic mechanisms.

The number of fragments and double fragments observed in the control group was minimal but consistently present, serving as a baseline. Under the influence of EMS, there was a clear and significant increase in these aberrations, starting even at the lowest concentration (0.025%), with substantial differences between the varieties. At EMS 0.025%, a significant increase in the number of fragments occurred in both varieties. The values measured 51.0 in Spivanka and 60.0 in Altigo. At EMS 0.05%, the number of fragments further increased in both varieties, measuring 86.0 in Spivanka and 103.0 in Altigo. This suggests a dose-dependent response to

EMS, with Altigo exhibiting higher susceptibility. At EMS 0.1%, the number of fragments plateaued, with values varying between 92.0 in Altigo and 96.0 in Spivanka. Unlike the previous concentrations, the difference between the varieties at this concentration was negligible, suggesting a threshold effect.

The differences between each EMS concentration and the control group, as well as between successive concentrations, were statistically significant in all the cases except for the plateau effect observed at the highest concentration (0.1%). Altigo ($F = 7.89$; $F_{0.05} = 2.48$; $P = 0.004$) exhibited a consistently higher number of fragments at lower EMS concentrations, indicating greater sensitivity to mutagenic stress compared with Spivanka. At the highest concentration (0.1%), the mutagenic impact seems to have reached a saturation point, with minimal further differentiation between the varieties. The significant increase in fragments and double fragments, especially at lower concentrations, underscores the efficacy of EMS in inducing chromosomal damage and highlights the dose-dependent nature of its action.

The analysis of fragment induction under sodium azide (SA) revealed the following trends and statistical outcomes: the number of fragments significantly increased with SA concentration in both varieties, in particular, the number of fragments significantly increased with SA concentration in both varieties, in particular, measuring 38.0 in Spivanka and 59 in Altigo at SA 0.01%, demonstrating a notable increase from the control; 71 and 105, respectively, at SA 0.025%, indicating a sharp rise in fragment induction; 91 and 113, respectively at SA 0.05%, marking the highest induction rates; and 68 and 94 Altigo at SA 0.1%, showing a decline compared with the previous concentration. The difference in fragment numbers between the control and each concentration was statistically significant in all the cases. The differences between successive concentrations were also significant, except for the last concentration (0.1%), where Spivanka was observed to have a decrease in fragment induction. The varieties exhibited distinct responses to SA treatment. Altigo consistently showed the highest fragment induction across all the concentrations, indicating greater susceptibility. Spivanka exhibited the lowest induction rates, reflecting reduced sensitivity to SA mutagenesis.

Table 5

Chromosomal abnormalities in properly dividing mitotic cells of root tips, EMS action ($x \pm SD$, $n = 800–1000$)

Variety	Variant	Fragments (single + double)		Bridges (chromosomal + chromatid)		Fragments / bridges	Other (micronucleus, lagging chromosomes)		Double and more	
		n	%	n	%		N	%	n	%
Spivanka	water	2.0 ± 0.3^a	50.0	2.0 ± 1.0^a	50.0	1.0	0.0 ± 0.0^a	0.0	0.0 ± 0.0^a	0.0
Spivanka	EMS 0.025%	51.0 ± 0.5^b	59.3	25.0 ± 1.5^b	29.1	2.0	10.0 ± 1.1^b	11.6	11.0 ± 1.0^b	12.8
Spivanka	EMS 0.05%	86.0 ± 1.0^c	52.8	58.0 ± 2.1^c	35.6	1.5	19.0 ± 1.3^c	11.7	26.0 ± 1.3^c	16.0
Spivanka	EMS 0.10%	96.0 ± 1.1^d	48.0	79.0 ± 2.4^d	39.5	1.2	25.0 ± 2.1^d	12.5	44.0 ± 1.9^d	22.0
Altigo	water	4.0 ± 0.2^a	57.1	2.0 ± 0.4^a	28.6	2.0	1.0 ± 0.3^a	14.3	0.0 ± 0.0^a	0.0
Altigo	EMS 0.025%	60.0 ± 1.2^b	61.9	28.0 ± 0.8^b	28.9	2.1	9.0 ± 1.0^b	9.3	14.0 ± 0.9^b	14.4
Altigo	EMS 0.05%	103.0 ± 1.8^c	63.2	44.0 ± 2.1^c	27.0	2.3	16.0 ± 1.4^c	9.8	21.0 ± 1.4^c	12.9
Altigo	EMS 0.10%	92.0 ± 1.8^c	49.2	66.0 ± 2.3^d	35.3	1.4	29.0 ± 2.0^d	15.5	41.0 ± 1.9^d	21.9

Note: see Table 1.

Sodium azide is highly effective in inducing fragments, with concentrations up to 0.05% causing the most pronounced effects. The decline at 0.1% suggests a potential threshold where cytotoxic effects outweigh mutagenic induction. The variability in response in between Spivanka and Altigo highlights the importance of considering genetic background when evaluating mutagenic efficiency. The consistent statistical significance of differences confirms the reliability of fragment induction as a metric for evaluating mutagenic activity. Sodium azide demonstrated a strong mutagenic effect, with optimal fragment induction observed at concentrations of 0.05%. The observed differences across genotypes and concentrations reinforce the need for tailored mutagenesis protocols to maximize genetic variability while minimizing cytotoxicity.

The analysis of fragment induction provided valuable insights into the effects of genotype, mutagen concentration, and their interaction. The effect of genotype on fragment induction was statistically significant ($F = 9.19$; $F_{0.05} = 2.35$; $P = 0.002$). This indicates that the inherent genetic makeup of the varieties (Spivanka and Altigo) significantly influences the extent of chromosomal fragmentation under mutagenic stress. An increase in concentration had a highly significant impact on the total number of fragments observed ($F = 207.01$; $F_{0.05} = 2.71$; $P = 5.17 \times 10^{-16}$). 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 ($F = 11.54$; $F_{0.05} = 4.87$; $P = 4.34 \times 10^{-4}$). 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 Spivanka and Altigo. The consistent increase in fragments with higher concentrations reaffirms the dose-dependent mutagenic effects of mutagens (without last critical concentrations for both mutagens). The interaction term emphasizes that the extent of fragment induction in the genotypes was not uniform, pointing to complex interactions that depend on both the genetic material and the mutagen concentration.

The analysis of chromatid and chromosomal bridges induction under EMS revealed important trends and statistical outcomes regarding the dose-dependent response and genotypic variability. All genotypes exhibited a minimal but consistent number of bridges in the control group, establishing a baseline for comparison. For EMS 0.025%, significant increases in bridge numbers were observed in both varieties, in particular, 25 in Spivanka and 28 in Altigo; and EMS 0.05% also marked increase in bridges, 44 in Altigo and 58 in Spivanka. The most pronounced increase in the bridges was caused by the highest concentration of EMS, 0.1%, accounting for 66 in Altigo and 79 in Spivanka.

Spivanka demonstrated higher bridges induction at higher EMS concentrations, particularly at 0.05% and 0.10%, suggesting greater sensitivity. Altigo exhibited a relatively lower increase in the bridges compared with Spivanka at higher concentrations, suggesting slight resistance to bridge formation under EMS action. The results indicate a clear dose-dependent response, with bridge induction increasing progressively with EMS concentration. This pattern aligns with the mechanism of EMS as a mutagen causing chromosomal damage, particularly at higher doses. The higher susceptibility of Spivanka to bridge formation suggests it is more prone to chromosomal missegregation and damage under EMS treatment. Altigo's relatively lower bridge induction may indicate differences in chromosomal structure or repair mechanisms between the two varieties. The differences between each EMS concentration and the control group, as well as between successive concentrations, were statistically significant in all the cases. Altigo ($F = 8.34$; $F_{0.05} = 2.48$; $P = 0.003$) exhibited a consistently lower number of chromatid and chromosomal bridges at third-second EMS concentrations, indicating less sensitivity to mutagenic action compared with Spivanka.

The analysis of chromatid and chromosomal bridges induction under sodium azide (SA) revealed the following trends and statistical outcomes: the number of fragments was significantly increased by SA in both the varieties, in particular, 25 in Altigo and 28 in Spivanka at SA 0.01%, demonstrating a notable increase compared with the control; 45 in Altigo and 50 in Spivanka at SA 0.025%, indicating a sharp rise in bridges induction; 63 in Altigo and 76 Spivanka at SA 0.05%, marking the highest induction rates; and 53 in Spivanka and 55 in Altigo at SA 0.1%, showing a decline compared with the previous concentration for both the varieties. Between SA 0.05% and SA 0.10%, the number of bridges plateaued. This suggests a threshold concentration, beyond which SA's mutagenic efficiency diminishes, potentially due to cytotoxic effects or cell death. Altigo displayed consistently lower bridge numbers compared with Spivanka across all the concentrations and exhibited a milder decline at SA 0.10%, suggesting better tolerance to higher concentrations. The plateau and decline at SA 0.10% suggest a potential cytotoxic threshold where SA's mutagenic effects are counterbalanced by its harmful

impact on cellular viability. Spivanka is better suited for studies requiring high mutagenic variability, whereas Altigo may be advantageous for maintaining plant viability while inducing variability.

The analysis of percentage of the total number of aberrations and bridge induction revealed consistent trends and highlights the interplay between mutagen concentration and genotype variability. The effect of the genotype factor on bridge induction was not significant ($F = 5.91$; $F_{0.05} = 2.35$; $P = 0.01$), indicating limited variability among genotypes for this specific trait. A significant effect of increasing mutagen concentration was observed ($F = 205.17$; $F_{0.05} = 2.71$; $P = 5.01*10^{-12}$), confirming a strong dose-dependent response in bridge induction. The interaction of genotype and mutagen was also significant ($F = 12.99$; $F_{0.05} = 1.87$; $P = 4.32*10^{-3}$), suggesting that while genotypic differences were less pronounced for the bridges, specific combinations of genotypes and concentrations influenced the outcomes. The observed trend in the percentage of total aberrations suggests that chemical supermutagens like SA and EMS are effective at moderate concentrations. At higher concentrations, the mutagens lose some site specificity, possibly affecting broader genomic regions and leading to resurgence in total aberrations. The genetic background plays a critical role in determining the sensitivity and response to mutagenic factors, as seen in the differences between Spivanka and Altigo. The number of this type of aberrations was significantly lower than that of the previous type.

In the varieties, there was an initial increase in the proportion of fragments, followed by a slight decline. This dynamic suggests that the mutagenic effect is influenced by specific genomic loci, which might vary in their susceptibility to mutagenic agents at different concentrations. For the variety Spivanka, the ratio of fragments to bridges remained relatively stable across all the concentrations. This consistency suggests lower susceptibility of Spivanka to sodium azide, as its chromosomal architecture appears less prone to site-specific variability under mutagenic stress. The dominance of fragments over bridges reflects the typical mechanism of action of SA and EMS, likely involving single-strand breaks or other localized chromosomal effects. The observed site-specific variability in the varieties could be leveraged to target particular genomic regions for inducing variability, while the stability of Spivanka might necessitate alternative strategies or higher mutagenic doses to achieve comparable effects.

The number of micronuclei and lagging chromosomes observed in the control group was minimal (sometimes zero) but consistently present, serving as a baseline. Under the influence of EMS, there was a clear and significant increase in these aberrations, starting even at the lowest concentration (0.025%), with substantial differences between the varieties. As with EMS, 0.025% concentration caused a significant increase, the values accounting for 9 in Altigo and 10 in Spivanka. At EMS 0.05%, the number further increased in both the varieties, accounting for 16 in Altigo and 19 in Spivanka. At EMS 0.1%, the number of other chromosomal abnormalities further increased in both the varieties, measuring 25 in Spivanka and 29 in Altigo. The Altigo variety appeared more susceptible to higher concentrations, showing slightly higher levels of aberrations compared with Spivanka. The induction of micronuclei and lagging chromosomes serves as a reliable indicator of mutagenic activity and highlights the cytogenetic impact of EMS on the tested genotypes. Moderate EMS concentrations (0.05%) provided a balance between inducing significant genetic variability and avoiding excessive cytotoxic effects.

Other chromosomal abnormalities (e.g., micronuclei, lagging chromosomes) were minimal or absent in the control group, establishing a reliable baseline for comparison. The number of aberrations in Spivanka and Altigo was 11 and 13, respectively, at SA 0.01%; 16 in both the varieties at SA 0.025%; 22 and 31, respectively, at SA 0.05% (marking the highest induction rates for both the varieties); and 16 and 25 in Spivanka and Altigo, respectively, at SA 0.1% (showing a decline compared with the previous concentration for both the varieties). Altigo consistently showed higher levels of chromosomal abnormalities at each SA concentration compared with Spivanka, indicating greater susceptibility. The decline in abnormalities at the highest concentration (0.1%) was more pronounced in Spivanka than in Altigo, suggesting potential genotype-specific thresholds for mutagenic tolerance. A clear dose-dependent resonse was observed up to SA 0.05%, at which point abnormalities peaked. The decline at SA 0.1% suggests potential cytotoxic effects or saturation of the mutagenic process at higher concentrations. Altigo exhibited a higher susceptibility to sodium azide across all the concentrations, particularly at the 0.05% level, which induced the greatest number of abnormalities.

In both the varieties, the percentage of rare chromosomal aberrations (e.g., micronuclei, lagging chromosomes) gradually increased with concentration. At higher concentrations, stabilization or a slight decrease in the proportion of these aberrations was observed, indicating the onset of critical concentrations where the mutagen's effectiveness began to plateau or decline due to cytotoxic effects.

The genotype factor was not significant ($F = 4.51$; $F_{0.05} = 2.35$; $P = 0.03$), suggesting that the induction of rare aberrations was relatively consistent in both the varieties. Concentration had a highly significant impact on the total number of rare aberrations ($F = 342.17$; $F_{0.05} = 7.62$; $P = 7.44\times10^{-16}$), confirming a strong dose-dependent relationship. The interaction between genotype and mutagen was also significant ($F = 2.19$; $F_{0.05} = 1.87$; $P = 0.04$), indicating some variability in how different genotypes responded to increasing concentrations. The data confirm a dose-dependent increase in the proportion of rare aberrations, peaking before reaching a critical concentration where cytotoxicity limits further induction. While genotype was not the primary factor influencing the induction of rare aberrations, the significant interaction with mutagen concentration suggests that, certain genotypes might display subtle, concentration-dependent differences in their response. The slight decline at high concentrations underscores the importance of identifying an optimal concentration range to maximize mutagenic effectiveness without inducing excessive cytotoxicity.

Table 6

Spectra of chromosomal abnormalities in properly dividing mitotic cells of root tips, SA action (x ± SD, n = 800–1000)

Variety	Variant	Fragments (single + double)		Bridges (chromosomal + chromatid)		Fragments / bridges	Other (micronucleus, lagging chromosomes)		Double and more	
		n	%	n	%		N	%	n	%
Spivanka	water	2.0 ± 0.3^{a}	50.0	2.0 ± 1.0^{a}	50.0	1.0	0.0 ± 0.0^{a}	0.0	0.0 ± 0.0^{a}	0.0
Spivanka	SA 0.01%	38.0 ± 1.4^{b}	41.3	28.0 ± 1.7^{b}	30.4	1.4	11.0 ± 1.1^{b}	12.0	11.0 ± 1.1^{b}	12.0
Spivanka	SA 0.025%	71.0 ± 2.1^{c}	44.1	50.0 ± 2.4^{c}	31.1	1.4	16.0 ± 1.2^{c}	9.9	28.0 ± 2.0^{c}	17.4
Spivanka	SA 0.05%	91.0 ± 2.4^{d}	42.3	76.0 ± 3.0^{d}	35.4	1.2	22.0 ± 1.6^{d}	10.2	41.0 ± 2.4^{d}	19.1
Spivanka	SA 0.10%	68.0 ± 2.4^{c}	41.7	53.0 ± 2.4^{c}	32.5	1.3	16.0 ± 1.5^{c}	9.8	41.0 ± 2.3^{d}	25.2
Altigo	water	4.0 ± 0.2^{a}	57.1	2.0 ± 0.4^{a}	28.6	2.0	1.0 ± 0.3^{a}	14.3	0.0 ± 0.0^{a}	0.0
Altigo	SA 0.01%	59.0 ± 1.3^{b}	60.8	25.0 ± 1.5^{b}	25.8	2.4	13.0 ± 1.5^{b}	13.4	14.0 ± 2.1^{b}	14.4
Altigo	SA 0.025%	105.0 ± 2.7^{c}	62.5	45.0 ± 2.6^{c}	26.8	2.3	16.0 ± 2.4^{b}	9.5	20.0 ± 2.0^{b}	11.9
Altigo	SA 0.05%	113.0 ± 2.4^{c}	51.8	63.0 ± 3.1^{d}	28.9	1.8	31.0 ± 3.1^{c}	14.2	42.0 ± 3.0^{c}	19.3
Altigo	SA 0.10%	94.0 ± 2.2^{c}	53.4	55.0 ± 3.0^{cd}	31.3	1.7	25.0 ± 3.1^{c}	14.2	34.0 ± 3.1^{c}	19.3

Note: see Table 1.

No cells with complex chromosomal aberrations were observed in the control group, confirming the reliability of the baseline for comparative analysis. A clear and significant increase in the number of cells with complex chromosomal aberrations was observed at all the EMS concentrations, with unsubstantial differences between the varieties. Following treatment with EMS 0.025%, a significant increase in the number was observed in both the varieties. The values measured 11 in Spivanka and 14 in Altigo. At EMS 0.05%, the number further increased in both the varieties, equaling 21 in Altigo and 26 in Spivanka. At EMS 0.1%, the number of other chromosomal abnormalities further increased in both the varieties, accounting for 41 in Altigo and 44 in Spivanka. The trend demonstrates a dose-dependent response, with the highest EMS concentration inducing the greatest number of complex chromosomal aberrations. Differences between genotypes (varieties) were relatively minor, indicat-

ing that EMS-induced complex chromosomal aberrations are primarily driven by concentration rather than genotype-specific sensitivity. While all concentrations effectively induce complex chromosomal aberrations, 0.05% EMS appeared to strike a balance between effectiveness and potential cytotoxicity, making it a suitable concentration for mutagenesis programs.

A notable increase in cells with two or more aberrations was observed in both the varieties compared with the control with the following values in Spivanka and Altigo: 11 and 14 at SA 0.01%, 20 and 28 at SA 0.025%; and 41 and 42, respectively, at SA 0.05%. At SA 0.1%, the parameter measured 41 and 34 in Spivanka and Altigo, respectively, showing the same level for Spivanka and a decline for Altigo, compared with the previous concentration. Spivanka displayed a consistent increase in the number of cells with two or more aberrations, peaking at 0.05% SA, and remaining stable at 0.10%. Altigo exhibited a peak at 0.05% SA, followed by a decline at 0.10%, suggesting potential cytotoxic effects at higher concentrations. The increase in SA concentration significantly influenced the induction of cells with multiple aberrations, with 0.05% SA eliciting the most pronounced effect. The genotypic differences were evident, particularly at higher concentrations, highlighting a variable response to SA in the varieties. The decline in aberrations at 0.1% SA for Altigo suggests potential cytotoxic effects, reducing the efficiency of mutagenesis at this concentration. Spivanka demonstrated greater tolerance to SA, maintaining high induction levels even at the highest concentration, whereas Altigo exhibited susceptibility to higher SA levels.

As with the indicator of the number of cells with complex aberrations, the genotype factor was not significant in influencing the number of cells with complex aberrations ($F = 2.15$; $F_{0.05} = 2.35$; $P = 0.06$), concentration had a highly significant effect ($F = 397.18$; $F_{0.05} = 4.67$; $P = 7.17*10^{-19}$), and the interaction effect between genotype and mutagen concentration was not significant ($F = 1.31$; $F_{0.05} = 1.87$; $P = 0.07$). This suggests that the response to increasing mutagen concentration in the genotypes was similar, with no significant interaction between the two factors. The concentration of the mutagen is the dominant factor influencing the induction of cells with complex aberrations. This highlights the dose-dependent nature of the mutagenic effect.

The discriminant analysis performed for the traits associated with mutagenic effects under both EMS and SA treatments provided the following key insights: confirmation of model characteristics (Tables 7 and 8) for most traits, there was a clear, model-consistent relationship with the increase in mutagen concentration. This was evident in pollen sterility, total rate of chromosomal rearrangements, number of fragments and double fragments. The presence of bridges was an exception. While it showed a distinct trend, it did not fully conform to the model character observed in other traits. The significant impact of the genotype (variety) was noted for pollen sterility, total rate of rearrangements and number of fragments. This indicates that the genetic background of the winter wheat varieties influenced their response to mutagenic treatments, especially for these traits. The interaction of mutagen concentration and genotype was significant for presence of the bridges and cells with two or more types of chromosomal changes (complex changes). This suggests that these traits are influenced by a combination of the mutagen's intensity and the inherent genetic predisposition of the variety.

Comparative similarity in action of EMS and SA are presented in Fig. 1 and 2. Despite some differences in their specific effects on traits such as pollen sterility or the presence of the bridges, the overall model parameters indicate that EMS and SA are broadly similar in their mutagenic actions. Both mutagens exhibited predictable effects with increasing concentration, supporting their reliability in inducing mutations under controlled conditions. The discriminant analysis validated the use of these mutagens for controlled genetic modification, with a clear understanding of which traits respond predictably to changes in concentration. The significant role of genotype-mutagen interactions highlights the necessity of selecting appropriate genotypes for specific mutagenic studies to optimize outcomes (variety Altigo in our case). The exceptionality of the bridges as a trait suggests a potential for further study into its unique response mechanisms. This analysis supports the broader application of EMS and SA in mutation and cytogenetic research, with a focus on leveraging genotype-specific responses for optimized results.

The analysis derived from Figure 1 further supports the following observations regarding the effects of EMS concentrations: contrasting effects of the concentrations 0.025% and 0.05%, whereas the 0.025% and 0.05% concentrations exhibited clear and significant contrasting effects on the studied traits. These concentrations appear to optimize the balance between inducing mutagenic effects and maintaining plant material viability. The 0.10% EMS concentration differed significantly from the lower concentrations, exerting more pronounced mutagenic effects. However, this concentration tended to approach a threshold of harmfulness, leading to excessive cellular damage and reduced viability of the plant material. Despite its higher mutagenic activity, the 0.10% concentration was less favorable due to increased cell death, diminished plant material survival, and regeneration capacity. This makes it less suitable for generating viable mutant populations compared with the lower concentrations. Concentrations of 0.025% and 0.05% EMS are optimal for mutagenic studies as they maximize biodiversity and mutational changes while maintaining health of the plant material.

Table 7
Trait in model after discriminant analysis of EMS

Parameter	Genotype			Concentration		
	Wilks' - Lambda	F_{remove} (10.15)	p-level	Wilks' - Lambda	F_{remove} (4.22)	p-level
Pollen fertility	0.021	11.11	0.04	0.041	28.17	0.01
General rates	0.020	10.44	0.05	0.022	12.92	0.01
Fragments	0.003	3.14	0.10	0.022	12.51	0.01
Bridges	0.003	3.12	0.22	0.011	3.33	0.07
Other	0.002	2.91	0.11	0.019	5.10	0.03
Double and more	0.002	2.92	0.11	0.055	30.38	0.01

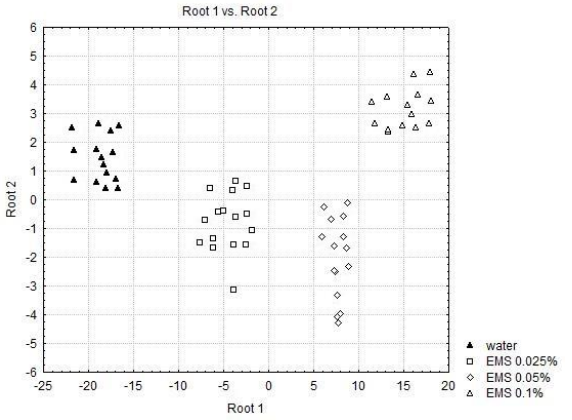

Fig. 1. Classification by canonical functions (discriminant analysis of EMS as a factor, by Mahalanobis distances)

Table 8
Trait in model after discriminant analysis of SA

Parameter	Genotype			Concentration		
	Wilks' - Lambda	F_{remove} (10.15)	P-level	Wilks' - Lambda	F_{remove} (3.89)	P-level
Pollen fertility	0.019	9.90	0.06	0.049	34.27	0.01
General rates	0.023	14.00	0.02	0.024	14,17	0.01
Fragments	0.002	2.34	0.11	0.024	14.31	0.01
Bridges	0.002	2.40	0.11	0.012	2.75	0.07
Other	0.002	2.46	0.11	0.031	22.19	0.01
Double and more	0.001	2.02	0.12	0.054	41.17	0.01

From Figure 2, it is evident that the sodium azide (SA) concentrations of 0.01% and 0.025% exhibited clearly contrasting effects, providing distinct responses in terms of mutagenic activity. By contrast, the higher concentrations of 0.05% and 0.10% demonstrated overlapping or mixed group characteristics, which raises questions about their utility in specific applications. This may lead to diminished efficiency in selecting for desired traits, as these concentrations might trigger more generalized or extreme changes, including excessive cell death or aberrant forms.

The classification results based on the influence of mutagen concentrations and varietal characteristics reflect a high level of precision and reliability in distinguishing the effects of these factors. The classification accuracy for different mutagen concentrations is robust. Even at the third and fourth concentrations, classification errors are minimal and do not significantly affects the results. This highlights the suitability of the chosen parameters for delineating the effects of varying concentrations of the mutagens. The varietal response to mutagen exposure is also effectively classified, demonstrating that the chosen indicators can reliably capture the interaction between genotype and mutagenic factors. The first three

studied parameters (e.g., pollen fertility, total frequency of rearrangements, and number of fragments) show high discriminative power. On average, 80% or more of the objects were accurately classified, confirming the resolution and reliability of these parameters in capturing the mutagenic effects and varietal differences.

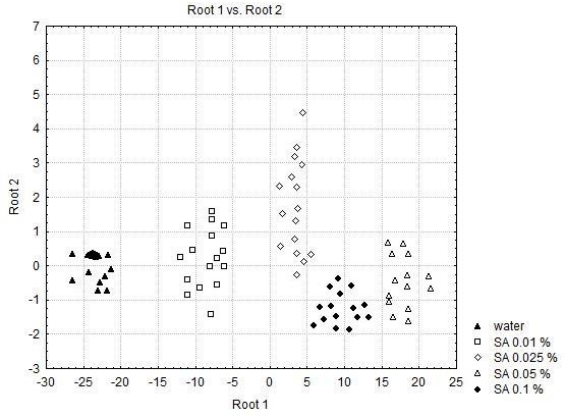

Fig. 2. Classification by canonical functions (discriminant analysis of SA as a factor, by Mahalanobis distances)

The resulting model provides a comprehensive framework for predicting the efficacy of SA as a genetically active compound in generating high levels of variability. The model effectively incorporates the variability of individual factors, offering a reliable means to forecast the outcomes of SA-induced mutagenesis. The ability to predict the potential genetic variability demonstrates the robustness of the parameters and experimental design used in this study. Altigo showed a higher suitability for SA exposure, with the results indicating a significant level of genetic variability and a strong potential for mutant form development. Spivanka - while less suitable for SA exposure (not EMS) - still falls within the confidence interval for yielding significant levels of mutant forms. However, the variability induced by SA in Spivanka is comparatively lower than in Altigo. Sodium azide demonstrated exceptionally high levels of potential genetic variability, affirming its utility as a mutagen for inducing variability in plant breeding programs. The results suggest that SA can be a valuable tool in creating diverse genetic forms, especially when targeting varieties like Altigo.

Discussion

Identifying the key components of cytogenetic variability allows for a significant simplification in selecting optimal doses and concentrations of mutagenic factors (Amri-Tiliouine et al., 2018). This process focuses on achieving specific milestones, such as maximizing activity or reaching a plateau for certain indicators (Shabani et al., 2022). In the case of chemical supermutagens, these milestones often include the induction of chromosomal fragments and double fragments, as well as the overall frequency of chromosomal rearrangements. These indicators have repeatedly been considered reliable markers for assessing the activity and efficiency of mutagenic treatments (Abdullah et al., 2018).

Studies have frequently highlighted the potentially pivotal role of micronuclei as a foundation for qualitative tests of ecogenetic activity and environmental pollution monitoring. However, while such changes are well-suited for evaluating low doses and concentrations of genetically active factors, they are less effective for primary screening in studies focused on inducible biodiversity (Surakshitha et al., 2017). This often necessitates using factor values far exceeding those encountered even in highly polluted environments (Horshchar & Nazarenko, 2024). Therefore, it is important to recognize that while rarer types of chromosomal aberrations can serve as indicators of increasing concentrations up to the point where overall mitotic activity is critically suppressed they do not adequately reflect the genotype-specific features in cytogenetic studies. Furthermore, they are insufficient for characterizing genotype interactions with particular chemical agents, highlighting the need for tailored approaches in such analyses (Cabahug et al., 2020; Shabani et al., 2022).

The use of the number of cells with complex aberrations (two or more) and less common bridge-type rearrangements shows promise for identifying genotype-mutagenic interactions in specific varieties and groups as a whole (El Oualkadi et al., 2019; Pathirana, 2021). These indicators can provide valuable insights into the nuanced effects of mutagens across different genotypes. However, questions remain regarding their practicality and reliability when compared with more established and robust indicators. Reliable markers that effectively reflect the influence of individual genotypes with a high degree of confidence may offer a more straightforward and efficient approach (Murthy et al., 2024). Further comparative studies could clarify whether these alternative methods add significant value or if they should be used as supplementary indicators in genotype-specific mutagenesis research (Kiani et al., 2022).

Reliable indicators for assessing the significance of genotype-mutagenic interactions and capturing varietal response characteristics include pollen fertility, the total frequency of chromosomal rearrangements, and the occurrence of fragments and double fragments (Rozman, 2015). These metrics serve as robust markers for evaluating the impact of mutagens on different genotypes. Moreover, increased variability in these traits among certain genotypes could justify isolating those varieties (Spencer-Lopes et al., 2018) as promising candidates for generating valuable and diverse changes through the use of mutagens such as sodium azide (Shabani et al., 2022; Von Well et al., 2023). This approach supports the targeted selection of genotypes for inducing beneficial mutations, optimizing the outcomes of mutagenic treatments.

The findings align with previous studies on this mutagen in other cereal crops, particularly regarding depressive effects in the first generation and the induction of mutations. The concentrations proposed for practical use are sufficiently distinct to generate a broad range of variability across a large group of genotypes (Muhammad et al., 2021; Oprica et al., 2023). While other effects may occur, these tend to be more limited, typically in groups with significant translocations from wild relatives or specific local forms and landraces (Surakshitha et al., 2017). Given the similar outcomes observed at high concentrations of sodium azide (0.05% and 0.1%), it might be more practical to choose one of these concentrations, with a preference for the lower one (Spencer-Lopeset et al., 2018). However, as evidenced by the response of certain variants, this choice could narrow the potential for discovering promising forms in the future, especially in less variable starting subjects (Nazarenko et al., 2019). Thus, the approach of slightly expanding the range of mutagenic factor concentrations remains valid, as even within this study, at least two forms out of eight (roughly a fourth of the sample) exhibited significant results, underscoring the importance of this variability (Nazarenko, 2020; Von Well et al., 2022).

The study of cytogenetic activity has proven its value not only as a testing system for evaluating the mutagenic potential of specific factors and determining optimal concentrations or doses but also as a means of assessing the compatibility of these factors with particular genotypes and subjects of exposure. This approach holds significant promise for enhancing the efficiency of mutation-based breeding systems, potentially yielding valuable modified materials with optimized traits (Yan et al., 2021). Over years of research, advancements in selecting appropriate concentrations, mutagenic substances, and genotypes have increased the efficiency of the mutation process by an order of magnitude (Spencer-Lopes et al., 2018). This methodology is particularly promising for generating complex biochemical mutations. Such mutations can contribute to developing food products enriched with essential microelements and biologically active compounds. Additionally, these methods support the creation of plant forms with physiologically enhanced growth and development characteristics, providing a foundation for high tolerance to adverse environmental factors (Nazarenko et al., 2022). This potential extends to innovations such as developing cereal forms with extended nitrogen reutilization periods, demonstrating the possibility of entirely new mechanisms for stress adaptation and resource efficiency.

Conclusion

To optimize the yield of promising valuable forms, the use of the variety Altigo with this ecogenetic factor is recommended, as the tested concentrations appear generally effective. However, considering the data regarding mutational variability, the third and fourth concentration ranges are likely to yield greater biodiversity. For the efficient production of economically valuable forms, the second or third concentration of SA and first or second concentrations of EMS are more favorable. At these levels, the genotype-mutagenic interaction is ascending, promoting desirable changes. Higher concentrations, although reaching a plateau in interaction effects, are more prone to induce complex mutations with additional negative traits or significant, abrupt genetic changes. These higher-dose

mutations may have limited utility, primarily for studies on genetic control mechanisms or as preliminary material for further selection. Future work will aim to validate these forecasts by assessing the emergence of desired forms in the second - third generations, particularly focusing on complex, valuable biochemical and physiological traits.

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