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Action of new epimutagen factor on winter wheat at cytogenetic level

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This study evaluated the epimutagenic potential of Nonidet P-40 (NP-40) in winter wheat by examining its ability to induce chromosomal aberrations, elucidating genotype-mutagen interactions, and assessing its predictive value at the cellular level for inducing epigenetic mutations at the plant level. Four winter wheat (*Triticum aestivum* L.) varieties – Perspektyva Odeska, Sonata Poltavska, Shpalivka, and MIP Lada – were treated with NP-40 at concentrations of 0.01%, 0.05%, 0.1%, and 0.5%. Cytogenetic effects were evaluated through pollen sterility and the frequency and spectrum of chromosomal aberrations during mitosis in root-tip cells. Results demonstrated significant genotype-specific responses to NP-40 treatment, particularly highlighting the variety MIP Lada as highly responsive. This variety showed the greatest genotype-specific sensitivity, making it a strong candidate for targeted induction of genetic variability and selection of mutant forms. Among tested concentrations, 0.5% NP-40 proved most effective, inducing substantial chromosomal aberrations while maintaining acceptable viability, thus optimizing mutation induction and limiting adverse effects. Conversely, moderate NP-40 concentrations (0.01–0.10%) were less effective, striking an inadequate balance between beneficial mutation induction and viability. Key cytogenetic indicators of genotype susceptibility included pollen fertility rates, overall chromosomal aberration frequencies, and the incidence of rare cytological anomalies such as micronuclei and lagging chromosomes. In contrast, the abundance of fragments and bridges was less analytically informative. Notably, the chromosomal aberration induction patterns by NP-40 were distinct from those observed previously with classical chemical supermutagens, varying significantly according to genetic background. These findings provide critical insights into the epimutagenic properties of NP-40, emphasizing the importance of genotype selection and concentration optimization for effective breeding strategies. Further research will integrate these cytogenetic findings with studies on hereditary variability in biochemical and physiological traits, thereby refining epimutagenic strategies and optimizing breeding programs for winter wheat improvement.

Keywords: winter wheat; chromosomal abberations; Nonidet P-40; genotype; cells; site-specific action; chemical epimutagen.

Introduction

Contemporary research in ecological genetics and genotoxicology emphasizes studying hereditary variability at both the whole-plant and cellular levels. Cytogenetic analyses, specifically evaluating chromosomal aberrations, serve as precise and objective indicators for assessing variability initiated by mutagenic exposure. Such investigateons are integral to understanding the initial effects observed in the first generation following mutagenic treatment (Oladosu et al., 2016; Nazarenko et al., 2022).

The analysis of chromosomal rearrangements provides insights into diverse genetic outcomes driven by various causative agents. These include exposure to carcinogenic substances as well as spontaneous ontogenetic mutations, both of which are primary drivers of genetic diversity and evolutionary change. Although the detailed interactions between chromosomal and gene-level mutations remain under investigation, chromosomal abnormalities have already proven critical in identifying specific chromosomes, genes, and nuclear components. Chromosomal aberrations, therefore, represent a fundamental biomarker for assessing the cellular-level impacts of natural and induced agents on living organisms (Yuan et al., 2021).

In general, spontaneous chromosomal aberrations occur at frequencies around 0.6%, though this rate strongly depends on genome structure. Cytogenetic analyses indicate that spontaneous aberrations significantly contribute to embryonic mortality (approximately 50% of embryo abortions), and certain genomic regions demonstrate higher susceptibility to structural rearrangements (Nazarenko, 2020). Contemporary studies have established a correlation between the frequency of spontaneous chromosomal aberrations and overall genomic mutability within populations, highlighting the importance of elucidating the underlying mechanisms of chromosomal alterations (Ergün et al., 2023).

Changes in chromosome structure and number result from both internal and external factors and lead directly to mutations. Investigations into plant chromosome rearrangements, including deletions, duplications, inversions, and translocations, demonstrate complex patterns of inheritance and variability (Shabani et al., 2022). Early cytogenetic studies, particularly those examining chromosomes at the pachytene stage, have highlighted the complex nature of such rearrangements, with inversions and translocations commonly observed as primary causes of hereditary variation. While paracentric inversions often occur more frequently, translocations typically hold greater significance for crop improvement efforts, owing to their beneficial impacts in cultivated species (Horshchar & Nazarenko, 2022a). These chromosomal aberrations are recognized as pivotal evolutionary mechanisms, profoundly shaping the genetic landscapes of organisms (Álvarez-Holguín et al., 2019; Yuan et al., 2021).

Two notable phenomena directly linked to chromosomal aberrations are the adaptive response and genomic instability. The adaptive response, initially demonstrated in bacterial systems and subsequently in higher organisms, is particularly evident with physical mutagens. Although hypotheses have been proposed regarding its mechanism, definitive conclusions remain elusive (Nazarenko et al., 2019; Yuan et al., 2021). Genomic instability, conversely, appears intrinsically connected to the genotype of an individual organism, particularly involving highly mutable loci. This instability remains inadequately explained by conventional principles of radiobiology or genotoxicology, such as mutagen dosage, mutagen specificity, or genotype–mutagen interactions (Nazarenko, 2020; Oprica et al., 2023).

Plants serve as advantageous model organisms for cytogenetic studies, uniquely enabling direct observation of chromosomal rearrangements during the initial mitotic divisions following mutagenic exposure. Factors influencing plant responses to mutagens include genotype differences, chromosome size, DNA repair system efficiency, and mitotic cycle duration (Oprica et al., 2023). Additionally, genetic mutability in cereal species can vary significantly, influenced by intrinsic genetic resistance mechanisms, of which only a limited number are currently understood. The capacity to monitor these variables closely in plants thus provides invaluable insights for advancing

mutateon breeding and genotoxicology research (Nazarenko et al., 2023). The establishment of stable, highly productive agrocenoses for grain crops fundamentally depends on integrating ecological and genetic variability. The ecological component primarily addresses the adaptability of newly developed forms and crop communities to specific environmental conditions. It involves the expression of novel traits and properties under diverse ecological scenarios. Employing an ecological-geographical approach is particularly essential for selecting parental materials capable of generating new forms adapted to targeted ecological niches. The genetic component defines the range and limits of variability at both the individual gene and gene-association levels (Jankowicz-Cieslak et al., 2022). This genetic variability critically influences how new traits are manifested, maintained, and stabilized during pre- and post-mutation stages. Additionally, it encompasses the intricate interactions between altered genes and their wild-type counterparts within the original genotype (Nazarenko et al., 2019). Ensuring the stable inheritance of these introduced traits across generations is vital for maintaining consistency and functionality. The interplay between ecological adaptability and genetic potential facilitates the development of grain crop varieties optimized for productivity under varied environmental conditions (Xiong et al., 2018; Chernysky & Gumentyk, 2020). Striking a balance between ecological responsiveness and genetic stability enables the creation of sustainable agricultural systems capable of adapting to evolving environmental challenges and enhancing crop performance. This integrated perspective highlights the importance of combining ecological insights with genetic innovation to improve agrocenosis productivity, stability, and resilience (Nazarenko et al., 2023).

The primary aim of this study was to investigate induced variability and biodiversity in modern winter wheat (*Triticum aestivum* L.) genotypes using an epimutagen with relatively low damaging potential. Specifically, the study focused on the several objectives: evaluating the variability and biodiversity generated by epimutagen treatment in winter wheat genotypes, particularly assessing both general variability and changes within specific trait categories; investigating the extent to which genetic background influences responses to epimutagen treatment, examining genotype-specific differences in mutation frequencies and trait variability; determining the effect of epimutagen type and dosage on the total rate of chromosomal aberrations, identifying threshold concentrations beyond which higher dosages may not proportionally increase variability (plateau effects); assessing the capability of the epimutagen to induce novel phenotypic forms exhibiting diverse and valuable agronomic traits, and evaluating how these new forms distribute across distinct trait groups; exploring the interactive relationship between wheat genotype and epimutagen dosage, focusing on identifying optimal conditions that yield maximum beneficial variability and minimize detrimental effects. This research provides critical insights into optimizing epimutagenic treatments for enhancing biodiversity and improving winter wheat breeding outcomes. Special emphasis is placed on genotype-epimutagen interactions, identifying ideal conditions to reliably generate promising wheat lines suitable for practical agricultural use. The next aim of this research was to determine the cellular-level effects of an epigenetic substance in plants and to compare its impacts with those induced by conventional physical mutagens and chemical supermutagens. The study specifically sought to characterize the variability of individual cytological parameters, evaluate the feasibility of modeling and predicting mutagenic processes, and assess the suitability of classical cytogenetic and mutagenesis methodologies for investigating epimutagenic agents.

Materials and methods

Field experiments were conducted at the experimental field of the Department of Breeding and Seed Production, Dnipro State Agrarian and Economic University. Seeds from four winter wheat varieties Perspektyva Odeska, Sonata Poltavska, Shpalivka, and MIP Lada were selected to comprehensively evaluate genotype-epimutagen interactions, accounting for the known genotype-specificity of chemical epimutagens.

For each variety, 1000 seeds per treatment were soaked in aqueous solutions of the epimutagen Nonidet P-40 (NP-40; Merck KGaA, Darmstadt, Germany) at concentrations of 0.01%, 0.05%, 0.1%, and 0.5%. Seeds soaked in distilled water served as controls. Treatment duration was maintained at 36 hours, consistent with standard chemical mutagenesis protocols. The selected concentration range was informed by previous studies to identify threshold effects on seed survival, mutation induction and variability. A total of 20 treatment variants were established to comprehensively evaluate genotype-specific mutagenic interactions, considering the pronounced genotype-specificity typically exhibited by chemical epimutagens.

Pollen samples for fertility analysis were collected from well-developed main spikes of first-generation (M_1) plants during the flowering phase. Each genotype-treatment combination involved analyzing at least 25 anther preparations. Pollen sterility was assessed using acetocarmine staining and observed under a Micromed XS-3330 microscope (Micromed, Poltava, Ukraine) equipped with a 5-megapixel camera.

Cytological analyses were conducted on mitotic cells from primary wheat roots harvested during late metaphase and early anaphase. Treated seeds were germinated on filter paper moistened with distilled water in Petri dishes, incubated at 25 °C. Root tips (0.8–1.0 cm) were fixed in Clark's fixative (3 parts 96% ethanol and 1 part glacial acetic acid) for 24 hours, followed by storage in 70% ethanol at 2 °C. Approximately 25–30 roots per treatment were processed. Temporary squash preparations were prepared using acetocarmine staining after maceration in 45% acetic acid, following standard cytogenetic techniques. Microscopic examination (600× magnification) was performed on at least 1000 cells per treatment to identify chromosomal aberrations, including fragments, dicentric chromosomes, micronuclei, and lagging chromosomes (Spencer-Lopes et al., 2018).

Statistical analyses were performed using Statistica 10.0 software (TIBCO, Palo Alto, USA). Data are presented as means ± standard deviations (x ± SD). Differences between treatments were evaluated through one-way ANOVA, with Tukey's HSD post-hoc test for pairwise comparisons, considering differences statistically significant at $P < 0.05$. Data normality was verified using the Shapiro-Wilk W-test.

Results

The initial phase of research on the cellular effects of the epimutagen NP-40 involved selecting mature anthers from medium spikelets of normally developed main spikes, followed by microscopic examination to determine the ratio of fertile to sterile pollen grains (Table 1). The fertility analysis of 16 mutant wheat populations exposed to varying concentrations of NP-40 revealed a significant, positive linear correlation between increased NP-40 concentration and pollen sterility ($r = 0.73$). Statistical analysis confirmed that fertility decreased significantly with increasing NP-40 concentrations ($F = 77.55$; $F_{0.05} = 3.25$; $P = 1.81 \times 10^{-8}$). Nonetheless, even the highest NP-40 concentration (0.5%) did not reach the lethal dose threshold (LD_{50}), maintaining a relatively high plant viability of 75–80%. At lower concentrations (0.01% and 0.05%), viability remained consistently high (90–99%), aligning with the international classification of low mutagenic doses.

Significant varietal differences in sensitivity to NP-40-induced sterility were detected ($F = 5.96$; $F_{0.05} = 3.49$; $P = 0.01$), confirmed by post-hoc Tukey tests. Specifically, the variety MIP Lada exhibited heightened sensitivity, showing a statistically significant reduction in fertility even at the lowest NP-40 concentration. However, fertility levels at this concentration remained within acceptable viability limits, suggesting that the observed effect, although statistically significant, is biologically tolerable.

Notably, the first concentration (0.01%) did not induce a statistically significant decrease in fertility in the varieties Perspektyva Odeska, Sonata Poltavska, and Shpalivka. Concentrations of 0.01%, 0.05%, and 0.1% maintained relatively high fertility (80–90%), classifying them as low-impact concentrations suitable for subsequent genetic variability studies. The highest concentration (0.5%) remained at an optimal threshold for maximizing induced variability without sig-

nificantly compromising viability, consistent with international standards (viability ≥ 75–80%). This concentration thus provides sufficeent plant material for evaluating hereditary variability in subsequent generations. Moreover, a significant genotype-by-mutagen interaction was observed ($F = 5.95$; $F_{0.05} = 5.10$; $P = 0.04$), highlighting genotype-dependent variability in response to NP-40 treatment. These results underscore the importance of genotype selection when using NP-40 epimutagen treatments to optimize induced genetic variability in wheat breeding programs.

Table 1
NP-40 action on pollen fertility trait of first-generation spikes (x ± SD, n = 25)

Variety	Control	NP-40 0.01%	NP-40 0.05%	NP-40 0.1%	NP-40 0.5%
Perspektyva Odeska	99.17 ± 0.64^{a}	98.34 ± 0.78^{a}	91.34 ± 1.00^{b}	88.75 ± 1.17^{c}	80.41 ± 1.34^{d}
Sonata Poltavska	99.14 ± 0.66^{a}	99.01 ± 0.89^{a}	91.13 ± 0.69^{b}	88.62 ± 0.52^{c}	80.60 ± 1.90^{d}
Shpalivka	99.11 ± 0.92^{a}	98.83 ± 0.66^{a}	90.99 ± 0.77^{b}	88.13 ± 0.98^{c}	81.21 ± 1.55^{d}
MIP Lada	99.43 ± 0.95^{a}	97.34 ± 0.61^{b}	89.91 ± 0.99^{c}	84.14 ± 1.16^{d}	76.44 ± 2.05^{e}

Note: values within a row followed by different letters differ significantly ($P < 0.05$), as determined by Tukey's HSD test with Bonferroni correction; comparisons were made separately for each variety across NP-40 treatments.

NP-40 epimutagen treatment resulted in a clear dose-dependent reduction in pollen fertility across all studied winter wheat varieties. The variety MIP Lada exhibited the greatest sensitivity to NP-40, with pollen fertility sharply declining to 76.4% at the highest concentration (0.5%), significantly lower than other genotypes. In contrast, Perspektyva Odeska, Sonata Poltavska, and Shpalivka demonstrated moderate yet consistent decreases in pollen fertility at increasing NP-40 concentrations. Statistically significant differences emerged predominantly at concentrations ≥0.05%, highlighting genotype-specific thresholds for epimutagen-induced fertility disruption. These findings emphasize the importance of genotype selection in breeding programs employing epimutagenic agents and underline the value of carefully managing NP-40 concentrations to balance induced genetic variability with plant viability.

The analysis of chromosomal rearrangement frequencies following exposure to NP-40 (Table 2) revealed distinct, concentration-dependent effects of this epimutagen, clearly establishing threshold concentrations associated with the onset and progression of chromosomal abnormalities. Factor analysis revealed a statistically significant effect of genotype, with clear differences observed between the varieties ($F = 7.11$; $F_{0.05} = 3.49$; $P = 0.007$). A strong concentration-dependent response to NP-40 was confirmed, as increasing concentrations of the epimutagen consistently led to higher frequencies of chromosomal rearrangements ($F = 73.12$; $F_{0.05} = 3.25$; $P = 1.01 \times 10^{-8}$). However, the genotype × concentration interaction was not significant ($F = 3.79$; $F_{0.05} = 5.10$; $P = 0.08$), indicating that the mutagenic effect of NP-40 was relatively consistent across different wheat genotypes.

In control samples, the frequency of chromosomal rearrangements was low, ranging from 0.9% to 1.2%. At 0.01% NP-40, rearrangement frequencies increased and varied from 1.69% in Perspektyva Odeska to 3.49% in MIP Lada. With a 0.05% concentration, frequencies further increased, ranging from 3.19% in Shpalivka to 4.67% in MIP Lada. At 0.1%, frequencies ranged from 4.67% (Sonata Poltavska) to 5.89% (MIP Lada), demonstrating a significant rise with concentration. At the highest tested concentration (0.5%), rearrangement frequencies peaked at 5.59% (Sonata Poltavska) and 7.76% (MIP Lada), approaching a cytotoxic threshold, as reflected by substantial increases in cell mortality.

The variety MIP Lada consistently showed higher susceptibility to NP-40, with cytogenetic damage at 0.5% potentially compromising plant viability in subsequent generations. Despite these differences, all tested varieties remain suitable for further mutagenesis studies, provided that NP-40 concentration is carefully controlled to optimize induced genetic variability while minimizing cytotoxic effects.

Exposure to NP-40 resulted in a clear dose-dependent increase in chromosomal abnormalities across all studied varieties. At the highest NP-40 concentration (0.5%), the variety MIP Lada displayed the most substantial increase (7.76%), confirming its heightened sensitivity to NP-40 treatment. Conversely, Perspektyva Odeska, Sonata Poltavska, and Shpalivka showed moderate yet significant increases in aberration rates at equivalent concentrations. The identified threshold concentration for significant chromosomal damage appeared to be between 0.05% and 0.1% for all varieties. These results confirm the capacity of NP-40 as an effective epimutagenic agent to induce quantifiable genetic variability while maintaining acceptable viability levels. The observed genotype-dependent responses highlight the importance of careful genotype selection to maximize the effectiveness of NP-40-based mutation breeding programs.

Table 2
Overall frequency of chromosomal abnormalities in mitotically dividing root-tip cells following NP-40 Treatment (x ± SD, n = 1000)

Variety	Variant	Cells analyzed (n)	Aberrant cells	
			number	%
Perspektyva Odeska	water	1001	12	1.20 ± 0.09^{a}
	NP-40 0.01%	1007	17	1.69 ± 0.10^{b}
	NP-40 0.05 %	1006	38	3.78 ± 0.14^{c}
	NP-40 0.1 %	1005	51	5.07 ± 0.21^{d}
	NP-40 0.5 %	1000	59	5.90 ± 0.22^{e}
Sonata Poltavska	water	1003	9	0.90 ± 0.09^{a}
	NP-40 0.01%	1002	28	2.79 ± 0.11^{b}
	NP-40 0.05 %	1010	39	3.86 ± 0.12^{c}
	NP-40 0.1 %	1007	47	4.67 ± 0.17^{d}
	NP-40 0.5 %	1001	56	5.59 ± 0.22^{e}
Shpalivka	water	1006	9	0.89 ± 0.08^{a}
	NP-40 0.01%	1004	23	2.29 ± 0.14^{b}
	NP-40 0.05 %	1002	32	3.19 ± 0.17^{c}
	NP-40 0.1 %	1007	50	4.97 ± 0.20^{d}
	NP-40 0.5 %	1008	59	5.87 ± 0.22^{e}
MIP Lada	water	1003	9	0.90 ± 0.08^{a}
	NP-40 0.01%	1004	35	3.49 ± 0.17^{b}
	NP-40 0.05 %	1006	47	4.67 ± 0.20^{c}
	NP-40 0.1 %	1001	59	5.89 ± 0.23^{d}
	NP-40 0.5 %	1005	78	7.76 ± 0.27^{e}

Note: values within a row followed by different letters differ significantly ($P < 0.05$), as determined by Tukey's HSD test with Bonferroni correction. Comparisons were made separately for each variety across NP-40 treatments.

The analysis of chromosomal alterations induced by mutagens yielded comprehensive insights into the types and frequencies of aberrations present in mitotic cells (Table 3). The following categories of chromosomal abnormalities were identified: fragments and double fragments, these were the most frequent and quantifiable indicators of chromosomal damage, representing breaks in the chromosomal structure ; Bridges observed in various morphological forms, bridges signified improper segregation of chromosomes during anaphase, often resulting from dicentric chromosomes or unresolved recombination events ; Micronuclei, these structures indicated the presence of lagging chromosomal material that failed to incorporate into the daughter nuclei, serving as a sensitive marker of genotoxic stress ; Lagging chromosomes, detected during anaphase, they highlighted disruptions in chromosomal movement and spindle attachment during cell division. Complex aberrations – cells exhibiting two or more distinct chromosomal rearrangements were classified as complex, reflecting a higher degree of cytogenetic instability and a compounded mutagenic effect. To further elucidate the underlying mechanisms of mutagenic action, the ratio of fragments to bridges was calculated. This ratio served as a diagnostic metric to assess the dominant mode of chromosomal disruption induced by different mutagens, thereby offering additional insights into their specific impact on chromosomal integrity.

The number of chromosomal fragments and double fragments in the control group was minimal but consistently detectable, providing a reliable baseline for assessing epimutagenic effects. Each concentration of NP-40 resulted in a significant increase in chromosomal fragment numbers compared to the control group. Even at the lowest NP-40 concentration (0.01%), a distinct and statistically significant increase in chromosomal fragmentation was observed, indicating the early onset of mutagenic activity. A similar trend was noted with NP-40. At 0.01% NP-40, fragment numbers increased across all genotypes, ranging from 8.0 in Perspektyva Odeska to 16.0 in Sonata Poltavska. When the concentration was raised to 0.05%, fragmentation levels rose further, with values between 14.0 (Shpalivka) and 21.0 (Perspektyva Odeska and MIP Lada), suggesting a more pronounced epimutagenic effect. At 0.1% NP-40, fragment numbers plateaued, ranging from 21.0 (Sonata Poltavska) to 26.0 (Perspektyva Odeska), while at 0.5% NP-40, values stabilized further between 24.0 (Sonata Poltavska) and 30.0 (MIP Lada).

These data suggest a threshold effect, whereby increasing the NP-40 concentration beyond 0.1% does not proportionally elevate chromosomal fragmentation. The variety MIP Lada consistently exhibited the highest sensitivity to NP-40-induced chromosomal breakage across all concentrations. Lada showed a notably higher induction of fragments at lower NP-40 concentrations, reflecting greater sensitivity to mutagenic stress ($F = 4.17$; $F_{0.05} = 2.48$; $P = 0.02$). For all varieties , lower concentrations (0.01% and 0.05%) were effective in inducing substantial chromosomal damage, highlighting their utility in inducing variability without exceeding toxicity thresholds. However, at 0.1% NP-40 and above, these differences became negligible, likely due to the saturation of the mutagenic response.The consistent and significant elevation in both fragments and double fragments across treatments reinforces the epimutagenic potential of NP-40 and its applicability for controlled induction of chromosomal changes in mutation breeding programs.

Table 3

Spectra of chromosomal abnormalities (%) observed in mitotically dividing root-tip cells treated with different NP-40 concentrations ($x \pm SD$, $n = 1000$)

Variety	Variant	Fragments (single + double)		Bridges (chromosomal + chromatid)		Fragments / bridges	Other (micronuclei, lagging chromosomes)		Double and more	
		n	%	n	%		n	%	n	%
Perspektyva Odeska	water	7.0 ± 0.1^{a}	70.0	5.0 ± 0.1^{a}	50.0	1.4	0.0 ± 0.0^{a}	0	0.0 ± 0.0^{a}	0.0
	NP-40 0.01%	8.0 ± 0.5^{a}	50.0	6.0 ± 0.3^{a}	37.5	1.3	3.0 ± 0.3^{b}	18.8	2.0 ± 0.1^{b}	12.5
	NP-40 0.05 %	21.0 ± 0.8^{b}	52.5	10.0 ± 0.4^{b}	25.0	2.1	7.0 ± 0.6^{c}	17.5	2.0 ± 0.1^{b}	5.0
	NP-40 0.1 %	26.0 ± 0.9^{c}	50.9	13.0 ± 0.5^{c}	25.5	2.0	12.0 ± 0.8^{d}	23.5	4.0 ± 0.3^{c}	7.8
	NP-40 0.5 %	26.0 ± 0.9^{c}	42.6	13.0 ± 0.5^{c}	21.3	2.0	20.0 ± 1.0^{e}	32.8	3.0 ± 0.3^{bc}	4.9
Sonata Poltavska	water	4.0 ± 0.1^{a}	40.0	5.0 ± 0.1^{a}	50.0	0.8	0.0 ± 0.0^{a}	0.00	0.0 ± 0.0^{a}	0.0
	NP-40 0.01%	16.0 ± 0.6^{b}	76.2	9.0 ± 0.3^{b}	42.9	1.8	3.0 ± 0.3^{b}	14.3	3.0 ± 0.2^{b}	14.3
	NP-40 0.05 %	18.0 ± 0.9^{c}	64.2	11.0 ± 0.5^{c}	39.3	1.6	10.0 ± 0.5^{c}	35.7	5.0 ± 0.3^{c}	17.9
	NP-40 0.1 %	21.0 ± 1.0^{d}	47.7	13.0 ± 0.6^{d}	36.4	1.3	10.0 ± 0.5^{c}	22.7	4.0 ± 0.3^{bc}	9.1
	NP-40 0.5 %	24.0 ± 1.0^{e}	48.0	17.0 ± 0.7^{e}	34.0	1.4	15.0 ± 0.7^{d}	30.0	6.0 ± 0.4^{d}	12.0
Shpalivka	water	5.0 ± 0.2^{a}	62.5	4.0 ± 0.1^{a}	50.0	1.3	0.0 ± 0.0^{a}	0.0	0.0 ± 0.0^{a}	0.0
	NP-40 0.01%	13.0 ± 0.5^{b}	68.4	7.0 ± 0.2^{b}	36.8	1.9	3.0 ± 0.3^{b}	15.8	1.0 ± 0.1^{b}	5.3
	NP-40 0.05 %	14.0 ± 0.6^{b}	50.0	10.0 ± 0.6^{c}	35.7	1.4	8.0 ± 0.5^{c}	28.6	4.0 ± 0.2^{c}	14.3
	NP-40 0.1 %	22.0 ± 1.0^{c}	44.9	18.0 ± 0.7^{d}	36.7	1.2	10.0 ± 0.6^{d}	20.4	7.0 ± 0.4^{d}	14.3
	NP-40 0.5 %	25.0 ± 1.0^{d}	46.3	20.0 ± 0.9^{d}	37.0	1.3	14.0 ± 0.7^{e}	25.9	7.0 ± 0.4^{d}	12.9
MIP Lada	water	4.0 ± 0.2^{a}	57.1	4.0 ± 0.1^{a}	57.1	1.0	1.0 ± 0.0^{a}	14.3	0.0 ± 0.0^{a}	0.0
	NP-40 0.01%	14.0 ± 0.8^{b}	38.9	11.0 ± 0.3^{b}	30.6	1.3	10.0 ± 0.5^{b}	27. 8	4.0 ± 0.2^{b}	11.1
	NP-40 0.05 %	21.0 ± 1.0^{c}	46.7	15.0 ± 0.4^{c}	33.3	1.4	11.0 ± 0.5^{b}	24.4	5.0 ± 0.3^{c}	11.1
	NP-40 0.1 %	22.0 ± 1.0^{c}	36.1	20.0 ± 0.9^{d}	32.8	1.1	17.0 ± 0.7^{c}	27.9	10.0 ± 0.5^{d}	16.4
	NP-40 0.5 %	30.0 ± 1.2^{d}	37.5	29.0 ± 1.2^{e}	36.3	1.0	19.0 ± 0.9^{c}	23.8	12.0 ± 0.7^{e}	15.0

Note: values within a row followed by different letters differ significantly ($P < 0.05$), as determined by Tukey's HSD test with Bonferroni correction; comparisons were made separately for each variety across NP-40 treatments.

The analysis of chromosomal fragment induction provides valuable insights into the individual and interactive effects of genotype, mutagen concentration, and their interaction. The effect of genotype on fragment formation was statistically significant ($F = 3.34$; $F_{0.05} = 2.40$; $P = 0.03$), indicating that the inherent genetic constitution of the studied varieties significantly influences the extent of chromosomal fragmentation under mutagenic stress. A highly significant effect of mutagen concentration was also observed ($F = 26.14$; $F_{0.05} = 2.90$; $P = 3.34 \times 10^{-4}$), confirming a clear dose-dependent relationship – with higher concentrations of NP-40 consistently inducing greater levels of chromosomal fragmentation across all genotypes tested. Notably, the interaction between genotype and mutagen concentration was also statistically significant ($F = 5.34$; $F_{0.05} = 5.11$; $P = 0.05$). This finding demonstrates that genotypes responded differently to increasing NP-40 concentrations, underscoring the importance of genotype-specific sensitivity in mutagenesis research. These results reinforce the conclusion that genetic background plays a critical role in determining the extent and nature of chromosomal damage under epimutagenic influence. While the overall trend confirmed a dose-dependent increase in fragment induction, the non-uniform response across genotypes points to complex genotype-mutagen interactions that must be considered when optimizing mutagenesis protocols for specific breeding objectives.

The analysis of chromatid and chromosomal bridge induction under NP-40 treatment reveals clear dose-dependent trends and notable genotypic variability in response to mutagenic exposure. At 0.01% NP-40, bridge formation increased significantly compared to the control, ranging from 6.0 in Perspektyva Odeska to 11.0 in MIP Lada. A marked rise in bridge induction was observed at 0.05% NP-40, with values ranging from 10.0 (Perspektyva Odeska and Shpalivka) to 15.0 (MIP Lada), indicating enhanced chromosomal instability. At 0.1% NP-40, the highest bridge induction rates were recorded for most varieties, with counts ranging from 13.0 (Perspektyva Odeska and Sonata Poltavska) to 20.0 (MIP Lada), suggesting a peak in mutagenic activity. However, at 0.5% NP-40, a plateau in bridge frequency was observed (for two varieties), with bridge numbers ranging from 13.0 (Perspektyva Odeska) to 29.0 (MIP Lada). This reversal indicates the onset of a cytotoxic threshold, beyond which the mutagenic efficiency of NP-40 is likely diminished. The plateau in bridge formation may be attributed to increased cell mortality or a suppression of mitotic activity, which limits the occurrence of further chromosomal rearrangements. Across all concentrations, MIP Lada consistently exhibited higher frequencies of chromosomal bridges, suggesting a greater susceptibility to chromosomal mis-segregation and mitotic disruption. These findings underscore the genotype-dependent nature of NP-40-induced chromosomal abnormalities and point to MIP Lada as a sensitive model for evaluating mutagen-induced genomic instability.

The analysis of the percentage of total chromosomal aberrations and bridge induction reveals consistent trends, emphasizing the inter-

play between mutagen concentration and genotypic variability in winter wheat. The effect of genotype on bridge induction was not statistically significant ($F = 2.11$; $F_{0.05} = 2.40$; $P = 0.06$), indicating that differences between varieties in bridge formation were relatively minor. In contrast, the effect of mutagen concentration was statistically significant ($F = 13.19$; $F_{0.05} = 2.90$; $P = 0.007$), confirming a clear dose-dependent relationship – with higher concentrations of NP-40 leading to increased bridge induction. The interaction between genotype and mutagen concentration was also not significant ($F = 2.99$; $F_{0.05} = 5.11$; $P = 0.08$), suggesting that specific combinations of genotype and mutagen dosage did not significantly alter the pattern of bridge formation. This implies that, although individual genotypes may vary slightly in response, mutagen concentration remains the primary driver of bridge induction, with limited genotype-specific effects in this context. These results reinforce the conclusion that bridge induction is largely concentration-dependent, and that genotypic variation plays a secondary role in determining the frequency of this specific type of chromosomal aberration.

At higher mutagen concentrations, there is a reduction in site specificity, wherein mutagens may lose their targeted action and begin affecting broader genomic regions. This shift results in a general increase in total chromosomal aberrations, diminishing the precision of mutagenic effects. The genetic background plays a crucial role in modulating the response to mutagenic agents, as evidenced by distinct differences in bridge induction levels between varieties. Although bridge formation occurs less frequently than other types of chromosomal aberrations, it remains a critical marker of chromosomal instability, particularly related to mis-segregation events during mitosis. The overall lower incidence of bridges, relative to fragments, underscores the dominant mode of action of NP-40, which primarily induces single-strand breaks or localized chromosomal disruptions.

Moderate NP-40 concentrations appear to be optimal for inducing targeted genetic variability, striking a balance between mutagenic effectiveness and the avoidance of widespread genomic damage. This concentration-dependent behavior suggests that careful calibration of epimutagen dosage is essential for maximizing mutation efficiency while minimizing cytotoxic effects. Importantly, genotypic selection is a key factor in the design of mutagenesis experiments, as some varieties exhibit lower chromosomal stability under mutagenic stress. For instance, MIP Lada showed heightened sensitivity across several parameters, making it a suitable model for assessing mutagenic efficiency and tolerance thresholds. Across all tested genotypes, the fragment-to-bridge ratio remained relatively stable across concentrations, indicating that chromosomal architecture is less prone to variability in terms of this ratio under NP-40 treatment. The initial increase in chromosomal fragments, followed by a plateau (for one variety) at higher concentrations, suggests that certain genomic loci are more responsive to epimutagenic activity at moderate doses, while higher concentrations may suppress further induction due to toxicity or cellular arrest. These findings highlight the strategic potential of using site-specific variability in mutagen responses to enhance targeted genetic modification, ultimately improving the efficacy of mutation breeding programs.

Rare chromosomal aberrations were infrequent in the control group, with only a single case recorded in MIP Lada, thereby establishing a reliable baseline for comparative analysis. A clear concentration-dependent increase in rare aberration frequency was observed under NP-40 treatment. At 0.01% NP-40, aberration frequencies increased notably compared to the control, ranging from 3.0 in three varieties to 10.0 in MIP Lada. With 0.05% NP-40, the induction further rose, ranging from 7.0 (Perspektyva Odeska) to 11.0 (MIP Lada). At 0.1% NP-40, all varieties exhibited elevated levels of rare aberrations, ranging from 10.0 (Sonata Poltavska) to 17.0 (MIP Lada), reflecting a continued upward trend. The highest frequency of rare aberrations was observed at 0.5% NP-40, with values from 14.0 (Shpalivka) to 20.0 (Perspektyva Odeska), representing the sharpest increase across concentrations. However, a slight decline in some varieties at this concentration suggests the onset of cytotoxicity, which may limit further mutagenic efficiency. Although MIP Lada consistently exhibited higher levels of rare chromosomal abnormalities across most NP-40 concentrations, it did not show the highest frequency at 0.5%, marking an exception to the trend. Interestingly, the greatest number of abnormalities in MIP Lada was recorded at 0.05% NP-40, suggesting a concentration-specific peak in mutagenic responsiveness. The genotype effect was not statistically significant ($F = 2.17$; $F_{0.05} = 2.40$; $P = 0.06$), indicating that rare aberration induction was relatively consistent across varieties. The effect of NP-40 concentration was highly significant ($F = 12.17$; $F_{0.05} = 2.90$; $P = 0.008$), confirming a strong dose-dependent relationship. The genotype × concentration interaction was significant ($F = 5.19$; $F_{0.05} = 5.11$; $P = 0.05$), suggesting non-uniform genotypic responses to NP-40 and indicating subtle genotype-specific sensitivities under different mutagen doses.

The proportion of rare aberrations increased consistently with rising NP-40 concentrations, peaking near a critical threshold. Beyond this point, cytotoxic effects may limit further induction, highlighting the importance of balancing mutagenic efficiency and cellular viability. While genotype was not the dominant factor, the significant interaction term emphasizes the need for genotype-aware mutagenesis strategies, particularly when working near cytotoxic limits. In conclusion, NP-40-induced rare aberrations exhibit a robust dose-dependent pattern, with the highest mutagenic efficiency occurring before the onset of cytotoxicity. Although genotypic differences are relatively minor, they may influence outcomes under specific concentrations. Thus, precise concentration selection remains essential for optimizing epimutagenic protocols and maximizing genetic variability without compromising cell survival.

No complex chromosomal aberrations were observed in the control group, confirming a stable cytogenetic baseline for comparison. A statistically significant increase in cells exhibiting two or more aberrations was recorded following NP-40 treatment, with the frequency rising in a dose-dependent manner across all tested genotypes. At 0.01% NP-40, the frequency of complex aberrations ranged from 1.0 in Shpalivka to 4.0 in MIP Lada. At 0.05%, values increased to 2.0 (Perspektyva Odeska) and 5.0 (MIP Lada), indicating enhanced cytogenetic instability. A sharp increase was observed at 0.1% NP-40, with frequencies ranging from 4.0 (Perspektyva Odeska and Sonata Poltavska) to 10.0 (MIP Lada). At 0.5%, a further rise was noted in MIP Lada (12.0), while some varieties showed a decline, suggesting the onset of cytotoxic effects at higher concentrations. MIP Lada consistently demonstrated the highest frequency of complex aberrations, peaking at 0.5% NP-40, and maintaining elevated levels even at 0.1%, suggesting both higher susceptibility and resilience to mutagenic stress. In contrast, other varieties exhibited a plateau or decline in aberration frequency at elevated concentrations, likely due to cytotoxic thresholds limiting further chromosomal damage.

From these results, 0.05% NP-40 appears to be the most effective concentration for inducing complex chromosomal aberrations across genotypes, balancing mutagenic efficiency with minimal cytotoxicity. Concentrations beyond 0.1% may reduce mutagenic efficiency due to increased cell lethality. Statistical analysis supported next trends: the genotype factor was not significant ($F = 1.56$; $F_{0.05} = 2.40$; $P = 0.09$), indicating that genotypic differences do not significantly influence the frequency of complex aberrations. The effect of mutagen concentration was highly significant ($F = 67.99$; $F_{0.05} = 2.90$; $P = 1.98 \times 10^{-19}$), confirming it as the dominant factor influencing complex chromosomal instability. The genotype × concentration interaction was not significant ($F = 1.99$; $F_{0.05} = 5.11$; $P = 0.09$), suggesting a uniform response across varieties to increasing mutagen concentrations. These findings reinforce the dose-dependent impact of NP-40 on chromosomal complexity, and highlight the importance of carefully selecting mutagen concentrations in future protocols to optimize efficiency while minimizing cytotoxic effects. Although genotype had minimal influence on complex aberration induction, its role in broader mutagenic responses warrants further investigation in genotype-specific breeding strategies.

The cytogenetic analysis of wheat root-tip cells exposed to NP-40 revealed a clear, concentration-dependent increase in chromosomal abnormalities across all studied varieties. The spectrum of induced aberrations included chromosomal fragments, chromosome and chro-

matid bridges, micronuclei, lagging chromosomes, and complex aberrations (cells with multiple aberrations). Fragments consistently represented the predominant type of aberration across most treatments, with their proportion typically increasing at moderate concentrations (0.05–0.10%) before stabilizing at the highest NP-40 concentration (0.5%). Bridges also increased significantly but generally to a lesser extent, resulting in an increased fragment-to-bridge ratio at moderate NP-40 concentrations, particularly noticeable in Perspektyva Odeska.

Micronuclei and lagging chromosomes, typically indicators of significant cellular stress, markedly increased at higher NP-40 concentrations (0.1% and 0.5%). This indicates enhanced genotoxic stress, particularly at the highest concentration, where their occurrence represented a substantial portion (up to ~33%) of total aberrations.

Complex aberrations, indicative of severe chromosomal damage, were observed primarily at higher NP-40 concentrations, with MIP Lada demonstrating the highest frequency (15% at NP-40 0.5%). This variety showed exceptional sensitivity to NP-40-induced chromosomal damage across all aberration types.

Overall, varieties showed genotype-specific responses: MIP Lada consistently exhibited greater susceptibility, whereas Perspektyva Odeska, Sonata Poltavska, and Shpalivka demonstrated intermediate yet distinct sensitivity patterns. These genotype-dependent responses underscore the importance of tailored selection of wheat varieties in breeding programs utilizing NP-40 as an epimutagenic agent. These findings emphasize that NP-40 induces a unique spectrum of chromosomal aberrations distinct from traditional mutagens, highlighting its potential for targeted induction of genetic variability in mutation breeding strategies.

The discriminant analysis presented in Table 4, conducted for traits associated with mutagenic effects under NP-40 treatment, revealed several key findings. A clear and consistent relationship was observed between increasing NP-40 concentrations and several key cytogenetic traits, including pollen sterility, total frequency of chromosomal rearrangements, number of chromosomal fragments, other types of aberrations (e.g., micronuclei and lagging chromosomes). These traits exhibited predictable, concentration-dependent trends, confirming their reliability as indicators of mutagenic activity. Their strong loading in the discriminant model underscores their value in assessing mutagenic response and classifying treatment levels. Although chromosomal bridges (both chromatid and chromosome types) also showed a concentration-related trend, their behavior deviated from the model-consistent pattern exhibited by the other traits. This inconsistency suggests that bridge formation may be influenced by mechanisms less directly tied to mutagen concentration, such as delayed chromatid separation, telomere fusion, or differences in DNA repair fidelity. Therefore, bridges may not serve as robust standalone indicators of mutagenic load under NP-40 treatment. The variety (genotype) factor did not significantly influence most cytogenetic traits analyzed, with the notable exception of pollen fertility, which exhibited a statistically significant genotype effect ($P < 0.05$). This implies that NP-40-induced effects are generally consistent across wheat genotypes in terms of chromosomal damage, though fertility-related sensitivity may be more genotype-dependent. A significant interaction was identified between genotype and mutagen concentration for "other" chromosomal changes, specifically micronuclei and lagging chromosomes. This suggests that the formation of such complex aberrations is influenced by both the intensity of the mutagenic factor and the genetic background of the variety. It highlights a unique genotype-mutagen interplay, possibly linked to differences in chromatin structure, spindle checkpoint function, or DNA repair systems. These results affirm that most chromosomal aberration traits induced by NP-40 follow predictable, dose-dependent patterns and can be effectively used as cytogenetic markers of mutagenic exposure. While genotypic effects are generally minimal, their role in modulating more complex aberrations – such as micronuclei and laggards – should not be overlooked, especially in breeding contexts. The findings support the strategic application of NP-40 in mutation breeding and cytogenetic screening, with emphasis on selecting appropriate marker traits for reliable assessment of mutagenic impact.

The discriminant analysis (Fig. 1) clearly separates the treatment groups based on chromosomal aberration profiles induced by NP-40 treatments. The first discriminant root (Root 1) effectively differentiates the highest NP-40 concentration (0.5%, black symbols) from all other treatments, indicating its markedly distinct cytogenetic effect. Lower NP-40 concentrations (0.01%, 0.05%, and 0.1%) form intermediate and partially overlapping clusters, illustrating their comparatively moderate yet progressively increasing mutagenic effects.

Table 4
Model traits for discriminant functions NP-40

Parameter	Genotype action			Concentrations action		
	Wilks' - Lambda	F_{remove} (3.53)	P-level	Wilks' - Lambda	F_{remove} (4.52)	P-level
Pollen fertility	0.016	11.17	0.01	0.15	9.34	0.01
General rates	0.06	1.37	0.26	0.13	5.74	0.02
Fragments	0.06	2.11	0.11	0.09	1.14	0.34
Bridges	0.06	1.72	0.18	0.09	1.55	0.19
Other	0.002	2.01	0.14	0.11	5.10	0.03
Double and more	0.06	1.12	0.35	0.09	0.75	0.56

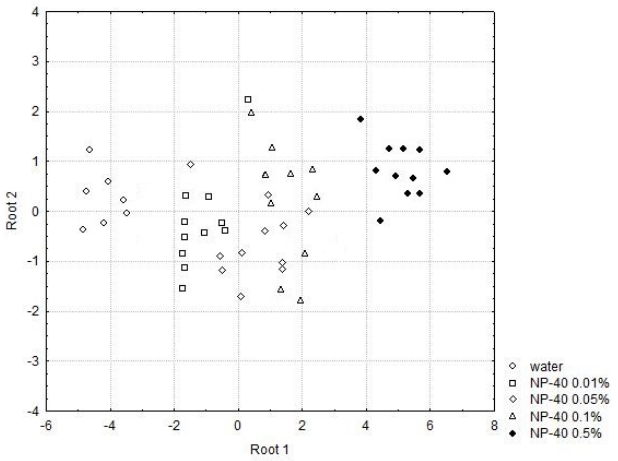

Fig. 1. Model for canonical functions (for concentrations of NP-40 action)

The control group (water treatment, open circles) is distinctly separated on the negative side of Root 1, reflecting minimal chromosomal aberrations. This pattern emphasizes a clear, dose-dependent cytogenetic response to NP-40. Thus, discriminant analysis confirms that NP-40 concentrations produce distinct chromosomal aberration patterns, supporting its potential use in controlled mutagenesis strategies.

The discriminant analysis (Fig. 2) distinctly separates the winter wheat varieties based on their chromosomal aberration responses to NP-40 treatments. The genotype MIP Lada (triangles) is clearly isolated from the other varieties along Root 1, underscoring its markedly different and higher susceptibility to NP-40-induced cytogenetic effects. In contrast, the genotypes Perspektyva Odeska, Sonata Poltavska, and Shpalivka exhibit overlapping distributions near the center of the plot, reflecting their relatively similar intermediate responses to NP-40 exposure.

This genotype-specific clustering emphasizes significant differences in chromosomal aberration patterns among wheat varieties and highlights MIP Lada as uniquely responsive, which could be strategically beneficial in targeted mutation breeding programs.

The comparative analysis of genotype and mutagen concentration revealed several important aspects of their mutagenic effects. Despite minor differences in specific outcomes – such as the presence of chromatin bridges – both factors demonstrated broadly similar mutagenic profiles (Fig. 1 and 2). These similarities indicate that, while individual responses may vary, the overall pattern of mutagenic action remains consistent across treatments. Model parameters confirmed the predictability of genotype and concentration effects, supporting their reliability for inducing genetic changes under controlled experimental conditions. Trends observed with increasing mutagen concentration and variations in initial genotypic material showed stable, reproducib-

le patterns, validating the efficacy of these parameters for directed mutagenesis. This predictability makes both factors valuable tools for targeted genetic modification and systematic experimentation. Discriminant analysis further emphasized the utility of NP-40 as a tool for controlled mutagenic studies by identifying specific cytogenetic and reproductive traits that respond consistently to changes in genotype and concentration. Traits such as pollen sterility, chromosomal rearrangements, and other nuclear anomalies were particularly responsive. However, the behavior of bridges deviated from the general trend, suggesting that their formation may involve more complex or genotype-specific mechanisms. The findings highlight the critical role of genotype selection in mutagenesis research, as genotype–mutagen interactions significantly influence mutagenic outcomes. In particular, the variety MIP Lada demonstrated heightened responsiveness to NP-40, making it a promising candidate for further mutagenic studies. Investigating the mechanisms underlying its susceptibility or tolerance could yield deeper insights into the site-specific action of epimutagens and guide the development of optimized breeding strategies.

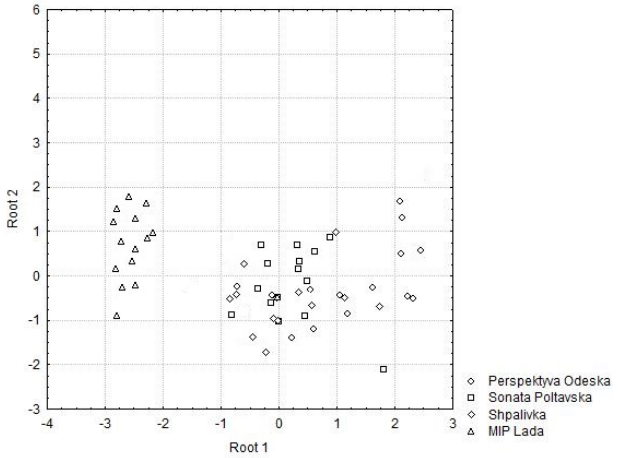

Fig. 2. Model for canonical functions (for initial material influence)

The classification results based on the influence of mutagen concentration and varietal characteristics demonstrate a high degree of precision and reliability in distinguishing the effects of NP-40 across experimental conditions. The accuracy of classification across different NP-40 concentrations, including the highest (fourth) level, was consistently high. Minimal classification errors affirm the robustness of the selected parameters in differentiating the mutagenic impact of varying concentrations. The varietal response to NP-40 exposure was also effectively classified, confirming that the chosen indicators successfully captured the interaction between genotype and mutagenic influence. Among the assessed traits, pollen fertility, total frequency of chromosomal rearrangements, and the number of other (non-fragment and non-bridge) rearrangements exhibited the highest discriminative power. These three parameters explained the majority of the observed variability and were critical in assessing both mutagenic intensity and genotype-specific responses.

Overall, the classification accuracy exceeded 75%, underscoring the reliability and resolution of the selected parameters for distinguishing mutagenic effects and varietal responses. The predictive model developed in this study provides a robust framework for assessing the efficacy of NP-40 in inducing high levels of genetic and epigenetic variability. By incorporating the variability of individual genotypes and concentration levels, the model serves as a reliable forecasting tool for predicting NP-40-induced epimutagenic outcomes. The experimental design and parameter selection contribute significantly to the model's robustness and applicability. Variety MIP Lada demonstrated a high suitability for NP-40 treatment, exhibiting elevated levels of genetic variability and strong potential for the development of mutant lines. These findings confirm NP-40's reliability as a mutagenic agent capable of generating diverse and desirable genetic modifications. In summary, the developed model offers a predictive and practical tool for optimizing NP-40 use in mutation breeding programs, supporting its application in generating genetically diverse and agronomically valuable mutant forms.

Discussion

A key challenge in generating valuable genetic forms through ecological-genetic methods lies in balancing induced variability against damage and lethality resulting from mutagenic treatment. In ecological genetics, continuous efforts are made to minimize adverse effects, such as severe DNA damage and associated reductions in viability, while maintaining the level of variability necessary to select beneficial traits (Kiani et al., 2022). Achieving an optimal balance typically involves identifying effective doses or concentrations and selecting appropriate genetic starting materials. However, further advancements in this optimization often require introducing novel mutagenic agents with fundamentally different mechanisms of action (Muhammad et al., 2021; Von Well et al., 2022). Adverse effects encountered in classical mutagenesis primarily stem from direct DNA damage, which offers limited control over its severity. Although generating numerous minor genetic alterations is generally more desirable for breeding purposes, traditional mutagens often cause irreparable DNA damage leading to permanent adverse or lethal effects. Cytogenetic analyses have proven valuable for quickly assessing these impacts, providing a reliable measure of induced genetic damage more efficiently than field-based evaluations (Andrew et al., 2021).

Historically, mutagenic treatments have often led to sharp declines in pollen fertility, initially showing gradual decreases until a critical dose threshold is reached, after which sterility sharply increases. Interestingly, the epimutagen under study exhibited milder impacts on pollen fertility across genotypes, implying subtler cellular disruptions. This milder response further suggests involvement of genetically mediated adaptive response mechanisms, differing markedly among genotypic groups (Ghasemi-Soloklui et al., 2023). Crucial indicators for evaluating chromosomal damage include overall rates of chromosomal aberrations, frequencies of specific aberration types (such as double fragments and bridges), and ratios of aberration categories that reveal underlying mechanisms of mutagen action. Particularly noteworthy was the unprecedented observation of significant numbers of micronuclei and lagging chromosomes paired with relatively few complex chromosomal rearrangements. Such cytogenetic profiles typically arise from high doses of gamma radiation or potent chemical supermutagens (Pathirana, 2021; Nazarenko et al., 2022). Remarkably, despite this cytological marker of severe mutagenic influence, treated materials demonstrated substantially higher viability compared to treatments with similarly potent conventional mutagens. This suggests the potential emergence of novel forms identifiable by distinct cytological signatures (Hong et al., 2022).

While similar chemical mutagens traditionally induce chromosomal fragments, typically dominating the aberration spectrum, the epimutagen investigated here exhibited an unusual ratio of fragments to bridges (Horshchar & Nazarenko, 2024). Additionally, genotype-specific dynamics were observed, particularly regarding pollen sterility rates and aberration spectra, aligning with prior research on weaker mutagenic agents like nitrosoureas. However, the absolute number of chromosomal aberrations remained notably lower relative to pollen sterility rates than expected based on conventional mutagen exposure (Pathirana, 2021). The cytogenetic behavior observed suggests fundamentally distinct genotype-mutagen interactions at the cellular level. These interactions likely arise from reduced DNA damage intensity and qualitatively distinct patterns of damage, evidenced by unique aberration frequencies. Notably, these effects consistently appeared across diverse genotypes, irrespective of geographic origin. Given that such cytogenetic effects have not previously been documented with classical physical or chemical mutagens, it strongly indicates an epimutagenic mode of action. This conclusion is indirectly supported by observed alterations in chromosomal behavior and nuclear architecture (Horshchar & Nazarenko, 2022a).

The established cytogenetic monitoring parameters thus remain applicable and effective, expanded somewhat by the unique rearrangement spectrum. Traditional cytogenetic measures retain their utility,

facilitating continued use of established experimental protocols to accurately detect and quantify mutagenic effects (Pathirana, 2021; Pathirana et al., 2023; Von Well et al., 2023). Despite variability in response strength, genotype-specific differences in epimutagen susceptibility were significant. One genotype demonstrated substantially higher sensitivity, suggesting either increased genomic affinity to the epimutagen or differential activity in adaptive response systems (Mahanish & Kin, 2025). Such significant genotype-mutagen interactions provide critical insights into epimutagen impacts at the chromosomal level, potentially yielding novel genetic variations valuable in crop breeding programs (Cabahug et al., 2020; Živković et al., 2024). Ultimately, these findings indicate the potential for qualitatively distinct hereditary changes, impacting trait variability frequencies and genetic mechanisms underlying desirable agronomic characteristics. Such advancements could transition previously challenging or limited genetic forms into practically valuable resources, enhancing biodiversity induction and supporting sustainable crop improvement strategies (Muhammad et al., 2021).

The frequency of chromosomal aberrations observed in this study closely corresponds with the rate of visually identifiable mutations. This correlation reinforces the utility of cytogenetic markers as reliable indicators for predicting the efficiency and effectiveness of epimutagenic agents. Although previous research has reported similar correlations, the relationship is not consistent across all mutagenic agents (Horshchar & Nazarenko, 2022b). The distinctive patterns noted here underscore the unique specificity of the investigated mutagen, which induces genetic changes detectable both cytogenetically and phenotypically (Voss-Fels et al., 2019; Lethin et al., 2022).

Our findings highlight the substantial value of the epimutagen in driving diverse genetic alterations. While these mutations may not immediately translate into economically valuable cultivars or lines, they offer essential insights and foundational genetic variation that is critically valuable for future breeding initiatives. Consequently, the studied epimutagen serves as an important tool for dissecting genetic control mechanisms and facilitating targeted improvements within existing wheat varieties (Yan et al., 2021; Živković et al., 2024). The epimutagen's capability to induce a broad spectrum of genetic modifications underscores its significance for investigating complex trait control mechanisms and genetic interactions (Cabahug et al., 2020; Bora et al., 2024; Kryshyn & Nazarenko, 2025).

In contrast, classical mutagens such as chemical ones are typically better suited for generating pre-breeding materials rather than direct commercial varieties. Sodium azide, however, demonstrates considerable potential in gene pyramiding approaches aimed at integrating multiple favorable traits into elite wheat cultivars (Xiong et al., 2018; Shabani et al., 2022). Ultimately, by associating phenotypic changes with genetic and physiological markers, the research aims to improve the prediction and identification of valuable mutant lines. Future studies will prioritize establishing robust correlations between induced phenotypic alterations and their genetic inheritance, thereby highlighting unique traits that can enhance the breeding value of selected lines for specific agricultural objectives.

Additionally, this study identifies at least one scenario demonstrating clear potential for achieving a higher frequency of valuable mutant forms, consistent with prior research outcomes observed across other wheat varieties. The detection of microchanges, although subtle and not dramatically altering overall mutation frequency trends, refines our understanding of mutagenic effects and provides supplemental data valuable for improving the predictability of mutational outcomes (Bilgın et al., 2022; Turaeva et al., 2024). Nevertheless, these microchanges, while informative, do not significantly shift broader patterns in mutagen-induced variability, as supported by recent literature (Von Well et al., 2023).

The adoption of novel substances with more complex modes of action compared to classical chemical supermutagens offers significant potential for enhancing genetic variability while reducing undesirable genotoxic effects (Murthy et al., 2024; Spisak et al., 2024). Cytogenetic analyses of these newer agents demonstrate their capacity to induce predominantly minor, less severe chromosomal changes, leading to substantially lower genotoxicity. Initial evidence suggests that the primary mechanisms of these substances involve epigenetic pathways influencing hereditary regulation and variability, rather than direct DNA damage alone. Consequently, this class of compounds promises higher efficiency in generating beneficial biodiversity, significantly accelerating the development of valuable crop forms (Hong et al., 2022; Didenko & Nazarenko, 2025). Future research aims to link cellular-level changes induced by these agents to observable phenotypic outcomes at the organismal level. Detailed phenological, biometric, and biochemical assessments of selected plant families and lines will elucidate the relationship between cytogenetic events and heritable trait variability (Pathirana et al., 2023). Despite their novel action mechanisms, classical cytogenetic methodologies retain their efficacy in identifying the type and optimal dosage of these substances, reinforcing their suitability for genetic improvement programs.

Conclusion

The studied epimutagen, NP-40, exhibited marked site-specificity, effectively differentiating among genotypes even within a relatively limited sample. This characteristic underscores its potential in mutation breeding programs, allowing targeted selection of highly responsive genotypes. In this context, the variety MIP Lada was particularly notable for its pronounced responsiveness, emerging as a promising candidate for programs prioritizing mutagen-induced variability. The analysis strongly supports the application of higher concentrations of NP-40 (0.1–0.5%), as these concentrations provided the optimal balance between beneficial trait induction and acceptable levels of collateral damage. Lower concentrations generally yielded fewer beneficial changes, reducing both the quantity and quality of mutant material obtained. Notably, the interaction between genotype and NP-40 concentration was less pronounced compared to previous studies, yet sufficiently robust to identify optimal genotype-specific responses. While the mutagen demonstrated clear potential for inducing valuable traits, challenges such as increased sterility rates at higher concentrations and occasional associations between desirable and undesirable traits were observed. Subsequent research will therefore prioritize evaluating selected genotypes for tolerance to critical environmental stressors, including winter hardiness, drought resistance, and improved technological grain qualities, as well as enhancements in beneficial microelement content. These studies will provide essential insights to harness the full potential of NP-40 and related epimutagenic agents in sustainable crop improvement strategies.

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