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Cytogenetic analysis of the effects of a new epimutagenic agent on chromosomal stability in winter wheat (*Triticum aestivum*)

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Abstract. This study evaluated the epimutagenic potential of Nonidet P-40 (NP-40) in winter wheat (*Triticum aestivum* L.) by assessing its capacity to induce chromosomal aberrations, elucidating genotype–mutagen interactions, and determining its predictive value at the cellular level for inducing heritable epigenetic mutations. Four winter wheat varieties (Farell, NE 12443, Ronin, and Seilor) were exposed to NP-40 concentrations of 0.01%, 0.05%, 0.1%, and 0.5%. The cytogenetic analyses included assessments of pollen sterility and the frequency and spectrum of chromosomal aberrations in root-tip meristematic cells during mitosis. The results demonstrated significant genotype-specific responses to NP-40 treatment, with varieties Farell and, to a lesser extent, Ronin identified as particularly sensitive. These varieties exhibited a pronounced susceptibility, highlighting their suitability for targeted induction of genetic variability and subsequent mutant selection. Of the tested NP-40 concentrations, 0.5% NP-40 was observed to be the most effective, inducing substantial chromosomal aberrations while maintaining acceptable plant viability, thereby optimizing mutation induction and minimizing adverse cytotoxic effects. Conversely, moderate NP-40 concentrations (0.01–0.1%) were less effective, yielding a suboptimal balance between induced genetic variability and viability. Key cytogenetic indicators of genotype-specific sensitivity included pollen fertility, the total frequency of chromosomal rearrangements, and the number of chromosomal bridges. By contrast, parameters such as fragment abundance and the occurrence of rare aberrations were analytically less informative. Notably, the NP-40-induced chromosomal aberration patterns differed markedly from those typically observed with classical chemical supermutagens, with responses significantly influenced by genetic background. These findings offer valuable insights into the epimutagenic properties of NP-40, emphasizing the critical role of genotype selection and concentration optimization in effective mutation breeding strategies. Future research will integrate these cytogenetic observations with analyses of hereditary variability in biochemical and physiological traits, enhancing epimutagenic methodologies and improving breeding programs aimed at winter wheat cultivar advancement.

Keywords: cereals; chromosomal abnormalities; Nonidet P-40; cytogenetic; spectra; site-specific action; epimutagen.

Introduction

The use of chemical factors as agents of ecogenetic improvement in cultivated plants plays a vital role in enhancing and stabilizing food production, addressing the needs of an expanding global population. Recent advances in ecological genetics are shaping multiple areas of science, including species conservation, ecological management, and the development of adaptation strategies to mitigate the impacts of climate change. Understanding the genetic basis of ecological interactions provides a strong foundation for biodiversity conservation and sustainable resource management. The genetic regulation of organismal responses to environmental stress is central to adaptive potential, and investigating these mechanisms offers critical insights into how populations respond to dynamic environmental conditions (Álvarez-Holguín et al., 2019; Pathirana & Carimi, 2023).

The analysis of chromosomal damage frequency serves as a valuable tool for assessing both genetic and epigenetic activity of mutagenic agents. Studying the spectrum of chromosomal rearrangements helps associate the nature of future mutations with the induction of rare genetic events (Bilgin et al., 2022). Although no direct test system links organism-level traits with specific DNA damage patterns, recurring associative patterns are increasingly being recognized (Chatterjee & Walker, 2017; Álvarez-Holguín et al., 2019). Chemical mutagens or epimutagens, which are gaining prominence in mutation breeding, demonstrate enhanced site specificity, resulting in elevated frequencies of specific mutational outcomes – such as rare mutations, dwarf phenotypes, and sterility – as reported in multiple plant species (Jalal et al., 2021; Bilgin et al., 2022; Horshchar & Nazarenko, 2022a).

Test systems based on chromosomal aberrations are widely utilized in environmental monitoring to evaluate pollution levels and identify its causes, characteristics, and potential biological consequences (Gupta et al., 2019; Nazarenko et al., 2023). These methods are well-standardized, internationally recognized, and offer high reliability for detecting mutagenic activity (Ghasemi-Soloklui et al., 2023).

In mutagenicity testing, high-dose treatments often result in cellular elimination, particularly at concentrations that exceed the threshold for physiological tolerance (Pathirana & Carimi, 2023). Typically, chromosomal aberration frequency peaks at a specific concentration, followed by a decline, which marks the practical upper limit for effective mutagen application (Horshchar & Nazarenko, 2022b). This behavior often results in a plateau effect, beyond which additional increases in concentration yield diminishing or adverse effects, and further experimentation becomes impractical. Importantly, maximum nuclear damage does not always correlate with lethal dose thresholds (LD_{50}) or reductions in morphometric parameters (RD_{50}) (Bora et al., 2024). In certain cases, doses beyond the mutagenic plateau are used deliberately to induce rare or high-impact mutations, aiding in the dissection of genetic control mechanisms for specific traits (Hong et al., 2022). This approach is common in modern mutation breeding, particularly when employing chemical supermutagens (Álvarez-Holguín et al., 2019).

The subject of epimutagenic influence – especially in chemical mutagenesis – is of critical importance, as genotype-specific responses directly affect the pattern of DNA damage, location of mutable sites, and stability transitions of genomic regions. Selecting optimal genotype combinations and mutagen or epimutagen concentrations can maximize the induction of desired genetic changes while minimizing unwanted effects (Nazarenko et al., 2023). This targeted approach enhances the efficiency of trait improvement and stress resistance.

Variation in epimutagen response among individual genotypes – particularly in terms of rate and type of chromosomal rearrangements – is essential for identifying genetically determined features of DNA structure (Nazarenko et al., 2019). These differences also reflect genotypic susceptibility or resistance to mutagenic stress, which may arise from innate tolerance mechanisms (Ergün et al., 2023).

In chemical mutagenesis, the study of genotype–mutagen interactions is especially crucial due to the high variability in responses to even closely related mutagenic compounds (Nazarenko et al., 2019). Chromosomal-level changes provide faster and more reliable diagnostic markers compared to conventional wheat mutagenesis methods, which typically require analysis of later generations (M_2–M_3). Notably, Nonidet P-40 remains underexplored in this context, particularly in domestic winter wheat varieties, where comparative genotype-specific analyses are scarce. Furthermore, Nonidet P-40 exhibits a fundamentally different mechanism of action compared with earlier-generation chemical mutagens (Bora et al., 2024), warranting further investigation of its unique mutagenic properties and applications.

The study aimed to evaluate the potential of Nonidet P-40 (NP-40) as an epimutagen, focusing on its ability to induce chromosomal aberrations, its interaction with different winter wheat genotypes, and the specific characteristics of genotype–mutagen interactions. A key objective was to assess the feasibility of applying NP-40 in future breeding programs by examining its predictive value at the cellular level for forecasting mutation-inducing capacity at the whole-plant level. In addition, the study investigated the effect of NP-40 on the fertility of first-generation (M_1) mutant plants, identifying this as a critical limitation for its practical application in generating stable and fertile mutant lines. The findings contribute to understanding NP-40's potential role in controlled epimutagenesis and its suitability for incorporation into plant improvement strategies.

Materials and methods

The study on pollen viability and the frequency and spectrum of chromosomal rearrangements was conducted by the Laboratory of the Department of Breeding and Seed Production at Dnipro State Agrarian and Economic University. Pollen and cytogenetic samples were obtained from first-generation mutant populations grown at the experimental field station of the Science-Education Center. A total of 1,000 grains per treatment were exposed to Nonidet P-40 (NP-40) (Sigma-Aldrich, Germany) at concentrations of 0.01%, 0.05%, 0.1%, and 0.5%. Water-treated grains served as controls. These concentrations were selected based on the previous mutagenesis studies in cereals (Spencer-Lopes et al., 2018). The grains were soaked for 24 hours, following standard mutagenesis protocols for the respective wheat cultivars.

The experiment included 18 treatment variants, using four winter wheat (*Triticum aestivum* L.) varieties: Farell, NE 12443, Ronin and Seilor. All agronomic practices followed regional cultivation standards for generating viable M_1 populations. Pollen viability was assessed during the flowering stage. An average of 25 spike samples per treatment was collected, selecting spikelets with no visible morphoses and well-developed yellow anthers located in the central part of the spike to ensure sampling consistency. Acetocarmine staining was used to differentiate viable and non-viable pollen, with fertility evaluated based on staining intensity under light microscopy.

Cytogenetic evaluation of NP-40 activity was conducted using light microscopy (Micromed XS-3330, Poltava, Ukraine) at 600× magnification, equipped with a 5 MP camera for image capture and documentation. Mitotic cells were observed from the primary root tips of germinated seedlings, focusing on stages from late metaphase to anaphase. The following chromosomal abnormalities were recorded: fragments and double fragments, chromatid and chromosomal bridges, micronuclei, lagging chromosomes, and complex rearrangements (cells with two or more simultaneous abnormalities).

To prepare the samples, germinated seeds (20–22 °C) were grown until primary roots reached 1.1 cm in length. Root tips were excised and fixed in Clarke's fixative (1 part glacial acetic acid : 3 parts 96% ethanol) for 24 hours, then stored in 70% ethanol at refrigeration temperature. Temporary squashed preparations (minimum of 25 per treatment) were stained with acetocarmine and examined microscopically. Up to 1,000 cells per treatment were evaluated; fewer were assessed at higher NP-40 concentrations due to cytotoxicity. Rigid root tips were pretreated in 45% glacial acetic acid to enhance tissue softening and visualization (Spencer-Lopes et al., 2018).

All statistical analyses were performed using Statistica 10.0 software (TIBCO, Palo Alto, USA). Analysis of variance (ANOVA) was conducted to evaluate the effects of genotype, mutagen concentration, and their interaction, using a significance threshold of $P < 0.05$. Data were visualized graphically for interpretation. Pairwise comparisons were performed using the Tukey HSD test. Descriptive statistics included the arithmetic mean ($\bar{x}$) and standard deviation (SD). Normality of data distribution was verified using the Shapiro–Wilk W-test. To assess the predictive value of cytogenetic and fertility traits, discriminant analysis was conducted using standard software modules to identify traits with high discriminatory power and to model classification accuracy.

Results

The initial phase of the study on the cellular effects of the epimutagen NP-40 involved assessing the pollen viability by selecting mature anthers from medium spikelets of normally developed main spikes. The microscopic analysis was used to determine the ratio of fertile to sterile pollen grains (Table 1).

The fertility analysis of 16 mutant winter wheat populations treated with varying NP-40 concentrations revealed a significant positive linear correlation between increasing NP-40 concentration and pollen sterility ($r = 0.71$). The statistical analysis confirmed a significant decrease in pollen fertility with increasing concentrations ($F = 71.15$; $F_{0.05} = 3.25$; $P = 3.21 \times 10^{-7}$). Despite the increasing sterility, even the highest concentration (0.5%) did not reach the lethal dose threshold (LD_{50}), maintaining relatively high plant viability (75–80%). At lower concentrations (0.01% and 0.05%), the viability remained consistently high (88–99%), aligning with international classifications of low mutagenic doses.

Significant varietal differences in the sensitivity to NP-40-induced sterility were also detected ($F = 11.12$; $F_{0.05} = 3.49$; $P = 0.004$), as confirmed by Tukey post-hoc tests. The variety NE 12443 showed the highest sensitivity, with a statistically significant reduction in fertility even at 0.01% NP-40. However, the fertility levels at this concentration still fell within biologically tolerable limits, preserving adequate viability for further breeding. The variety Seilor ($F = 5.96$; $F_{0.05} = 3.49$; $P = 0.01$) demonstrated an intermediate sensitivity, more tolerant than NE 12443 but less tolerant than Farell and Ronin.

At 0.01%, no statistically significant reduction in fertility was observed for Farell and Ronin, and across 0.01%, 0.05%, and 0.1% concentrations, the fertility levels remained between 80–90%, indicating these are low-impact concentrations suitable for inducing genetic variability without compromising plant development.

Importantly, the 0.5% NP-40 concentration, while approaching the upper threshold, still maintained the plant viability above 75%, meeting international standards for mutagenic treatments. This concentration thus represents a practical upper limit for maximizing mutagenic efficiency while ensuring sufficient viable material for the evaluation of hereditary variability in subsequent generations.

A statistically significant genotype×mutagen interaction was observed ($F = 6.11$; $F_{0.05} = 5.10$; $P = 0.03$), highlighting the genotype-dependent nature of NP-40 responses. These results emphasize the importance of genotype selection in designing epimutagenesis experiments and optimizing induced variability for wheat breeding applications.

Table 1

Dose-dependent effect of NP-40 epimutagen on the pollen fertility of first-generation (M_1) winter wheat spikes across different genotypes ($x \pm SD$, $n = 25$)

Variety	Control	NP-40 0.01%	NP-40 0.05%	NP-40 0.1%	NP-40 0.5%
Farell	99.15 ± 0.62[a]	98.23 ± 0.78[a]	92.44 ± 0.44[b]	88.67 ± 0.57[c]	80.99 ±1.04[d]
NE 12443	99.13 ± 0.63[a]	97.03 ± 0.89[b]	88.22 ± 0.49[c]	86.12 ± 0.52[d]	75.10 ± 1.00[e]
Ronin	99.12 ± 0.63[a]	98.33 ± 0.66[a]	92.59 ± 0.47[b]	89.08 ± 0.68[c]	81.16 ± 1.05[d]
Seilor	99.40 ± 0.67[a]	97.94 ± 0.61[b]	91.51 ± 0.49[c]	88.14 ± 0.56[d]	78.14 ± 1.05[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 performed separately for each variety across NP-40 treatment concentrations.

Treatment with NP-40 epimutagen resulted in a clear, dose-dependent reduction in pollen fertility across all the studied winter wheat varieties. Among them, the variety NE 12443 exhibited the greatest sensitivity, with pollen fertility declining sharply to 75.10% at the highest NP-40 concentration (0.5%), a value significantly lower than those observed in other genotypes. By contrast, Farell and Ronin showed moderate but consistent reductions in pollen fertility with increasing NP-40 concentrations, main-

taining relatively higher levels of viability. The variety Seilor occupied an intermediate position in terms of sensitivity ($F = 5.96$; $F_{0.05} = 3.49$; $P = 0.01$), being more tolerant than NE 12443 but less resistant than Farell and Ronin. Statistically significant differences in pollen fertility emerged predominantly at concentrations ≥ 0.05%, indicating genotype-specific thresholds for epimutagen-induced fertility disruption.

These results underscore the importance of genotype selection in mutation breeding programs that utilize epimutagenic agents. They also highlight the necessity of careful management of NP-40 concentrations to ensure an optimal balance between induced genetic variability and preservation of plant viability.

Further analysis of chromosomal rearrangement frequencies following NP-40 exposure (Table 2) revealed distinct concentration-dependent effects, clearly delineating threshold concentrations associated with the onset and progression of chromosomal abnormalities. These findings support the use of NP-40 as a potent tool in controlled mutagenesis, provided its application is tailored to genotype-specific responses and physiological tolerances.

Factor analysis demonstrated a statistically significant effect of genotype ($F = 9.97$; $F_{0.05} = 3.49$; $P = 0.005$), highlighting clear differences in sensitivity among winter wheat varieties exposed to NP-40. A robust, dose-dependent response to NP-40 was confirmed, as increasing concentrations consistently resulted in elevated frequencies of chromosomal rearrangements ($F = 80.12$; $F_{0.05} = 3.25$; $P = 1.34 \times 10^{-7}$). However, the genotype × concentration interaction was not statistically significant ($F = 2.11$; $F_{0.05} = 5.10$; $P = 0.11$), suggesting that the mutagenic effects of NP-40 remained broadly consistent across different wheat genotypes.

In the control samples, chromosomal rearrangement frequencies were consistently low, ranging 0.79% to 0.99%. Treatment with 0.01% NP-40 increased rearrangement frequencies, which ranged 2.39% (Seilor) to 3.60% (Ronin). At 0.05% NP-40, frequencies further rose to 3.60% (NE 12443) and reached as high as 5.18% (Farell). The concentration of 0.1% NP-40 yielded frequencies between 5.16% (NE 12443) and 6.49% (Farell), clearly demonstrating a significant increase relative to the lower doses. At the highest concentration tested (0.5% NP-40), rearrangement frequencies peaked, ranging 6.67% (NE 12443) to 8.14% (Farell). These high frequencies approached cytotoxic thresholds, as evidenced by noticeable increases in cell mortality and potential reductions in cell viability.

Table 2

Overall frequency of the chromosomal abnormalities in mitotically dividing root-tip cells following NP-40 Treatment (x ± SD)

Variety	Variant	Cells analyzed (n)	Aberrant cells	
			number	%
Farell	water	1009	10	0.99 ± 0.08^{a}
Farell	NP-40 0.01%	1008	34	3.37 ± 0.13^{b}
Farell	NP-40 0.05 %	1004	52	5.18 ± 0.19^{c}
Farell	NP-40 0.1 %	1001	65	6.49 ± 0.29^{d}
Farell	NP-40 0.5 %	1007	82	8.14 ± 0.31^{e}
NE 12443	water	1002	8	0.80 ± 0.08^{a}
NE 12443	NP-40 0.01%	1004	29	2.89 ± 0.10^{b}
NE 12443	NP-40 0.05 %	1001	36	3.60 ± 0.14^{c}
NE 12443	NP-40 0.1 %	1007	52	5.16 ± 0.18^{d}
NE 12443	NP-40 0.5 %	1004	67	6.67 ± 0.29^{e}
Ronin	water	1009	8	0.79 ± 0.06^{a}
Ronin	NP-40 0.01%	1001	36	3.60 ± 0.13^{b}
Ronin	NP-40 0.05 %	1005	44	4.38 ± 0.18^{c}
Ronin	NP-40 0.1 %	1001	57	5.69 ± 0.22^{d}
Ronin	NP-40 0.5 %	1005	76	7.56 ± 0.30^{e}
Seilor	water	1006	8	0.80 ± 0.08^{a}
Seilor	NP-40 0.01%	1004	24	2.39 ± 0.14^{b}
Seilor	NP-40 0.05 %	1001	38	3.80 ± 0.19^{c}
Seilor	NP-40 0.1 %	1013	56	5.53 ± 0.21^{d}
Seilor	NP-40 0.5 %	1008	68	6.75 ± 0.28^{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 performed separately for each variety across NP-40 treatment concentrations.

Across all varieties, a clear dose-dependent increase in the frequency of aberrant cells was observed, with statistically significant differences ($P < 0.05$) between treatments. Farell exhibited the highest frequency of aberrant cells, progressively increasing from 0.99% (control) up to 8.14% (NP-40 0.5%). The variety NE 12443 showed the lowest aberration frequency among the varieties at lower concentrations (0.01% and 0.05%), but its sensitivity notably increased at the highest concentration (0.5%), reaching 6.67% aberrant cells. Ronin displayed intermediate responsiveness, increasing from 0.79% (control) to 7.56% (0.5% NP-40). Seilor was initially less sensitive at low concentrations (2.39% at 0.01% NP-40), but approached intermediate levels at higher doses (6.75% at 0.5% NP-40).

Based on maximum aberration frequency (0.5% NP-40), the varietal sensitivity ranked (from most to least sensitive) as follows: Farell (8.14%), Ronin (7.56%), Seilor (6.75%), and NE 12443 (6.67%). Although differences between varieties at the highest concentration were moderate, the consistent ranking suggests intrinsic genotypic differences influencing susceptibility to NP-40-induced chromosomal damage.

The concentrations tested (0.01–0.5%) remained within a range that induced significant chromosomal aberrations but not exceeding critical cytotoxic thresholds (typically 10–15% aberrant cells or higher). At the highest tested concentration (0.5%), aberration frequency remained below 10%, indicating that NP-40 doses used here are suitable for generating genetic variability without severely impairing plant viability.

Statistical significance ($P < 0.05$, Tukey's HSD test) confirms that the observed differences among treatments within each variety were robust and consistent. Each incremental increase in NP-40 concentration yielded statistically distinct aberration frequencies, indicating reliability and reproducibility of the cytogenetic response.

Epimutagen NP-40 showed a strong dose-dependent cytogenetic activity across all the wheat varieties tested, confirming its effectiveness as an epimutagenic factor. The variety Farell exhibited the greatest susceptibility, making it highly suitable for generating mutant populations with high genetic variability. Ronin also appears responsive, suitable as a secondary genotype choice. The varieties Seilor and NE 12443 demonstrated lower initial sensitivity but were effectively responsive at higher NP-40 concentrations. The optimal NP-40 concentration range appears between 0.05–0.1%, where substantial genetic variability can be induced without significantly compromising cell viability. Higher concentrations (0.5%) are valuable for intensive mutagenesis but must be carefully managed due to approaching cytotoxic limits. Overall, NP-40 treatment provides a reliable and effective method for controlled induction of chromosomal variability, with genotype-specific considerations essential for optimizing outcomes in breeding and mutagenesis programs.

The data presented (Table 3) illustrates the cytogenetic impact of varying concentrations of the epimutagen NP-40 across four winter wheat varieties (Farell, NE 12443, Ronin, and Seilor). The cytogenetic analysis identified several distinct categories of chromosomal abnormalities induced by the mutagenic treatments. Fragments and double fragments, which represented the most frequent and easily quantifiable indicators of chromosomal damage, corresponding to direct breaks within the chromosome structure. Bridges, that observed in diverse morphological forms, chromosomal and chromatid bridges indicated improper chromosome segregation during anaphase. Typically, these arise from dicentric chromosomes, unresolved recombination events, or telomere dysfunction. Micronuclei, which are small nuclear structures, signify lagging chromosome fragments or entire chromosomes that fail to incorporate into daughter nuclei. They serve as highly sensitive markers for genotoxic stress and chromosomal missegregation. Lagging chromosomes, detected primarily during anaphase, illustrated disruptions in chromosomal movement and inadequate spindle attachment during cell division, highlighting mitotic dysfunction. Complex aberrations, cells demonstrating two or more simultaneous chromosomal rearrangements, were categorized as complex aberrations, indicative of elevated cytogenetic instability and cumulative mutagenic effects. To further investigate mutagenic mechanisms, the fragment-to-bridge ratio was calculated as a diagnostic metric. This ratio clarified the dominant mode of chromosomal disruption caused by the applied mutagen, providing insights into its specific impact on chromosomal integrity and stability.

In the control group, the frequency of chromosomal fragments and double fragments was minimal but consistently detectable, providing a reliable baseline for evaluating NP-40-induced epimutagenic effects. Even at the lowest tested NP-40 concentration (0.01%), a statistically significant ($P < 0.05$) increase in fragment numbers was observed, clearly indicating the onset of mutagenic activity. Specifically, at 0.01% NP-40, fragment counts increased notably across all the genotypes, ranging from 10.0 in Seilor to 18.0 in Ronin. Raising the concentration to 0.05% NP-40 further intensified fragmentation, resulting in fragment counts ranging 12.0 (Seilor) to 21.0 (Farell), thus confirming a pronounced dose-dependent effect. At 0.1% NP-40, fragment numbers increased further, varying between 23.0 (Seilor and Ronin) and 25.0 (Farell). Finally, at the highest

concentration (0.5% NP-40), fragment counts stabilized at elevated levels, ranging 28.0 (Seilor) to 32.0 (Farell and NE 12443), suggesting a possible onset of a mutagenic saturation threshold or a maximal achievable effect at this dosage. These observations clearly demonstrate a robust and consistent dose-dependent relationship between NP-40 concentration and chromosomal fragmentation, reinforcing the efficacy of NP-40 as an epimutagen in wheat genotypes.

Table 3

Cytogenetic effects induced by NP-40 treatment in four winter wheat varieties (x ± SD, n = 1000)

Variety	Variant	fragments (single + double)		bridges (chromosomal + chromatid)		fragments / bridges	other (micronucleus, lagging chromosomes)		double and more	
		n	%	n	%		n	%	n	%
Farell	water	6.0 ± 0.2^{a}	60.0	4.0 ± 0.2^{a}	40.0	1.5	0.0 ± 0.0^{a}	0.0	0.0 ± 0.0^{a}	0.0
Farell	NP-40 0.01%	17.0 ± 0.6^{b}	50.0	10.0 ± 0.4^{b}	29.4	1.7	7.0 ± 0.3^{b}	20.5	3.0 ± 0.2^{b}	8.8
Farell	NP-40 0.05 %	21.0 ± 0.7^{c}	40.3	18.0 ± 0.7^{c}	34.6	1.1	13.0 ± 0.5^{c}	25.0	9.0 ± 0.3^{c}	17.3
Farell	NP-40 0.1 %	25.0 ± 1.0^{d}	38.4	23.0 ± 0.9^{d}	35.3	1.0	17.0 ± 0.7^{d}	26.1	13.0 ± 0.5^{d}	20.0
Farell	NP-40 0.5 %	32.0 ± 1.2^{e}	39.0	31.0 ± 1.1^{e}	37.8	1.0	19.0 ± 0.9^{d}	23.1	16.0 ± 0.5^{e}	19.5
NE 12443	water	4.0 ± 0.1^{a}	50.0	4.0 ± 0.1^{a}	50.0	1.0	0.0 ± 0.0^{a}	0.0	0.0 ± 0.0^{a}	0.0
NE 12443	NP-40 0.01%	13.0 ± 0.7^{b}	44.8	10.0 ± 0.4^{b}	34.4	1.3	6.0 ± 0.2^{b}	20.6	4.0 ± 0.2^{b}	13.7
NE 12443	NP-40 0.05 %	14.0 ± 0.8^{b}	38.8	10.0 ± 0.5^{b}	27.7	1.4	12.0 ± 0.4^{c}	33.3	7.0 ± 0.2^{c}	19.4
NE 12443	NP-40 0.1 %	23.0 ± 1.1^{c}	44.2	16.0 ± 0.7^{c}	30.7	1.4	13.0 ± 0.4^{c}	25.0	10.0 ± 0.2^{dc}	19.2
NE 12443	NP-40 0.5 %	29.0 ± 1.1^{d}	43.2	19.0 ± 0.9^{d}	28.3	1.5	19.0 ± 0.6^{d}	28.3	16.0 ± 0.5^{e}	23.8
Ronin	water	4.0 ± 0.2^{a}	50.0	3.0 ± 0.1^{a}	37.5	1.3	1.0 ± 0.1^{a}	12.5	0.0 ± 0.0^{a}	0.0
Ronin	NP-40 0.01%	18.0 ± 0.4^{b}	50.0	11.0 ± 0.4^{b}	30.5	1.6	7.0 ± 0.3^{b}	19.4	3.0 ± 0.1^{b}	8.3
Ronin	NP-40 0.05 %	20.0 ± 0.8^{b}	45.4	15.0 ± 0.5^{c}	34.0	1.3	9.0 ± 0.4^{b}	20.4	5.0 ± 0.2^{b}	11.3
Ronin	NP-40 0.1 %	23.0 ± 1.1^{c}	40.3	20.0 ± 0.8^{d}	35.0	1.1	14.0 ± 0.5^{c}	24.5	9.0 ± 0.3^{c}	15.7
Ronin	NP-40 0.5 %	32.0 ± 1.1^{d}	42.1	27.0 ± 1.1^{e}	35.5	1.1	17.0 ± 0.7^{d}	22.3	11.0 ± 0.6^{c}	14.4
Seilor	water	4.0 ± 0.2^{a}	50.0	4.0 ± 0.1^{a}	50.0	1.0	0.0 ± 0.0^{a}	0.0	0.0 ± 0.0^{a}	0.0
Seilor	NP-40 0.01%	10.0 ± 0.9^{b}	41.6	8.0 ± 0.4^{b}	33.3	1.2	6.0 ± 0.4^{b}	25.0	2.0 ± 0.1^{a}	8.3
Seilor	NP-40 0.05 %	12.0 ± 1.1^{b}	31.5	13.0 ± 0.5^{c}	34.2	0.9	13.0 ± 0.6^{c}	34.2	7.0 ± 0.3^{b}	18.4
Seilor	NP-40 0.1 %	23.0 ± 1.0^{c}	41.0	16.0 ± 0.7^{d}	28.5	1.4	17.0 ± 0.9^{d}	30.3	8.0 ± 0.4^{b}	14.2
Seilor	NP-40 0.5 %	28.0 ± 1.1^{d}	41.1	20.0 ± 1.0^{e}	29.4	1.4	20.0 ± 0.9^{e}	29.4	14.0 ± 0.6^{c}	20.5

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 performed separately for each variety across NP-40 treatment concentrations.

Each tested concentration of NP-40 led to a statistically significant increase in the frequency of chromosomal fragments compared with the control group. Incremental increases in concentration generally resulted in significantly elevated fragment counts. However, between 0.1% and 0.5% NP-40, a plateau effect was observed, suggesting that the epimutagenic response reached a saturation threshold. Notably, the variety Farell exhibited a significantly higher frequency of chromosomal fragments at lower NP-40 concentrations, reflecting heightened sensitivity to mutagenic stress ($F = 4.02$; $F_{0.05} = 2.48$; $P = 0.03$). Nonetheless, at 0.1% NP-40 and above, differences among the genotypes became statistically insignificant, reinforcing the presence of a mutagenic saturation point beyond which additional increases in concentration did not proportionally enhance cytogenetic damage.

Lower NP-40 concentrations (0.01% and 0.05%) proved effective for inducing considerable chromosomal aberrations without surpassing toxicity thresholds. The consistent and significant elevation in fragment and double-fragment counts across all treatments further substantiates the effectiveness and reliability of NP-40 as an epimutagenic agent suitable for controlled chromosomal mutagenesis in plant breeding programs. Factorial analysis provided deeper insights into individual and interactive effects of genotype and NP-40 concentration. The effect of genotype on chromosomal fragment formation was statistically significant ($F = 2.44$; $F_{0.05} = 2.40$; $P = 0.05$), clearly demonstrating that inherent genetic differences among the studied wheat varieties significantly influence their cytogenetic response under mutagenic stress conditions.

The effect of mutagen concentration was highly significant ($F = 28.17$; $F_{0.05} = 2.90$; $P = 1.45 \times 10^{-4}$), confirming a robust, dose-dependent relationship. Higher NP-40 concentrations consistently produced elevated levels of chromosomal fragmentation across all the genotypes. Crucially, the genotype × concentration interaction was also statistically significant ($F = 6.95$; $F_{0.05} = 5.11$; $P = 0.03$). This interaction indicates that genotypes responded differently to increasing NP-40 concentrations, emphasizing the importance of genotype-specific sensitivity and the complexity of genotype-mutagen interactions. These findings underline the critical role of genetic background in determining both the extent and the type of chromosomal damage induced by epimutagenic treatments. In conclusion, while a general dose-dependent increase in fragment induction was evident, the observed non-uniform genotype-specific responses highlight the need for careful consideration of genotype selection and mutagen concentration in the design of optimized mutation breeding protocols.

The analysis of chromatid and chromosomal bridges induced by NP-40 revealed distinct dose-dependent trends and notable variability among the winter wheat varieties. At the lowest concentration (0.01% NP-40), bridge frequencies increased significantly compared with the control, ranging 8.0 (Seilor) to 11.0 (Ronin). At an intermediate concentration (0.05% NP-40), a substantial rise in bridge induction was observed, varying from 10.0 (NE 12443) to 18.0 (Farell), indicating increased chromosomal instability. At 0.1% NP-40, bridge induction peaked for most varieties, ranging 16.0 (NE 12443 and Seilor) to 23.0 (Farell). Finally, at the highest concentration (0.5% NP-40), bridge numbers further increased, ranging 19.0 (NE 12443) to 31.0 (Farell), approaching cytotoxic thresholds and suggesting diminished mutagenic efficiency at higher concentrations. This observed stabilization or slight decrease in bridge formation at higher concentrations is likely due to increased cell mortality or mitotic arrest, limiting the accumulation of additional chromosomal rearrangements.

Throughout the range of tested concentrations, the variety Farell consistently exhibited higher bridge frequencies, underscoring its enhanced susceptibility to chromosomal missegregation and mitotic disruption. These genotype-dependent differences highlight the Farell's utility as a sensitive model for evaluating NP-40-induced genomic instability. Factor analysis of total chromosomal aberrations and bridge induction frequencies revealed consistent trends, emphasizing a clear interplay between NP-40 concentration and genotypic variability. The effect of genotype alone on bridge induction was not statistically significant ($F = 2.01$; $F_{0.05} = 2.40$; $P = 0.07$), suggesting minor genotypic differences in absolute bridge formation frequencies. However, the effect of mutagen concentration was statistically significant ($F = 19.44$; $F_{0.05} = 2.90$; $P = 0.001$), confirming a robust, dose-dependent increase in bridge induction with elevated NP-40 concentrations. The genotype × concentration interaction was also not significant ($F = 2.19$; $F_{0.05} = 5.11$; $P = 0.11$), implying that the observed patterns of bridge formation were predominantly driven by NP-40 concentration, with limited differential responses attributable to genotype–concentration combinations. These statistical outcomes reinforce the conclusion that chromosomal bridge induction primarily depends on NP-40 concentration, with genotype playing a secondary yet discernible role. Thus, while genotypic variation should not be ignored, mutagen concentration remains the dominant determinant of chromosomal instability outcomes.

At higher NP-40 concentrations, the specificity of mutagenic action diminishes, resulting in broader genomic damage and an overall rise in total aberrations. This reduction in specificity potentially limits the precision of induced mutations. Nonetheless, chromosomal bridges remain critical markers of mitotic dysfunction, despite their lower frequency compared to chromosomal fragments. The predominance of fragment-type

aberrations highlights NP-40's primary mode of action - inducing localized DNA strand breaks rather than complex structural rearrangements such as dicentric bridges.

Moderate NP-40 concentrations (0.05% to 0.1%) appear optimal, providing effective induction of targeted genetic variability while avoiding excessive cytotoxic effects. Careful calibration of epimutagen concentrations is essential to balance mutation induction with cell viability. Importantly, genotype selection emerges as a key consideration, since certain varieties, notably Farell, display reduced chromosomal stability under mutagenic stress, making them suitable models for evaluating mutagenic efficiency and establishing cytotoxic thresholds. Interestingly, the fragment-to-bridge ratio remained relatively stable across concentrations, suggesting that chromosomal architecture and specific modes of DNA damage response remain consistent under varying NP-40 doses. The observed pattern – an initial increase in fragment frequency followed by a plateau or slight decline at higher doses – indicates a saturation of mutagenic potential at elevated concentrations due to increased cytotoxicity or cellular arrest. Such insights underscore the potential of strategically utilizing concentration-dependent, site-specific cytogenetic responses to refine mutation breeding methodologies, ultimately enhancing genetic modification efficiency and breeding outcomes.

Rare chromosomal aberrations were infrequent in control treatments, with only a single case detected in the variety MIP Lada, establishing a reliable baseline for comparative analyses. Treatment with NP-40 elicited a clear, statistically significant ($P = 0.001$) dose-dependent increase in the frequency of these rare aberrations. Specifically, at the lowest NP-40 concentration (0.01%), aberration frequencies rose substantially, ranging 6.0 in Seilor and NE 12443 to 7.0 in Farell and Ronin. Increasing the concentration to 0.05% NP-40 further elevated the frequency of rare aberrations, varying between 9.0 (Ronin) and 13.0 (Farell and Seilor). At the intermediate concentration of 0.1%, all the varieties demonstrated further elevation, ranging 13.0 (NE 12443) to 17.0 (Farell and Seilor), clearly indicating sustained mutagenic activity.

The highest frequency of rare aberrations occurred at the maximal tested concentration (0.5% NP-40), reaching values between 17.0 (Ronin) and 19.0 (Seilor), representing the sharpest rise across the tested doses. However, the slight plateau or decline observed in some varieties at this highest concentration suggests the onset of cytotoxicity or cellular lethality, potentially restricting further accumulation of chromosomal damage. Statistical analysis indicated that the effect of genotype on the rare aberration frequency was not statistically significant ($F = 1.97$; $F_{0.05} = 2.40$; $P = 0.07$). Thus, overall, the induction of rare aberrations was relatively uniform across the tested wheat genotypes. By contrast, the effect of NP-40 concentration was highly significant ($F = 17.17$; $F_{0.05} = 2.90$; $P = 0.001$), confirming a robust concentration-dependent relationship. Although the genotype × concentration interaction was not statistically significant ($F = 3.19$; $F_{0.05} = 5.11$; $P = 0.09$), the observed variation suggests subtle genotype-specific sensitivities to NP-40 treatment at certain doses. This interaction underscores the importance of genotype selection when applying NP-40 concentrations near cytotoxic thresholds.

The consistent increase in the proportion of rare aberrations with rising NP-40 concentrations demonstrates a reliable, dose-dependent mutagenic pattern. The highest mutagenic efficiency appears to occur before cytotoxicity becomes limiting, underscoring the need for carefully balancing mutagenic potency and cellular viability. While genotype did not significantly influence the overall rare aberration frequency, minor genotype-specific variations observed highlight the importance of precise genotype selection, especially when approaching concentrations associated with higher toxicity. In conclusion, NP-40 effectively induces rare chromosomal aberrations in a predictable, dose-dependent manner, achieving maximal mutagenic efficiency below cytotoxic thresholds. Selecting optimal NP-40 concentrations – generally in moderate ranges (0.05–0.1%) – is crucial for maximizing genetic variability while preserving sufficient cell viability, thus enhancing the effectiveness of mutation breeding strategies.

No complex chromosomal aberrations were detected in the control group, confirming a stable cytogenetic baseline for comparative analysis. Treatment with NP-40 resulted in a statistically significant, dose-dependent increase in the frequency of cells exhibiting two or more simultaneous aberrations across all the tested winter wheat genotypes. At 0.01% NP-40, the frequency of complex aberrations ranged 2.0 (Seilor) to 4.0 (NE 12443). Increasing NP-40 concentration to 0.05% led to a further rise, ranging 5.0 (Ronin) to 9.0 (Farell), indicative of heightened cytogenetic instability. A pronounced increase was observed at 0.1% NP-40, with frequencies varying from 8.0 (Seilor) to 13.0 (Farell). The highest tested concentration (0.5% NP-40) further elevated the complex aberration frequencies, ranging 11.0 (Seilor) to 16.0 (Farell). Notably, Farell consistently exhibited the highest levels of complex aberrations, particularly at 0.5% NP-40, suggesting greater susceptibility as well as resilience to high mutagenic stress. The 0.5% NP-40 concentration emerged as the most effective for inducing complex chromosomal aberrations, providing optimal mutagenic efficacy while maintaining acceptable cell viability across genotypes.

The effect of genotype alone was not statistically significant ($F = 1.96$; $F_{0.05} = 2.40$; $P = 0.07$), indicating that intrinsic genotypic differences did not significantly influence the overall frequency of complex aberrations. By contrast, the effect of mutagen concentration was highly significant ($F = 77.99$; $F_{0.05} = 2.90$; $P = 4.11 \times 10^{-11}$), confirming concentration as the dominant factor driving complex chromosomal instability. The genotype × concentration interaction was not significant ($F = 1.43$; $F_{0.05} = 5.11$; $P = 0.12$), suggesting that all the tested varieties displayed relatively uniform responses to ascending mutagenic concentrations. These findings underscore the dominant role of NP-40 concentration in inducing complex chromosomal damage, with limited genotype-specific variation. Consequently, concentration selection remains the primary consideration in mutagenesis protocols aimed at inducing complex cytogenetic changes, whereas genotype selection may play a secondary but nuanced role, particularly in fine-tuning targeted breeding outcomes.

All the tested wheat varieties exhibited clear, statistically significant ($P < 0.05$) dose-dependent increases in chromosomal aberrations (fragments, bridges, micronuclei, lagging chromosomes, and cells with multiple aberrations) relative to the control (water). Chromosomal fragments (single and double) were consistently the most prevalent aberration type induced by NP-40 across all the varieties. The ratio of fragments to bridges generally decreased with increasing NP-40 concentration, indicating that bridges become proportionally more significant at higher doses, possibly due to cumulative chromosomal damage affecting segregation processes.

The variety Farell consistently displayed the highest absolute numbers of fragments, bridges, and complex aberrations at most NP-40 concentrations, suggesting a higher intrinsic sensitivity to NP-40-induced chromosomal damage. The variety Ronin also demonstrated significant susceptibility, particularly at higher concentrations (0.1% and 0.5%), though slightly lower than Farell. The varieties Seilor and NE 12443 generally exhibited lower overall levels of aberrations, indicating a comparatively moderate susceptibility. However, NE 12443 showed notable increases at 0.5% NP-40, suggesting its vulnerability becomes pronounced at higher concentrations.

The transition from lower concentrations (0.01% and 0.05%) to higher concentrations (0.1% and 0.5%) resulted in marked increases in the aberration frequency. Specifically, the largest jumps in aberration rates frequently occurred between 0.05% and 0.1% NP-40, highlighting a critical concentration threshold for cytogenetic damage induction. At the highest concentration (0.5% NP-40), the number of aberrations generally peaked or plateaued, potentially indicating the approach of a cytotoxicity limit, beyond which cells experiencing extensive damage may no longer successfully divide or survive.

The percentage of cells exhibiting multiple aberrations increased notably with NP-40 concentration, suggesting progressive genomic instability at higher doses. Farell showed the highest percentages of complex aberrations (19.5% at 0.5% NP-40), reinforcing its elevated sensitivity. All the varieties displayed minimal or no complex aberrations in the controls, emphasizing the direct mutagenic effect of NP-40 treatment.

Statistically significant differences between the treatments (letters "a-e") across all parameters indicate the reliability of chromosomal aberrations as robust cytogenetic markers of NP-40 exposure. The presence of clear statistical separation (Tukey's HSD test, $P < 0.05$) demonstrates that cytogenetic responses reliably distinguish between mutagen doses, confirming their utility in experimental mutagenesis and environmental monitoring protocols.

The epimutagen NP-40 shows strong dose-dependent cytogenetic activity, making it suitable for generating genetic variability in mutation breeding. Variety-specific responses underline the importance of genotype selection, with Farell being the most responsive genotype, suitable for generating diverse genetic forms. The optimal concentration for inducing maximal genetic variability without excessive cytotoxicity is likely around 0.1% NP-40, as higher concentrations (e.g., 0.5%) begin to approach cytotoxic thresholds. These findings support the strategic use of NP-

40 in mutation breeding, recommending concentration levels around 0.05–0.1% to balance genetic variability induction and plant viability.

The discriminant analysis presented in Table 4 and illustrated in Figure 1 identified several key findings related to cytogenetic traits affected by NP-40 treatment. A clear and consistent relationship emerged between increasing NP-40 concentrations and the key cytogenetic parameters, notably pollen sterility, total frequency of chromosomal rearrangements, number of chromosomal fragments and bridges, and frequency of cells with multiple aberrations. These traits exhibited predictable, concentration-dependent trends, affirming their reliability as markers of mutagenic activity. Their strong discriminant loadings underscore their effectiveness in assessing mutagenic responses and classifying treatment intensity.

However, chromosomal bridges (both chromatid- and chromosome-type) displayed concentration-related trends but deviated from the general, consistent pattern observed in other traits. This discrepancy suggests that bridge formation may involve mechanisms less directly linked to mutagen concentration – such as delayed chromatid separation, telomere fusion, or differential DNA repair fidelity. Thus, bridges alone might not serve as robust standalone indicators of NP-40-induced mutagenicity.

The genotype factor (variety) did not significantly affect the most analyzed cytogenetic traits, with the notable exceptions of the pollen fertility, overall frequency, and number of chromosomal bridges ($P < 0.05$). This indicates that, although NP-40-induced chromosomal damage is generally consistent across genotypes, certain fertility-related traits and bridge formation exhibit greater genotype-dependent sensitivity. Contrary to prior findings, no significant genotype × mutagen interaction was detected for "other" chromosomal rearrangements, reinforcing that genotype-specific responses are primarily restricted to fertility and bridge traits. This interaction likely reflects differences in chromatin structure, spindle checkpoint function, or DNA repair mechanisms inherent to the specific genotypes.

Table 4

Traits in model traits for canonical factors space 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	0.72	9.34	0.01	0.07	13.74	0.01
General rates	0.63	3.83	0.05	0.07	11.73	0.01
Fragments	0.63	3.75	0.05	0.04	3.18	0.07
Bridges	0.67	5.45	0.03	0.05	4.54	0.01
Other	0.12	1.21	0.15	0.11	1.11	0.19
Double and more	0.64	4.34	0.04	0.04	2.58	0.04

The discriminant analysis (Fig. 1) clearly separated the treatment groups based on their chromosomal aberration profiles. The first discriminant root (Root 1) distinctly isolated the highest NP-40 concentration (0.5%, black symbols) from all other groups, highlighting its markedly stronger cytogenetic impact. Similarly, the 0.1% NP-40 concentration was clustered separately but adjacent to the 0.5% treatment, demonstrating intermediate yet distinct mutagenic effects. The lower concentrations (0.01% and 0.05% NP-40) formed partially overlapping, intermediate clusters, reflecting progressively moderate levels of mutagenesis. The control group (water-treated, open circles) was clearly distinguished on the negative side of Root 1, confirming minimal baseline aberration frequencies.

These results emphasize a clear, dose-dependent cytogenetic response to NP-40 treatment. The discriminant analysis conclusively confirms that NP-40 concentrations of 0.1% and 0.5% induce distinct, characteristic chromosomal aberration patterns, supporting their strategic use in controlled mutagenesis experiments. Although genotype-specific effects are modest, their significance regarding pollen fertility and chromosomal bridge formation underscores the importance of genotype selection in NP-40-based mutagenesis programs.

The discriminant analysis (Fig. 2) effectively separates the winter wheat varieties based on their distinct chromosomal aberration responses to NP-40 treatments. The varieties Farrel and Ronin (represented by triangles) are clearly differentiated from the other genotypes along Root 1, indicating their significantly elevated susceptibility to NP-40-induced cytogenetic damage. By contrast, the genotypes NE 12443 and Seilor exhibit partially overlapping distributions near the center of the discriminant plot, reflecting their comparatively similar intermediate responses to NP-40 exposure.

This genotype-specific clustering underscores the significant differences in chromosomal aberration patterns among wheat varieties, highlighting Farrel as uniquely responsive, and therefore strategically beneficial for targeted mutation breeding programs. Ronin also exhibited higher-than-average responsiveness, positioning it as a potentially valuable genotype for mutagenesis studies, although less responsive than Farrel.

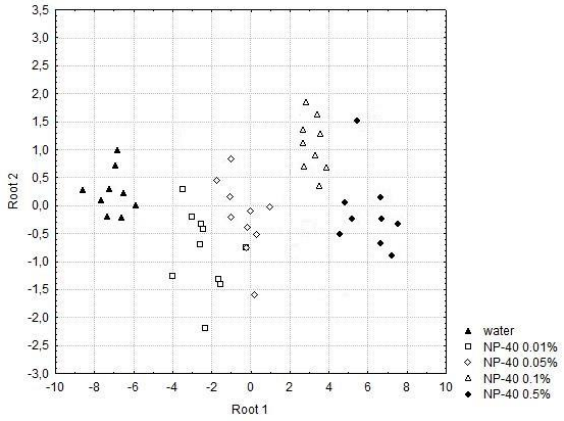

Fig. 1. Model for factors space (for concentrations)

The comparative analysis of genotype and mutagen concentration revealed important characteristics of their combined mutagenic action. Although minor genotype-specific variations – particularly in the formation of chromatin bridges – were noted, both genotype and NP-40 concentration demonstrated broadly similar mutagenic profiles (Figs. 1 and 2). These results indicate that, despite individual variations, the general patterns of cytogenetic response remain consistent across treatments. Model parameters confirmed the predictability and stability of both genotype and concentration effects, reinforcing their reliability in controlled genetic modification experiments.

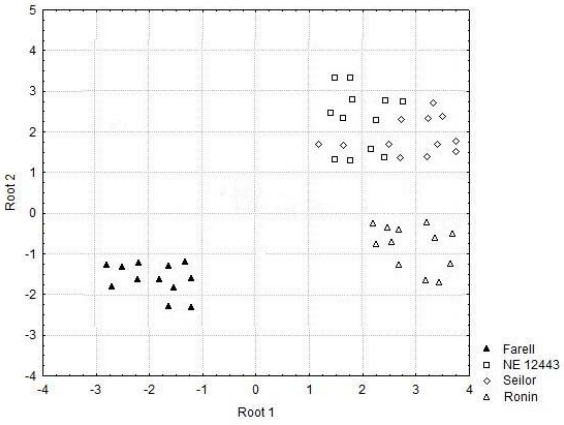

Fig. 2. Model for factors space
(for initial material, winter wheat varietyvariety)

The clear trends observed with increasing NP-40 concentrations and varying initial genotypes validated these parameters as stable, reproducible indicators, essential for targeted mutagenesis applications. The discriminant analysis further highlighted the utility of NP-40 as an effective mutagenic tool, identifying cytogenetic and reproductive traits – such as pollen sterility, chromosomal rearrangements, and complex nuclear anomalies – that respond consistently to both genotype and concentration changes. However, the behavior of chromosomal bridges deviated from this general trend, suggesting involvement of more intricate or genotype-dependent mechanisms, possibly linked to variations in chromosome architecture, telomere integrity, or DNA repair fidelity.

Overall, these findings emphasize the critical importance of genotype selection in mutation breeding programs, as genotype–mutagen interactions substantially influence mutagenic outcomes. Specifically, the pronounced responsiveness of Farrel and, to a lesser extent, Ronin, positions these genotypes as promising candidates for further mutagenic investigations. Understanding the underlying mechanisms driving their enhanced susceptibility or tolerance to NP-40 treatment could yield deeper insights

into the site-specific effects of epimutagens and contribute to the optimization of targeted breeding strategies.

The classification analysis based on the effects of NP-40 concentration and varietal characteristics demonstrated high precision and reliability in differentiating mutagenic outcomes across experimental conditions. Classification accuracy remained consistently high across all NP-40 concentrations, including the highest (fourth) concentration, highlighting the robustness and sensitivity of the selected parameters for distinguishing mutagenic impacts at various levels. Minimal classification errors further confirmed the suitability of these parameters as reliable discriminators.

The varietal responses to NP-40 exposure were effectively differentiated, indicating that the chosen traits accurately captured genotype–mutagen interactions. Among the evaluated cytogenetic and fertility traits, pollen fertility, total frequency of chromosomal rearrangements, and the number of chromosomal bridges exhibited the highest discriminative power. These three traits accounted for the majority of the observed variability and were essential for evaluating both the intensity of mutagenic effects and genotype-specific sensitivities. Their significance underscores their value as primary indicators for predicting mutagenic responses and assessing genotype-specific vulnerability to NP-40 treatment.

Overall, the classification accuracy achieved by the model exceeded 80%, highlighting the robustness and discriminative capability of the selected cytogenetic and fertility parameters in differentiating mutagenic effects and varietal responses. The predictive model developed in this study provides a reliable framework for evaluating the efficacy of NP-40 as an epimutagen capable of inducing substantial genetic and epigenetic variability. By effectively integrating the variability associated with specific genotypes and mutagen concentrations, the model serves as a dependable tool for forecasting NP-40-induced outcomes at the cellular and organismal levels.

The experimental design and selection of key cytogenetic parameters significantly enhance the model's predictive power and practical applicability. Among the tested genotypes, the variety Farell exhibited high responsiveness to NP-40 treatment, demonstrating substantial genetic variability and strong potential for generating valuable mutant lines. The variety Ronin, although less predictable, also presented adequate levels of induced variability and thus can serve as suitable initial breeding material. Collectively, these findings confirm the utility and reliability of NP-40 as an effective mutagenic agent for creating diverse and desirable genetic modifications in winter wheat. The developed predictive model offers breeders a valuable and practical resource for optimizing NP-40 applications in mutation breeding programs, ultimately facilitating the development of genetically diverse, agronomically advantageous mutant cultivars.

Discussion

Identifying the key components of cytogenetic variability significantly streamlines the process of selecting optimal doses and concentrations of mutagenic agents (Amri-Tiliouine et al., 2018; Khalil et al., 2018). Effective mutagenesis protocols typically aim to maximize the activity of certain cytogenetic indicators or to achieve a plateau effect in response to increasing mutagen doses (Desai et al., 2022; Shabani et al., 2022). Chemical supermutagens commonly induce prominent chromosomal aberrations, such as fragments and double fragments, and elevate the overall frequency of chromosomal rearrangements, establishing these as reliable markers for evaluating mutagenic efficacy (Abdullah et al., 2018; Jung & Till, 2021).

Fragements have frequently been identified as valuable markers for qualitative assessments of environmental genotoxicity and low-dose mutagen exposure (Surakshitha et al., 2017). However, their utility diminishes in primary screening protocols intended to induce significant genetic diversity, as such research typically requires mutagen concentrations far exceeding typical environmental contamination levels (Horshchar & Nazarenko, 2024). Moreover, while rare chromosomal aberrations effectively indicate increasing mutagen concentrations up to critical levels of mitotic suppression, they inadequately reflect genotype-specific cytogenetic responses or detailed genotype-mutagen interactions, highlighting the necessity of tailored, genotype-aware approaches (Cabahug et al., 2020; Shabani et al., 2022).

By contrast, parameters such as frequency of complex aberrations (cells exhibiting two or more simultaneous chromosomal changes) and rarer bridge-type rearrangements offer potential in evaluating nuanced genotype–mutagen interactions (El Oualkadi et al., 2019; Pathirana, 2021). Although promising, the practicality and reliability of these indicators, compared with more established parameters, remain under investigation. Additional comparative studies could clarify whether complex aberrations and bridge-type rearrangements should serve as primary indicators or complementary tools in genotype-specific mutagenesis research (Kiani et al., 2022; Murthy et al., 2024).

Established and reliable indicators for evaluating genotype–mutagen interactions include pollen fertility, total frequency of chromosomal rearrangements, and fragment-type aberrations (single and double fragments) (Rozman, 2015; Spencer-Lopes et al., 2018). These parameters effectively capture varietal responses and reflect the differential impacts of epimutagen treatments. The observed variability in these traits across certain genotypes justifies their targeted selection for mutation breeding programs using epimutagenic agents like NP-40 (Shabani et al., 2022). Such strategic genotype selection optimizes the induction of beneficial genetic and epigenetic changes, maximizing the breeding efficiency.

The findings presented in this study align well with previous reports of NP-40 mutagenesis in other cereal crops, notably concerning early-generation depressive effects and the induction of desirable mutations (Muhammad et al., 2021; Oprica et al., 2023). The tested NP-40 concentrations provided sufficient contrast to generate a broad spectrum of variability among the evaluated genotypes. While certain other effects are possible, they are usually restricted to genotypes containing translocations from wild relatives or specific landrace traits (Surakshitha et al., 2017). Given the observed outcomes at higher concentrations (0.05% and 0.1% NP-40), selecting the lower of these concentrations could prove more practical for general mutation breeding protocols. However, focusing exclusively on the lower concentration might inadvertently narrow the scope of potentially beneficial mutations, especially when working with genotypes inherently exhibiting limited variability (Nazarenko et al., 2019; Kryshyn & Nazarenko, 2025). Hence, a moderately broad concentration range remains advisable, as even within this study, approximately 25% of the tested forms demonstrated a notably valuable variability (Nazarenko, 2020; Von Well et al., 2022).

Cytogenetic evaluation has proven invaluable not only as a screening tool to determine epimutagenic potential and optimal dosing but also as an effective method for evaluating genotype compatibility with mutagenic treatments (Yan et al., 2021; Apio et al., 2024). Over successive research cycles, advances in selecting appropriate mutagenic agents, concentrations, and responsive genotypes have markedly improved the efficiency of mutation induction (Spencer-Lopes et al., 2018; Li et al., 2022). This methodology is particularly promising for generating complex biochemical epimutations, with applications ranging from the development of nutritionally enriched food crops containing essential microelements and bioactive compounds to creating plant lines exhibiting enhanced physiological performance and resilience to environmental stresses (Nazarenko et al., 2022; Didenko & Nazarenko, 2025). Potential innovations include cereal genotypes exhibiting prolonged nitrogen reutilization periods, highlighting the possibility of fundamentally novel stress adaptation mechanisms and improved resource efficiency in cultivated crops.

Conclusion

The studied epimutagen, NP-40, exhibited a 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 varieties Farrel and Ronin were particularly notable for their pronounced responsiveness, emerging as promising candidates 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. The lower concentrations generally yielded fewer beneficial changes, reducing both the quantity and quality of the mutant material obtained. Notably, the interaction between genotype and NP-40 concentration was less pronounced compared with the 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 the 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 es-

sential insights to harness the full potential of NP-40 and related epimutagenic agents in sustainable crop improvement strategies.

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