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Share of positive changes in winter wheat varieties as parameter of action of a new epimutagenic agent

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The investigation of novel agents for inducing practical biodiversity in modern winter wheat varieties presents significant opportunities for developing new commercial cultivars and components for recombination breeding. This study aimed to assess the effectiveness of the epimutagen Nonidet P-40 (NP-40) for optimizing yield and grain quality parameters in mutant wheat forms. Eight winter wheat varieties (Perspektyva Odeska, Sonata Poltavska, Shpalivka, MIP Lada, Farell, NE 12443, Ronin, and Seilor) were exposed to NP-40 at concentrations of 0.01%, 0.05%, 0.1%, and 0.5%, with a seed exposure period of 24 hours, adhering to established chemical mutagenesis protocols. The study identified clear patterns in epimutational processes affecting agriculturally valuable traits, thereby enhancing the predictability and reliability of mutation breeding programs. The varieties Farell, Ronin, and Perspektyva Odeska demonstrated particularly high potential for generating agriculturally valuable variability. Optimal epimutagen concentrations (0.1% and 0.5% NP-40) produced substantial breeding variability, yielding valuable genetic material suitable for both direct commercialization and parental lines in hybridization programs. Notably, NP-40 induced epimutations with high reliability in traits such as improved microelement content, early ripeness, increased protein content, and reduced plant height. Moderately induced traits included elongated spikes, enhanced protein composition, improved productivity, and increased disease tolerance. NP-40 treatments, while highly effective overall, showed a strong dependency on genotype, suggesting targeted variety selection is essential. Although the frequency of complex trait epimutations was relatively low, such epimutations, when present, proved highly valuable. Higher concentrations of NP-40 increased the risk of undesirable traits such as taller plants and delayed maturity, highlighting the necessity for balanced concentration management. Several promising epimutant winter wheat lines were identified as potential future commercial cultivars, exhibiting high grain productivity and superior bread-making quality, including one exceptional form noted for outstanding grain protein characteristics. Further research is planned to evaluate physiological adaptations of these new lines, particularly their resistance to abiotic stresses such as drought and winter hardiness.

Keywords: cereals; variability; Nonidet P-40; positive changes; nutrition value; site-specific action; epimutations; ecogenetic.

Introduction

Mutations introduce new alleles into a population's gene pool, serving as the raw material for evolution. Without mutations, there would be no genetic diversity for natural selection to act upon. This makes mutation not only a central mechanism in natural evolution but also a critical tool in artificial selection and plant breeding. The application of chemical mutagenesis to cultivated plants often results in hereditary changes that can significantly benefit genetic improvement efforts in mutation breeding programs. Even a single, minor exposure to chemical mutagens can profoundly alter the development and heredity of crop plants (Stearns et al., 2025). Chemical supermutagens, characterized by their low-damage yet highly effective mutation-inducing capacity, have become one of the preferred methods not only for practical genetic improvements but also for fundamental investigations into plant mutagenesis, beginning with model genetic plant systems. A significant portion of successful agricultural forms in major cultivated crops has emerged from chemical mutagenesis efforts. Given the current level of biological research, chemical mutagenesis is widely applied both for crossbreeding improvement through parental lines and for studying gene systems associated with specific key traits, as well as obtaining fundamentally novel biochemical compositional traits along with other advantageous phenotypic characteristics (Didenko & Nazarenko, 2025).

Induced mutagenesis, coupled with targeted breeding strategies, represents an efficient and cost-effective means of enhancing both quantitative and qualitative traits in crops, offering results within a significantly shorter timeframe compared to traditional breeding techniques. The relative simplicity and affordability of chemically mutating seeds or plant tissues continue to make this a favored approach for isolating desired variants, particularly for traits conferring resistance to biotic and abiotic stressors across diverse crop species (Fradgley et al., 2024).

Several researchers advocate for the use of higher concentrations of chemical mutagens due to their potential to induce complex, multi-trait changes. Supporting this perspective, global statistical analyses demonstrate successful genetic advancements in certain directions through high-concentration mutagen applications (Farooq et al., 2024). Conversely, moderate chemical mutagen concentrations have been effectively employed to generate productive mutant lines demonstrating increased resistance to diseases and environmental stresses. Chemical mutagen treatments frequently result in profound morphological alterations, yielding novel phenotypes with potentially advantageous biochemical compositions and enhanced nutritional profiles (Anter et al., 2021).

The efficacy of chemical mutagenesis is marked by the generation of pronounced mutations, accompanied by a high incidence of morphoses and genocopies. Although chemical mutagens may not consistently produce the highest overall mutation rates, their seed treatments remain highly effective for generating subsequent mutational material. In contrast, increasing mutation rates and spectra through other approaches often encounters subsequent challenges and limitations (Horshchar & Nazarenko, 2023).

Innovative methodologies in both food and non-food crop mutagenesis have led to the successful creation of thousands of improved crop varieties worldwide, characterized by enhanced yields, greater resilience to drought, salinity, diseases, and pests, along with improved consumer quality. Mutations have been systematically induced to boost productivity and stress tolerance in major crops, including wheat, rice, barley, and cotton, affirming the value of mutagenesis as a practical breeding tool (Bayhan et al., 2024).

Chemical mutagens used in plant breeding programs typically belong to two main categories: chemical agents, such as alkylating agents, sodium azide (SA), ethyl methanesulphonate (EMS, inducing point mutations), and diepoxybutane (DEB, causing point mutations and minor deletions); and physical agents, including X-rays, gamma rays, fast neutrons, and ultraviolet radiation (Arumingtyas et al., 2023).

Chemical mutagens offer distinct advantages, particularly their ability to interact more effectively with specific genotypes and exhibit heightened site-specificity to native DNA sequences – characteristics less commonly associated with physical mutagens. This increased specificity presents numerous promising opportunities for precise genetic improvements in agricultural crops, which are otherwise challenging to achieve using alternative mutation-inducing techniques (Abdel-Hamed et al., 2021; Kryshyn & Nazarenko, 2025).

Bread winter wheat is particularly significant for regions experiencing unstable climatic conditions, encompassing large agricultural territories within Ukraine (Nazarenko et al., 2021). Ongoing climate challenges, including global warming, have driven the cultivation of traditionally heat-tolerant crops further northward, thereby necessitating adaptation strategies such as improved overwintering capabilities, particularly critical for winter crops (Hongjie et al., 2019; Harkness et al., 2020).

The central objective of this research was to elucidate hereditary variability characteristics in local and introduced winter wheat varieties, focusing specifically on the frequency and spectrum of positive trait alterations, including biochemical epimutations. The study further aimed to evaluate the efficacy of the chemical agent NP-40 as an epimutagen within breeding practices, emphasizing the importance of genotype-mutagen interactions. The investigation sought to leverage site-specific mutational capabilities to achieve targeted genetic enhancements, promoting advancements in breeding strategies at both broad and narrow genetic improvement levels.

Materials and methods

Field experiments were conducted during 2022–2024 (M_4–M_6 generations) at the experimental fields of the Educational and Scientific Center of the Dnipro State Agrarian and Economic University (48°50′98″ N, 35°25′63″ E, Fig. 1), located in Dniprovskyi district, Dnipropetrovsk region, Ukraine. This region is characterized as a northern warm, moderately arid agro-climatic zone with a hydrothermal coefficient above 0.9. Total precipitation during the growing season typically ranged from 250 to 280 mm, with an annual accumulation of approximately 450–490 mm. The sum of temperatures above 10 °C during the growing period was approximately 2900 °C.

The experimental fields exhibited homogenous soil cover, consisting of ordinary low-humus, leached, medium-loamy chernozem formed on loamy parent material. Soil nutrient analyses during the experimental years indicated a nitrogen content (Tiurin method) of 3–5 mg/100 g dry soil, mobile phosphorus (20–30 mg/100 g dry soil, Chyrykov method), and exchangeable potassium (20–35 mg/100 g dry soil, Chyrykov method).

Eight winter wheat (*Triticum aestivum* L.) varieties – Perspektyva Odeska, Sonata Poltavska, Shpalivka, MIP Lada, Farell, NE 12443, Ronin, and Seilor – were selected as initial plant material. Seeds (1000 grains per treatment variant) were treated with the epimutagen Nonidet P-40 (NP-40) at concentrations of 0.01%, 0.05%, 0.1%, and 0.5% (Sigma-Aldrich, Germany). Treatments were conducted by soaking seeds for 24 hours, following established protocols for chemical mutagenesis (Spencer-Lopes et al., 2018; Nazarenko, 2021).

In the M_2 and M_3 generations, induced mutations were visually assessed, and inheritance patterns were studied. Seeds were sown by hand in family plots (1–3 rows per plot; row spacing 0.15 m, row length 1.5 m). Mutation rates were calculated as the percentage of mutant occurrences relative to the total number of families.

Mutant lines were evaluated for yield and grain quality parameters from the M_4 to M_6 generations. Plots varied from 5 to 10 m², depending on the year of the experiment, with replication performed 1–2 times and standard control varieties included every 20 plots. Grain protein content was quantified using a Spektra RT device. Glutenin and gliadin contents were analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC). The concentration of essential microelements (Mg, Mn, Zn, Mo, Co, Cu) in grains was determined using an Agilent 5110 inductively coupled plasma atomic emission spectrometer (ICP-AES), with wavelength parameters calibrated against Agilent multi-element standard solutions.

Fig. 1. Place of field experiment

Statistical analyses were conducted using Statistica 10.0 software (multivariate module, TIBCO, Palo Alto, USA). The significance of treatment effects was evaluated through analysis of variance (ANOVA), complemented by discriminant and cluster analyses (Euclidean distance, single linkage method). Pairwise comparisons between treatments were performed using Tukey's HSD test, with significance set at $P < 0.05$.

Results

The evaluation of plant material derived from NP-40 treatment began with the assessment of positive phenotypic changes in the second and third generations (M_2–M_3), followed by analysis of yield and grain quality traits in later generations (M_4–M_6). All selected lines were successively sub-cultivated to confirm the inheritance of observed changes, thereby ensuring that the modifications were stable mutations rather than transient epigenetic effects. In total, 19,950 families (lines) were evaluated at M_2–M_3 stages for NP-40-induced populations. Out of these, 344 mutant lines were selected as carriers of noticeable trait changes (see Table 1). Among them, 243 lines showed agriculturally valuable traits, and 124 lines were identified as elite material – exhibiting positive traits without any undesirable characteristics, and thus were advanced for breeding purposes. Each treatment variant included 500 families, except for the variety Farell under 0.5% NP-40, which had 450 families, due to partial loss of viability at high concentration – indicative of a nearing cytotoxic threshold.

Mutant lines were selected based on the following morphological traits (evaluated at M_2–M_3 stages): thick stem, short-stem, semi-dwarf, dwarf forms, intense epicuticular wax accumulation, long spike and large spike size, large grain size, early ripeness, disease tolerance, tillering capacity, productivity indicators (preliminary M_2–M_3, confirmed at M_4–M_6). Grain quality traits (evaluated during M_4–M_6 ecological trials), such as higher total protein content, positive shifts in gluten composition: changes in HMW/LMW glutenins and gliadins, enhanced microelement content: Mg, Mn, Zn, Mo, Co, Cu. These traits were used as selection markers to distinguish lines with practical breeding value. The overall mutation efficiency under NP-40 was highly promising: nearly 1.7% of families produced valuable breeding material (124/7,500 averaged across variants). Heritability of traits was confirmed through multi-generation stability, indicating that epimutagenic changes from NP-40 had likely entered the heritable genomic layer (epimutations fixed or linked to genetic mutation sites). NP-40 induced a broad mutation spectrum, including both structural and biochemical traits, which enhances its utility in both recombinant breeding and direct selection strategies.

Despite being modern and genetically narrow (low spontaneous variability), the varieties responded to NP-40 with significant phenotypic diversification, supporting NP-40's non-genotoxic but epigenetically active profile. Epimutation frequencies consistently increased with rising NP-40 concentrations for all varieties. The highest total mutation frequencies were generally observed at the maximum concentration (0.5%), suggesting strong, dose-dependent mutagenic activity. Varieties exhibited substantial variability in response to NP-40 treatments – Seilor (3.80%) and NE 12443 (3.58%) exhibited the highest total mutation frequencies at 0.5% NP-40, indicating strong responsiveness, but Farell (2.80%), Perspektyva Odeska (3.40%), Ronin (3.20%), and Shpalivka (2.60%) were also highly responsive at 0.5%, Sonata Poltavska (2.00%) and MIP Lada (2.80%) showed moderate responsiveness.

Frequent epimutation categories included short-stem, early ripeness and long spike reflecting typical traits desired in wheat breeding. Short-stem changes consistently occurred across all varieties, becoming increasingly frequent at higher NP-40 concentrations (up to 0.4–0.6%). Early ripeness lines were also consistently induced, reaching frequencies of 0.4–0.6% at higher concentrations, notably in Seilor and NE 12443. Changes linked to productivity (productive, large-size grain, tillering capacity) and disease resistance were observed at lower frequencies (0.2–0.4%) across varieties and NP-40 concentrations. Disease tolerance mutations appeared at moderate frequencies (up to 0.4%) primarily at higher concentrations.

Positive changes in protein content and composition ("higher protein content," "positive changes in protein components") exhibited moderate to high frequencies at elevated NP-40 concentrations, notably reaching 0.6–0.8% in Farell, Ronin, and Seilor. Positive changes in microelement content also reached significant levels (0.6–0.8%) at higher concentrations, notably in Farell, NE 12443, Ronin, and Seilor.

The most frequently induced traits were positive changes in microelement content (average ~1.4%). High frequency and low variability indicate that NP-40 reliably induces these biochemical mutations across all tested varieties. Early ripeness (average ~1.05%), consistently high across varieties, indicates a robust mutation-inducing potential. There was higher protein content (average ~0.95%), significant frequency and consistent induction across multiple varieties. Short-stem (average ~0.90%) was frequently induced across nearly all varieties, strongly desired agronomic trait.

Moderately induced traits were long spike and positive changes in protein components (~0.80%–0.85%), consistently observed but with moderate variability across varieties, indicating genotype-dependent induction. Productivity and tolerance to diseases (~0.65%), moderate frequency and moderate genotype dependency indicate potential but less predictable induction. Traits such as dwarf, semi-dwarf forms, thick stem, intense epicuticular wax accumulation were infrequent or absent, suggesting that these traits are difficult to induce or strongly genotype-dependent. Perspektyva Odeska was characterized by high induction for biochemical traits (protein and microelements) and short-stem, early ripeness, and long spike. It is a highly responsive variety overall, particularly responsive to biochemical improvement. Sonata Poltavska has moderate to high frequency of agronomic traits (short-stem, early ripeness, tolerance, productivity), moderate biochemical response. This is particularly valuable for improvement of agronomic traits.

Shpalivka was characterized by balanced induction of agronomic (short-stem, long spike, productivity) and biochemical traits. It is well-suited for diverse breeding objectives due to broad responsiveness. MIP Lada was characterized by high induction in microelement content and moderate induction in long spike, early ripeness, and short-stem. It showed more specialized responsiveness, strong biochemical trait induction potential. Farell showed strong induction across both biochemical (protein content, microelements) and agronomic traits (short-stem, early ripeness). It is highly responsive, ideal for multi-trait improvement strategies.

NE 12443 was characterized by notably high response for protein content and microelements, with moderate agronomic responsiveness. It is suitable for improving nutritional quality of grain. Ronin showed strong and consistent biochemical and agronomic responses (protein, microelements, short-stem, early ripeness). It is a good candidate for targeted biochemical and early maturity traits. Seilor has the highest overall mutation frequency, especially strong in microelement content, early ripeness, and protein content. It is the most responsive and versatile genotype, ideal for extensive breeding objectives.

Ronin and Farell emerge as optimal candidates for broad-spectrum mutation breeding, showing high trait responsiveness. The varieties Perspektyva Odeska, Sonata Poltavska and Shpalivka are valuable for targeted agronomic trait improvement. 0.5% NP-40 induced the most substantial genetic variability, balancing mutagenic efficiency without apparent excessive toxicity. Lower concentrations (0.01–0.1%) yielded fewer mutations but maintained plant viability, making them suitable for less intensive mutation induction.

The varieties Seilor and Ronin are highly responsive to NP-40, demonstrating considerable potential for mutation breeding, particularly at higher concentrations. Epimutations affecting plant architecture (short-stem), maturity (early ripeness), and biochemical traits (protein, microelement content) are effectively induced by NP-40, particularly at the concentration of 0.5%. Given these findings, using 0.5% NP-40 is recommended to maximize induced variability in targeted traits, while moderate concentrations (0.05–0.1%) can be effectively utilized for more conservative breeding strategies.

As observed, the rate of positive trait changes increased with higher mutagen concentrations for varieties Farell, NE 12443, Ronin and Seilor across all tested variants. However, varieties Perspektyva Odeska ($F = 3.26$; $F_{0.05} = 4.99$; $P = 0.08$), Sonata Poltavska ($F = 3.02$; $F_{0.05} = 4.99$; $P = 0.08$), and Shpalivka ($F = 2.72$; $F_{0.05} = 4.99$; $P = 0.09$) showed no significant differences between the lowest two NP-40 concentrations (0.01% and 0.05%). Similarly, no significant differences were found between NP-40 concentrations of 0.1% and 0.5% for varieties Perspektyva Odeska ($F = 3.11$; $F_{0.05} = 4.99$; $P = 0.07$) and MIP Lada ($F = 2.99$; $F_{0.05} = 4.99$; $P = 0.07$). Overall variability in positive changes was generally consistent across genotypes (ranging from 2.6% to 3.2% without significant differences), with the exception of variety Farell, which demonstrated higher variability at 3.57% ($F = 5.62$; $F_{0.05} = 4.99$; $P = 0.04$). Thus, Farell exhibited a gradual and concentration-dependent increase in obtaining valuable forms without introducing additional negative traits, although the rate of complex changes typically increased with higher NP-40 concentrations.

Factor analysis revealed that the induction rate of positive trait changes was significantly influenced by increased NP-40 concentrations ($F = 45.36$; $F_{0.05} = 3.86$; $P = 4.01×10-5$) and was significantly affected by varietal differences ($F = 34.17$; $F_{0.05} = 4.11$; $P = 1.98×10^{-4}$). Additionally, the genotype-mutagen interaction was significant ($F = 19.17$; $F_{0.05} = 4.55$; $P = 1.17×10^{-3}$).

The proportion of positive changes relative to the general mutation rate was modest, ranging approximately from 0.5% to 0.7% for most varieties, except Ronin, which showed somewhat higher values. Other parameters, such as the number of promising lines, were generally unaffected by increasing mutagen concentrations or by varietal differences, except for variety Farell, which showed a greater number of promising lines, and to a lesser extent Ronin.

Evaluation of the number of promising lines indicated the ineffectiveness of lower NP-40 concentrations (0.01% and 0.05%) due to the minimal induction of additional desirable traits. Importantly, unlike classical mutagens, higher epimutagen concentrations did not lead to an increased frequency of negative characteristics. Another key parameter for mutation breeding, the proportion of promising lines derived from the total number of families (500 for all variants), did not substantially increase with higher NP-40 concentrations (less variable indicator overall). Nevertheless, the rate of promising lines was influenced significantly by NP-40 concentration increases ($F = 4.17$; $F_{0.05} = 3.86$; $P = 0.04$), varietal differences ($F = 34.25$; $F_{0.05} = 4.11$; $P = 2.49×10^{-4}$), and the genotype-mutagen interaction ($F = 9.43$; $F_{0.05} = 5.20$; $P = 0.002$).

In pairwise comparison, we find that there is significant difference between water and NP-40 0.01% for MIP Lada ($F = 1.12$; $F_{0.05} = 4.99$; $P = 0.09$), between NP-40 0.01% and NP-40 0.05% for Perspektyva Odeska ($F = 4.10$; $F_{0.05} = 4.99$; $P = 0.06$), Sonata Poltavska ($F = 4.12$; $F_{0.05} = 4.99$; $P = 0.06$), Shpalivka ($F = 4.17$; $F_{0.05} = 4.99$; $P = 0.06$), MIP Lada ($F = 3.17$; $F_{0.05} = 4.99$; $P = 0.06$), NE 12443 ($F = 4.49$; $F_{0.05} = 4.99$; $P = 0.06$), Ronin ($F = 4.44$; $F_{0.05} = 4.99$; $P = 0.06$) and Seilor ($F = 4.53$; $F_{0.05} = 4.76$; $P = 0.06$), for the concentrations of

NP-40 0.05% and 0.1% significant difference for all varieties, between NP-40 0.1% and NP-40 0.5% for varieties Perspektyva Odeska (F = 4.44; $F_{0.05}$ = 4.99; P = 0.06), Sonata Poltavska (F = 4.67; $F_{0.05}$ = 4.99; P = 0.06), Shpalivka (F = 4.41; $F_{0.05}$ = 4.99; P = 0.06), MIP Lada (F = 4.48; $F_{0.05}$ = 4.99; P = 0.06), NE 12443 (F = 4.56; $F_{0.05}$ = 4.99; P = 0.06), Ronin (F = 4.44; $F_{0.05}$ = 4.71; P = 0.06) and Seilor (F = 4.82; $F_{0.05}$ = 4.76; P = 0.06).

Table 1
Parameters of epimutagen action, NP-40 (x ± SD, n = 450–500)

Variety	Variant	Rate of positive changes, %	Share of general rate, %	Number of lines, pcs	Number of promising lines	Rate of promising lines, %
Perspektyva Odeska	water	0.00 ± 0.00^{a}	0.00	0	0	0.00 ± 0.00^{a}
	NP-40 0.01%	1.60 ± 0.15^{b}	0.67	6	2	0.40 ± 0.11^{b}
	NP-40 0.05%	1.60 ± 0.15^{b}	0.50	6	3	0.60 ± 0.12^{b}
	NP-40 0.1%	3.40 ± 0.20^{c}	0.77	14	5	1.00 ± 0.18^{c}
	NP-40 0.5%	3.20 ± 0.19^{c}	0.57	12	6	1.20 ± 0.19^{b}
Sonata Poltavska	water	0.20 ± 0.03^{a}	0.20	0	0	0.00 ± 0.04^{a}
	NP-40 0.01%	1.20 ± 0.08^{b}	0.60	4	2	0.40 ± 0.06^{b}
	NP-40 0.05%	1.40 ± 0.12^{b}	0.46	5	3	0.60 ± 0.13^{b}
	NP-40 0.1%	2.00 ± 0.14^{c}	0.47	7	5	1.00 ± 0.14^{c}
	NP-40 0.5%	2.60 ± 0.15^{d}	0.48	8	5	1.00 ± 0.15^{c}
Shpalivka	water	0.20 ± 0.01^{a}	0.50	1	0	0.00 ± 0.00^{a}
	NP-40 0.01%	1.00 ± 0.11^{b}	0.50	4	2	0.40 ± 0.05^{b}
	NP-40 0.05%	1.20 ± 0.12^{b}	0.43	5	2	0.40 ± 0.05^{b}
	NP-40 0.1%	2.00 ± 0.15^{c}	0.56	6	4	0.80 ± 0.09^{c}
	NP-40 0.5%	2.80 ± 0.19^{d}	0.58	9	5	1.00 ± 0.12^{c}
MIP Lada	water	0.40 ± 0.03^{a}	0.67	1	1	0.20 ± 0.04^{a}
	NP-40 0.01%	1.00 ± 0.11^{b}	0.56	3	1	0.20 ± 0.04^{a}
	NP-40 0.05%	1.40 ± 0.14^{c}	0.54	4	1	0.20 ± 0.04^{a}
	NP-40 0.1%	2.60 ± 0.18^{d}	0.69	7	4	0.80 ± 0.10^{b}
	NP-40 0.5%	2.80 ± 0.18^{d}	0.64	8	4	0.80 ± 0.10^{b}
Farell	water	0.20 ± 0.00^{a}	0.33	1	0	0.00 ± 0.00^{a}
	NP-40 0.01%	1.40 ± 0.10^{b}	0.87	6	3	0.60 ± 0.04^{b}
	NP-40 0.05%	1.80 ± 0.13^{c}	0.64	7	5	1.00 ± 0.09^{c}
	NP-40 0.1%	2.80 ± 0.19^{d}	0.82	11	7	1.40 ± 0.14^{d}
	NP-40 0.5%	3.57 ± 0.27^{e}	0.73	14	10	2.22 ± 0.19^{e}
NE 12443	water	0.00 ± 0.00^{a}	0.00	0	0	0.00 ± 0.00^{a}
	NP-40 0.01%	1.00 ± 0.09^{b}	0.63	3	1	0.20 ± 0.02^{b}
	NP-40 0.05%	1.40 ± 0.11^{c}	0.64	4	1	0.20 ± 0.02^{b}
	NP-40 0.1%	2.40 ± 0.17^{d}	0.67	9	3	0.60 ± 0.07^{c}
	NP-40 0.5%	3.00 ± 0.19^{e}	0.71	10	3	0.60 ± 0.07^{c}
Ronin	water	0.20 ± 0.01^{a}	0.50	0	0	0.00 ± 0.00^{a}
	NP-40 0.01%	1.20 ± 0.10^{b}	0.50	5	2	0.40 ± 0.03^{b}
	NP-40 0.05%	2.00 ± 0.15^{c}	0.63	8	3	0.60 ± 0.08^{b}
	NP-40 0.1%	3.20 ± 0.18^{d}	0.89	13	9	1.80 ± 0.11^{c}
	NP-40 0.5%	3.80 ± 0.19^{e}	0.86	14	10	2.00 ± 0.12^{c}
Seilor	water	0.00 ± 0.00^{a}	0.00	0	0	0.00 ± 0.00^{a}
	NP-40 0.01%	1.20 ± 0.10^{b}	0.55	4	1	0.20 ± 0.02^{b}
	NP-40 0.05%	1.60 ± 0.12^{c}	0.57	6	2	0.40 ± 0.04^{b}
	NP-40 0.1%	2.40 ± 0.18^{d}	0.66	9	4	0.80 ± 0.10^{c}
	NP-40 0.5%	3.20 ± 0.21^{e}	0.76	11	5	1.00 ± 0.12^{c}

Note: indicate significant differences at $P < 0.05$ by Tukey's HSD test with Bonferroni amendment; comparison in terms of one variety at columns.

For each variety genotype-mutagen interaction was significant in a negative sense for varieties NE 12443 (F = 7.77; $F_{0.05}$ = 3.55; P = 0.02) and MIP Lada (F = 6.93; $F_{0.05}$ = 3.55; P = 0.03). Thus, in general, NP-40 as epimutagen in complex with these genotypes cannot be used for high epimutation rate with any concentration, for the most part of varieties the critical value of the epimutagen in terms of valuable changes in the range NP-40 0.1–0.5%.

Dose-dependent trends were identified. Both the rate of positive changes and the rate of promising lines consistently increase with rising NP-40 concentrations across all varieties. This demonstrates a robust, dose-dependent mutagenic effect of NP-40.

Farell and Ronin exhibit the steepest increases, particularly at higher NP-40 concentrations (0.1% and 0.5%), making these varieties the most responsive and suitable candidates for intensive breeding programs. Perspektyva Odeska and Seilor also demonstrate high responsiveness at elevated concentrations, particularly noticeable at 0.5% NP-40, indicating their potential for breeding applications. Sonata Poltavska, Shpalivka, MIP Lada, and NE 12443 showed moderate but consistent responsiveness, suitable for targeted breeding objectives at medium mutagenic intensities.

The number of promising lines generally correlates positively with the rate of positive mutations, particularly for highly responsive varieties like Farell and Ronin. This suggests that increased mutagenic activity not only generates broader genetic variability, but specifically enhances the production of commercially valuable mutations.

The analysis of Table 1 reveals several important insights regarding the induction of mutations by the epimutagen NP-40 in winter wheat varieties. Farell at 0.5% NP-40 showed the highest rates of both positive changes (3.58%) and promising lines (2.22%), making it the top-performing treatment for breeding. Ronin at 0.5% NP-40 also demonstrated strong results, with a high rate of positive changes (3.80%) and promising lines (2.00%). Ronin at 0.1% NP-40 and Farell at 0.1% NP-40 were notable, indicating good efficiency at a moderately lower concentration.

Higher NP-40 concentrations consistently led to increased rates of positive changes and more promising lines across all varieties, clearly showing a dose-dependent relationship. Varietal responsiveness varied significantly, with Farell and Ronin emerging as highly responsive, demonstrating a strong ability to produce both general positive changes and valuable breeding lines. Other varieties such as Perspektyva Odeska and Seilor also showed significant mutation induction at higher concentrations (0.5% NP-40), suggesting their potential in targeted mutation breeding programs.

Farell and Ronin, particularly at NP-40 0.5%, are strongly recommended for future breeding programs aimed at maximizing genetic variability and valuable mutations. Moderate concentrations (0.1%

NP-40) are also effective, providing a balance between mutation efficiency and viability, particularly for Farell and Ronin. Varieties such as Seilor and Perspektyva Odeska show potential at higher NP-40 concentrations and should be considered for targeted trait improvement strategies. These insights support strategic decision-making regarding variety and concentration selections to optimize the outcomes of mutation breeding efforts. Continued monitoring of promising lines at higher concentrations is suggested to ensure a balance between genetic diversity and plant viability.

To assess the complex, site-specific response of different genotypes to the mutagenic action of NP-40, a cluster analysis was performed based on the cumulative trait response across all concentrations and traits (Fig. 2). In accordance with the known site-specific and genotype-dependent effects of mutagens of different nature, a cluster analysis was conducted to classify the winter wheat genotypes based on their integrated response to NP-40 treatment. The clustering incorporated key phenotypic, biochemical, and cytogenetic parameters across all tested concentrations.

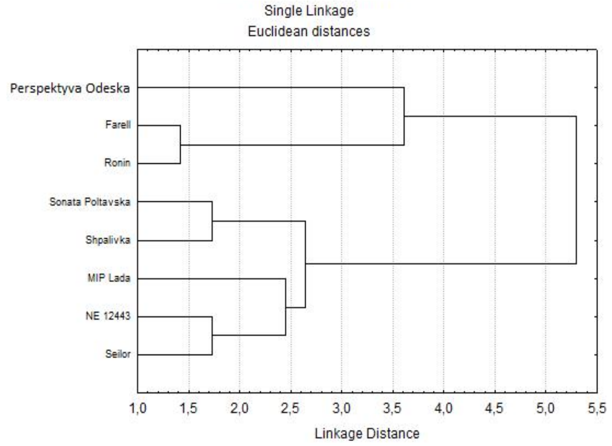

Fig. 2. Cluster analysis of winter wheat varieties based on the rate of positive changes under different concentrations of NP-40

Three distinct genotype groups were identified through cluster analysis based on their response to NP-40 treatments. The first group included the varieties Farell and Ronin, which exhibited similar reactions to NP-40. Consequently, the potential for these genotypes to serve as highly effective initial forms for epimutagenesis with substances was characterized by low damaging potential. The overall efficacy of mutation induction is expected to be high.

The second group consisted solely of the variety Perspektyva Odeska, which demonstrated a unique, genotype-specific response to NP-40 treatment. Given its genetic structure, this variety is likely to yield intermediate effectiveness, contributing partially to enhanced mutation frequencies for beneficial traits. The third group encompassed five varieties: Sonata Poltavska, Shpalivka, MIP Lada, NE 12443, and Seilor. These varieties exhibitted low responsiveness to NP-40 treatment, suggesting a relatively limited number of positive trait mutations when treated with this agent. Discriminant analysis results (Fig. 3) indicated that NP-40 concentrations of 0.1% and 0.5% had comparable effectiveness across all genotypes. In contrast, the lower NP-40 concentrations of 0.01% and 0.05% demonstrated reduced variability and effectiveness in inducing mutations.

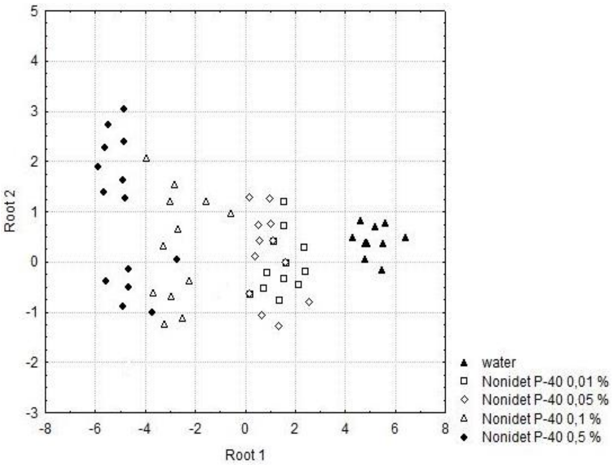

Fig. 3. Discriminant analysis of winter wheat varieties based on the rate of positive changes under different concentrations of NP-40

In the spectrum of induced changes, traits were classified using discriminant analysis to evaluate their relative mutability depending on the nature of the mutagen. This analysis enabled the identification of traits most susceptible to NP-40 treatment, such as early ripeness, spike architecture, and biochemical grain quality parameters. Conversely, traits related to tillering capacity and plant height demonstrated lower variability across treatments. A complementary classification analysis was conducted to further characterize the mutation process across genotypes and treatment levels (Table 2). This classification allowed a more detailed interpretation of the mutation spectra, highlighting genotype-specific responses and the site-specificity of the epimutagenic factor. For NP-40 epimutations by such traits as positive changes in microelement content, early ripeness, higher protein content, short-stem are probable with sufficient reliability. In this regard, NP-40 is a highly effective epimutagen for this initial material. Moderate traits by occurrence were long spike and positive changes in protein components, productivity and tolerance to diseases. Traits such as dwarf and semi-dwarf form , thick stem, intense epicuticular wax, large-size grain and spike accumulation were infrequent or absent, suggesting that these traits are difficult to induce or strongly genotype-dependent. In conclusion, action of NP-40 in complex is the most practical suitable variant for breeding process, but strongly dependent on genotype.

Table 2
Positive changed traits. Results of discriminant analysis

Trait at model	Wilks lambda λ	Concentration at model	$F_{0.05}$	P-level
Thick stem	0.445	none	1.01	0.145
Short-stem	0.118	NP-40 0.1%, NP-40 0.5%	12.45	0.002
Semi-dwarf	0.299	none	1.98	0.074
Dwarf	0.396	none	2.76	0.083
Intense epicuticular wax accumulation	0.390	none	2.72	0.087
Large-size grain	0.363	none	2.88	0.082
Long spike	0.165	NP-40 0.5%	6.65	0.032
Large-size spike	0.396	none	2.46	0.097
Early ripeness	0.092	NP-40 0.1%, NP-40 0.5%	19.45	0.001
Tolerance for diseases	0.145	NP-40 0.1%, NP-40 0.5%	7.82	0.022
Productive	0.149	NP-40 0.1%, NP-40 0.5%	7.57	0.023
Tillering capacity	0.444	none	1.11	0.132
Higher protein content	0.110	NP-40 0.1%, NP-40 0.5%	12.01	0.003
Positive changes at protein components	0.154	NP-40 0.1%, NP-40 0.5%	8.05	0.021
Positive changes at microelement content	0.121	NP-40 0.1%, NP-40 0.5%	11.67	0.005

Characteristics of lines obtained under the action of the studied epimutagen were evaluated in terms of productivity and grain technological quality traits, which influence bread-making parameters (Tables 3 and 4). Among the more productive lines derived, all initial varieties except NE 12443 and MIP Lada contributed positively. Three varieties, Sonata Poltavska, Shpalivka and Seilor were represented by a single form resulting exclusively from NP-40 0.5% treatment. Varieties Farell, Ronin, and Perspektyva Odeska, treated with NP-40 concentrations of 0.1% and 0.5%, each contributed two or three lines. All selected lines exceeded the standard cultivar's productivity based on three years of evaluation, though the degree of this excess varied among the lines. In 2022, the evaluated lines formed three productivity groups relative to the standard: lines 91, 98, 101, and 109 comprised the most productive group, other forms occupied intermediate positions, variety Podolyanka alone formed the least productive group. In 2023, productivity differentiation was significantly more pronounced. Lines 91, 98, and 109 again stood out clearly as the highest productivity group. The intermediate groups were statistically distinct, with a clear separation from the top group. In the third testing year, despite conditions favoring semi-intensive rather than highly intensive genotypes (Fig. 4), productivity differences were even more distinct. Line 91 remained in the top group, while lines 98 and 109 occupied a statistically separate second group with significant yield differences from subsequent groups.

Table 3
Yield capacity of agrocenosis of mutant lines of winter wheat during the ecological estimation (DSAEU, 2022–2024, t/ha)

Variant	Origin	2022	2023	2024	Average
1	Podolyanka, standard	5.47 ± 0.09^{a}	6.11 ± 0.11^{a}	5.54 ± 0.13^{a}	5.71 ± 0.10^{a}
12	Perspektyva Odeska, NP-40 0.1%	5.98 ± 0.12^{b}	6.78 ± 0.15^{b}	5.76 ± 0.14^{a}	6.17 ± 0.11^{b}
21	Perspektyva Odeska, NP-40 0.5%	6.02 ± 0.12^{b}	7.23 ± 0.17^{c}	6.13 ± 0.15^{ab}	6.46 ± 0.14^{c}
45	Sonata Poltavska, NP-40 0.5%	5.79 ± 0.11^{b}	6.77 ± 0.15^{b}	6.12 ± 0.15^{ab}	6.23 ± 0.15^{bc}
51	Shpalivka, NP-40 0.5%	5.91 ± 0.11^{b}	6.65 ± 0.13^{b}	5.86 ± 0.14^{a}	6.14 ± 0.11^{b}
87	Farell, NP-40 0.1%	6.21 ± 0.13^{bc}	6.40 ± 0.13^{a}	5.99 ± 0.15^{ab}	6.20 ± 0.12^{bc}
91	Farell, NP-40 0.5%	6.43 ± 0.14^{c}	7.55 ± 0.18^{c}	7.26 ± 0.17^{c}	7.08 ± 0.17^{d}
98	Farell, NP-40 0.5%	6.39 ± 0.14^{c}	7.47 ± 0.18^{c}	6.76 ± 0.16^{d}	6.87 ± 0.16^{d}
101	Ronin, NP-40 0.5%	6.27 ± 0.13^{c}	6.45 ± 0.12^{ab}	5.80 ± 0.14^{a}	6.17 ± 0.11^{b}
109	Ronin, NP-40 0.5%	6.45 ± 0.14^{c}	7.52 ± 0.18^{c}	6.60 ± 0.16^{d}	6.86 ± 0.16^{d}
112	Seilor, NP-40 0.1%	5.99 ± 0.11^{b}	6.22 ± 0.11^{a}	6.19 ± 0.15^{ab}	6.13 ± 0.11^{b}

Note: see Table 1.

Fig. 4. Promising lines at ecological estimation, 2023 year:
samples for yield structure analysis, prevalence of semi-intensive forms (14) over intensive (9)

Aggregating results from the three-year testing period, three productivity groups were distinctly identified: lines 91, 98, and 109 formed the most productive and stable group ($F = 11.34$; $F_{0.05} = 4.23$; $P = 0.005$), line 21 occupied an intermediate productivity level ($F = 7.41$; $F_{0.05} = 4.23$; $P = 0.01$), and lines 12, 45, 51, 87, 101, and 112 exceeded the standard yield ($F = 6.01$; $F_{0.05} = 3.71$; $P = 0.02$) but significantly trailed the first group ($F = 8.17$; $F_{0.05} = 4.01$; $P = 0.01$). Lines 91, 98, and 109 demonstrated consistent trait stability, whereas other lines exhibited considerable annual variability.

Regarding technological grain quality, lines 51 and 112 notably excelled in protein content, while lines 12, 51, 91, and 112 stood out in gluten content, highlighting a close association between these two traits. Overall, most lines, except line 87, possessed good grain quality suitable for baking. However, line 112, despite its productivity, was deemed unpromising due to variability under NP-40 0.1% treatment.

Positive grain quality traits included high concentrations of high molecular weight glutenins (highly variable trait), low levels of low molecular weight glutenins, and moderate variability in gliadin content. Specifically, lines 45, 51, 87, 109 and 112 excelled in high molecular weight glutenins; lines 45, 51, 101 and 109 exhibited lower low molecular weight glutenin content; and lines 45, 51, 87, 91, 109 and 112 demonstrated high gliadin levels. Notably, line 51 combined multiple positive quality traits but exhibited lower yield, making it suitable primarily for quality improvement through traditional cross-breeding. Among consistently high-yielding lines, lines 91, 98, and 109 displayed the best balance of productivity and grain quality (high protein, gluten, and high molecular weight glutenins), while line 87 was excluded due to suboptimal grain quality.

Table 4
Technological properties of wheat grain (DSAEU, 2024)

Variety/line	Protein, %	Gluten, %	Glutenins		Gliadins
			HMW	LMW	
Podolyanka	13.98^{a}	25.22^{a}	0.15863^{a}	0.45442^{a}	0.4567^{a}
12	14.11^{a}	$26{,}00^{b}$	0.16005^{a}	0.48546^{b}	0.4499^{a}
21	14.03^{a}	25.05^{a}	0.15953^{a}	0.46177^{a}	0.4495^{a}
45	14.09^{a}	25.45^{a}	0.16996^{b}	0.43411^{c}	0.4865^{b}
51	15.21^{b}	28.32^{c}	0.20935^{c}	0.43001^{c}	0.4867^{b}
87	13.44^{c}	23.61^{d}	0.20831^{c}	0.45154^{c}	0.4857^{b}
91	13.99^{a}	26.08^{b}	0.16223^{a}	0.45313^{a}	0.4850^{b}
98	14.03^{a}	25.23^{a}	0.15722^{a}	0.48760^{b}	0.4550^{a}
101	14.12^{a}	25.19^{a}	0.15453^{a}	0.43300^{c}	0.4548^{a}
109	14.15^{a}	25.78^{a}	0.20953^{c}	0.43977^{c}	0.4946^{b}
112	15.17^{b}	28.30^{c}	0.21173^{c}	0.45009^{a}	0.4948^{b}

Note: see Table 1; HMW – high molecular weight glutenins, LMW – low molecular weight glutenins.

Discussion

The regularity of beneficial mutational processes has been well-described in previous studies (Lal et al., 2020; Nazarenko et al., 2021), enabling the use of induced variability to produce new genetic materials with enhanced manageability, reliability, and predictability (Nazarenko et al., 2022). Epimutations, in particular, represent a consistent and heritable source of variability for local genetic resources (Hongjie et al., 2019). Traits such as high-intensity stunting, elongated and grain-rich spikes (Bayhan et al., 2024), and early ripeness

(Kartseva et al., 2023; Naserian Khiabani et al., 2025) have been reliably achieved using local genotypes and targeted mutagenesis (Winkler et al., 2023).

Based on our experimental outcomes, NP-40 is recommended as an effective epimutagen for both local and international wheat germplasm (Chakraborty et al., 2023). Data suggest that NP-40 primarily serves as a tool to develop lines intended as parental components for subsequent improvement of established varieties through recombinant breeding approaches (le Roux et al., 2021; Han et al., 2025). While direct commercial application of these mutant lines as varieties is less common, their value as novel sources of genetic diversity remains high (Horshchar & Nazarenko, 2024).

NP-40 demonstrates significant dependency on genotype-specific interactions, attributable to structural features of DNA within the initial variety. The clear subdivision observed in variety responsiveness underscores NP-40's pronounced genomic site-specificity. This specificity has beneficial implications for enhancing grain quality traits, as mutant lines generated under NP-40 treatment frequently exhibit improved biochemical profiles, making them particularly suitable for meeting agricultural requirements and boosting grain nutritional value (Beiko & Nazarenko, 2022).

Our results also highlight that certain economically important traits – traditionally considered relatively stable or challenging to modify – showed modest yet meaningful variability under NP-40 influence, with minimal accompanying negative effects. This outcome contrasts notably with chemical supermutagens, emphasizing the critical role of appropriate genotype selection to leverage NP-40's unique site-specific effects effectively. Previous studies reinforce the findings that active concentrations (especially 0.1% and 0.5%) enhance this variability significantly (le Roux et al., 2021; OlaOlorun et al., 2021).

Importantly, as concentrations of epimutagenic substances increased, the likelihood of encountering combinations of negative and positive trait changes was significantly lower compared to classical supermutagens (Liu et al., 2025). Even at the highest tested NP-40 concentrations (0.1% and 0.5%), the frequency of adverse trait combinations remained notably limited, thus avoiding the scenario where half or more of promising mutant lines are typically discarded due to undesirable traits (Mangi et al., 2021; Hassine et al., 2023). Genotype selection remains essential, as varieties exhibiting high variability in initial generations (reflecting mutagenic depression or diverse hereditary alterations) are not always ideal for practical breeding applications (Nazarenko, 2020). In contrast, genotypes demonstrating relative resistance to negative effects were more successful in producing economically valuable lines with greater commercial potential (Mahmood et al., 2023; Rebouh et al., 2023; Stearns et al., 2025).

Additionally, we observed a stronger correlation between agriculturally valuable traits and the overall rate and spectrum of visually identified epimutations specifically within the three responsive varieties (Farell, Ronin, and Perspektyva Odeska). Notably, such a relationship is distinctive to epimutagens and was less pronounced or absent in earlier experiments involving chemical supermutagens (Yali & Mitiku, 2022; Liu et al., 2025).

Although direct selection of a commercially viable mutant line via epimutagenesis remains relatively improbable, epimutational processes offer considerable potential for targeted improvement in key economically significant traits. Specifically, epimutagens effectively enhance biochemical characteristics such as protein quality and micronutrient content (OlaOlorun et al., 2021; Arumingtyas et al., 2023). Changes in biochemical profiles, particularly reducing unfavorable low-molecular-weight glutenins and increasing the content of essential microelements, commonly deficient in wheat, significantly elevate grain nutritional value, thus positively influencing agricultural and dietary outcomes (Spencer-Lopes et al., 2018).

Conclusion

The winter wheat varieties Farell, Ronin and Perspektyva Odeska emerged as particularly favorable initial materials for developing new, high-yielding, and high-quality breeding lines, characterized by positive modifications in plant architecture, timing of critical developmental stages, and grain biochemical composition, including protein content, structure, and microelement complexes. When these genotypes are combined with optimal concentrations of the epimutagen NP-40 (0.1% and 0.5%), the resulting mutation-induced variability is significantly enhanced. This approach facilitates the creation of genetically valuable parent lines for breeding as well as commercially promising cultivars. Notably, morphometric traits (such as plant stature and spike architecture) and developmental traits (including maturity timing) exhibited a higher responsiveness to induced mutation than grain technological properties, like protein quality or microelement content. Consequently, it remains strategically important to prioritize genetic improvement efforts during the initial selection stages (M_2–M_3 generations) for traits related to yield and plant morphology. Nevertheless, biochemical traits, despite being less mutable through classical mutagenesis, demonstrated significantly enhanced variability under epimutagenic treatments compared to traditional chemical mutagens, highlighting the superior efficiency of epimutagens in targeting grain biochemical properties. Additionally, traditional breeding methods, particularly classical crossing approaches, can leverage these epimutation-derived parental forms to successfully develop novel cultivars possessing optimized grain yield and quality parameters. Compared to previously studied chemical supermutagens, NP-40 exhibits considerably greater genomic site specificity and a heightened genotype-by-mutagen interaction, underscoring the critical role genotype selection plays in determining the effectiveness of the epimutagenic breeding strategy. From the present investigation, mutant lines numbered 91, 98, and 109 stand out, demonstrating sufficiently high grain yields coupled with satisfactory or superior grain quality traits. These lines are recommended for inclusion in formal state varietal evaluation trials. Future research will delve deeper into critical adaptive traits such as drought resistance and winter hardiness, evaluating parameters including photosynthetic efficiency at key growth stages and winter survival rates. Moreover, studies on nutrient uptake and accumulation profiles will be conducted to confirm and elucidate the physiological mechanisms underpinning observed improvements in grain yield and quality, as well as their interactions with environmental conditions. Moving forward, additional chemical substances with potential epimutagenic activity will be screened to generate further beneficial variability in these local and international winter wheat varieties, maintaining a consistent focus on yield, grain quality traits, and adaptive responses.

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