AGROLOGY
ISSN 2617-6106 (print)
ISSN 2617-6114 (online)
Agrology, 2025, 8(4), 236–242
doi: 10.32819/202527

Original researches

Received: 16.09.2025
Revised: 11.10.2025
Accepted: 30.11.2025

Dnipro State Agrarian
and Economic University,
Serhii Efremov st., 25,
Dnipro, 49600, Ukraine.
Tel.: +38-095-848-53-86.
E-mail: nik_nazarenko@ukr.net

Cite this article: Kryshyn, R., & Nazarenko, M. (2025). Positive changes under action of classical supermutagens in winter wheat local plant material. Agrology, 8(4), 236–242. doi: 10.32819/202527

Positive changes under action of classical supermutagens in winter wheat local plant material

R. Kryshyn, M. Nazarenko
Dnipro State Agrarian and Economic University, Dnipro, Ukraine

Abstract. Developing reliable sources of novel variation for winter wheat remains a priority for both direct varietal release and for developing stronger parental pools in recombination programs. In this work, we systematically tested two classical chemical mutagens, ethyl methanesulfonate (EMS 0.025%, 0.05%, 0.10%) and sodium azide (SA 0.01%, 0.025%, 0.05%, 0.10%), to determine dose ranges that generate useful phenotypic and quality shifts without unacceptable fitness impairments. Seeds of two contrasting cultivars, Spivanka and Altigo, were soaked for 24 hours following established mutagenesis protocols and subsequently selected for agronomically relevant traits. Clear, dose-dependent patterns emerged. The most informative and practically valuable spectra were obtained at mid-low levels: EMS at 0.05–0.10% and SA at 0.025–0.050%. By contrast, increasing SA to 0.10% reliably elevated the gross mutation rate but diluted efficiency, increasing neutral or undesirable changes. Genotype shaped outcomes in predictable ways. Spivanka consistently produced stature-reduced selections, short-stem variants arose under both agents, making it a reliable source for height reduction and lodging resistance. Altigo, while less responsive for simple short-stem types, excelled in semi-dwarf, dwarf categories, and generated more long-spike phenotypes. It also yielded a higher frequency of disease-tolerant selections and showed stronger quality-oriented responses. For programs prioritizing spike architecture, robust main-spike contribution to yield, and micronutrient enhancement, Altigo paired with SA 0.025% was the most productive combination. EMS at 0.05–0.10% provided a complementary approach in Altigo when the target ideotype emphasized semi-dwarf and dwarf stature together with improved head traits. Earliness varied in the two genotypes: Spivanka responded well to accelerated maturity under SA 0.025–0.050%, with EMS 0.05% as a conservative alternative when risk needs to be minimized. Importantly, the same Spivanka treatments that advanced maturity also continued to produce short-stem variants, allowing simultaneous progress regarding cycle length and canopy architecture. Across all scenarios, SA 0.10% was uniformly counterproductive; its apparent activity came with reduced selection efficiency and should be avoided in practical breeding. From these screens, multiple mutant lines were advanced to candidate status for pre-registration testing, combining high grain productivity with favorable bread-making qualities. Ongoing work will profile physiological resilience, especially drought tolerance and winter survival.

Keywords:bread wheat; sodium azide; ethylmethanesulfonate; rate of changes; microelement; grain quality; mutations; ecogenetic.

Introduction

Genetic mutation is the ultimate source of new alleles in any population, and therefore the foundational engine of evolutionary change. In the absence of spontaneous or induced mutations, selection, natural or artificial, would have nothing to act upon. For plant improvement, this principle translates into a practical toolset: By generating novel heritable variation, breeders can uncover or create phenotypes that conventional crossing alone may never reveal. Decades of studies of crops show that carefully applied chemical mutagenesis can trigger stable, transmissible alterations that are immediately useful for selection schemes aimed at yield, adaptation, and end-use quality. Remarkably, even short, mild exposures to active agents may set off enduring developmental and genetic shifts in crop plants, providing a rich source of variation for breeders to explore (Stearns et al., 2025).

Among the wide array of mutagenic approaches, the use of chemical supermutagens has earned a special place. These compounds are valued for two complementary properties: They tend to induce mutations with high efficacy while keeping collateral damage, such as lethality or severe morphological disruption, comparatively low. Because of this balance, supermutagens are used not only for direct breeding applications but also as precision tools for probing the biology of mutagenesis itself, often beginning with model genotypes and then extending to elite germplasm. A substantial fraction of successful modern cultivars of major crops has derived from mutagenesis-driven variability. In contemporary breeding pipelines, chemical mutagenesis is therefore deployed in two strategic modes. First, it generates parent lines with desirable alleles or allele combinations for subsequent recombination breeding. Second, it enables targeted discovery and study of gene networks governing traits of high agronomic value, including the biochemical features that determine grain functionality and nutritional quality (Didenko & Nazarenko, 2025).

When paired with purposeful selection, induced mutagenesis offers a time- and cost-efficient route to improvement across both quantitative and qualitative traits. In practical terms, treating seeds or tissues is technically straightforward and inexpensive, yet it can deliver measurable gains within a few breeding cycles. This is especially advantageous for traits linked to resistance, biotic (pathogens, pests) and abiotic (temperature extremes, drought, salinity), where naturally occurring variation is limited or genetically complex. Numerous studies demonstrate that moderate, well-controlled treatments consistently yield useful variants, while minimizing the risk of deleterious pleiotropy (Fradgley et al., 2024).

Optimal dosage is a subject of ongoing debate. Some researchers argue for higher concentrations because they broaden the mutation spectrum and increase the chance of complex, multi-trait changes arising in a single background. Meta-analyses indeed document notable successes at elevated doses in certain species and trait classes (Farooq et al., 2024). However, a substantial body of work also shows that intermediate levels are often sufficient to produce lines with stronger stress tolerance and robust agronomic performance - without the liabilities that can accompany heavy mutagenic loads. Morphological novelties are common, and many of them are favorable, from architecture shifts to improvements in grain composition and nutritional attributes (Bharathi et al., 2025).

It is useful to recognize that chemical mutagenesis typically produces conspicuous, easily observed phenotypes alongside less visible molecular changes. Pronounced mutants, morphoses, and genocopies frequently appear, indicating that the underlying biological systems have been perturbed in meaningful ways. While physical mutagens or alternative technologies sometimes generate broader raw mutation rates, chemical seed treatments remain particularly effective as a pipeline for downstream, selection-ready variability. Attempts to produce even higher rates through other means can encounter practical constraints, including excessive damage, unstable inheritance, or developmental impairments that undercut breeding utility (Horshchar & Nazarenko, 2023).

The global footprint of induced mutagenesis is already large. Thousands of crop varieties, across both food and industrial uses, owe part of their performance to mutation-derived alleles. Gains span yield potential, resilience to drought and salinity, disease and pest resistance, and consumer-facing quality. Cereals such as wheat, rice, and barley, as well as fiber crops such as cotton feature prominently in these successes, underscoring mutagenesis as a mature, reliable breeding tool (Bayhan et al., 2024).

From a methodological perspective, breeding programs commonly group mutagens into two broad families. Chemical agents include alkylating compounds, sodium azide (SA), ethyl methanesulphonate (EMS), well known for creating point mutations, and diepoxybutane (DEB), which induces point changes and small deletions. Physical agents include X-rays, gamma irradiation, fast neutrons, and ultraviolet light (Arumingtyas et al., 2023). Each class has distinct advantages, but chemical agents frequently stand out for their capacity to interact in a more genotype-dependent and sequence-specific manner with chromosomal DNA. This relative site-specificity can be exploited to make finer, more predictable genetic edits at the population level, opening avenues for precise trait enhancement that can be more difficult to achieve with less targeted approaches (Bharathi et al., 2025; Kryshyn & Nazarenko, 2025).

Nowhere is the need for adaptable breeding tools more evident than in winter wheat, a crop integral to food security in regions facing increasingly erratic climates, including large swathes of Ukraine. Warming trends have nudged heat-tolerant crops poleward, and winter cereals must now combine strong overwintering with flexibility during volatile springs. Breeding for better winter hardiness, along with stability under drought and heat pulses, is therefore a central priority (Nazarenko & Okselenko, 2025).

Within this context, our research focused on characterizing heritable variation in a group of local and introduced winter wheat varieties, placing special emphasis on the occurrence and breadth of beneficial changes (morphological, physiological, and biochemical). A key element was the evaluation of classical chemical supermutagens, assessed for its ability to generate heritable, site-directed alterations that enhance breeding value. Because the effectiveness of any mutagen depends not only on dose and exposure but also on the recipient genotype, we paid particular attention to genotype × mutagen interactions in order to determine how different genotypes respond in both frequency and spectrum of induced changes (Okselenko et al., 2025).

Our aims were as follows: first, quantifying the rates at which favorable phenotypes emerged across the test group, mapping response as a function of treatment level: second, delineating the mutation spectrum, identifying which traits, including protein architecture and micronutrient composition, were most amenable to alteration; and third, determining whether classical chemical supermutagens (SA and EMS) provided a degree of genomic or chromatin site-specificity consistent with a mutagenic mode, thereby enabling a more directed manipulation of biochemical quality traits, compared with classical physical mutagens (like as gamma rays) (Phadungsawat et al., 2025).

By assembling this evidence, we sought to inform two complementary breeding strategies. The first is direct selection, identifying mutant lines that meet performance thresholds for yield, adaptability, and grain quality, and advancing them as candidate varieties. The second is recombination breeding using mutation-derived lines as parents to introgress favorable alleles and epialleles into elite backgrounds, thereby accumulating gains while buffering against negative linkages. In both strategies, the predictable targeting of biochemical traits (e.g., glutenin subunit balance or microelement enrichment) would represent a meaningful step beyond the largely stochastic outcomes of traditional mutagenesis (Khare et al., 2025).

This study is positioned at the intersection of practical breeding and functional genetics. It leverages the power of induced variation while acknowledging the necessity of genotype-aware deployment. By evaluating classical chemical capacity of supermutagens to trigger beneficial, heritable changes with a plausible site-specific component and by documenting how local genotypes differ in responsiveness, compared with introduced genotypes, we aimed at providing an empirical basis for tailoring mutagen protocols for breeding goals. Ultimately, in a climate of rising uncertainty, tools that deliver targeted variability in key adaptive and quality traits can accelerate progress toward winter wheat ideotypes that are both higher yielding and more resilient.

Materials and methods

Field trials were carried out in 2023–2025 (generations M_2–M_4) at the Educational and Scientific Center of Dnipro State Agrarian and Economic University (48°50′99″ N, 35°25′65″ E; Dniprovskyi District, Dnipropetrovsk Oblast, Ukraine). The site falls within a northern warm, moderately arid zone (hydrothermal coefficient >0.9). During the study, in-season rainfall typically totaled 250–280 mm, with annual precipitation of 450–490 mm, and heat accumulation above 10 °C reached 2,900°C. Soils were uniform across the area and classified as regular, low-humus, leached, medium-loam chernozem developed on loam. Routine diagnostics indicated 3–5 mg N per 100 g dry soil (Tiurin), 20–30 mg mobile P per 100 g (Chyrykov), and 20–35 mg exchangeable K per 100 g (Chyrykov).

Two bread wheat (*Triticum aestivum* L.) cultivars Spivanka and Altigo served as the starting material. Seeds (1,000 kernels per treatment) were primed for 24 h in aqueous solutions of two mutagens (Sigma-Aldrich): ethyl methanesulfonate (EMS) at 0.025%, 0.05%, and 0.10% and sodium azide (SA) at 0.01%, 0.025%, 0.05% and 0.10% concentrations. Water-soaked seeds were used as controls. Mutagen application followed widely adopted protocols for chemical mutagenesis (Spencer-Lopes et al., 2018).

In the M_2–M_3 generations, plants were assessed visually for induced variants and analyzed to confirm inheritance. Families were hand-sown in short plots (1–3 rows per family; 0.15 m interrow spacing; 1.5 m row length). Mutation frequency was expressed as the proportion of mutant families relative to the total examined.

Performance testing of selected mutants took place at M_4. Depending on the year, plot size ranged from 2 to 10 m² with one or two repetitions; the references were inserted after every twenty plots. Grain quality assessments included total protein (Spektra RT), glutenin and gliadin profiling by RP-HPLC, and microelement quantification (Mg, Mn, Zn, Mo, Co, Cu) using an Agilent 5110 ICP-AES. Instrument wavelengths were set and verified against Agilent multi-element standards.

Data processing was conducted in Statistica 10.0 (TIBCO, Palo Alto, USA). Treatment effects were tested by factorial ANOVA, and multivariate structure was explored with cluster (Euclidean distance, single-linkage) and discriminant analyses. Where ANOVA indicated significance ($P < 0.05$), mean separation was performed using Tukey's HSD.

Results

Across the EMS populations, 3,000 families were examined in M_2–M_3 generations. From these, 222 lines exhibited discernible modifications and were kept for further study. Within that subset, 91 lines expressed traits of clear agronomic value and 28 met elite criteria, i.e., favorable changes without accompanying negative effects, which qualifies them for breeding use. Each EMS treatment level comprised 500 families. Across the SA populations, 4,000 family rows were inspected in M_2 – M_3 generations. A total of 384 lines of them demonstrated notable changes and were examined further. Thus, 93 lines were found agronomically valuable and 30 met elite criteria. Each SA treatment level comprised 500 families, except for the variety Altigo under 0.1% SA, which had 350 families, due to partial loss of viability at high concentration (Tables 1 and 2).

Selection was focused on clearly recognizable agronomic targets early and on end-use quality later. In M_2–M_3, candidates were flagged for architectural and developmental shifts such as thicker culms, reduced height (short-stem, semi-dwarf, dwarf), stronger epicuticular wax, elongated or bulkier spikes, larger kernels, earliness, visible disease tolerance, improved tillering, and preliminary productivity (verified subsequently in M_3–M_4). In M_4, lines were screened for technological grain quality, i.e. elevated total protein, favorable remodeling of the gluten network (higher HMW-glutenin signal with restrained LMW fractions, along with calibrated changes in gliadins), and enrichment in key microelements (Mg, Mn, Zn, Mo, Co, Cu). Together, these criteria served as a marker suite to identify specimens with genuine breeding utility.

From EMS treatments, approximately 3.0% of families yielded lines with actionable value (91 of 3,000 across concentrations). Sodium azide (SA) produced a comparable, though slightly lower, rate of 2.3% (93 of 4.000). Stability checks across generations confirmed inheritance of target phenotypes, indicating that the induced states are fixed, either as true genomic mutations or as epigenetic changes that have become coupled to genetic mutations, ensuring reliable transmission.

Rate of changes (total mutations) increased with dose of both agents: 4.60% to 8.80% in case of EMS and 4.40% to 12.40% in case of SA at the highest concentration of 0.1%. Rate of positive change also from 2.60% to 4.00% under EMS action, and from 2.20% to 4.82% under SA action. As for efficiency of positives changes (part from general rate), it declined as concentration of mutagen was increased, i.e., more mutations, but a smaller number of favorable ones. Therefore, best efficiencies were produced by EMS 0.025% (0.57) and SA 0.05% (0.58).

As for lines selected (with one or more positive changes) and perspective lines (without negative changes and with potential for future varieties directly or as components in winter wheat improvement process), the number of lines produced by EMS hardly changed with increasing concentration (10 to 11), while it increased under increasing concentration of SA (9 – 16 under first three concentrations and then to 17 at the highest concentration), indicating over-dose effect. The number of promising was quite stable at moderate doses, but only three at the highest SA concentration. Rate of perspective lines peaked at EMS 0.025% (1.00%) and SA 0.025% (1.00% too); and declined at higher concentrations (EMS 0.1% (0.60%), SA 0.1% (0.60%).

If the goal is maximum favorable-mutation count, SA 0.05% (4.40%) and SA 0.1% (4.82%) would be more prefferable. If the goal is efficiency and utility for further breeding, the best choises would be EMS 0.025% and SA 0.025% (high rate of positive changes and the highest rate of perspective lines). Thus, EMS 0.05% – 0.1% and SA 0.1% are workable when large populations could be screened: more total changes but less beneficial ones should be expected.

Table 1

Parameters of mutation rates types under mutagen action for variety Spivanka (x ± SD, n = 500)

Variant	Rate of changes, %	Rate of positive changes, %	Share from general rate, %	Number of lines, pcs	Number of promising lines	Rate of promising lines, %
Spivanka	0.40 ± 0.02^{a}	0.20 ± 0.02^{a}	0.50	1	0	0.00 ± 0.00^{a}
Spivanka, EMS 0.025 %	4.60 ± 0.14^{b}	2.60 ± 0.12^{b}	0.57	10	5	1.00 ± 0.13^{b}
Spivanka, EMS 0.05 %	6.60 ± 0.17^{c}	3.20 ± 0.18^{c}	0.48	12	4	0.80 ± 0.12^{b}
Spivanka, EMS 0.1 %	8.80 ± 0.20^{d}	4.00 ± 0.21^{d}	0.45	11	3	0.60 ± 0.11^{bc}
Spivanka, SA 0.01%	4.40 ± 0.15^{b}	2.20 ± 0.11^{e}	0.50	9	4	0.80 ± 0.11^{b}
Spivanka, SA 0.025%	6.60 ± 0.18^{c}	3.80 ± 0.19^{d}	0.58	14	5	1.00 ± 0.14^{b}
Spivanka, SA 0.05%	10.60 ± 0.23^{e}	4.40 ± 0.22^{d}	0.42	16	4	0.80 ± 0.13^{b}
Spivanka, SA 0.1%	12.40 ± 0.27^{f}	4.82 ± 0.24^{f}	0.39	17	3	0.60 ± 0.11^{bc}

Note: indicate significant differences at $P < 0.05$ by Tukey HSD test with Bonferroni amendment.

In general, according to changed traits in the variety Spivanka, EMS is more suitable for strong architecture remodeling, especially at 0.1%; SA was superior for earliness, disease tolerance, and quality traits (protein profile, micronutrients), with the best balance at 0.05–0.10%. SA 0.05% can be used for quality and moderate rate of morphological traits, and EMS 0.1% can be used for plant architecture-driven ideotypes.

As for the variety Altigo (Table 2), total mutation rate evenly increased with dose of both agents, from 5.6% to 10.2% under EMS action and from 5.8% to 17.6% under SA action at the highest listed concentration (SA 0.1%). Favorable mutations rate (rate of positive changes) also increased with dose, but with diminishing returns at the top level: under from 1.8% to 4.4% under EMS action with concentration increase, and from 3.0% to 5.4% under SA (at the highest SA concentration (0.1%), the parameter was low (4.8%), despite the largest total mutation rate).

The proportion of beneficial changes was produced at an optimal level at SA 0.01% and SA 0.025% (0.50–0.52,). SA 0.1% was inefficient, while the best EMS action, measuring 0.43, was provided by 0.1% concentration. Number of lines generally increased with concentration of EMS, and under SA it declined the most at 0.1% concentration. Rate of promising ranged lines from 0.4% to 0.8% under EMS, and from 1.0% to 1.4% under SA. SA 0.025% produced the highest number of promising lines (7) and their highest rate (1.4%).

In comparison to the absolute number of beneficial changes, SA 0.05% action was the best among non-extreme doses (5.40%). Best efficiency in breeding yield (usable lines) was achieved by SA 0.025% (0.50, 7 promising lines, 1.4%). As with EMS, 0.05% concentration provided good balance (part 0.46, 4 perspective lines, 0.8%).

Table 2

Parameters of mutation rates types under mutagen action for variety Altigo (x ± SD, n = 350–500)

Variant	Rate of changes, %	Rate of positive changes, %	Share from general rate	Number of lines, pcs	Number of promising lines	Rate of promising lines, %
Altigo	0.40 ± 0.02^{a}	0.40 ± 0.03^{a}	0.50	2	0	0.00 ± 0.00^{a}
Altigo, EMS 0.025 %	5.60 ± 0.15^{b}	1.80 ± 0.10^{b}	0.32	8	2	0.40 ± 0.10^{b}
Altigo, EMS 0.05 %	7.40 ± 0.18^{c}	3.40 ± 0.18^{c}	0.46	11	4	0.80 ± 0.12^{c}
Altigo, EMS 0.1 %	10.20 ± 0.23^{d}	4.40 ± 0.22^{d}	0.43	15	4	0.80 ± 0.12^{c}
Altigo, SA 0.01%	5.80 ± 0.15^{b}	3.00 ± 0.17^{c}	0.52	12	5	1.00 ± 0.13^{c}
Altigo, SA 0.025%	8.00 ± 0.19^{e}	4.00 ± 0.21^{d}	0.50	14	7	1.40 ± 0.14^{d}
Altigo, SA 0.05%	12.00 ± 0.25^{f}	5.40 ± 0.25^{e}	0.45	19	5	1.00 ± 0.13^{c}
Altigo, SA 0.1%	17.56 ± 0.32^{h}	4.80 ± 0.24^{e}	0.27	12	3	0.86 ± 0.12^{c}

Note: see Table 1.

The best variant for Altigo was SA 0.025%, which provided the best conversion of mutational events into selectable changes, promising lines, strong positive rate with solid efficiency. A highly productive alternative was SA 0.05%, which maximized changes, but the efficiency and perspective yield were lower than at 0.025%. Conservative choice is EMS 0.05%, which provided a reliable increase in beneficial forms and promising lines.

SA was generally more effective in enhancing structural stature (semi-dwarf and dwarf), producing long and larger spikes, and promoting earliness (0.01%) and disease tolerance (0.05%). However, SA 0.1% had some detrimental effects on the quality parameters (protein and protein components). EMS was better at promoting large grains and microelement enrichment, and was better than SA at enhancing tillering.

A good range of SA for earliness and disease tolerance was 0.01–0.05%, and that for height reduction was 0.05–0.10%. For the latter parameter, EMS showed good results at 0.05–0.1% concentration. For grain size and microelements, we recommend using EMS 0.1%, and for positive changes in protein complex it would be best to use SA 0.025–0.05%. For EMS 0.05–0.10% and all SA concentrations, Altigo delivered equal or higher rate of positive changes (peaking at SA 0.05% – 5.40%). In Spivanka, the best positive changes were induced by low EMS dose; whereas Altigo responded better to higher doses, especially under SA action.

Spivanka gave more short-stem forms (under both mutagens), while Altigo was observed to be less promising for this trait. However, Altigo delivered higher parameters of semi-dwarf or dwarf forms, long spikes, disease tolerance, and microelement content (at SA 0.025%), therefore being a more suitable donor for plant architecture and grain quality changes. Effective variants for Altigo was observed to be SA 0.025% for grain quality, long-spike forms and tolerance to diseases. For Spivanka, SA 0.05% or SA 0.025% were good for earliness. Altigo and Spivanka, under EMS 0.05–0.10% and EMS 0.1% respectively, delivered high numbers of semi-dwarf and dwarf forms. As with content of microelements, Altigo provided best results under EMS 0.1% or SA 0.025–0.05%. Factor analysis showed a strong effect of dose on the induction of beneficial changes, with higher concentrations increasing the rate of positive traits ($F = 21.17$; $F_{0.05} = 3.86$; $P = 3.11\times10^{-3}$). Varietal background also mattered ($F = 8.07$; $F_{0.05} = 4.11$; $P = 0.01$), and a significant genotype × mutagen interaction was detected ($F = 5.17$; $F_{0.05} = 4.55$; $P = 0.04$).

To determine how individual genotypes react to EMS in a locus-specific manner, we applied linear discriminant analysis using the pooled response across doses and traits (Fig. 1). Rather than treating traits in isolation, the classifier summarized each variety's integrated reaction to EMS, reflecting the well-documented genotype- and site-dependent behavior of chemical mutagens. The discriminant model was built from a panel of informative variables spanning morphology, grain-quality biochemistry, and cytogenetic markers, aggregated across all EMS concentrations. This multivariate approach enabled separation of varieties according to their composite EMS sensitivity profiles.

Discriminant analysis showed that EMS at 0.05% and 0.1% produced similar numbers of positive changes, high levels of response across varieties, whereas the lowest dose (0.025%) generated noticeably less variation and a weaker mutagenic effect.

Regarding SA (Fig. 2), discriminant analysis showed that the concentrations clearly split into three groups. The first (lowest) concentration was rather weak at inducing changes. The second and third concentrations acted similarly and were much higher in genetic activity. The fourth (highest) concentration was also highly active, but its action was less effective in terms of generating useful changes. Thus, the second and third concentrations, SA 0.025% and 0.05%, were the most effective.

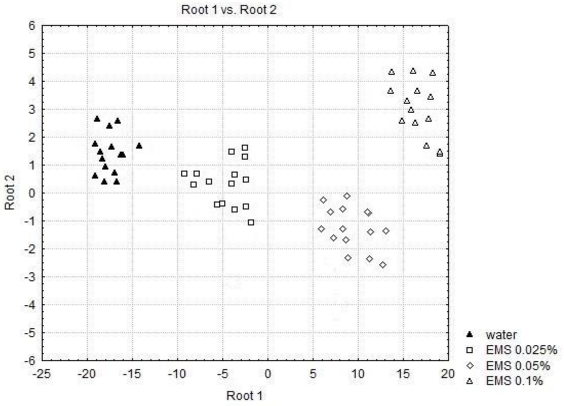

Fig.1. Discriminant analysis of winter wheat, evaluating the rate of beneficial changes across sodium azide (EMS) concentrations

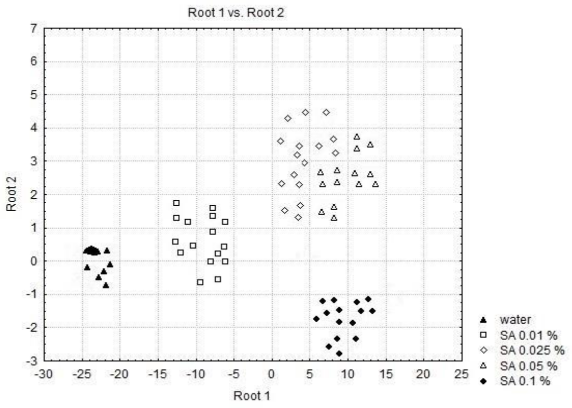

Fig.2. Discriminant analysis of winter wheat, evaluating the rate of beneficial changes across sodium azide (SA) concentrations

Discriminant analysis was used to rank traits by how readily they responded to mutagen treatment, taking into account both the mutagen and the applied dose. This multivariate approach separated traits into groups of high, moderate, and low mutability and therefore clarified where the strongest breeding leverage lies. Traits associated with accelerated development and grain-quality enhancement showed the greatest responsiveness across the group, indicating that phenology and end-use quality are particularly tractable targets under chemical mutagenesis. By contrast, disease tolerance, semi-dwarf form, and long-spike architecture exhibited intermediate, but still useful, levels of induced variation. Truly recalcitrant traits were rare, yet the analysis underscored notable genotype-by-mutagen contingencies that should guide choice of initial material.

Additional classification (Table 3) refined these patterns by linking trait categories with specific varieties and concentrations. Altigo emerged as a stronger donor for quality-oriented changes (e.g., favorable shifts in glutenin/gliadin balance and protein profile) and also performed well for semi-dwarf expression and disease-tolerance shifts. Spivanka was observed as a better starting genotype for advancing earliness, repeatedly producing earlier-maturing selections at effective doses. For spike morphology, particularly the generation of longer spikes, both varieties yielded mutant classes with practical frequencies, suggesting that spike-length modification is comparatively accessible in this background regardless of variety.

As with EMS, the 0.1% concentration consistently produced broader and more distinct phenotypic and biochemical shifts than 0.025% or 0.05%, while still maintaining a workable balance between mutation yield and line recoverability. For sodium azide (SA), an optimal window was detected at 0.025–0.05%. Within this interval, the rate of agriculturally valuable changes rose markedly without the undesirable escalation of non-target or deleterious variants that accompanies the highest dose. Notably, the highest SA concentration was genetically active, but was less effective according to the proportion of useful, advanceable mutants, an efficiency criterion that matters most in breeding.

Taken together, these findings point to a pragmatic scheme for using induced variability. When the goal is early maturity, we recommend using Spivanka and EMS 0.1%. To obtain grain-quality features, semi-dwarf frequency, or disease-tolerance, success can be achieved by treating Altigo with SA 0.025–0.05%. For spike elongation, either genotype can be used at the aforementioned optimal doses. Across all targets, outcomes remained strongly genotype-conditioned; therefore, mutagen choice and concentration should be paired deliberately with the variety whose baseline genetics aligns with the breeding objective.

We evaluated the candidate lines produced by the mutagen treatments for both agronomic performance and grain-quality parameters relevant to breadmaking (Tables 3 and 4). Every source variety contributed at least one elite line. From Spivanka, two lines were advanced: one derived from EMS at 0.1% and one from SA at 0.025%. From Altigo, three lines were selected: one produced using EMS 0.1% and two using SA at 0.025% and 0.05%. Across three seasons of testing, all selected lines surpassed the standard check for grain yield, although the size of the advantage differed among entries.

Seasonal grouping relative to the standard revealed clear leaders. In 2024, lines 39 and 43 formed the top productivity class. The remaining selections distributed into two intermediate tiers, one consisting solely of line 31 and another comprising the other lines, while the reference variety Podolianka constituted the lowest class. The pattern sharpened in 2025: Productivity contrasts became more pronounced, and the elite cluster was again readily separable from the rest. Lines 39 and 43 continued to perform the best, with lines 37 and 51 also aligning with the top cluster under that year's conditions, which favored semi-intensive rather than highly intensive ideotypes. Lines 19 and 31 were grouped as a statistically distinct second tier, clearly separated from both the leaders and the lower-yielding group.

Taken together, the multi-year data indicate that mutagen-derived selections can reproducibly outperform the standard, and that the advantage is robust to moderate shifts in seasonal intensity. Spivanka contributions were associated with the low-mid concentrations (SA 0.025%, EMS 0.1%), while Altigo yielded multiple high performers particularly when paired with SA at 0.025–0.05% or EMS at 0.1%. The stability of lines 39 and 43 across the years, and the recurrent ranking of lines 37 and 51 among the leaders, provides evidence of strong underlying genetic gains rather than transient environmental effects. Meanwhile, lines 19 and 31, although not elite, consistently ranked as a reliable second echelon and may serve as useful parental material or as candidates for targeted improvement in specific environments.

Lines 39 and 43 consistently were top performers, high-stability class ($F = 12.11$, $F_{0.05} = 4.01$, $P = 0.001$). Lines 37 and 51 formed a mid-performing group ($F = 8.91$, $F_{0.05} = 4.01$, $P = 0.009$). Lines 19 and 31 outyielded the standard ($F = 5.55$, $F_{0.05} = 4.01$, $P = 0.03$) yet remained statistically below the elite pair (contrast with the first group: $F = 7.64$, $F_{0.05} = 4.01$, $P = 0.02$). Yield expression in lines 39 and 43 was notably stable across seasons, whereas the remaining specimens showed greater year-to-year fluctuation.

Table 3
Mutability of traits: discriminant functions

Trait at model	Wilks Lambda λ	Concentration at model	Genotype at model	$F_{0.05}$	P-level
Thick stem	0.523	Non	non	0.98	0.167
Short-stem	0.313	Non	non	3.05	0.067
Semi-dwarf	0.172	EMS 0.1%, SA 0.025–0.05%	Altigo	7.09	0.036
Dwarf	0.399	Non	non	2.66	0.084
Intense epicuticular wax accumulation	0.395	Non	non	2.68	0.085
Large-size grain	0.423	Non	non	1.48	0.135
Long spike	0.174	SA 0.025–0.05%	both	6.98	0.039
Large-size spike	0.421	Non	non	1.46	0.137
Early ripeness	0.122	SA 0.025–0.05%	Spivanka	12.05	0.005
Tolerance for diseases	0.147	SA 0.025–0.05%	Altigo	7.34	0.031
Productive	0.449	Non	non	1.27	0.147
Tillering capacity	0.442	Non	non	1.31	0.141
Higher protein content	0.127	SA 0.025–0.05%	Altigo	11.11	0.006
Positive changes in protein components	0.121	SA 0.025–0.05%	Altigo	12.15	0.005
Positive changes in microelement content	0.119	SA 0.025–0.05%	Altigo	13.49	0.003

Table 4
Grain productivity of winter wheat mutant lines at field estimation (DSAEU, 2024 – 2025, t/ha)

Variant	Origin	2024	2025	Average
1	Podolianka, standard	6.16 ± 0.11^{a}	5.59 ± 0.13^{a}	5.88 ± 0.10^{a}
15	Spivanka, EMS 0.1%	7.00 ± 0.15^{b}	6.01 ± 0.14^{b}	6.50 ± 0.15^{b}
19	Spivanka, SA 0.025%	6.81 ± 0.14^{b}	6.02 ± 0.14^{b}	6.40 ± 0.14^{b}
31	Altigo, EMS 0.1%	6.70 ± 0.12^{c}	6.16 ± 0.14^{b}	6.43 ± 0.15^{b}
37	Altigo, SA 0.025%	6.99 ± 0.12^{b}	6.49 ± 0.12^{c}	6.74 ± 0.12^{c}
39	Altigo, SA 0.025%	7.45 ± 0.15^{d}	7.01 ± 0.15^{d}	7.23 ± 0.15^{d}
43	Altigo, SA 0.05%	7.44 ± 0.15^{d}	7.06 ± 0.16^{d}	7.25 ± 0.16^{d}
51	Altigo, SA 0.05%	6.95 ± 0.13^{b}	6.51 ± 0.12^{c}	6.73 ± 0.13^{c}

Note: see Table 1.

For end-use quality, lines 31, 37, 39, and 43 led for both grain protein and gluten, underscoring the strong relationship between these traits. All evaluated lines met baking suitability thresholds, but lines 15, 19, and 51, despite respectable productivity, were comparatively less favorable regarding technological quality criteria.

Grain-quality gains were driven by elevated high-molecular-weight (HMW) glutenins, suppressed low-molecular-weight (LMW) glutenins, and only moderate shifts in gliadins. Lines 31, 37, and 43 were outstanding for HMW glutenins; the same three lines also showed reduced LMW fractions. Gliadin content was comparatively stable overall, though lines 31 and 43 tended toward higher values.

From a breeding-use perspective, line 37 aggregated several favorable qualities, but delivered only moderate yield, making it best suited as a donor in conventional crosses aimed at strengthening baking quality. Line 43 combined strong quality (high protein, robust gluten, high HMW and low LMW profile) with competitive yield, offering a practical balance for advancement. By contrast, line 39, despite solid field performance, showed weaknesses in grain-quality composition and would require quality-oriented improvement before varietal release.

Discussion

Decades of work have established that beneficial mutation patterns are not random but follow reproducible tendencies that breeders can harness (Nazarenko et al., 2021; Hekal et al., 2025). When managed well, induced variation yields genetic resources that are easier to guide, evaluate, and deploy in breeding pipelines (Nazarenko et al., 2022). For regional germplasm, mutation is a dependable, heritable source of new alleles (Chetto et al., 2025), and it has repeatedly produced target phenotypes, ranging from strong stature reduction to long, grain-dense spikes (Bayhan et al., 2024) and earliness (Hilukwa et al., 2025; Naserian Khiabani et al., 2025), when applied to appropriate local backgrounds (Winkler et al., 2023).

Within this context, our results position sodium azide (SA) and ethylmethanesulfonate (EMS) as complementary tools rather than interchangeable agents. SA, in our material, functioned as an effective epimutagen for both domestic and introduced wheat lines, meaning it frequently triggered stable, heritable adjustments in performance and quality, often with a favorable ratio of useful to neutral changes (Harb et al., 2025). EMS, by contrast, was especially valuable for generating parent material: It reliably broadened the allelic landscape and produced lines that serve well as donors in subsequent recombination and selection schemes, even if immediate, variety-level release was less common (Chetto et al., 2025; Han et al., 2025). In other words, SA more often delivered near-ready material, whereas EMS expanded the palette for strategic crossing; both, however, contributed meaningfully to diversity pools (Horshchar & Nazarenko, 2024).

The core finding of the study is that mutagen efficacy is not a property of the chemical alone. Response is closely associated with genotype, likely reflecting DNA sequence context, chromatin state, and repair pathway architecture in the initial variety. We observed clear, repeatable differences among varieties in how quickly useful changes appeared and at what dose adverse effects began to accumulate. This aligns with the idea of pronounced site-specificity and genotype-mutagen interaction: The same dose can be near-optimal for one background and inefficient or mildly harmful for another. This specificity is not merely an academic curiosity; it has practical upside. Where SA matched the genotype, we documented consistent improvements in end-use traits, particularly grain quality parameters, with lines showing more favorable biochemical signatures for food use (Beiko & Nazarenko, 2022).

Dose windows were decisive. Medium SA concentrations, most notably 0.025% and 0.05%, were the optimal levels for inducing measurable, agronomically beneficial shifts while minimizing collateral negative effects (Nanggia & Manimozhi, 2025; Nazarenko & Okselenko, 2025). At these levels, traits traditionally regarded as slow-moving, protein quality attributes or mineral micronutrient enrichment, showed modest but consistent movement in the desired direction. Compared with EMS, SA at these intermediate concentrations produced a cleaner action/responce ratio for quality enhancement in our material. The best agent still depended on the breeding objective and the recipient genome.

It is also notable that increasing the dose did not simply scale benefits. At the upper end of our tested range (0.1% for both agents), we did not see the typical pattern when half the lines are discarded (Liu et al., 2025). The frequency of strongly negative trait combinations remained relatively restrained, although proportion of promising lines among all mutants flattened or declined. In operational breeding terms, pushing the dose higher raised total mutation counts but did not increase the fraction of genuinely useful outcomes. Variety choice remained paramount: backgrounds that tolerated mutagenic stress with minimal depression yielded more commercially realistic selections, whereas highly labile genotypes produced diversity at the cost of stability and agronomic reliability (Mahmood et al., 2023; Rebouh et al., 2023; Stearns et al., 2025).

A particularly informative responce emerged from one responsive genotype (Altigo). In that variety, the relationship between the visually scored mutation spectrum and the appearance of traits with direct production value significantly increased This stronger coupling, more apparent with SA than in our earlier supermutagen work, implies that certain mutagens can aim the spectrum more effectively, or at least bias it toward useful categories, when the genetic substrate is receptive (Okselenko et al., 2025; Liu et al., 2025). For breeders, this suggests a two-step logic: first, identifying responsive backgrounds; second, pairing them with the agent and dose that best align with the target trait class.

From a trait standpoint, the clearest opportunities under SA were biochemical. We saw consistent upward movement in metrics that underpin baking performance, not just crude protein or bulk gluten, which can be poorly responsive or variety-dependent, but the composition of the glutenin complex itself. Gains in high-molecular-weight (HMW) glutenins without inflation of low-molecular-weight (LMW) fractions were common at productive SA doses, shifting dough strength in a favorable direction (Arumingtyas et al., 2023; Phadungsawat et al., 2025). Parallel trends in micronutrient content (e.g., Zn, Fe, Cu, Mn) also appeared, addressing nutritional gaps typical for wheat and adding food-quality value (Spencer-Lopes et al., 2018). These biochemical improvements did not always require visible architectural changes, which makes them attractive for upgrading established varieties with minimal disruption to farmer-preferred phenotypes (Nanggia & Manimozhi, 2025).

In summary, the operational guidance emerging from this work is to treat mutagen choice and dose as a genotype-specific decision. SA can be used at 0.025–0.05% when the priority is to improve quality gains and micronutrients with a high probability of retaining agronomic fitness. EMS can be applied to diversify donor pools, especially for complex, polygenic targets, and then gains can be consolidated through recombination. Rather than using the highest dose, the mid-low window should be chosen, as it maximizes the proportion of promising lines rather than sheer mutation count. Finally, candidate varieties for tolerance and response should be prescreened, because the pairing of “right background × appropriate agent × tuned concentration” is what converts mutagenesis from a stochastic perturbation into a predictable breeding instrument.

Conclusion

Altigo and Spivanka differed markedly in how they translated mutagen exposure into useful breeding outcomes. Altigo was more responsible genetic background overall, generated a broader spectrum of heritable changes and converted a larger share of those changes into lines worth advancing, most clearly when seeds were treated with sodium azide (SA) at 0.025–0.05% concentration. By contrast, Spivanka showed strong sensitivity at the low end of the dose range (notably EMS 0.025% and SA 0.025%), but reached its toxicity ceiling sooner: Performance deteriorated sharply at SA 0.1%, where the proportion of desirable mutants declined despite higher raw mutation counts. Dose-response windows were distinctly agent- and genotype-specific. For Altigo, SA 0.025% consistently turned out to be the most efficient single variant, balancing breadth of mutation spectra with a high fraction of agronomically valuable selections. Spivanka also benefited at SA 0.025%, though its best “conservative” chemical fallback was EMS 0.05%, which delivered reliable gains with fewer collateral negative effects. Across both varieties, the mid-low ranges, EMS 0.05–0.10% and SA 0.025–0.05%, yielded the richest, most usable spectra. Increasing the dose to the highest (e.g., SA 0.1%) raised the overall mutation frequency but eroded effectiveness by amplifying neutral or adverse changes and increasing stand/fitness losses. Trait profiles also diverged in informative ways. Spivanka was a dependable source of reduced stature, and short-stem selections appeared readily under both mutagens, making it a practical donor for plant height reduction and associated lodging resistance. Altigo was less promising for short-stem forms, but it outperformed Spivanka for semi-dwarf and dwarf classes, produced more long-spike phenotypes, and showed higher rates of disease-tolerance variants. Quality-oriented responses favored Altigo, with shifts in the glutenin-gliadin complex and increases in micronutrients (e.g., Zn, Mn, Cu) occurring at notable frequencies under SA 0.025%. For mineral traits, similar effects were observed under EMS 0.1%. Spivanka could contribute to quality improvement, especially at SA 0.025%, but the effects were less consistent compared with Altigo. If the breeding objective prioritizes quality and architecture (long spike, stronger main spike, micronutrient enrichment), Altigo under SA 0.025% is the first choice; and EMS 0.05–0.10% offers a complementary path, particularly for semi-dwarf and dwarf ideotypes. For early maturity, Spivanka responds well at SA 0.025% – 0.05% (with EMS 0.05% as a risk-managed alternative), and can provide short-stem variants under both agents. In all cases, SA 0.1% should be avoided: It consistently marked a toxicity threshold with lower conversion to elite selections. Lines 37 and 43 combined robust yield with satisfactory or superior grain quality across evaluation cycles, indicating balanced improvement rather than single-trait shifts. Line 39 also performed well agronomically, although its protein-fraction profile showed minor imbalances. These did not preclude its potential as a varietal candidate and may be addressed through targeted crossing. Priorities include drought-response phenotyping (photosynthetic efficiency at key stages, canopy temperature, and stay-green behavior); winter-hardiness screening (overwinter survival and crown carbohydrate dynamics); nutrient uptake/partitioning analyses to validate the mechanistic basis of observed micronutrient enhancements.

References

Arumingtyas, E. L., Atiaturrochmah, & Kusnadi, J. (2023). Confirmation of mutation and genetic stability of the M_4 generation of chili pepper’s (*Capsicum frutescens* L.) ethyl methane sulfonate (EMS) mutant based on morphological, physiological and molecular characters. Biodiversitas, 24(1), 531–538.

Bayhan, M., Özkan, R., Albayrak, Ö., Akinci, C., & Yildirim, M. (2024). Effects of ethyl methanesulfonate on growth and yield parameters of wheat and tolerance to imazamox. Bangladesh Journal of Botany, 53(3), 487–493.

Beiko, V., & Nazarenko, M. (2022). Early depressive effects of epimutagen in the first generation of winter wheat varieties. Agrology, 5(2), 43–48.

Bharathi, S., Nageswari, K., Rajesh, S., Anand, G., Geetharani, P., Rajangam, J., & Williams, M. (2025). M_4 generation of moringa (*Moringa oleifera* Lam.) mutants revealing genetic diversity for leaf traits based on SSR markers. Plant Science Today, 12(2), 4241.

Chetto, O., Belqadi, L., Kouighat, M., Bucher, E., El Fechtali, M., Ndiaye, R. I., & Nabloussi, A. (2025). Unveiling new insights into faba bean sensitivity and genetic responses to the mutagen agent EMS (ethyl methanesulfonate). International Journal of Agronomy, 2025(1), 2738022.

Diallo, S., Badiane, F. A., Gueye, M. D., Diouf, M., & Diouf, D. (2025). Determining the optimal gamma irradiation dose for developing novel cowpea (*Vigna unguiculata*) genotypes. International Journal of Radiation Biology, 101(2), 174–185.

Didenko, V., & Nazarenko, M. (2025). Impact of ecogenetic factors on cytogenetic variability of winter wheat. Agrology, 8(1), 16–24.

Fradgley, N. S., Gardner, K. A., Kerton, M., Swarbreck, S. M., & Bentley, A. R. (2024). Balancing quality with quantity: A case study of UK bread wheat. Plants People Planet, 6(5), 1000–1013.

Han, D., Wang, P., Tang, J., Li, Y., Wang, Q., & Ma, Y. (2025). Enhancing crop yield forecasting performance through integration of process-based crop model and remote sensing data assimilation techniques. Agricultural and Forest Meteorology, 372, 110696.

Harb, A. H., Awaly, S. B. H., Elsayed, M. I. E., & El-Maaty, S. A. (2025). Effect of gamma irradiation on yield components and molecular marker traits in desi and kabuli chickpea. Egyptian Journal of Botany, 65(1), 68–84.

Hekal, M. A., Abbas, M. H. H., & Abdelhafez, A. A. (2025). Enhancing durum wheat growth and productivity in arid soils via seed irradiation with gamma rays and the foliar application of nano Cu. Applied Radiation and Isotopes, 221, 111821.

Hilukwa, R., Franke, A. C., Labuschagne, M., Wanga, M. A., Hukununa, R.-M. K., Hangula, M. N., Hasheela, E. B. S., Zorrilla, C., & Sarsu, F. (2025). Radio-sensitivity of selected Namibian landrace groundnut (*Arachis hypogaea* L.) genotypes to gamma radiation. Journal of Environmental Radioactivity, 282, 107618.

Horshchar, V., & Nazarenko, M. (2023). Induction of positive changes for winter wheat under the action of a group of ecogenetic factors with lower damaging ability. Agrology, 6(3), 60–66.

Horshchar, V., & Nazarenko, M. (2024). Peculiarities of the sodium azide action as a factor of variability on winter wheat. Agriculture and Forestry, 70(2), 61–76.

Khare, V., Gupta, S. K., & Manjaya, J. G. (2025). Exploring differential radiosensitivity in soybean genotypes exposed to gamma rays and determining optimal doses for induced mutagenesis. Applied Radiation and Isotopes, 220, 111778.

Kryshyn, R., & Nazarenko, M. (2025). Cytogenetic effect of highly active ecogenetic factors for winter wheat. Agrology, 8(1), 40–47.

Liu, C., Frascarelli, G., Stec, A. O., Heinen, S., Lei, L., Wyant, S. R., Legg, E., Spiller, M., Muehlbauer, G. J., Smith, K. P., Fay, J. C., & Morrell, P. L. (2025). Sodium azide mutagenesis induces a unique pattern of mutations. PLoS Genetics, 21(6), e1011634.

Mahmood, S. A., Karampoiki, M., Hammond, J. P., Paraforos, D. S., Murdoch, A. J., & Todman, L. (2023). Embedding expert opinion in a Bayesian network model to predict wheat yield from spring–summer weather. Smart Agricultural Technology, 4, 100224.

Nanggia, A. R., & Manimozhi, V. (2025). Cytogenetic effects of gamma radiation-induced seed extracts of *Panicum miliaceum* L. on the somatic chromosomes of *Allium cepa* L. Research Journal of Biotechnology, 20(6), 25–30.

Naserian Khiabani, B., Alizadeh, B., Borzouei, A., Rahemi, M. R., & Mirkhani, R. (2025). Evaluation of genetic diversity and selection of superior mutant lines in rapeseed (*Brassica napus* L.): The impact of mutations on yield and agronomic traits. Euphytica, 221(5), 53.

Nazarenko, M., & Okselenko, O. (2025). Peculiarities of the new epimutagen action on variability of winter wheat. Agriculture and Forestry, 71(3), 87–101.

Nazarenko, M., Izhboldin, O., & Izhboldina, O. (2022). Study of variability of winter wheat varieties and lines in terms of winter hardness and drought resistance. AgroLife Scientific Journal, 11(2), 116–123.

Nazarenko, M., Semenchenko, O., Izhboldin, O., & Hladkikh, Y. (2021). French winter wheat varieties under Ukrainian North Steppe condition. Agriculture and Forestry, 67(2), 89–102.

Okselenko, O., Nazarenko, M., & Horshchar, V. (2025). Cytogenetic analysis of the effects of a new epimutagenic agent on chromosomal stability in winter wheat (*Triticum aestivum*). Agrology, 8(1), 132–139.

Phadungsawat, B., Wiangsamut, B., Sasivatchutikool, P., Yeemin, J., Picha, R., & Orpong, P. (2025). Determination of median lethal dose and effects of gamma radiation on seed germination in *Viola cornuta* L. Trends in Sciences, 22(1), 8705.

Rebouh, N. Y., Khugaev, C. V., Utkina, A. O., Isaev, K. V., Mohamed, E. S., & Kucher, D. E. (2023). Contribution of eco-friendly agricultural practices in improving and stabilizing wheat crop yield: A review. Agronomy, 13(9), 2400.

Spencer-Lopes, M., Forster, B., & Jankuloski, L. (2018). Manual on mutation breeding. Third edition. Food and Agriculture Organization of the United Nations, Rome.

Stearns, F. W., Zhou, J., & Fenster, C. B. (2025). Scaling the fitness effects of mutations with respect to differentially adapted *Arabidopsis thaliana* accessions under natural conditions. Evolution, 79(6), 951–961.

Winkler, H. M., Cereijo, A. E., Scarpin, G. J., Dileo, P. N., Muchut, R. J. W., Roeschlin, R. A., Lorenzini, F. G., Paytas, M. J., & Landau, A. M. (2023). Phenotypic effects of different doses of physical and chemical mutagens in cotton plants. Revista de Investigaciones Agropecuarias, 49(2), 71–85.