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ASSESSMENT OF POSITIVE CHANGES IN WINTER WHEAT VARIETIES UNDER MUTAGEN TREATMENT

SUMMARY

The exploration of novel agents capable of inducing practical genetic diversity in modern winter wheat varieties represents a significant avenue for enhancing both commercial wheat breeding and recombination-based selection methods. This study investigated the effectiveness of a relatively novel chemical mutagen, 1,4-bisdiazoacetylbutane (DAB), as an inducer of beneficial variability in grain yield and quality attributes, as well as in other agriculturally significant traits. Eight varieties of winter wheat (Perspektyva Odeska, Sonata Poltavska, Shpalivka, MIP Lada, Farell, NE 12443, Ronin, and Seilor) were exposed to the mutagen DAB at three concentrations: 0.1%, 0.2%, and 0.3%. Among the tested varieties, Farell, Ronin and Seilor exhibited the highest responsiveness, making them particularly favorable candidates for targeted mutagenesis aimed at enhancing beneficial traits. Optimal results, characterized by substantial genetic variability and an increased proportion of agriculturally beneficial forms, were consistently achieved at the highest DAB concentration tested (0.3%). Two particularly promising mutant lines emerged from the study, exhibiting the most favorable combination of high grain productivity, consistent yield stability and desirable grain quality traits suitable for bread-making purposes. Furthermore, several other mutant lines derived in this study exhibit significant potential as valuable parental lines for cross-breeding.

Keywords: cereals; variability; 1,4-bisdiazoacetylbutane; rate of positive changes; nutrition value; site-specific action; mutations; *Triticum aestivum* L.

INTRODUCTION

Changes in environmental conditions increasingly require agricultural crops to adapt to new and sometimes challenging circumstances. Climate change, in particular, has introduced a broad array of stress factors, including altered

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precipitation patterns, rising temperatures, increased incidence of extreme weather events, and shifts in pest and disease dynamics (Bezie *et al.*, 2020)

Among various methods of mutagenesis, chemical mutagenesis has demonstrated significant potential due to its unique ability to induce specific genetic alterations in plant genomes, thereby enhancing variability in beneficial traits. According to Lal *et al.* (2020), chemical mutagens have been notably effective in triggering genome mutations that lead to heritable phenotypic changes, enhancing crop adaptation and resilience. However, mutations induced by chemical mutagens are inherently random, lacking precise predictability regarding their outcomes. As J. Chaudhary *et al.* (2019) have reported, many mutations induced by chemical mutagenesis do not confer immediate advantages, and in fact, some mutations might prove detrimental, negatively impacting plant growth, survival, and productivity. Nevertheless, within these randomly generated genetic changes, breeders can discover and isolate beneficial mutations, leading to varieties characterized by improved yield (Nazarenko, 2016). W. Yali and T. Mitiku (2022) emphasized that while chemical mutagenesis significantly influences the heredity and developmental processes in crops.

Chemical supermutagens, characterized by their pronounced DNA-damaging capacity, are particularly notable within mutation breeding, as they typically induce more extensive and complex genetic alterations. M. Spencer-Lopes *et al.* (2018) highlighted the effectiveness of chemical supermutagens in major crops, demonstrating that they could successfully produce mutant forms with complex and highly valuable trait combinations. These supermutagens are routinely used not only in applied breeding programs but also in fundamental research aiming to elucidate the genetic basis of complex traits (Kartseva *et al.*, 2023).

Specific chemical mutagens exhibit preferential affinities toward particular genetic structures or sequences, resulting in higher mutation frequencies at these loci. G. Abaza *et al.* (2020) emphasized two critical components necessary for effective site-specific mutagenesis: the genetic constitution of the treated variety and the chemical properties of the mutagen itself. The effects induced by high concentrations of chemical mutagens can be challenging to predict accurately, as different crop varieties may respond differently to the same mutagenic agent (Ahumada-Flores *et al.*, 2021). The interaction between genotype and mutagen concentration plays a critical role in the outcome of mutagenesis experiments. Consequently, breeding programs incorporating chemical mutagenesis must carefully consider these factors through comprehensive preliminary studies, as suggested by Ram *et al.* (2019) and Abdel-Hamed *et al.* (2021).

Winter wheat (*Triticum aestivum* L.) represents a critically important crop for Ukraine and countries with comparable agroecological conditions. The changing climate conditions necessitate the urgent development of novel winter wheat genotypes adapted to emerging environmental challenges, either through introducing genetic diversity from different ecological and geographic sources or

by generating new variability directly from local genetic resources (Cann *et al.*, 2022).

To address these challenges, chemical mutagenesis methods are increasingly applied in breeding programs focused on winter wheat. These programs aim to induce genetic variability sufficient to create novel genotypes capable of sustaining high productivity under changing climatic conditions (Nazarenko *et al.*, 2021). Recent research has demonstrated that properly selected mutagenic agents and optimal treatment concentrations can significantly enhance adaptive traits in winter wheat, particularly those related to stress tolerance, disease resistance, and grain quality attributes essential for baking and human nutrition (Jalal *et al.*, 2021).

In summary, the purpose of this study was to comprehensively investigate the specific patterns of induced heritable variability in winter wheat varieties, emphasizing the frequency and range of beneficial mutations arising from genotype-by-mutagen interactions.

MATERIAL 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), located in the Dniprovskiy district, Dnipropetrovsk region, Ukraine. This region is characterized as a northern warm, moderately arid agro-climatic zone with a hydro-thermal 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.

Eight winter wheat varieties (*Triticum aestivum* L.), Perspektiyya Odeska, Sonata Poltavska, Shpalivka, MIP Lada, Farell, NE 12443, Ronin, and Seilor, were selected as initial plant material. Seeds (1000 grains per treatment variant, 14 % seeds moisture) were treated with the mutagen DAB (1,4-bisdiazoacetylbutane) in concentrations of 0.1, 0.2 and 0.3% (Sigma-Aldrich, Germany). After treatment, the seeds wrapped in gauze were rinsed under running water; all procedures were performed in a laboratory hood using appropriate personal protective equipment. Sowing was carried out under field conditions immediately after rinsing. Treatments were conducted by soaking seeds for 18 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.

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 AND DISCUSSION

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, 16,000 families (lines) were evaluated at M_2 – M_3 stages for DAB -induced populations. Out of these, 481 mutant lines were selected as carriers of noticeable trait changes (see Table 1). Among them, 205 lines showed agriculturally valuable traits, and 76 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.

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 DAB was moderate promising: nearly 1.2% of families produced valuable breeding material (205/16,000 averaged across variants).

For most varieties and traits, the frequency of induced changes increases as DAB concentration increases from 0.1% to 0.3%. Most responsive traits across varieties were early ripeness, tolerance for diseases, protein-related traits (content

and composition) and productivity. Less responsive traits were thick stem, dwarf types, intense wax accumulation. Other traits had average variability.

Table 1. Parameters of mutagen action, DAB, ($\bar{x} \pm SD$, n=500)

Variety	Variant	RPC, %	PFGR, %	NL, pcs	NPL, pcs	RPL, %
Perspektyva Odeska	water	0.00 ± 0.00 ^a	0.00	0	0	0.00 ± 0.00 ^a
	DAB 0.1%	2.00 ± 0.15 ^b	0.59	9	3	0.60 ± 0.08 ^b
	DAB 0.2 %	1.60 ± 0.12 ^c	0.42	7	2	0.40 ± 0.07 ^b
	DAB 0.3 %	3.40 ± 0.21 ^d	0.65	12	3	0.60 ± 0.08 ^b
Sonata Poltavska	water	0.20 ± 0.03 ^a	0.20	0	0	0.00 ± 0.04 ^a
	DAB 0.1%	1.60 ± 0.13 ^b	0.57	7	2	0.40 ± 0.08 ^b
	DAB 0.2 %	1.40 ± 0.12 ^b	0.39	5	2	0.40 ± 0.08 ^b
	DAB 0.3 %	2.80 ± 0.20 ^c	0.56	10	4	0.80 ± 0.14 ^c
Shpalivka	water	0.20 ± 0.01 ^a	0.50	0	0	0.00 ± 0.00 ^a
	DAB 0.1%	1.40 ± 0.12 ^b	0.47	5	1	0.20 ± 0.04 ^b
	DAB 0.2 %	2.20 ± 0.17 ^c	0.58	7	3	0.60 ± 0.04 ^c
	DAB 0.3 %	3.40 ± 0.22 ^d	0.68	10	4	0.80 ± 0.09 ^c
MIP Lada	water	0.40 ± 0.03 ^a	0.67	1	1	0.20 ± 0.04 ^a
	DAB 0.1%	1.60 ± 0.13 ^b	0.62	7	2	0.40 ± 0.05 ^a
	DAB 0.2 %	2.20 ± 0.18 ^c	0.61	8	3	0.60 ± 0.05 ^{ab}
	DAB 0.3 %	2.80 ± 0.21 ^d	0.58	10	5	1.00 ± 0.10 ^c
Farell	water	0.20 ± 0.00 ^a	0.33	1	0	0.00 ± 0.00 ^a
	DAB 0.1%	1.40 ± 0.12 ^b	0.47	6	2	0.40 ± 0.05 ^b
	DAB 0.2 %	2.40 ± 0.18 ^c	0.60	9	4	0.80 ± 0.08 ^c
	DAB 0.3 %	3.40 ± 0.21 ^d	0.68	12	7	1.40 ± 0.15 ^d
NE 12443	water	0.00 ± 0.00 ^a	0.00	0	0	0.00 ± 0.00 ^a
	DAB 0.1%	1.20 ± 0.10 ^b	0.40	5	1	0.20 ± 0.03 ^b
	DAB 0.2 %	1.60 ± 0.14 ^c	0.38	7	1	0.20 ± 0.03 ^b
	DAB 0.3 %	3.80 ± 0.23 ^d	0.79	12	3	0.60 ± 0.08 ^c
Ronin	water	0.20 ± 0.01 ^a	0.50	0	0	0.00 ± 0.00 ^a
	DAB 0.1%	1.80 ± 0.13 ^b	0.75	8	3	0.60 ± 0.03 ^b
	DAB 0.2 %	2.20 ± 0.16 ^c	0.69	8	4	0.80 ± 0.07 ^c
	DAB 0.3 %	3.80 ± 0.23 ^d	0.79	14	7	1.40 ± 0.12 ^d
Seilor	water	0.00 ± 0.00 ^a	0.00	0	0	0.00 ± 0.00 ^a
	DAB 0.1%	1.60 ± 0.11 ^b	0.62	7	3	0.60 ± 0.02 ^b
	DAB 0.2 %	2.00 ± 0.14 ^c	0.50	8	3	0.60 ± 0.04 ^b
	DAB 0.3 %	3.00 ± 0.21 ^d	0.65	11	4	0.80 ± 0.11 ^b

Note: indices (a, b, c ...etc.) indicate that the data belong to different groups with significant differences at $P < 0.05$ by ANOVA-analyze with Bonferroni amendment. Comparison in terms of one variety. RPC - rate of positive changes; PFGR - part from general rate; NL - number of lines; NPL - number of perspective lines; RPL - rate of perspective lines.

As a result recommendations in general by variety based on variability for variety Farell were traits changed broadly and consistently across all DAB levels, ideal for inducing high trait diversity, DAB 0.3% preferred. For variety Ronin DAB was high-effective in early ripeness, productivity and biochemical changes, variety suitable for high-stability breeding in terms of agriculture-value forms produce, DAB at concentrations 0.2–0.3% more optimal. Variety Shpalivka was moderate to response in spike and grain traits changes, good for quality

improvement, 0.2% and 0.3% more effective for practical purpose. Variety Sonata Poltavska focused on spike-related and early ripeness traits, effective DAB 0.2% for spike improvements, 0.3% for protein traits. Seilor was less responsive to protein changes, more for spike morphology and ripeness traits, prefer DAB 0.3%. Variety Perspektyva Odeska, variability limited to a few key traits, may be used in background improvement for specific goals, DAB 0.2–0.3%. NE 12443 was low or moderate by variability, some traits respond at higher concentration, consider only DAB 0.3% for nutritional or disease-resistance traits improvement. Genotype MIP Lada, low in variability, few traits triggered, more stable genome, use for minor trait enrichment only, not suitable for DAB action as initial form.

Summary of varietal responses were for varieties Ronin and Farell were strong concentration-dependent response. Highest rates of positive changes and perspective lines at elevated concentrations. The most effective for creating viable lines with new traits. Varieties NE 12443 and Perspektyva Odeska have good responsiveness in terms of positive changes, but moderate perspective rates, indicating beneficial mutations occurred but fewer resulted in clearly advantageous lines.

Varieties Shpalivka, Sonata Poltavska and Seilor were intermediate responses, balanced between rate of positive changes and perspective line generation. Suitable for mutation breeding but might require more extensive selection. MIP Lada was moderate overall response, though notable improvement at higher concentration (0.3%), indicating usefulness at targeted higher doses for obtaining valuable lines.

Some differences in traits can be identified early ripeness induced for all varieties from DAB 0.1%; tolerance for diseases for Ronin, NE 12443, Shpalivka, DAB 0.2% and above; for productivity all varieties (except Perspektyva), clearly at DAB 0.3%; for protein content Farell, Ronin, Shpalivka Peak at DAB 0.3%; for positive protein changes Farell, Ronin, Shpalivka, significant from DAB 0.2%; for microelement enrichment Ronin, Farell, Shpalivka, higher at 0.3%; spike architecture, Sonata, Shpalivka, Seilor, modest at DAB 0.2–0.3%; for dwarfism/semi-dwarfism, varieties Ronin, Sonata, Seilor, low frequency, specific.

As observed, the rate of positive trait changes increased with higher mutagen concentrations for all varieties across all tested variants with one exception for Sonata Poltavska. Variety Sonata Poltavska ($F=3.11$; $F_{0.05}=4.23$; $P=0.07$) showed no significant differences between the lower two DAB concentrations (0.1% and 0.2%). Overall variability in positive changes was generally consistent across genotypes (ranging from 2.8% to 3.8% with some significant differences for varieties), less variable were Sonata Poltavska and MIP Lada (3.8%), more NE 12443 and Ronin 12443 (3.8%) ($F=7.89$; $F_{0.05}=4.23$; $P=0.009$), Perspektyva Odeska and Farell (up to 3.4%) ($F=5.14$; $F_{0.05}=4.23$; $P=0.03$). Thus, NE 12443, Ronin, Perspektyva Odeska, 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 DAB concentrations.

Factor analysis revealed that the induction rate of positive trait changes was significantly influenced by increased DAB concentrations ($F=42.17$; $F_{0.05}=3.86$; $P=3.41\times 10^{-5}$) and was significantly affected by varietal differences ($F=36.14$; $F_{0.05}=4.11$; $P=2.42\times 10^{-4}$). Additionally, the genotype-mutagen interaction was significant ($F=21.01$; $F_{0.05}=4.55$; $P=3.44\times 10^{-3}$).

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 NE 12443 and 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 DAB concentrations (0.1% and 0.2%) due to the minimal induction of additional desirable traits. Importantly, unlike classical mutagens, higher mutagen 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 DAB concentrations (less variable indicator overall). Nevertheless, the rate of promising lines was influenced significantly by DAB concentration increases ($F=4.94$; $F_{0.05}=3.86$; $P=0.03$), varietal differences ($F=22.17$; $F_{0.05}=4.11$; $P=1.34\times 10^{-3}$), and the genotype-mutagen interaction ($F=7.44$; $F_{0.05}=5.20$; $P=0.02$).

For each variety genotype-mutagen interaction was significant in negative sense for varieties NE 12443 ($F=5.34$; $F_{0.05}=3.55$; $P=0.03$) and MIP Lada ($F=5.93$; $F_{0.05}=3.55$; $P=0.02$). Thus, in general, DAB as mutagen in complex with these genotypes not can be used for high mutation rate with any concentration, for the most part of varieties the critical value of the mutagen in terms of valuable changes in the range DAB 0.2 % – DAB 0.3 %. For rate of positive changes for all varieties, the highest concentration (0.3%) consistently induced the greatest positive change, demonstrating a clear concentration-dependent effect. The varieties Ronin and NE 12443 reached the highest values (3.8%), indicating a significant responsiveness to higher mutagen concentrations. For the part from general rate, generally increased with higher mutagen concentrations across all varieties, suggesting a proportionally higher influence of mutagen concentration on the induction of positive traits. For the number of lines clearly increased with increasing concentrations of DAB for all varieties, Sonata Poltavska and Seilor produced slightly fewer promising lines, indicating moderate responsiveness. Number of perspective lines increased with concentration for all varieties, with the strongest response in Farell and Ronin (7 perspective lines at 0.3%).

To assess the complex, site-specific response of different genotypes to the mutagenic action of DAB, a cluster analysis was performed based on the

cumulative trait response across all concentrations and traits (Fig. 1). Each group showed a different response to DAB in terms of mutational variability.

Five distinct genotype groups were identified through cluster analysis based on their response to DAB. The first group included the varieties Farell, Seilor and Ronin, which exhibited similar reactions to DAB. Consequently, the potential for these genotypes to serve as highly effective initial forms for mutagenesis with substances characterized by low damaging potential. The second minor group consisted solely of the variety Perspektiyya Odeska, which demonstrated a unique, genotype-specific response to DAB treatment. Given its genetic structure, this variety is likely to yield intermediate effectiveness, contributing partially to enhanced mutation frequencies for beneficial traits.

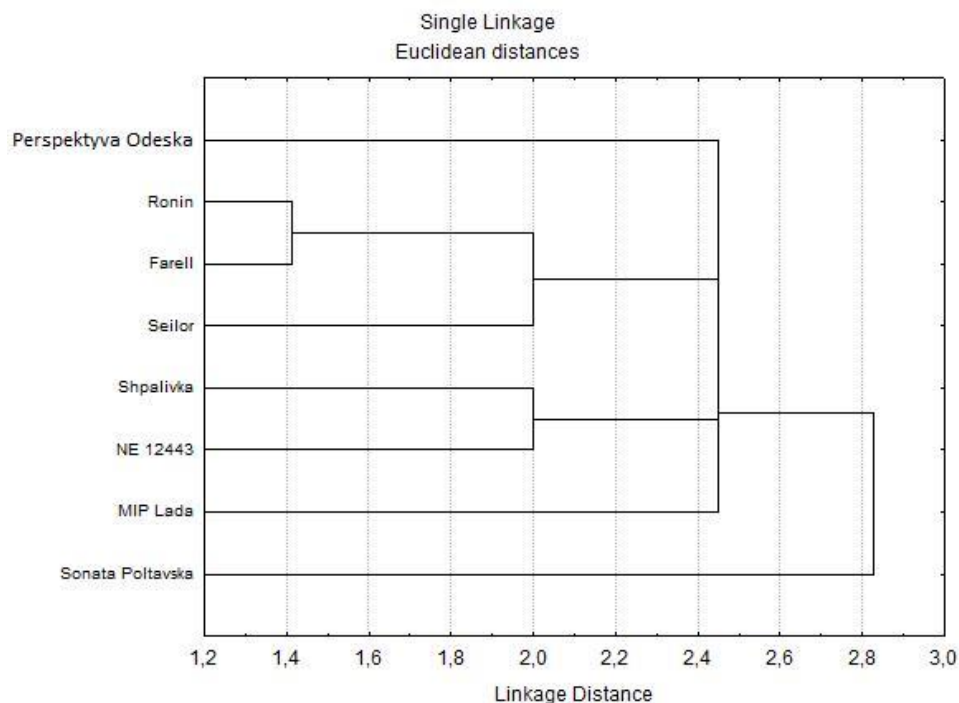


Figure 1. Cluster analysis of winter wheat varieties based on the rate of positive changes under different concentrations of DAB

The third group encompassed two varieties: Shpalivka, NE 12443. These varieties exhibited low-average responsiveness to DAB treatment, suggesting a relatively limited number of positive trait mutations when treated with this agent. The fourth minor group encompassed one variety Sonata Poltavska. These varieties exhibited average responsiveness to DAB treatment. The fifth minor group encompassed one variety MIP Lada. These varieties low average responsiveness to DAB treatment.

Discriminant analysis results (Fig. 2) indicated that DAB concentrations of 0.1% and 0.2% had comparable effectiveness across all genotypes. In contrast,

the higher DAB concentration of 0.3% demonstrated increased variability and effectiveness in inducing mutations.

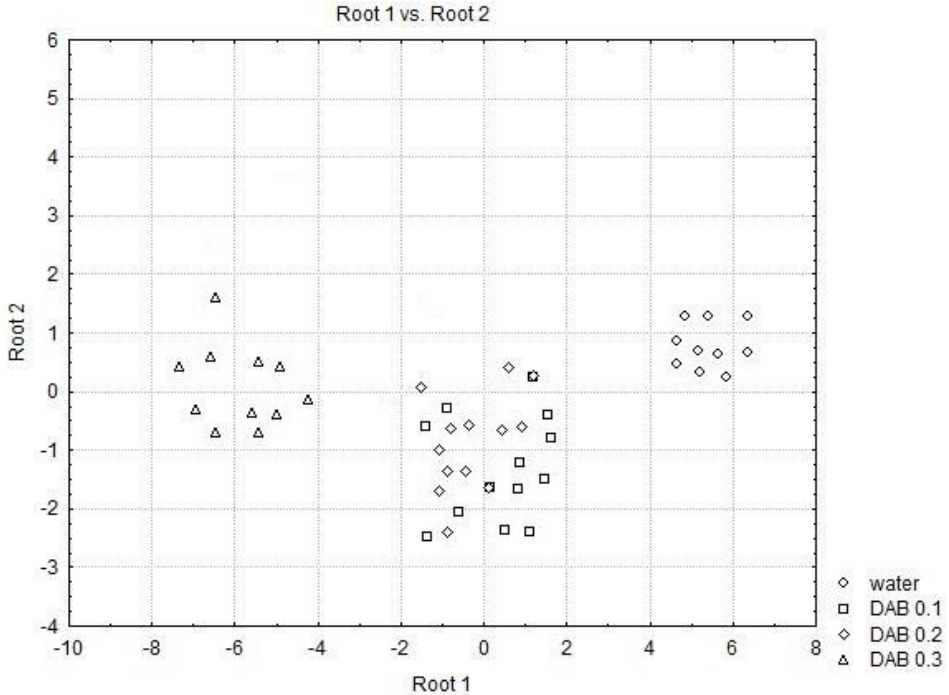


Figure 2. Discriminant analysis of winter wheat varieties based on the rate of positive changes under different concentrations of DAB

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 DAB 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 for a more detailed interpretation of the mutation spectra, highlighting genotype-specific responses and the site-specificity of the mutagenic factor. For DAB mutations by so traits as early ripeness, tolerance for diseases, productive are probable with sufficient reliability. Regarding this DAB as mutagen high effective for this initial material (Ronin, Farell). Moderately traits by occurrence were long spike and positive changes in microelement content, positive changes at protein components, higher protein content, semi-dwarf. Traits such as dwarf, short-stem, thick stem, intense epicuticular wax, large-size grain and spike, long spike, tillering capacity accumulation were infrequent or absent, suggesting that these traits are difficult to induce or strongly genotype-dependent. As conclusion,

action of DAB in complex is the most practical suitable variant for breeding process, but strongly dependent from genotype.

Table 2. Positive changed traits. Results of discriminant analyses.

Trait at model	Wilks Lambda λ	Concentration at model	F _{0.05}	P-level
Thick stem	0.467	non	2.01	0.085
Short-stem	0.411	non	2.45	0.065
Semi-dwarf	0.311	DAB 0.3%	4.12	0.047
Dwarf	0.396	non	2.76	0.083
Intense epicuticular wax accumulation	0.490	non	1.72	0.112
Large-size grain	0.463	non	1.88	0.102
Long spike	0.465	non	1.79	0.093
Large-size spike	0.496	non	1.46	0.137
Early ripeness	0.097	DAB 0.2 – 0.3%	22.14	0.001
Tolerance for diseases	0.151	DAB 0.2 – 0.3%	7.45	0.024
Productive	0.159	DAB 0.3%	7.11	0.025
Tillering capacity	0.451	non	2.16	0.072
Higher protein content	0.270	DAB 0.3%	5.01	0.036
Positive changes at protein components	0.254	DAB 0.2 – 0.3%	5.05	0.035
Positive changes at microelement content	0.291	DAB 0.3%	4.92	0.038

Characteristics of lines obtained under the action of the studied mutagen 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, Sonata Poltavska and MIP Lada contributed positively. Three varieties Perspektivya Odeska, Shpalivka and Seilor were represented by a single form resulting exclusively from DAB 0.2 – 0.3 % treatment. Varieties Farell and Ronin, treated with DAB concentration of 0.3%, each contributed two lines. In 2022, the evaluated lines formed three productivity groups relative to the standard: lines 89, 90, 98, 100 and 105 comprised the most productive group, one form 10 occupied intermediate positions, variety Podolyanka and line 41 alone formed the least productive group. In 2023, productivity differentiation was significantly more pronounced. Lines 89, 100, and 105 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, productivity differences were even more distinct. Lines 89, 100 and 105 remained in the top group, while lines 10, 41, 90 and 98 occupied a statistically separate second group with significant yield differences from subsequent groups.

Aggregating results from the three-year testing period, three productivity groups were distinctly identified: lines 89, 100 and 105 formed the most productive and stable group ($F=10.17$; $F_{0.05}=4.23$; $P=0.009$), lines 90, 98 occupied an intermediate productivity level ($F=7.14$; $F_{0.05}=4.23$; $P=0.02$) and line 10

exceeded the standard yield ($F=4.01$; $F_{0.05}=3.71$; $P=0.04$) but significantly trailed the first group ($F=9.11$; $F_{0.05}=4.01$; $P=0.01$). Lines 89, 100 and 105 demonstrated consistent trait stability, whereas other lines exhibited considerable annual variability. Line 41 on the level of variety Podolyanka.

Table 3. Grain yield of mutant lines of winter wheat during the ecological estimation (DSAEU, 2022–2024), $t\ ha^{-1}$

Number	Origin	2022	2023	2024	Average
1	Podolyanka, standard	5.48 ± 0.09^a	6.12 ± 0.11^a	5.55 ± 0.13^a	5.72 ± 0.10^a
10	Perspektyva Odeska, DAB 0.3 %	5.78 ± 0.10^b	6.97 ± 0.17^b	6.12 ± 0.15^b	6.29 ± 0.12^b
41	Shpalivka, DAB 0.2 %	5.56 ± 0.10^a	6.67 ± 0.14^b	6.04 ± 0.14^b	6.09 ± 0.11^{ab}
89	Farell, DAB 0.3 %	6.29 ± 0.12^c	7.52 ± 0.18^c	6.72 ± 0.14^c	6.84 ± 0.15^c
90	Farell, DAB 0.3 %	6.11 ± 0.12^c	7.12 ± 0.16^d	6.14 ± 0.13^b	6.46 ± 0.17^{bc}
98	Ronin, DAB 0.3 %	6.17 ± 0.12^c	7.02 ± 0.16^{bd}	6.44 ± 0.13^{bc}	6.54 ± 0.17^{bc}
100	Ronin, DAB 0.3 %	6.34 ± 0.12^c	7.44 ± 0.18^c	6.98 ± 0.16^c	6.92 ± 0.18^c
105	Seilor, DAB 0.3 %	6.38 ± 0.12^c	7.56 ± 0.18^c	6.89 ± 0.15^c	6.94 ± 0.18^c

Note: indices (a, b, cetc.) indicate that the data belong to different groups with significant differences at $P<0.05$ by ANOVA-analyze with Bonferroni amendment.

Regarding technological grain quality, none of the evaluated lines notably excelled in protein content; however, lines 10, 90, and 100 stood out positively in gluten content, highlighting a close association between these two technological parameters. Overall, the majority of the studied lines exhibited good grain quality, maintaining a satisfactory protein content of around 14%, suitable for bread-making purposes, with the exception of line 105.

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

Variety/line	Protein, %	Gluten, %	Glutenins		Gliadins
			HMW	LMW	
Podolyanka	13.97 ^a	25.20 ^a	0.15861 ^a	0.45441 ^a	0.4563 ^a
10	14.02 ^a	26.01 ^b	0.15675 ^a	0.45541 ^a	0.4519 ^a
41	14.05 ^a	25.35 ^a	0.15753 ^a	0.46007 ^a	0.4505 ^a
89	14.10 ^a	25.48 ^a	0.16946 ^b	0.42401 ^b	0.4911 ^b
90	14.01 ^a	26.12 ^b	0.19915 ^c	0.43126 ^b	0.4567 ^a
98	14.04 ^a	25.61 ^a	0.19831 ^c	0.44144 ^c	0.4759 ^c
100	14.05 ^a	26.18 ^b	0.16113 ^a	0.44513 ^{ac}	0.4867 ^{bc}
105	13.45 ^b	23.60 ^c	0.20821 ^c	0.45134 ^a	0.4837 ^{bc}

Note: indices (a, b, cetc.) indicate that the data belong to different groups with significant differences at $P<0.05$ by ANOVA-analyze with Bonferroni amendment. HMW - high molecular weight glutenins, LMW - low molecular weight glutenins

Positive grain-quality characteristics observed among the tested lines included a relatively high concentration of high molecular weight glutenins, a trait exhibiting substantial variability, as well as lower levels of low molecular weight glutenins and moderate variability in gliadin content. Specifically, lines 89, 90, 98, and 105 excelled in the accumulation of high molecular weight glutenins. In addition, lines 89, 90, and 98 presented advantageous reductions in low molecular weight glutenin content. Furthermore, lines 89, 98, 100, and 105 demonstrated elevated gliadin levels, beneficial from a baking-quality perspective. Notably, none of the evaluated lines demonstrated a complete combination of multiple positive quality traits alongside consistently high grain yield. However, lines 89 and 98 exhibited a favorable complex of optimal storage protein structures. Among lines consistently exhibiting higher yields, 89 and 100 represented the best overall balance between productivity and desirable grain quality characteristics. Conversely, line 105 was excluded from further breeding consideration due to suboptimal grain quality attributes. Thus, lines 89 and 100 are recommended as promising candidates for further advancement in breeding programs aiming at combined productivity and grain-quality enhancement.

The systematic exploration and utilization of induced genetic variability have become pivotal strategies in modern agricultural breeding programs. The beneficial mutational processes have been extensively characterized in previous studies (Nazarenko *et al.*, 2021; Lal *et al.*, 2020), facilitating their application in generating novel genetic materials. These newly derived genotypes exhibit enhanced manageability, reliability, and predictability regarding economically valuable traits (Hongjie *et al.*, 2019).

Through targeted mutagenesis, traits typically challenging to improve, such as high-intensity dwarfism, grain-rich elongated spikes (Bayhan *et al.*, 2024), and early maturity (Kartseva *et al.*, 2023; Naserian Khiabani *et al.*, 2025), have been successfully modified, particularly utilizing locally adapted genetic backgrounds.

Our empirical data highlight the suitability and effectiveness of DAB as a mutagen, applicable not only to local but also to international wheat germplasm collections (Chakraborty *et al.*, 2023). DAB treatments predominantly generate genetic material valuable as parental components for recombinant breeding strategies, rather than producing directly deployable commercial cultivars (le Roux *et al.*, 2021; Horshchar and Nazarenko, 2024; Han *et al.*, 2025).

Crucially, our findings indicate that certain traits traditionally considered genetically stable or difficult to manipulate displayed meaningful variation under DAB exposure, with minimal negative side effects (OlaOlorun *et al.*, 2021; le Roux *et al.*, 2021). Significantly, the risk of combining beneficial with negative trait alterations remained low even as concentrations of the mutagen increased, a marked improvement over traditional mutagens (Liu *et al.*, 2025). At the highest tested DAB concentration (0.3%), adverse trait combinations were notably infrequent. This result markedly reduces the frequency of discarding promising mutant lines (Mangi *et al.*, 2021; Hassine *et al.*, 2023; Ergun *et al.*, 2023b).

Varieties exhibiting extreme initial variability, both in terms of mutagenic depression in the first generation and diverse heritable changes in subsequent generations, do not necessarily yield the most agriculturally valuable mutant lines (Nazarenko, 2017). Instead, genotypes demonstrating greater resistance to negative mutational effects produced economically superior lines more reliably (Rebouh *et al.*, 2023; Stearns *et al.*, 2025).

Notably, we observed a stronger correlation between agriculturally significant traits and the overall frequency and spectrum of visually identifiable mutations. This distinctive genotype-specific response was particularly evident under mutagenic treatments, contrasting earlier observations involving classical chemical supermutagens (Yali and Mitiku, 2022; Liu *et al.*, 2025).

While direct selection of commercially viable mutant lines via chemical mutagenesis remains challenging due to inherent genetic randomness, mutational processes provide substantial opportunities for targeted improvement (OlaOlorun *et al.*, 2021; Arumingtyas *et al.*, 2023). The observed biochemical alterations - particularly reductions in low-molecular-weight glutenins and simultaneous increases in essential microelement content, positively impact grain nutritional and commercial value (Spencer-Lopes *et al.*, 2018; Shimelis *et al.*, 2019).

Analysis of our experimental data (Nazarenko *et al.*, 2022) has revealed interesting mutational dynamics. Typically, increased mutagenic potency and dosage correlate negatively with mutational site specificity. However, when applying DAB, the situation differed markedly. Previous research by A. Anter (2021) corroborates that mutations induced by chemical mutagens, including DAB, constitute a reliable and continuous source of valuable local genetic diversity, beneficially impacting traits such as spike morphology, early maturation, and improved disease resistance profiles. Additionally, prior studies by Hussain *et al.* (2021) support the strategic use of chemically-induced mutants primarily as parental genetic resources, contributing to the subsequent enhancement of existing commercial varieties through recombination and cross-breeding approaches (Ergun *et al.*, 2023a; Hassine *et al.*, 2023; Fradgley *et al.*, 2024).

The results reaffirm the importance of careful genotype selection to maximize beneficial outcomes while minimizing the risk of adverse genetic modifications (Farooq *et al.*, 2024). Despite inherent challenges, the experimental results strongly advocate the combined use of responsive genotypes and optimized DAB concentrations to enhance trait-specific genetic variability effectively (Sala and Herbei, 2023).

CONCLUSIONS

The winter wheat varieties Farell, Ronin, and Seilor proved to be strong donors for breeding, showing useful mutation-derived variation in plant architecture, phenology, and grain quality (protein content and microelement profiles). Obtaining breeding-valuable material is substantially more likely when DAB is applied at a concentration of 0.3%, provided that appropriate initial

(source) material is selected. Among the derived material, mutant lines 89 and 100 combined consistently high yield with grain quality at or above target levels, making them priority candidates for inclusion in state varietal testing.

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