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INDUCED MUTAGENESIS FOR YIELD COMPONENT VARIABILITY IN WINTER WHEAT: EVALUATION OF GAMMA IRRADIATION DOSES IN THE M3 GENERATION

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ABSTRACT

Wheat (*Triticum aestivum* L.) is a major staple crop that plays a crucial role in global food security. Induced mutagenesis represents an effective approach for generating novel genetic variability in well-adapted cultivars and expanding the genetic base available for breeding. The present study aimed to evaluate the effects of gamma ray irradiation on the winter wheat variety "Nova Bosanka" by assessing dose-dependent responses in mutant populations and identifying promising lines for further selection. A total of 6 000 seeds were irradiated with three doses (150, 200 and 300 Gy) and advanced to the M3 generation under field conditions using the pedigree method. The number of spikes and seed yield were recorded for mutant lines based on five plants per line. Welch's one-way ANOVA revealed a significant effect of irradiation dose on spike number ($p \leq 0.05$), with the highest mean value observed at 300 Gy, while seed yield did not differ significantly among treatments. However, higher doses markedly increased phenotypic variability, particularly for seed yield, where the coefficient of variation reached 59.2% at 300 Gy. The identification of extreme high-yielding mutant lines and genotypes combining high spike number and seed productivity demonstrates the effectiveness of gamma irradiation for generating useful agronomic variation. Moderate doses (200 Gy) provided a favorable balance between mutagenic effectiveness and survival, whereas the highest dose produced the most promising elite lines. The selected mutants represent valuable genetic material for further evaluation in replicated and multi-environment trials and for use in wheat improvement programs.

Keywords: gamma irradiation, induced mutagenesis, winter wheat, spike number, seed yield, mutant lines, genetic variability

INTRODUCTION

Wheat (*Triticum aestivum* L.) plays the key role in global food security and is grown on more than 219 million hectares with the production of over 798 million tons per year globally (FAO STAT, 2024). It has a relatively high protein content (around 13%) and represents a dietary staple food for more than 35% of the global population, while providing around 20% of the world's protein and caloric intake (Sharma and Sharma, 2025). The biggest challenge for wheat breeders is not only to improve the yield, but also to improve the grain quality in order to reach the requirements of the consumers and the processing industry. While conventional breeding based on hybridization is time-consuming and often constrained by limited genetic variability, induced mutations provide a powerful and efficient approach for generating novel genetic variation, enabling the rapid introduction of desirable agronomic traits into well-adapted crop varieties (Abaza et al., 2020; Duveiller et al., 2007). Gamma rays are one of the most frequently used physical mutagens due to their convenience as well as its capability of penetrating deeper in the tissues (Suprasanna et al., 2015). Because of the large genome size and high genomic redundancy of the wheat, single-gene mutations are unlikely to produce observable phenotypic changes, necessitating the development of large mutant populations from polymorphic wild types to effectively capture phenotypic variation (Guo et al., 2017).

The Mutant Variety Database (FAO/IAEA-MVD), managed by the Food and Agriculture Organization of the United Nations in collaboration with the International Atomic Energy Agency, which represents a long-established repository of voluntarily submitted data on officially released mutant crop varieties, currently lists 3 502 officially released mutant crop varieties, out of which 276 are wheat mutants. This highlights the substantial contribution of mutation breeding to wheat improvement worldwide.

Various physical and chemical mutagens have been demonstrated to influence phenotypic variations in wheat (Abaza et al., 2020). In the research performed by Tarigan et al. (2023) mutant wheat plants exhibited significant morphological changes in plant height, number of productive tillers, and seed number per plant, with these traits varying depending on genotype and gamma irradiation dose. Also, Balkan (2018) reported changes in grain yield, the number of grains per spike and the grain weight per spike in wheat mutants irradiated with gamma rays. Furthermore, following gamma irradiation treatment, in the M2 generation of mutant wheat, a wide spectrum of chlorophyll mutations was observed, including complete or partial loss of chlorophyll, altered pigment distribution, and abnormal pigmentation patterns, resulting in seedlings with albino, yellow, light purple, striped, spotted, variegated, dark green, or orange-stem phenotypes (Ahumada-Flores et al., 2021). Chemical mutagenic treatment induced a range of morphological changes in wheat, including alterations in stem height (high, short, dwarf and semi-dwarf forms), stem thickness, and waxy bloom characteristics (Nazarenko, 2020), spike morphology (spikelet density and spike length), and grain characteristics (colour, size, weight, and shape) (Guo et al., 2017), awn presence and sterility (Rakszegi et al., 2010).

Despite the global importance of mutation breeding, the development and evaluation of gamma-induced wheat mutants have not yet been reported in Bosnia and Herzegovina. Therefore, the aim of this study was to evaluate the effects of gamma ray induced mutagenesis on the winter wheat (*Triticum aestivum* L.) variety “Nova Bosanka” by assessing the extent of induced genetic variability and phenotypic alterations across mutant generations. Specifically, the study focused on determining dose-dependent effects on spike number and seed yield in the M3 generation and identifying promising mutant lines as potential genetic resources for future wheat improvement programs.

MATERIAL AND METHODS

Wheat (*Triticum aestivum* L.) seeds were obtained from the Gene Bank of the Republic of Srpska, Bosnia and Herzegovina. The variety “Nova Bosanka” is winter wheat created by hybridization of parental varieties “BL 1-94” and “Proteinka” at the Department for small grains in the Agricultural Institute of Republic of Srpska, Bosnia and Herzegovina (Mandić et al., 2011).

Using the pedigree method with continuous selection of typical plants, the variety "Nova Bosanka" was selected from phenotypically homogeneous F6 generation, subsequently evaluated in preliminary and comparative trials against multiple standards, and finally registered in 2010 by the Variety Registration Commission of the Federal Institute of Agriculture (Sarajevo, Bosnia and Herzegovina). The variety is characterized by a moderately compact spike with 18–20 spikelets bearing 3–4 grains per spikelet, an erect golden-yellow spike without awns at maturity, a plant height of approximately 75 cm with medium-sized light-green leaves, and a medium-early maturity type (Mandić et al., 2011).

A total of 6 000 dry and healthy seeds were subjected to gamma irradiation to induce genetic variability. Gamma ray treatments were performed at the Plant Breeding and Genetics Laboratory in the Seibersdorf of International Atomic Energy Agency (IAEA) by using a gamma radiation source. Seeds were irradiated with three different doses in order to evaluate dose dependent mutagenic effects: 2 000 seeds were treated with a dose of 150 Gy, 2 000 seeds were treated with a dose of 200 Gy, while 2 000 seeds were exposed to a dose of 300 Gy. The selected doses were based on previous studies demonstrating their effectiveness in inducing mutations while maintaining acceptable levels of seed viability (Ahumada-Flores et al., 2021; Kiani et al., 2022; Tarigan et al., 2023).

Following irradiation, the treated seeds were sown under open field conditions to generate successive mutant generations. The M1 plants were grown and harvested, and their progeny were advanced through subsequent generations using the pedigree method. Field sowing was conducted in 2022 to obtain the M2 generation, and in 2023 for the M3 generation. Advancing generations allowed for the fixation and stabilization of induced mutations, as phenotypic variation becomes more clearly expressed and heritable from the M2 generation onward (Balkan, 2018). Throughout the advancement of generations, plants were evaluated for viability, growth performance, and visible morphological variation.

For each mutant line, phenotypic values were recorded based on five plants. Missing observations were excluded from analyses for the respective trait. The effect of gamma irradiation dose (150, 200 and 300 Gy) on the number of spikes and seed yield was evaluated using the mutant line as the experimental unit. Descriptive statistics, including mean, standard deviation (SD), coefficient of variation (CV%), minimum, and maximum values, were calculated for each irradiation treatment in order to quantify the extent of induced variability. In addition, the number of evaluated mutant lines per dose was recorded to estimate the survival percentage following irradiation.

Prior to analysis of variance, homogeneity of variances among treatments was tested using Levene's test. As this assumption was not met, Welch's one-way analysis of variance (ANOVA) was applied to assess the effect of irradiation dose on the number of spikes and seed yield. Pairwise comparisons among doses were performed using variance-robust tests (Games–Howell type) at the 0.05 probability level. Statistical significance was declared at $p \leq 0.05$. All analyses were conducted using standard statistical procedures for unbalanced designs.

The identification of superior mutant lines was based on a combined selection index integrating spike number and seed yield. It was calculated as the sum of standardized (Z) values for spike number and seed yield, where Z represents the deviation of each line from the overall mean expressed in standard deviation units. Lines with the highest index values were considered primary candidates for advancement to subsequent generations and further evaluation under replicated and multi-environment conditions, following standard mutation-breeding selection procedures recommended by FAO/IAEA guidelines (Spencer-Lopez et al., 2018). All statistical analyses were performed using R software (R Core Team, 2024).

RESULTS AND DISCUSSION

Survival of mutant lines and mutagenic effectiveness

The number of evaluated mutant lines differed among treatments due to missing or non-viable lines, which is a common consequence of irradiation damage. The reduction in the number of surviving lines at higher doses indicates increased biological injury, confirming the strong mutagenic effect of gamma radiation. Such dose-dependent reductions in survival have been widely documented in wheat and other cereals and are considered an important indicator of mutagenic efficiency (Balkan, 2018; Kaina et al., 2022; Kumar et al., 2022).

The combination of increased variability and reduced survival at 300 Gy suggests that this dose was highly effective in inducing genetic changes. However, from a breeding perspective, moderate doses such as 200 Gy may provide a more favorable balance between variability and population size, as also reported in recent studies on induced wheat mutants (Tarigan et al., 2023).

Effect of gamma irradiation dose on number of spikes

The irradiation dose had a statistically significant effect on the number of spikes per mutant line in the M3 generation. The mean number of spikes increased progressively with dose, from 24.07 at 150 Gy to 26.22 at 200 Gy and 27.80 at 300 Gy. Pairwise comparisons indicated that the difference between 150 Gy and 300 Gy was significant ($p \leq 0.05$), whereas 200 Gy did not differ significantly from either 150 or 300 Gy (Table 1).

Table 1. Descriptive statistics of number of spikes per mutant line across gamma irradiation doses in the M3 generation

Dose (Gy)	**n**	**Mean**	**SD**	**CV%**	**Min**	**Max**
150	135	24.07b	8.41	34.9	4.00	45.00
200	142	26.22ab	9.29	35.4	5.00	56.00
300	137	27.80a	11.28	40.6	3.00	53.00

Means followed by the same letter within a column are not significantly different at $p \leq 0.05$

This trend suggests a dose-dependent stimulation of tillering or spike formation in a subset of mutant lines. Similar responses have been reported in recent wheat mutagenesis studies, where moderate to high gamma doses increased variation in spike number and facilitated the identification of high-tillering mutants (Ahumada-Flores et al., 2021; Tarigan et al., 2023). Increased spike number is often associated with altered hormonal balance and changes in meristem activity induced by mutagenic treatments, leading to the expression of novel phenotypes in segregating generations.

Despite the increase in mean spike number, the coefficient of variation remained high across all treatments (34.9–40.6%), indicating substantial within-dose variability. Such high phenotypic dispersion is typical for early mutant generations and reflects the heterogeneous genetic background of induced populations (Balkan, 2018). The highest variability observed at 300 Gy confirms that higher doses generate a broader range of phenotypes, including both beneficial and deleterious mutations.

The boxplot for spike number (Figure 1) shows a gradual upward shift in the median with increasing irradiation dose, indicating a higher central tendency at 300 Gy compared to 150 Gy. The interquartile range is slightly wider at 300 Gy, reflecting increased variability among mutant lines. Several high-value outliers are present in the 200 and 300 Gy treatments, representing individual genotypes with markedly higher spike numbers. These graphical patterns are consistent with the statistical results, where a significant difference was detected between 150 and 300 Gy, confirming the stimulatory effect of higher irradiation doses on spike formation in a subset of mutants.

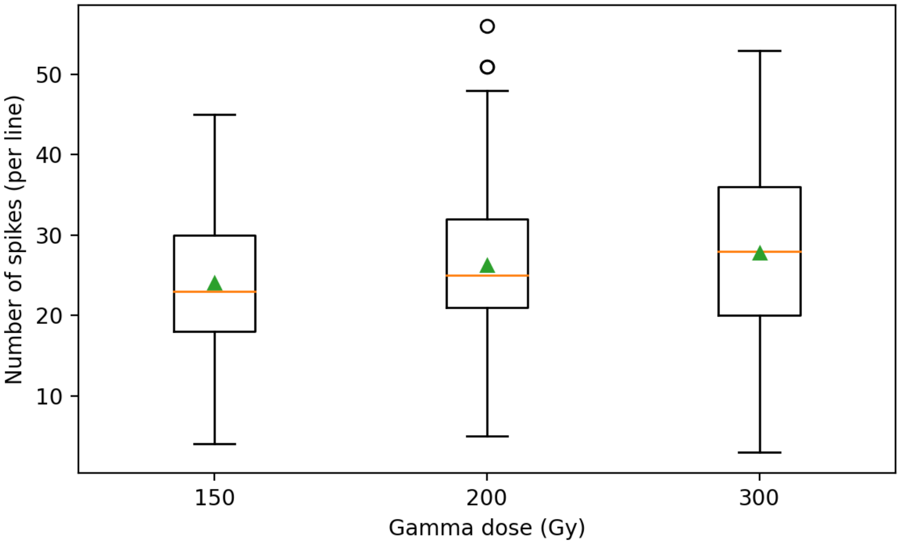

Figure 1. The effect of gamma ray dose on number of spikes

Effect of gamma irradiation dose on seed yield

In contrast to spike number, seed yield per mutant line did not differ significantly among irradiation doses. Mean yield values were relatively similar across treatments (44.45 g at 150 Gy, 47.65 g at 200 Gy, and 46.95 g at 300 Gy). However, a pronounced increase in variability was observed with increasing dose, particularly at 300 Gy, where the coefficient of variation reached 59.2% and the maximum yield value (150.99 g) greatly exceeded that recorded at lower doses Table 2).

Table 2. Descriptive statistics of seed yield per mutant line across gamma irradiation doses in the M3 generation

Dose (Gy)	n	Mean	SD	CV%	Min	Max
150	135	44.45a	15.19	34.2	11.42	76.08
200	142	47.65a	19.58	41.1	6.87	105.00
300	137	46.95a	27.78	59.2	5.98	150.99

Means followed by the same letter within a column are not significantly different at $p \leq 0.05$

The absence of a significant difference in mean yield among doses is consistent with previous reports indicating that mutagenesis often affects yield components more strongly than total yield in early generations (Ahumada-Flores et al., 2021). Yield is a complex trait controlled by multiple genes and influenced by strong environmental interactions; therefore, significant differences in mean performance are rarely detected in M2–M3 mutant populations (Tarigan et al., 2023). Instead, the primary value of mutagenesis lies in expanding phenotypic variation and enabling the identification of superior individual lines.

The markedly higher variability observed at 300 Gy suggests increased mutagenic effectiveness, resulting in a wider distribution of phenotypes and the occurrence of extreme high-yielding mutants. Similar findings have been reported in recent wheat mutation studies, where higher irradiation doses produced broader yield distributions and allowed the selection of promising lines despite reduced population means or survival rates (Arain et al., 2022; Kumar et al., 2022). This pattern reflects the balance between mutagenic effectiveness and biological damage, with higher doses generating both useful variability and increased lethality.

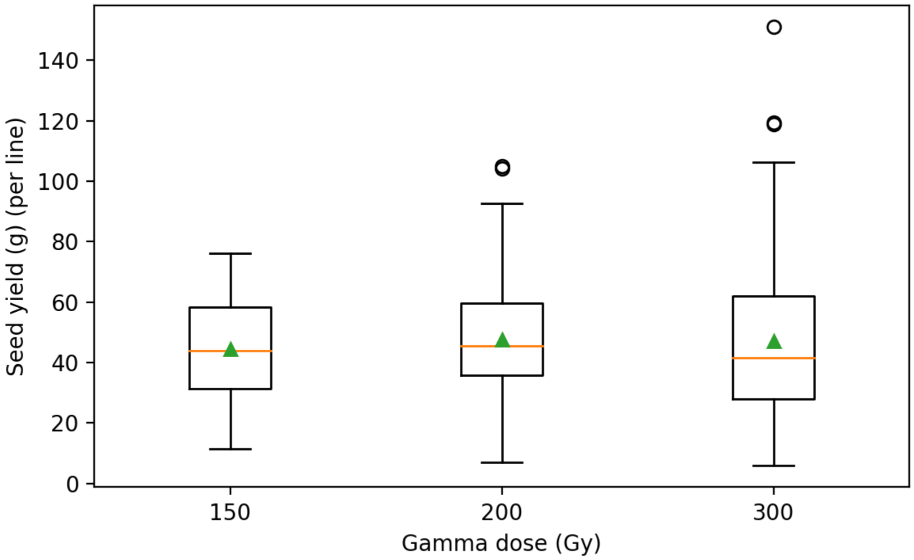

Figure 2. The effect of gamma ray dose on seed yield

The boxplot for seed yield (Figure 2) reveals similar median values across all irradiation treatments, which is in agreement with the non-significant ANOVA results. However, a pronounced increase in dispersion is observed at 300 Gy, as indicated by a wider interquartile range and the presence of extreme high-yield outliers. This suggests that higher irradiation doses did not shift the population mean but substantially increased phenotypic variability, enabling the identification of superior individual mutant lines. The presence of extreme high-value outliers at 300 Gy highlights the usefulness of higher irradiation doses for identifying elite mutant lines in early generations.

Identification of superior mutant lines using a combined selection index

A combined selection index based on standardized values of spike number and seed yield was applied to identify mutant lines with superior overall performance (Table 3). This approach enabled the simultaneous evaluation of both traits and facilitated the detection of genotypes combining high productivity with increased tillering capacity.

The highest index values were observed predominantly in the 300 Gy treatment, confirming that the highest irradiation dose generated the most extreme and potentially valuable phenotypes. Line M3-300-119 exhibited the highest selection index (6.14), characterized by a high spike number (53) and elevated seed yield (119.28 g). Similarly, line M3-300-107 showed the highest seed yield (150.99 g) combined with above-average spike production, indicating its potential as a high-yielding genotype. Other promising lines from the 300 Gy treatment included M3-300-53 and M3-300-115, both combining high spike number and yield.

Table 3. Mutant lines with the highest combined selection index based on standardized values of spike number and seed yield in the M3 generation

Line	**Dose**	**Spikes**	**Yield (g)**	**Selection index**
M3-300-119	300	53	119.28	**6.14**
M3-300-107	300	36	150.99	**5.88**
M3-300-53	300	53	103.46	**5.40**
M3-200-103	200	51	105.00	**5.27**
M3-300-115	300	52	102.74	**5.26**
M3-200-71	200	56	86.79	**4.93**
M3-200-148	200	48	104.11	**4.92**

Notably, several superior lines were also identified at the intermediate dose of 200 Gy, including M3-200-103, M3-200-71, and M3-200-148. These genotypes exhibited high spike numbers and stable yield values, suggesting that moderate irradiation doses can produce agronomically favorable mutants while maintaining higher population survival. This supports previous findings that intermediate doses often provide an optimal balance between mutagenic effectiveness and biological damage.

The predominance of elite lines in the 300 Gy treatment reflects the increased phenotypic variability observed at higher doses and highlights the value of strong mutagenic pressure for generating rare but highly productive genotypes. However, the presence of promising lines at 200 Gy indicates that useful variability can also be obtained at moderate doses, which is advantageous for breeding programs aiming to maintain larger population sizes.

From a breeding perspective, the identified lines represent primary candidates for advancement to replicated and multi-environment trials. Their superior performance in both spike number and seed yield suggests the presence of favorable genetic alterations that may contribute to yield improvement. The use of a combined selection index is consistent with mutation-breeding strategies, where simultaneous selection for multiple yield components enhances the efficiency of early-generation screening.

Identification of high-yielding mutant lines within irradiation treatments

High-yielding mutant lines were identified using a 95th percentile threshold within each irradiation treatment (Table 4). This approach enabled the detection of superior genotypes independently of differences in population size and variability among doses.

The highest seed yield was recorded for line M3-300-107 (150.99 g), followed by M3-300-119 (119.28 g) and M3-300-79 (118.94 g), all originating from the 300 Gy treatment. These values substantially exceeded the maximum yields observed at 150 Gy, confirming that the highest irradiation dose generated the most extreme phenotypes. The 300 Gy treatment also showed the greatest range of yield values, which is consistent with the elevated coefficient of variation observed for this dose.

Several high-yielding lines were also identified at the intermediate dose of 200 Gy, including M3-200-103 (105.00 g) and M3-200-148 (104.11 g). These genotypes exhibited high productivity while originating from a treatment with higher survival compared to 300 Gy, indicating that moderate irradiation doses can produce agronomically valuable mutants with reduced biological damage.

In contrast, the top-yielding lines from the 150 Gy treatment showed lower absolute yield values, with a maximum of 76.08 g. This suggests that lower irradiation doses induced less phenotypic variability and produced fewer extreme high-yielding genotypes.

The predominance of superior lines in the 300 Gy treatment reflects the strong mutagenic effect of higher irradiation doses, which increases the probability of generating rare favorable mutations. However, the identification of promising lines at 200 Gy highlights the importance of moderate doses for maintaining a larger effective population size while still producing useful variability. Similar trends have been reported in mutation breeding studies of wheat, where higher doses broaden phenotypc distributions and enable the selection of elite individuals despite reduced mean performance and survival.

Table 4. High-yielding mutant lines selected from the top 5% of each irradiation treatment

Line	Seed yield (g)	Line	Seed yield (g)	Line	Seed yield (g)
M3-150-104	76.08	M3-200-103	105.00	M3-300-107	150.99
M3-150-141	74.56	M3-200-148	104.11	M3-300-119	119.28
M3-150-9	72.63	M3-200-27	92.69	M3-300-79	118.94
M3-150-18	72.07	M3-200-18	91.59	M3-300-51	106.36
M3-150-37	69.57	M3-200-25	91.29	M3-300-53	103.46
M3-150-144	68.85	M3-200-106	88.98	M3-300-115	102.74
M3-150-33	67.58	M3-200-107	86.85	M3-300-62	102.26

From a breeding perspective, the identified high-yielding lines represent primary candidates for advancement to replicated trials. Their superior performance within treatments indicates the presence of beneficial genetic alterations that may contribute to yield improvement and can serve as valuable genetic resources for further selection and hybridization.

The induced mutant population of the winter wheat variety “Nova Bosanka” represents a valuable source of novel genetic variability for yield components and productivity. Although no significant differences in mean seed yield were detected among irradiation treatments in the M3 generation, the substantial increase in phenotypic variability, particularly at 300 Gy, enabled the identification of superior individual mutant lines, which corresponds with the results presented by Rana et al. (2024). The presence of genotypes combining high spike number and elevated seed yield indicates that gamma irradiation effectively generated useful recombinational-like variation within an otherwise genetically uniform background. This confirms that early-generation mutant populations should be evaluated primarily for variability and extreme phenotypes rather than population mean performance. Similar conclusions have been drawn in recent mutagenesis research, where the identification of superior individual lines rather than shifts in population means represents the primary outcome of early-generation evaluation (Arain et al., 2022; Rana et al., 2024).

From a practical breeding perspective, the moderate dose of 200 Gy provided a favorable balance between mutagenic effectiveness and survival, while the highest dose (300 Gy) produced the most promising elite lines despite increased biological damage. Selected high-performing mutants identified through the combined selection index will be advanced to replicated and multi-environment trials to assess yield stability and agronomic value. These lines may serve either as candidate cultivars following further selection or as donors of favorable alleles for spike productivity and yield improvement in conventional breeding programs.

Given that the study was conducted in a single environment without replications, the results should be interpreted as an assessment of induced variability rather than definitive yield improvement. Further evaluation of selected high-performing mutant lines in replicated and multi-environment trials will be necessary to confirm their agronomic potential and stability.

CONCLUSIONS

Gamma irradiation proved to be an effective tool for generating phenotypic variability in the winter wheat variety “Nova Bosanka”. A significant dose effect was observed for spike number, while seed yield showed no significant differences in mean values among treatments, which is typical for early mutant generations. However, higher irradiation doses markedly increased phenotypic variability and enabled the identification of superior individual mutant lines. The 300 Gy treatment produced the widest range of variation and the highest-yielding mutants, indicating high mutagenic effectiveness, although accompanied by reduced survival of lines. Moderate doses (200 Gy) provided a more balanced combination of variability and population size, which is advantageous for practical selection. These findings are consistent with FAO/IAEA mutation-breeding principles, where the primary objective in early generations is the creation of broad genetic diversity and the identification of promising individual genotypes rather than immediate population mean improvement.

Selected high-yielding mutant lines should be advanced to replicated and multi-environment trials to confirm their agronomic performance and stability. The identified mutants represent valuable genetic resources for future wheat breeding programs.

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