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BREAD WHEAT (*TRITICUM AESTIVUM* L.) YIELD AND QUALITY IMPROVEMENT THROUGH MUTAGENESIS

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SUMMARY

This study induced phenotypic diversity in bread wheat (*Triticum aestivum* L.) using gamma rays, EMS (ethyl methane sulfonate), and their combined application to improve grain yield and end-use quality. Healthy seeds of three well-adapted cultivars received treatments with the mutagens and advanced through four generations with standard agronomic practices. The M₁ and M₂ generations exhibited reduced germination, stunted growth, and altered spike morphology, confirming the effectiveness of mutagenic treatments. Based on phenotypic evaluation, the selection of 30 promising mutant lines ensued in the M₃ generation for detailed characterization. Among these, mutant line SE4/12-1 exhibited the shortest plant height, SG2/12-26 had the highest numbers of tillers per plant, and SE5/12-7 showed the longest spike length. Mutant lines SG4/12-35 and SE2/12-29 appeared with the highest number of spikelets per spike, whereas SE5/12-15 produced the highest number of grains per head spike. SE5/12-13 recorded the maximum head spike yield. Considerable variation was also evident in grain quality traits, with grain hardness index, seed diameter, moisture content, and gluten content. The highest Zeleny sedimentation value (87.3 ml) resulted in the mutant line SG2/12-27, and the mutant line SE4/12-5 displayed the highest falling number (729 sec). Overall, the selected mutant lines provide valuable genetic resources for breeding high-yielding wheat varieties with improved grain and processing quality.

Keywords: Bread wheat (*T. aestivum* L.), gamma rays, ethyl methane sulfonate, ideotypes, grain texture, falling numbers, gluten content, Zeleny sedimentation value

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Key findings: Gamma rays, EMS, and their combined treatments generated substantial phenotypic diversity for the development of new ideotypes in bread wheat (*T. aestivum* L.). Promising wheat lines showed desirable agronomic traits, and these useful genetic resources can be beneficial in developing high-yielding wheat cultivars with improved quality.

INTRODUCTION

Global climate change continues to threaten food security worldwide by increasing the frequency and intensity of heat waves, drought, and extreme weather events. Recent estimates indicate that drought alone can reduce global crop production by approximately 10.1% in rainfed systems and 6.8% in irrigated systems (Tuninetti and Davis, 2026), highlighting the urgent need to develop climate-resilient crop varieties through induction of new genetic diversity.

Bread wheat (*Triticum aestivum* L.) is a staple food crop, contributing over 20% of global protein and calorie intake. In 2022–2023, global wheat production reached 765.3 MMT (Sendhil *et al.*, 2023). In Pakistan, hexaploid wheat accounts for 95% of cultivated area, contributing 8.7% to agricultural value and 1.7% to GDP, with an average yield of 2.8 t/ha (Zawar *et al.*, 2024). The common yield of wheat is pretty low due to drastic changes in climatic conditions, and its consumption is also increasing for the growing population. Hence, the improvement and genetic manipulation are still the best tools to increase production.

Breeders primarily rely on existing related cultivars as parents by hybridization to develop desirable traits, limiting genetic diversity and leading to genetic erosion over time. This restricts the adaptability of modern wheat genotypes under climate stress. Therefore, as a method of introducing new alleles for crop improvement, inducing new genetic variation through physical and chemical mutagens promises to be an important approach for sustainable agriculture (Bibi and Arshad, 2020). Gamma rays are applicable to induce breaks in DNA strands, whereas EMS is commonly effective to introduce point mutations (Wang *et al.*, 2022). Their combined use enhances phenotypic diversity, aiding the development of new ideotypes and resistant varieties.

It has been a widely used technique to alter plant architecture, flowering time, stress tolerance, and grain nutritional content (Kumar *et al.*, 2022; Lethin *et al.*, 2022; Raina *et al.*, 2023; Rana *et al.*, 2024). Notably, mutagenesis led to the release of high-yielding crops like the cotton variety 'NIAB-78' and six wheat varieties in Pakistan (Sarsu *et al.*, 2020). Mutants with altered glutenin and gliadin profiles have been successful in their identification, offering improved baking quality (Saripalli *et al.*, 2024). Chen *et al.* (2022) generated novel germplasm relevant for grain yield and protein content. EMS mutagenesis has also succeeded in its employment to increase micronutrient content (Zn and Fe), addressing hidden hunger in developing nations (Singh, 2023).

However, using induced mutagenesis has caused the generation of genetic variability in wheat; comprehensive evaluation of advanced mutant lines for grain quality characteristics is so far insufficient. In particular, the combined assessment of key grain quality traits, including grain hardness, gluten content, Zeleny sedimentation value, and falling number, has limited exploration. Therefore, the presented study aimed to characterize the advanced wheat mutant lines, as well as end-use quality traits, and identify viable mutant lines with improved yield and flour quality for future wheat breeding programs.

MATERIALS AND METHODS

Study setting

This study commenced at the Nuclear Institute of Agriculture (NIA), Tando Jam, Sindh, and Nuclear Institute for Agriculture and Biology (NIAB), Punjab, Pakistan. This study received acceptance from the Research and Ethical Committee of the University of Sindh,

Jamshoro, and NIA, Tando Jam, Pakistan, to generate the genetic variation in wheat, with the resulting material suitable for use in future wheat varieties.

Plant material and variant identification

Three wheat (*T. aestivum* L.) cultivars named Sarsabz, Kiran-95, and TD1 were the selected materials for induced genetic variation using gamma rays, EMS, and combined mutagens following Abaza *et al.* (2020). A total of 500 seeds (14% moisture) per treatment (sourced from NIA, Tando Jam, and Agriculture Research Institute, Tando Jam, Pakistan) sustained exposure to irradiation doses of 50, 100, 150, 200, 250, and 300 Gy. The seeds' irradiation was in a 137 Cesium source (Nigo 5, Belgaria) with a 30.86 Gy/minute dose rate from the Nuclear Institute of Medical and Radiotherapy (NIMRA), Jamshoro, at room temperature (22 °C–25 °C). Non-irradiated seeds of each variety served as the control.

The 500 presoaked seeds had their immediate transfer to a freshly prepared aqueous solution of EMS (0.4%, 0.8%, 1.2%, 1.6%, and 2.0%) at 25 °C ± 2 °C with shaking. Thereafter, seeds received thorough rinsing with tap water for two hours to remove excess mutagens, and afterward, drying of seeds continued on blotting paper. Non-treated seeds of each variety became specimens for control.

In the combined treatment, the 500 seeds of each variety obtained initial exposure to gamma rays before soaking in the respective concentrations of EMS as mentioned above for eight hours. Thereafter, seeds incurred thorough rinsing with tap water for two hours to remove excess mutagens before being dried on blotting paper. The control was the non-treated seeds of each variety.

Sowing of seeds used a randomized complete block design (RCBD) (10 cm intra-row, 30 cm inter-row spacing) with three replications per treatment. The M₁ generation received a basal fertilizer dose (20:40:40 kg/ha as N:P₂O₅:K₂O) and standard agronomic practices. M₂ seeds entailed harvesting from 256–780 M₁ plants before growing in RCBD with three replications (2 m rows spaced 30 cm apart and 10 cm between plants). From this,

510 high-yielding M₂ plants attained successful selection for M₃.

M₃ progenies underwent evaluation in a compact family block (CFB) design with treatments as main plots and progenies as subplots (progeny sown in 3 m rows) using standard production practices. High-yielding homozygous lines succeeded in their selection for carrying out to M₄ to M₆ generations. Slight differences in rainfall and temperature conditions were notable between generations, with generally moderate precipitation (30–70 mm) and temperatures between 5 °C and 27 °C day per night. Ten random plants per line reached assessment for phenotypic traits. These were plant height, the number of tillers per plant, spike length, the number of spikelets per spike, the number of grains per head spike, head spike yield, 50% DH, and 50% DM. Likewise, their assessment for end-use grain quality traits comprised seed diameter, moisture content, hardness index, gluten content, Zeleny sedimentation value, and falling number. Changes in mean and variance entailed a comparison between mutant lines and control populations.

Grain quality assessment

Grain hardness of 30 advanced mutant and parental lines incurred analysis using the single kernel characterization (SKCS) 4100 system at 11%–13% moisture. The SKCS provided average hardness, standard deviation, and distribution across eight classes, from extra soft (< 10) to extra hard (> 90). Seed diameter, moisture content, gluten content, falling number, and Zeleny sedimentation value measurements were according to AACC (American Association of Cereal Chemists, 2000) guidelines using standard grain analyzers and milling equipment (Perten, 3100).

Statistical analysis

All experimental data first underwent examination for compliance with the assumptions of analysis of variance, including normality of residuals and homogeneity of variances. The data presentation was as mean

± standard error (SE). Differences among mutant lines, upon evaluation, used the one-way analysis of variance (ANOVA). When the ANOVA indicated significant treatment effects, mean separation continued using the Least Significant Difference (LSD) test at a 5% probability level ($p < 0.05$) using Statistics X software. The further investigation of the genetic diversity and relationships among the mutants based on morphological and grain quality traits, performing the principal component analysis (PCA) and correlation analysis, used Minitab 19. PCA sought to identify the major sources of variation and trait contributions according to their overall phenotypic similarity.

RESULTS

Evaluation of advanced mutant lines

Immediate effects of mutagens received assessment by the seedlings' damage (Figure S1), and M_2 generation showed variations in growth pattern, leaf, spike morphology, and heading traits of the bread wheat (*T. aestivum* L.) (Figure S2). The germination percentage for gamma rays, EMS, and combined treatments ranged from 42% to 100%, 34% to 100%, and 12% to 99%, respectively. In total, 510 promising M_2 mutant lines reached selection (Figure S3). Among these, obtaining 30 stable M_3 mutant lines (~5.88%) (Figure 1) advanced to M_4 for homozygosity confirmation and further stabilized in M_5 and M_6 generations.

Analysis of variance revealed significant ($p < 0.05$) variations among the advanced wheat mutant lines for all yield and yield-related traits, confirming the efficiency of induced mutagenesis in producing considerable phenotypic diversity (Table 1). The observed variation is due to the induction of new genetic changes that can be favorable to improve plant architecture and yield potential.

Plant height ranged from 51.5 to 103.9 cm. The study identified two dwarf (≤ 60 cm), 26 semi-dwarf (60–86 cm), and two tall (≥ 100 cm) mutant lines. The semi-dwarf phenotype is especially useful in wheat breeding because it has an association with

improved lodging resistance and improved partitioning of assimilates to grain production. The tallest mutant lines, TGE4/12-2 (103.9 cm) and TGE4/12-1 (103.2 cm), contrasted with the dwarf mutant SE4/12-1 (51.5 cm), demonstrating the broad spectrum of mutation-induced variation.

Several mutant lines, SG2/12-26, SG3/12-20, SE5/12-15, and SE5/12-8, produced more tillers per plant than their respective parents, indicating increased sink capacity and potential for elevated spike production. Likewise, mutant lines with longer peduncle length and spike length, particularly SE5/12-7, SE4/12-4, and SE4/12-3, could have better assimilate translocation and spike development, which leads to better grain formation. The increased number of spikelets per spike in mutant lines, such as SG4/12-35 (25.7) and SE2/12-29 (25.7), also indicates enhanced potential to produce fertile florets—an important component of grain yield.

The significant performance in the number of grains per head spike and head spike yield was evident in a small group of elite mutant lines. The mutant line SE5/12-15 recorded the highest number of grains per head spike (95.7 g). The maximum head spike yield resulted from SE5/12-13 (4 g), followed by SE5/12-15 (3.57 g), SG4/12-35 (3.40 g), and SE2/12-29 (3.37 g). Days to 50% heading ranged from 68 days to 88 days, with the earliest heading recorded in the mutant line TGE4/12-2. Days to 50% maturity varied from 100 days to 115 days, with the earliest maturity observed in mutant lines SG3/12-25 and SG1/12-38. The concurrent improvement of multiple yield components in these mutant lines indicates that mutagenesis successfully generated favorable genetic combinations, influencing source-sink relationships and assimilate allocation. These elite lines represent promising genetic resources for wheat breeding programs focused on developing high-yielding cultivars with improved agronomic performance.

Multivariate analysis of agronomical traits

Principal component analysis (PCA) resulted in nine components, with PC1 and PC2 explaining



Figure 1. Phenotypic variation of advanced wheat mutant lines in M4 generation, *1-25 show variation in height, plant, and the number of tillers; 25a, b, and c show a compact spike mutant with 10 cm of spike length; 25e is a bold seeded mutant line; 26-30 show variations in spike morphology, the number of spikelets per spike, the number of grains per spikelets, and Amber spike and grain color.

Table 1. Comparative performance of advanced wheat mutant lines for yield and yield-related traits.

Mutants	PH (cm)	NT per plant	PL (cm)	SL (cm)	Spikelets per Spike	NG per HS	HSY (g)	50% DH	50% DM
SE4/12-1	51.53±4.23	9.33±1.5	12.67±3.1	15.17±1.8	23±2.0	83.67±7.1	2.67±0.4	88.33±3.51	114.7±3.06
SE4/12-2	60.60±3.92	8.33±1.5	8.67±1.15	13.2±1.0	21±1.0	65±7	2.87±0.3	75.33±4.5	110.0±14.0
SE4/12-3	60.40±7.19	10.67±2.08	22.1±3.85	15.4±1.4	21.33±1.5	76.7±7.6	3.2±0.2	76.33±4.5	105.7±10.5
SE4/12-4	60.93±2.51	10±2.00	15.0±4.0	15.53±1.55	22.7±2.5	68.67±4.0	2.5±0.3	82.33±10.0	103.0±7.6
SE4/12-5	58±6.32	10±2.00	9.67±2.52	14.70±1.87	22.3±1.2	62.67±2.5	2.57±0.4	73.67±1.5	107±8.5
SE4/12-6	60.23±2.97	8.67±0.6	13.67±4.5	13.9±1.7	21±1.0	76.7±3.5	3.0±0.2	78.0±7.2	103.1±7.7
SE5/12-7	63.17±6.33	10.33±0.6	13.33±1.76	16.0±2.0	23.3±1.5	83.3±7.6	3.0±0.2	71.33±3.1	105.3±11.0
SE5/12-8	64.40±4.75	11±2.6	17.0±3.6	14.0±1.5	23.3±1.5	92.67±7.0	3.30±0.3	76.0±5.0	105.2±10.1
SE5/12-9	73.53±9.29	8.33±0.58	13.67±2.52	12.83±1.4	23±1.0	83.3±8.1	2.73±0.4	85.0±5.57	104.78±7.8
SE5/12-10	85.1±10.5	8.00±2.6	18.53±2.25	13.37±0.8	20.67±1.5	73.7±10.9	2.53±0.5	76.0±4.0	113.3±13.6
TGE4/12-1	103.23±7.31	7±1.0	17.67±3.06	11.70±0.8	25±2.0	74±8.7	3.20±0.2	74.0±4.0	101.67±5.77
TGE4/12-2	103.87±7.96	6.33±1.53	17.0±2.0	11.30±1.13	24.67±1.5	65.67±6.0	2.73±0.3	68.0±4.0	103.3±11.4
SE5/12-12	75.05±2.95	9±1.0	11.33±3.06	13.67±0.4	21.67±1.5	81.3±3.2	2.63±0.2	80.0±9.17	106.4±11.1
SE5/12-13	83.8±3.2	10±1.0	18.03±2.30	14.90±1.28	25±1.0	92.67±13.0	3.63±0.25	71.33±4.16	102.0±7.55
SE5/12-15	89.20±6.8	11±2.0	20.0±2.0	13.90±1.6	24.33±1.2	95.67±17.8	3.57±0.5	74.33±5.5	104.33±8.3
SE5/12-17	78.15±1.65	8.33±1.5	9.33±2.08	12.17±1.3	20.67±1.5	86.33±15.3	3.17±0.6	75.33±7.5	102.33±7.64
SE5/12-19	87.5±2.5	8.67±1.5	10.0±2.0	14.33±1.2	24±1.0	84±10.58	3.10±0.5	75±3.6	108.2±9.46
SG3/12-20	85.10±2.1	11.33±3.5	18.33±1.76	12.33±1.5	22±2.6	73±7.2	2.93±0.3	84±8.0	102.7±11.4
SG3/12-21	80.50±4.5	7.0±1.0	17.63±19	13.47±0.5	20.67±2.1	69.67±7.0	2.67±0.42	69.67±1.5	108.0±14.4
SG3/12-23	94.30±6.8	9.67±2.5	16.33±3.06	12.40±0.6	23.33±2.1	65.67±10.0	2.63±0.4	69.33±5.8	103.33±8.3
SG3/12-25	96.85±4.6	8±1.0	15.2±4.25	14.53±1.0	21.33±1.5	76±13.4	3.0±0.5	71.0±5.57	100.0±8.2
SG2/12-26	86.55±1.6	13.33±2.5	14.83±5.3	12.33±0.58	21.33±2.5	73±8.0	2.80±0.3	74.0±6.0	101±7.0
SG2/12-27	89.90±4.0	6±1.0	10.83±3.3	12.63±1.1	23±2.0	81±9.2	2.67±0.25	75.33±9.0	105.0±10.0
SE2/12-29	81.73±3.6	7.0±1.0	15.3±5.03	15±1.1	25.67±1.15	84±10.6	3.37±0.3	76.67±4.2	107.0±8.3
SG4/12-35	91.87±4.0	7.33±1.5	15.3±4.16	14.67±0.6	25.67±0.58	76.7±5.0	3.40±0.2	75.33±0.58	101.0±3.6
SG1/12-38	85.67±10.6	8.33±1.5	17.67±3.51	14.90±1.6	24±1.0	87±5.3	3.1±0.3	74.33±3.5	100±6.0
SG1/12-41	81.27±4.6	9.67±1.53	13.3±4.16	15.13±0.8	21.67±3.1	69.33±3.1	2.90±0.17	74.0±10.0	101.33±8.7
SG1/12-43	85.80±5.8	11±2.0	22.0±2.0	15±1.0	21.33±1.5	72±9.17	2.90±0.5	73.67±6.03	101.67±8.0
KGE7/12-44	81.50±7.5	8.0±2.0	14.3±3.06	13.07±0.5	23.3±2.5	87.67±3.1	3.27±0.2	73.33±10.1	103.67±8.1
SGE6/12-9	83.50±4.5	7.33±1.5	15.67±3.1	12.03±1.7	21.33±0.6	68.67±6.7	2.87±0.3	78±12.5	105.33±10.3
Sarsabz	101.83±3.2	4.0±1.0	14.7±0.58	12.67±0.6	21.33±0.6	57±4.0 ^j	2.60±0.10	75.33±0.58	100.0±1.0
Kiran-95	103.10±7.39	5±1.0	12.33±0.58	12.47±0.9	20±0.00	58±2.0 ^j	2.60±0.17	72.67±1.15	100.33±0.58
TD-1	79.10±7.40	6.33±1.5	9.33±1.15	10.67±0.6	15.33±1.15	63.33±3.1	2.87±0.15	73.33±1.15	99.33±1.15
Grand Mean	80.828	8.616	14.873	13.615	22.374	76	2.938	75.455	104.26
LSD 5%	9.12	2.705	4.889	1.734	2.259	11.58	0.05	6.490	7.632
C.V.	6.92	19.25	20.15	7.81	6.19	9.34	10.13	5.27	4.49

**PH, Plant height; NT per Plant, Number of tillers per plant; PL, Peduncle length; SL, Spike length; NG per HS, Number of grains per Head spike; HSY, Head spike Yield; 50% DH, Days to 50% heading; and 50% DM, Days to 50% maturity.

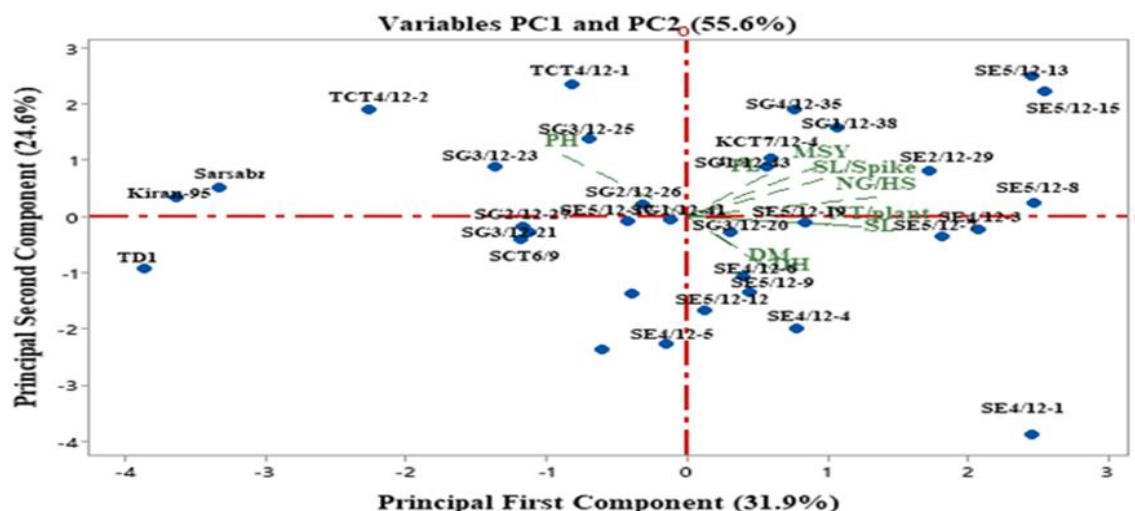


Figure 2. Classification of wheat mutants based on yield and its associated traits through PCA.

Table 2. Correlations among yield and its associated traits among advanced wheat mutant lines.

Variables	PH	NT per plant	PL	SL	Spikelets per Spike	NG per HS	HSY	50% DH
NT per plant	-0.456							
PL	0.230	0.300						
SL	-0.494	0.411	0.203					
Spikelets per Spike	0.149	0.129	0.295	0.364				
NG per HS	-0.214	0.380	0.178	0.367	0.502			
HSY	0.060	0.221	0.300	0.235	0.460	0.689		
50% DH	-0.491	0.180	-0.110	0.146	-0.004	0.182	-0.174	
50% DM	-0.514	0.102	-0.115	0.233	0.096	0.172	-0.218	0.366

**PH, Plant height; NT per plant, Number of tillers per plant; PL, Peduncle length; SL, Spike length; NG per HS, Number of grains per Head spike; HSY, Head spike yield; 50% DH, Days to 50% heading; and 50% DM, Days to 50% maturity.

31.9% and 25% of the genetic variation, respectively, and together accounted for a combined 56.5%. The biplot analysis (Figure 2) proceeded to assess the relationships between variables among 30 wheat mutant lines. It revealed that the positive components on both axes contained high-yielding mutant lines, i.e., SE5/12-13, SE5/12-15, SG4/12-35, SG1/12-38, KCT7/12-4, SG1/12-43, SE2/12-29, SE5/12-8, SE4/12-3, SE5/12-19, and SG1/12-41. The genotypes selected from components A and C may be beneficial for trait improvement in future breeding programs.

Correlation of agronomic traits

The correlation between yield and yield-related traits appears in Table 2. Plant height exhibited

a highly significant and positive association with peduncle length, the number of spikelets per spike, and the number of grains per head spike. Inversely, it displayed a negative association with the number of tillers per plant, spike length, the number of grains per head spike, days to 50% heading, and days to 50% maturity. The number of tillers per plant had a positive correlation with all attributes, except plant height, whereas a similar trend resulted in spike length. Peduncle length was with a negative correlation with days to 50% heading and days to 50% maturity. All traits, except days to 50% heading, expressed a positive association with the number of spikelets per spike. The number of grains per head spike negatively correlated with plant height only, while head spike yield showed a negative

association with days to 50% heading and days to 50% maturity. Days to 50% heading positively correlated with the number of tillers per plant, spike length, the number of grains per head spike, and days to 50% maturity.

Assessment of milling and baking traits

Comparative evaluation of the 30 advanced wheat mutants and their parental cultivars showed significant improvements in several milling and baking quality traits (Table 3), which indicates the effectiveness of induced mutagenesis in broadening genetic variability for end-use quality. Desirable mutants with improved seed size for enhancing milling efficiency and flour recovery, representing a favorable change in grain development, are the best selections.

Results indicated the seed diameter of evaluated genotypes ranged from 2.51 to 2.86 mm, with the boldest grains observed in the mutant SE4/12-5. Moisture content varied from 10.4% to 11.7%, and protein content ranged from 15.53% to 16.33%. Mutant lines SE5/12-8, TGE4/12-2, and SG2/12-27 showed the highest protein content, suggesting their potential for enhanced nutritional and processing quality. Variation among grain

texture was also evident, with the highest grain hardness index exhibited by mutant lines SE5/12-19 and SGE6/12-9. Meanwhile, the lowest hardness index was notable with the parental cultivar TD-1.

The gluten content ranged from 37% to 45% with the mutant line SG2/12-27, and Kiran-95 showed the highest gluten content. Similarly, the maximum Zeleny sedimentation value (87.3 mL) resulted in mutant lines TGE4/12-2 and SG2/12-27 and was lowest in TD-1 (82.3). The falling number ranged from 425 to 729 seconds, with the mutant line SE4/12-5 exhibiting the highest number, indicating lower α -amylase activity and better resistance to pre-harvest sprouting.

Principal component analysis (PCA) explained seven components, where PC1 and PC2 accounted for 32.1% and 23.5% of the genetic variation, respectively, jointly explaining 55.6% of the total variability in grain quality traits (Table 3). Each variable's distance from its component indicated its contribution to mutant line distinctness. The biplot analysis based on the first two PCs (Figure 3) was effective in highlighting mutants for the relationships between the variables. It was notable that both positive components with better milling quality contained mutants

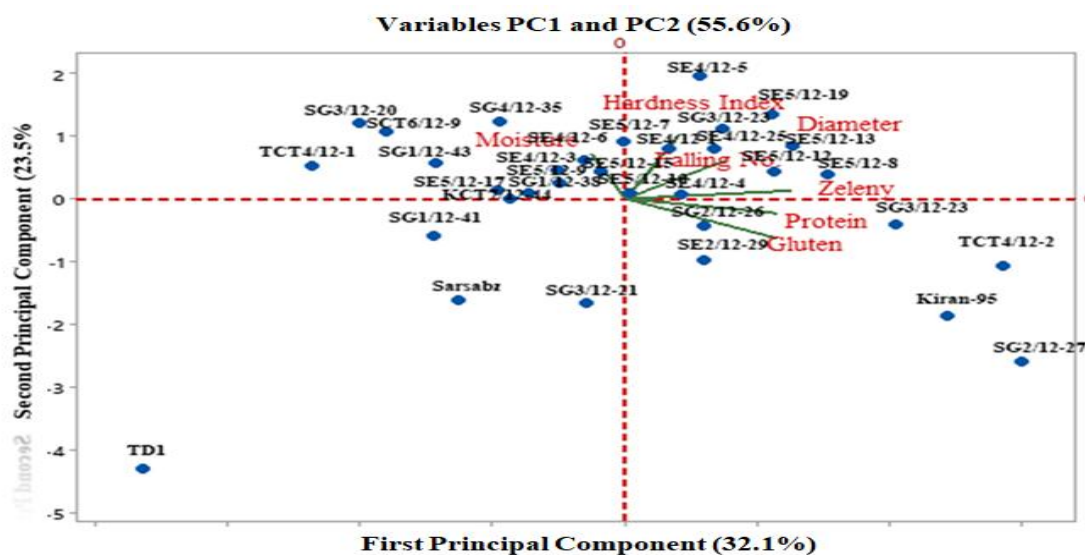


Figure 3. Classification of advanced wheat mutants based on grain milling quality and its associated traits through PCA.

Table 3. Comparative performances of advanced wheat mutant lines and parent varieties for milling quality characteristics, using LSD and P values (≤ 0.05 are significant).

Mutant	Seed Diameter	Moisture Content	Protein Content	Grain Hardness Index	Gluten Content	Zeleny Sedimentation Value	Falling Number
SE4/12-1	2.8	10.90	16.10	83.3	38.0	85.0	588.0
SE4/12-2	2.77	11.00	16.13	85.3	39.0	85.3	575.0 ^j
SE4/12-3	2.77	10.80	15.9	82.0	37.3	85.0	554.0
SE4/12-4	2.73	10.93	16.13	82.0	38.0	86.0	516.0
SE4/12-5	2.86	11.00	16.0	82.0	38.0	85.3	729.0
SE4/12-6	2.81	11.20	16.13	79.0	38.0	84.0	562.0
SE5/12-7	2.81	11.10	15.83	80.0	38.0	86.3	562.0
SE5/12-8	2.72	11.40	16.3	84.0	41.0	86.3	520.0
SE5/12-9	2.80	11.10	15.93	78.0	38.0	85.0	516.0
SE5/12-10	2.65	11.20	16.2	84.0	40.3	84.0	561.0
TGE4/12-1	2.72	11.30	15.53	78.0	37.3	84.3	498.0
TGE4/12-2	2.82	11.00	16.3	75.0	44.0	87.0	569.0
SE5/12-12	2.78	11.40	16.27	77.3	39.3	86.3	560.0
SE5/12-13	2.80	11.20	16.17	81.0	40.0	86.0	614.0
SE5/12-15	2.67	11.13	16.13	82.0	38.0	85.0	575.0
SE5/12-17	2.5733	11.50	16.13	81.0	39.0	84.0	541.0
SE5/12-19	2.61	11.20	16.17	89.0	40.0	86.3	671.0
SG3/12-20	2.60	11.43	15.8	84.0	37.0	84.0	572.0
SG3/12-21	2.64	11.10	15.9	71.0	40.3	86.3	473.0
SG3/12-23	2.78	10.70	16.2	76.0	42.3	86.0	717.0
SG3/12-25	2.69	11.70	16.1	83.0	41.0	86.0	581.0
SG2/12-26	2.57	11.20	16.0	80.0	42.0	86.3	597.0
SG2/12-27	2.75	10.70	16.333	77.0	45.0	87.3	425.0
SE2/12-29	2.72	10.93	16.0	78.0	42.0	85.3	532.0
SG4/12-35	2.76	11.30	15.8	81.0	39.0	84.0	627.0
SG1/12-38	2.58	11.30	15.73	81.3	39.7	86.3	527.0
SG1/12-41	2.58	11.20	15.93	76.0	39.0	84.0	563.0
SG1/12-43	2.65	11.10	15.83	83.0	38.0	84.0	572.0
KGE7/12-44	2.76	10.90	15.8	78.3	39.3	84.0	585.0
SGE6/12-9	2.72	10.90	15.7	88.0	37.3	83.3	545.0
Sarsabz	2.51	10.73	15.83	76.0	39.3	85.0	547.0
Kiran-95	2.68	10.40	16.2	82.0	45.0	86.0	584.0
TD-1	2.44	10.7	16.0	56.0	38.3	81.0	542.7
Grand Mean	2.70	11.08	16.02	79.81	39.61	85.16	568.55
C.V.	1.32	2.18	0.90	1.64	2.70	1.61	0.69
LSD 5%	0.1	0.394	0.235	2.136	1.742	2.2405	6.367

SE4/12-5, SE5/12-19, SG3/12-23, SE5/12-7, SE4/12-1, SE4/12-25, SE5/12-13, SE5/12-12, SE5/12-8, SE4/12-4, and SE5/12-38. Some selected genotypes from components A and C showed potential for use in breeding programs to improve desirable traits.

Correlation analysis of grain quality traits revealed the seed diameter had a high positive correlation with protein content, grain hardness index, gluten content, Zeleny sedimentation value, and falling number. However, it was in a negative correlation with

moisture content (Table 4). Gluten content displayed a positive correlation with all traits, except moisture content and falling number. The falling number showed a negative correlation with moisture content, gluten content, and Zeleny sedimentation value. The grain hardness index expressed a positive correlation with all traits, except gluten content. Protein content showed a negative correlation only with moisture content. The value of Zeleny sedimentation exhibited a negative association with falling number.

Table 4. Correlations among grain milling quality traits among advanced wheat mutant lines

Variables	Seed diameter	Moisture content	Protein content	Hardness index	Gluten content	Zeleny sedimentation value
Moisture content	-0.057					
Protein content	0.212	-0.082				
Hardness index	0.323	0.293	0.023			
Gluten content	0.022	-0.296	0.578	-0.113		
Zeleny sedimentation value	0.379	0.033	0.442	0.301	0.580	
Falling number	0.210	-0.019	0.109	0.244	-0.056	-0.018

DISCUSSION

Induced mutagenesis is a well-established strategy to broaden the genetic base of self-pollinated crops, such as wheat (*T. aestivum* L.), through the generation of new allelic variation often absent from the natural germplasm. Physical mutagens, such as gamma rays, tend to cause chromosomal rearrangements and DNA double-strand breaks, while chemical mutagens, viz., EMS, tend to cause point mutations through G/C to A/T transitions. The use of these mutagens simultaneously increases the frequency and spectrum of mutations and therefore raises the chances of recovering superior genotypes with desirable agronomic and quality traits.

In the presented study, gamma rays, EMS, and their combination effectively produced significant phenotypic heterogeneity among three wheat cultivars in the current investigation (Figure 1, S2, and S3). In wheat mutation breeding experiments, Sarsabz's higher responsiveness in comparison to TD-1 indicates that mutation efficiency had a strong influence from the genetic background. DNA repair capacity and mutagen sensitivity of individual cultivars had previous reports stated in wheat mutation breeding studies (Wang *et al.*, 2022; Raina *et al.*, 2023).

The reduction in seed germination following mutagenic treatments reflects the physiological damage caused by induced DNA damage. High doses of mutagens disrupt normal cell division, affect enzyme activity, and reduce embryo viability before DNA repair mechanisms can restore the integrity of the genome. The promising study has shown an

inverse relationship between mutagen dose and germination. This observation is therefore

consistent with the classical principle of mutation breeding that increasing mutagen intensity boosts the mutation frequency but simultaneously reduces biological efficiency due to greater cellular injury (Kumar *et al.*, 2022). Therefore, the optimum dose of mutagen is still important to maximize useful genetic variability and, at the same time, to ensure the survival of the plant.

The broad spectrum of morphological variants identified, including dwarf, semi-dwarf, and tall plants, altered spike architecture, bold grains, chlorophyll-deficient seedlings, and sterile mutants (Figures 1 and S2), demonstrates induced mutations affected multiple developmental pathways controlling plant architecture and reproductive growth. Such diversity is to be the expected mutagens that cause random changes in the genome, which may influence regulatory genes, transcription factors, hormone biosynthesis, and signaling pathways controlling plant development. In particular, variation in plant height probably reflects modifications in genes regulating gibberellins biosynthesis or signaling, whereas changes in spike morphology could result from mutations altering meristem identity and spikelet development. The predominance of semi-dwarf mutants is important as reduced plant height generally improves lodging resistance (Sao *et al.*, 2022). Visible phenotypes are reliable indicators of successful mutagenesis, which have had reports as variation in wheat morphology by Thomas (2017), Gatta *et al.*

(2021), Kumar *et al.* (2022), and Chen *et al.* (2024).

Interestingly, the double head spike had the same grain number per head spike as its single spikes (Figure S2). Genetically, stability of all the induced phenotypes across generations did not succeed, except in selected mutant lines in the M₃ generation. The disappearance of the double head spike mutant line in subsequent generations suggests that this phenotype had the control of recessive alleles, influenced by epigenetic modifications, or resulted from chimeric tissues produced during the early stage of mutagenesis. Such instability is typical for mutation breeding since many mutations in the early generation segregate before homozygosity is achieved. Thus, the stabilization of desirable mutants in later generations (M₄) is a critical step to distinguish heritable genetic changes from transient developmental abnormalities. Reports on similar observations have emerged in wheat and other cereals where many M₂ mutants fail to stabilize due to segregation, pleiotropic effects, or reduced reproductive fitness (Kumar *et al.*, 2022; Chen *et al.*, 2024).

The high variability among selected mutant lines for quantitative agronomic traits further confirms that mutagenesis had effectively generated new allelic combinations, influencing yield and yield-related characteristics (Rana *et al.*, 2024). These characteristics indicate source-sink relations and partitioning of assimilate during reproductive development for flour production. Improvement of photosynthates partitioning to developing grains increased harvest index and head spike yield (Thomas, 2017; Gatta *et al.*, 2021). These findings were consistent with the mutation breeding, where small genetic variations accumulated at multiple loci can result in larger improvement in complex polygenic traits.

Principal component analysis further confirmed that induced mutants possessed broad multivariate genetic diversity, indicating that mutagenesis altered multiple traits simultaneously rather than producing isolated phenotypic changes. Similar applications of multivariate analyses have been successfully useful to characterize mutant populations and

identify elite breeding materials in wheat and other cereals.

These results also show induced mutagenesis can enhance end-use quality in addition to agronomic performance, as evidenced by significant improvements in grain quality traits (Table 3). The moisture content, an essential factor in grain milling and storage stability, ranged from 11% to 13%, similar to findings by Mutwali *et al.* (2018). Lower moisture content increases storage time and reduces spoilage risks (Kubik *et al.*, 2021). The grain hardness index, an important trait for milling and marketing, was medium to strong among mutant lines, and the mutant line SE5/12-19 exhibited an ultra-hard texture. The increased grain protein content in several mutant lines is probably due to the improved nitrogen uptake, remobilization efficiency, and storage protein biosynthesis during grain filling. Similarly, higher grain hardness has a linkage to alterations in endosperm texture, which may be in relation to genes controlling starch-protein interactions, such as those in the puroindoline-associated pathways, influencing the milling properties and flour particle characteristics (Singh *et al.*, 2024).

The gluten content of the mutant lines ranged from 37 to 45, sufficient for good baking performance. Similarly, Rafiq *et al.* (2023) reported an increase in wet gluten in wheat varieties across two consecutive years. The Zeleny sedimentation value, which is a key trait of baking quality, ranged from 82 to 87 in the latest study. Similar findings have been in previous reports from Akkaya *et al.* (2018) and Brankovic *et al.* (2021). The improvements in gluten content and Zeleny sedimentation values indicate favorable modifications in gluten, protein composition, and polymerization, leading to stronger dough, increased gas retention, and improved bread-making quality.

The selected mutant lines consistently showed high falling number values (> 425 sec), indicating the reduced α -amylase activity and better resistance to pre-harvest sprouting, and thereby, preserving starch integrity and flour quality. The mutants identified in this study exhibited desirable traits for both commercial milling and food processing

industries, as they had low α -amylase activity, which is a negative factor for dough stability and baking performance. Akbar et al. (2022) have also found similar results and confirmed that falling number is a reliable indicator of wheat processing quality.

Overall, study findings indicate the development of advanced mutant lines via induced mutagenesis is a viable strategy for generating new variations to improve current wheat varieties. The simultaneous improvement of yield and yield-related components, grain protein content, gluten content, hardness index, and processing characteristics highlights the ability of mutation breeding to broaden the genetic base of elite wheat cultivars while preserving their overall adaptation. The superior mutant lines identified in this study therefore represent valuable pre-breeding resources that can attain direct exploration in cultivar development, gene discovery, and climate-resilient wheat improvement programs.

CONCLUSIONS

Wheat (*T. aestivum* L.) mutant lines, such as SE5/12-8, SE4/12-1, and SG2/12-26, showed desirable traits, including dwarfism, longer spikes, more spikelets, compact spikes, and amber grains. The mutant line SE5/12-19 exhibited an ultra-hard grain texture, while selected mutants also showed optimal gluten content, Zeleny sedimentation value, and falling numbers ≥ 425 seconds, confirming excellent milling and baking quality. These mutant lines can further proceed for exploration as donor parents to broaden wheat genetic diversity. This can be through introgression of new alleles and the support of gene discovery, quantitative trait locus (QTL) mapping, and the identification of genes controlling yield, grain quality, and stress adaptation.

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