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GAMMA IRRADIATION-INDUCED VARIATION IN GRAIN PROTEIN CONTENT AND DAYS FROM SOWING TO HEADING IN M₅ MUTANT LINES OF SPRING WHEAT (CV. ERITROSPERMUM-35)

Abstract: Bread wheat (*Triticum aestivum* L.) is a major staple crop providing essential calories for human diets. However, intensive breeding for yield has reduced genetic diversity for quality traits, including grain protein content (GPC). Induced mutagenesis offers an effective strategy to broaden the genetic base and generate novel alleles affecting agronomic traits. In this study, M₅ mutant lines of the spring wheat cultivar EritrospERMUM-35 were developed through gamma irradiation at 100 Gy and 200 Gy. Lines were evaluated for variation in GPC and days from sowing to heading under controlled greenhouse conditions. GPC was measured using near-infrared reflectance spectroscopy (NIR), and allelic variation at the candidate gene *Eps-A^m1* was analyzed using PCR-based markers. Substantial variation in GPC was observed. The 100 Gy lines showed GPC values ranging from 12.60% to 14.43% (mean $13.56 \pm 0.57\%$), whereas the 200 Gy lines had a mean GPC of $13.76 \pm 0.63\%$. Eleven mutant lines (37%) exhibited significantly higher GPC (5.7–11.0%) than the parent. Importantly, the increase in GPC was not associated with a reduction in thousand kernel weight; TKW values were higher in irradiated lines compared with the parent. Days to heading differed between treatments: 100 Gy lines headed earlier, while 200 Gy lines showed delayed heading. Molecular screening identified new alleles of *Eps-A^m1*, with allele carriers generally exhibiting earlier heading. Overall, gamma irradiation generated valuable genetic variation for improving grain protein content and adaptive traits in spring wheat.

Keywords: gamma irradiation, grain protein content, days to heading, thousand kernel weight, spring wheat, *Eps-A^m1*, mutation breeding.

Introduction

Bread wheat (*Triticum aestivum* L.) is a major cereal crop of global importance and plays a central role in food and nutrition security (Shewry & Hey, 2015). In many developing countries, wheat-based foods dominate the diet, providing a substantial portion of daily caloric intake as well as essential plant proteins and micronutrients (Guzmán et al., 2019). However, wheat-based diets often lack sufficient protein quality and micronutrients, leading to “hidden hunger” and contributing to malnutrition-related diseases and impaired development (Bouis & Saltzman, 2016).

Grain protein content (GPC) represents a key determinant of wheat grain quality, as it influences both nutritional characteristics and processing performance, particularly baking properties (Sun et al., 2025). However, efforts to enhance GPC through

conventional breeding remain constrained by substantial environmental effects (Rybacki et al., 2016), the well-documented inverse relationship between protein concentration and grain yield, limited genetic diversity in modern germplasm (Simmonds, 1995), and the complex polygenic nature of this trait. Together, these factors complicate the development of high-protein wheat cultivars (Welch & Graham, 2004), and complex polygenic inheritance involving genotype-by-environment interactions (Marcotuli et al., 2025). These factors make selection for enhanced grain protein particularly challenging (Shewry, 2009).

In addition to protein concentration, thousand kernel weight (TKW) represents a key yield-related trait reflecting grain size and overall productivity in wheat. Improvement of grain protein content is frequently associated with a potential trade-off with

kernel weight and yield components. Therefore, simultaneous evaluation of GPC and TKW is essential to determine whether enhanced protein levels are accompanied by unintended reductions in grain weight.

Adaptation to diverse or changing environments requires flexible control of developmental traits such as heading time (Kamran et al., 2014). In wheat, heading time is primarily regulated by three genetic systems: vernalization response mediated by VRN genes (Allard et al., 2012), photoperiod sensitivity controlled by PPD genes (Kitagawa et al., 2012), and earliness per se (*Eps*) genes that fine-tune heading independently of vernalization and photoperiod (Ochagavía et al., 2019). *Eps* genes allow wheat to synchronize reproductive development with favorable environmental windows, supporting yield stability (Alvarez et al., 2016). Among these, the *Eps-Aml* locus has been identified as an important determinant of heading time, spike development, and yield components (Appendino & Slafer, 2003). In the present study, *Eps-Aml* was selected as a candidate gene because it represents a well-characterized intrinsic earliness locus regulating heading time independently of vernalization and photoperiod pathways. Since the experiments were conducted under controlled greenhouse conditions minimizing environmental variation, differences in heading time were expected to reflect underlying genetic factors. Targeting *Eps-Aml* allowed us to examine whether gamma irradiation induced allelic variation at a major developmental regulator associated with intrinsic earliness. Additional studies have shown that *Eps* genes contribute significantly to local ecological adaptation (Horváth et al., 2023; Zhang et al., 2022).

Induced mutagenesis is a powerful approach for expanding genetic diversity by creating novel alleles not available in natural germplasm (Shu et al., 2012). Both physical and chemical mutagens are widely used to generate beneficial mutants for crop improvement (Chaudhary et al., 2019). Gamma irradiation, especially using a 60 Co source, is one of the most effective and widely applied physical mutagens in plant breeding (Spencer-Lopes et al., 2018). It has been responsible for developing numerous improved crop varieties across the world, including cereals (Ahloowalia et al., 2004). In wheat, gamma irradiation has successfully increased variability in agronomic traits (Kenzhebayeva et al., 2017), yield components (Maree et al., 2025), grain micronutrient content (Kenzhebayeva et al., 2018b), and grain quality traits (Kenzhebayeva et al., 2013). The biological effects of gamma rays, including increased mutation frequency and altered gene expression,

have been well documented (Zulfiqar et al., 2021). Gamma-induced mutants are often screened in early generations, and promising genotypes are advanced for further evaluation (Tahir et al., 2023).

Several studies have also demonstrated increases in protein content, gluten strength, and other quality parameters in wheat mutants developed using gamma irradiation (Köksel et al., 1998; von Well et al., 2023). Mutation breeding continues to play an important role in wheat research, especially for traits with limited natural variation (Holme et al., 2019). Evaluation of mutant lines under diverse environments provides opportunities to identify novel alleles affecting grain quality and developmental traits (Oury et al., 2006).

The local spring wheat cultivar Eritrospermum-35 is an important variety in Kazakhstan, but like many modern cultivars, it has limited genetic variability for some agronomic and quality traits. Previous research in Kazakhstan has emphasized the need to expand genetic diversity through modern breeding and mutagenesis approaches (Kenzhebayeva et al., 2018a). Studies on wheat adaptation in Central Asia further highlight the importance of improving both nutritional quality and resilience traits. Kazakhstan wheat breeding programs continue to explore new genetic sources for improving grain quality, stress tolerance, and yield potential (Kenzhebayeva et al., 2022; Kenzhebayeva et al., 2015).

Recent advances in wheat genomics have provided new tools for understanding complex traits such as protein content and flowering time (Song et al., 2023). The use of molecular markers accelerates the identification of key alleles and supports marker-assisted selection in breeding programs (Appels et al., 2018). Wheat genome research has shed light on the structure and evolution of polyploid wheat, improving the efficiency of genetic analysis (Uauy et al., 2006). Regulatory genes, including transcription factors influencing grain protein content, have also been identified (Velu et al., 2018). Improving wheat nutritional quality remains a global objective, particularly in the context of food security challenges (Gupta et al., 2020).

Biofortification through genetic and breeding approaches is recognized as a promising strategy to address micronutrient deficiencies worldwide (Velu et al., 2011). Wheat is a primary target for biofortification due to its central role in human diets (Velu et al., 2014). Enhancing grain zinc and iron concentrations, along with improving protein quality, is essential for combating hidden hunger. Wheat adaptation studies in Kazakhstan further support the need for breeding

nutritionally enhanced cultivars suited to local conditions. Consequently, enhancing grain nutritional traits continues to be a central objective of both international and regional wheat improvement programs (Wan Teng et al., 2022).

Therefore, the objective of this study was to evaluate gamma irradiation-induced variation in grain protein content and heading time in M_5 mutant lines of spring wheat (cv. Eritrospermum-35), and to assess allelic variation in the candidate gene *Eps-A^m1* associated with heading traits.

Materials and methods

Development of M_5 mutant lines through gamma irradiation. The experimental material included M_5 mutant lines obtained from the local spring bread wheat cultivar Eritrospermum-35 (*Triticum aestivum* L.). Dry seeds were exposed to gamma radiation at doses of 100 and 200 Gy using a ^{60}Co source at the Kazakh Nuclear Centre in accordance with established protocols for mutation induction in cereal crops (Ahloowalia et al., 2004; Zhang et al., 2022). The irradiated seeds were then sown to produce the M_2 generation.

Subsequent generations (M_2 – M_4) were cultivated under field conditions at the Kazakh Research Institute of Agriculture and Plant Growing (Almaty, Kazakhstan). Selection of superior plants was performed mainly based on yield-related parameters, including grain weight per plant (GWP) and grain weight per main spike (GWS), in comparison with the non-irradiated parental cultivar grown under identical conditions. Following harvest of the M_5 generation, fifteen lines from each radiation treatment (100 and 200 Gy) were selected for further evaluation of grain protein content, Thousand kernel weight and flowering time. These mutant lines, together with the parental cultivar Eritrospermum-35, were used for subsequent analyses

Field and greenhouse experiments. To evaluate yield performance and grain quality traits, the M_5 mutant lines together with the parental cultivar Eritrospermum-35 were cultivated in replicated field trials. The experiment was conducted at the Institute for Cereal Crops Improvement, Department of Molecular Biology and Ecology of Plants, Tel Aviv University. A randomized design with three replications was applied. Each experimental unit consisted of three rows (2 m in length and 1.2 m in width) with 20 cm spacing between rows and a planting density of 30 seeds per row. Standard agronomic management practices, including fertilization, weed control

and plant protection, were applied according to local recommendations. Flowering time assessment was performed under controlled greenhouse conditions to reduce environmental variation. Seeds were initially germinated on moist filter paper, and four-day-old seedlings were transferred to plastic pots (16 cm upper diameter, 12 cm lower diameter, 18 cm height) containing 2 kg of soil substrate.

Plants were maintained at 23°C under a 16 h photoperiod in a naturally illuminated greenhouse. Soil moisture was maintained at approximately 70% of field capacity. Thirty plants were grown per pot, and each genotype was represented by three independent biological replicates.

Grain protein content determination. The GPC was measured on whole grains using near-infrared reflectance (NIR) spectroscopy. For each M_5 line and the Eritrospermum-35 parent, three subsamples were analyzed using a ZX50 Portable Grain Analyzer (Zeltex, Hagerstown, MD, USA) with proprietary calibration software. Each subsample consisted of 25 grains per line. Results are expressed as percentage of protein in the grain on a mass basis. For the Eritrospermum-35 mutant population, summary statistics (minimum, maximum, mean and standard deviation) of GPC were calculated separately for the 100 Gy and 200 Gy treatments. Superior high-protein lines were identified using analysis of variance (ANOVA) followed by pairwise comparisons.

Thousand kernel weight determination. Thousand kernel weight (TKW) was determined according to standard grain analysis procedures. For each genotype, mature and healthy grains were randomly selected after harvest, and measurements were performed in three biological replicates. TKW values were expressed in grams as mean $\pm$ standard deviation (SD). All genotypes were grown under field conditions during the same growing season and harvested at physiological maturity to ensure reliable comparison of TKW values.

Molecular analysis of *Eps-A^m1*. To investigate the genetic basis of flowering time and the earliness per se candidate gene *Eps-A^m1* was analyzed by PCR. Total genomic DNA was extracted from young leaf tissue of 10-day-old seedlings using a modified CTAB method adapted from previously published protocols and optimized according to Kenzhebayeva et al. (2016). DNA pellets were dried and resuspended in TE buffer, and DNA concentration was determined using a Bio Photometer (Eppendorf AG, Hamburg, Germany). For *Eps-A^m1*, candidate gene-specific primers *Eps-A^m1* -F (5'-CACATATCTGGCACCCACA-3') and *Eps-A^m1* -R (3'-TGCTCAT-

GAGTTTTCC-TCTGAA-5') (Eurofins Genomics, Germany) were used to amplify a fragment of around 780 bp associated with the presence of the *Eps-A^m1* allele (Kenzhebayeva et al., 2016). PCR amplification was performed under the following conditions: initial denaturation at 95°C for 5 min; followed by 37 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 30 s; with a final extension at 72°C for 5 min. PCR products were separated on 1% agarose gels and visualized under UV light using a Gel Documentation System (the ENDURO™ GDS, Labnet International, Inc). The presence or absence of the diagnostic bands for *Eps-A^m1* was recorded for each Eritrospermum-35 M₅ mutant line and the parent, and the frequency of alleles in the mutant populations was compared between the 100 Gy and 200 Gy treatments.

Recording of heading time. Heading time was recorded in the greenhouse experiment as the number of days from sowing to spike emergence (heading). Pots were inspected every two days, and heading date was defined as the day when spikes first emerged from the flag leaf sheath. For each genotype, the mean heading time (days from sowing) was calculated based on three replicated pots. In addition, the number of main spikes per pot at heading was recorded as an indicator of tillering and reproductive development. For Eritrospermum-35, summary statistics of heading time (mean ± standard deviation) were calculated for the parent, the 100 Gy mutant lines, and the 200 Gy mutant lines.

Statistical analysis. All data were analyzed using standard statistical procedures. The GPC data were evaluated by one-way analysis of variance (ANOVA) to test the effect of irradiation dose (parent vs 100 Gy vs 200 Gy) within the Eritrospermum-35 background. Coefficients of variation (CV, %) were calculated to assess the relative variability of GPC in each group of lines. Multiple comparisons of mutant lines with the parent were performed using appropriate post hoc tests (e.g. Dunnett's test), and a significance level of $p \leq 0.05$ was considered statistically significant. For flowering time, mean values and standard deviations were calculated for the parent and the 100 Gy and 200 Gy mutant populations. Differences between means were interpreted considering the presence or absence of *Eps-A^m1* alleles. Frequency distributions and boxplots of GPC and flowering time are presented to visualize the response of the parent and mutant populations and to facilitate interpretation of irradiation effects on the studied traits.

Results and discussion

Gamma-Induced Variation in Grain Protein Content of Eritrospermum-35 M₅ Mutant Line. The population derived from the 100 Gy treatment displayed protein values between 5.60% and 14.43%, with a mean of $13.56 \pm 0.57\%$ ($n = 45$). Lines exposed to 200 Gy showed a marginally higher average GPC ($13.76 \pm 0.63\%$). Compared with the parental cultivar ($13.27 \pm 0.31\%$), eleven mutant genotypes (37%) demonstrated an increase in protein concentration ranging from 5.7% to 11.0%.

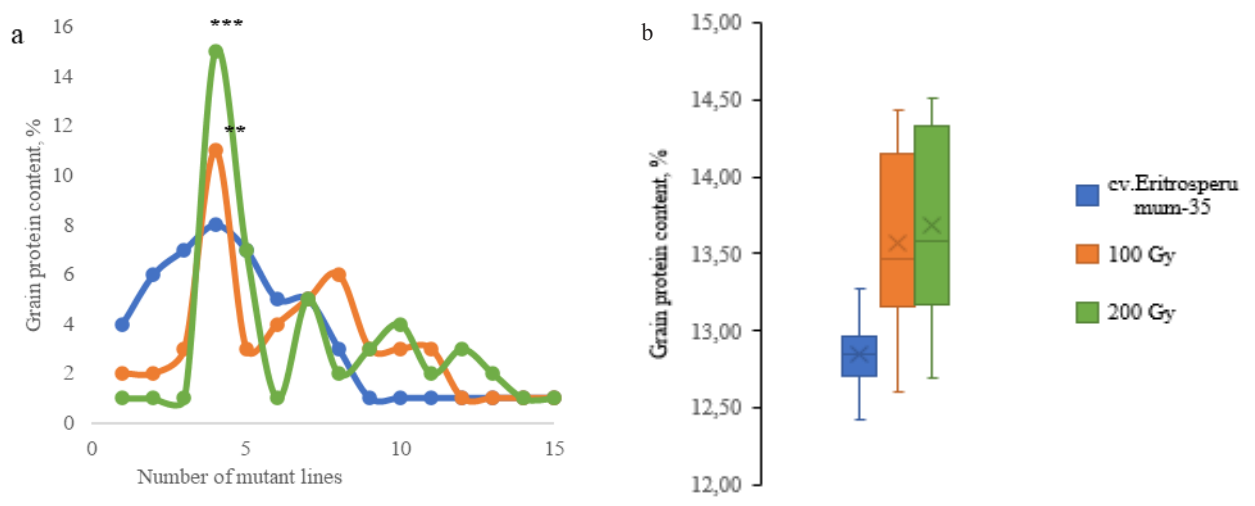
Among the most promising high-protein genotypes were lines 108(1), 118(3), 135(1), 156(1), 140(2), 149(2), 150(7), 152(5), 153(6), 153(7) and 153(8). The coefficient of variation for GPC in Eritrospermum-35 mutant lines was 4.2% for the 100 Gy population and 4.58% for the 200 Gy population, indicating a moderate level of variability in GPC within each Gy group. The boxplots (Figure 1. a, b) clearly illustrate that both irradiation doses increased the spread of GPC values relative to the parent, with several outlier lines exceeding the parental mean by more than one standard deviation.

Statistical evaluation using one-way ANOVA demonstrated that irradiation dose significantly influenced GPC levels ($p \leq 0.05$). Pairwise comparisons indicated that both irradiated populations differed from parental control, and irradiation explained a considerable proportion of total phenotypic variation in protein content (Figure 1).

Gamma irradiation induced substantial variation in grain protein content (GPC) among M₅ lines, consistent with earlier findings that mutagenesis generates novel alleles affecting grain quality. Both 100 Gy and 200 Gy groups showed increased mean GPC relative to the parent (Table 1). In addition to protein content, thousand kernel weight (TKW) was evaluated to assess the potential impact of irradiation on grain size and yield-related traits. The mutant populations exhibited higher mean TKW values (47.19 ± 8.54 g for 100 Gy and 48.68 ± 5.95 g for 200 Gy) compared with the parent cultivar (34.19 ± 0.32 g). Importantly, the increase in GPC was not accompanied by a reduction in kernel weight, suggesting that protein enhancement did not result in an evident yield penalty. Analysis of variance indicated a significant effect of irradiation dose on TKW ($p \leq 0.05$). These results indicate that gamma irradiation generated favorable combinations of quality and agronomic traits in the Eritrospermum-35 mutant lines.

Figure 1

Grain protein content (GPC) distribution in the parental cultivar *Eritrospermum-35* and in M_5 mutant lines generated after exposure to 100 Gy and 200 Gy gamma irradiation (b) shows box-and-whisker plots representing median values, interquartile ranges and extreme data points for each treatment group

**Table 1**

Mean values ($\pm$ SD) of grain protein content (GPC) and thousand kernel weight (TKW) in parental cultivar and M_5 mutant lines of *Eritrospermum-35* developed under different gamma irradiation doses

Genotype	Grain protein content, % on db	TKW, g
<i>Eritrospermum-35</i> (parent)	13.27 ± 0.31	34.19 ± 0.32
<i>Eritrospermum-35</i> M_5 mutant lines (100 Gy)	13.56 ± 0.57	47.19 ± 8.54
<i>Eritrospermum-35</i> M_5 mutant lines (200 Gy)	13.76 ± 0.63	48.68 ± 5.95

Gamma irradiation induced substantial variation in GPC among M_5 lines, consistent with earlier findings that mutagenesis generates novel alleles affecting grain quality. Both 100 Gy and 200 Gy groups showed increased mean GPC relative to the parent (Table 1).

Variation in days to heading in *Eritrospermum-35* mutant lines. Days to heading showed clear differences between the parental cultivar and the *Eritrospermum-35* mutant populations (Table 2). The parent exhibited a mean heading time of 106 ± 8.13 days from sowing to spike emergence under greenhouse conditions. The 100 Gy-treated M_5 lines headed substantially earlier, with a mean of 88 ± 10.41

days, corresponding to an advance of approximately 18 days relative to the parent. In contrast, the 200 Gy-treated lines headed later, with a mean of 114 ± 5.45 days, about 8 days later than the parent.

Table 2

Days to heading (mean $\pm$ SD) in the parental cultivar and M_5 mutant lines of *Eritrospermum-35* developed under different gamma irradiation doses

Genotype	Number of lines	Days to heading (mean $\pm$ SD)
<i>Eritrospermum-35</i> (parent)	1	106 ± 8.13
<i>Eritrospermum-35</i> M_5 mutant lines (100 Gy)	15	88 ± 10.41
<i>Eritrospermum-35</i> M_5 mutant lines (200 Gy)	15	114 ± 5.45

Eritrospermum-35 mutants showed strong shifts in days to heading. Early heading at 100 Gy aligns with observations that moderate irradiation doses often generate beneficial developmental mutations (Chaudhary, 2019; von Well et al., 2023). In contrast, delayed heading at 200 Gy corresponds to findings that higher irradiation doses cause broader genomic perturbations.

Molecular characterization of *Eps-A^m1* alleles. Molecular analysis revealed differences in allelic

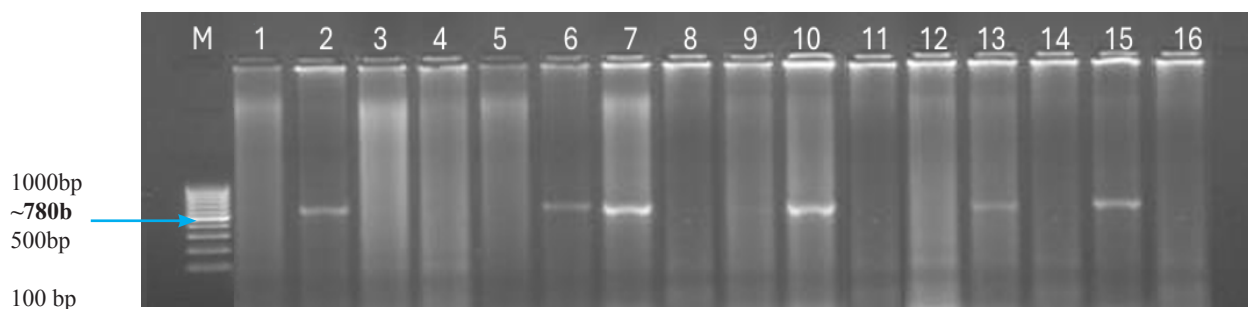
composition at the *Eps-A^m1* locus between the parent cultivar Eritrospermum-35 and its M₅ mutant lines. For the *Eps-A^m1* candidate gene, a relatively high frequency of the diagnostic PCR fragment was found in the Eritrospermum-35 100 Gy-dose mutant lines. Sex out of fifteen 100 Gy lines (66.7%) carried the *Eps-*

A^m1 allele, including lines 105(1); 118(1); 118(2); 136(1); 140(3) and 232(1). In the 200 Gy mutant lines, the *Eps-A^m1* allele was not present. These results indicate that 100 Gy dose was more efficient than the 200 Gy dose in generating allelic variation at *Eps-A^m1* in Eritrospermum-35 mutant populations.

Figure 2

Agarose gel electrophoresis of PCR amplification products of the *Eps-Am1* gene.

M – 100 bp DNA ladder; lane 1 – Eritrospermum-35 (parent); lanes 2–16 – M₅ mutant lines (200 Gy) [105(1), 108(1), 113(1), 113(5), 118(1), 118(2), 118(3), 135(1), 136(1), 138(6), 140(2), 140(3), 140(4), 232(1) and 242(2)]



The frequency distribution of days to heading (not shown) demonstrated a clear shift toward earlier heading in the 100 Gy population and toward delayed heading in the 200 Gy population. Lines carrying the *Eps-Am1* allele tended to head 12–28 days earlier than the parent, depending on genotype and irradiation dose, confirming the contribution of this locus to intrinsic earliness. The presence of early-heading *Eps-A^m1* alleles, combined with enhanced GPC, supports their potential breeding value, especially in environments prone to heat and drought stress (Appendino & Slafer, 2003).

Treatment of Eritrospermum-35 seeds with gamma radiation at 100 and 200 Gy led to the formation of M₅-derived mutants characterized by substantial variation in both grain protein content and heading time. These findings confirm that induced mutagenesis is an effective strategy to broaden genetic variability for important agronomic and quality traits in locally adapted wheat varieties (Chaudhary et al., 2019).

An increased grain protein content was detected in a considerable number of M₅ mutant lines, particularly within the 100 Gy and 200 Gy treatments, compared with the parental cultivar. The occurrence of these high-protein genotypes indicates that gamma irradiation may have induced genetic alterations influencing nitrogen assimilation, remobilization efficiency, or protein accumulation in the grain (Maree et al., 2025). Although breeding for high GPC is often hampered by its negative correlation with yield,

the mutant lines in this study were selected from earlier generations based on yield components, indicating that at least some of the high-protein lines may maintain acceptable productivity.

The observed variation in days to heading among Eritrospermum-35 mutant lines has important implications for adaptation and yield stability. Earlier heading can help wheat escape terminal heat and drought stress in continental climates, whereas slightly later heading may be advantageous in cooler or more humid regions (Alvarez et al., 2016; Ochagavía et al., 2019). The 100 Gy mutant population showed generally earlier heading than the parent, whereas the 200 Gy lines tended to head later, suggesting that different sets of mutations affecting developmental regulators were recovered in the two irradiated populations. The molecular data suggest that allelic variation at *Eps-A^m1* contributed to these differences. In particular, the higher frequency of the *Eps-A^m1* allele in the 100 Gy population is consistent with the reduced days to heading in these lines.

The *Eps* genes are believed to control intrinsic earliness by affecting developmental rates after vernalization and photoperiod requirements are satisfied (Appendino & Slafer, 2003). Thus, the appearance of novel *Eps-A^m1* alleles in the Eritrospermum-35 mutant lines likely enabled fine-tuning of heading time independently of external cues. The PCR products observed in Figure 2 confirm that irradiation generated additional allelic variants at this locus.

From a breeding perspective, the combination of enhanced GPC, favorable heading time and novel alleles at *Eps-Am1* makes the Eritrospermum-35 M₅ mutant lines valuable genetic resources. High-protein, early-heading lines are particularly attractive for regions where short growing seasons and climatic stresses limit wheat productivity and quality. Such lines could be used directly as mutant varieties after agronomic evaluation or as donors in crossing programs to transfer desirable alleles into elite breeding lines (von Well et al., 2023).

Overall, the study highlights the potential of gamma irradiation and mutation breeding for improving both grain quality and adaptation traits in spring wheat. Further work should focus on (i) detailed phenotypic characterization of the most promising mutant lines in multi-environment field trials, (ii) fine mapping of the novel *Eps-Am1* alleles and (iii) integration of mutation-derived alleles into marker-assisted selection schemes for spring wheat improvement.

Conclusions

Gamma irradiation of Eritrospermum-35 seeds at 100 Gy and 200 Gy generated M₅ mutant lines exhibiting significant variation in grain protein content (GPC), thousand kernel weight (TKW), and flowering time. A considerable proportion of mutant lines showed GPC values 5.7–11.0% higher than the parental cultivar, demonstrating the effectiveness of induced mutagenesis for improving wheat grain nutritional quality.

Importantly, the increase in GPC was not accompanied by a reduction in grain weight. Both irradiation treatments resulted in significantly higher mean TKW compared with the parent (34.19 ± 0.32 g), reaching 47.19 ± 8.54 g at 100 Gy and 48.08 ± 5.95 g at 200 Gy. Analysis of variance confirmed a significant effect of irradiation dose on TKW, indicating favorable combinations of quality and yield-related traits. The 100 Gy population headed earlier (88 ± 10.41 days) than the parent (106 ± 8.13 days), whereas the 200 Gy population

headed later (114 ± 5.45 days), suggesting dose-dependent shifts in developmental timing. Molecular analysis revealed novel allelic variation at the *Eps-Am1* locus, with the 100 Gy treatment showing greater allelic diversity. Lines carrying the *Eps-Am1* allele tended to head 12–28 days earlier than the parental genotype.

Overall, M₅ mutant lines combining enhanced GPC, increased TKW, modified days to heading, and favorable allelic variation represent promising material for breeding high-quality and well-adapted spring wheat cultivars for Kazakhstan and similar environments.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Author contributions

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Saule D. Atabayeva: Methodology, Formal Analysis

Sabina A. Shoinbekova: Data Curation, Formal Analysis

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Malika A. Abdulzhanova: Visualization, Formal Analysis

Adel Rsatayeva: Data Curation, Methodology

Assaf Distelfeld: Writing – Resources, Analysis, Methodology

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