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## Mutant Resources of Spring Wheat to Improve Grain Quality and Morphology

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Additional information is available at the end of the chapter

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### Abstract

The objective of this study was to broaden genetic variation of spring common wheat, evaluate and identify among  $M_5$  mutant lines those with high-yield and improved grain quality characteristics. New lines were generated by initial treatment of variety of Eritrospermum-35 adapted to conditions of Kazakhstan by irradiation with 100-Gy and 200-Gy doses from a  $^{60}\text{Co}$  source. Yield-associated traits including grain number and weight per main spike, grain weight (GW) per plant, and the thousand-kernel weight; grain size and grain shape variations; as well as quality characteristics such as grain protein content (GPC), iron, and zinc concentrations were evaluated. Mutant lines with high iron and zinc concentrations and high protein content were identified as those which have 1.6–3.4 and 1.4–2.9 times more as well as 3.7–16.9% more higher data of target concentrations than parental variety had, respectively. Several mutant lines showed significant increase in both grain iron and zinc concentrations. The positive correlation of grain quality parameters with grain area, length, and width suggest that they are related to each other. Wheat grain can be biofortified with micronutrients without negative impact on crop productivity using new mutant lines. Mutation breeding can significantly contribute to human health malnutrition and improve nutrition quality diet.

**Keywords:** biofortification, high productivity, new mutation resources, spring wheat, micronutrients

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## 1. Introduction

Bread wheat (*Triticum aestivum* L.) is a main crop with global importance for food safety and one of the major cereal source of nutrients for both humans and animals. This balance of consumption of required nutrient for human metabolic needs generally resulted in serious metabolic violations leading to sickness, poor health, suppressing of children development, and high economic expenses for society [1]. It is necessary for agricultural systems to ensure proper products, which will balance quantity of nutrients to support healthy life. However, in many developing countries, agriculture does not meet these requirements [2].

Presently, over three billion people suffer due to micronutrient malnutrition and the numbers are increasing [3]. There are many human diseases associated with nutritional deficiency, and around two-thirds of all children's deaths are related to malnutrition [4, 5].

Improvement of many agronomic traits including grain quality requires their genetic variation, which should be separable from non-genetic impacts. Over the years, the main focus of wheat breeding programs was the replacement of traditional varieties with modern high-yield ones that led to reduction of its genetic diversity mainly by end-use quality characteristics and nutrition quality (FAO Document Repository, 2015). The key desired traits for breeding were high yield and disease resistance. It is also known that the genetic variability of major crops currently have systematically decreased due to the repeated utilization of the local adapted genotypes in breeding processes which leads to the decreasing of the wide genetic recombination which may posse the novel traits [6].

Crops' genetic variability and therefore crop improvement could be powerfully generated through mutation breeding. Over the past 80 years, this approach has been applied for development of new mutant varieties of both seeds and vegetatively propagated crops [7–9]. According to the FAO/IAEA Mutant Variety Database, in 2014, there were 3220 mutant plant varieties of 214 plant species all round the world [10] (<http://mvgs.iaea.org/>). Application of mutagenesis in crop breeding has two main steps: the selection of individual mutants with improved traits after the mutagenic treatment and utilization of selected plants or lines in breeding programs [7].

One of the greatest contributions into crop yield is grain weight trait [11] which is directly related to two important morphometric characteristics such as grain size and grain shape. Grain size and grain shape are essential breeding traits because they are phenotypically the most stable of the yield components. They also greatly influence the grinding process, yield of flour, which is the main source for human consumption with a lot of other end-use utilizations, and starch damage [12]. Larger grains have strong contribution to higher grain weight in addition to increase in seedling vigor and production of dry matter of the raw material in the field [12, 13]. Grain size can be generally characterized by grain width (GW) and grain area (GA), however grain shape also determines along the grain's main growth axis. Grain shape is measured by grain length (GL), grain width (GW), vertical perimeter, sphericity, and determination along the horizontal axis [12, 14]. It is very substantial to improve the wheat grain morphometry according to the demand of grain market and processing industries by various breeding approaches. Therefore, the estimation of genetic diversity of the

traits related to grain morphometry will be very important for feather genetic improvement of wheat varieties according to their end-use quality demand.

The grain protein content (GPC) is an economically valuable trait which plays one of the key roles in the determination of the wheat grain nutrition quality and has strong impact on the bread-making and end-use quality [15]. Despite the great importance of GPC, advancement in wheat breeding for high GPC is quite poor. One of the explanations of this situation is low variations by GPC among commercial cultivars; in addition, breeding for high GPC is very difficult and time consuming due to the trait complicated genetic control. The GPC is routinely screened in wheat breeding programs and selected the accessions with high protein content being for bread making and accessions with low protein content being for feed and other directions of industrial utilizations. One more limitation in wheat breeding programs for high GPC is the strong negative correlation between GPC and grain yield [16–18]. Nevertheless, it was shown that in common and spelt wheat populations, there are lines with simultaneous high GPC and yield components [19].

The evidence of correlation between high GPC and grain morphometry such as grain size and grain shape has not been pointed out. The wild tetraploid wheat (*Triticum turgidum* L. var. *dicoccoides*) has gene for GPC as a promising source for wheat improvement [20], which is located on chromosome 6B [21]. The high protein content gene from *T. dicoccoides* has been transferred into hexaploid wheat [22]. However, the exclusive milling and baking properties of bread wheat are not found among the diploid and tetraploid wheats. Mainly because of the fact that only the hexaploid wheats have the subgenome D chromosomes derived from diploid *T. tauschii* which determine the quality parameters of bread making of common wheat as widely suggested by wheat scientists [23]. In order to improve the modern wheat cultivars by GPC characteristics without reductions in yield, it is very important to develop wheat genotypes with more high N-use efficiency which involves improved N-uptake and/or N-remobilization [16]. The significant variations in GPC was related to post-anthesis N uptake independently of anthesis date and total N at anthesis [24, 25]. One of the last results on bread wheat highlights the correlation with GPC of post-flowering N uptake occurring early during grain development [26].

Iron and zinc deficiency is a widespread food-related health problem and affects over half of the world population [27, 28]. Wheat is the cheapest and primary source, which supplies the bulk of nutrients for the human diet. If compared to cultivated wheat species, the wild species appear to be a rich genetic resource for high Fe and Zn concentrations [29]. Meanwhile, despite cultivated wheat had mainly lower Fe and Zn concentrations that it was indicated for wild species, more their screening is required in order to find out elite germplasm with high Fe and Zn concentrations already possessing good agronomic productivity [30]. Wheat grain has low content micronutrients. For this reason, there is need for genetic enhancement with more of this nutrient being one of the most cost-effective and powerful method of diminishing global micronutrients malnutrition [31].

Biofortification of cereal grains is one of the most economic effective ways for solving the global micronutrients malnutrition issue. Biofortification has multiplicative advantages [31], and it is considered to be a promising and cost-effective approach for decreasing malnutrition and human health improvement [32].

The aims of present studies were: (1) to generate  $M_5$  mutant lines of spring common wheat in genetic background of cv. Eritrospermum-35; (2) to evaluate variability in components of productivity, including grain number and weight per main spike (GNS and GWS), grain weight per plant (GWP) and 1000-grain weight (TKW), variability in grain morphometry (size and shape), and quality parameters, namely GPC, grain Fe (GIC), and grain Zn (GZnC) concentrations in parent and  $M_5$  mutant lines from generation developed by irradiation treatment of seeds with 100 Gy and 200 Gy and identify those that have high-yield characteristics and improved grain quality traits; and (3) to estimate relationship between two sets of data, including grain quality and agronomic performance parameters.

## 2. Materials and methods

### 2.1. Plant material and application of induced mutagenesis

Grains of spring bread wheat variety cv. Eritrospermum-35 (*Triticum aestivum* L.) were irradiated by doses of 100 Gy and 200 Gy from a  $Co^{60}$  source at the Kazakh Nuclear Centre. Grains were planted immediately after irradiation in order to obtain  $M_1$  plants. The  $M_1$  generation was grown in the experimental field of the Kazakh Institute of Agricultural and Farming near Almaty according to the standard agricultural practice. Single spikes were harvested from each plant in order to develop the  $M_2$  generation. Selection of the best lines from  $M_1$  to  $M_5$  was carried out based on individual plants. The plants of  $M_3$  and  $M_4$  generations were planted in randomized blocks in three replications. The best lines were tested with their parent variety in order to select advanced mutants. The selection criteria for these lines were GWS and GWP, which were applied in the  $M_3$  and  $M_4$  generations (2011 and 2012) and based on the values for the parent cv. Eritrospermum-35 grown under the same trial conditions. In 2011 and 2012, the parent line had a mean of GWS of  $0.79 \pm 0.24$  g and  $0.80 \pm 0.26$  g, a mean of GWP of  $2.06 \pm 0.06$  g, and  $1.41 \pm 0.43$  g yield values, respectively. The threshold criteria for selection in the  $M_3$  and  $M_4$  generation were  $GWS > 1.1$  g and  $GWP > 2.2$  g for mutant lines. Grains of the best mutants were individually selected in each generation. After harvesting the  $M_5$  plants, 15 lines from the original 100-Gy radiation dose were selected. These lines were numbered as follows: 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)). Another 15 lines were selected from the 200-Gy radiation dose, which were numbered: 144(1), 144(2), 149(2), 150(7), 152(1), 152(4), 152(5), 152(6), 152(7), 152(8), 153(4), 153(5), 153(6), 153(7) and 153(8).

Samples of grains of each mutant line and the parent were tested. Grain samples from each mutant line, together with the parent Eritrospermum-35, were planted in a field trial for further evaluation. Each line was grown in three replicate three-row plots, 2 m long, 1.20 m wide with 20 cm between rows, and 30 seeds planted per row. The trial was managed according to the local recommendations for agronomic practices [33]. The following productivity parameters, GWP, GNS, GWS, and TGW calculated as the mean weight of three sets of 100 grains per line were estimated.



## 2.2. Grain morphometric analysis

Morphometric analysis was performed with the WinRHIZO and image analysis system ((version 1.38 2007, Reagent Instruments Inc., Canada) for GL, GW, and GA on 50–60 grains per line and the GL:GW ratio was also calculated.

## 2.3. Estimation of grain protein content, iron and zinc concentrations

Grain protein content was estimated with near infrared reflectance (NIR) spectroscopy of whole grains (ZX50 Portable Grain Analyzer, USA) using respective calibration software provided by Zeltex. Three repetitions were studied using 25 grains per line.

The measurements of grain iron and zinc concentrations were described in detail [33]. Briefly, grain samples (100-Gy- and 200-Gy-dosed  $M_5$  mutant lines and cv. Eritrosporum-35) were washed with sodium dodecyl sulfate (0.1%) with the following several times washings in deionized water, dried, ground with a mixer mill (Retsch MM400 GmbH) and digested (0.2 g) with a mixture of nitric acid (65%, analytical grade) and hydrogen peroxide (30%) (5:1, v/v) using digestion automat K-438 and scrubber K-415 model triplescrub (BÜCHI Labortechnik AG CH-9230 Flawil 1/Switzerland). The sample was diluted to 20 mL with twice-distilled water. The Fe and Zn concentrations were analyzed using flame atomic absorption spectroscopy (Analytic Jena NovAA350, Germany). Estimation of mineral nutrients were checked against the certified reference values from the State standard samples LLC “HromLab,” Zn 7837–2000, Fe 7835–2000 diluted by 0.3% HNO<sub>3</sub>. Three extracts and analysis repetitions were performed.

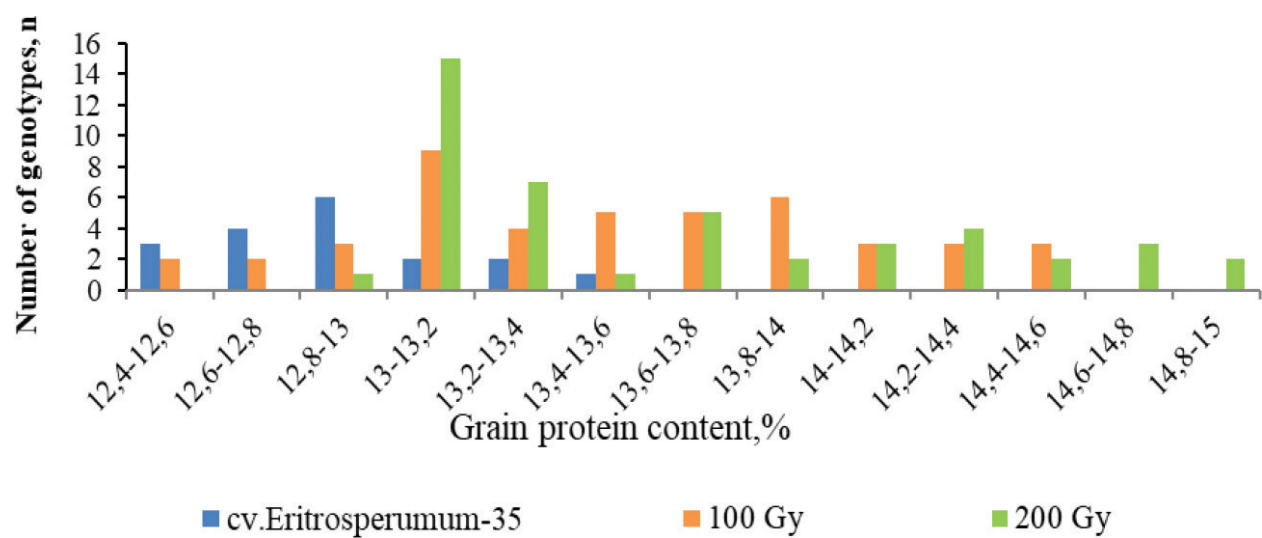
## 2.4. Statistical analysis

All data were evaluated in R 3.0.2 (R Core Development Team 2013). The simultaneous tests of general linear hypotheses, Dunnett Contrasts, were used for multiple comparisons of the means. Summarized data are reported as a mean values  $\pm$  standard deviations. Correlation coefficients between productivity components and grain-quality parameters and  $p$  values were calculated using the GenStat software (10th edition). A  $p$ -value  $\leq 0.05$  was considered statistically significant.

## 3. Results and discussion

In the current study, GPC showed considerable variation from 12.60 to 14.73% with a mean of  $13.62 \pm 0.60\%$  (**Figure 1**). It identified 11 advanced  $M_5$  mutant lines (37.7%), of which 5 lines were from 100-Gy gamma-irradiated lines, which demonstrated significant differences from the parent up to 5.7–11.0% GPC higher.

The screening of mutant lines ( $n = 90$ ) for Fe concentration (GIC) showed great variations in this grain quality parameter (**Figure 2**). The GIC varied from 13.49 to 65.53 mg/kg with a mean



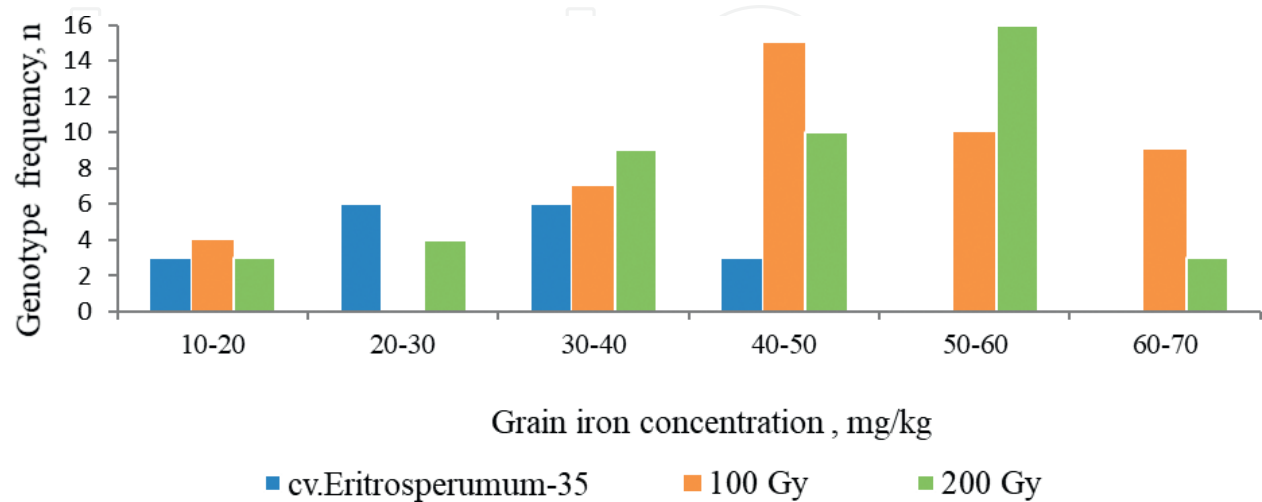
**Figure 1.** Frequency distribution for range of GPC in 100-Gy- and 200-Gy-dosed  $M_5$  wheat mutant lines and parent cv. Eritrospermum-35.

of  $44.95 \pm 13.95$  mg/kg. The 16  $M_5$  lines (53%) had significantly enhanced GIC with regard to cv. Eritrospermum-35 such that it exceeds the parent by 1.3 to 1.9 times. The highest values of GIC were revealed in 200-Gy mutant germplasm.

Considerable increase in GIC of mutant lines if compared to parent is the useful tool for further crop improvement.

The ranges of GZnC in mutant lines ( $n=90$ ) were more higher comparing with that of GIC, from 25.97 to 106.23 mg/kg with a mean of  $65.73 \pm 26.39$  mg/kg (**Figure 3**).

Therefore, identification of genetically determined high GIC and GZnC in mutant germplasm and afterward development of iron and zinc biofortified varieties is a very important and



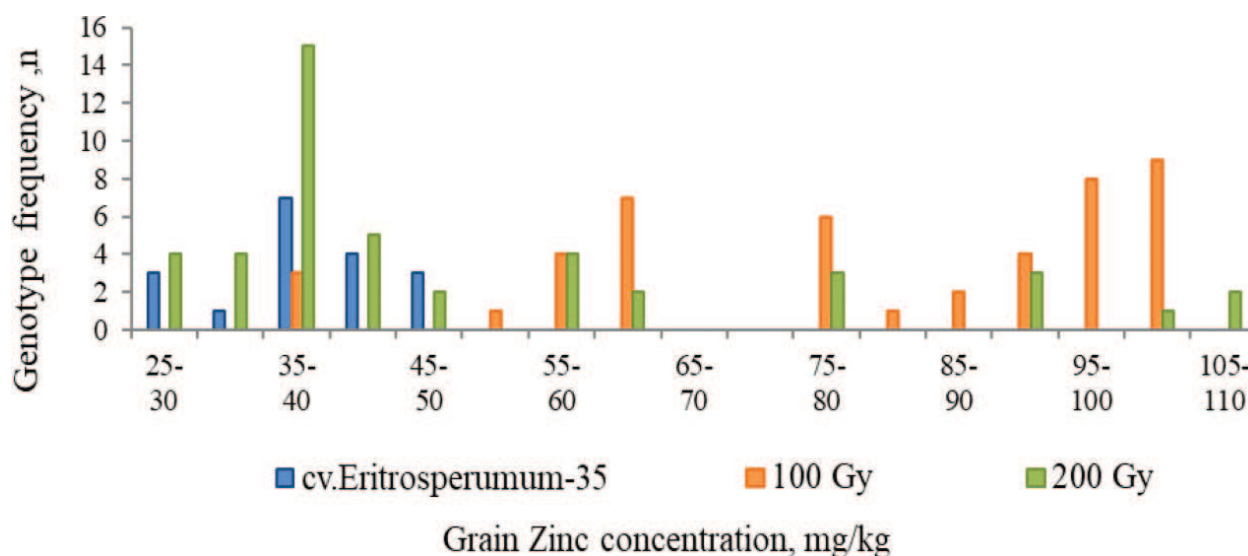
**Figure 2.** Frequency distribution for wheat grain Fe concentration (GIC) in 100- and 200-Gy-dosed  $M_5$  wheat mutant lines and parent cv. Eritrospermum-35.

promising approach. Varieties and lines with high GIC and GZnC can be used to reduce the human nutrition deficiencies in iron, zinc, and other micronutrients [34, 35].

The key basis of crops improvement is the range of their genetic variability. These ranges of values define the genetic variability that exists in the pooled parent and gamma-irradiated M<sub>5</sub> lines under one set of environmental conditions. We revealed that 19 M<sub>5</sub> mutant lines (63%) of which 14 lines were from 100 Gy treatment had significantly higher GZnC by 1.24-3.62 times than that of the parent variety. The great variation in GIC and GZnC among wheat mutant lines suggests that it is possible to identify and develop cultivars with high metal concentration. These grain metal accumulations can occur without adversely affecting plant biochemical and physiological functions and they indicate the potential to induce mutations in genes involved in mineral homeostasis processes. The advantage of wild emmer over cultivated wheat for higher grain nutrient concentrations has been previously consistently demonstrated [28]. However, because of sexual incompatibility between the crop and its wild relative, it may require embryo rescue or use tissue culture to recover fertile embryos.

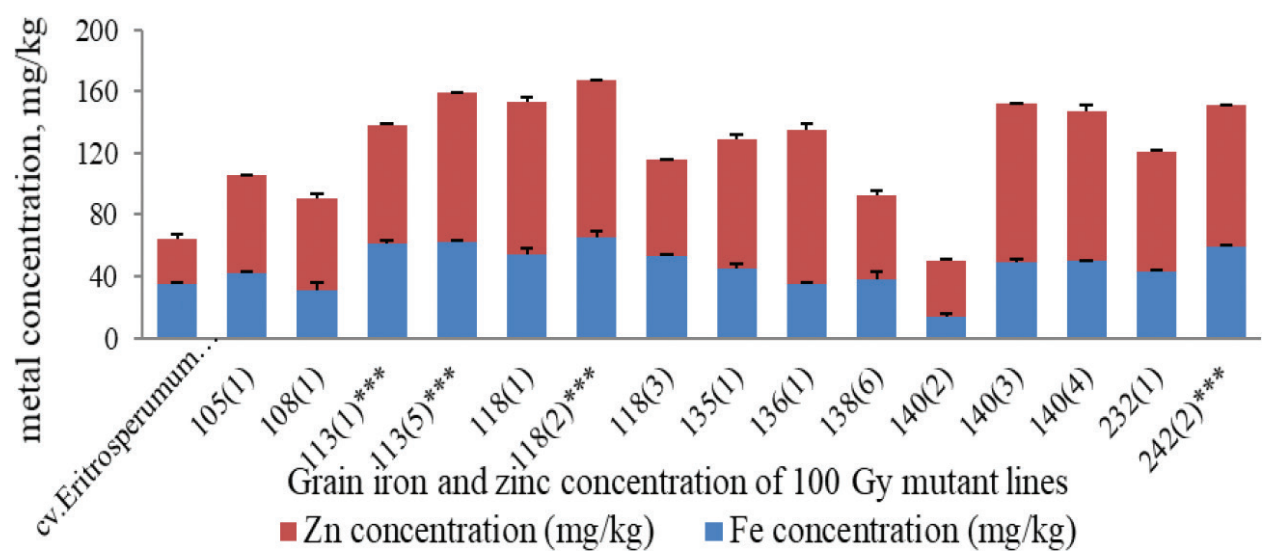
The results of comparison of mutant lines with significantly enhanced grain Fe and Zn concentrations pointed out that eight lines of 100- and 200-Gy-gamma-irradiated M<sub>5</sub> (generation 26.7%, the same number for each) had concomitant increase in both GIC and GZnC (**Figures 4 and 5**). Identification of genetically determined high GIC and GZnC in mutant germplasm and afterward development of Fe and Zn biofortified varieties is very important and promising approach. Resources with high GIC and GZnC can be used to reduce the human nutrition deficiencies in Fe, Zn, and other micronutrients [34, 35].

The effect of gamma irradiation on averages of GIC and GZnC of 100- and 200-Gy-dosed M<sub>5</sub> mutant wheat lines indicated that irradiation treatment of 100-Gy induced greater variations



**Figure 3.** Frequency distribution for wheat grain Zn concentration (GZnC) in 100- and 200-Gy-dosed M<sub>5</sub> mutant lines and parent cv. Eritrosporum-35.

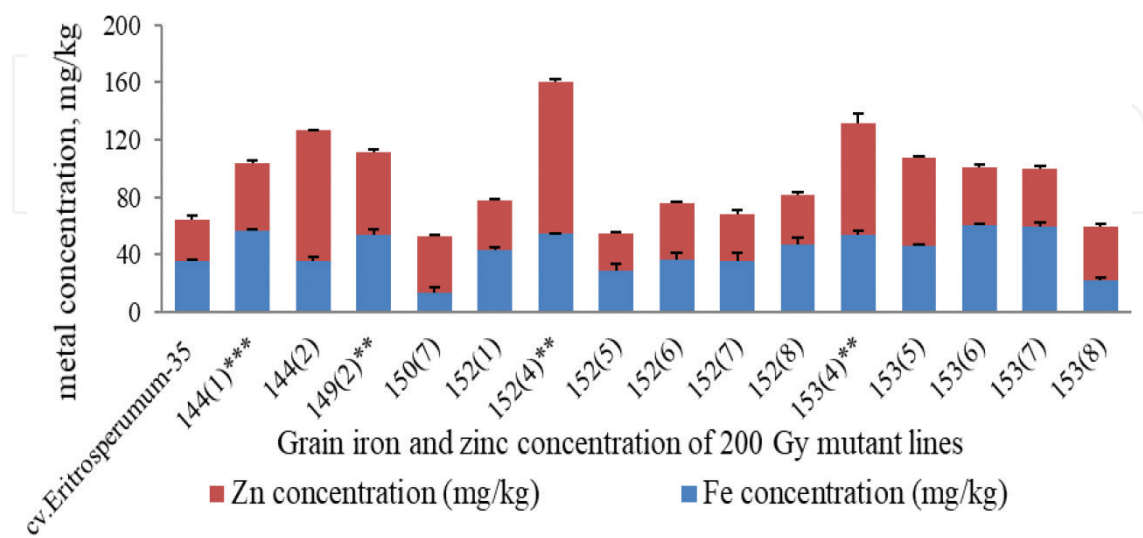




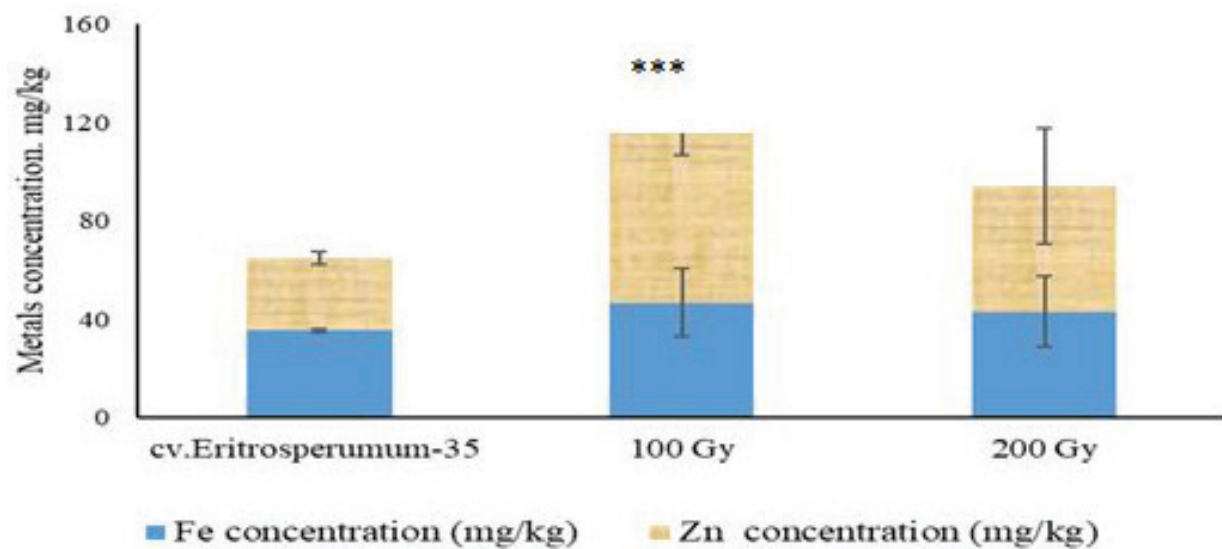
**Figure 4.** Comparison of 100 Gy-dosed  $M_5$  wheat mutant lines and parent (cv. Eritrosporum-35) with simultaneous enhancement of grain Fe and Zn concentrations.

for GZnC in comparison to GIC, and there is significant difference in GZnC between the dose of irradiation (**Figure 6**).

Spring wheat mutant lines generated by irradiation treatments were characterized by grain morphometric parameters, namely grain area (GA), grain length (GL), grain width (GW), and the grain length to width ratio (GL:GW ratio) with comparison to the parent. The GA of 100- and 200-Gy-treated mutant lines from 16.74 to 23.46 mm<sup>2</sup> and from 19.65 to 23.31 mm<sup>2</sup> with means of  $19.68 \pm 2.31$  mm<sup>2</sup> ( $n = 45$ ) and  $21.27 \pm 1.28$  mm<sup>2</sup> ( $n = 45$ ), respectively (**Figure 7A**). Among 20 genotypes (67%), mostly 200-Gy-treated lines had significantly higher GA than that of the parent on intervals between 10.95% and 34.4%.



**Figure 5.** Comparison of 200 Gy-dosed  $M_5$  wheat mutant lines and parent (cv. Eritrosporum-35) with significantly enhanced grain Fe and Zn concentrations.

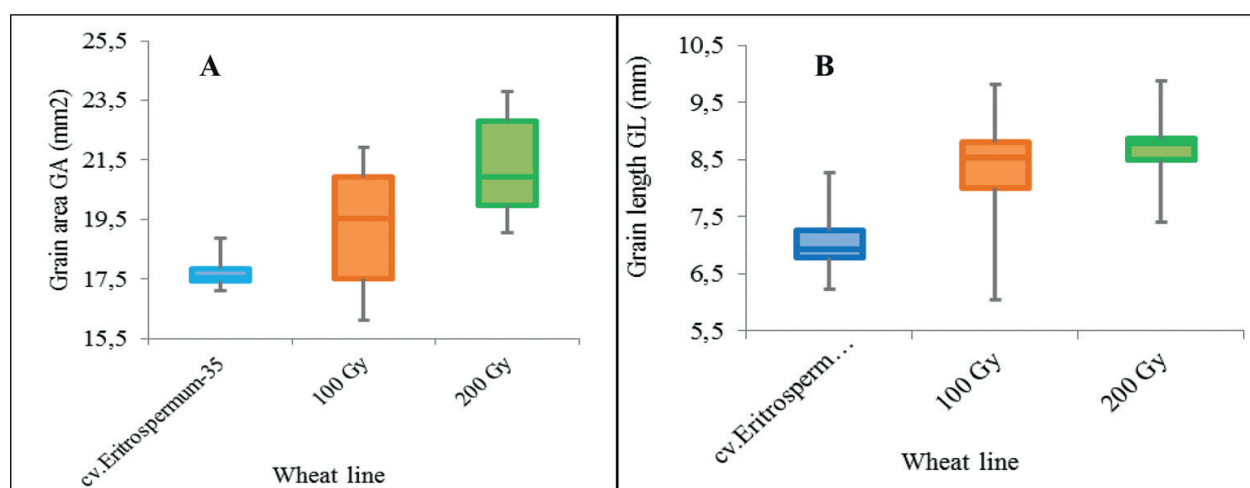


**Figure 6.** The effect of gamma irradiation on averages of grain Fe and Zn concentrations of 100 Gy and 200-Gy-dosed  $M_5$  and the parent cv. Eritrospermum-35.

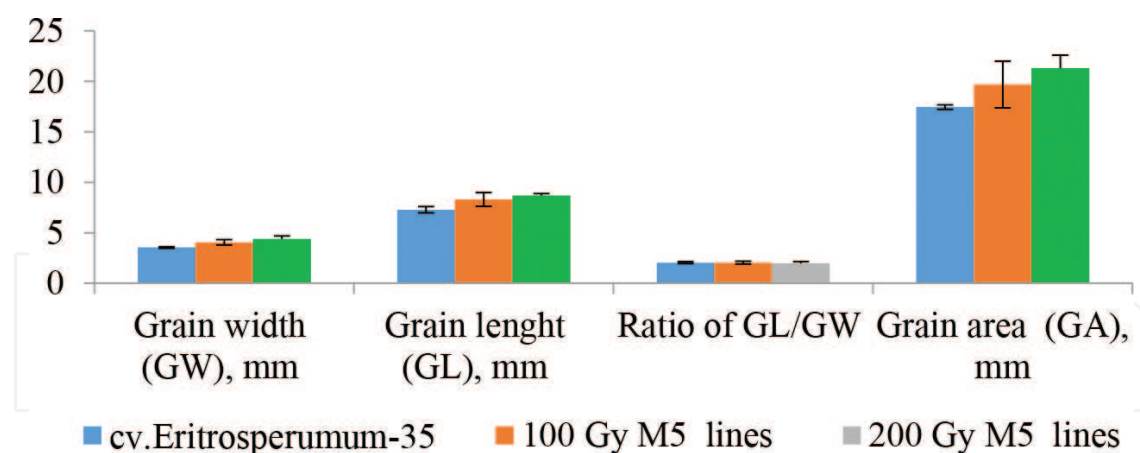
Grain length (GL) of 100- and 200-Gy-dependent mutant lines varied from 6.81 to 9.37 mm with a mean of  $8.68 \pm 0.21$  mm ( $n = 90$ ) (**Figure 7B**). The majority, 21  $M_5$  lines (77.0%), showed significantly longer grains by intervals of 12.09% and 28.0%.

The GW data of 100- and 200-Gy-dosed mutant lines ranged from 3.24 to 4.77 mm with  $4.06 \pm 0.26$  mm and from 3.65 to 4.79 mm ( $n = 225$ ) with a mean of  $4.43 \pm 0.27$ , respectively. With the exception of four 100-Gy-dependent radiation mutant lines (26.67%), most of them had significantly lower GW than the parent cv. Eritrospermum-35. Opposite, in 200-Gy-dosed lines, 12 lines (80.0%) showed significant wider grains by 12.9–13.5% than the parent.

The means of GW, GL, GL/GW ratio, and GA of  $M_5$  mutant lines generated by gamma irradiation with 100- and 200-Gy doses and the parent cv. Eritrospermum-35 are shown in **Figure 8**.



**Figure 7.** Phenotypic screening grain area (GA) (A) and grain length (GL) (B) of 100- and 200-Gy-dosed  $M_5$  lines and the parent cv. Eritrospermum-35.



**Figure 8.** Phenotypic variation in grain morphometric parameters (GW, GL, ratio of GL/GW, and GA) of  $M_5$  mutant lines generated by irradiation with 100- and 200-Gy doses and the parent cv. Eritrospermum-35. Means of GL, GW, GL/GW, and GA with standard error bars.

These results indicate that among grain morphometric parameters of mutation resources, phenotypic variation in GA and to a lesser degree GL were the most variable phenotypic traits. Variations in GW and the GL:GW ratio were moderate and less variable. Moreover, most of the longer and wider grains were found in lines developed by 200-Gy dose treatment. We did not find out the dose-dependent pattern for all grain morphometric parameters. Meanwhile, in our previous study with mutant germplasm generated on the base of cv. Almaken, we revealed that GW is a dose-dependent pattern and the 200-Gy gamma irradiation induced significant higher variation in this shape-characterizing parameter [33].

The generated by 100 Gy and 200 Gy treatments on the base of the cv. Eritrospermum-35 mutant lines were evaluated on the following yield-associated parameters: grain weight per plant (GWP), grain number per main spike (GNS), and grain weight per main spike (GWS) (**Table 1**). Among these productivity components, TGW of mutant lines showed the highest number of genotypes with significantly greater means in comparison to the parent (total 17 lines) and followed by a GWP trait (total 14 lines). The TGW in the 100- and 200-Gy-treated germplasm varied from 27.64 to 58.54 g with mean of  $46.98 \pm 8.27$  ( $n = 225$ ) and from 33.58 to 57.18 g with mean of  $46.98 \pm 8.27$  ( $n = 225$ ), respectively.

Another productivity element such as GWP in mutant lines was the most variable trait (**Table 1**). Its range was from 2.34 to 6.53 g in the 100-Gy-dosed lines with 8 genotypes (53.3%), having significant higher GWP in comparison to the parent. The variability in GWP of 200-Gy-dosed germplasm was from 1.84 to 5.37 g with mean of  $3.75 \pm 1.18$  ( $n = 225$ ). The 14 lines (47.0%) were identified as those which had significant higher GWP by 1.86–3.36 times than that of the parent.

The GNS means were  $46.98 \pm 8.27$  and  $48.69 \pm 5.95$  ( $n = 225$ ) in the 100- and 200-Gy-dosed mutant lines with their ranges of 30.33–56.67 and of 38.58–58.67, respectively (**Table 1**). When compared to parent and other mutant lines, 11 lines of 100- and 200-Gy-generated lines were identified as those having significant high GNS. Concerning the GWS trait, the differences were not significant between each of  $M_5$  lines and the parent cv. Eritrospermum-35.

Genotypes	Grain number per main spike	Grain weight per main spike (g)	Grain weight per plant (g)	1000 Grain weight (g)
cv. Eritrospermum-35	30.33 ± 8.21	1.55 ± 0.52	1.94 ± 0.07	34.12 ± 1.17
100-Gy-dosed M <sub>5</sub> mutant lines				
105(1)	38.33 ± 7.51	2.26 ± 1.25	2.34 ± 0.71	41.86 ± 1.02
108(1)	37.33 ± 5.13	1.69 ± 0.27	2.42 ± 0.51	42.17 ± 2.05
113(1)	43.67 ± 7.37	2.01 ± 0.34	2.72 ± 0.56	39.63 ± 0.78
113(5)	43.00 ± 4.36	2.22 ± 0.33	3.28 ± 1.39	45.34 ± 0.82
118(1)	40.33 ± 8.01	1.42 ± 0.53	2.70 ± 0.27	39.32 ± 1.74
118(2)	44.67 ± 1.16	2.20 ± 0.07	2.85 ± 0.34	49.97 ± 0.84**
118(3)	46.33 ± 6.25*	2.70 ± 0.47	3.61 ± 0.72*	49.88 ± 0.58**
135(1)	56.33 ± 4.47***	2.64 ± 0.45	6.53 ± 0.52***	58.54 ± 1.49***
136(1)	42.33 ± 6.11	2.53 ± 0.12	6.13 ± 0.18***	53.54 ± 2.27***
138(6)	45.66 ± 5.06	2.05 ± 0.49	6.37 ± 0.63***	50.11 ± 2.83***
140(2)	56.67 ± 3.61***	2.77 ± 0.67	4.01 ± 0.73**	57.23 ± 1.78***
140(3)	40.00 ± 2.03	1.91 ± 0.16	3.13 ± 1.04	56.94 ± 2.16***
140(4)	42.33 ± 4.93	1.88 ± 0.17	3.68 ± 0.32*	48.51 ± 2.38***
232(1)	30.33 ± 5.51	1.46 ± 0.38	2.48 ± 0.46	27.64 ± 1.56
242(2)	47.67 ± 7.02**	2.27 ± 0.49	2.61 ± 0.23	43.98 ± 2.32
200-Gy-dosed M <sub>5</sub> mutant lines				
144(1)	44.33 ± 3.79	2.22 ± 0.21	4.32 ± 2.28***	49.67 ± 1.89**
144(2)	41.10 ± 3.52	1.80 ± 0.03	1.84 ± 0.41	33.58 ± 2.49
149(2)	55.34 ± 2.31***	2.63 ± 0.04	5.37 ± 0.64***	57.18 ± 1.23***
150(7)	52.34 ± 3.65***	2.29 ± 0.38	3.01 ± 0.23	49.89 ± 1.24**
152(1)	47.33 ± 6.66**	2.37 ± 0.35	3.75 ± 0.42*	46.21 ± 2.75*
152(3)	43.33 ± 1.53	2.12 ± 0.53	2.59 ± 0.96	39.85 ± 2.16
152(4)	42.00 ± 3.01	1.88 ± 0.30	2.48 ± 0.60	46.86 ± 2.33*
152(5)	50.43 ± 4.04**	2.43 ± 0.12	4.28 ± 0.96***	51.77 ± 1.39***
152(6)	42.67 ± 4.62	2.22 ± 0.16	3.07 ± 0.21	49.18 ± 2.42
152(7)	41.33 ± 1.53	1.92 ± 0.04	2.24 ± 0.65	45.59 ± 1.88
152(8)	38.00 ± 4.58	1.73 ± 0.23	3.14 ± 0.47	43.23 ± 1.68
153(4)	39.67 ± 2.52	1.83 ± 0.07	3.22 ± 0.23	45.87 ± 1.42
153(5)	58.67 ± 4.02***	2.87 ± 0.58	4.43 ± 0.87***	53.77 ± 1.86***
153(6)	51.75 ± 3.42**	2.48 ± 0.78	5.97 ± 1.37***	54.56 ± 1.38***
153(7)	44.30 ± 3.69	2.07 ± 0.32	5.03 ± 0.74***	54.17 ± 2.36***
153(8)	51.00 ± 3.84**	2.21 ± 0.49	4.16 ± 1.16***	49.79 ± 1.86

\*, \*\*, and \*\*\* denote significance at 0.05, 0.01, and 0.001 probability levels, respectively. The lines are significantly different from parent line. Grain number and weight per main spike, grain weight per plant are a means of 15 randomly selected spikes/plants.

**Table 1.** Comparing yield-associated traits of advanced spring wheat M<sub>5</sub> mutant lines developed using 100 Gy and 200 Gy and the parent cv. Eritrospermum-35.

Comparing yield-associated traits of Eritrosporum-35 spring wheat  $M_5$  mutant lines generated by treatments of 100 Gy and 200 Gy showed that 8 lines numbered by 118(3), 135(1), 140(2), 149(2), 152(1), 152(5), 153(5), 153(6) and accounting for 26.7% of the total number of mutated genotypes had simultaneous significant higher GNS, GWP, and TGW than the parent (**Table 1**). In our previous study with mutant germplasm developed on cv. Almaken, we were able to generate only three lines which had high GNS, GWP, and TGW [33]. This number is less than the Eritrosporum-35 mutant lines.

Thus, the radiation doses of 100 Gy and 200 Gy had generated mutations with respect to components of productivity such as GWS, GWP, and TGW in comparison to the parent, with the greatest variation being for GWP and TGW. High GWP and TGW of these mutant populations indicate that genetic background of variety selected for irradiation treatment is of great importance to generate broad and valuable variability in productivity traits (**Table 1**).

One of the important outputs revealed in the present study was the correlation between concentrations of grain nutrients, grain characteristics, and plant productivity. Parent variety showed a significant correlation of TGW with GNS and grain morphometric parameter of GW with yield-associated components such as TGW and GWS (**Table 2**). There was no relation between grain quality characteristics (GPC, GIC, and GZnC) and plant productivity and grain morphometry.

In 100-Gy-dosed  $M_5$  mutant lines, there was significant correlation of productivity components such as GNS with GWS, GNS with GWP (**Table 2**). Both grain metals, namely GZnC and GIC, were related to each other indicating that metal accumulation may be controlled by the same loci. These results which describe positive correlation between grain Zn and Fe concentrations are similar to those which were observed in domesticated wheat and synthetic hexaploids [31]. This fact suggested that the alleles for Zn and Fe deposition co-segregate or are pleiotropic effect and therefore grain metal concentrations possibly improve metals accumulation simultaneously. It was also found that another nutritional value parameter such as GPC was significantly correlated with one of the grain morphometric parameter such as GW.

This finding suggests that this grain quality character may be genetically linked to grain morphometry. In our studies reported for Almaken mutant lines such kind of relation was revealed for GA, GL, and GW at 200-Gy gamma irradiation treatment [33]. Concerning correlations between parameters characterizing grain size and shape, we determined that there was a high relation of GA with GL and GW for 200-Gy-gamma irradiated population.

Similar to 100-Gy-dosed  $M_5$  mutant germplasm, GNS was significantly related to GWS with high  $r^2$  mean as well as grain metals, GZnC and GIC, were correlated to each other in 200-Gy-dosed  $M_5$  mutant lines (**Table 2**). The interesting fact, which was only revealed for these lines, is that GIC except GZnC significantly related to GPC. Thus, in mutant populations, both metals, GIC and GZnC, are associated with each other. For parameters characterizing grain morphometry, we revealed that there were high correlations of GW with GA and GL.



	GWS (g)	GWP (g)	TGW (g)	GPC (%)	GIC (mg/kg)	GZnC (mg/g)	GL (mm)	GW (mm)	GA (mm <sup>2</sup> )
cv. Eritrospermum-35									
GNS	0.003	0.115	0.171*	0.056	0.001	0.012	0.011	0.006	0.008
GWS		0.089	0.021	0.007	0.000	0.000	0.078	0.142*	0.004
GWP			0.155	0.005	0.000	0.007	0.120	0.042	0.096
TGW				0.011	0.003	0.065	0.008	0.163**	0.000
GPC					0.021	0.008	0.004	0.001	0.000
GIC						0.000	0.001	0.014	0.008
GZnC							0.030	0.045	0.008
GL								0.115	0.017
GW								—	0.008
GNS	0.470***	0.176**	0.051	0.093	0.000	0.001	0.003	0.043	0.036
GWS)		0.099	0.002	0.038	0.000	0.017	0.019	0.073	0.110
GWP			0.030	0.098	0.083	0.002	0.062	0.071	0.087
TGW				0.050	0.033	0.044	0.010	0.046	0.079
GPC					0.025	0.031	0.063	0.107*	0.020
GIC						0.417***	0.000	0.006	0.064
GZnC							0.000	0.025	0.038
GL								0.211*	0.048
GW									0.181*
GNS	0.870***	0.070	0.018	0.048	0.014	0.038	0.011	0.074	0.016
GWS		0.071	0.064	0.011	0.011	0.050	0.088	0.028	0.020
GWP			0.004	0.043	0.072	0.122	0.021	0.006	0.002
TGW				0.059	0.034	0.000	0.018	0.006	0.015
GPC					0.251*	0.036	0.019	0.002	0.144
GIC						0.224*	0.035	0.004	0.000
GZnC							0.045	0.000	0.044
GL								0.400***	0.451***
GW									0.503***

\*, \*\*, and \*\*\* denote significance at 0.05, 0.01, and 0.001 probability levels, respectively.

**Table 2.** R2 correlation between yield-associated traits (TWG, GNS, GWS, and GWP) grain protein content and micro-elements concentrations, and grain morphometric parameters (GA, GL, GW, and GL:GW ratio) in cv. Eritrospermum-35 parent and 100 Gy-(orange) and 200-Gy-(gray) M<sub>5</sub> mutant lines.

## 4. Conclusions

This study aimed to develop  $M_5$  mutant lines of spring wheat by 100- and 200-Gy gamma irradiation treatment and identify genetic variability in grain nutritional value including grain protein content, grain Fe and Zn concentrations, and yield-associated traits such as grain number and weight per main spike, grain weight per plant, and 1000-grain weight and grain morphometric parameters, namely grain area, length, and width and  $M_5$  mutant lines in comparison to the cv. Eritrospermum-35 parent. We identified mutant lines with high Fe and Zn concentrations and grain protein content as those which have 1.6–3.4 and 1.4–2.9 times more as well as 3.7–16.9% than target parameters of parent variety, respectively. Several mutant lines showed significantly simultaneous increase of metals concentrations. The positive correlation of grain protein content with grain width suggests that they are genetically related. This relation could be a good candidate for evaluating genotypes for improvement of grain protein content. Wheat grain can be biofortified by micronutrients without negative impact on crop productivity using new mutant lines. Mutation breeding can significantly contribute to human health malnutrition and improve nutrition quality diet.

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## Conflict of interest

The authors have declared that no competing interests exist.

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