TEST Engineering & Management

May – June 2020
ISSN: 0193-4120 Page No. 9400 - 9409

Relations of Mutation of Spring Wheat Resources with Improved Grain Bioavailability of Iron and Zinc

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Article Info
Volume 83
Page Number: 9400 - 9409
Publication Issue:
May - June 2020

Article History
***Article Received**: 19 November 2019*
***Revised**: 27 January 2020*
***Accepted**: 24 February 2020*
***Publication**: 18 May 2020*

Abstract:
Wheat varieties have low bioavailability of grain micronutrients for human balanced nutrition. Therefore, there is a need a genetic enhancement by micronutrient of crops (bio fortification) to provide a cost-effective way of diminishing malnutrition. In our study, genetic variability of spring common wheat was broaden by treatment of gamma radiation at 100 Gy and 200 Gy using ^{60}Co source and Kazakhstan variety of the parent Almaken. Mutant lines (M_5 generation) were evaluated for grain phytic acid content (PhyC) and molar rations of Phy: Fe and Phy: Zn to estimate microelements bio availabilities and correlations between quality characteristics and grain size and grain shape parameters. Significant variations in grain PhyC among mutant lines were found. Several mutant lines with significantly decreased PhyC by 1.6–4.62 times, than that the parent was identified. There was wide variation in grain Fe and Zn bioavailability calculated by grain molar rations of Phy: Fe and Phy: Zn in mutant lines. The highest microelements bio availability revealed in mutant lines exceeded that the parental cultivar by 2.87- and 3.06- fold, respectively. The molar ratio of Phy: Fe significantly correlated with grain width but not with grain length and area only in 200 Gy-dosed mutants, suggesting there is considerable scope to improve Fe bio availability and grain width simultaneously and also with grain ZnC.

***Keywords**: Grain content of phytic acid,Spring wheat M_5 mutant lines,Grain Bioavailability of Iron and Zinc*

Introduction:

Bread wheat as key cereal has overall significance in food security. Wheat grain is a principle micronutrients source at human nutrition (Shewry and Hey, 2015). However, cultivated wheat varieties, generally, have grain low content of micronutrients and their bioavailability including Kazakh varieties for balanced nutrition. Therefore, cereal crop genetic improvement of micronutrient (bio fortification) is needed which provide a cost-effective way of diminishing global malnutrition.

Micronutrients malnutrition is widespread throughout the world. The iron (Fe) and zinc (Zn) shortage is especially acute, affecting the half population in the world (Welch and Graham, 2004). The solution of Fe deficit has become one of priorities of ministry of health of Central Asia and Kazakhstan and the states as a whole. Insufficient consumption of needed micronutrients for human activity leads to disturbance conditioned to illness, low health, repressing various types of children abilities and high economic and social costs (De Benoist et al., 2008). For agricultural production, it is necessary to guarantee expedient products with good quantity of micronutrients in order to maintain the human vital activity. Wherein, a majority of developing countries agriculture could not correspond those demands **(**Godfrayet al. 2010**)**.

Wheat products are abundant by anti-nutrients, primarily, phytic acid (Phy), which inhibites the micronutrients intake or utilization by humans (FAO, 2014. FAOSTAT. www.fao.org**)**. Phy-tic acid (Phy, myo-inositol 1,2,3,4.5,6-hexakisphosphate) presents a basic keeping type of phosphate (P), characteristically composing 1/3 of the seed total P level. The Phy strongly affects the bioavailability of metals due to the powerful chelated ability with each other, forming phytate salt (Shahzadet al. 2015). High bioavailability micronutrients could be attained by the decline of anti nutritional compounds. High phy content is one of the most significant restrictive feature decreasing bioavailability of macro- and microelements by chelation (Bouis and Saltzman, 2017). Micronutrients absorption from wheat foods are substantially conditioned by amount obtainable for human intake. On the whole, cereal crop grains have very small Fe and Zn bio availabilities, about 5% and around 25%, respectively.

Prosperous breeding for yield related features and improvement of nutritional magnitude of crops foods are in need genetic variability which have to be discernible from environments. A variability of cereals dramatically declines as a result of the frequent utilization of native accessions and a spread of cultivation design locally developed which are not advantage for genetic recombination (http://mvgs.iaea.org). Mutagenesis is a strong approach to widen genetic variability and was utilized for yield magnification but has been used less for increasing nutritional quality.

For generating new genetic variability in most of cereal crops mutagenesis is particularly effective due to they are characterized by restricted genetic variability (Parry et al. 2009). In cereals breeding, mutant resources are not reflected by public concern and not considered as GMO and are widely allocated in many countries. Mutagenesis expands the scores of genetic improvement in cereals grain quality. Bio fortification is a productive tool that decreases the break between agriculture production and health, and adds other supplements corresponding fortification, ration diversification and complement schedules (Gomez-Galera et al., 2010; Zhao and Shewry , 2011). New, bio fortified varieties have increased level of micronutrients, in additions, they agronomical competitive in comparison with non-biofortified cultivars on yield, biotic and abiotic resistance, and market ability characteristics. Biofortification is a relatively cheap and valuable way for diminishing micronutrient malnutrition in most of countries (Borrill et al., 2014).

Seeds and grains with low PhyC is reasonable goal e to enhance nutritional bioavailability of any crop. Significant efforts to reduce Phy have been made through mutagenesis of cereals. Mutants with low PhyC (*lpa*) of several cereals were developed through mutagenesis by chemical and physical treatments (Guttieri et al., 2004; Liy et al., 2007; Raboy et al., 2010, Badone et al., 2012).

Grain characteristics such as size and shape are substantial breeding goal due to they are the stable of productivity parameters in terms of phenotypical response (Gegas et al. 2010). These grain characteristics have also significant impact on the many end-use properties such as milling yield, flour yield and starch breaking. Bigger grains have powerful impact into greater weight of grains. Grain weight is widely used in improvement of wheat varieties and it is regarded as dependent on grain size or shape as the parameters, which define individual grain packages.

Addressing all of these issues, our previous efforts were centered on developing new spring wheat M_5 mutant lines on the genetic basis of cv. Almaken released in Kazakhstan to identify the valuable genotypes with significantly higher components of productivity, such as weight and number of grains on major ear, weight of grains on whole plant, 1000 grains weight, morphometrical characteristics of grain and some quality parameters. Several mutant lines developed mostly through 200 Gy irradiation dose showed 2 to 4 times larger grain Fe and Zn content and increase in protein content by 7-11% relative to the parent. Some irradiated lines had significantly greater, grain area, length and width (Kenzhebayeva et al., 2017).

This study aimed at evaluating: (1) the variability in grain Phy content (Phy C), molar rations of Phy: Fe and Phy: Zn to estimate the metals bioavailability, (2) the correlations between PhyC and Phy: Fe and Phy: Zn with grain size and shape parameters among Almaken spring wheat M_5 mutant lines which were produced after low (100 Gy) and high (200 Gy) gamma treatments relatively parent, cv. Almaken.

2. Plants material and methods

2.1. Plants and use of mutagenesis by gamma irradiation treatments

The grains of spring bread wheat variety Almaken (*T. aestivum* L.) were treated by low (100 Gy) and high (200 Gy) dosages using ^{60}Co source (Kazakh Nuclear Center). To raise M_2 plantswheat irradiated grains were planted. The M_2- M_5 plants along with cv. Almaken were field grown near Almaty (43°15′N, 76°54′E, and elevation 550 m above mean sea level) (kazakh research institute of agriculture and plant growing) (Kenzhebayevaet al., 2017). The M_5 plants were harvested, then 15 advanced 100 Gy treated mutant lines were tested. We numbered these Almaken M_5 mutant lines as: 75/2, 76/2, 76/3, 79(1), 79/5, 81/1, 82/2, 82/4, 82/5, 84/2, 84/4, 89/5, 89(8) 91/1 and 91/2. The other 200 Gy-irradiated 15 lines selected had the following number: 94/4, 95/2/, 95/3, 95/5, 95/7, 95/8, 98/1, 98/2, 98/4, 98/6, 101/1, 101/3, 101/5 and 101/6. These two mutant populations, 100 Gy and 200 Gy levels of radiation, were analyzed for grain PhyC, molar rations of Phy: Fe and Phy: Zn characterizing metals bioavailability, and the correlations between molar rations of Phy: Fe and Phy: Zn and grain morphometry analysis including length of grain (GL), width of grain (GW) and area of grain (GA).

2.2. Determination of grain morphometric parameters and analysis of grain FeC and ZnC

Grain morphology measurements were made with the Win RHIZO, system of image measurement, version 1.38, 2007 (Reagent Instruments Inc., Canada), the results of analysis data of GA, GL and GW are presented (Kenzhebayeva et al. 2017). The digestion, extraction procedures and the following estimation of FeC and ZnC were carried out on grain sample (0.2 g) and described (Kenzhebayeva et al. 2017).

2.3. Determination of phytic acid content in grain sample

For PhyC analysis, we used a Megazyme kit (Megazyme, Ireland 2016). Some modifications to the protocol were provided. Whole-meal flour from 2,0 g sample of grains was obtained by use of a mixer mill (Retsch MM400 GmbH). Half of the resulting sample was subjected to digestion with 10 mL of HCl (0.66 M) for overnight (15 h) at RT by constant stirring. The extract (1 mL) was centrifuged (13 000 r.p.m, 10 min). The neutralization of extract was carried out by the addition of the equal volume of NaOH (0.75 M). The solution obtained was used for reaction of dephosporylation according to the official Megazyme kit protocol. The P calibration curve was prepared following the protocol. The P free, total P (phosphorus) and PhyC levels of the grain samples were calculated based on Megazyme protocol. Grain P free, total P and PhyC were expressed as g/100 g.

2.4. Measurement of grain phosphorus content

The extraction of grains free and total phosphorus (P) were done by 0.66 M HCl according to Megazyme method. The both P leves were analyzed using colour reagent containing 10 % w/v of ascorbic acid, M of sulphuric acid (1 M) and 5% w/v of solution of ammonium molybdate, (5:1) (McKie and Mccleary, 2016). The complex of resulting colored solution was determine at 655 nm.

2.4. Calculation of graqin molar ratio of Phy: Fe and molar ratio of Phy: Zn

To determine grain molar ratio of Phy: Fe and molar ratio of Phy: Zn, the contents of grain Phy, microelements (Fe and Zn) were presented in moles by using molar mass and weight of Phy (660.04), Fe (55.82) and Zn (65.4) g/mol.

2.5. Statistical Analysis

Assay of all results was conducted by r core team, 2013. The Dunnett contrasts, simultaneous tests of general linear hypotheses were applied to multiply compare the means. Summary data are presented as means ± standard deviation. The R-squared value R^2correlation coefficients were used to test for a linear relationship between grain parameters. When testing significance, R^2 values are more meaningful as they describe the amount of covariation explained by an R value and these are shown as % values. A *P* significantly was statistical value ≤ 0.05. One way Anova (single factor) analysis using excel 2016 and the mean values were calculated by test of Tukey at significance level, $P<0.05$.

3. Results and Discussion

Micronutrients absorption from products of wheat is basically definited by their presence in diet intake of people. Acceptable bioavailability of micronutrients could be reached through diminution of anti-nutritional compounds. The Phy being the most basic components declines bioavailability of micronutrients due to their strong chelated ability (Shahzad et al. 2014). The anti-diet impact of Phy is especially considered as key point in terms of microelements (Fe and Zn), due to Phy is a crucial anti-nutritional agent decreasing the bioavailability of these metals. In plant seeds Phy is found in the form of phytin being an insoluble salt complex with many cations. In cereal crops grain, in storage vacuoles, phytin exist as globoids presenting crystalloid globular inclusions. The composition of the mature grain globoids includes phosphorous (P), potassium, K, calcium, Ca and magnesium, Mg; microelements: copper, Cu, Fe and Zn, sulfur, S, sodium, Na, protein (Bohn et al. 2007).

Evaluation of spring wheat Almaken mutant M_5 lines obtained by low (100 Gy) and high (200 Gy) irradiation treatments for grain PhyC showed considerable genetic variation among genotypes (Figure 1). The ranges of grain PhyC were in intervals of 0.43 to 1.54 g/100 g (3.58-time variation) in all tested mutant lines. The identified grain PhyC values ranges determine the variability that obtain for the pooled cv. Almaken and gamma-treated lines irradiated lines grown at same environmental conditions. Relative to parent, cv. Almaken, PhyC was significantly lower by 1.43 to 3.12-time in 11 mutant lines (37%).

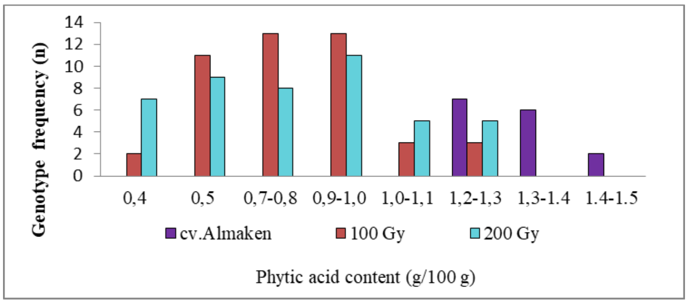

Figure 1. Frequency distribution for spring wheat grainphytic acid content (PhyC) in the parent, cv. Almaken, mutant M_5 lines obtained by low (100 Gy) and high (200 Gy) irradiation treatments

Low PhyC is a key breeding goal to increase grain bioavailability of nutrients. For the decline of PhyC, low phytate cereal and soybean mutants (*lpa*) were generated using chemical and physical mutagenesis (Raboy, 2002, 2009, Punjabi et al. 2018, Qamar et al. 2019). Mutagenesis by chemical agent was applied to develop *lpa* wheatmutant (Guttieri et al. 2004). For breeding high inorganic phosphate (HIP) phenotypes maize *lpa* mutants have been utilized (Badoneet al. 2012). The *lpa* mutations found in a number of cereal crops pleiotropically affected on plant growth displaying as decreased emergence and germination, reduced productivity, and susceptibility to stress factors (Badoneet al. 2012). Recently, to conceive this objective the construct of inositol polyphosphate 6-/3-/5-kinase gene-specific RNAi was designed and expressed in the seeds of soybean cultivar of Pusa-16 (Punjabi et al. 2018). Our experiments indicated that spring wheat M_5 lines having low Phy level developed on background of cv. Almaken through low (100 Gy) and high (200 Gy) treatments did not significantly differ in grain germination ability, shoots and roots growth of 7-days seedlings comparing to parent (the results not present).

A great difference of Phy level was shown in a number of studies in which PhyC of wheat natural variation ranged from 5.9 to 45.4 mg/g (Ahmad et al., 2013; Liu et al., 2017). Apparently, these results are inconsistent related to the methodological differences applied for analysis of PhyC. In this context as noted by Gibson et al., 2010, the choice of suitable method for PhyC measurement is decisive factor.

We estimated total and free phosphorus (P) levels of screened spring wheat grains of cv. Almaken and low (100 Gy) and high (200 Gy) treated mutant M_5 lines. Table 1 presents the means and ranges of these measurements. The highest total P content was revealed in cv. Almaken. Its PhyC is amounted 27.7±1.3 % of grain total P level. Total P content varied from 1.35–4.53 g/100 g with mean of 3.42 ± 0.88 in all mutant M_5 lines (n = 90). Percent Phy out of total Pi accounted for the ranges of 16.0-43.51 (Table 1). Noted earlier for cereals and legumes, near 60-90 % of total phosphorus were presented in the form of Phy phosphorus (Wu et al. 2009, Madsen and H. Brinch-Pedersen, 2019).

Considerable variation in free P levels was also found among mutant M_5 spring wheatlines generated through treatments of low (100 Gy) and high (200 Gy) dosages and cv. Almaken (Table (1).

Table (1): Comparison between free and total phosphorus (P) means and ranges for spring wheat parent, cv. Almaken and M_5 100 Gy and 200 Gy – developed mutant lines. The measurements are presented as averages and ranges (n=90).

Grain characteristics	cv. Almaken		low (100 Gy) lines		high (200 Gy) lines	
	average	range	average	range	average	range
Free (P), g/100 g of dry matter	1.75	1.70–1.90	1.20	0.44–2.43	1.11	0.13–1.86
Total P, g/100 g of dry matter	4.55	4.30–4.70	3.46	1.35–4.17	3.37	1.40–4.53
% Phy out of total P	27.7	26.3-28.5	30.7	17.30-35.08	31.3	16.00-43.51

Note. Analysis of each line was carried out by Each line was triplicate analyzed .

The micronutrients bioavailability for human intake is defined as molar rations of Phy: metals As a rule, molar ratio with low value determines high mineral bioavailability and the same on the contrary (Gibsonet al. 2010). Based on the results of PhyC (Figure 1) and measurements of grain FeC and ZnC shown earlier (Kenzhebayeva et al. 2017)for parent, cv. Almaken and mutant M_5 lines of low (100 Gy) and high (200 Gy) irradiation treatments, we calculated molar rations of Phy: microelements (Fe and Zn). These data are presented on figures 2 - 3, respectively. The molar rations of Phy: microelements significantly varied for cv. Almaken, and spring wheat mutant M_5 lines with great ranges of 0.47-7.05 for grain Phy: Femolar ratios and 0.47-5.24 for grain Phy: Zn molar ratios. The significantly lower means of PA:Fe comparing to cv. Almaken and therefore meaning high metals bioavailability, were revealed in 11 genotypes (73.0%) and 9 genotypes (60 %)of low (100 Gy) and high (200 Gy) lines, respectively. Accordingly, significantly enhanced metals bioavailability related to the low means of Phy: Fe molar ratios exceededthe parent, cv. Almaken, in the ranges of 1.71-6.43-fold for both doses. For PA: Zn molar ratios we identified 6 genotypes (40.0%) and 9 genotypes (60.0 %) of 100 Gy- and 200 Gy-dosed lines, respectively, with significantly lower means than cv. Almaken, in the ranges of 1.78-6.02-fold.

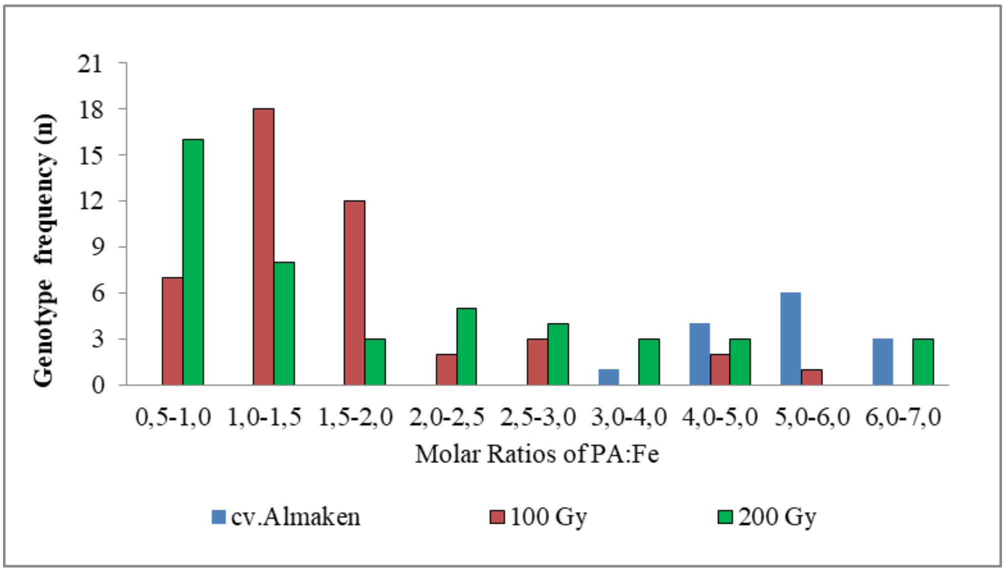

Figure 2. Distribution of frequency of grain Phy: Femolar ratios in parent, cv. Almaken, low (100 Gy) and high (200 Gy) dosages developed mutant M_5 spring wheat lines.

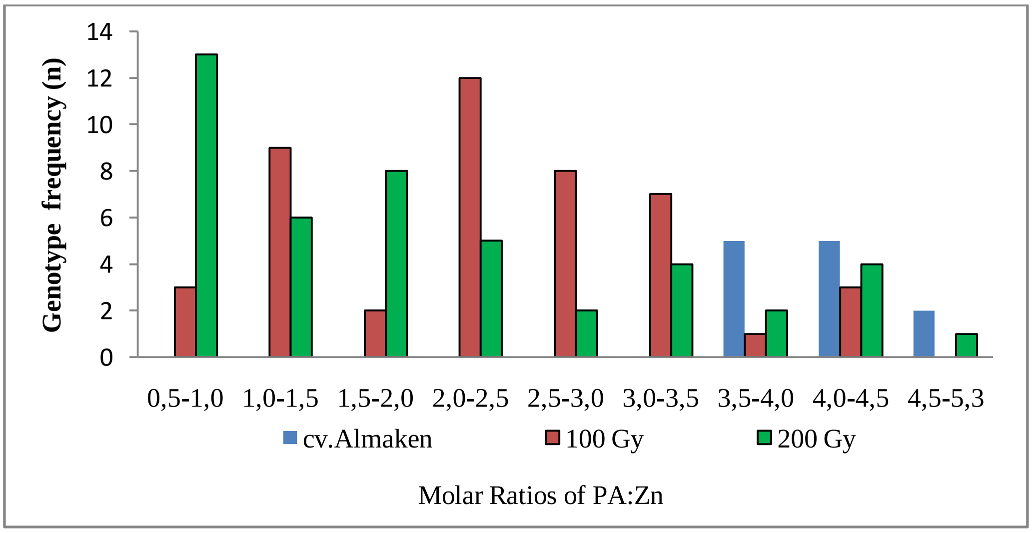

Figure 3. Distribution of frequency of grain Phy: Zn molar ratios in parent, cv. Almaken, low (100 Gy) and high (200 Gy) dosages developed mutant M_5 spring wheat lines.

Analysis of variance (one way ANOVA) for differences in PhyC, grain Phy: Femolar ratios, and grain Phy: Zn molar rationsamong cv. Almaken and irradiated mutant M_5 lines is shown in Table 2. These assessments indicate that the cv. Almaken and 100 Gy and 200 Gy developed mutant lines significantly differed in terms of investigated grain characteristics (Table 2). The interaction between low (100 Gy) and high (200 Gy) treated mutant M_5 lines was no significant on all traits. The greater impact of 200 Gy radiation than that of 100 Gy for PhyC and Phy: Fe molar rations possibly shows it’s more influence for their improvement and may indicate it’s more efficiency to induce mutations in genes related to these grain characteristics.

Table (2): Comparison of grain phytic acid content (PhyC) and molar rations of Phy: Fe and Phy: Zn of mutant M_5 spring wheat lines and parent, cv. Almaken.

Variation of source	cv. Almaken x low (100 Gy) developedmutant lines	cv. Almaken x high (200 Gy) developed mutant lines	low (100 Gy) x high (200 Gy) developed mutant lines
df	89	89	89
PhyC (g/100 g)	28.21***	20.20***	0.0
Phy:Fe	38.22***	113.59***	0.88
Phy:Zn	94.56***	105.68***	2.19

Note. The results are shown by percent of total sum from analysis of variance (ANOVA). The significant difference of the lines from the parent, cv. Almaken. Asterisks signify: *, ** and ***, $P < 0.05$, 0.01 and 0.001, respectively.

To evaluate the relationshipsbetween grain metals content, PhyC, molar rations of Phy: Fe and Phy:Zn and grain characteristics related to morphometry (grain lenght, width and area), the R^2 coefficient of determination as statistical measurements was carried out. These data are shown in Table 3. In parent, cv. Almaken, the correlations between PhyC and molar rations of Phy: Zn ($R^2 = 0.65$, $P < 0.001$) and ZnC with Phy: Zn ($R^2 = 0.73$, $P < 0.001$) were highly significant. There were no significant relationships of all the quality parameters with grain morphometric parameters (grain length, width and area).

In low (100 Gy) dosed spring wheat M_5 mutant lines, PhyC significantly correlated with FeC ($R^2 = 0.12$, $P < 0.001$), with ZnC ($R^2 = 0.38$, $P < 0.001$) and more highly with Phy: Fe ($R^2 = 0.66$, $P < 0.001$) and with Phy:Zn ($R^2 = 0.73$, $P < 0.001$) (Table 3). Similar to the parent, cv. Almaken, no significant correlation was observed between nutritional traits and grain morphometric characteristics.

For high (200 Gy) treated mutant lines it was revealed, regarding grain metals analysis, PhyC was highly significant related to ZnC ($R^2 = 0.81$, $P < 0.001$) and molar rations of Phy: Fe and molar rations of Phy: Zn ($R^2 = 0.48$, $P < 0.001$ and ($R^2 = 0.72$, $P < 0.001$), respectively) (Table 3). At 200 Gy irradiation dose there were significant relationships of Phy: Fe molar rations with ZnC and also with molar rations of Phy: Zn. Notably, only in this dose response lines Phy: Fe molar ration significantly correlated with grain shape morphometric characteristic such as GW. The last finding as significant correlation between Phy: Fe and GW ($R^2 = 0.216$, $P < 0.05$) suggest that wider grain may be related to increase of Fe bio availability.

Table (3): R^2 correlation coefficients between grain metals concentrations, PhyC, molar rations of Phy: Fe and Phy: Zn and morphometric grain characteristics (length of grain, width and area) in parent, cv. Almaken, and low (100 Gy) and high (200 Gy) developed spring wheat mutant M_5 lines with *P* values denoted by asterisks.

Grain traits	PhyC , g/100 g	Molar rations of Phy: Fe	Molar rations of Phy: Zn	Length of grain GL, mm	Grain width, GW, mm	Grain area, GA, mm^2	FeC, mg/kg
cv. Almaken							
Phy:Fe	0.33						
Phy:Zn	0.65***	0.20					
GL	0.08	0.0012	0.0266				
GW	0.01	0.0005	0.0006	0.95			
GA	0.18	0.05	0.01	0.99	0.9		

		31	76		9		
FeC, mg/kg	0.04	0.46	0.03	0.14	0.43	0.27	
ZnC, mg/kg	0.18	0.09	0.73 ***	0.91	0.75	0.85	0.04
low (100 Gy) developed mutant M_5 lines							
Phy:Fe	0.66 ***						
Phy:Zn	0.81 ***	0.73 ***					
GL	0.005	0.013	0.030				
GW	0.07	0.070	0.0479	0.001			
GA	0.02	0.102	0.02	0.04	0.34*		
FeC, ng/kg	0.12 ***	0.52	0.25 **	0.001	0.04	0.10	
ZnC, ng/kg	0.38 ***	0.30 ***	0.71 ***	0.01	0.02	0.04	0.15**
high (200 Gy) developed mutant M_5 lines							
Phy:Fe	0.48 ***						
Phy:Zn	0.72 ***	0.54 ***					
GL	0.09	0.1442	0.0868				
GW	0.20	0.216*	0.1969	0.25 ***			
GA	0.16	0.184	0.1563	0.37 ***	0.19**		
FeC, ng/kg	0.16	0.66	0.16	0.02	0.05	0.05	
ZnC, ng/kg	0.81 ***	0.40 ***	0.82 ***	0.07	0.06	0.19**	0.05

CONCLUSION

The results presented in the given study revealed the considerable variability in grain anti nutrient content (PhyC) and molar rations of Phy: Fe and Phy: Zn in new mutant M_5 spring wheat lines that were developed through low (100 Gy) and high (200 Gy) dosages on cv. Almaken background. This finding suggest that donors for grain low PhyC and high Fe and Zn bio availability to be address among mutant resources and also that the genetic backgrounds and the factors by which they are created play an important role. Overall, the relations between the tested grain quality characteristics were identified as higher for low (100 Gy) and high (200 Gy) developed mutant M_5 lines than cv. Almaken. We found that in developed mutant M_5spring wheat lines by 200 Gy treatment, molar rations of Phy: Fe significantly and positively correlated with grain shape parameter such as GW only in the 200 Gy-dosed lines suggesting there is considerable scope to improve Fe bioavailability and grain width simultaneously. The distinguishing significant ability in both low (100 Gy) and high (200 Gy) developed mutant M_5 lines was the relation between molar rations of Phy: Fe with ZnC and Phy:Zn which was not revealed in the parent cv. Almaken. The advanced mutant lines identified could have the contribution to develop mutant varieties with adequate contents of bioavailable microelements, which could result in to the creation of varied products abundant by the target metals to overcome micronutrients malnutrition in a people diet.

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