Applied Biological Research 19 (2): 138-145; (2017)
DOI: 10.5958/0974-4517.2017.00020.9

GENETIC VARIATION FOR GRAIN ZINC AND IRON CONCENTRATIONS AND QUALITY PARAMETERS IN WHEAT (*Triticum aestivum* L.) GENOTYPES

Ramandeep Kaur Jhinjer*, Gurvinder Singh Mavi, Neerja Sood, Akhil Malhotra, Harinderjeet Kaur and Virinder Singh Sohu

Wheat Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana – 141 004, Punjab (India)
*e-mail: *ramandeepkaur175@pau.edu*

(Received 11 August, 2016; accepted 31 May, 2017)

ABSTRACT

Grains of fifty lines of bread wheat (*Triticum aestivum* L.) representing diverse genotypes were analyzed in the year 2015 for variation in their micronutrient concentrations. Both grain Zn and Fe concentrations correlated negatively with grain yield and 1000-grain weight. Zn concentration also correlated significantly but negatively with phenol reaction score and plant height. Grain Fe concentration correlated significantly but positively with plant height, grain Zn and grain protein concentration. However, the correlations of Fe content with days to heading, grain appearance score, sodium dodecyl sulphate (SDS) sedimentation value and phenol reaction score and correlations of Zn content with days to heading, grain appearance score, SDS and grain protein were weak. Both Fe and Zn concentration showed negative direct on grain yield. Significant differences between bread wheat genotypes were found for grain Fe and Zn concentrations. Five entries namely 448, 412, 420, 414 and 419 showed high values for both Fe and Zn concentration. For Zn, an increment of 33.3% (54.4 ppm) over check (40.8 ppm) was observed. For Fe, an increment of 28.1% (45.1 ppm) was observed over check (35.2 ppm). The information generated could be used for developing micronutrient biofortification strategies.

Key words: Biofortification, grain protein, iron, yield, zinc

INTRODUCTION

Globally more than three billion people are affected by micronutrient malnutrition, a serious health problem worldwide (Kennedy *et al*., 2003; Welch and Graham, 2004). The most widespread deficiencies are those of Fe, Zn and vitamin A, which are prevalent in women and children in developing countries (WHO, 2002). About one-third of the world's population is affected by Fe and Zn deficiencies (Hotz and Brown, 2004). The proportion of global population suffering from micronutrient malnutrition has increased during last four decades (Welch and Graham, 2002; Graham *et al*., 2007). Two reasons might be responsible for this distressing trend. First, the cultivation of pulse crops in South Asia has decreased as compared to high yielding varieties of rice and wheat in last 2-3 decades. This trend may be the main cause for rise in micronutrient deficiency in South Asia; because edible legume seeds are better source of micronutrients than cereals, especially after the cereal grains have been processed before consumption (Welch and Graham, 2002). Second, new plant breeding programmes are mainly focused on attaining high agronomic yield rather than on nutritional quality (Morris and Sands, 2006). Increased grain yield may have resulted in low mineral content in grains (Graham *et al*., 1999; Garvin *et al*., 2006; Oury *et al*., 2006). Biofortification, which aims at to improve micronutrient concentrations and bioavailability in plant-based foods through genetic enhancement, is a cost-effective way of solving micronutrient deficiency problem (Bouis, 2002; Nestel *et al*., 2006). Micronutrient fertilizers are required to meet biofortification targets where micronutrient concentrations in foods are low because of soil supply (Graham *et al*., 2007; Cakmak, 2008).

Cereals are the main resource of micronutrient minerals for human. Knowledge of variation in traits among the available germplasm is essential for developing cereal crops with improved micronutrient concentration. Many screening trials on wheat germplasm for mineral content have indicated the existence of considerable variation in micronutrient concentrations in grain (Graham *et al*., 1999; Liu *et al*., 2006; Morgounov *et al*., 2007).

The acceptable varieties at farmer level and those commercially competitive ones should be acceptable to millers, manufacturers and consumers and must possess acceptable processing quality. For instance, in South Asia where chapatti is main local unleavened flat bread, biofortified wheat products should have medium-to-hard grain texture, as well as extensible and medium-strength gluten, to produce flat bread with acceptable (long-lasting) texture. Lines showing superior dough extensibility combined with medium to high gluten strength can also be used for products such as yeast leavened bread, thereby promoting small-to-intermediate-scale local industry. There exists a strong positive correlation between grain Zn and protein content (Feil and Fossati, 1995; Morgounov *et al*., 2007) suggesting that breeding for high Zn content may not affect processing and end-use quality. Conversely, Morgounov *et al*. (2007) have reported a significant but negative correlation between glutenin content and Zn and Fe concentrations among central Asian wheat varieties. More studies are needed to define this relationship in wheat germplasm. The objective of present study was to characterize 50 bread wheat lines of diverse genotypes to assess variations in micronutrient for their processing and end-use quality traits and to determine whether this breeding material has processing quality suitable to produce different end-use products.

MATERIALS AND METHODS

Plant material

Fifty lines of bread wheat (*Triticum aestivum* var. *aestivum*) of 5^{th} HPYT (5^{th} harvest plus yield trial) including one control cultivar 'WH 1105' were grown in field at PAU, Ludhiana, Punjab (India) in 2014-2015. Each line was sown in two replicate plots of 5 m long with 6 rows spaced 20 cm apart in a randomized block design. All the recommended management and agronomic practices for the area were followed to raise the plant material and realize full yield potential of crop. Observations were recorded on plots on 4 quantitative characters. Plant height and days to heading were recorded at seedling stage while 1000-grain weight and grain yield were recorded after harvest. The data was analyzed for variability (Singh and Chaudhry, 1977), correlation (Johnson *et al*., 1955) and path coefficient study (Dewey and Lu, 1959).

Grain analysis

The concentration of elements Fe and Zn in wheat grains was determined using a bench-top, non-destructive, energy-dispersive X-ray fluorescence spectrometry (EDXRF) instrument (model X-Supreme 8000, Oxford Instruments plc, Abingdon, UK), previously standardized for high throughput screening of Zn and Fe in whole wheat grain (Paltridge *et al*., 2012). Grain appearance score (GAS) was evaluated subjectively out of a maximum score of ten (Kaur *et al*., 2013), giving due weightage to the grain size (3), shape (2), colour (2) and luster (3). The grain protein content was estimated using the whole grain analyzer, Infratec 1241 (Foss Analytical AB, Sweden). The SDS sedimentation value of whole meal samples was determined as per Axford *et al*. (1979). The phenol reaction score (PRS) was evaluated by treating about 100 grains soaked overnight, with 1%

phenol solution for 4 h. The grains are evaluated for extent of darkness out of a score of 10, half an hour after draining off the phenol solution (Kaur *et al*., 2013).

Data analysis

Correlation and regression analysis were performed on bread wheat data from the diversity screen trial. Analysis of variance was calculated according randomized block design analysis with CPCS-1 software (Cheema and Singh, 1991) to evaluate the significance of differences between the lines.

RESULTS AND DISCUSSION

Estimate of genetic variability

Genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2) and genetic advance as percent of mean (GA) are given in Table 1. PCV and the GCV were close for most traits, except Fe and grain yield. High estimates of GCV and PCV were observed in phenol reaction score. GCV and PCV in Fe and Zn concentrations were observed as low. Plant height and days to heading had low GCV values.

High heritability was recorded for plant height, days to heading, 1000-grain weight, protein, grain appearance score, phenol reaction score and SDS sedimentation value and high GA was recorded for grain yield. Grain yield had lowest heritability followed by Fe content. Plant height, days to heading, 1000-grain weight, grain protein, grain appearance score, phenol reaction score and SDS sedimentation value had high h^2 but low GA values. Phenol reaction score showed high heritability coupled with high PCV. PCV and GCV were close for most traits, except Fe and grain yield, indicating primarily the genetic control of these traits rather than environment effect alone. High estimates of GCV and PCV were noticed in phenol reaction score thus suggesting that the selection based on these characters would facilitate the isolation of desirable types. GCV and PCV for Fe and Zn were low thereby suggesting that the selection based on these characters would not be successful in isolation of yield trait. However, the genetic variability along with heritability estimates would give a better idea on the amount of GA expected from selection (Burton, 1952).

The traits having high heritability and high genetic advance are supposed to be under the control of additive genes; hence these can be improved by selection based on phenotypic performance (Fonseca and Patterson, 1958; Dewey and Lu, 1959; Anwar *et al*., 2009). Traits like plant height, days to heading, 1000-grain weight, grain protein, grain appearance score, phenol reaction score and SDS sedimentation value had high h^2 but low GA values suggesting the involvement of non-additive gene action in their inheritance. The traits like phenol reaction score

Table 1: Estimates of genetic parameters for different traits in wheat

Characters	h^2 (%)	GA	%GA	PCV	GCV	GM	CV
Plant height (cm)	85.01	8.29	8.39	4.79	4.42	98.80	1.86
Days to heading	81.82	5.90	5.71	3.39	3.06	103.36	1.44
1000 grain weight (g)	93.99	7.02	18.20	9.40	9.11	38.60	2.30
Fe (ppm)	27.98	2.19	6.12	10.62	5.62	35.85	9.01
Zn (ppm)	65.76	6.67	16.18	11.94	9.68	41.24	6.99
Grain protein	99.11	2.15	18.83	9.22	9.18	11.41	0.87
Grain appearance score	97.87	0.60	10.86	5.38	5.33	5.48	0.79
Phenolic reaction score	98.25	1.33	43.75	21.62	21.43	3.04	5.86
SDS sedimentation value	96.37	8.19	16.89	8.51	8.35	48.48	1.62
Grain yield (g $plot^{-1}$)	18.52	139.58	4.68	12.26	5.27	2985.16	11.06

h^2 = heritability (broad sense); GA = Genetic advance; %GA = Genetic advance as percentage of mean; PCV = Phenotypic coefficient of variability; GCV = Genotypic coefficient of variability; GM = Grand mean showed high heritability coupled with high PCV suggesting greater scope for selection of these traits on analytical basis.

Genotypic and phenotypic correlation coefficient

The studies revealed that Fe concentration correlated highly significantly ($p<0.001$) with Zn concentration, plant height, days to heading, grain protein and grain appearance score among the bread wheat lines but the correlation coefficient between Zn concentration with days to heading, grain protein and grain appearance score was lower than Fe concentration (Table 2). The correlation coefficient of both Fe and Zn concentrations with SDS sedimentation value was also lower. Fe concentration correlated negatively but significantly ($p<0.001$) with 1000-grain weight and grain yield. Zn concentration correlated negatively and significantly with plant height ($p<0.005$) and grain yield ($p<0.001$). Zn concentration also correlated negatively and non-significantly with 1000-grain weight and phenol reaction score. Apart from Fe and Zn concentrations, days to heading, grain appearance score and SDS sedimentation exhibited a negative and significant correlation with grain yield.

Grain Fe and Zn concentrations are complex characters controlled by several components which reflect positive and negative effects on these traits. Thus, for achieving rational improvement in grain Fe and Zn concentrations and its components, the knowledge about the mechanism of association, cause and effect relationship provides a basis for formulating suitable selection methods for these components. Both grain Fe and Zn concentrations correlated positively with grain protein content among 50 lines of wheat. Our findings are similar with other wheat genotypes trials and indicate a feasible link between grain protein and concentrations of two trace elements (Morgounov *et al.*, 2007). Other studies have shown that Gpc-B1 (grain protein content-B1) locus of wild wheat affects both grain protein and Fe and Zn concentration in grains (Distelfeld *et al.*, 2007). This locus encodes an NAC transcription factor (NAM-B1) that accelerates senescence and enhanced remobilization of nutrients (N, Fe and Zn) from leaves to developing grains (Uauy *et al.*, 2006). The wheat genome contains three NAM genes, but modern wheat varieties carry a non-

Table 2: Genotypic and phenotypic correlation coefficients among various traits of wheat

Characters		Plant height	Days to heading	1000-grain weight	Fe	Zn	Grain protein	GAS	PRS	SDS SV
Days to heading	G	0.23*								
	P	0.18								
1000-grain weight	G	0.12	-0.18							
	P	0.13	-0.17							
Fe	G	0.28**	0.37**	-0.26**						
	P	0.16	0.08	-0.11						
Zn	G	-0.20*	0.09	-0.04	0.40**					
	P	-0.07	0.06	-0.03	0.30**					
Grain protein	G	-0.03	-0.30**	-0.15	0.25**	0.16				
	P	-0.02	-0.27**	-0.15	0.14	0.14				
GAS	G	0.06	0.10	0.0673	0.25**	0.10	-0.35**			
	P	0.04	0.10	0.06	0.10	0.06	-0.35**			
PRS	G	-0.42**	-0.41	-0.06	0.05	0.00	0.11	-0.15		
	P	-0.39**	-0.35**	-0.06	0.02	-0.01	0.11	-0.14		
SDS SV	G	0.08	-0.08	-0.27**	0.13	0.02	0.32**	-0.21*	0.05	
	P	0.09	-0.07	-0.25**	0.07	0.01	0.31**	-0.20*	0.04	
Grain yield	G	0.08	-0.59**	0.59**	-1.37**	-0.95**	0.19	-0.08	0.18	-0.25**
	P	0.02	-0.18	0.23*	-0.15	-0.34**	0.07	-0.06	0.06	-0.10

GAP = Grain appearance score; PRS = Phenolic reaction score; SDS SV = SDS sedimentation value; Critical value of 'r' at 5% level = 0.20 and 1% level = 0.25; G = Genotypic correlation coefficient; P = Phenotypic correlation coefficient functional NAM-B1 allele, which causes delayed leaf senescence, and low levels of grain protein, Fe and Zn in modern wheat varieties as compared to wild emmer wheat. The positive relationship between grain protein, Fe and Zn concentrations may be a pleiotropic effect of NAM genes (Cakmak, 2008). The positive correlations between Fe and Zn concentrations and protein content would be helpful information for wheat breeders, and further studies included a larger set of modern varieties with high protein content (Zhao *et al*., 2009).

Wheat lines producing lower grain yields may contain higher levels of trace elements and this is because of concentration-dilution effect by the dry matter accumulated in grain. It is not helpful in breeding for biofortification of high-yielding wheat. It is reported in some studies that grain yield and trace element concentrations were correlated negatively (Garvin *et al.*, 2006; McDonald *et al*., 2008; Zhao *et al*., 2009). Increasing grain yield by N fertilization resulted in high micronutrient concentrations in wheat grain (McGrath, 1985). Zhao *et al*. (2009) showed that high yielding varieties contained significantly low levels of Zn in grain than old varieties. In present study, a significant negative relationship existed between grain yield and both Fe and Zn concentrations (Table 2), which is in line with Zhao *et al*. (2009). Garvin *et al*. (2006) and Zhao *et al*. (2009) who showed a significant decreasing trend in grain Zn concentration with the release date of 26 wheat lines tested in multiple site trials. This means that improved grain yield as a result of genetic enhancement may have resulted in decreased Zn content in grains. The negative effect of yield enhancement was less visible on grain Fe concentration. Other study has shown a decreasing pattern in wheat grain mineral concentrations (including Zn, Cu, Mn, Mg) over last 160 years in England, with reduction coinciding with the introduction of short-straw, high-yielding modern varieties of wheat (Fan *et al*., 2008). It is significant to break this negative relationship and breed genotypes that have high levels of trace elements without any yield penalty, which help in biofortification programs.

Aleurone layer and germ of wheat grain, which are separated as bran part during milling, contain majority of trace elements like Fe and Zn (Ozturk *et al*., 2006; Liu *et al*., 2007; Zhao *et al*., 2009). Therefore, it was assumed that the contents of Fe and Zn in whole grain may associate negatively with grain size, because larger grain would have a relatively smaller bran portion. Though because of this localization pattern, the correlation among grain size and grain Fe and Zn content was either weak or insignificant, indicating that smaller grain does not necessarily lead to smaller trace element concentration (Table 2). Calderini and Ortiz-Monasterio (2003) reported that the contents of Zn and Fe reduced at grain positions more distal from rachis within ear. Therefore, higher number of grains per ear may be associated with low average content of these minerals.

Positive genotypic correlation values of traits like plant height (0.08), 1000 grain weight (0.59), grain protein (0.19) and phenol reaction score (0.18) with grain yield and their high direct effect values on grain yield i.e., 1.019, 0.137, 1.512 and 1.136, respectively, indicated a true picture of association between these traits (Table 2 & 3).

Path coefficient analysis for direct and indirect effects on grain yield

Partitioning of total correlation coefficient into direct and indirect effects for grain yield per plot showed a positive direct effect of many yield contributing traits like plant height, days to heading, 1000-grain weight, grain protein, grain appearance score and phenol reaction score (Table 3). Traits like Fe and Zn contents and SDS sedimentation value showed negative direct effect on grain yield. However, plant height, days to heading, grain protein and grain appearance score contributed more positive indirect effects than negative indirect effects. Traits like 1000-grain weight, Fe and Zn content, phenol reaction score and SDS sedimentation value showed more negative indirect effects.

Analysis of variance

Among the 50 bread wheat lines, grain Fe concentration varied by 1.5-fold, ranging from 30.6 to 45.1 ppm and grain Zn by 1.7-fold, from 32.0 to 54.4 ppm. Wheat entry 428 had highest mean Fe concentration (45.1 ppm), followed by entries 435 (43.0 ppm), 441 (40.9 ppm), 431 (40.0 ppm) and 412 (39.6 ppm). In case of Zn mean concentration, entry '409' had the highest value (54.4 ppm),

Table 3: Genotypic path coefficient analysis for direct (bold) and indirect effects on grain yield plot^{-1} in wheat

Characters	Plant height	Days to heading	1000-grain weight	Fe	Zn	Grain protein	GAS	PRS	SDS SV
Plant height	**1.0191**	0.2210	0.0163	-0.7300	0.0268	-0.0446	0.0609	-0.4764	-0.0151
Days to heading	0.2307	**0.9762**	-0.0250	-0.9706	-0.0125	-0.4467	0.1088	-0.4625	0.0155
1000-grain weight	0.1214	-0.1782	**0.1367**	0.6771	0.0053	-0.2247	0.0732	-0.0711	0.0504
Fe	0.2849	0.3629	-0.0354	**-2.6108**	-0.0540	0.3773	0.2716	0.0527	-0.0236
Zn	-0.2022	0.0904	-0.0054	-1.0457	**-0.1349**	0.2478	0.1125	-0.0038	-0.0036
Grain protein	-0.0301	-0.2883	-0.0203	-0.6513	-0.0221	**1.5124**	-0.3794	0.1281	-0.0591
GAS	0.0570	0.0976	0.0092	-0.6519	-0.0140	-0.5274	**1.0879**	-0.1731	0.0383
PRS	-0.4272	-0.3972	-0.0086	-0.1212	0.0005	0.1704	-0.1657	**1.1365**	-0.0087
SDS SV	0.0825	-0.0812	-0.0369	-0.3295	-0.0026	0.4791	-0.2233	0.0529	**-0.1866**

GAP = Grain appearance score; PRS = Phenolic reaction score; SDS SV = SDS sedimentation value

followed by entries 432 (48.5 ppm), 447 (48.2 ppm), 448 (47.9 ppm) and 449 (47.8 ppm). Five entries 448, 412, 420, 414 and 419 showed high values for both Fe and Zn concentration. Analysis of variance showed a highly significant difference between wheat lines for plant height, days to heading, 1000 grain weight, grain Fe and Zn concentrations, grain protein and SDS sedimentation value (Table 4). Mean data of replications showed that 14 entries (417, 405, 430, 425, 410, 423, 406, 450, 427, 426, 416, 403, 449 and 422) yielded more than the mean of check. Entry 417 showed an increment of 18% (61.7 q ha^{-1}) over the check (52.3 q ha^{-1}). Element data showed an increment of 33.3% (54.4 ppm) over the check for Zn (40.8 ppm). Twenty two entries showed more than half of the target Zn (4 ppm) and 2 entries with more than full target Zn (8 ppm). For Fe, an increment of 28.1% (45.1 ppm) was observed over the check (35.2 ppm).

It is evident from the range of mean values for various traits among the genotypes evaluated that these had diverse genetic background. Analysis of variance showed a highly significant difference between wheat lines for plant height, days to heading, 1000-grain weight, grain Fe and Zn contents, grain protein and SDS sedimentation value (Table 4). But wheat lines were non-significantly differed for traits like grain appearance score, phenol reaction score and grain yield. Nutritionists have established that in South Asia the target is to increase Zn and Fe levels by 8 and 25 ppm, respectively, above baseline in mega-varieties such as ‘PBW343’, grown on 8 million ha area in India, and Pakistani mega-variety ‘Inqalab 91’, which possess about 25 ppm Zn and Fe. This means a total Zn and Fe levels of 33 and 50 ppm, respectively, in grain. The absolute target level for Fe is significantly higher than for Zn, due to lower bioavailability of Fe (Velu *et al*., 2012). In general, grain Fe content showed greater variation across genotypes than Zn. Identifying the best lines with wide adaptation across locations as well as the best site-specific lines (adapted to favorable environment) is essential for promoting candidate varieties to specific environments.

Table 4: Analysis of variance for experimental design for various traits in wheat

Source of variation	d.f.	Mean square									
		Plant height	Days to heading	1000-grain weight	Fe	Zn	Grain protein	GAS	PRS	SDS SV	GY
Replications	1	46.24	168.92	46.24	36.86	4.01	1.44	0.81	0.23	57.77	2616.49
Genotypes (A)	49	41.49**	22.27**	25.53**	18.54*	40.21**	2.21**	0.17	0.86	33.41**	43.99
Error A	49	3.36	2.23	0.79	10.43	8.31	0.98	0.19	0.76	0.62	30.26

GAP = Grain appearance score; PRS = Phenolic reaction score; SDS SV = SDS sedimentation value
The values with *and ** are significant at 5 and 1% level of significant, respectively

Acknowledgments: Financial support for this study was provided by HarvestPlus (www.HarvestPlus.org), a global alliance of agriculture and nutrition research institutions working to increase the micronutrient density of staple food crops through biofortification. The views expressed here do not necessarily reflect those of HarvestPlus. The authors sincerely acknowledge the receipt of experimental plant material from CIMMYT, Mexico in form of 5th HPYT.

REFERENCES

Anwar, J., Ali, M.A., Hussain, M., Sabir, W., Khan, M.A., Zulkiffal, M. and Abdullah, M. 2009. Assessment of yield criteria in bread wheat through correlation and path analysis. *The Journal of Animal and Plant Science*, **19**: 185-188.

Axford, D.W.E., McDermott, E.E. and Redman, D.G. 1979. Note on the sodium dodecyl sulphate test of bread making quality comparison with Pelenske and Zeleny tests. *Cereal Chemistry*, **56**: 582-584.

Bouis, H.E. 2002. Plant breeding: A new tool for fighting micronutrient malnutrition. *Journal of Nutrition*, **132**: 491-494.

Burton, G.N. 1952. Quantitative inheritance in grasses. pp 277-283. **In**: *Proceedings of 6th International Grass Congress*. Pennsylvania, USA.

Cakmak, I. 2008. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant and Soil*, **302**: 1-17.

Calderini, D.F. and Ortiz-Monasterio, I. 2003. Grain position affects grain macronutrient and micronutrient concentrations in wheat. *Crop Science*, **43**: 141-151.

Cheema, H.S, and Singh, B. 1991. *Software Statistical Package CPCS-1*. Department of Statistics, Punjab Agricultural University, Ludhiana, Punjab (India).

Dewey, D.R. and Lu, K.H. 1959. A correlation and path analysis of components of created wheat grass seed production. *Agronomy Journal,* **51**: 515-518.

Distelfeld, A., Cakmak, I., Peleg, Z., Ozturk, L., Yazici, A.M., Budak, H., Saranga, Y. and Fahima, T. 2007. Multiple QTL-effects of wheat Gpc-B1 locus on grain protein and micronutrient concentrations. *Physiologia Plantarum,* **129**: 635-643.

Fan, M.S., Zhao, F.J., Fairweather-Tait, S.J., Poulton, P.R., Dunham, S.J. and McGrath, S.P. 2008. Evidence of decreasing mineral density in wheat grain over the last 160 years. *Journal of Trace Elements in Medicine and Biology*, **22**: 315-324.

Feil, B. and Fossati, D. 1995. Minerals composition of *Triticale* grains as related to grain yield and grain protein. *Crop Science*, **35**: 1426-1431.

Fonseca, S. and Patterson, F.L. 1958. Yield components heritabilities and interrelationship in winter wheat (*T. aestivum* L.). *Crop Science*, **8**: 614-617.

Garvin, D.F., Welch, R.M. and Finley, J.W. 2006. Historical shifts in the seed mineral micro-nutrient concentration of US hard red winter wheat germplasm. *Journal of the Science of Food and Agriculture*, **86**: 2213-2220.

Graham, R., Senadhira, D., Beebe, S., Iglesias, C. and Monasterio, I. 1999. Breeding for micro-nutrient density in edible portions of staple food crops: conventional approaches. *Field Crops Research*, **60**: 57-80.

Graham, R.D., Welch, R.M., Saunders, D.A., Ortiz-Monasterio, I., Bouis, H.E., Bonierbale, M., de Haan, S., Burgos, G., Thiele, G., Liria, R., Meisner, C.A., Beebe, S.E., Potts, M.J., Kadian, M., Hobbs, P.R., Gupta, R.K. and Twomlow, S. 2007. Nutritious subsistence food systems. *Advances in Agronomy,* **92**:1-74.

Hotz, C. and Brown, K.H. 2004. Assessment of the risk of zinc deficiency in populations and options for its control. *Food and Nutrition Bulletin,* **25**: 94-203.

Johnson, H.W., Robinson, H.F. and Comstock, R.E. 1955. Estimates of genetic and environment variability in soybean. *Agronomy Journal,* **46**: 314-318.

Kaur, H., Jha, J., Sohu, V.S., Mavi, G.S. and Singh, R.P. 2013. Evaluation of Ug99 resistant elite CIMMYT wheat lines for chapatti and grain quality. *Crop Improvement*, **40**: 144-149.

Kennedy, G., Nantel, G. and Shetty, P. 2003. The scourge of ''hidden hunger'': Global dimensions of micronutrient deficiencies. *Food, Nutrition and Agriculture*, **32**: 8-16.

Liu, Z.H., Wang, H.Y., Wang, X.E., Zhang, G.P., Chen, P.D. and Liu, D.J. 2006. Genotypic and spike positional difference in grain phytase activity, phytate, inorganic phosphorus, iron and zinc contents in wheat (*Triticum aestivum* L.). *Journal of Cereal Science*, **44**: 212-219.

Liu, Z.H., Wang, H.Y., Wang, X.E., Zhang, G.P., Chen, P.D. and Liu, D.J. 2007. Phytase activity, phytate, iron, and zinc contents in wheat pearling fractions and their variation across production locations. *Journal of Cereal Science,* **45**: 319-326.

McDonald, G.K., Genc, Y. and Graham, R.D. 2008. A simple method to evaluate genetic variation in grain zinc concentration by correcting for differences in grain yield. *Plant and Soil*, **306**: 49-55.

McGrath, S.P.,1985. The effects of increasing yields on the macroelement and microelement concentrations and offtakes in the grain of winter wheat. *Journal of the Science of Food and Agriculture,* **36**: 1073-1083.

Morgounov, A., Gomez-Becerra, H.F., Abugalieva, A., Dzhunusova, M., Yessimbekova, M., Muminjanov, H., Zelenskiy, Y., Ozturk, L. and Cakmak, I. 2007. Iron and zinc grain density in common wheat grown in Central Asia. *Euphytica*, **155**: 193-203.

Morris, C.E. and Sands, D.C. 2006. The breeder's dilemma – yield or nutrition? *Nature Biotechnology,* **24**: 1078-1080.

Nestel, P., Bouis, H.E., Meenakshi, J.V. and Pfeiffer, W. 2006. Biofortification of staple food crops. *Journal of Nutrition*, **136**: 1064-1067.

Oury, F.X., Leenhardt, F., Remesy, C., Chanliaud, E., Duperrier, B., Balfourier, F. and Charmet, G. 2006. Genetic variability and stability of grain magnesium, zinc and iron concentrations in bread wheat. *European Journal of Agronomy,* **25**: 177-185.

Ozturk, L., Yazici, M.A., Yucel, C., Torun, A., Cekic, C., Bagci, A., Ozkan, H., Braun, H.J., Sayers, Z. and Cakmak, I. 2006. Concentration and localization of zinc during seed development and germination in wheat. *Physiologia Plantarum*, **128**: 144-152.

Paltridge, N.G., Milham, P.J., Ortiz-Monasterio, J.I., Velu, G., Yasmin, Z., Palmer, L.J., Guild, G.E. and Stangoulis, J.C.R. 2012. Energy-dispersive X-ray fluorescence spectrometry as a tool for zinc, iron and selenium analysis in whole grain wheat. *Plant and Soil*, **361**: 251-260.

Singh, R.K. and Chaudhary, B.D. 1977. *Biometrical Methods in Quantitative Genetics Analysis*. Kalyani Publishers, Ludhiana (India).

Uauy, C., Distelfeld, A., Fahima, T., Blechl, A. and Dubcovsky, J. 2006. A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science*, **314**: 1298-1301.

Velu, G., Singh, R.P., Huerta-Espino, J., Pena, R.J., Arun, B., Mahendru-Singh, A., Mujahid, M.Y., Sohu, V.S., Mavi, G.S., Crossa, J., Alvarado, G., Joshi, A.K. and Pfeiffer, W.H. 2012. Performance of biofortified spring wheat genotypes in target environments for grain zinc and iron concentrations. *Field Crops Research*, **137**: 261-267.

Welch, R.M. and Graham, R.D. 2002. Breeding crops for enhanced micronutrient content. *Plant and Soil*, **245**: 205-214.

Welch, R.M. and Graham, R.D. 2004. Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany*, **55**: 353-364.

WHO, 2002. *The World Health Report 2002. Reducing Risks, Promoting Healthy Life.* World Health Organization, Geneva, Switzerland.

Zhao F.J, Su Y.H, Dunham S.J, Rakszegi M, Bedo Z, McGrath S.P. and Shewry P.R. 2009. Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. *Journal of Cereal Science*, **49**: 290–295.