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EXPLORATION OF BREEDING POTENTIAL FOR GENETIC BIOFORTIFICATION AND YIELD IN SPRING WHEAT (*Triticum aestivum* L.)

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The emphasis of this research was to assess the genotypes which were rich in iron and zinc content in their grains. Genetic diversity was estimated for zinc and iron content in grains along with agro-morphological traits directly related to yield in 85 diverse wheat genotypes. The experiment was carried out in the experimental field of Plant Breeding and Genetics Department, University of Agriculture, Faisalabad using replicated randomized complete block design during 2013-14. The results depicted highly significant differences among all the traits i.e., iron & zinc content in grains, flag leaf area, peduncle length, spikelets per spike, grains per spike, thousand grain weight and grain yield per plant. Mean values of all the traits indicated the presence of genetic variability. WHEAR-TUKURU-WHEAR (83.30 mg/kg), MARVI-2000 (80.98 mg/kg) and PRL'S'/PVN (80.27 mg/kg) had highest means among other genotypes for iron and 89.77 mg/kg, 85.14 mg/kg and 88.49 mg/kg for zinc, respectively. Grain content for iron (Fe) and zinc (Zn) showed positive and significant correlation among them and with yield related traits as well. Cluster analysis grouped germplasm into six clusters on the basis of studied mineral content and agro-morphological traits. Cluster 4 contained 10 genotypes showing the best performance for most of the traits contributing for high grain mineral content and high yield. It was suggested that ample genetic diversity was present in the germplasm for the uptake of iron and zinc along with high yield potential. Moreover, it was concluded that selection for micronutrient efficiency could be achieved without affecting yield potential.

Keywords: Genetic biofortification, iron, zinc, genetic diversity, spring wheat.

INTRODUCTION

Wheat is widely cultivated and utilized as staple food for large population of the world. It is the most important cereal on the basis of its consumption, production, trade and nutritional value. The economy of Pakistan is mainly dependent on agriculture sector. So, wheat is also called the backbone of Pakistan's agriculture as it provides staple food to millions of residents. It contributes 9.1% to the value addition of agriculture and 1.7% share in GDP. Average wheat yield has been reported 3.21 tonnes per hectare during 2017-18, which needs to be improved (Pakistan Economic Survey, 2017-18). Nearly 2000 million people of the world suffer from hidden hunger caused by iron and zinc deficiency. The victims of these micronutrient deficiencies are generally pregnant woman and the infants or the growing children. The deficiency of these minerals kills about 0.5 million children who are below the age of 5 years all over the world (Velu *et al*., 2012). Certain disorders such as maternal mortality, hemoglobin dysfunction, ineffective physical and mental development and anemia are caused by deficiency of iron. Similarly, decreased bone growth, contagious diseases, improper immune system and certain cancers are caused by deficiency of zinc (Liu *et al*., 2014).

To cope with the problem, certain strategies have been tried which could fight the hidden hunger such as dietary diversification, industrial fortification, agronomic fortification and pharmaceutical fortification. These strategies have been showing limited success due to improper delivery systems, inadequate infrastructure, lack of consumer acceptance, high recurrent costs and affordability (Kanatti *et al*., 2014). The most stable and economical approach has been genetic manipulation for biofortification. The products of genetic biofortification can reach the remote areas and deserving rural masses (Rai *et al*., 2012). It is usually assumed that modern advances in plant breeding are becoming the root cause for the reduced genetic diversity. According to a study carried out by Reif *et al*., (2005), genetic diversity of spring wheat has been reduced over 50 years of international breeding for crop improvement. Breeding for high yielding cultivars is leading towards ignorance for nutritional quality of wheat (Morris and Sands, 2006). Therefore, minerals and vitamins along with essential micronutrients are not present in staple foods and posing a threat to human population globally, especially in developing countries (Stein, 2010).

The regions of the world with a high consumption rate of cereals as the staple food, face the major outbreak of mineral deficiency and malnutrition (Velu *et al*., 2014). The major reason is the lack of food diversification in daily dietary intake. Mineral malnutrition can be overcome through the biofortification of staple cereal crops such as wheat, rice and maize. Mineral biofortification of iron and zinc in the wheat crop could be the tremendous landmark for the people utilizing it as their mainstay staple. It could improve the healthcare of billions of people around the globe. Complete understanding of genetics for the improvement of present wheat varieties for iron and zinc content in the grains requires the exploration of genetic diversity and analysis of their potential (Badakhshan *et al*., 2013). Improvement of wheat genotypes for their mineral content in grains requires the selection procedures targeting the uptake, mobilization across the plant tissues and deposition in the grains. These mechanisms are controlled by the genes and need to be improved through gene manipulations (Yang *et al*., 2007).

Identification of such genotypes which are efficient in uptake of micronutrients from the soil and their translocation to the grains is useful for fighting the hidden hunger and this trend is developing by the time (EL-Bendary *et al*., 2013). Therefore, identification of the germplasm having higher content of iron and zinc in grains for biofortification is the first step in conventional plant breeding. Selection of such genotypes could be achieved only with the presence of genetic diversity (Ficco *et al*., 2009). Some researchers reported genetic diversity in the germplasm with regard to minerals uptake, especially iron and zinc (Welch and Graham, 2004). It was indicated that improvement of grain iron and zinc content alongwith agro-morphological traits was possible and could be practiced alongside (Welch *et al*., 2005). Keeping in view the scenario of mineral deficiency in human population and above discussion, a study was designed to evaluate 85 wheat genotypes comprised of advance lines and commercial cultivars in Pakistan for the identification of cultivars having ability of higher grain iron and zinc content alongwith high productivity.

MATERIALS AND METHODS

The material used for exploring the biofortification potential consisted 85 diverse wheat genotypes (Table 1). The germplasm was collected from Ayyub Agricultural Research Institute and Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. The germplasm was selected on the morphological basis, depicting a diverse nature of the germplasm. The experiment was conducted using randomized complete block design with three replications. Each experimental unit comprised of one accession of the length of 4 meters. Seed was sown with the help of drill. The inter row and inter plant distance was 20cm and 5cm to maintain the plant population, respectively. Two seeds per hill were sown and thinned out to single seedling to ensure healthy plant stand after germination. Nine plants per genotype were randomly taken to collect the data for grain iron and zinc content alongwith agronomic traits. Normal cultural practices were carried out to maintain the healthy crop stand.

Grain iron and zinc content of 85 genotypes was assessed in two steps i.e. sample preparation for digestion and sample analysis through atomic absorption spectrophotometer (AAS). Electronic grinder was used to grind the grains obtained from all genotypes. One gram of each sample was obtained from the whole ground material of each genotype. These samples were then transferred to labeled flasks separately. The samples containing flasks were poured with 10 ml of perchloric acid, sulfuric acid and nitric acid in a ratio of 2:1:4, respectively in each flask. The flasks were given a treatment of hotplate for the purpose of heating. After getting a transparent solution in the flasks, each sample was subjected to dilution and distillation with water. Each flask was filled up to the volume of 100ml. After dilution and distillation, each flask was filtered and poured empty in separate plastic bottles. The prepared digested samples were then subjected to atomic absorption spectrophotometer used for micro nutrient analysis. This instrument provided the results of iron and zinc content in PPM which were converted into mg/kg afterwards. Proper protocol was followed for the screening of grain mineral content, given by Piper (1942) and Lindsay and Norvell (1978).

Data regarding grain mineral content and yield related traits were analyzed under analysis of variance (Steel *et al*., 1997) for the differences among genotypes using Statistix 8.1. Interaction of the traits under study was determined through RStudio (R-3.4.1) using Pearson's Correlation and corrplot. The genotypes were further grouped into clusters on the basis of similarities and dissimilarities between them using Minitab 17 Statistical software and dendrogram was constructed.

RESULTS

Analysis of variance: All the germplasm was evaluated for following traits; grain iron (Fe) and zinc (Zn) content with standard error (SE-1.77) and (SE-1.51), flag leaf area (FLA) with (SE 0.42), peduncle length (PdL) with (SE 0.50), spikelets per spike (SpS) with (SE 0.90), grains per spike (GPS) with (SE 1.63), thousand grain weight (TGW) with (SE 0.67) and grain yield per plant (GYP) with (SE 0.33) (Table 2). Mean values for all traits are depicted through a scatter plot (Fig. 1). Grain Fe content ranged between 27.95 mg/kg to 83.30 mg/kg with an average of 55.63 mg/kg. The genotypes; BFT-72 (83.30), BFT-49 (80.98), BFT-46 (80.27) had the highest means. Grain Zn content showed a range between 29.71 mg/kg to 89.77 mg/kg with an average of 59.74 mg/kg. Similar genotypes as of grain Fe content were the best performers in case of grain Zn content as well i.e. BFT- 72 (89.77), BFT-46 (88.49) and BFT-49 (85.14). All the germplasm showed a range of 27.35 cm^2 to 48.24 cm^2 for FLA with an average of 37.80 cm^2. A range of 27.75 cm to

Table 1. Names and coding of the wheat germplasm.

Coding	Names of genotypes	Coding	Names of genotypes
BFT1	KOHSAR 95	BFT44	V-06117
BFT2	MANTHAR	BFT45	KASYON//PVN'S'/SPRW'S'
BFT3	WEEBILL-1=V-3158	BFT46	PRL'S'/PVN
BFT4	BABAX/LR42//BABAX*2/3VIVITSI	BFT47	V-03007
BFT5	SA 75	BFT48	PF70402/ALD'S'//P80 72/160//ALD'S'/3/PEW'S'
BFT6	PAVON 76	BFT49	MARVI-2000
BFT7	WATAN (V-87094)	BFT50	NING 8319
BFT8	HD2236//SA.42/HARRIER'S'=V.97088	BFT51	V-07178
BFT9	ZA-77/NOR/7C	BFT52	NAEEM 82
BFT10	OASIS F 86	BFT53	V-07189
BFT11	T.J-83	BFT54	BAVIACORA M 92 =V97097
BFT12	HOOSAM3	BFT55	TAN/PEW//SARA/3/CBRD
BFT13	INQ-91*2/TUKURU	BFT56	PVN//CAR422/ANA/3/KAUZ*2/TRAP//KAUZ
BFT14	PAK 81	BFT57	V-4022:INQ/AUQAB
BFT15	V-04188	BFT58	V-07200
BFT16	PFAU/WEAVER	BFT59	CHILERO=CHIL'S'
BFT17	V-05100	BFT60	KAKATSI
BFT18	UP 262	BFT61	WH542
BFT19	V-05115	BFT62	SASSI
BFT20	SONOITA=SNI	BFT63	BLS//F3.71/TRM/3/SKA/PRL'S'//YDING=V-1034
BFT21	V-05121	BFT64	TRAP#1
BFT22	FAISALABAD 85	BFT65	PBW 343*2/CHAPIO
BFT23	V-056132	BFT66	V-07155
BFT24	T.D-1	BFT67	KHIRMAN
BFT25	PARULA=PRL	BFT68	PEWEE'S'
BFT26	SOGHAT-90=PVN	BFT69	V-06007
BFT27	V-02192	BFT70	TUC'S'/MON'S'//VEE'S'/3/LIRA'S'
BFT28	LYP 73	BFT71	SNADAL/CMH912//CMH76A.769/3/F3.71/TRM73
BFT29	V-06067	BFT72	WHEAR-TUKURU-WHEAR
BFT30	TRAP#1/PBW65/3/KAUZ*2/TRAP//KAUZ	BFT73	76309
BFT31	PBW 343*2/KUKUNA	BFT74	WHEAR/KUKUNA/WHEAR
BFT32	GAR 422/ANA//TRAP#1/3/KAUZ*2//TRAP//KAUZ	BFT75	05BT014
BFT33	PAS 90/SH.88=V-96059	BFT76	PFAU/MILAN/5/CHEN/A. SQ
BFT34	CHAKWAL 97	BFT77	D-7663
BFT35	LU 26S	BFT78	KIRITATI//PBW65/2*SERI.1B
BFT36	V-06068	BFT79	V- 08171
BFT37	BYRSA-87 = SUNBIRD	BFT80	YANAC/3/PRL/SARA/TSI/VEE#5/4/CROC_1/AE/SQUARR
BFT38	V-06096	BFT81	V-07102
BFT39	MH 97 = ATILLA	BFT82	V-08164
BFT40	V-06103	BFT83	V-07076
BFT41	CHAM-6=NESSER	BFT84	NR381
BFT42	V-06111	BFT85	V-07032
BFT43	AS-2002=WD97603		

Table 2. Mean square values of traits understudy in spring wheat.

SOV	df	Fe	Zn	FLA	PdL	SpS	GpS	TGW	GYP
Replication	2	21.34	23.44	0.73	2.44	35.83	100.14	29.94	2.19
Genotype	84	684.18**	723.47**	61.12**	129.19**	21.61**	281.99**	140.20**	106.13**
Error	168	9.48	6.86	0.54	0.77	2.48	8.07	1.38	0.35

df = degree of freedom, Fe = Iron, Zn = Zinc, FLA= Flag leaf area, PdL = Peduncle length, SpS = Spikelets per spike, GpS = Grains per spike, TGW = Thousand grain weight and GYP = Grain yield per plant

66.23 cm with an average of 46.99 cm was observed for PdL. SpS had a range of 13.00 to 23.67 with an average of 18.33. A range of 36.33 to 77.67 with an average of 57.00 was observed for GPS. TGW had a similar trend with a range of 29.40 g to 61.59 g with an average of 45.50 g. GYP had a range of values between 21.98 g to 44.61 g with an average of 33.30 g.

***Correlation*:** Correlation between grain iron and zinc content and all other yield contributing traits is depicted in (Fig. 2). A positive and highly significant correlation (0.95) was observed for iron (Fe) content in grains with zinc (Zn) content in grains. It was also evident that Fe content in grains showed good positive correlation with peduncle length (PdL) while Fe content showed slightly positive correlation with all other traits like spikelets per spike (SpS), grains per spike (GPS), thousand grain weigh (TGW) and grain yield per plant (GYP).

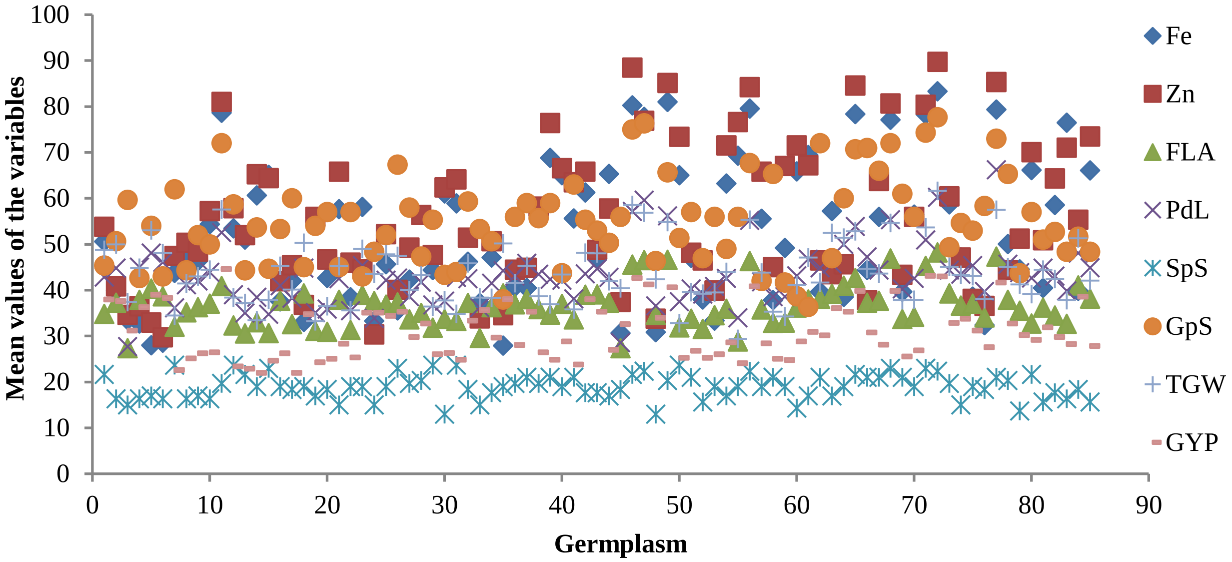

Figure 1. Scatter plot of all the mean values of eight variables of wheat genotypes.
Fe = Iron, Zn = Zinc, FLA= Flag leaf area, PdL = Peduncle length, SpS = Spikelets per spike, GpS = Grains per spike, TGW = Thousand grain weight and GYP = Grain yield per plant

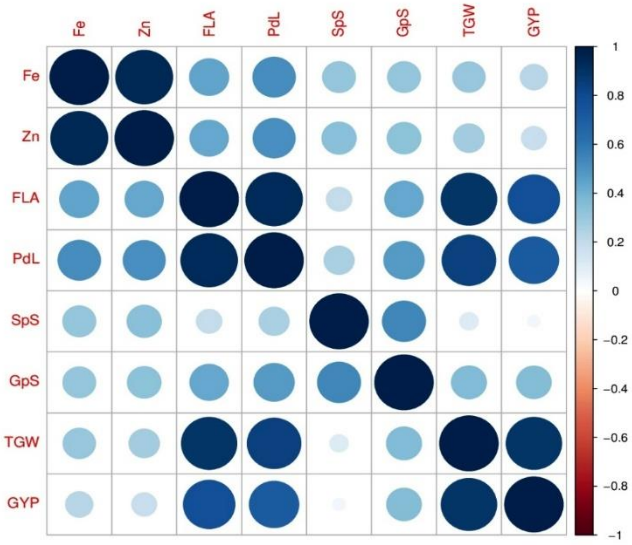

Figure 2. Correlation between eight variables studied in this experiment.
Fe = Iron, Zn = Zinc, FLA= Flag leaf area, PdL = Peduncle length, SpS = Spikelets per spike, GpS = Grains per spike, TGW = Thousand grain weight and GYP = Grain yield per plant

There was no association between Fe and other traits which could be negative. Zinc (Zn) content in grains was found positively and moderately correlated with peduncle length (PdL), flag leaf area (FLA), grains per spike (GPS), spikelets per spike (SpS) and slightly correlated with thousand grain weight (TGW). The trait flag leaf area (FLA) showed strong significant and positive correlation (0.93) with PdL. The number of spikelets per spike (SpS) had significant correlation (0.54) with grains per spike. The thousand grain weight (TGW) showed highest and positively significant correlations and association with grain yield per plant (GYP) (0.90). Grain iron and zinc content had positive correlation with all other yield contributing traits.

Cluster analysis**:** The cluster analysis placed 85 genotypes into six clusters based upon the cumulative effects of all the eight traits under study. The graphical presentation of these genotypes was obtained in the form of Dendrogram (Fig. 3). Once dendrogram was constructed, the differences between the clusters were observed by the average distance from centroid. The clusters were made based on central centroids which is the indicator of mean value of the data of all the variables (Table 3). It was observed that members of all clusters were of diverse nature and had great genetic variability between clusters. Thirty seven genotypes were grouped in cluster number 1, five in cluster number 2, twenty six genotypes in cluster number 3, ten in cluster number 4, four in cluster number 5 and three in cluster number 6. This distribution showed the genetic divergence of the genotypes. Cluster number 1, 2 ,3 5 and 6 showed greater average distance from the centroid indicating most of the variability among genotypes. All the genotypes grouped in cluster number 4 showed minimum genetic differences among themselves due to least average distance from centroid. Members of the cluster number 4 (BFT11, BFT46, BFT47, BFT49, BFT56, BFT65, BFT68, BFT71, BFT72 and BFT77) showed positive values for all the studied traits, hence suggesting their use as parents in further breeding programs of wheat biofortification.

Table 3. Cluster centroids for eight variables.

	Cluster Centroids					
Variable	**Cluster 1**	**Cluster 2**	**Cluster 3**	**Cluster 4**	**Cluster 5**	**Cluster 6**
Fe	-0.28	-1.27	-0.06	1.80	-0.65	1.03
Zn	-0.29	-1.40	0.03	1.80	-0.87	0.81
FLA	0.19	-1.15	-0.80	2.02	0.25	-0.51
PdL	0.12	-1.61	-0.66	2.07	0.30	-0.45
SpS	-0.48	-1.38	0.61	1.02	0.88	-1.65
GpS	-0.59	-0.22	0.10	1.80	1.44	-1.17
TGW	0.30	-0.46	-0.99	1.86	0.16	-0.69
GYP	0.18	0.43	-0.99	1.78	0.18	-0.57

Fe = Iron, Zn = Zinc, FLA= Flag leaf area, PdL = Peduncle length, SpS = Spikelets per spike, GpS = Grains per spike, TGW = Thousand grain weight and GYP = Grain yield per plant

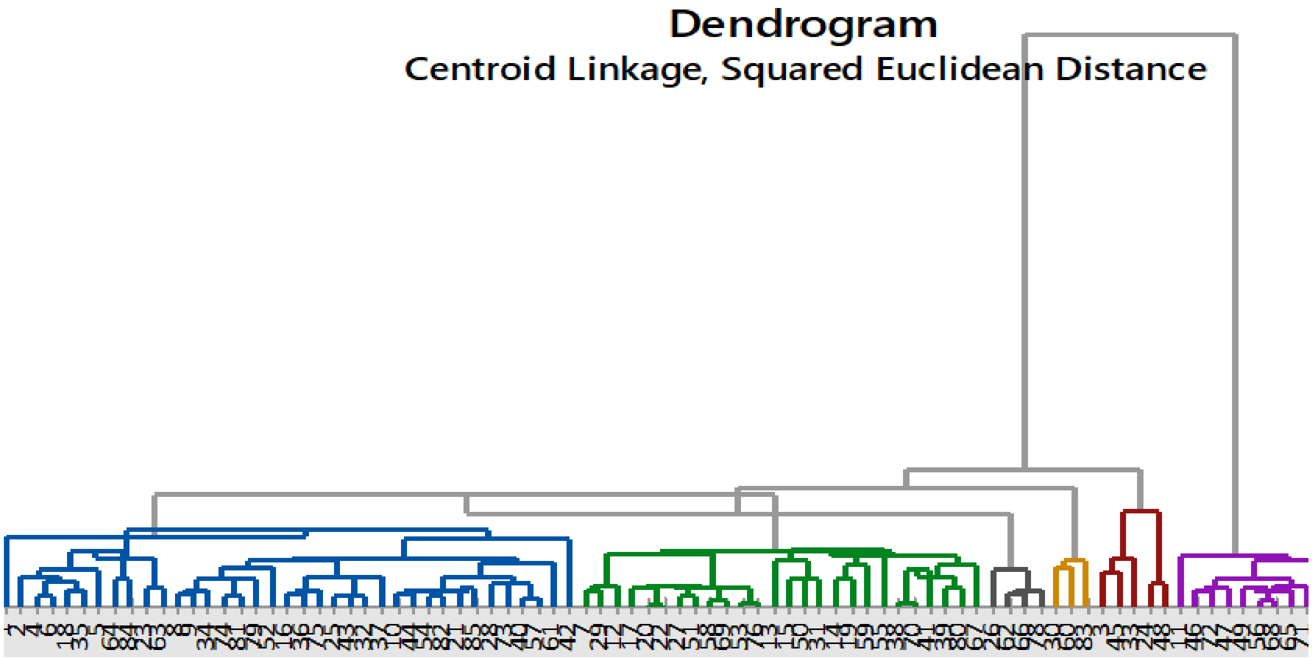

Figure 3. Dendrogram of wheat genotypes showing cluster membership.

DISCUSSION

The study exhibited considerable genetic variation in wheat genotypes with regard to mineral content i.e. iron (Fe) and zinc (Zn) in grains. The genetic variability for mineral content among genotypes has also been reported by several scientists (Amiri *et al*., 2015; Zhao *et al*., 2009; Welch *et al*., 2005). The grain iron concentration for 85 spring wheat accessions ranged between 27.95-83.30 mg/kg and grain zinc concentration ranged between 29.71-89.77 mg/kg. A number of studies have been carried out in the past to check the potential of wide range of wheat genotypes for their Fe and Zn concentration. The researchers reported similar trend of Fe and Zn concentration in their germplasm (Teklic *et al*. 2013; Monasterio and Graham, 2000). According to a research done by Graham *et al*. (1999), it was observed that Fe and Zn concentration of wheat genotypes ranged between 28.8 mg/kg - 56.5 mg/kg and 25.2 mg/kg - 53.3 mg/kg. Another research carried by Welch (2003) reported a similar trend of 33-73 mg/kg of grain iron and 27-85 mg/kg of grain zinc content. Ficco *et al*. (2009) reported a range of 33.6-65.6 mg/kg of iron content in grains and 28.5-46.3 mg/kg of zinc content in grains.

The reason behind such ranges of iron and zinc concentration in grains could be due to the presence of genetic variability, soil condition and environmental factors. The presence of genetic variability can be due to the diverse nature of germplasm and further testing like cluster analysis can be carried out. There could possibly be a role of increased uptake of minerals from soil. This information could possibly be utilized to improve the nutritional value of the edible grains through classical or modern plant breeding approach of genetic biofortification (Rawat *et al*., 2013). Presence of strong positive correlation between grain mineral content and yield related traits provides a platform for the role of pleiotropy which supports the idea of genes controlling many traits simultaneously (Zhao *et al*., 2009; Morgounov *et al*., 2007). So, improvement in micronutrient profile of edible grains can also improve the productivity of the crop. It could be useful for selection of such breeding material that could produce mineral enriched genotypes without disturbing the yield potential. Negative correlations have been found in previous studies between iron content and thousand grain weights as well (Zhao *et al*., 2009; Ficco *et al*., 2009). The outcome of our research suggested the presence of positive correlation between iron and zinc content and yield related traits which is a positive indicator for the improvement of high yielding cultivars in terms of genetic biofortification. Some researchers reported that negative association between grain iron and zinc content with thousand grain weight was due to the small size of grains (Ozturk *et al*. 2006). The research carried out by Amiri *et al*. (2015) and Velu *et al*. (2011) negated this idea and reported that grain mineral content was not dependent on size of the grain.

In this research, it was found that genotypes having broader genetic background had a broad range for grain iron and zinc content, confirmed by cluster analysis. Strong positive correlation was found between mineral content and most of the yield attributing traits. This is a positive sign for the improvement of wheat varieties for their micronutrient profiles. These interactions of the traits suggested the role of plant breeding efforts for high yield instead of micronutrients, ultimately depleting the levels of these nutrients in the recent cultivars (Fan *et al*., 2008). This loss of nutritional value of wheat is impacted by genetic and environmental patterns (Davis, 2005). It was observed that yield was the main focus of breeders in past and attention on genetic biofortification was not well paid. Improvement in nutrition of the staple crops by improving their quality can be combined with yield enhancement (Anandan *et al*., 2011).

***Conclusions*:** The breeding potential of the genotypes for the purpose of genetic biofortification was assessed in this study. It was concluded that a huge genetic potential for the improvement of cultivars was present in the germplasm to provide the starving population with mineral enriched food. The evaluated material had a great genetic potential for Fe and Zn concentration in grains along with yield, which could further be utilized for nutritional improvement of wheat crop. WHEAR-TUKURU-WHEAR (83.30 mg/kg), MARVI-2000 (80.98 mg/kg) and PRL'S'/PVN (80.27 mg/kg) had highest means among other genotypes for iron and 89.77 mg/kg, 85.14 mg/kg and 88.49 mg/kg for zinc, respectively. The positive aspect of this study was positive and significant correlation between micronutrient content and yield related traits. The information obtained through cluster analysis was also very useful as the best performing genotypes were grouped in one cluster and can be differentiated from others.

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