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Evaluation of biofortified spring wheat genotypes for yield and micronutrients

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Abstract: Advanced wheat genotypes were tested for agronomic as well as grain iron and zinc content traits. The analysis of variance indicated variation for all traits except iron (Fe) and zinc (Zn). The grain Fe content ranged from 39-58 mg/kg whereas grain Zn ranged from 32-47 mg/kg among the tested lines. A significant positive correlation (0.45) was observed between grain Fe and Zn content. There was no association between yield and grain Fe and Zn content indicating that improvement in these micronutrients will not have any undesirable affect on yield. The data was further analysed for principal component analysis and genotype by trait association. The first five principal components viz., PC1 (0.3149), PC2 (0.2198), PC3 (0.1461), PC4 (0.10) and PC5 (0.0923) accounted for 0.87 of the total variation. The major traits contributing to the PC1 are days to heading, days to maturity, grain iron content and yield. The cluster analysis revealed significant variation among the tested germplasm thus providing opportunities for increasing the micronutrient content along with yield through hybridization with high micronutrient content lines.

Keywords: Correlation, diversity, Grain Fe and Zinc content, Wheat

INTRODUCTION

Wheat is widely grown cereal crop occupying around 17% of the world's total cultivated land and a staple crop for 35 % of world's population. Wheat like other staple cereals contains low levels of iron and Zinc (Cakmak *et al.,* 2010). During the last 50 years breeding, efforts are mainly focussed towards increasing food production and countries became self sufficient from satisfactory dietary intake of staples but deficiency of Fe, Zn and vitamin A were widespread (Cakmak, 2008). Micronutrient deficiencies particularly of Fe and Zn are becoming a worry where wheat is consumed as staple crop particularly in developing countries. Biofortification through conventional and modern breeding approaches can address these problems in a cost effective manner as other approaches like supplementation, fortification, agronomic fortification etc. adds additional cost for fortification (Velu *et al*., 2014).

Wheat is staple food of south Asia and its consumption by an individual is around 100–150 g/day (Joshi *et al.*, 2010). Increasing micronutrient concentration in seeds requires the availability of sufficient variation of these nutrients in seeds. Wide variation for iron and Zinc has been reported by several authors in different studies (Graham *et al.,* 1999; Morgounov *et al.,* 2007; Rawat *et al.*, 2008), but the availability of micronutrients in modern day varieties is partial. Wild relatives of wheat like *Triticum boeoticum*, *T. monococcum*, *T. dicoccoides*, *Aegilops tauschii*, and *Ae. speltoides* have been evaluated and found to be promising donors for Fe and Zn (Cakmak *et al*., 2000; Monasterio and Graham, 2000; Chhuneja *et al.,* 2006; Rawat *et al.,* 2008). Cakmak *et al.,* (2000) identified *T. dicoccoides* having both high concentration and range of variability for Fe and Zn. High yielding and micronutrient nutrient rich wheat genotypes can be developed by genetic manipulations, since seeds could reach a larger number of people without necessarily changing consumer's behaviour (Ortiz-Monasterio *et al.,* 2007; Cakmak, 2008).

Correlation analysis helps to identify effective traits in order to make indirect selection for selecting superior genotypes. On the other hands, principal component analysis is suitable multivariate technique in identify and determination of independent principal components that are effective on plant traits separately. Therefore, correlation and principal component analysis help in the genetic improvement of traits such as yield that have low heritability specifically in early generations *via* indirect selection for traits effective on this (Beheshtizadeh *et al.,* 2013). An International group, HarvestPlus aimed at developing new plant

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genotypes with high micronutrient concentrations of micronutrients utilizing both landraces and wild crop relatives and supplies fixed breeding material to associated partner countries (Velu *et al*., 2014). The present investigation was carried out to estimate genetic variability for agronomic traits and micronutrient traits (Fe & Zn) as well as genotype by trait analysis in a set of 3rd HarvestPlus Yield Trial (HPYT) received from International Maize and Wheat Improvement Center (CIMMYT), Mexico.

MATERIALS AND METHODS

Plant material: The plant material consisted of 50 wheat genotypes of which 47 genotypes were numbered (HP404- HP450), one local check DPW 621-50 and two international checks BAJ#1 and MUNAL#1 (Table 1) received from CIMMYT, Mexico as 3rd HPYT. The genotypes which are used in this study were having *T. dicoccum* and *Aegilopes squarrosa* in their pedigree which are identified donors of high Fe and Zinc. These 50 genotypes were tested in randomised complete block design with two replications at ICAR-Indian Institute of Wheat and Barley Research, Karnal Haryana (Latitude 29˚ 43'N, longitude 76˚ 58'E and altitude 245m) during 2012-13. Genotypes were planted in plots (6 rows of six metre with a row spacing of 20cm). All the package of practices were followed for raising the wheat crop. Data was recorded for the days to heading, days to maturity, plant height, grains/spike, thousand grain weight and yield.

Micronutrient sampling and analysis: Before harvesting, 50 spikes from each plot were plucked and threshed manually using wooden sticks in cotton bags. Cleaning of grains was carried out in plastic trays and a random sample of each genotype was taken for Fe and Zn estimation. Fe and Zn estimation was carried out using, an Oxford Instruments X-Supreme 8000 (XRF) fitted with a 10 place auto-sampler holding 40 mm Al cups. For each sample, Al cups were prepared using fresh Poly-4 film and cups having samples were shaken to distribute grains evenly (Paltridge *et al.,* 2012).

Statistical analysis: The descriptive statistics Analysis of variance (ANOVA) and heritability estimates were

Table 1. Pedigree of biofortified genotypes evaluated for grain iron and zinc content.

Entry Name	Pedigree
DPW 621-50	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES
BAJ #1	WAXWING/4/SONOITAF81/TRAP#1/3/KAUZ*2/TRAP//KAUZ
MUNAL #1	WAXWING*2/KIRITATI
HP404	CROC_1/AE.SQUARROSA (210)//INQALAB 91*2/KUKUNA /3/PBW343*2/KUKUNA
HP405, 406, 407	QUAIU #1/3/T.DICOCCON PI94625/AE.SQUARROSA (372)// 3*PASTOR/4/QUAIU #2
HP408	VILLA JUAREZ F2009/SOLALA//WBLL1*2/BRAMBLING
HP409	VILLA JUAREZ F2009/SOLALA//WBLL1*2/BRAMBLING
HP410, 411, 412, 413	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1/4/T.DICOCCON PI94625/AE.SQUARROSA(372)//SHA4/ CHIL/5/ WHEAR/ KUKUNA / 3/C80.1/3*BATAVIA//2*WBLL1
HP414	
HP415	T.DICOCCON CI9309/AE.SQUARROSA (409)/3/MILAN/S87230//BAV92/4/2*MILAN/S87230//BAV92
HP416	KVZ/PPR47.89C//TACUPETO F2001*2/BRAMBLING/3/2*TACUPETO F2001*2/BRAMBLING
HP417	HGO94.7.1.12/2*QUAIU #1
HP418	
HP419	QUAIU #1/3/T.DICOCCON PI94625/AE.SQUARROSA (372)//3*PASTOR/4/QUAIU #2
HP420	DANPHE #1*2/3/T.DICOCCON PI94625/AE.SQUARROSA (372)//SHA4/CHIL
HP421, 422,423	T.DICOCCONCI9309/AE.SQUARROSA(409)/3/MILAN/S87230//BAV92/4/2*MILAN/S87230//BAV92
HP424	COAH90.26.31/4/2*BL2064//SW89-5124*2/FASAN/3/TILHI
HP425	QUAIU #1/SOLALA//QUAIU #2
HP426	
HP427	T.DICOCCON CI9309/AE.SQUARROSA (409)/3/MILAN/S87230//BAV92/4/2*MILAN/S87230//BAV92
HP428	CROC_1/AE.SQUARROSA (210)//WBLL1*2/BRAMBLING/3/2*VILLA JUAREZ F2009
HP429	KIRITATI/3/2*SERI.1B*2//KAUZ*3/BOW/4/CMH81.530
HP430	KIRITATI/3/2*SERI.1B*2//KAUZ*3/BOW/4/CMH81.530
HP431	
HP432	WHEAR/KIRITATI/3/C80.1/3*BATAVIA//2*WBLL1/4/CMH75A.66/SERI
HP433	KINDE/4/CMH75A.66//H567.71/5*PVN/3/SERI
HP434	KINDE/4/CMH75A.66//H567.71/5*PVN/3/SERI
HP435	VILLA JUAREZ F2009/SOLALA//WBLL1*2/BRAMBLING
HP436	QUAIU #1/3/T.DICOCCON PI94625/AE.SQUARROSA (372)//3*PASTOR/4/QUAIU #2
HP437	PAURAQ//RL6043/4*NAC
HP438	PAURAQ//RL6043/3*GEN
HP439	PAURAQ//RL6043/3*GEN
HP440, 441, 442,443	PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/4/CMH75A.66/SERI
HP444	HUW234+LR34/PRINIA//INQALAB91*2/KUKUNA/4/FRET2*2/3/SNI/TRAP#1//KAUZ*3/TRAP/5/ CMH73A.497/3*CNO79
HP445	
HP446	DANPHE #1*2/3/T.DICOCCON PI94625/AE.SQUARROSA (372)//SHA4/CHIL
HP447	VILLA JUAREZ F2009/SOLALA//WBLL1*2/BRAMBLING
HP448, 449	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1/4/T.DICOCCON PI94625/AE.SQUARROSA(372)//SHA4/ CHIL/5/WHEAR /KUKUNA /3/C80.1 /3*BATAVIA//2*WBLL1
HP450	KVZ/PPR47.89C//TACUPETO F2001*2/BRAMBLING/3/2*TACUPETO F2001*2/BRAMBLING

Table 2. Analysis of variance of biofortified genotypes along with checks for different traits.

Source of Variation	df	Mean squares							
		DTH	**DTM**	**PH**	**GSPK**	**TGW**	**YIELD**	**Fe (mg/kg)**	**Zn (mg/kg)**
Replication	1	0.16	0.09	36.00**	23.04	27.04*	181987.56**	14.44	135.03**
Genotypes	49	9.56**	8.30**	37.69**	81.55**	23.72**	41649.13**	34.58	23.17
Error	49	0.36	1.56	12.18	30.09	5.55	19423.27	22.72	18.17

** Significant at 1% level, * Significant at 5% level of significance.

calculated using PBTools, version 1.4 (2014). Euclidean distances calculated using Wards method and dendrogram was constructed to observe the relationship among different genotypes. Principal component analysis, genotype by trait and cluster analysis was done using STAR version 2.0.1(2014).

RESULTS AND DISCUSSION

The present study was carried out for evaluating the 50 wheat genotypes for yield potential, character association, cluster analysis and principal component analysis and the results obtained were presented as below.

Analysis of variance: Analysis of variance indicated variation in almost all the traits days to heading (DTH), days to maturity (DTM), plant height (PH), grains per spike (GSPK), thousand grain weight (TGW) and yield except iron (Fe) and Zinc (Zn) (Table 2). Most of the genotypes were comparable to the check varieties (Table 3). For DTH, significant variation was observed in the evaluated genotypes. The mean value of the trait was 82 days with a range of 78 days (HP 404) to 88 days (MUNAL#1). The local check variety DPW 621-50 showed average 86 days to heading as compared to other checks Munal#1(88 days) and BAJ#1 (80 days). The trial mean for days to maturity was 115 days with a range of 112 days (HP404) to 120days (MUNAL#1).The local check DPW 621-50 showed days to maturity of 118 days. The plant height ranged from 91 cm (HP438) to 110 cm (HP423) with an average of 101 cm. However, the local check DPW 621-50 showed a height of 93cm as compared to other two checks 98 days(BAJ#1) and 103 days (MUNAL#1). For GSPK trait, a range of 45 (HP433) to 70 (HP427) grains/spike with a mean of 56 GSPK was observed. The checks DPW 621-50, BAJ#1 and MUNAL#1 had a 55, 51 and 52 GSPK respectively. A range of 33g (HP429) to 46g (HP417) with an average of 39 g was observed for thousand grain weight. Among the checks, MUNAL#1 had highest TGW of 39g as followed by BAJ#1 (36g) and DPW 621-50 (35g). Genotypic variation for yield was observed among the test entries evaluated for yield. The genotype HP 404 (1375g) showed minimum yield whereas the genotype HP422 (1875g) showed highest yield among the test entries. The overall mean of the trial yield was 1607g. Among the test entries only one entry HP422 (1875) out yielded all the checks DPW 621-50 (1850g), BAJ#1 (1665g) and MUNAL#1

Table 3. Descriptive statistics of biofortified genotypes for agronomic and micronutrient traits.

Trait	Range	Mean	CV	Heritability
DTH	77-87	82	2.7	0.96
DTM	112-120	115	1.8	0.82
HT	93-110	101	4.3	0.68
GSPK	44-69	56	11.5	0.63
TGW	33-46	39	8.6	0.75
YLD (g)	1372-1875	1607	9.0	0.53
Fe(mg/kg)	39-58	46	9.0	0.35
Zn(mg/kg)	32-47	40	8.5	0.22

*DTH- days to heading, DTM- days to maturity, HT-plant height, GSPK- grains/spike, TGW- thousand grains weight, YLD- grain yield, Fe-grain iron content and Zn- grain zinc content.

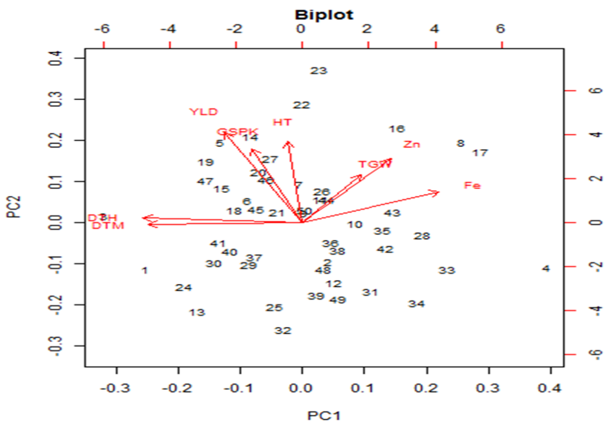

Fig. 1. *Biplot between first and second principal component.*

Table 4. Correlation matrix for different agronomic and micronutrient traits.

	DTM	HT	GSPK	TGW	YLD	Fe	Zn
DTH	0.81**	0.07	0.12	-0.18	0.29*	-0.42**	-0.21
DTM		-0.03	0.00	-0.03	0.30*	-0.47**	-0.21
HT			0.24	0.13	0.30*	-0.05	0.19
GSPK				-0.14	0.48**	-0.09	0.02
TGW					0.13	0.28*	0.25*
YLD						-0.08	0.07
Fe							0.45**

*DTH- days to heading, DTM- days to maturity, HT-plant height, GSPK- grains/spike, TGW- thousand grains weight, YLD- grain yield, Fe-grain iron content and Zn- grain zinc content.

Table 5. Vector loadings and percentage variation explained by the first five principal components (PC).

Traits	PC1	PC2	PC3	PC4	PC5
DTH	-0.5369	0.0295	-0.2570	0.1571	-0.2720
DTM	-0.5217	-0.0102	-0.4275	0.1375	-0.0900
HT	-0.0517	0.4912	0.0907	-0.7359	-0.3269
GSPK	-0.1731	0.4448	0.5424	0.2750	0.1795
Fe	0.4590	0.1860	-0.1439	0.4114	-0.0501
Zn	0.2972	0.3852	-0.2411	0.2662	-0.6075
TGW	0.1957	0.2874	-0.6079	-0.2225	0.5538
Yield	-0.2638	0.5425	0.0217	0.2228	0.3172
		Loadings			
Standard Deviation	1.5872	1.3260	1.0812	0.9015	0.8591
Eigen Value	2.5192	1.7583	1.1690	0.8126	0.7381
Individual Percentage	0.3149	0.2198	0.1461	0.1016	0.0923
Cumulative variance	0.3149	0.5347	0.6808	0.7824	0.8747

*DTH- days to heading, DTM- days to maturity, HT-plant height, GSPK- grains/spike, TGW- thousand grains weight, YLD-grain yield, Fe-grain iron content and Zn- grain zinc content

Table 6 Mean Value of traits in four Clusters.

	Cluster Ia			**Cluster Ib**			**Cluster IIa**			**Cluster IIb**		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
DTH	83	88	85	81	86	84	78	83	81	78	82	81
DTM	115	120	118	114	118	116	112	115	114	112	115	114
HT	93	106	98	97	110	104	96	109	101	95	104	101
GSPK	46	61	54	48	70	59	46	64	56	45	58	52
TGW	33	44	38	36	45	41	34	41	38	37	46	43
YLD	1383	1850	1563	1580	1875	1746	1410	1668	1542	1373	1730	1518
Fe (mg/kg)	40	49	44	42	49	46	41	48	45	45	58	52
Zn (mg/kg)	34	41	38	36	45	41	33	42	39	36	48	44

*DTH- days to heading, DTM- days to maturity, HT-plant height, GSPK- grains/spike, TGW- thousand grains weight, YLD-grain yield, Fe-grain iron content and Zn- grain zinc content

(1818g).

A wide variation range of 40mg/kg (HP424) to 58mg/kg (HP404) with a mean of 46 mg/kg was observed for Fe among the tested genotypes. The check variety BAJ#1 had 45mg/kg Fe as compared to other two checks DPW 621-50 (42mg/kg) and MUNAL#1 (41mg/kg). Similarly for Zn, a variation range of 33mg/kg (HP439) to 48mg/kg (HP408) with an average of 40mg/kg was observed among test genotypes. The local check DPW 621-50 had lowest Zn (36mg/kg) as compared to international checks MUNAl#1 (37mg/kg) followed by BAJ#1 (40mg/kg). However, the differences among genotypes were found non-significant for both Fe and Zn. Different studies showed wide variation for grain Fe and Zn content (Oury *et al.,* 2006; Zhao *et al.,* 2009; Velu *et al.,* 2011; Badakhshan *et al.,* 2013) among wheat genotypes as well as among core collection of accessions of diverse origin. Heritability estimates are limited to experimental material and may differ widely in the same crop

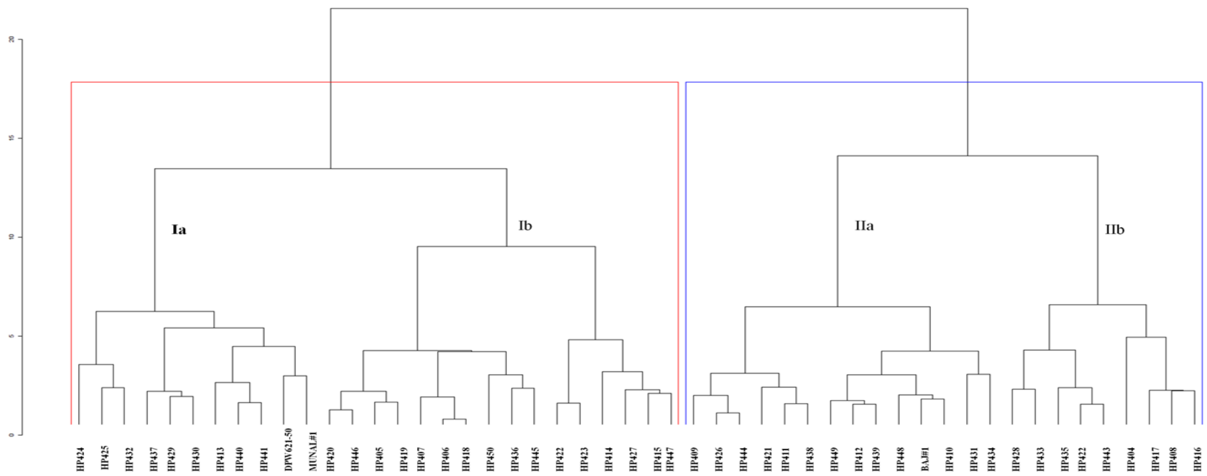

Fig. 2. *Clustering of genotypes based on Agglomerative clustering method.*

and same trait (Garcia- Oliveira *et al.,* 2009). High heritability was found for DTH (0.96) followed by DTM (0.82), TGW (0.75), HT (0.68), GSPK (0.63), Yield (0.53), Fe (0.35) and Zn (0.22). Broad sense heritability estimates ranged from 14.96% for Fe to 55% for Zn reported in wheat by Badakhshan *et al.,* (2013). The mean, range, coefficient of variation and Heritability estimates were given in Table 2.

Pearson correlation coefficient and principal component analysis (PCA): A significant positive correlation (r= 0.45) was observed between Fe and Zn indicating that if one stable component Zn is improved then Fe content is also improved (Table 4). Similar results have been reported by Velu *et al* 2011 and Ortiz - Monasterio *et al (,* 2007) in wheat TGW positively correlated with Fe (r = 0.28) and Zn (r= 0.25). However, there was no correlation was found between yield between and Fe (r= -0.08) and Zn (r= 0.07), indicating yield improvement do not have any negative influence on grain Fe and Zn content. No negative association of grain Fe and Zn with grain yield was observed in wheat by Graham *et al.,* (1999), Welch and Graham (2004) and Velu *et al.,* (2012). However, few reports revealed slightly negative correlation between Zn and grain yield in wheat (Zhao *et al.,* 2009; Gomez-Becerra *et al.,* 2010).

The PCA analysis indicated that the first five components (PC1, PC2, PC3, PC4 and PC5) explained maximum cumulative variances of 0.8747% are important (Table 6). Among all PCs, the first PC (0.3149) contributed maximum to the total variance. The major traits contributing to the first PC are DTH, DTM, Fe and Yield. Similarly for second PC, Yield, HT and GSPK were the major contributors. TGW, GSPK and DTM were the diversity contributor traits in the third PC. In fourth PC, max variation was explained by height followed by iron. In the last fifth PC, the maximum variation contributors were Zn and TGW. The biplot explains the relationship of 50 wheat genotypes with component traits (Fig. 1). Across the 50 genotypes, grain yield was positively associated with GSPK, DTM, GSPK, HT, TGW and Zn and negatively with Fe.

Cluster analysis: The mean values of clusters are presented in Table 5. The dendrogram constructed using STAR version 1.0.2 revealed two major clusters I (with two sub clusters Ia, Ib) and II (with two clusters IIa and IIb) (Fig. 2). Sub-cluster Ia consists of eleven genotypes viz., HP424, HP425, HP432, HP437, HP429, HP430, HP413, HP440, HP441, DPW 621-50 and MUNAl#1. This cluster represents the tall genotypes (93-106cm) having yield range (1383-1850g) accompanied by lowest Fe (44mg/kg) and Zn (38mg/kg) grain content among all sub-clusters (Table 6). The genotypes HP420, HP664, HP405, HP419, HP407, HP406, HP418, HP450, HP436, HP445, HP422, HP423, HP414, HP427, HP415 and HP447. The sub-cluster Ib had the genotypes with high grain yield (1580-1875g), GSPK (48-70 grains) and TGW (36-45g).The cluster IIa comprised of 14 genotypes HP409, HP426, HP444, HP421, HP411, HP438, HP449, HP412, HP439, HP448, BAJ#1, HP410, HP431 and HP434. Tall genotypes (96-109cm) with average values for all traits under study were presented in sub-cluster IIa. The nine genotypes viz., HP428, HP433, HP435, HP422, HP443, HP404, HP417, HP408 and HP416 formed the sub-cluster IIb. The sub -cluster IIb represents the genotypes which are early in flowering (78-82 days) and maturity (112-115 days) as well as high in Fe (45-58 mg/kg) and Zn (36-48) content. The minimum, maximum and mean values of each trait for each cluster are presented in Table 6.

Conclusion

In this study, genotypic variation was found among all the tested genotypes the genotypes for different agronomic traits. The variation for Zn (32-57 mg/kg) and Fe (39-58 mg/kg) was observed among the entries. Genotypes having high Fe and Zn grain content along with yield comparable to checks were identified indicating that these lines can be utilized for breeding high micronutrient genotypes.

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