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Genotype-environment interaction for grain iron and zinc concentration in recombinant inbred lines of a bread wheat (*Triticum aestivum* L.) cross

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Abstract

A set of 306 recombinant inbred lines (RILs) along with the two parents and hitherto popular wheat variety in India, PBW 343, were evaluated in three environments over two years for grain iron (Fe) and zinc (Zn) concentration. Considerable genetic variation for both grain iron and zinc concentration exists. The environment effect was the most important source of variation for grain Fe and Zn concentration, explaining 37.42% and 57.78% of the total sum of squares respectively. Genotype-environment interaction (G x E) for Fe and Zn accounted for 29.46% and 23.24% of the total sum of squares, respectively. The magnitude of G x E interaction was relatively high. High heritabilities were observed for iron (0.81) and zinc (0.71) concentrations reflecting non-crossover type of interaction. The positive and moderately high correlation (0.677) between Fe and Zn concentration suggest good prospect of simultaneous improvement of both the micronutrients. Additive main effects and multiplicative interaction biplot and environmental indices indicated the most favorable environment for Fe to be at Delhi, which was the second most favorable environment after Samastipur in Bihar for Zn. A poor environment for grain Fe and Zn accumulation was at Pantnagar. Four stable RILs each for grain Fe and grain Zn concentration were identified.**

Key words: Grain Fe and Zn concentration, genotype-environment interactions, AMMI analysis, *Triticum aestivum*

Introduction

Micronutrients play an important physiological role in plants as well as in human body. Iron and zinc are two of the three nutrients (i.e. Fe, Zn and Vitamin A) that are recognized by World Health Organization (WHO) as limiting (Ortiz-Monasterio et al. 2007). Micronutrient deficiency can be overcome by consuming a diverse diet, which is not afforded by majority, particularly in developing and underdeveloped countries where malnutrition is a major problem. The other alternatives are supplementation and fortification but these are also difficult to sustain on a long-term basis. Biofortification has been recognized as an economical and sustainable strategy that can be useful as a complementary solution to the problem of malnutrition. Improving livelihoods through increased level of micronutrients in food is considered second only to controlling HIV/AIDS (http://siteresources. worldbank.org; Joshi et al. 2010) and biofortification has appeared among the five topmost solutions to the problem of micronutrient malnutrition in the Copenhagen consensus of the world's top economists (http://www. copenhagenconsensus.com/sites/default/files/cc08_results_final_0.pdf). Enrichment of food crops with nutrients is one of the high priority research area for the scientific community. High iron beans, high zinc rice, Pro-vitamin A rich cassava, high iron pearl millet etc. are some examples that speak of the feasibility and success of this strategy (www.harvestplus.org).

Cereal based foods represent the largest proportion of the daily diet in countries where high incidence of micronutrient deficiencies is prevalent (Bouis and Welch 2010). Wheat is an important staple food for humans which contributes 20% of the world's dietary energy (http://faostat.fao.org). Therefore, development of genetically enriched wheat varieties

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with higher micronutrient levels is a sustainable and economic approach for eliminating malnutrition across the globe (Welch and Graham 2004). To find solution to prevailing micronutrient malnutrition, a widespread exploration of genetic resources coupled with understanding of the physiological and genetic basis of mineral accumulation in seed is essential; critical also is an appreciation of the importance of environment and its interaction with genotype (Ficco et al. 2009; Peterson et al. 1986). Various reports have indicated significant effect of the genotype-environment interaction on the final grain iron and zinc concentrations (Daniel et al. 2011; Velu et al. 2012; Oury et al. 2006; Gomez-Becerra et al. 2010a and b). The assessment of environmental stability of the genotypes for micronutrient concentrations in grains is important for a reliable and useful enhancement of the nutritional quality of crops.

The present study was aimed to evaluate the magnitude and nature of genotypic variation for grain Fe and Zn concentration, environment and genotype-environment interaction effects for grain Fe and Zn concentration in the RIL population and to identify promising and stable genotypes for future wheat breeding programme.

Materials and methods

Plant material and environments

Materials consisted of 309 wheat entries of which 306 were the RILs developed from a cross between an Indian bread wheat cultivar WH542 (termed as parent 1 or P1) and a synthetic derivative (*Triticum dicoccon* PI94624/*Aegilops sqarrosa* (409)//BCN (termed as parent 2 or P2) from CIMMYT, Mexico. The remaining three genotypes included the two parents of the RILs and one hitherto popular wheat variety in India, PBW 343. The RILs were developed and maintained at Grain Quality Laboratory, IARI, New Delhi, India. The selected genotypes were evaluated at three different locations, two belonging to North Western Plains Zone (NWPZ) and one to North Eastern Plains Zone (NEPZ) during the winter (*rabi*) season of the years 2012-13 and 2013-14. The crop was timely sown under irrigated conditions in first fortnight of November at all the locations in both the years. The locations selected from NWPZ include, Delhi (Indian Agricultural Research Institute, Research Farm, New Delhi, 28^{o} 38'N, 77^{o} 9'E, 228.6 m amsl) and Pantnagar (GB Pant University of Agriculture and Technology, Research Farm, Uttarakhand, 29^{o} 'N, 79^{o} 31'E, 243.8 m amsl). The third location, (IARI, Regional Station, Research Farm, Samastipur, Bihar, 25^{o} 14'N, 87^{o} 2'E, 62.5 m amsl) belonged to the NEPZ. The genotypes were planted in a randomized complete block design (RCBD) with two replications per entry and two rows (5 m length) per replication with a plant-to-plant spacing of 10 cm and row-to-row spacing of 25 cm. Standard agronomic practices were followed for raising the crop.

Grain sampling and micronutrient analysis

A random sample of 20 spikes per entry was harvested after physiological maturity, the spikes were threshed in a clean cloth and the grain was separated from husk in a plastic *chaaj*. The grain was sampled for iron and zinc analysis. Care was taken at every step to avoid metal contamination. The grain samples were analyzed at Grain Quality Laboratory, IARI, New Delhi, India, using a new cost-effective, high throughput method called Energy Dispersive X-ray Fluorescence (ED-XRF). The XRF machine model X-Supreme 8000 (M/S Oxford Inc) was calibrated with glass standards in collaboration with Flinders University (James Stangoulis; Technical coordinator, HarvestPlus).

Statistical analysis

The data were subjected to combined analysis of variance (ANOVA) using the GLM procedure of Statistical Analytical System (SAS) facility available at IASRI, New Delhi, to determine the significance of the main effects and interactions. Stability analysis was undertaken following the Eberhart and Russell model (Eberhart and Russell 1966) and Additive Main and Multiplicative Interaction (AMMI) (Zobel et al. 1988; Gauch and Zobel 1996) model using Windostat (Version 8.0, Indostat Services, University of Agricultural Sciences, Bangalore, India) software. The biplots [main effect means *vs* first Interaction Principal Component Axis (IPCA1)] from AMMI analysis were used to study the pattern of response of genotype, environment and genotype by environment and to identify genotypes with broad or specific adaptation to target environments for Fe and Zn concentration. Pearson's correlations of the means were determined using the software OPSTAT (CCS, Haryana Agricultural University, Hisar, India). Heritability ($h^2_{b.s}$) was estimated from the ANOVA (Comstock and Robinson 1952a).

Results and discussion

Genetic variability

ANOVA revealed significant variation for both grain Fe and Zn concentrations in all the three locations

and two years, suggesting the presence of wider genetic variability to be used for the genetic improvement of grain iron and zinc concentration in wheat. In the present study, grain Fe ranged from 27.95-51.55 mg/kg (mean 41.09 ± 0.13 mg/kg) at Delhi, 27.85-54.60 mg/kg (mean 37.67 ± 0.14 mg/kg) at Pantnagar, and 27.90-47.75 mg/kg (35.36 ± 0.11 mg/kg) at Bihar, whereas pooled mean across the locations and years for iron was 38.04 ± 0.13 mg/kg and its range was between 30.28-40.00 mg/kg. The range for grain Zn concentration was 29.65-58.85 mg/kg (mean 41.06 ± 0.21 mg/kg) at Delhi, 19.30-55.10 mg/kg (mean 29.51 ± 0.17 mg/kg) at Pantnagar, and 26.45-70.55 mg/kg (42.56 ± 2.2 mg/kg) at Bihar, while pooled mean across the locations and years for zinc was 37.71 ± 0.19 mg/kg and its range was between 30.31-48.36 mg/kg.

Broad sense heritability and grain mineral association

Broad sense heritability based on an entry mean basis was 0.81 for iron and 0.71 for zinc. Similar results with high broad sense heritability have been reported (Velu et al. 2012; Gomez-Becerra et al. 2010a) while one author observed low broad sense heritability (Joshi et al. 2010). Correlation analysis of the genotypes tested resulted in highly significant correlation coefficients (P<0.01) for iron and zinc. The association was strong (r=0.677**) suggesting that improving one of the micronutrients would allow the other to be improved simultaneously. Positive correlation among the two traits have been reported by others also (Oury et al. 2006; Gomez-Becerra et al. 2010a and b) while Daniel et al. (2011) observed absence of any association.

Genotype-environment interactions

The pooled ANOVA (Table 1) was carried out after conducting Bartlett's test. The test was non-significant for both iron and zinc. The ANOVA indicated that the effects of genotype, year, location, and their first order interactions (year × location, genotype × year and genotype × location) and that of second or higher order interaction (genotype × year × location) were highly significant ($p < 0.0001$) for Fe and Zn concentration except year x location for Zn. The environmental factors as a whole explained 37.42% (for Fe) and 57.78% (for Zn) of total variance. Similar results have been reported for bread wheat (Peterson et al. 1986) and for durum wheat (Ficco et al. 2009). The environment accounts for a substantial part of total variance. The magnitude of the total genotype-environment interactions (G × Y, G × L and G × Y × L) was 29.46 % for Fe and 23.24 % for Zn of the total variance. These results are in conformity with most of the multi-environment studies on mineral concentration in wheat and other related species, that stress the importance of the genotype-environment interaction (Ficco et al. 2009; Peterson et al. 1986; Oury et al. 2006), thus pointing to a possible but potentially slow

Table 1. Combined analysis of variance (ANOVA) for grain iron and zinc concentration of 306 RILs, two parents and one check variety grown for two years at three locations

Source of variation	DF	Fe		Zn	
		MSS	Pr > F	MSS	Pr > F
Replication	1	35.13	0.0265	239.54	<.0001
Year (Y)	1	546.02	<.0001	264.85	<.0001
Location (L)	2	10281.82	<.0001	63027.8	<.0001
Y x L	2	650.72	<.0001	30.88	0.1079
Genotype (G)	308	64.26	<.0001	133.97	<.0001
G x L	616	11.87	<.0001	37.17	<.0001
G x Y	308	11.01	<.0001	31.2	<.0001
G x Y x L	616	11.27	<.0001	29.78	<.0001
Pooled error	1853	7.12		13.85	
C.V. (%)		7.01		9.87	
% GSS/total sum of squares		33.05		18.87	
% (Y+L+Y x L)		37.42		57.78	
%(Y × G +G × L+G × Y × L) SS/Total SS		29.46		23.24	

genetic progress during breeding for these traits because of substantial influence by the environment.

Stability analysis and identification of stable genotypes

The genotype-environment interaction component was further elaborated by using the joint regression model of stability analysis proposed by Eberhart and Russell (Eberhart and Russell 1966). The ANOVA (Table 2) for stability using Eberhart and Russell model revealed that the variation due to genotype-environment (linear) was non-significant for both iron and zinc concentration, however other components *i.e.,* both the main effects and its interaction effects were found to be significant. Magnitude of environmental effect was more pronounced than genotypes, this suggests that environment plays a major role in these trait's expression, which emphasizes the need to select genotypes with specific adaptation to particular environment/set of locations with similar environment particularly soil availability (Feila et al. 2005; Trethowan et al. 2007). Another reason for greater genotype-environment interaction for iron and zinc concentration could be their quantitative inheritance as reported in maize and rice (Gregorio 2002; Long et al. 2004) and in wheat (Trethowan et al. 2007; Yunfeng et al. 2012; Yuanfeng et al. 2014). Based on stability parameters *viz*., linear response (bi=1), minimum deviation from linearity (S^2d_i= 0), and higher trait mean value (Eberhart and Russell 1966), a few stable genotypes for Fe (MP 97-8, MP 97-24, MP 97-36, MP 97-272) and for Zn (MP 97-2, MP 97-138, MP 97-169, MP 97-312) were identified as stable across the environments despite the profound effect of environment on the expression of Fe and Zn observed in this study (Table 3). No genotype stable for both the traits could be identified. It is important to analyse the genotype-environment interactions for individual crops as genotype-environment interactions in the same environments for different crops have been found to vary (Chakraborti et al. 2011).

AMMI analysis

Since the initial genotype-environment interaction was found to be significant for both the grain Fe and Zn concentration, AMMI was used for further estimation of various variance components. ANOVA (Table 4) revealed significant role of the additive main effects i.e., genotype and environment on the total variation of the traits. In the multiplicative analysis, the first two interaction principal components were significant for both the traits. The first two interaction principal

Table 2. ANOVA for stability of grain iron and zinc concentration

Sources of variation	DF	MSS	
		Iron	Zinc
Genotypes	308	32.13***	66.98***
Environment	5	2241.11***	12638.22***
Environment + (Genotype × Environment)	1545	12.96***	57.36***
Environment (Linear)	1	11205.56***	63191.12***
Genotype × Environment (Linear)	308	4.75	17.74
Pooled deviation	1236	5.95**	16.15**
Pooled Error	1848	3.41	6.4

*** Significance at P<0.001, ** Significance at P<0.01

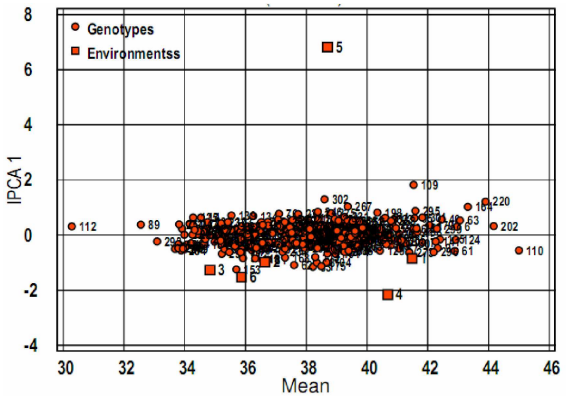
Table 3. Identification of stable RILs for iron and zinc concentration across six environments

RIL	Fe			RIL	Zn		
	Mean	bi	S^2di		Mean	bi	S^2di
MP 97-8	41.83	1.04	-0.79	MP 97-2	42.23	1.06	0.22
MP 97-24	41.15	1.03	0.10	MP 97-138	40.91	1.09	2.06
MP 97-36	41.06	1.06	0.95	MP 97-169	40.56	1.08	-0.4
MP97-272	40.96	0.98	0.47	MP 97-312	41.12	1.10	0.71

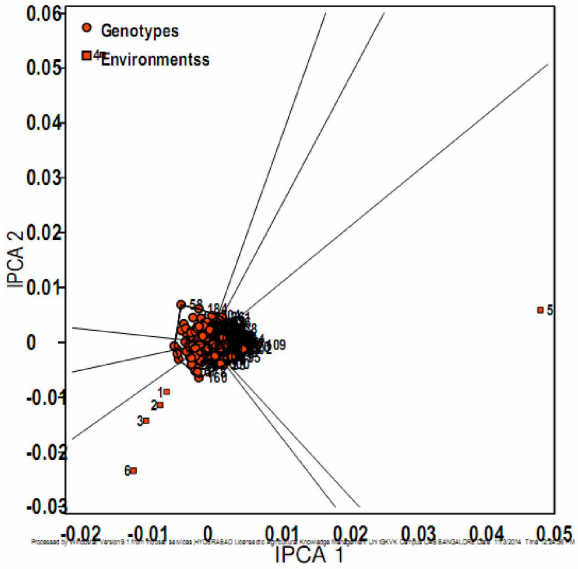
Table 4. AMMI stability ANOVA of 306 RILs, two parents and one check variety for grain iron and zinc concentration in six environments

Sources of variation	DF	Iron		Zinc	
		MSS	Variance (%)	MSS	Variance (%)
Treatments	1853	16.15***		58.96***	
Genotypes	308	32.13***		66.98***	
Environment	5	2241.11***		12638.22***	
G*E interaction	1540	5.73		16.51	
PCA I	312	10.37***	36.69	22.41***	27.5
PCA II	310	6.36***	22.36	19.69*	24.01
PCA III	308	4.55	15.9	16.29	19.73
PCA IV	306	4.14	14.37	13.63	16.4
Residual	304	3.1	10.68	10.33	12.36
Pooled residual	1228	4.55		16.51	

*,** Significance at P<0.001, * Significance at P<0.05



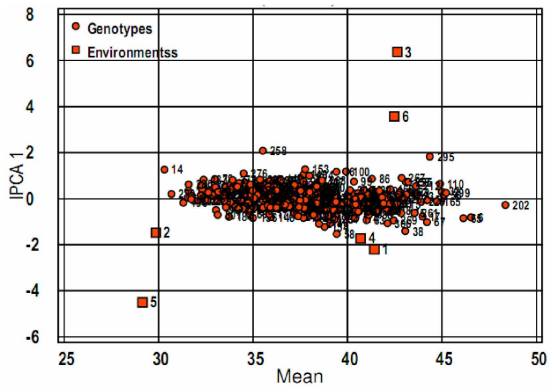
(a)



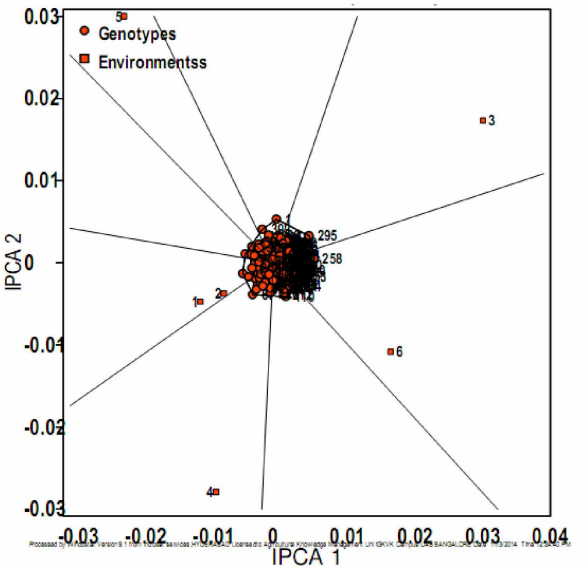
(b)

Fig. 1. AMMI biplots for Fe: (a) unadjusted mean and first principal component (b) between first and second principal components

components cumulatively explained 59.05% of interaction variance for Fe, whereas it was 51.51% for Zn. Graphical representation (biplot) summarizes information on main effects and interactions (PCA1) of environment and genotypes simultaneously. AMMI biplot analysis between the mean and the first PCA of genotype-environment interactions (Fig. 1a and 2a) indicated the distinct behavior of the environment. The biplot was also employed by using PCA 1 and PCA 2 to ascertain environmental variation and to interpret the genotype-environment interactions for Fe (Fig. 1b) and for Zn (Fig. 2b). Genotypes which have PCA 1 scores near zero allow very less interaction across environments. Similarly environments with PCA 1 scores near zero display little interaction across genotypes and generally show less discrimination among the genotypes (Crossa et al. 1991). For both Fe and Zn, location in the environment component was more variable than the year (Figs. 1a, 2b), environments 1 and 4 are two different years at Delhi, 2 and 5 are two different years at Pantnagar, 3 and 6 are two years at Bihar. Genotypes overlapped in interaction biplots constructed for first and second PCA axis (Figs. 1b and Fig. 2b). The genotypes which are lying near the origin were stable across the test environments. The environmental indices for grain Fe were 3.45, –1.40 and -3.20 during 2012-13 at Delhi, Pantnagar and Bihar respectively and 2.65, 0.67 and –2.16 during 2013-14 at Delhi, Pantnagar and Bihar respectively. The environmental indices for Zn were 3.71, –7.85, and 4.9 during 2012-13 at Delhi, Pantnagar and Bihar respectively. The values during 2013-14 for Zn were 2.9, –8.55 and 4.77 at Delhi, Pantnagar and Bihar respectively. Environmental indices and biplot suggest that Delhi was the most favorable environment



(a)



(b)

Fig. 2. AMMI biplots for Zn: (a) unadjusted mean and first principal component (b) between first and second principal components

for the expression of grain Fe, while Delhi was next best environment after Bihar for grain Zn. Pantnagar represented a poor environment for grain Fe and more so for Zn accumulation. Pantnagar is a well-known poor soil zinc phytoavailability environment and the lowest grain zinc values in genotypes in this study corroborate this fact.

This study emphasizes that environment and its interaction with genotypes have greater effects than genotypes per se on the grain Zn and Fe concentration in wheat. This study also implies that selecting broad based genotypes with stability for enhanced grain micronutrients over environments may not be very effective due to greater role of environment and genotype-environment interactions. Development of suitable genotypes for targeted environment/s would be better option than stable genotypes for grain iron and zinc concentration. This study also revealed that selection for either iron or zinc could be practiced as it would lead to simultaneous improvement of the other trait due to positive and highly significant association. The promising genotypes identified in the present study could be used as donors for developing grain micronutrient-enriched wheat cultivars.

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