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Phenotypic Evaluation of Grain Zinc Enhanced Wheat Lines for Agronomic and Quality Traits

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ABSTRACT

Background: Biofortification of wheat (*Triticum aestivum*) with increased micronutrients *via* conventional plant breeding is a potential mechanism for alleviating micronutrient deficiencies. As the quantitative inheritance for zinc concentration adds towards greater G x E interactions, the study for significant genotype x location interactions for zinc is an important target towards the production of biofortified wheat.

Methods: The present study was conducted to evaluate a set of promising zinc elevated wheat backcross lines containing introgressions from *T. monococcum* and *T. boeoticum* for grain zinc (ppm) and iron concentration (ppm), protein content (%) and other agronomic and quality traits. Eighteen promising wheat backcross derivatives (BC_1F_5 and BC_2F_5) were evaluated along with donor and recipient parents in a randomized complete block design with three replications at two different locations i.e. Ludhiana and Gurdaspur (Punjab) during 2016-17.

Result: Pooled analysis of variance revealed genotype x location interactions were non significant for grain zinc (P>0.05) and protein (P>0.05) which indicated ranking of lines for these traits did not change with location and hence the increase for these traits in wheat lines were stable and not influenced by the environment. The data also indicated a BC_2F_5 derivative belonging to cross PBW703/ BF14//2*PBW703 possessed significantly higher grain zinc concentration at both the locations exhibiting 18% and 16% zinc enhancement over check variety PBW725 at Ludhiana and Gurdaspur, respectively.

Key words: Biofortification, Genotype x location interactions, Grain zinc concentration, *Triticum aestivum.*

INTRODUCTION

Zinc deficiency has become a global issue in recent years affecting health of over three billion people worldwide (Welch and Graham, 2004). In India, 26% of the population is at risk for zinc deficiency with India's current population of 1.2 billion, means 312 million people in India are deficient in zinc (Das and Green, 2013). Wheat, being a widely cultivated food crop, represents more than 50 percent of the daily caloric intake for the majority of world population (Cakmak, 2008). However, wheat inherently contains lower amount of zinc and iron (20-35 mg/kg) (Cakmak *et al*., 2004) which is unable to meet a person's recommended dietary allowances. Thus, increasing grain zinc concentration in wheat, as it is the major staple food crop, is an important global challenge in order to minimize nutrient deficiency-related health problems for over a billion people.

Biofortification of wheat with increased micronutrients is a promising but long term strategy which includes the conventional plant breeding and modern biotechnology for the development of high micronutrient cultivars. This holds great opportunity to achieve measurable impact on health of poor populations in the developing world (Velu *et al*., 2014; Bouis and Saltzman, 2017; Garg *et al*., 2018). But environmental conditions, mainly soil composition complicates the breeding for high Zn concentration (Trethowan, 2007). One of the important aspect includes the significant genotype x location interactions which have been observed for zinc and iron in wild and improved wheat cultivars (Velu *et al*., 2012; Gopalareddy *et al*., 2015; Saleem *et al*., 2015). Moreover, quantitative inheritance for Zn concentration further added towards greater G x E interaction (Long *et al*., 2004; Zhi-en *et al*., 2014; Gopalareddy *et al*., 2017). The present study was, therefore, conducted to test the genotype x location interactions for grain zinc on wheat backcross derivatives which were derived from different cross combinations using hexaploid wheat as recipient parents and advanced backcross tetraploid derivatives carrying high grain zinc concentration introgressed from *Triticum monococcum* and *Triticum boeoticum* as donors. *T. monococcum* and *T. boeoticum* from diploid 'A' genome progenitor gene pool of hexaploid wheat, were earlier identified to be among the most promising sources of high

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Fe and Zn grain concentration (Cakmak *et al*., 2000). At Punjab Agricultural University, Ludhiana, under wheat biofortification program, these backcross derivatives were available for screening and evaluation for grain zinc concentration as well as other agronomic and quality traits at different locations.

MATERIALS AND METHODS

Donor plant material and introgression lines development

The backcross introgression lines in hexaploid background were developed earlier. These lines were developed at the Punjab Agricultural University in a two-step process. First the target region for grain zinc concentration was introgressed from diploid (*T. monococcum* and *T. boeoticum*) to tretraploid (*T. durum*) wheat background. Eight *T. durum* lines with high zinc concentration (>60ppm) represented as BF13, BF14, BF18, BF20, BF21, BF22 (BC_1F_7 developed from *T. monococcum*W49-27-1 backcrossed to *T. durum* cv Aconchi89) and BF25 (*T. boeoticum* 49992 backcrossed to *T. durum* cv PDW274) were shortlisted. These were used as donor for the introgression of target region for grain zinc concentration into the hexaploid wheat *i.e.* HD2967 and WH1105 (high yielding wheat cultivars of the North West Plain Zone of India); PBW698 and PBW703 (gene pyramided versions of PBW343 carrying major rust resistance genes *Yr10, Yr15, Lr24* and *Lr28*). The F_1's were backcrossed two times with high yielding wheat cultivars to sufficiently recover the genetic background of elite cultivars (Singh C., 2016). The development of material as well as further breeding work was carried out in the Department of Plant Breeding and Genetics at Punjab Agricultural University, Ludhiana and Off season station Keylong (Himachal Pradesh).

Material studied in present experiment

The material used in the present investigation comprises of eight agronomically superior high zinc BC_1F_5 lines and ten high zinc BC_2F_5 derivatives in the background of high yielding wheat varieties and advanced lines. The field experiments were laid out in a randomized block design with three replications in at two locations *i.e.* Punjab Agricultural University, Ludhiana (30°54' N and 75°51' E.) and PAU Regional Research Station, Gurdaspur (32°2'N and 75°24') for testing the effect of environment on grain iron and zinc concentration during main season 2016-17. Experimental units consisted of 4 rows of 1.75m length spaced 20 cm apart. Total entries used in this experiment were thirty including 18 backcross derivatives, 7 donor parents (BF13, BF14, BF18, BF20, BF21, BF22 and BF25), 4 high yielding recipient parents (HD2967, WH1105, PBW698 and PBW703) and one high yielding variety PBW725 as check.

Observations were recorded on days to flowering, plant height, days to maturity, tillers per meter row length, spike length, number of spikelets per spike, grains per spike, thousand grain weight, grain yield per plot (g), grain protein content (%), grain zinc concentration (ppm), grain iron concentration (ppm) and grain yellow pigment content.

Grain protein content (%)

The grain protein content was assessed using the whole grain analyzer "Infratec1241" supplied by M/S Foss Analytical AB, Sweden. It utilizes the near infrared light which is transmitted through the grains. The grain samples were scanned in the range of 850 to 1050 nm with a bandwidth of 7 nm and there were 100 data points per scan. The results were displayed as percent protein content along with percent moisture.

Grain iron and zinc concentration (ppm)

Energy dispersive X ray fluorescence (EDXRF) method was used to determine iron and zinc concentrations in wheat grains. EDXRF was carried out utilizing an Oxford Instruments X-Supreme 8000 fitted with a 10 place auto-sampler. Estimation conditions were as recommended by the producer for grain zinc and iron analysis in a cellulose matrix.

Yellow pigment content (ppm)

Yellow pigment content in the wheat wholemeal was estimated using a standard AACC colorimetric method. Four gram wholemeal was weighed and put into a 125 ml reagent bottle to which, 20ml water saturated n-butanol was added. Bottle was shaken properly to mix the contents and kept in dark for 16 hours. The extract was obtained after filteration of the contents into standard test tubes. The intensity of colour of extract was read at 440 nm using Spectronic20$^+$D spectrophotometer and recorded as optical density (O.D.). Yellow pigment content was calculated using the following formula:

Yellow pigment content (ppm) = [(O.D. X 23.5366) + 0.0105]

RESULTS AND DISCUSSION

The pooled analysis of variance over the two locations (data not given) revealed significant location x genotype interactions for all characters but non significant for grain protein and zinc concentration. The non significant genotype x environment interactions indicated that the comparative ranking of lines for protein and zinc concentration did not change with change in location and the lines performing superior for these traits at one location were also performing superior at another location. Hence, the increased grain zinc concentration is stable in these wheat lines.

The pooled analysis of variance also revealed significant variation among the lines for days to flowering, days to maturity, plant height, thousand grain weight, yield per plot, grain protein content, iron concentration and yellow pigment content but genotypes differed non significantly for grain zinc concentration.

Location effect was significant for all the studied traits. Mean values of genotypes for investigated characters exhibited some variations between the two locations (Ludhiana and Gurdaspur). The mean values for almost all the characters (Table 1) were higher in Gurdaspur trial as compared to Ludhiana except for grain zinc and protein content. For important agronomic traits, overall mean for thousand grain weight and grain yield were lower for Ludhiana trial (37g and 1005g/plot) as compared to Gurdaspur trial (45g and 1275g/plot). The inverse trend was obtained for grain zinc concentration where overall mean of Ludhiana trial was 53.6ppm as compared to 45.1 ppm in Gurdaspur trial. The mean value obtained for iron was on higher side for Gurdaspur (35.9ppm) and on lower side for Ludhiana trial (30.0ppm). The significant effect of location may be ascribed to differences in the environmental and soil nutritional factors between both locations.

Evaluation over the two locations *viz*., Ludhiana and Gurdaspur was carried out to identify the entries performing better than recipient/check varieties with respect to grain zinc concentration, protein content and yellow pigment content along with acceptable agronomic performance consistent at both locations. Among BC_1F_5 progenies, the entry no. 2 (Table 2) belonging to cross WH1105/BF13//WH1105 (65.4ppm), entry no. 4 from cross BF20/PBW703//PBW 703 (67.8ppm) and entry no. 5 from cross BF21/PBW703//PBW 703 (64.6ppm) recorded significant enhancement for grain zinc concentration (24%-39% over check PBW725) compared to the recipient range of 43.3ppm (PBW698) to 48.2ppm (WH1105) and 48.5ppm for check variety PBW725 at Ludhiana only whereas none of the BC_1F_5 progenies outperformed for zinc concentration at Gurdaspur. These lines exhibited grain yield of 757 g/plot, 653 g/plot and 877 g/plot as compared to 1136 g/plot for PBW703 and 1272 g/plot for WH1105 with 1257 g/plot for check PBW 725.The decrease in yield shows the constraint for the enhancement of grain zinc concentration. The component which contributed to low yield of high zinc lines was thousand grain weight. High zinc lines displayed significantly low grain weight (39, 30 and 34g) in contrast to thousand grain weight of 45g registered by PBW725. Along with high zinc, some BC_1F_5 progenies also recorded significant enhancement for the grain yellow pigment content (upto 30 and 31%) over check PBW725 possessing 3.36 and 3.19 ppm for Ludhiana and Gurdaspur respectively.

Among the whole set of BC_2F_5 derivatives, entry no.9 (Table 2) from cross PBW703/BF14//2*PBW703 was found to be promising which had significantly higher grain zinc and protein content over the parental and check varieties at both the locations along with superior agronomic performance. At Ludhiana, this line recorded 57.3 ppm of zinc and 11.50% protein along with acceptable grain yield (1137 g/plot) and other desirable agronomic traits *i.e.* days to flowering (93 days), plant height (98 cm), days to maturity (150 days) and thousand grain weight (39 g). In Gurdaspur trial, among all BC_2F_5 derivatives, same line (entry no.9) exhibited highest grain yield (1560 g/plot) with rest of the lines also at par to check PBW725 (1717g/plot). It possessed

Table 1: Various agronomic and quality traits recorded at two locations *viz*., Ludhiana (LDH) and Gurdaspur (GDP) during 2016-17.

Traits	Range		Mean±SE		CD (0.05)		Donor		Recipient/check	
	LDH	GDP	LDH	GDP	LDH	GDP	LDH	GDP	LDH	GDP
Days to flowering (days)	79-93	79-103	91±1.31	95±1.51	2.0	1.77	95-107	104-110	92-98	96-100
Days to maturity (days)	133-152	147-153	149±0.63	152±0.33	1.72	1.54	149-153	153-157	148-153	150-155
Plant height (cm)	87-113	90-114	99±1.1	101±0.87	5.02	3.52	88-101	95-107	96-106	98-105
Thousand grain weight (gm)	30-42	38-53	37± 0.68	45±0.98	8.18	4.81	36-41	36-45	37-45	41-46
Grain yield (g/plot)	653-1337	611-1560	1005±38.2	1275±52	181.9	281	639-1029	642-1376	773-1272	1400-1778
Grain protein content (%)	9.22-12.46	8.85-10.71	10.6±0.21	10±0.14	1.53	1.07	8.90-14.33	8.71-12.77	9.03-10.85	8.78-10.49
Grain iron concentration (ppm)	28.1-32.4	34.6-42.1	30± 0.33	35.9±0.64	7.28	4.98	27.0-35.2	28.8-40.6	27.0-29.7	32.6-37.7
Grain zinc concentration (ppm)	46.5-67.8	39.9-50.0	53.6 ±1.2	45.1±0.62	8.76	6.74	54.5-63.1	41.2-56.2	43.3-48.5	39.1-45.4
Grain yellow pigment content (ppm)	3.31-4.43	2.97-4.15	4.36±0.23	4±0.27	0.41	0.34	6.15-7.03	5.63-7.27	3.40-3.76	3.05-3.77

Table 2: Genotypic means for four promising lines for various agronomic and quality traits recorded at two locations *viz.*, Ludhiana (LDH) and Gurdaspur (GDP) during 2016-17.

Line No.		Days to flowering		Days to maturity		Plant height (cm)		1000 grain weight (g)		Grain yield (g/plot)		Grain protein content (%)		Grain iron conc. (ppm)		Grain zinc conc. (ppm)		Yellow pigment conten (ppm)	
		LDH	GDP	LDH	GDP	LDH	GDP	LDH	GDP	LDH	GDP	LDH	GDP	LDH	GDP	LDH	GDP	LDH	GDP
	BC_1F_5																		
2	WH1105/BF13//WH1105	91	103	148	151	104	103	39	41	757	611	9.22	8.85	28.2	38.0	65.4	45.3	3.31	3.67
4	BF20/PBW703//PBW 703	79	79	133	147	87	90	30	50	653	748	10.13	10.71	30.8	37.4	67.8	49.5	4.02	4.15
5	BF21/PBW703//PBW 703	89	89	147	150	99	101	34	50	877	1216	10.50	9.03	30.1	39.7	64.6	45.4	4.43	4.05
	BC_2F_5																		
9	PBW703/BF14//2*PBW703	93	95	150	152	98	101	39	53	1137	1560	11.50	10.23	31.7	38.8	57.3	49.4	3.37	3.05
	PBW725 (Check)	98	100	153	155	103	105	45	43	1257	1717	10.85	10.49	28.7	37.7	48.5	42.5	3.36	3.19

49.4ppm zinc and 10.23% protein along with other traits in acceptable range.

Correlation coefficient among traits pooled for both the locations (Ludhiana and Gurdaspur) (data not given) showed that days to flowering recorded significant positive correlation with grain yellow pigment content at both the locations. Significant negative association was observed for grain yield with grain zinc and yellow pigment content. Though the negative association of yield with grain zinc concentration is well established (Fan *et al*., 2008; Liu *et al*., 2014; Ning *et al*., 2019), its association with yellow pigment needed further understanding. Positive association was observed for grain protein with grain iron concentration whereas positive association was observed for grain protein content with grain zinc only in Gurdaspur trial.

From the present study, it may be inferred that location effects were significant for all the studied traits including the target traits i.e grain iron and zinc concentration whereas genotype x location effect were non significant for grain zinc which is contradictory to the earlier studies (Khokhar *et al*. 2018; Velu *et al*. 2018). This indicated that ranking of lines for grain zinc concentration did not change with location and hence its increase in wheat lines was stable and not influenced by the environment. The entry no.9 from cross PBW703/BF14//2*PBW703 was superior performing for grain zinc concentration at both the locations which showed the stability of the genotype for this trait exhibiting 18% and 16% enhancement at Ludhiana and Gurdaspur respectively, over the best released cultivar PBW725. Furthermore, out of the total promising high zinc progenies identified, maximum numbers of progenies were derived from the crosses in which PBW703 and WH1105 were used a recipient parents. It was also evident from the data where the BC_1F_5 progenies possessing enhanced zinc concentration were low yielding which suggested that for the proposed end use of the material and making a way for increased iron and zinc concentration, yield/yield component may be sacrificed.

CONCLUSION

This study aimed to test the genotype x environment interactions on grain zinc concentration in advanced wheat backcross lines across two locations. Advanced wheat backcross lines, derived from crossing of zinc enhanced durum parents carrying introgression from *T. monococcum* and *T. boeoticum* with high yielding hexaploid wheat, having enhanced zinc concentrations were needed to be evaluated at different locations to carry out the selection which is a step nearer towards the dissemination of superior wheat varieties with significantly enhanced grain zinc concentrations. Four entries were found to be promising which had significantly higher grain zinc concentration over the parental and check varieties at both the locations. The selected germplasm lines are under further testing at multilocation in the region. Therefore, in further testing, the zinc biofortified germplasm may come out as a candidate for varietal nomination or for the stock registration which could be further disseminated to the wheat breeders for use in their program.

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