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Nutritional diversity in spring wheat with chronological perspective and its association with grain yield

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Abstract

The population of world is accelerating fast with increased number of malnourished people having deficiency of micronutrients, particularly in developing countries. Mineral malnutrition is considered to be the most serious among the global challenges for humans. Biofortification of wheat grain through genetics is a powerful methodology for altering the balance of nutrients in the human food on a large scale. In this study, concentration of mineral nutrients, protein and grain yield were studied to find out potential source of minerals in historical and present spring wheat varieties of Pakistan with the objective to strengthen the hybridization programme and to develop high nutritive wheat. Fifty eight genotypes were sown according to randomized complete block design with three replications. Total nitrogen in wheat grain samples was determined by the Kjeldahl method and grain nitrogen value in percentage was multiplied by 6.25 to get grain protein concentration. Potassium was measured with the help of Jenway Flame Photometer and phosphorus was determined colorimetrically using spectrophotometer. Zn^{2+}, Fe^{2+}, Cu^{2+} and Mn^{2+} were determined using an atomic absorption spectrophotometer. A wide range of diversity was observed among the studied varieties for grain yield, protein and seven mineral in wheat grain. The grain yield was increased and nitrogen concentration in the endosperm diluted over time of green revolution. While mineral concentration of Zn^{2+}, Fe^{2+}, Cu^{2+}, NO_3^- and protein was significantly low in present local varieties as compared to the mean of pre-green revolution local varieties. Fe^{2+} concentration was significantly increased in present local varieties than the local varieties of green revolution period. The mean of grain yield, Zn^{2+}, Mn^{2+} and $H_2\,PO_4^-$ concentration of present International Maize and Wheat Improvement Center (CIMMYT) varieties (GA-2002, Seher-2006, Chakwal-50 and FSD-2008) were significantly increased (33.8%, 22.8%, 60% and 30.8 %, respectively) than CIMMYT varieties of green revolution era (Mexipak-65, Blue Silver, Arz, Sandal, Lyallpur-73, Pari-73, Pothowar-73, Noori, Pavon, WL-711 and Bahawalpur-79). The present (local & CIMMYT) varieties have a significant edge for yield, Zn^{2+}, Fe^{2+}, Mn^{2+}, $H_2\,PO_4^-$ and K^+ over varieties of all other groups. Grain yield had positive and significant phenotypic correlation with Mn^{2+}, $H_2\,PO_4^-$ and K^+ (0.247, 0.364 and 0.140, respectively). Nitrogen had negative genotypic/ phenotypic correlations with the minerals (Zn^{2+}, Fe^{2+}, Mn^{2+}, $H_2\,PO_4^-$ and K^+) and the yield. Therefore, variation of mineral concentration and grain yield present in studied genotypes can be utilized to develop high yielding wheat varieties without affecting the nutritional quality of grain.

Keywords: Association; genetic diversity; grain yield; minerals; *Triticum aestivum*.
Abbreviations: CIMMYT_ International Maize and Wheat Improvement Center, Cu^{2+}_ Copper, FAO_ Food and Agriculture Organization, Fe^{2+}_ Iron, Mn^{2+}_ Manganese, NO_3^-_ Nitrogen, $H_2\,PO_4^-$_ Phosphorus, PCA_ Principal component analysis, K^+_ Potassium, Zn^{2+}_ Zinc.

Introduction

Wheat (*Triticum aestivum*) is the third largest crop in the world with current annual production of 674.88 million tons (FAO, 2013) lagging after corn and rice. It is an essential source of daily diet for billions of people. The population of world is escalating fast with increased number of malnourished and micronutrient deficient people, particularly in developing countries. White and Broadley (2009) reported that over three billion people suffered from micronutrient malnutrition worldwide. Zinc and Iron deficiencies cause severe health problems including losses in the immune system, growth, mental and cognitive development and increased incidence of anemia, mortality and morbidity (Black, 2003; Boccio and Iyenger, 2003; Holtz and Brown, 2004; Sanchez and Swaminathan, 2005; Cakmak, 2008). Micronutrients deficiency is linked to reduced work efficiency and decreased in gross national product in developing countries (Bouis, 2003). Minerals have important roles in physiological and biochemical functions of any biological system. In plants, proper availability of minerals is essential for most of the developmental phases, from seed germination to grain development and mineral deposition in grains (Yilmaz et al., 1998; Welch and Graham, 1999). Minerals are also necessary nutrients for animal and human security. Mineral malnutrition is considered to be the most serious among global challenges for humans (Copenhagen Consensus, 2004; http://www.copenhagenconsensus.com).

The availability of proper quantity of nutrients in the human food depends primarily on their composition in higher plants (Grusak and Cakmak, 2005; Cakmak et al., 2010; Sands et al., 2009), predominantly on mineral contents of daily food such as grains of cereals. So enrichment of grain nutrients (biofortification), either agronomically through the use of mineral fertilizers or genetically through breeding, is considered to be the most encouraging and cost-effective method to improve malnutrition and related health problems (Welch and Graham, 2004; Bouis, 2007; Cakmak, 2008; Peleg et al., 2009). Wheat is the principal food crop in many countries of the world with respect to cultivated area and food source, which contributes 28 percent of the world's edible dry matter and up to 60 percent of the daily caloric intake in many developing countries (FAOSTAT, 2008; http://faostat.fao.org). Hence, the nutritional quality of grains has a substantial effect on human health and well-being globally. While world's cereal grain yields have increased dramatically since the Green Revolution (i.e. breeding for semi-dwarf varieties), a cereal-based food is deficit in providing adequate amount of nutrients (Welch and Graham, 2004); most agricultural systems in the developing world do not provide enough nutrients from grain for a balanced human diet (Graham et al., 2001; Cakmak et al., 2010). In past, higher grain yields, resistance to diseases, lodging and low farming cost have been the major objectives of breeding (Bouis and Welch, 2010). Resultantly, newly developed and high yielding wheat varieties were low in essential minerals especially in iron and zinc (Fan et al., 2008). Biofortification of wheat grain through genetic approaches is a powerful tool for altering the nutrient balance in the human diet on a large scale. The genetic diversity of crop plants has been expressively eroded by domestication and breeding processes (Tanksley and McCouch, 1997; Ladizinsky, 1998). Murphy et al. (2008) and Hussain et al. (2011) reported highly significant differences among the studied genotypes for yield and mineral concentrations. They also observed that grain yield had increased over time of green revolution, while amount of mineral nutrients reduced. While Chatzav et al. (2010) reported that concentration of grain zinc, iron and protein in wild emmer accessions were two-fold higher than the domesticated genotypes. The studied genotypes of spring wheat showed variation in mineral concentration, which can be utilized through breeding for improving the nutritional quality of wheat grain (Hussain et al., 2010). Genetic approach to enhance nutritional status of wheat is efficient as once mineral elements are improved no further cost is required every year. Genetic approach, however, needs a complete knowledge about the potential genetic sources and a comprehensive understanding of the physiological and genetic basis of nutrient-accumulation processes in wheat grains. Therefore, the aims of the studies were to compare the macronutrients' concentrations (nitrogen; phosphorous; potassium), micronutrients (copper; iron; zinc; magnesium), protein and grain yield in historical and present spring wheat varieties of Pakistan, to search the potential source of minerals for strengthening the hybridization programme and to develop high yielding and nutritive wheat.

Results

Analysis of variance and genetic variability

Analysis of variance, genotypic coefficient of variability (GCV%), phenotypic coefficient of variability (PCV%), broad sense heritability (h^2_{bs}), and genetic advance as percentage of mean (GA % of mean) for grain yield, protein and seven mineral concentrations is presented in Table 1.

Grain yield and protein contents

Highly significant differences were observed among 58 genotypes for grain yield and protein contents. Phenotypic coefficient of variability was higher than genotypic coefficient of variability for protein contents while grain yield had same values for GCV% and PCV%. Highest values of broad sense heritability (h^2_{bs}) and least genetic gain (GA as % of mean) were noted for grain yield. Protein contents had moderate heritability and GA as % mean (Table 1).

Grain yield varied from 1715 to 4948 kg ha^{-1} in 58 genotypes of wheat (Suppl. Table 1) with mean value of 3376 kg ha^{-1} (Fig. 1). In pre-green revolution varieties (G-1) grain yield ranged from 1715 to 2182 kg ha^{-1} having mean value of 1979 kg ha^{-1} (Fig. 1). Grain yield of locally developed varieties during green revolution period (G-2) ranged from 2195 to 3436 kg ha^{-1} with mean value of 2919 kg ha^{-1}. While the yield of CIMMYT varieties of green revolution period (G-3) ranged from 2600 to 3781 kg ha^{-1} with a mean of 3058 kg ha^{-1}. During post green revolution period, mean yield of local and CIMMYT varieties was 3282 and 3185 kg ha^{-1} and range was 2999 to 3564 kg ha^{-1} and 2546 to 4060 kg ha^{-1}, respectively. The grain yield of recently discontinued local varieties (G-6) ranged from 4017 to 4578 kg ha^{-1} with a mean of 4205 kg ha^{-1}. Whereas mean yield of recently discontinued CIMMYT varieties (G-7) was 3635 kg ha^{-1} and ranged from 3172 to 4476 kg ha^{-1}. The yield of present local varieties (G-8) ranged from 4060 to 4386 kg ha^{-1} with a mean value of 4225 kg ha^{-1}. Grain yield of present CIMMYT varieties (G-9) ranged from 3282 to 4948 kg ha^{-1} and mean yield was 4093 kg ha^{-1} (Fig. 1).

Protein contents of wheat grain ranged from 6.5 to 13.8 percent (Suppl. Table 1), with an average of 11.7 percent of 58 studied genotypes (Fig. 1). Pre-green revolution local varieties had maximum protein contents (12.1 %) as compared to other groups of local varieties. Generally means of protein contents of all groups of local varieties were less than groups of CIMMYT varieties (Fig. 1) hence maximum protein contents 13.81% were recorded in variety Bahawalpur 79 belonging to CIMMYT varieties of green revolution era (Suppl. Table 1).

Micronutrients

The differences among the 58 wheat varieties were highly significant for micronutrients (Zn^{2+}, Fe^{2+}, Mn^{2+}, and Cu^{2+}). The phenotypic coefficient of variability was higher than genotypic coefficient of variability for all micronutrients. The highest value of GCV% was noted for Mn^{2+} followed by Zn^{2+}, Fe^{2+} and Cu^{2+}. Mn^{2+}, Zn^{2+} and Fe^{2+} exhibited the highest broad sense heritability ((h^2_{bs}) and Cu showed moderate heritability. Maximum genetic gain (GA as % of mean) was noted for Zn^{2+} followed by Cu^{2+}, Mn^{2+}, and Fe^{2+} (Table 1). The amount of grain Zn^{2+} ranged from 9 to 56 µg g^{-1} in the studied varieties (Suppl. Table 1) having mean value of 23.6 µg g^{-1} (Fig. 2). Zinc concentration was 27.1 µg g^{-1} in varieties of pre-green revolution and ranged from 10 to 56 µg g^{-1} (Fig. 2). Local as well as CIMMYT varieties of green revolution period had less Zn^{2+} concentration than the pre-green revolution varieties (Fig. 2). Zn^{2+} concentration was further decreased in post green revolution varieties while mean value of Zn^{2+} in recently discontinued local varieties was 31.0 µg $g^{-1}$with range of 19.7 to 37.3 µg g^{-1}. In present

Table 1. Analysis of variance, genotypic coefficient of variability (GCV%), phenotypic coefficient of variability (PCV%), heritability in broad sense (h^2_{bs}), and genetic advance as percentage of mean (GA % of mean).

Traits	Mean Squares			Coefficient of variation		h^2_{bs}	GA % of mean
	Replication (df = 2)	Genotype (df = 57)	Error (df = 114)	GCV%	PCV%		
Grain yield	27188	1765340**	851	22.7	22.7	99.9	1.5
Protein	0.515	3.925**	0.847	8.7	11.7	57.3	7.9
Zn^{2+}	7.603	267.502**	10.615	39.2	41.6	89.0	21.6
Fe^{2+}	53.241	235.104**	10.119	24.7	26.4	88.1	14.1
Mn^{2+}	4.707	711.462**	16.374	41.5	42.9	93.3	18.1
Cu^{2+}	0.421	4.257**	0.739	23.5	30.1	61.3	20.1
NO_3^-	1770172	10260000**	2180172	8.8	11.8	58.2	8.1
$H_2PO_4^-$	3517299	741525**	119053	16.5	20.8	66.7	14.7
K^+	440057	2852305**	380233	19.8	24.0	68.9	16.3

** = Significant at 0.01 level of probability.

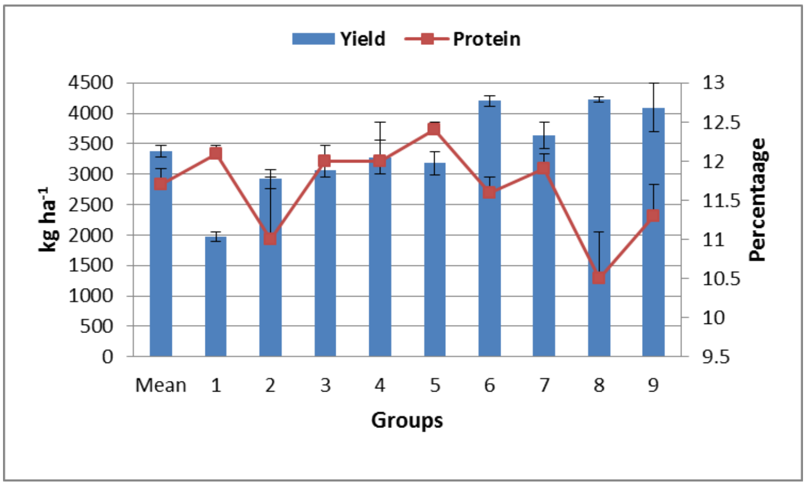

Fig 1. Overall mean of 58 genotypes and mean of groups for yield and protein contents in wheat.

local varieties, the Zn^{2+} concentration was 23.0 µg g^{-1} while in CIMMYT varieties it was 28.0 µg g^{-1} (Fig. 2). In 58 studied wheat genotypes, Fe^{2+} concentration ranged from 17.0 to 57.7 µg g^{-1} (Suppl. Table 1) having a mean value 35.0 µg g^{-1} (Fig. 2). The mean value of pre-green revolution varieties was higher than mean of all other groups. It is also depicted from Fig. 2 that Fe^{2+} concentration was high in CIMMYT varieties than local varieties of all groups. The concentration of Mn^{2+} ranged from 18.7 to 68.3 µg g^{-1} (Suppl. Table 1) in the studied cultivars, with mean value of 36.7 µg g^{-1} (Fig 2). The Mn^{2+} concentration was low in local and CIMMYT varieties of post green revolution. In contrast Mn^{2+} concentration was very high in present CIMMYT varieties (59.2 µg g^{-1}). Copper concentration in wheat grains ranged from 2.6 to 9.1 µg g^{-1}, with an average of 58 wheat genotypes (4.6 µg g^{-1}). The concentration of Cu^{2+} 5.07 µg g^{-1} was noted in local varieties of pre-green revolution period and 5.27 µg g^{-1} was in recently discontinued varieties (Fig. 2).

Macronutrients

The differences among the 58 wheat varieties were highly significant for NO_3^-, $H_2PO_4^-$ and K^+ concentrations. The phenotypic coefficient of variability was higher than genotypic coefficient of variability for macronutrients. The highest value of GCV% was noted for K^+ followed by $H_2PO_4^-$ and NO_3^-. Moderate to high broad sense heritability (h^2_{bs}) and moderate GA as % of mean were observed for these three macronutrients (Table 1). Grain nitrogen ranged from 10467 to 22100 µg g^{-1} (Suppl. Table 1) with an average of 18657µg g^{-1} (Fig. 3). It was also observed that present local varieties have low nitrogen concentration (16790 µg g^{-1}). Grain phosphorus ranged from 1933 to 4067 µg g^{-1} with an average of 2753 µg g^{-1} (Fig. 3). Phosphorus concentrations were 3050 and 3267 µg g^{-1} in recently discontinued local varieties and present CIMMYT varieties, respectively (Fig. 3). Potassium concentration in grains ranged from 3200 to 6867 µg g^{-1}, with mean value of 4577 µg g^{-1}. Potassium contents were 4213 µg g^{-1} in local varieties of pre-green revolution era while its concentration was high (5389 µg g^{-1}) in recently discontinued local varieties. Potassium concentration 4248 µg g^{-1} was observed in present local and 5350 µg g^{-1} in CIMMYT varieties (Fig. 3). It is depicted from Suppl. Table 1 that current local and CIMMYT varieties have high grain yield and mineral concentration. Highest grain yield 4386 kg ha^{-1} was produced by Lasani 08 among current local varieties while Seher 06 produced maximum grain yield 4948 kg ha^{-1} followed by FSD 08 (4581 kg ha^{-1}) among present CIMMYT varieties. These three varieties are being grown on large scale in Pakistan. Lasani 08 had maximum concentrations of Zn^{2+} and K^+ while Faisalabad 08 had highest concentrations of Zn^{2+} and $H_2PO_4^-$ (Suppl. Table 1).

Contrast comparison analysis

The contrast comparison analysis for grain yield, protein and minerals is presented in Table 2. G-1 and G-8 were significantly

Table 2 Contrast effects of different group comparisons of varieties for yield, protein and mineral concentrations in wheat.

Set No.	Contrasts	Yield	Protein	Zn^{2+}	Fe^{2+}	Mn^{2+}	Cu^{2+}	NO_3^-	$H_2PO_4^-$	K^+
		Differences/ probability levels / percent increase (+) or decrease (-)								
I	-1,0,0,0,0,0,0,1,0	1123.0 [a]	-0.797	-2.138	-4.052	4.765	-0.538	-1271.5	359.50	17.00
		(0.000) [b]	(0.000)	(0.001)	(0.000)	(0.000)	(0.002)	(0.000)	(0.000)	(N.S)
		113.5 [c]	-13.2	-15.8	-18.8	28.5	-21.3	-13.1	32.7	1.0
ii	0,-1,0,0,0,0,0,1,0	653.4	-0.280	0.453	3.192	8.070	-0.012	-447.83	93.00	109.5
		(0.000)	(0.048)	(N.S)	(0.000)	(0.000)	(N.S.)	(0.069)	(0.237)	(N.S)
		44.8	-3.7	4.1	22.2	60.1	-0.6	-3.7	7.0	5.5
iii	0,0,0,-1,0,0,0,1,0	471.9	-0.737	3.262	4.048	10.082	-0.288	-1179.83	326.17	248.67
		(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.072)	(0.000)	(0.001)	(0.056)
		28.8	-12.3	39.9	30.0	88.3	-12.6	-12.3	28.6	13.3
iv	0,0,0,0,0,-1,0,1,0	10.167	-0.540	-4.045	-1.618	-3.473	-0.648	-863.17	-65.33	-570.83
		(0.165)	(0.001)	(0.000)	(0.013)	(0.001)	(0.001)	(0.001)	(N.S)	(0.000)
		0.5	-9.3	-26.1	-8.4	-13.9	-24.2	-9.3	-4.3	-21.2
v	-1,-1,0,-1,0,-1,0,4,0	225.831	-0.235	-0.247	0.157	1.944	-0.148	-376.23	71.33	-19.57 (N.S)
		(0.000)	(0.000)	(0.149)	(N.S)	(0.000)	(0.007)	(0.000)	(0.009)	-2.2
		36.5	-9.8	-5.1	2.3	29.2	-15.6	-9.8	14.0	
vi	0,0,-1,0,0,0,0,0,1	517.430	-0.347	2.613	-0.028	11.098	-0.032	-655.00	1.83	56.83
		(0.000)	(0.018)	(0.000)	(0.002)	(0.000)	N.S	(0.008)	(0.000)	(N.S)
		33.8	-5.8	22.8	-0.2	60.0	-1.3	-6.8	30.8	2.1
vii	0,0,0,0,-1,0,0,0,1	453.85	-0.563	5.862	4.612	18.325	0.260	-1003.33	272.33	758.33
		(0.000)	(0.001)	(0.000)	(0.000)	(0.000)	(0.101)	(0.000)	(0.002)	(0.000)
		28.5	-9.1	71.3	32.8	162.8	12.4	–10.1	20.2	39.7
viii	0,0,0,0,0,0,-1,0,1	228.730	-0.300	0.558	-0.667	9.343	-0.100	-579.17	11.332	308.33
		(0.000)	(0.037)	(0.294)	(0.265)	(0.000)	N.S	(0.016)	(0.129)	(0.021)
		12.6	-5.0	4.1	-3.4	46.2	-4.0	-6.0	7.9	13.1
ix	0,0,-1,0,-1,0,-1,0,3	200.00	-0.201	1.506	0.653	6.461	0.021	-372.92	129.25	187.252
		(0.000)	(0.002)	(0.000)	(0.014)	(0.000)	N.S	(0.001)	(0.001)	(0.002)
		24.3	-6.7	27.2	7.5	77.6	1.9	-7.7	18.9	16.3
x	0,-1,1,-1,1,-1,1,-1,1	-82.52	0.316	0.314	1.545	2.063	0.113	479.0	68.42	217.292
		(0.000)	(0.000)	(0.240)	(0.000)	(0.000)	0.151	(0.000)	(0.090)	(0.002)
		-4.5	5.9	2.7	9.6	11.6	5.0	5.7	5.0	9.9
xi	0,-1,-1,-1, -1,-1,-1,3,3	194.61	-0.230	0.725	0.795	4.454	-0.068	-394.03	94.11	75.903
		(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.137)	(0.000)	(0.001)	(0.044)
		23.0	-7.6	12.8	9.6	53.6	-5.8	-8.1	13.9	6.8
xii	-2,-2,-2,-2,-2,-2,-2,7,7	108.74	-0.107	0.251	0.192	1.976	-0.037	-181.86	50.02	38.206
		(0.000)	(0.000)	(0.001)	(0.018)	(0.000)	(0.068)	(0.000)	(0.000)	(0.023)
		30.8	-7.9	9.7	5.0	53.4	-7.0	-8.4	17.1	7.8

a =differences, b = probability level and c = percentage increase or decrease

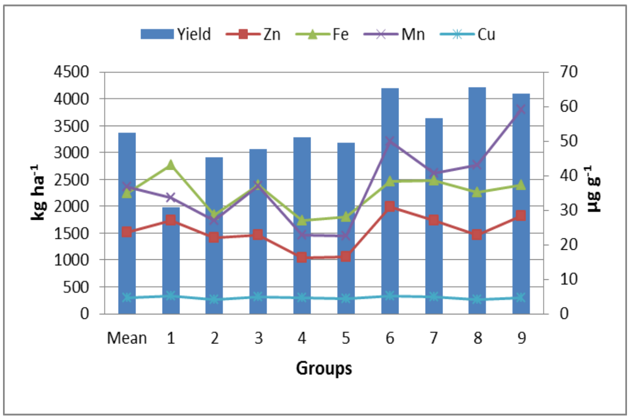

Fig 2. Overall mean of 58 genotypes and mean of groups for yield and micronutrient concentration in wheat grain.

different and G-8 had 113.5 percent greater mean yield than the G-1. G-8 also had significant differences with G-2 (44.8%) and G4 (28.8). While the differences of G-8 between G-6 were non-significant and showed only 0.5 percent increase over G-6. The differences were significant when mean yield of present CIMMYT varieties (G-9) compared to G-3, G-5, and G-7 and showed 34, 29 and 13 percent greater mean yield, respectively. Present CIMMYT varieties also had 24 percent higher yield than all earlier CIMMYT varieties. All current varieties developed from both hybridization programmes had significant increase over all varieties belonging to remaining groups and showed 30.8% increase in grain yield while these varieties had 7.9% lesser protein contents. It is revealed from contrast comparison analysis that G-8 had significant and negative differences with G-1 and G-6 for Zn^{2+} (Table 2). Zn^{2+} concentration of current local varieties had non-significant and negative differences with all groups of local varieties. Current CIMMYT varieties had significant and positive differences over past CIMMYT varieties for Zn^{2+} concentration. Current varieties developed from CIMMYT material showed significant improvement in grain yield, Zn^{2+}, Fe^{2+}, Mn^{2+}, H_2PO_{4-} and K^+ concentration than all other CIMMYT varieties. Current varieties also showed significant increase in Zn^{2+} and Fe^{2+} concentrations (9.7% and 5.0%, respectively), which is a good source to combat malnutrition and development of nutritionally rich wheat varieties in future (Table 2).

Association of nutrients with protein and grain yield

Positive and non-significant genotypic association of grain yield was observed with Zn^{2+}, Cu^{2+}, Mn^{2+}, $H_2PO_4^-$ and K^+. Grain yield showed a negative and non-significant genotypic correlation with Fe^{2+}, protein and nitrogen (Table 3). Nitrogen had negative genotypic correlation with all other studied minerals (Zn^{2+}, Fe^{2+}, Mn^{2+}, $H_2PO_4^-$, $Cu^{2+}$and K^+) and grain yield. Nitrogen had significant and positive genotypic correlation with protein. All other minerals had positive and non-significant genotypic correlation among each other except between Fe^{2+} and $H_2PO_4^-$, which is negative and non-significant. Grain yield showed significant and positive genotypic/phenotypic correlations with Mn^{2+}, $H_2PO_4^-$ and K^+ (Table 3). Nitrogen had significant and positive phenotypic correlation with protein. Nitrogen showed negative and significant phenotypic correlation with Zn^{2+}, Fe^{2+}, Mn^{2+}, $H_2PO_4^-$ and yield. Other minerals (Zn^{2+}, Fe^{2+}, Mn^{2+}, Cu^{2+}, $H_2PO_4^-$ and K^+) had positive and significant phenotypic association with each other except between $H_2PO_4^-$ and Fe^{2+}, which was negative and non-significant (Table 3).

Principal component analysis

Principal component analysis was made to ascertain the relationship of grain yield, protein and all nutrients to categorize superior genotypes (Table 4). It is exhibited from the principal component analysis (PCAs) that there were two major clusters one consisting of Cu^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+}, K^+ and other of yield and $H_2PO_4^-$ (Fig.4). Nine components were defined with help of principal component analysis from nine studied parameters. The first five components had 86.9 percent of the total variation. The eigenvalues of fourth and fifth components were less than one and first three components had 71.2 percent of the total variation, therefore, only these were discussed (Table 4). The first component had 37.5 percent variation and the most effective traits of this component were Mn^{2+}, Zn^{2+}, Fe^{2+}, K^+, protein and NO_3^-, respectively (Table 4). The variation was 20.6 and 13.2 percent for second and third principal components, respectively. For second component NO^{3-}, protein, Cu^{2+}, Fe^{2+} and K^+ while for the third component grain yield and $H_2PO_4^-$ had the maximum effect.

Discussion

At present in most of the countries, cereals especially wheat is chief source of calories for human beings. The requirement for wheat continues to grow approximately 2% annually (Skavmond et al., 2001) due to the global increase of human population. So increased yield is the primary and major objective of plant breeding programme but nutritional value of staple food crops is mostly ignored in breeding programmes (Welch and Graham, 1999; Cakmak, 2008; Cakmak et al., 2010). Nutritionally improved cereal can contribute to health improvement, both directly by increasing the micronutrient availability and indirectly through better agronomic practices and crop yield (Gomez-Galera et al., 2010). It is depicted from the present studies that sufficient genetic variability is present in the studied genotypes for grain yield, protein and mineral concentration (Murphy et al., 2008; Hussain et al., 2010 & 2011). High coefficient values of Mn^{2+}, Zn^{2+}, Fe^{2+}, Cu^{2+} and grain yield played important role in genetic variation of these traits. High heritability of grain yield, Mn^{2+}, Zn^{2+} and Fe^{2+} indicated that these traits were under genetic control. Therefore, the improvement in these traits can be made through breeding programme without progeny test. Grain nitrogen concentration and protein exhibited the lowest heritability. Thus these traits were mostly under environmental control so improvement of these traits should be done through progeny test. Increase in grain yield and resistance/tolerance to biotic (rusts, smuts, bunts, insects) and abiotic stresses (heat, drought, salt) have been the main aims of wheat breeding in the world as well as in Pakistan. Pakistani varieties have great diversity in yield. The varieties released prior to green revolution period have low yield potential due to less response to fertilizer, having tall stature and weak stem. With the advent of dwarfing genes and their utilization in wheat breeding programme, it became possible to use high dose of fertilizer so varieties released thereafter have more yield potential. The present study depicted a complete analysis of grain protein, micronutrients (Zn^{2+}, Fe^{2+}, Cu^{2+} and Mn^{2+}) and macronutrients (NO_3^-, $H_2PO_4^-$, and K^+) concentration of 58 wheat varieties. About three billion world's population has deficiency of minerals.

Table 3. Genotypic (above diagonal) and phenotypic (below diagonal) correlations between grain yield, protein and minerals.

	Protein	Zn^{2+}	Fe^{2+}	Mn^{2+}	Cu^{2+}	NO_3^-	$H_2PO_4^-$	K^+	Yield
Protein		-0.306	-0.257	-0.418	-0.086	1.00*	-0.429	-0.114	0.266
Zn^{2+}	-0.219**		0.380	0.612	0.468	-0.311	0.220	0.356	0.026
Fe^{2+}	-0.180**	0.341**		0.432	0.547	-0.250	-0.060	0.498	-0.013
Mn^{2+}	-0.297**	0.549**	0.395**		0.430	-0.424	0.460	0.573	0.254
Cu^{2+}	-0.026	0.350**	0.411**	0.317**		-0.085	0.012	0.415	0.031
NO_3^-	0.997**	-0.225**	-0.176**	-0.305**	-0.025		-0.448	-0.124	-0.276
$H_2PO_4^-$	-0.239**	0.151*	-0.043	0.362**	0.062	-0.252**		0.218	0.457
K^+	-0.049	0.275**	0.411**	0.457**	0.240**	-0.058	0.174**		0.168
Yield	-0.197*	0.023	-0.014	0.247**	0.021	-0.205**	0.364**	0.140*	

*, ** = Significant at 0.05 and 0.01 level of probability, respectively.

Table 4. Principal component (PC) loadings for grain yield, protein and minerals.

Variable	PC1	PC2	PC3	PC4	PC5
Yield	0.198	-0.282	-0.552	-0.415	0.426
Protein	-0.377	0.418	-0.390	0.102	0.011
Zn^{2+}	0.360	0.221	0.079	0.616	0.090
Fe^{2+}	0.314	0.370	0.249	-0.447	-0.031
Mn^{2+}	0.445	0.119	-0.178	0.223	-0.172
Cu^{2+}	0.271	0.418	0.040	-0.025	0.666
NO_3^-	-0.380	0.422	-0.375	0.094	0.021
$H_2PO_4^-$	0.277	-0.310	-0.474	0.288	-0.065
K^+	0.313	0.312	-0.277	-0.310	-0.576
Eigenvalue	3.371	1.852	1.188	0.754	0.653
Proportion	37.5	20.6	13.2	8.4	7.3
Cumulative	37.5	58.0	71.2	79.6	86.9

Table 5. Nine categories of wheat varieties and their description.

Group No.	Category of varieties/ Breeding Program (Period of release)	Varieties	Description
1	Pre-green revolution / Local (1930-60)	5 (C-518, C-217, C-271, C-273, Dirk	Local varieties having tall stature, weak stem, lodging susceptible, low response to fertilizer and low yield while having excellent bread quality.
2	Green revolution / Local (1965-80)	7 (Chanab-70, SA-75, Punjab-76, LU-26, Bulbul, Indus-79, Chanab-79)	Local varieties having short stature, fertilizer responsive and high yielding.
3	Green revolution / CIMMYT (1965-80)	11 (Mexipak 65, Blue silver, ARZ, Sandal, Lyallpur-73, Pari-73, Pothwar-73, Noori, Pavon, Wl-711, Bahawalpur-79)	Varieties developed through CIMMYT program, having short stature, fertilizer responsive and high yielding. Start of green revolution in the world.
4	Post-green revolution / Local (1981-88)	2 (Punjab-81, Shalimar-88)	Varieties developed after green revolution period.
5	Post-green revolution / CIMMYT (1981-88)	9 (Pak-81, Barani-83, Kohinoor-83, Punjab-85, Faisalabad-85, Sutlej-86, Chakwal-86, Rawal-87, Punjnad-88)	
6	Recently discontinued / Local (1990-2002)	6 (Inqalab-91, Punjab-96, Kohistan-97, Iqbal-2000, SH-2002, AS-2002)	Cultivation of these varieties banned due to loss of resistance against rusts in the near past.
7	Recently discontinued/ CIMMYT (1990-2002)	7 (Rohtas -90, Pasban-90, Kohsar-95, Chakwal-97, Zartasha-99, Margalla-99, Wafaq-2001)	
8	Present / Local (2000-11)	7 (Auqab-2000, Shafaq-2006, Fareed-2006, Lasani-2008, AARI 2011, Punjab-11, Millat-11)	Under cultivation
9	Present/ CIMMYT (2000-11)	4 (GA-2002, Seher-2006, Chakwal-50, FSD-2008)	

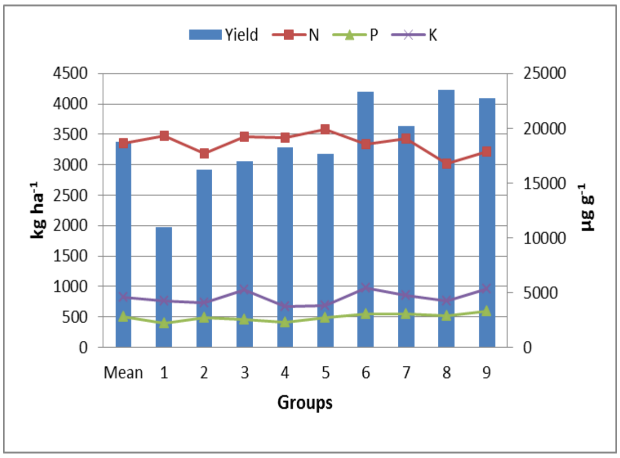

Fig 3. Overall mean of 58 genotypes and mean of groups for yield and macronutrient concentration in wheat grain.

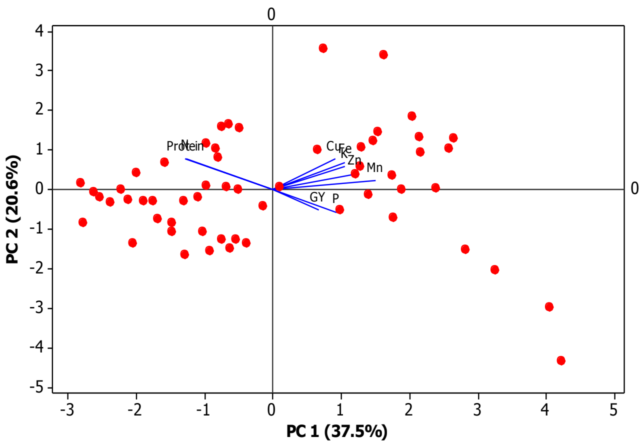

Fig 4. Biplot of principal component analysis of 58 wheat genotypes in relation to grain yield, protein, micronutrients and micronutrients.

Minerals deficiency decreases working efficiency and increases the rates of premature death as well as cost of healthcare (Welch and Graham, 2004). Population of developing countries is mainly at risk, because the people do not have sufficient money to purchase mineral rich food such as fruits, vegetables, meat, poultry and fish. Globally Zn^{2+} and Fe^{2+} are major deficient nutrients (Graham et al., 1999). The main factor for deficiency of Fe^{2+} and Zn^{2+} is the low concentration in cereals (Hurrell, 2000). Importance has been given on screening of wheat germplasm for high concentration of Fe^{2+} and Zn^{2+} under a mega project led by International Maize and Wheat Improvement Center, Mexico (Monasterio and Graham, 2000; Stangoulis, 2010). Under this project a comprehensive research work has been started for the development of Fe^{2+} and Zn^{2+} dense staple food crop. It is well known that the cultivars and production system interaction have a significant effect on the performance of a cultivar in a cropping system (Murphy et al., 2007). The great variation among genotypes and genotype groups specify the presence of genetic potential that can be utilized to improve the concentration of minerals in wheat grains. A negative correlation between more recent cultivars and amount of minerals was reported by earlier researchers (Fan et al., 2008; Murphy et al., 2008; Hussain et al., 2011).
This association has been attributed to a dilution effect of the minerals due to the increased yield of most recent varieties (Fan et al., 2008). The dilution effect, due to bran versus endosperm ratio change, may play a role in the negative association between yield and mineral content for cereal crops. Earlier studies depicted negative association between grain yield and amount of grain nitrogen (Cox et al., 1985; Heitholt et al., 1990) showing that the dilution of nitrogen compounds in high yielding grains was a consequence of the breeding process (Calderini et al., 1995). It is depicted from the present study that grain yield had positive association with all minerals except Fe^{2+} and NO_3^-. The similar findings have been reported by Graham et al. (1999); Zhao et al. (2009). In contrast negative relationship between yield and minerals have been reported in other studies (Garvin et al., 2006; McDonald et al., 2008; Zhao et al., 2009). From this study, it seems that genetic differences for wheat grain minerals are present in studied genotypes. Genetic differences for grain mineral concentration have also been reported in various varietal trials (Peterson et al., 1986; Graham et al., 1999; Zhao et al., 2009). This variation can be utilized in hybridization programme for developing high yielding and nutritive wheat varieties to reduce the number of malnourished and nutrient deficient people. Further studies are underway to find out the genetic basis and inheritance of mineral nutrients in wheat. Molecular markers may be tagged to screen the highly nutritive genotypes for strengthening breeding programme.

Materials and Methods

Plant material

Fifty eight genotypes including locally and CIMMYT hybridized material were sown in field during 2011-12 for grain yield, protein and mineral nutrients (Suppl. Table 2). These genotypes were grouped into nine categories according to the period, status, year of release and link with hybridization program (Table 5).

Experimental site and details

The genotypes were sown on 10 November 2011, at Wheat Research Institute, Faisalabad located in South East of Pakistan (31° 39`; 73° 04`; 183.35 meters a.s.l). Mean minimum and mean maximum temperature and rainfall during cropping season 2011-12 are presented in Suppl. Table 3. The soil texture was clay-loam, with pH (7.8-8.0) and less than one percent organic matter. Each genotype was sown in six rows of 6 meters long and 27 cm apart, seeded at an average rate of 100 kg ha^{-1} with self-propelled mechanical planter and maintained 5 meters length of each plot after germination. The whole dose of nutrients i.e. nitrogen 120 kg ha^{-1} and P_2O_5 90 kg ha^{-1} was used at the time of seedbed preparation and sowing. Five irrigations were applied to the wheat crop at critical growth stages i.e. crown root development and tiller initiation, stem elongation, booting, anthesis and grain filling. Weeds were controlled by application of Clodinafop @ 250 g ha^{-1} and Bromoxynil+MCPA @ 750 ml ha^{-1}. For determining the final yield (kg ha^{-1}) at maturity 5.4 m^2 plot size was harvested and yield was recorded. A sample from grains of each genotype was kept for mineral and protein analysis.

Analytical methods

Total nitrogen (NO_3^-) in wheat grain samples was determined by the Kjeldahl method after acid digestion of 0.5g ground material (Anderson and Ingram 1993). Grain protein was determined by using proper protocols as mentioned by AACC (2000) i.e. Method No. 44-15A. Grain nitrogen value in percentage was multiplied by 6.25 to get grain protein concentration (Merrill and Watt, 1973). Dried ground grain material (1.0 g) was digested in a diacid mixture (9: 4 mixture of HNO_3: $HClO_4$) for the determination of other nutrients. Aliquots of this solution were used for the determination of zinc (Zn^{2+}), iron (Fe^{2+}), manganese (Mn^{2+}), copper (Cu^{2+}), phosphorus ($H_2PO_4^-$) and potassium (K^+). Potassium in the digested samples was measured with help of Jenway Flame Photometer (Model PFP-7) and $H_2PO_4^-$ was determined colorimetrically following Olsen and Sommers (1982) by using spectrophotometer (Model U2020). While Zn^{2+}, Fe^{2+}, Cu^{2+} and Mn^{2+} were determined using an atomic absorption spectrophotometer (Model Shimadzu AA-7000). Quantities of mineral nutrients were determined by using the certified values of the related minerals in reference grain samples.

Statistical analysis

Analysis of variance was carried out to find out the genetic variation for recorded parameters. Broad sense heritability (h^2_{bs}) was calculated as defined by Falconer and Mackay (1996). Broad sense heritability $h^2_{(bs)}$ is the ratio of genotypic variance (σ^2_g) and phenotypic variance ($\sigma^2_g + \sigma^2_e$) while σ^2_e is the error variance:

$$h^2_{(bs)} = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_e}$$

Genotypic correlation coefficients were calculated by the formula (Falconer and Mackay, 1996) to assess the correlation among grain yield, protein and minerals. Multivariate analysis of grain yield, protein and seven mineral contents was also performed. Principal component analysis (PCA) was used to access the association among grain yield, protein and seven mineral nutrients (Hardle and Simar, 2012). PCA was based on a correlation matrix and presented as biplot. Two components were taken out using Eigenvalues to ensure significant application of the data by each factor.

Conclusions

It is concluded from the present study that high genetic diversity was present in the studied varieties for grain yield, protein and mineral concentration. A consecutive increase in grain yield was observed since green revolution. So dilution of nitrogen concentration in high yielding varieties was weakness of the breeding process since green revolution. It is also noted that Seher-06, FSD-08 and Lasani-08 are the potential source of grain yield. Concentration of Zn^{2+} and Fe^{2+} was more in present varieties of CIMMYT material as compared to local varieties due to HarvestPlus biofortification programme of CIMMYT, Mexico. Therefore, mineral concentration in future varieties can be increased simultaneously without decreasing the grain yield by using the variation present in studied wheat varieties.

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