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Research Article

Genotypic variation for phytic acid, inorganic phosphate and mineral contents in advanced breeding lines of wheat (*Triticum aestivum* L.)

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Abstract
Wheat is a staple food of Indian population. Its nutritional and quality parameters have gained considerable importance over past few decades. In the present study, genotypic variation was studied in 100 advanced breeding lines developed for Indian peninsular zone by measuring phytic acid (PA), inorganic phosphate (IP), iron and zinc content in seeds and hundred kernel weight (HKW). Advanced breeding lines under investigation exhibited wide variation for the characters studied. The PA content ranged from 4.97 mg/g to 15.02 mg/g (mean of 9.58 mg/g). Iron and zinc content was in the range of 0.042 to 0.098 mg/g and 0.017 mg/g to 0.029 mg/g respectively. HKW ranged from 2.99 to 5.42 g. There was significant negative correlation between PA and HKW. Iron content showed very high genotypic coefficient of variation and heritability (h^2bs) as compared to zinc content and other traits. Low heritability of IP content indicated the environmental influence on the trait.

Key words
Triticum aestivum, Phytic acid, Inorganic phosphate, Iron, Zinc

Introduction

Wheat is a primary food grain crop for majority of world population including India. It was cultivated on an area of 30 mha with a production of 93.5 mt during 2012-2013 (Sharma, 2013). India accounts for 12.5% of world wheat acreage and 12.05% of world wheat production(http://dwd.dacnet.nic.in). In developing countries where majority of the population depends upon wheat and rice as staple food, nutritional and antinutritional factors present in wheat grains assume considerable importance as slight increase or decrease in one or the other nutritional quality parameters affects very large portion of population. Among the nutritional components, minerals like iron and zinc are very important and at the same time antinutritional compounds like phytic acid which adversely affect the availability of iron and zinc are equally important. Over three billion people suffer from micronutrient malnourishment (Zuzana *et al.,* 2009).

Phytic acid (PA) is a myoinositol (1,2,3,4,5 and 6) hexakisphosphoric acid representing 65-80% of total phosphate content in seeds (Lott *et al.,* 2000, Centeno *et al.,* 2001). Non-ruminant animals can not digest the phytate bound phosphorus. PA binds with the minerals like Ca, Zn, B, and Fe forming respective salts and making them unavailable during digestion (Raboy, 2001). Human intestine can't digest these salts as it lacks phytase (Holm *et al.,*2002). Phytate is also reported to bind with proteins altering their structure, reducing their solubility or making them unavailable (Vikas *et al*., 2010). Besides these antinutritional effects, PA has anti-carcinogenic, antioxidant and other beneficial properties like lowering blood sugar level by reducing starch digestion rate. In developing countries, wheat and wheat based food products constitute a major ingredient of daily diet resulting in high intake of PA with very limited intake of minerals. In this scenario, it becomes important to increase the bio-availability of minerals in common man diet by developing low PA wheat varieties.

Phosphate, absorbed by plants from soil, is utilized for its own development. During ripening of seeds, it is stored in aleuronic layer of seed in the form of phytate (Lisbeth *et al.,* 2008). The absorbed phosphate is stored not only in PA form but also in inorganic form. Significant negative correlation has been reported between PA and inorganic phosphate (IP) content (Xinglin *et al.,* 2011) in crop plants.

Minerals such as iron and zinc though required in small quantity in diet, play very important role in metabolism and function as co-factor in many enzymes and as biological constituents. Iron is one of the important mineral element and deficiency of which leads to tissue hypoxia, heart failure and maternal mortality during child birth (Viteri *et al*., 1998 and Maberly *et al.,* 1994). With the increasing trend of including fiber component in diet for health benefits, whole wheat meal is being used in bread, biscuits, pasta, semolina, pizza, dough nuts and *Chapati*, resulting into increased intake of PA (Lisbeth *et al.*, 2008).

Addition of minerals and vitamins (biofortification) externally to the food as supplement is a costly and temporary solution for developing countries. Adjusting the pH of dough, adding phytase to food and feed from wheat meal is difficult to follow in everyday use. One of the suggested measures is; identifying wheat genotypes with low phytate and higher minerals content and incorporating them in various wheat hybridization programs. The present study was undertaken with 100 advanced breeding lines of wheat developed for peninsular zone of India, to evaluate for PA, IP, iron (Fe) and zinc (Zn) content. This study will be helpful in identifying genotypes possessing low PA and higher minerals content which can then be exploited in hybridization programme to develop nutritionally superior varieties.

Material and method

Plant material: One hundred advanced breeding lines of wheat (Table No 1) derived from various crosses involving diverse parents, were grown at Agriculture Research Station, Niphad, Dist. Nashik, Maharashtra during 2012-13. These breeding lines were grown in randomized block design with two replications. Seeds from 10 random plants per replication were harvested and bulked. Hundred kernel weight was recorded after drying the seeds. Hundred intact full grown seed from each advanced breeding line per replication were ground to make fine flour for biochemical analysis.

Estimation of phytic acid: For estimation of PA, finely ground 35 to 40 mg flour per sample per replication was taken in 1.5 ml centrifuge tube and extraction buffer (0.75N HCl) was added and kept overnight on rocker. Samples were centrifuged at 10,000 rpm for 10 min. at room temperature (25^0C). Supernatant was transferred to another tube to which 0.1 g NaCl was added and vortexed. Samples were kept at -20^0C for 20 min and then centrifuged at 10,000 rpm for 10 min. The supernatant was collected and diluted to 1:24 with glass distilled water. Thereafter, to 750 µl of the diluted supernatant, 250 µl of Wade reagent (0.03% $FeCl_3.6H_2O$ + 0.3% sulfosalicylic acid) was added. Calorimetric determination of phytic acid phosphate (PAP) was done as per the procedure described by Latta and Eskin (1980) with appropriate modifications (Gao *et al.*, 2007). Observations were recorded at 500 nm using spectrophotometer (Jasco, USA). Standard curve was obtained using series of calibration standards of sodium phytate (P content 18.38%) (Sigma, USA) following the above said procedure.

Estimation of inorganic phosphate: To determine the IP content, procedure given by Chen *et. al.* (1956) was followed with appropriate modifications. Fifty milligrams of powdered seed sample was taken in 2 ml centrifuge tube. To this, 400 µl of trichloro acetic acid (12.5%) containing 25 mM $MgCl_2$ was added and vortexed. For proper extraction, suspension was shaken overnight on rocker. Next day, the suspension was centrifuged at 10,000 rpm for 20 min. The supernatant was collected in another tube and diluted with equal volume of glass distilled water. 100 µl of this diluted supernatant was taken in another tube and 900 µl of Chen's reagent (6N H_2SO_4, 2.5% ammonium molybdate, 10% ascorbic acid and water in 1:1:1:2 proportion) was added. Suspension was incubated at 50^0C in hot waterbath for 1 hr. For determination of standard curve, a series of solutions of known concentrations of sodium dihydrogen phosphate were prepared and processed same as above. The absorbance of the reaction product was measured at 660 nm and the total IP content was expressed as mg/g of the sample.

Estimation of iron and zinc: Iron and zinc contents were determined by atomic absorption spectrophotometry (AAS). Sample digestion was done as per Perkin- Elmer (1976). One gram of finely powdered wheat seeds were taken in 100 ml conical flask and 20 ml of mixture of conc. nitric acid and perchloric acid (5:1) was added and kept overnight. Next day, the samples were digested on hotplate at 200^0C. Samples were then allowed to cool and volumes were made up to 50 ml using glass distilled water. The diluted samples were filtered through Whatman No. 42 filter paper and filtrate was used for the estimation of iron and zinc using GBC model 932-Plus atomic absorption spectrophotometer.

Statistical analysis: The analysis of variance for assessing diversity for PA and IP contents, Pearson's correlation coefficients and cluster analysis were carried out by using SAS 9.3.1(SAS Institute Inc., Cary, NC). Genotypic variance, genotypic coefficient of variation (GCV) and broad sense heritability were also calculated. Eigenvalues of the covariance matrix, which describe the proportion of total variance attributable to their respective principal components and the corresponding eigenvectors of the principal components, which describe the weight attributable to the measured traits for those principal components, were calculated using the PAST software (Hammer *et al*., 2001).

Result and discussion

Variation for phytic acid: The advanced breeding lines differed significantly for the PA content. The PA content ranged from 4.97 mg/g to 15.02 mg/g (Table 2). The average content of phytic acid was 9.59 mg/g of seed weight. Similar results were reported by others (Erdal *et al.*, 2002, Febles *et al.*, 2002 and Tavajjoh *et al.*, 2011). Lowest amount of phytic acid was observed in the genotype NIAW2968 (4.97 mg/g) followed by NIAW2761 (5.86 mg/g), NIAW2841 (6.24 mg/g), NIAW2828 (6.55 mg/g) and NIAW2927 (6.82 mg/g). Whereas highest PA content was found in genotype NIAW2805 (15.02 mg/g) followed by NIAW2740 (14.87 mg/g), NIAW2799 (14.03 mg/g), NIAW2798 (14.02 mg/g) and NIAW2803 (13.45 mg/g) (Table 1). Genotypic coefficient of variation for PA was 17.06%. Higher genetic variance for content of PA indicates the possibility of developing wheat genotypes with lower phytate content. Significant differences for PA content, total phosphate content and phytase activity have also been reported by Steiner *et al.* (2007). The differences between the phytic acid content observed in the present study and that reported by others may be due to the different choice of genotypes and also to variable agro-climatic conditions prevailing in the area where the study was conducted.

Variation for inorganic phosphate (IP): The IP content in 100 genotypes ranged from 0.127 to 0.234 mg/g. Low IP content was observed in genotypes NIAW2894 (0.127 mg/g), NIAW2871 (0.128 mg/g), NIAW2779 (0.132 mg/g), NIAW2942 (0.135 mg/g) and NIAW2878 (0.136 mg/g) while the genotypes NIAW2922 (0.234 mg/g), NIAW2927 (0.200 mg/g), NIAW2802 (0.197 mg/g), NIAW2930 (0.195 mg/g) and NIAW2916 (0.193 mg/g) recorded high IP content. Mean IP content was 0.16 mg/g (Table 2). Xinglin *et al.* (2011) reported IP content to range between 0.11 and 2.98 mg/g in wheat seeds. Genotypic coefficient of variation for IP content was 9.41% which is less than that for PA, indicating IP content is more influenced by the environmental factors. This may be due to differences in the available phosphorus in soil which is heterogeneous.

Variation for iron content: Wheat genotypes exhibited wide range of variability for iron content (0.042 to 0.098 mg/g) (Table 2). Lowest iron content was observed in genotype N2949 (0.042 mg/g) followed by NIAW2944 (0.045 mg/g), NIAW2791 (0.047 mg/g), NIAW2830 (0.047 mg/g) and NIAW2794 (0.047 mg/g) whereas genotypes NIAW2865 (0.098 mg/g), NIAW2937 (0.094 mg/g), NIAW2766 (0.090 mg/g), NIAW2841 (0.087 mg/g) and NIAW2788 (0.087 mg/g) exhibited high iron content. Average iron content was 0.059 mg/g of seed. Graham *et al.*, (1999) reported iron content to range between 28.8 and 56.5 mg/kg in the germplasm of CIMMYT, Mexico, with mean iron content of 37.2 mg/kg. The difference between the iron content observed in the present study and that reported earlier may be due to the genotypic lines having different genetic background used in both studies and different agro-climatic conditions. At the same time, within a cultivar, soil nutrient status may affect yield and grain micronutrient density (Graham and Welch, 1996). Genotypic coefficient of variation for iron content was found to be 19.71%, which is highest among the traits studied indicating very less environmental influence. This trait is suitable for improvement of germplasm by recombination breeding. The range of iron content also indicate the availability of enough genetic variation which can be utilized for improving the iron content and breeding nutritionally superior advanced genotypes.

Variation for zinc content: Genotypes showed wide range for zinc content (0.0172 to 0.0289 mg/g) (Table 2). Average zinc content was 0.023 mg/g. Low zinc content was observed in genotypes NIAW2791 (0.0172 mg/g), NIAW2758 (0.0183 mg/g). NIAW2865 (0.0185 mg/g), NIAW2878 (0.0185 mg/g) and NIAW2794 (0.0187 mg/g) whereas genotypes NIAW2922 (0.0291 mg/g), NIAW2887 (0.0289 mg/g), NIAW2802 (0.0278 mg/g), NIAW2766 (0.0275 mg/g) and NIAW2785 (0.0273 mg/g) were found to have high zinc content. Graham *et al.* (1999) reported wide range of zinc content between 25-53 mg/kg in the CIMMYT germplasm, with mean zinc content of 35 mg/kg. Genotypic coefficient of variation for zinc content was observed to be 10.07% which is less than the GCV for iron content.

Variation for hundred kernel weight:Values of HKW for genotypes under study ranged from 2.99 to 5.42g with average of 4.03g. Highest HKW was observed for the genotype NIAW2856 (5.41g) followed by the genotypes NIAW2887 (5.20 g), NIAW2886 (5.04 g), NIAW2859 (5.04 g) and NIAW2884 (5.00 g) while genotype NIAW2918 (2.98 g) recorded the lowest HKW followed by NIAW2903 (3.05 g), NIAW2721 (3.15 g), NIAW2904 (3.16 g) and NIAW2977 (3.20 g). The genotypes differed significantly for the contents of PA, IP, Fe, Zn and HKW. The correlation between PA and IP was found to be negative but non-significant.

Correlation and heritability: Significant differences among the genotypes for PA, IP, iron, zinc and HKW were observed in the present study. Phytic acid showed significant negative correlation with HKW (Table 3). Liu *et al.*,(2006) while studying 186 wheat genotypes from China, reported that grain weight with phytate, inorganic P and phytase activity were negatively correlated. Increased kernel weight may contain more accumulated starch and storage proteins than the small sized kernels which may be accountable for the negative correlation between seed weight and PA. PA also showed negative correlation with IP and zinc content however it was not significant. Xinglin *et al.*, (2011) reported significant negative correlation between PA and IP in wheat. Since 1990, several low phytic acid mutants have been isolated in different crops like soybean, maize, barley, wheat and *Arabidopsis thaliana.* In these crops, reduction in PA has always been accompanied with increase in IP content (Raboy, 2007). Vesna *et al.* (2010) found significant negative correlation between PA and IP in maize. Synthesis of PA and IP may be sharing some common pathway and as a result increase in amount of one will be reflecting into decrease of other. This may be evident from the study by Yuan *et al.* (2007) who observed increase in IP content due to substitution and deletions mutation in *MIPS1* gene involved in synthesis of PA in soybean.

IP content showed significant positive correlation with iron and zinc content (Table 3). Tavajjoh *et al.* (2011) observed significant negative correlation between PA/Zn ratio to zinc concentration.
No correlation was found between HKW and content of iron and zinc. Morgounov *et al.* (2007) also reported no correlation between 1000 kernel weight and content of iron and zinc. Graham *et al.* (1999) in their review concluded that there is no negative linkage between cultivar yield and micronutrient density, because of small amount of micronutrients in wheat. Ozturk *et al.* (2009) also observed no correlation between seed weight and Fe, Zn or protein concentration in wheat genotypes. Welch and Graham (2002) also reported no negative correlation between yield and Fe and Zn content. Positive correlations were observed between Fe and Zn content as reported by others (Monasterioand Graham, 2000; Morgounov *et al.*, 2007; Peleg*et al.*, 2008; Velu *et al.*, 2011). Our results are not in agreement with the earlier studies which may be due to the genotypes selected and the agro-climatic regions where the study was conducted.

Principal component analysis (PCA): PCA provides a tool to describe relationship between antinutritional properties and mineral content of wheat genotypes. Results of the initial PCA revealed that 99% of the variation among the lines was attributable to the first two principal components (Fig. 1). Based on the Euclidean distances, most of the wheat genotypes were fairly distinguished using PCA analysis. Genotypes such as, NIAW2805, NIAW2740, NIAW2799, NIAW2798 and NIAW2803 showed high PA and IP, while genotypes, NIAW2968, NIAW2761, NIAW2841, NIAW2927 and NIAW2828 showed low PA and high IP values. Among the genotypes studied, NIAW2968 had lowest PAwith better mineral content, compared to others. The low PA coupled with high mineral content is of much value for breeding wheat genotypes for better utilization of mineral nutrition by lowering antinutritional properties.

In conclusion, significant variability was observed for PA, IP, iron and zinc contents in advanced breeding lines of wheat developed for peninsular region. The parameters exhibiting higher estimates of GCV, coupled with high heritability indicate that considerable improvement in these parameters could be achieved through incorporation of desired genotypes in crossing programme followed by pedigree selection.

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Table 1.Genotype wise mean content of PA, IP, iron, zinc and HKW

Sr. No	Genotype code	Phytic acid (mg/g)	Inorganic phosphate (mg/g)	Iron (mg/g)	Zinc (mg/g)	HKW(g)
1	NIAW2709	11.043	0.185	0.054	0.0242	3.382
2	NIAW2710	9.768	0.169	0.058	0.0251	3.615
3	NIAW2711	8.498	0.152	0.055	0.0230	3.360
4	NIAW2712	9.172	0.192	0.051	0.0236	3.382
5	NIAW2713	8.906	0.156	0.051	0.0238	3.653
6	NIAW2718	8.699	0.158	0.054	0.0250	3.899
7	NIAW2721	9.281	0.167	0.049	0.0191	3.145
8	NIAW2724	10.983	0.175	0.054	0.0219	3.403
9	NIAW2725	9.122	0.151	0.050	0.0223	3.384
10	NIAW2734	8.500	0.168	0.054	0.0213	3.935
11	NIAW2735	8.582	0.158	0.053	0.0214	3.760
12	NIAW2740	14.876	0.167	0.050	0.0205	3.397
13	NIAW2746	9.167	0.155	0.074	0.0265	3.502
14	NIAW2748	7.788	0.188	0.052	0.0216	4.678
15	NIAW2750	10.213	0.149	0.056	0.0238	4.007
16	NIAW2751	8.881	0.170	0.058	0.0240	3.957
17	NIAW2757	7.601	0.160	0.068	0.0197	4.162
18	NIAW2758	10.511	0.170	0.055	0.0183	4.112
19	NIAW2761	5.866	0.188	0.084	0.0243	3.946
20	NIAW2762	7.644	0.180	0.063	0.0241	4.293
21	NIAW2763	8.230	0.185	0.068	0.0217	3.682
22	NIAW2765	8.798	0.189	0.064	0.0204	3.554
23	NIAW2766	8.297	0.162	0.090	0.0275	3.447
24	NIAW2779	9.002	0.132	0.072	0.0242	3.686
25	NIAW2782	12.128	0.161	0.068	0.0265	3.903
26	NIAW2785	8.541	0.156	0.063	0.0273	3.326
27	NIAW2788	9.171	0.152	0.087	0.0222	4.204
28	NIAW2791	9.775	0.139	0.047	0.0172	3.861
29	NIAW2792	9.577	0.158	0.057	0.0214	4.521
30	NIAW2794	9.453	0.165	0.047	0.0187	4.707
31	NIAW2796	9.619	0.144	0.056	0.0219	4.022
32	NIAW2798	14.022	0.153	0.050	0.0220	4.180
33	NIAW2799	14.033	0.147	0.053	0.0234	3.722
34	NIAW2802	11.876	0.197	0.064	0.0278	3.653
35	NIAW2803	13.459	0.174	0.057	0.0224	4.040
36	NIAW2804	10.373	0.144	0.067	0.0223	3.971
37	NIAW2805	15.020	0.169	0.066	0.0247	3.787
38	NIAW2806	11.984	0.191	0.055	0.0242	4.725
39	NIAW2827	8.574	0.141	0.052	0.0207	4.102
40	NIAW2828	6.559	0.179	0.049	0.0196	4.895
41	NIAW2830	9.632	0.187	0.047	0.0227	4.338
42	NIAW2832	7.651	0.168	0.057	0.0226	4.503
43	NIAW2841	6.245	0.172	0.087	0.0244	3.411
44	NIAW2845	7.864	0.171	0.086	0.0191	4.006
45	NIAW2846	8.339	0.163	0.076	0.0218	4.137
46	NIAW2847	10.120	0.190	0.063	0.0205	4.367
47	NIAW2848	9.219	0.154	0.075	0.0234	4.451
48	NIAW2849	9.637	0.148	0.063	0.0188	4.054
49	NIAW2853	8.685	0.164	0.061	0.0205	4.867
50	NIAW2855	7.837	0.142	0.052	0.0207	4.779
51	NIAW2856	7.996	0.192	0.067	0.0225	5.419
52	NIAW2857	9.287	0.141	0.061	0.0246	4.793
53	NIAW2859	11.384	0.162	0.067	0.0236	5.040
54	NIAW2862	8.835	0.179	0.080	0.0204	4.285
55	NIAW2863	10.626	0.144	0.069	0.0238	3.439
56	NIAW2864	9.512	0.174	0.080	0.0270	4.318
57	NIAW2865	11.893	0.182	0.098	0.0185	4.200
58	NIAW2866	11.857	0.179	0.086	0.0233	4.332
59	NIAW2867	11.216	0.150	0.066	0.0242	4.038
60	NIAW2871	9.509	0.128	0.055	0.0223	4.186

Table 1 Contd..

61	NIAW2874	9.475	0.156	0.054	0.0209	4.661
62	NIAW2876	11.094	0.137	0.052	0.0211	4.321
63	NIAW2878	9.197	0.136	0.058	0.0185	4.318
64	NIAW2884	7.136	0.163	0.053	0.0271	5.004
65	NIAW2885	7.951	0.159	0.048	0.0251	4.662
66	NIAW2886	7.702	0.166	0.055	0.0267	5.042
67	NIAW2887	8.034	0.168	0.050	0.0289	5.200
68	NIAW2894	11.706	0.127	0.053	0.0235	3.993
69	NIAW2896	11.264	0.142	0.055	0.0238	3.710
70	NIAW2898	9.252	0.157	0.051	0.0226	3.891
71	NIAW2899	10.440	0.156	0.053	0.0210	3.469
72	NIAW2900	9.158	0.164	0.048	0.0209	4.055
73	NIAW2901	9.573	0.159	0.054	0.0235	3.980
74	NIAW2903	11.456	0.177	0.055	0.0267	3.053
75	NIAW2904	9.611	0.177	0.049	0.0216	3.167
76	NIAW2907	11.591	0.159	0.052	0.0261	3.803
77	NIAW2908	10.662	0.143	0.056	0.0243	3.667
78	NIAW2910	9.686	0.186	0.053	0.0240	4.160
79	NIAW2914	9.298	0.162	0.053	0.0212	3.793
80	NIAW2915	11.780	0.150	0.055	0.0234	3.447
81	NIAW2916	10.535	0.193	0.054	0.0243	3.641
82	NIAW2918	11.639	0.160	0.054	0.0246	2.986
83	NIAW2920	9.419	0.173	0.053	0.0225	3.518
84	NIAW2922	8.256	0.234	0.052	0.0291	4.026
85	NIAW2926	10.008	0.156	0.056	0.0248	4.453
86	NIAW2927	6.820	0.200	0.072	0.0265	4.626
87	NIAW2930	8.330	0.195	0.051	0.0263	4.178
88	NIAW2933	8.628	0.181	0.050	0.0253	3.702
89	NIAW2937	9.917	0.181	0.094	0.0229	4.084
90	NIAW2939	9.470	0.141	0.054	0.0204	3.723
91	NIAW2940	9.394	0.161	0.057	0.0249	3.751
92	NIAW2941	8.856	0.158	0.051	0.0216	3.955
93	NIAW2942	9.450	0.135	0.051	0.0230	3.523
94	NIAW2944	9.352	0.155	0.045	0.0240	4.476
95	NIAW2945	8.446	0.175	0.049	0.0243	4.466
96	NIAW2946	11.099	0.165	0.050	0.0251	4.510
97	NIAW2948	8.676	0.172	0.052	0.0257	4.144
98	NIAW2949	8.430	0.153	0.042	0.0244	4.126
99	NIAW2968	4.967	0.165	0.047	0.0236	4.401
100	NIAW2977	9.843	0.143	0.047	0.0218	3.209
	CD (5%)	0.2618	0.0038	0.0003	0.0001	0.0626

Table 2. Mean, range, GCV, PCV and broad sense heritability (h^2_{bs}) for PA, IP, iron content, zinc content and 100 seed weight (HKW)

	PA (mg/g of seed)	IP (mg/g of seed)	Iron (mg/g of seed)	Zinc (mg/g of seed)	HKW (g)
Mean	9.59	0.16	0.059	0.023	4.03
Range	4.97-15.02	0.128-0.234	0.042-0.098	0.0172-0.0291	2.98-5.42
CV	9.72	8.29	4.18	3.79	5.53
Vg	2.68	0.0002	0.000135	0.000005	0.232
Vp	3.11	0.0003	0.000138	0.0000058	0.257
GCV (%)	17.06	9.41	19.71	10.07	11.96
PCV(%)	18.40	11.10	19.93	10.42	12.58
h^2_{bs}	0.86	0.72	0.98	0.93	0.90

***** CV: coefficient of variation; Vg: genotypic variance; Vp: phenotypic variance; GCV: genotypic coefficient of variation; PCV: phenotypic coefficient of variation; h^2_{bs}: heritability in broad sense

Table3. Pearson's correlation coefficient of PA, IP, 100 seed weight (HKW), Fe and Zn content

	PA	IP	HKW	Fe	Zn
PA	1	-0.1293	-0.235*	0.0491	-0.0438
IP		1	0.0995	0.133*	0.204**
HKW			1	0.0242	-0.0017
Fe				1	-0.0366
Zn					1

*,** significant at 5 and 1 percent respectively.

Fig 1. PCA analysis of wheat genotypes based on Euclidean distances (PCA1: 92.98% and PCA2: 6.99%) for all the traits