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Heritability and genetic diversity of iron, zinc and some morphological and physiological traits in some spring wheat genotypes (*Triticum aestivum* L.)

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Abstract

Wheat is one of the most important cereals and main source of food in Iran and many different countries. Thirty spring common wheat genotypes from Iran were assessed for grain concentrations of Iron and Zinc and some morphological and physiological traits in 2009 and 2010. Iron showed large variation among genotypes, ranging from 17.5 µg g^{-1} to 48.68 µg g^{-1} (mean 30.79 µg g^{-1}) in 2009 and from 22.74 µg g^{-1} to 45.60 µg g^{-1} (mean 30.53 µg g^{-1}) in 2010. Similarly, Zinc concentration varied among genotypes, ranging from 22.36 µg g^{-1} to 52.69 µg g^{-1} (mean 35.79 µg g^{-1}) in 2009 and from 20.74 µg g^{-1} to 78.60 µg g^{-1} (mean 42.45 µg g^{-1}) in 2010. The highest heritability was exhibited by days to 50% heading (0.96) in 2009 and by stem number (0.96), spike length (0.96) in 2010. Heritability of grain iron and zinc were 0.74 and 0.61 in 2009, 0.85 and 0.92 in 2010 respectively. Four clusters formed through cluster analysis. Sistan, Akbari, Roshan and Roshan Bahareh backcross genotypes are recommended to use in multiple crossing program to reach transgressive segregants with high genetic potential of studied traits especially for chlorophyll content, grain iron and grain zinc all together. The most squared Euclidean genetic distance (75.81) observed between Niknejad and Roshan genotypes. Therefore, these genotypes can be used to plan wide crosses, to reach genetic diversity and maximize expression of hetrosis. Mahdavi and Bahar genotypes had the most squared Euclidean genetic distance (556.45) for grain iron and zinc content.

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Introduction

Approximately 600 million metric tons of wheat are commercially milled annually and consumed in nearly every nation of the world (Akhtar and Ashgar, 2011). Whole wheat flour is a staple food of the Indian population and supply more than 50% of the total energy intake. Similarly, in countries such as Syria, Algeria, Turkey, and Iran, wheat consumption is approximately half the total caloric intake and ranges up to 600 g/day (Ranum, 2001). Zinc and iron are essential micronutrients for human nutrition and health. There are estimates that some three billion people worldwide are afflicted by iron deficiency, and up to half of the population in developing countries are at risk of zinc deficiency (Welch and Graham, 2004; Cakmak, 2008). Micronutrient malnutrition greatly increases mortality and morbidity rates, diminishes cognitive abilities of children and lowers their educational attainment, reduces labor productivity, stagnates national development efforts, contributes to continued high population growth rates and reduces the livelihood and quality of life for all those affected (Welch and Graham, 2002). Zinc and iron deficiencies are particularly known to be a common problem in populations that depend on cereals as the main staple food and with little or no access to animal products (White and Broadley, 2005). According to above mentioned subjects, wheat flour and its products are good cases for fortification with iron and zinc. But, fortification efforts are highly dependent on funding, and the scope is restricted to a single geographical area. Standard fortification programs must be sustained at the same level of funding year after year; and if the investments are not sustained, the benefits disappear, in contrast micronutrient improvement by plant breeding programs is sustainable successful (Bouis *et al.,* 2000). Consequently, genetically improvement of wheat grain Iron and Zinc concentration is a good approach. Previously, Morganov *et al.* (2007) studied on sixty-six spring and winter wheat cultivars and advanced lines from Central Asian national breeding programs of the Central Asia region comprises five countries (Kazakhstan, Kyrgyzstan, Turkmenistan, Tajikistan and Uzbekistan) to determine the levels of

Iron and Zinc in the grain of the target wheat genotypes and identify promising lines with higher Iron and Zinc concentrations in the grain. Also, cakmak *et al.* (2004) studied on 825 wild emmer accessions (*Triticum turgidum* ssp. *dicoccoides*) for natural variation of Iron and Zinc as an important genetic resource for improving zinc and iron concentration in cultivated wheat.

One of the important goals to wheat breeding is hybridization and subsequent selection. Thus select of parents with the most genetic distance is the first step in breeding program. However, genetic distance between parents is necessary to develop superior hybrid (Ilker *et al.*, 2010). Transgressive segregation occurs often when the difference between parents is small and the additive variance is high (Joshi *et al.*, 2004) and it is better that these parents selected from divergent cluster with high performance for under breeding traits (Kumar *et al.*, 2009).

In order to determine whether iron and zinc concentration in a specific crop or other traits can be improved by traditional breeding methods, it must be known to how much these traits are heritable (Courtney, 2007). Heritability is a measure of the extent to which observed phenotypic differences for a trait are due to genetic variations (Klug and Cummings, 2005). A study of genetic variability with the help of suitable parameters such as genotypic coefficient of variation and heritability are thoroughly necessary to start an efficient breeding program (Mishra *et al.*, 1988). Although direct selection for various traits could be misleading, indirect selection via related traits with high heritability might be more impressive than direct selection (Toker and Cigirgan, 2004). Targeted opt of physiological traits that limit yield and have a high heritability may be more impressive than direct selection for yield (Sayar *et al.*, 2007). Iron and zinc concentration, exists in the germplasm for the crop. Thus, an important initial step in developing a biofortified crop is the screening of germplasm to see if this genetic variation exists. Such germplasm screenings have been undertaken for a number of crops. Studies with common bean

(*Phaseolus vulgaris*), rice (*Oriza sativa*), and wheat (*Triticum spp.*) have all found large variation in iron and zinc concentration (Welch and Graham, 2004). The genetic variation of selected parents is not always based on factors such as geographic diversity place of release of ploidy level. So, characterization of genotypes should be based on statistical procedure such as hierarchical Euclidean cluster analysis (Kumar *et al.*, 2009). The objectives of this study was determine the level of grain iron and zinc content in the Iranian spring wheat genotypes and identify genotypes with the highest genetic distance for using in breeding programs.

Material and methods

Plant materials and experimental design

Thirty spring common wheat genotypes (Fig. 1) supplied by the Agricultural Research Institutes gene bank in Karaj were prepared and used for this research in the Research Field of Faculty of Agricultural Sciences, Tarbiat Modares University, Tehran, Iran in 2009 and 2010. The plot size was 1.5 m length with three rows, row spacing was 20 cm and seeding rate was 250 seeds m^{-2} based on a randomized complete block design with three replications. Nitrogen and phosphorus fertilizer, were consumed at 40 and 60 Kg ha^{-1}, respectively before planting and nitrogen fertilizer was also broadcast at the stages of tillering and stem elongation at 40 kg ha^{-1}. The measured traits include stem number, fertile stem number, flag leaf length (cm), flag leaf width (cm), leaf area (cm^2), main stem leaf number, spike length (cm), days to 50% heading, 1000 grain weight, chlorophyll content (measured by SPAD-502), grain iron concentration ($\mu g \ g^{-1}$) and grain zinc concentration ($\mu g \ g^{-1}$).

Grain iron and zinc were measured using atomic absorption method (Gupta, 2000) after digestion based on nitric (0.1 normal)/citric (10%) acid (Morgounov *et al.* 2007) and extraction by ion-chromatography method (Small *et al.*, 1975). Also, homogeneity of experimental field has confirmed and then trial was conducted.

Statistical analysis

Normality test using Shapiro-Wilk method, analysis of variance, means comparison by Duncan's new multiple range test (DMRT) at 5% level of probability and genotypes grouping using hierarchical Euclidean cluster analysis (Kumar *et al.*, 2009) were performed using the statistical software SPSS version 16.0 (SPSS, Chicago, USA) program. In addition, cophenetic correlation computed through Eta statistic using SPSS. For cluster analysis, ward's method based on squared Euclidean distance measure was used. Because of non-uniformity of measurement scale of traits date were standardized (Mohammadi and prasanna, 2003) using Z score method while in cluster analysis for gain iron and zinc original data were used. Some statistics including squared distance between cluster centroids, within cluster sum of square, average and maximum distance from centroid computed using Minitab 14.

The mean squares were used to estimate genotypic and phenotypic variance according to Johnson *et al.* (1955). The coefficient of variation was calculated according to the formula suggested by Burton (1952). The phenotypic and genotypic coefficient of variation and broadcast heritability were calculated according to Singh and Chowdhury (1985).

Results

Analysis of variance for studied traits

Results of normality test showed that data for all traits were normal except for grain iron and zinc in second year. Therefore, data transformation for these traits was done. Analysis of variance showed high significant difference among genotypes for all traits in both years (Table 1). Some descriptive statistics of studied traits were presented in Table 3. Sistan, Roshan Bahareh backcross, Chenab, Bayat, Kavir, Arta, Shiraz, Shiraz and Bayat, Dez, Verinak, Akbari and Akbari genotypes in 2009; Sholeh, Darya, Sistan, Roshan-Bahareh back cross, Roshan, Roshan and Bayat, Shiraz, Shiraz and Bayat, Akbari, Roshan, Marvdasht and Darab 2 genotypes in 2010 exhibited maximum means for traits presented in Table 3 with order from left to right.

Table 1. Analysis of variance for studied traits in 30 spring wheat genotypes.

Year	S.O.V	DF	Mean square											
			Stem No.	Fertile stem No.	Flag leaf length	Flag leaf width	Leaf area	Main stem leaf No.	Spike length	Days to 50% heading	1000 grain weight	Chlorophyll content	Grain iron	Grain zinc
2009	Replication	2	18.00^{*}	4.00^{ns}	27.00^{**}	0.02^{ns}	5.00^{ns}	1.00^{*}	0.35^{ns}	2.00^{ns}	8.98^{*}	138^{***}	145.21^{**}	0.47^{ns}
	Genotype	29	16.3^{***}	4.00^{***}	16.20^{***}	0.08^{***}	35.7^{**}	0.6^{***}	2.3^{***}	19.90^{***}	28.78^{***}	32.1^{***}	89.71^{***}	61.90^{***}
	Error	58	5.10	1.70	5.80	0.02	15.90	0.22	0.29	0.70	2.73	8.40	23.07	23.90
2010	Replication	2	0.27^{ns}	3.74^{***}	68.21^{***}	0.62^{***}	9.93^{ns}	0.08^{ns}	2.58^{***}	4.58^{*}	1.14^{ns}	5.43^{ns}	$1.73e^{-6\,ns}$	0.002^{ns}
	Genotype	29	8.52^{***}	4.78^{***}	28.86^{***}	0.07^{***}	48.17^{***}	0.31^{***}	3.11^{***}	27.39^{***}	64.02^{***}	32.81^{***}	$6.71e^{-5\,***}$	0.05^{***}
	Error	58	0.31	0.30	1.38	0.01	8.49	0.04	0.13	1.50	6.39	5.69	$1.02e^{-5}$	0.004

***, **, * and ns: significant at 0.1%, 1%, 5% level of probability and non-significant, respectively.

Table 2. Phenotypic (CVp) and genotypic (CVg) coefficient of variation and broadcast heritability (H_b^2) in 30 spring wheat genotypes.

Year	Statistic	Stem No.	Fertile stem No.	Flag leaf length	Flag leaf width	Leaf area	Main stem leaf No.	Spike length	Days to 50% heading	1000 grain weight	Chlorophyll content	Grain iron	Grain zinc
2009	CVp (%)	18.55	16.66	12.62	9.20	18.48	10.35	8.03	3.32	10.03	7	17.76	12.72
	CVg (%)	15.41	12.56	10.11	7.96	13.76	8.17	7.49	3.26	9.54	6.01	15.31	9.96
	H_b^2	0.69	0.57	0.64	0.75	0.55	0.62	0.87	0.96	0.91	0.74	0.74	0.61
2010	CVp (%)	22.08	22.09	15.41	9.03	16.44	6.29	8.97	4.46	13.17	6.64	14.99	30.72
	CVg (%)	21.67	21.39	15.03	8.67	14.92	5.84	8.78	4.34	12.50	6.03	13.85	29.47
	H_b^2	0.96	0.94	0.95	0.92	0.82	0.86	0.96	0.95	0.90	0.83	0.85	0.92

Table 3. Descriptive statistics of studied traits in 30 spring wheat genotypes.

Year	Statistic	Stem No.	Fertile stem No.	Flag leaf length	Flag leaf width	Leaf area	Main stem leaf No.	Spike length	Days to 50% heading	1000 grain weight	Chlorophyll content	Grain iron	Grain zinc
2009	Mean	12.58	6.93	18.41	1.72	18.66	4.32	10.84	77.50	30.89	46.8	30.79	35.72
	Min	7.20	3.60	11.20	1.34	9.00	2.80	8.40	73.00	22.80	34.96	17.50	22.36
	Max	20.40	11.00	25.80	2.16	30.00	5.80	13.70	81.00	41.6	56.80	48.65	52.69
	CV%	23.8	22.80	16.80	11.20	25.18	13.9	8.90	3.40	10.91	9.30	22.39	16.74
2010	Mean	7.63	5.72	20.13	1.76	24.37	5.16	11.36	67.71	35.07	49.83	30.53	42.45
	Min	4.00	3.20	11.30	1.30	12.50	5.00	8.80	61.00	17.74	37.72	22.74	20.74
	Max	12.00	9.40	27.58	2.16	35.00	6.00	13.90	75.00	48.44	56.84	45.60	78.60
	CV%	22.64	23.70	17.09	11.71	19.01	7.07	9.46	4.67	14.29	7.65	16.87	32.70

Estimates of genetic parameters

The phenotypic coefficient of variation ranged from 3.32 to 18.55 in 2009 and from 4.46 to 30.72 in 2010 and the genotypic coefficient of variation ranged from 3.26 to 15.41 in 2009 and from 4.34 to 29.47 in 2010 (Table 2). The highest heritability was exhibited by days to 50% heading in 2009 and by stem number, spike length in 2010 (Table 2).

Cluster analysis

Based on cluster analysis, thirty genotypes were grouped into four distinct clusters that group number of genotypes was shown in Fig. 1 suggesting considerable amount of genetic diversity in the material. The accuracy of grouping was confirmed by discriminant analysis and multivariate analysis of variance based on complete randomized design so that the genotypes correctly belonged in to their groups and the difference between group means were significant (P<0.001) based on Wilks' Lambda, Pillai's trace and Roy's Largest Root tests. The data presented in Table 4 showed significant (P<0.001)

difference among group means for all traits except for main stem leaf number, chlorophyll content, grain iron and grain zinc content.

Cluster 1 included 26.66% of genotypes (Fig. 1) and mean of flag leaf length, flag leaf width, leaf area and Spike length were lower than total mean and for other traits were higher than total mean (Table 5). Cluster 1 had the least average distance from centroid and within cluster sum of square for this cluster was 41.81 (Table 6). Cluster 2 included 20% of genotypes and mean of all traits except main stem leaf number and grain zinc were lower than total mean (Table 5). Cluster 2 had 2.70 and 3.40 average and maximum distance from centroid respectively and had 47.44 within cluster sum of square (Table 6). Cluster 3

included 13.33% of genotypes and mean of all traits in this cluster were higher than total mean and had the highest value for all traits except for grain zinc (Table 5). Cluster 3 had the most average and maximum distance from centroid and within cluster sum of square (Table 6). Also based on mean comparison test, cluster 3 had a rank for all traits. Cluster 4 included 40% of genotypes indicating overall genetic similarity among them and mean of this cluster in more cases were lower than total mean. Cluster 4 had the least maximum distance from centroid and within cluster sum of square (Table 6). The most squared Euclidean genetic distance (75.81) observed between Niknejad and Roshan genotypes. The least squared Euclidean genetic distance (2.71) observed between Shiroodi and Tajan genotypes.

Table 4. Analysis of variance based on Wilks' Lambda statistic.

Statistic	Stem No.	Fertile stem No.	Flag leaf length	Flag leaf width	Leaf area	Main stem No.	Spike leaf length	Days 50% heading	to 1000 grain weight	Chlorophyll content	Grain iron	Grain zinc
Wilks' Lambda	0.40***	0.34***	0.52***	0.42***	0.53***	0.93ns	0.46***	0.90nd	0.67**	0.82 ns	0.89ns	0.84ns

$DF_1=3$, Degree of freedom between groups; $DF_2= 26$, Degree of freedom within groups; ***, ** and ns: significant at 0.1%, 1% level of probability and non-significant, respectively.

Table 5. Results of group mean comparison and some statistics of clusters.

Cluster	Statistic	Stem No.	Fertile stem No.	Flag leaf length	Flag leaf width	Leaf area	Main stem No.	Spike leaf length	Days 50% heading	to 1000 grain weight	Chlorophyll content	Grain iron	Grain zinc
1	mean	10.48[b]	7.01[a]	17.98	1.73[b]	19.86[b]	4.77[a]	10.57[b]	72.92[a]	24.72[a]	48.50[ab]	31.44[a]	39.44[a]
	DfTM	0.37	0.69	-1.29	-0.01	-1.66	0.03	-0.53	0.29	0.50	0.18	0.77	0.35
	SD	0.64	0.35	1.63	0.13	1.65	0.19	0.37	2.53	0.59	2.37	2.26	4.39
2	mean	8.93[c]	5.72[b]	17.36	1.54[c]	19.82[b]	4.81[a]	10.28[b]	71.50[a]	22.55[b]	48.12[ab]	29.31[a]	42.53[a]
	DfTM	-1.17	-0.61	-1.91	-0.20	-1.70	0.07	-0.82	-1.12	-1.67	-0.20	-1.35	3.45
	SD	0.79	0.48	2.04	0.06	2.59	0.27	0.65	2.82	1.68	2.51	1.73	7.26
3	mean	12.20[a]	7.43[a]	20.58[a]	1.89[a]	23.57[a]	4.79[a]	11.80[a]	74.33[a]	25.81[a]	50.67[a]	32.78[a]	41.20[a]
	DfTM	2.09	1.11	1.30	0.15	2.05	0.05	0.70	1.71	1.60	2.35	2.12	2.11
	SD	0.37	0.21	1.36	0.06	2.13	0.32	0.62	2.92	1.59	2.64	3.64	4.41
4	mean	9.75[bc]	5.80[b]	20.65[a]	1.79[ab]	22.79[a]	4.66[a]	11.63[a]	72.42[a]	24.18[ab]	47.52[b]	30.11[a]	36.42[a]
	DfTM	-0.36	-0.52	1.38	0.06	1.27	-0.07	0.53	-0.21	-0.03	-0.80	-0.55	-2.66
	SD	1.07	0.68	1.53	0.10	1.29	0.25	0.72	2.79	1.69	2.10	4.26	6.57

DfTM: Deviation from total mean; SD: Standard Deviation.

Discussion

Analysis of variance and means comparison

Variation among genotypes is appropriate to breeding programs for studied traits. The results presented in Table 1 indicate that. Similarities between results of

both years indicate that the trial is replicable and its results are reliable. Results of previous studies on wheat are in accordance with this research, Fareed Khan *et al.* (2004) and Shafeeq *et al.* (2006) for stem number Kashif and Khaliq (2004) for fertile stem

number, leaf area and 1000 grain weight, Heidari *et al.* (2006) for flag leaf length, flag leaf width and spike length, days to 50% heading, Morganov *et al.* (2007) for grain iron and zinc concentration. In the previous studies obtained means were 12.72 for fertile stem number, 53.61 for flag leaf area, 14.40 for spike length and 37.96 for 1000 grain weight (Kashif and Khaliq, 2004); 162 for days to 50% heading and 38.3 for 1000 grain weight (Aycicek and Yildirim, 2006), 25.4 for flag leaf length and 2.03 for flag leaf width

(Dere and Yildirim, 2006), 46.84 for chlorophyll content (Awaad *et al.*, 2010); 38.0, 31.6 and 37.2 for grain iron and 28, 35.0 and 30.7 for grain zinc content (Welch and Graham 2002; Morgounov *et al.*, 2007) that in more cases, obtained results are in accordance and in a few cases are in contrast with those. Table 2 content suggesting that environment had no influence on the traits under study especially in 2010.

Table 6. Between cluster distance, within cluster sum of square and maximum and average distance from centroid for 30 spring wheat genotypes.

Squared distance between clusters			Within cluster sum of squares	Average distance from centroid	Maximum distance from centroid
	Cluster2	Cluster3	Cluster4			
Cluster1	35.66	43.34	62.34	41.81	2.18	3.07
Cluster2		109.78	59.23	47.44	2.70	3.40
Cluster3			44.92	108.63	2.91	4.12
Cluster4				25.84	2.50	2.88

Table 7. between cluster distances and some statistics of clusters for 30 spring wheat genotypes.

Squared distance between clusters			Within cluster sum of squares	Average distance from centroid	Maximum distance from centroid	Observation No. from	Grain iron	Grain zinc
	Cluster2	Cluster3	Cluster4						
Cluster1	7.35	14.45	8.20	26.72	2.15	3.41	5	35.98	41.10
Cluster2		16.75	10.54	97.96	3.12	4.41	9	30.80	46.31
Cluster3			6.51	15.60	1.82	2.89	4	26.61	30.09
Cluster4				133.68	2.89	7.65	12	29.69	35.83

Heritability

Heritability of all studied traits in both years was high therefore environmental conditions have little impact on the phenotypic differences observed in the studied population (Klug and Cummings, 2005) and these traits can be improved by breeding programs (Courtney, 2007). Heritability of studied traits in this study was estimated by some researchers previously; Kashif and Khaliq (2004) and Ajmal *et al.* (2009) estimated 0.41 and 0.98 for stem number respectively, Kahrizi *et al.* (2010) estimated 0.43 for stem number and 0.50 for flag leaf length, Smocek (1970) estimated 0.88 for flag leaf length and 0.94 for flag leaf width, Ejaz-ul-hassan and Khaliq (2008) estimated 0.88 for flag leaf area, Dere and Yildirim (2006) estimated 0.5 for flag leaf length and 0.48 for flag leaf width, Memon (2007) estimated 0.93 for fertile stem number and 0.64 for spike length, Ali *et*

al. (2008) estimated 0.99 for 1000 grain weight, 0.76 for spike length and 0.46 for fertile stem number, Zhang *et al.* (2009) estimated 0.72 for chlorophyll *a* and 0.75 for chlorophyll b content and 0.76 for days to heading time, Awaad (2010) estimated 0.70 for chlorophyll content and 0.76 for flag leaf area and Ghai *et al.* (1969) estimated 0.84 for chlorophyll content. In more cases, obtained results are in accordance with previous studies and in a few cases in contrast with those.*Breeding strategies based on cluster analysis*

Members of the second cluster had the highest grain zinc content. Therefore, genotypes of this cluster can be used in breeding programs with aim of grain zinc improving. As suggested by Kumar *et al.* (2009) who said hybridization between genotypes of divergent cluster will lead to accumulation of suitable genes in a

single variety and also suggested to create variability for creating the varieties including a large number of different lines instead of closely instead of closely related ones, the genotypes of the third cluster may be involve in a multiple crossing program to recover transgressive segregants with high genetic potential of studied traits especially for chlorophyll content, grain iron and grain zinc all together.
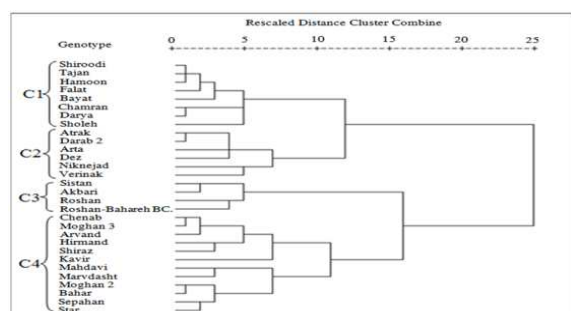

Fig. 1. Dendrogram showing the genetic relationships among 30 spring wheat using ward method and squared Euclidean distance, C1, C2, C3 and C4 contain members of clusters 1, 2, 3 and 4 respectively.

Because of the most squared Euclidean genetic distance between Niknejad and Roshan located in cluster 2 and cluster 3 respectively (Fig. 1) and these clusters had the most distance from together. Suggesting by Ilker *et al.* (2010) these genotypes can be used to plan wide crosses, to reach genetic diversity and maximize expression of hetrosis. Shiroodi and Tajan genotypes had the least squared Euclidean genetic distance with together and can be used in back cross program for gene transfer. The frequency distribution of genetic distance value for all 435 pairs of comparisons indicates that 95 % of the pair comparisons had values between 6.3 and 49.4. Only 10 pairs accounting 2.30 % of the total had genetic distance value smaller than 6.3 and 11 pair comparisons, which are only 2.53 % of the total, had values greater than 49.4. Cophenetic value of 0.75 or more are usually recommended for the best fit of the cluster analysis (Ribeiro Trindade *et al.*, 2010). The present study confirmed a good fit with the genetic distance matrix by having cophenetic correlation coefficient of r=0.793.

Because of grain iron and grain zinc importance and presence meaningful genetic diversity among genotypes for these traits (Table 1), genotypes were grouped based on these traits. The most squared Euclidean genetic distance (556.45) observed between Mahdavi and Bahar. Therefore, these genotypes can be used as parents in hybridization program. The least squared Euclidean genetic distance (0.07) observed between Shiroodi and Tajan genotypes. Some information about clusters, which extracted through cluster analysis based on grain iron and zinc content, presented in Table 7.

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