Australian Journal of Crop Science AJCS
AJCS 5(1):17-24 (2011) ISSN:1835-2707

Genetic diversity of wheat (*Triticum aestivum* L.) genotypes based on cluster and principal component analyses for breeding strategies

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Abstract

Determination of genetic diversity is useful for plant breeding and hence production of more efficient plant species under different conditions. Accordingly, the most common wheat (*Triticum aestivum* L.) genotypes including 36 winter wheat genotypes cultivated in different regions of Iran were selected, grown and analyzed for genetic diversity. The experiment was conducted at the Agricultural Research Farm of Shahed University, Tehran, Iran as a randomized complete block design with three replications. All traits, except emergence time and heading time were statistically significant among different genotypes. Cluster analysis based on squared Euclidean distance and ward's method, categorized the cultivars into seven groups. The highest genetic distance was observed between Sardari and Spn/Mcd/Cama/3/Nzr/4/Passarinho (SP) genotypes. Based on Principal Component Analysis (PCA), the first five components explained over 97% of genetic variation. Cluster analysis based on PCA using the first five principal components indicated six separate groups of genotypes, with the maximum genetic distance observed between Sardari and Vorona/Kauz (VO) genotypes. Such differences in genetic component of traits studied in this manuscript can be applied as a new source of variation in other breeding programs and crossing nurseries for wheat improvement.

Keywords: genetic variation; heritability; cluster analysis; wheat (*Triticum aestivum* L.)

Introduction

Genetic diversity of plants determines their potential for improved efficiency and hence their use for breeding, which eventually may result in enhanced food production. Plant uniformity, which can be resulted by the use of modern plant breeding techniques, can produce plants, which are more efficient by means of different goals including enhanced resistance under stress, however much more research must be performed to indicate the most optimized methods that can be used for the production of efficient plants. This is of significance for the production of food for the world increasing population (Fu and Somers, 2009). Accordingly, the increased attention to the production of resistant plant species for prolonged food production under different conditions indicate the necessity of performing breeding experiments (Martin et al., 2008; Van de Wouw et al., 2010). One of the important approaches to wheat breeding is hybridization and subsequent selection. Parents' choice is the first step in plant breeding program through hybridization. In order to benefit transgressive segregation, genetic distance between parents is necessary (Joshi et al., 2004). The higher genetic distance between parents, the higher heterosis in progeny can be observed (Joshi and Dhawan, 1966; Anand and Murrty, 1968). Benadeki (1992) investigated the genetic diversity of five local geographical regions across central provinces of Iran for bread wheat. It has been proposed that the differences for studied traits across regions were significantly (P=0.01) different and resulted in nine classes discriminated by geographical regions (Benadeki, 1992). Narouee Rad (2006) determined the genetic diversity of wheat landraces in the west of Iran and by using cluster analysis, six clusters were determined for different areas. Fang et al. (1996) clustered 120 genotypes of durum wheat into five groups based on maturity date, plant height, spike length, number of seed per spike, 1000-seed weight and spike seed yield. Jain et al. (1975) investigated the geographical patterns of phenotypic diversity of durum wheat using the world collection and achieved a developed program for the protection of genetic resources to identify and assess inter variation and intra societies. Genetic diversity could be the result of geographical impact through evolution and hence traits could be considered as a function of variety (Benadeki, 1992). Estimation of genetic distance is one of appropriate tools for parental selection in wheat hybridization programs. Appropriate selection of the parents is essential to be used in crossing nurseries to enhance the genetic recombination for potential yield increase (Islam, 2004). Some appropriate methods, cluster analysis, PCA and factor analysis, for genetic diversity identification, parental selection, tracing the pathway to evolution of crops, centre of origin and diversity, and study interaction between the environment are currently available (Bhatt, 1970; Carves et al., 1987; Mohammadi and Prasanna, 2003; Eivazi et al., 2007).Usually before calculating the genetic distance, the variables are standardized so that all variables are of similar

importance in determining the distance. Unfortunately, standardization decreases the differences among groups. The results of cluster analysis and PCA may have relative differences with each other. Therefore, before using cluster analysis, the principle components may be avoided. On the other hand, when the two first principal components account for high variation percentage, grouping according to these two components, can certainly be a useful method to find the clusters (Fotokian et al., 2002). Various algorithms have been used in studying of genetic diversity in cluster analysis of which, UPGMA and Ward's methods are the most popular approaches. Of the algorithms, UPGMA, Ward's, SLINK, and CLINK, applied for cluster analysis and exploring genetic diversity and grouping of plant materials in the past, , the UPGMA is the most valid method in accordance with the relationship of family based on their genetic material (Mohammadi and Prasanna, 2003). Chaining effect in UPGMA model is considered as the major drawback on application of this approach in cluster analysis and results in confusions in interpretation of the results (Mohammadi and Prasanna, 2003). Ward's approach is similar to UPGMA method but it without having chain effect issues. Results of using PCA showed that this method is limited when the pattern of variation is not based on a 0 and 1 scores. Therefore, combined PCA and other techniques can be appropriately used for grouping (Mohammadi and Prasanna, 2003). The cluster analysis is an appropriate method for determining family relationships (Mellingers, 1972). The main advantage of using PCA over cluster analysis is that each genotype can be assigned to one group only (Mohammadi, 2002). One of the issues with breeding projects based on hybridization is to estimate the relationship between parents before initiating the crossing. Euclidean distance can theoretically estimated the genetic distance between parents to maximize the trangressive segregation (Hoque and Rahman, 2006). Determination of genetic diversity is useful for plant breeding and hence production of more efficient plant species under different conditions. Accordingly, the most common wheat genotypes including 36 winter wheat genotypes cultivated in different parts of Iran were selected, grown and analyzed for their genetic diversity based on the studied traits explained in this paper. The main objective of this study is to capture the potential genetic diversity between wheat genotypes grown in Iran by using cluster analysis and cluster analysis-PCA-based methods. The results of present study have been used in selection of appropriate parents for breeding program based at Shahed University.

Materials and methods

Plant material

Thirty six winter wheat cultivars (Table 1) kindly provided by the Agricultural Research Institutes in East Azarbaijan and Karaj were cultivated in the research field of College of Agricultural Sciences, Shahed University, Tehran, Iran in 2005.

Field experiment

The seeds were planted using furrows on lines with 1.5 m in length and 20 cm in width on the basis of a randomized complete block design with three replications. Pre-emergence fertilizers (Nitrogen and Phosphorus at rate of 40 and 60 Kg ha^{-1}, respectively) applied in addition to nitrogen fertilizer which was applied at a rate of 40 Kg ha^{-1} at tillering and stem elongation wheat growth stages. During the growth season the traits including emergence time (days to 50% emergence), tillering time (days to 50% tillering), heading time (days to 50% heading), height at heading time (cm), total stem number, fertile stem number, flag leaf sheath distance to spike (cm), flag leaf length (cm), flag leaf width (cm), spike Length (cm), Plant height (cm) and number of seed per spike were measured.

Statistical analysis

Five plants per plot were collected and the mean data points were used for statistical analysis. Normality test using Shapiro-Wilk method, Analysis of variance, cluster analysis based on ward's method using squared Euclidian distance (Kumar et al., 2009) and identification the cutting point using discriminate analysis and Multivariate analysis of variance (Mohammadi and Prasanna, 2003) in both analysis were performed using the statistical software SPSS version 16.0 (SPSS, Chicago, USA) program. Because of non-uniformity of measurement scale of traits date were standardized (Mohammadi and prasanna, 2003). PCA was performed using Minitab 14 software and the values of the first five components were selected and analyzed using SPSS and the related clusters were plotted based on the main components.

Genetic and phenotypic variances were calculated according to the following formulas and applied in the analysis:

$$MS_T = rMS_G + MS_E$$

$$MS_G = \frac{(MS_T - MS_E)}{r}$$

$$MS_P = MS_G + \frac{MS_E}{r}$$

Where: MS_T , is treatment variance; MS_E , experimental error variance; MS_G , genotype variance; MS_P , phenotypic variance; and r is the number of replications. Phenotypic variance based on the mean value of the genotypes for each trait, were calculated. Genetic and environmental variances were estimated based the model proposed by (Johnson et al., 1955). Broad sense heritability (H_b^2) was estimated according to the following formula:

$$H_b^2 = \frac{MS_G}{MS_P}$$

Clusters generated through both approaches were manually compared by searching for the presence and absence of each genotype in each cluster generated by each model.

Results

Analysis of variance

There were significant differences between wheat genotypes for all traits measured with an exception of emergence time (Table 2). Flag leaf width and flag leaf sheath distance to the spike

Table 1. Thirty six winter wheat genotypes (A letter acronym in the parentheses represents the related genotype)

Genotypes			
PNR2548/STAR1 (PN)	OMID	KARAJ2	NEMURA/STAR1 (NE)
GFgy158/Zrn/4/Hys//Drc *2/7c/3/2*Rsh (GFG)	Spn/Mcd/Cama/3/Nzr/4/Passarinho (SP)	ALVAND	Uzer81/HD2206/Hork"S"/3/Lov24/Coc75/4/.. (UZ)
BEZOSTAYA	GFgy54/5/Gds/4/Anza/3/Pi/Nar//Hys/6/;1-66-76/.. (GF)	V-83035/1-67-78 (V)	DEIHIM
VORONA/KAUZ (VO)	ADL	BISTONE	130L1.11//F35.70/MO73/4/YMH/TOB//MCD/3/LIRA (130L)
NAVID	TOOS	SEBELAN	TAST/SPRW//BLL/3/NWT (TA)
MV17	GASPARD	Alvand//Aldan/Ias58/3/40-73-17 (AA)	SHAPASAND
KARAJ2	Ghk"s"/Bow"s"//Nning8201 (GH)	ZARIN	AGRI/NAC//ATTILA (AG)
SARDARIE	SHAHRIAR	Evwyt2/Azd/4/Azd//Rsh*2/10120/3/Ombu1/Alamo (EV)	KARAJ3
Alvand//NS732/Her (AN)	ALEMOOT	Falat//Shi#4414/Crow"s" (FA)	C-79-16 (C)

exhibited the highest H_b^2 and the least H_b^2 was estimated for emergence time and flag leaf length (Table 2).

Cluster analysis

By incision the dendrogram at 12 units distance, the genotypes categorized into six groups. Using discriminant analysis revealed that in this case 10% of the members of the second group were classified into the first Group. Then cutting point was determined at distance 7 and 7 clusters was obtained, in which the members completely belonged to the same group (Fig. 1).

Genotypes of the first cluster

Karaj2, Alvand, Toos, Zarin and GF genotypes were classified in the first cluster including 16.6% of total genotypes. The average values of genotypes in this cluster for height at heading time, flag leaf length, flag leaf width, number of seed per spike, spike length, and flag leaf sheath distance to spike and plant height is higher than the mean of all genotypes (Table 3). Standard deviation for all traits in this cluster is less than the total standard deviation and this subject is considerable for the height at heading time and plant height.

Genotypes of the second cluster

Bezostaya, 130L, V, GH, AN, NE and EV genotypes were classified in this cluster including 19.4% of total genotypes. The average values for plant height, heading time and fertile stem number in this cluster was less than the total mean and for other traits was in the range of total mean (Table 3). Standard deviation for the number of seed per spike and heading time in this cluster was less than the total standard deviation and was higher than the total for the other traits.

Genotypes of the third cluster

Sabalan, Adl, and Karaj1 genotypes were classified in this cluster accounting for 8.3% of the total genotypes. Values of flag leaf length, flag leaf sheath to spike distance, plant height and height at heading time in this cluster were greater than the total mean (Table 3). Genotypes had tall stem in this group and the number of seed per spike was less than total mean of this trait. Standard deviation of the traits in this group was more than the total standard deviation.

Genotypes of the fourth cluster

AG, C, Bistone, AA, Shahriar, Deihim and VO genotypes belonged into this cluster. In this group, mean of number of seed per spike was more than the total average and for other traits were approximately less than or equal to the total average (Table 3). Standard deviation in this group was less than the total average for all traits except the number of fertile tillers indicating that these genotypes are less subjected to variations.

Genotypes of the fifth cluster

Karaj3, GFG, Navid, Shahpasand and Omid genotypes were classified in this cluster. The average of flag leaf sheath distance to spike and plant height were less than the total mean in this group. There was a significant positive difference for plant height at heading time with the overall mean height (Table 3). Standard deviations of traits in this group except plant height and height at heading time were higher than the total standard deviation.

Genotypes of the sixth cluster

The only genotype was Sardari. This genotype showed significant differences in terms of number of seed per spike, relative to the total average. Accordingly, it can be expressed that because this genotype has high number of fertile tillers thus number of seeds in spike was reduced (Table 3).

Table 2. Analysis of variance, coefficient of variation and H_b^2 for the studied traits in wheat genotypes

		Mean of Square											
S.O.V	DF	Flag leaf length	Flag leaf width	No. seed per spike	Spike length	Flag leaf sheath distance to spike	Plant height	No. fertile stem	No. total stem	Tillering time	Emergence time	Heading time	Height at heading time
Replication	2	0.3^{ns}	2.37^{ns}	1.33^{ns}	2.19^{ns}	1.29^{ns}	1.84^{ns}	6.08^{**}	2.87^{ns}	0.81^{ns}	35.5^{**}	0.32^{ns}	4.40^{*}
Genotypes	35	1.60^{*}	10.50^{**}	3.60^{**}	7.20^{**}	8.50^{**}	5.01^{**}	2.10^{**}	2.20^{**}	2.40^{**}	1.4^{ns}	0.72^{**}	5.70^{**}
Error	70	3.3	0.008	27.50	0.40	4.20	30.90	0.05	0.29	4.43	5.19	16.80	24.60
CV%		10.5	6.2	14	7	29	7	19	27	2	10	2	9
H_b^2		0.38	0.91	0.72	0.86	0.88	0.80	0.53	0.56	0.51	0.28	0.39	0.83

*, **and ^{ns} significant at P<0.05, P≤0.01 and non significant, respectively

Table 3. The average of traits for each cluster (above number) and the difference between each cluster with the total mean (below number)

Clusters	Height at heading time	Heading time	Emergence time	Tillering time	No. total stem	No. fertile stem	Plant height	Flag leaf sheath distance to spike	Spike Length	No. seed per spike	Flag leaf width	Flag leaf length
1	57.66	166.00	21.50	106.11	1.60	1.05	80.62	7.06	10.10	41.60	1.50	17.14
	0.51	-1.16	-0.02	-0.43	-1.01	-0.05	4.87	0.42	0.57	3.47	0.09	0.07
2	53.24	168.22	21.86	107.48	2.30	1.10	75.84	9.47	9.67	39.44	1.48	18.57
	-3.94	1.45	0.33	0.35	0.27	-0.02	0.10	2.83	0.20	1.31	0.01	1.30
3	63.11	166.33	19.67	105.89	1.98	1.07	85.14	10.22	9.19	30.53	1.28	18.10
	5.96	-0.86	-1.86	-1.74	-0.07	-0.04	9.39	3.58	-0.27	-7.59	-0.17	0.84
4	55.86	166.43	21.00	105.76	2.46	1.23	75.14	6.65	8.97	39.25	1.37	16.50
	-1.29	-0.54	-0.53	-1.37	0.42	0.12	-0.61	0.01	-0.50	1.13	-0.09	-1.12
5	66.27	169.13	20.60	107.13	1.95	1.04	73.67	0.68	10.20	35.06	1.57	17.66
	9.12	1.97	-0.93	0.00	-0.09	-0.07	-2.73	-5.96	0.73	-0.06	0.13	0.39
6	60.33	165.67	21.00	107.68	2.93	1.87	91.60	9.80	9.57	23.40	0.74	19.59
	3.19	-1.50	-0.53	0.54	0.90	0.76	15.85	3.16	0.10	-14.73	-0.71	2.32
7	52.38	166.62	23.29	109.48	1.67	1.02	67.27	5.71	8.86	40.25	1.53	16.05
	-4.77	-0.55	1.76	2.35	-0.36	-0.09	-8.47	-0.93	-0.61	2.13	0.08	-1.21

Table 4. PCA analysis for studied traits in the 36 winter wheat genotypes

PCA	Height at heading time	Heading time	Emergence time	Tillering time	No. total stem	No. fertile stem	Plant height	Flag leaf sheath distance to spike	Spike length	No. seed per spike	Flag leaf width	Flag leaf length
PC1	0.61	0.04	-0.09	-0.05	0.002	0.003	0.64	0.006	0.01	-0.45	0.007	0.03
PC2	0.53	0.16	-0.02	0.09	-0.02	-0.005	-0.61	-0.53	-0.01	-0.19	0.008	-0.06
PC3	0.4	-0.004	0.02	-0.11	-0.03	-0.008	0.22	-0.18	0.14	0.85	0.02	-0.05
PC4	0.02	-0.84	0.24	0.07	-0.06	-0.01	-0.03	-0.13	-0.11	-0.04	-.003	-0.44
PC5	-0.26	-0.016	-0.41	-0.70	0.80	0.03	0.06	0.46	0.12	-0.09	-0.02	0.06

Genotypes of the seventh cluster

Gaspard, UZ, TA, PN, MV17 FA and SP genotypes were classified into this cluster. The average number of seed per spike, tillering time and emergence time was higher than the total mean while plant height in this group was much less compared to the total average (Table 3). In this group traits that are positively correlated with yield is higher and traits that have negative correlation with yield are lower than the total mean.
The highest genetic distance (11.2) was calculated between Sardari and SP genotypes and the lowest genetic distance (1.7) was calculated between Karaj2 and Alvand genotypes (Fig. 1). Results of mean comparison indicated that the meaningful genetic difference was between Sardari and SP genotypes. Hence, the most genes expressed for the traits appearance at the highest or lowest level are located in Sardari genotype and in some cases in the SP genotype.

Principal component analysis and cluster analysis based on principal component

Nine components were extracted from the 12 studied traits by PCA analysis. The first five components that explained 97% of total variation were used for clustering genotypes. In fact, with this method, 12 variables were reduced to five (Table 4). Using the discriminant analysis the best incision point was determined at distance seven. By incision at distance seven, six clusters were formed (Table 5). The most effective traits in the first component were plant height, height at heading time and number of seed per spike, respectively. For the second component plant height, height at heading time and flag leaf sheath distance to spike and for the third component number of seed per spike and height at heading time had the greatest effect. Heading time and flag leaf length had effective influence on the fourth component. The fifth component mostly affected by distance from flag leaf sheath to spike and emergence time. Comparing these results with the results of Table 2 indicated that the traits with the largest impact on the components showed the highest rate of variation and hence can be used for grouping genotypes, effectively. The degree of similarity between dendrogram (obtained from cluster analysis) and dendrogram obtained from the cluster analysis based on PCA was estimated at 71.5%.

Discussion

High heritability of flag leaf width and flag leaf sheath distance to the spike indicated that these traits were under genetic control and fewer genes control these (Feng et al., 2006; Fu and Somers, 2009; Mohammadi et al., 2010). Therefore, for improving these traits breeding program without progeny test can be used. Flag leaf length and emergence time exhibited the lowest heritability. Thus these traits were mostly under environment control and for improving these traits selection based on progeny test should be done. It should be noted that heritability estimates are always unique to the population under study, the growing conditions, and the experimental design used (Bergman et al., 1998). Considering the positive correlation between flag leaf width and spike length with the number of seed per spike and also that the amount of these two traits for the first group is higher than the average of all genotypes, members of this group can be used to increase yield in the breeding program. Similar results for these traits correlation was reported in the previous studies (Shahid et al., 2002; Saleem et al., 2006; Eivazi et al., 2007). The mean of heading time (a criterion for prematurity) in sixth group was significantly lower than the total average. Therefore, these genotypes can be used for prematurity breeding programs.
Because of a consistent relationship between the number of grains per unit of land area and the spike dry mass at anthesis, the impact of semi-dwarf genes indicates an increase in the number of grains per m^2 (Youssefian et al., 1992; Miralles and Slafer, 1995; Flintham et al., 1997; Miralles et al., 1998). Hence, genotypes of seventh group can be used for breeding program with hybridization for a dwarf stem and increase in yield. Generally, the traits that are positively correlated with yield were higher than the total mean and traits that have negative correlation with yield were lower in the seventh group. Thus, members of this group are suitable for breeding programs aimed at improving the yield (Nersting et al., 2006; Saleem et al., 2006; Figliuolo et al., 2007; Hysing et al., 2008; Mantegazza et al., 2008; Aghaee et al., 2010). The highest genetic distance was observed between Sardari and SP cultivars

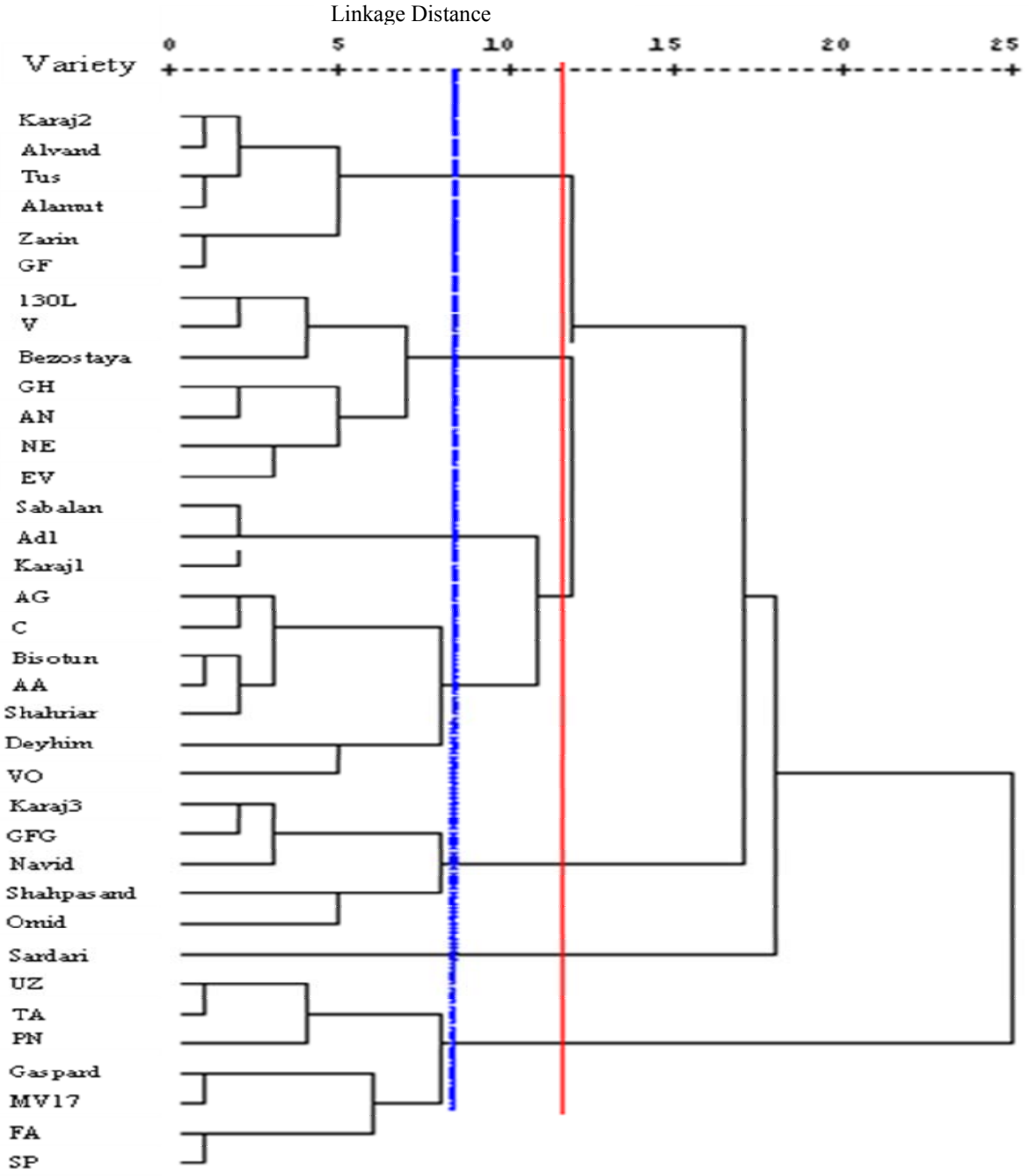

Fig 1. Tree diagram of 36 genotypes for 12 studied variables using hierarchical cluster analysis (ward's method and squared Euclidean distance)

Table 5. Grouping genotypes using cluster analysis based on principal component analysis

Clusters	Genotypes
1	TOSE, SHAHRIAR, NE, 130L, AG, C, V, AA
2	GH, GF, KARAJ2, ALVAND, BISTONE, ZARIN, ALMOOT
3	DEIHIM, NAVID, KARJ3, GFG, MV17, GASPARD
4	UZ, TA, EV, FA, SP, PN, VO
5	SHAHPASAND, OMID
6	SABALAN, ADL, BEZOSTAYA, KARAJ1, SARDARI

According to Rahim et al. (2010) who showed that the hybrids of genotypes with maximum distance resulted in high yield, the cross between these genotypes can be used in breeding programs to achieve maximum heterosis. Minimum distance was between Karaj2 and Alvand genotypes, which can be used for backcross breeding programs. Similar to the findings by Ali et al. (2008) who reported that cluster analysis can be useful for finding high yielding wheat genotypes and Singh and Dwivedi (2002), the results of this study showed the presence of a high genetic divergence among wheat genotypes. Considering the nine main components, the first five components explained 97% of total variations in data. PCA and cluster analysis allowed a natural grouping of the wheat genotypes. Accordingly, the use of different measurement techniques can be appropriately used for genotypes grouping (Bauer et al., 2007; Kraic et al., 2009). However, results showed that cluster analysis based on PCA is a more precise indicator of differences among wheat genotypes than cluster analysis (not based on PCA). Evaluation of genetic diversity can be useful for the selection of the most efficient genotypes. Accordingly, if such efforts result in the reduction of diversity, production of plants with higher uniformity may guarantee the production of enough food for the world increasing population. However, so far the breeding strategies have not resulted very much in the reduction of genetic (allelic) diversity (Reif et al., 2005; Fu et al., 2006; White et al., 2008).

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