Pakistan
Journal of Biological Sciences

Multivariate Analysis of Morphological Variation in Accessions of *Aegilops crassa* from Iran

[1]M. Ranjbar, [2]M.R. Naghavi, [3]A. Zali and [4]M.J. Aghaei

[1, 2, 3]Department of Agronomy and Plant Breeding, Agricultural College, Tehran University, Karaj, Iran
[4]Seed and Plant Improvement Institute, National Plant Gene Bank of Iran, Iran

Abstract: Principal component and cluster analyses were used to evaluate the pattern of morphological variation in 122 accessions of *Aegilops crassa* for 14 quantitative characters. With the principal component analysis, the first five principal components with eigenvalues more than 1 contributed 69.5% of the variability amongst accessions, whereas PC_6 to PC_{14} were less than unity. Plant height, stem diameter, spike length and number of spikelets per spike were the most important characters in the first principal component. The germplasm was grouped into five clusters using cluster analysis. Although each cluster had some specific characteristics of its own, but clusters were not clearly separated when plotted by the first two principal components. Mahalanobis distances (D^2) determined that plant height, stem diameter, spike length, number of spikelets per spike, node width, seed length, seed width and flowering date characters as the most important characters in differentiating the accessions. The morphological variation of *Aegilops crassa* accessions obtained in this study provides useful information for the future collection and makes these genetic resources more accessible to breeders.

Key words: *Aegilops crassa*, morphological diversity, principal component analysis, cluster analysis

INTRODUCTION

Aegilops is mainly distributed in West Asia and the Mediterranean region and its center of diversity follows the central part of the Fertile Crescent arc in West Asia (van Slageren, 1994; Hegde *et al.*, 2002). The genus *Aegilops* consists of approximately 21 species, classified into six sections or subgenera (Eig, 1929; van Slageren, 1994).

This genus has an important potential utilization in wheat improvement because of its resistance to different biotic and abiotic stresses and close relation with the cultivated wheat. Therefore, a better knowledge of the eco-geographical distribution of *Aegilops* species and their collection and conservation are required (Zaharieva *et al.*, 2003). Low within-species genetic variation was observed in polyploid *Aegilops* species compared with diploid *Aegilops* species (Hegde *et al.*, 2002).

Aegilops crassa BOISS. (tetraploid and hexaploid, genome symbols being DDM^cM^c and $DDD_2D_2M^cM^c$, respectively) is known to be distributed through Palestine, Syria, Iraq, Iran, Afghanistan and Turkestan (Eig, 1929). It was reported previously by Kihara *et al.* (1957) that all the strains of *Ae. crassa* from Iran and Iraq are 4x, while 6x forms grow mixed together with 4x forms in Afghanistan and that the strains from Turkestan are 6x.

Phenotypic identification based a description of the morphological and physiological traits can be provided a simple way of quantifying genetic variation and have been successfully used for genetic diversity analyses and cultivar development (Smith and Smith, 1992). There are some research regarding morphological variation in *Aegilops* species (Zaharieva *et al.*, 2003; Naghavi and Amirian, 2005; Knaggs *et al.*, 2000; Leilah and Al-Khateeb, 2005). But still there are some regions that little or no information is available for important agronomic and morphological traits of these species. Although rich germplasm of *Ae. crassa* has been reported from Iran (Eig, 1929), but little information is available about the diversity of their accessions. The objective of this study was to investigate the morphological variation in a collection of *Ae. crassa* accessions sampled from different geographical regions of Iran.

MATERIALS AND METHODS

A total of 122 accessions of *Aegilops crassa* provided by the National Plant Gene Bank of Iran (NPGBI) were used for their diversity evaluation. Each accession was grown in a single row plot of 1 m long and wide and plant to plant spacing was 5 cm in a row, with three replications in a randomized complete block design.

Corresponding Author: M. Ranjbar, Department of Agronomy and Plant Breeding, Agricultural College, Tehran University, Karaj, Iran Tel: +982612227608 Fax: +982612227607

Fourteen quantitative characters were recorded using descriptors established for *Aegilops* (IBPGR, 1981) with some modifications. Flowering date (days from sowing to appearance of 50% flowers) and maturity date (days from sowing to appearance of first maturity) were recorded by a single value for each row. Plant height (cm), stem diameter (mm), spike length (cm), spike diameter (mm), number of spikelets per spike, node length (cm), node width (mm), number of seeds per spikelet, culm length (cm), culm width (mm), seed length (cm) and seed width (mm) from 5 plants which had been randomly chosen in each row and mean of quantitative data sets were used for analysis.

The data recorded were analyzed for numerical taxonomic techniques using the procedure of principal component and cluster analyses (Hair *et al.*, 1992) with the help of computer software Statistica and Spss. As the characters were recorded on different scales, the data were standardized to a mean of zero and a variance of unity prior to principal components analyses to eliminate scale differences (Sneath and Sokal, 1973). The principal components analysis is a multivariate statistical technique for exploration and simplifying complex data sets and has been demonstrated by Everitt and Dunn (1992).

Cluster analysis was conducted on the basis average distance of k-means and the accessions in each cluster were analyzed for basic statistics. The accessions of each of the five clusters were plotted according to their first two principal component scores. The relationships among the clusters were assessed by measuring the inter-cluster distances using Mahalanobis distance (D^2).

RESULTS AND DISCUSSION

Principal component analysis was done to determine which of the characters more strongly contributed to the principal components. There are no tests to evaluate the significance of eigenvalues. Therefore, we follow the criterion established by Kaiser (1960), which adapts very well to the purpose of this analysis. This criterion is based on the selection of principal components whose eigenvalues are >1. Principal component analysis reduced the original 14 quantitative characters in experiment to 5 principal components. The first five principal components with eigenvalues >1 explained 69.5% of variation among 122 accessions (Table 1). Other Principal Components (PCS) had eigenvalues <1 and have not been interpreted. There are no clearly guidelines to determine the importance of a trait coefficient for each principal component. Johnson and Wichern (1988) regard a coefficient as significant that is greater than half divided by the square root of the standard deviation of the

Table 1: Principal components (PCS) for 14 characters in 122 accessions of *Aegilops crassa*

Characters	PC1	PC2	PC3	PC4	PC5
Flowering date	-0.02	-0.21	-0.02	0.84	-0.12
Maturity date	-0.06	0.05	-0.17	0.86	0.02
Plant height	0.69	-0.06	-0.18	-0.23	0.38
Stem diameter	0.53	0.45	0.36	-0.14	-0.01
Spike length	0.85	0.14	0.29	0.03	0.09
Spike diameter	-0.12	0.77	0.23	-0.25	0.08
No. of spikelets per spike	0.87	-0.05	0.14	0.1	-0.01
Node length	0.03	-0.07	0.55	-0.29	-0.10
Node width	0.24	0.17	0.72	-0.14	0.24
No. of seeds per spikelet	0.41	0.65	0.02	0.17	-0.09
Culm length	-0.03	0.85	0.06	-0.06	0.17
Culm width	0.09	0.07	0.75	0.09	0.14
Seed length	0.04	-0.03	0.22	-0.01	0.84
Seed width	0.18	0.41	0.01	-0.07	0.65
Eigenvalues	3.92	1.92	1.53	1.20	1.15
% of total variance	18.12	15.81	12.81	12.52	10.24
% cumulative variance	18.12	33.93	46.74	59.26	69.50

eigenvalue of the respective principal component. But we used coefficient greater than half to decide the importance of characters in the different principal components. The first PC, which is the most important component, explained 18.12% of total variation and was positively related to plant height, stem diameter, spike length and number of spikelets per spike, so PC1 is a weighted average of these four characters. PC2 accounted of 15.81% of the total variation and the characters with the greatest weight on this component were spike diameter, number of seeds per spikelet and culm length. PC3 was mainly related to node length, node width and culm width, while PC4 was related to flowering date and maturity date and PC5 was related to seed length and seed width. Leilah and Al-Khateeb (2005) also found that first three principal components contributed 74.4% of the variability for 10 quantitative traits in wheat. Their results showed that PC1 correlated moderately well with plant height, number of spikes/m^2, 100-grain weight and weight of grain/spike.

The entries in this study were grouped into five clusters based on average linkage, each cluster containing accessions that were highly similar. Cluster I consisted of 26 accessions, cluster II of 7, cluster III of 15, cluster IV of 32 and cluster V of 42 accessions. Mean value for each cluster (Table 2) revealed that accessions in cluster I characteristically had moderately late flowering and maturing and were low or medium for most characters, while accessions grouped in cluster II were tall and also the highest for stem diameter, spike length, number of spikelets per spike, node width, seed length and seed width. Accessions in cluster III and IV showed late flowering and maturity, respectively. Meanwhile accessions in cluster V were the highest for spike diameter and medium for other characters. The information on
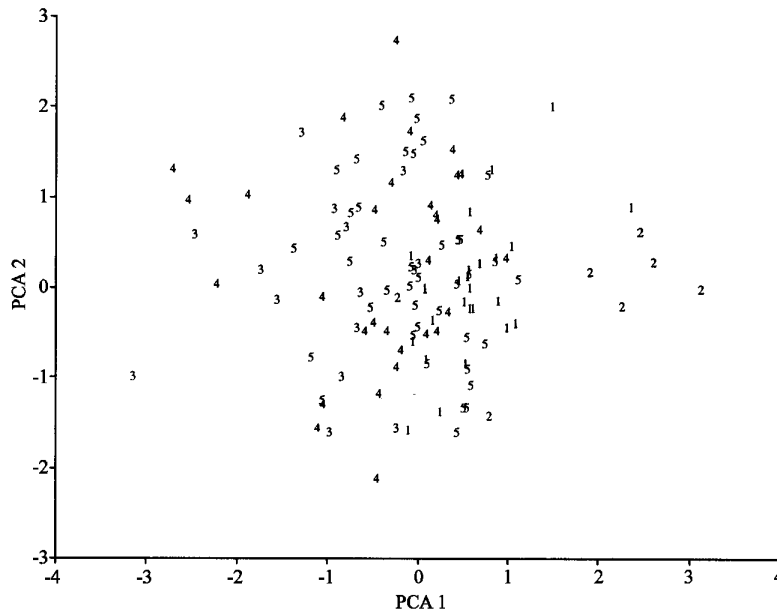
Fig. 1: Principal component analysis for 14 characters in Iranian accessions of *Aegilops crassa*. 1, 2, 3, 4 and 5 represent the cluster number

Table 2: Comparison profile of the five clusters of *Aegilops crassa* accessions classified by k-means clustering

Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
No. of accessions	26	7	15	32	42
Flowering date	116.85	113.71	117.53	116.09	114.45
Maturity date	146.50	143.86	146.27	147.06	143.93
Plant height	62.50	73.85	37.29	47.08	54.10
Stem diameter	1.77	1.86	1.64	1.74	1.75
Spike length	10.98	12.16	9.15	9.84	10.64
Spike diameter	6.15	6.08	6.17	6.26	6.43
No. of spikelets per spike	8.59	9.90	7.47	8.06	8.32
Node length	3.41	3.43	3.47	3.40	3.49
Node width	1.29	1.32	1.24	1.27	1.30
No. of seeds per spikelet	3.79	3.97	3.72	3.80	3.73
Culm length	4.96	4.95	4.80	5.04	5.03
Culm width	0.97	0.96	0.94	0.96	0.97
Seed length	0.73	0.77	0.69	0.73	0.74
Seed width	3.14	3.20	3.01	3.06	3.16

Table 3: Pairwise Mahalanobis distances (D^2) between five clusters of 122 accessions of *Aegilops crassa*

Cluster No.	1	2	3	4	5
1	0.0				
2	22.52	0.0			
3	76.8	169.48	0.0		
4	29.33	91.85	13.76	0.0	
5	10.46	48.85	39.32	9.34	0.0

clusters to which particular accessions with traits of interest belong will assist in looking extensively for more accessions with similar traits (Upadhyaya *et al.*, 2001).

The accessions of each cluster and the first two principal component scores were plotted to aid visualization of group differences (Fig. 1). Clusters were not clearly separated which may be due to mixture of accessions with different morphological traits. Moreover accessions from same or near geographical origins scattered in different parts of the plot. In contrast, Ayana and Bekele (1999) grouped accessions of sorghum according to similar agro-climatic conditions and suggested the relevance of environmental factors in ordering the structure of variation in sorghum accessions.

According to the Mahalanobis distances (D^2) among clusters (Table 3), the five clusters were statistically different from each other. The most similar clusters were, on the one hand, Clusters 4 and 5 (9.34 units) and, on the other, Clusters 1 and 5 (10.46 units). The largest inter-cluster distance (169.48 units) was between clusters 2 and 3. This result suggested that plant height, stem diameter, spike length, number of spikelets per spike, node width, seed length, seed width and flowering date are the most important characters in differentiating the accessions.

In our analysis, we were able to define groups of accessions that were significantly different from each other for characters of interest. Various multivariate methods, such as the principal components analysis and cluster analysis used here, have been shown to have utility in revealing germplasm grouping in numerous genetic resources such as blackgram (Ghaphor *et al.*, 2001), sorghum (Ayana and Bekele, 1999; Grenier *et al.*, 2001), quinoa (Ortiz *et al.*, 1998), sugarcane (Tie and Miller, 2002) cacti (Carmona and Casas, 2005) and chickpea (Ghafoor *et al.*, 2003; Naghavi and Jahansouz, 2005). In some of these studies suggested that morphological variation is strongly influenced by

environmental factors. While in some others, like our study, both effects of environmental differences and genetic factors were important.

CONCLUSIONS

An understanding of the genetic variability available in germplasm collections is a preliminary requirement for the exploitation of useful traits in plant breeding. In this research we found that there was no relationship between genetic divergence and geographical origins as accessions from one place entered into more than one cluster. Conversely, accessions from different geographical origins were relatively unique and tend to be clustered in one part of the dendrogram. This information suggests that the morphological variation analyzed is determined not only by environmental differences but also by genetic factors. The lack of regional differentiation observed by the cluster and principal component analyses could be due to gene flow between regions. Anyway this high amount of morphological variation in Iranian accessions of *Aegilops crassa* makes these genetic resources more accessible for the future collection and provides these genetic resources more available to breeders.

REFERENCES

Ayana, A. and E. Bekele, 1999. Multivariate analysis of morphological variation in sorghum (*Sorghum bicolor* (L.) Moench) germplasm from Ethiopia and Eritrea. Genet. Resour. Crop Evol., 46: 273-248.

Carmona, A. and A. Casa, 2005. Management, phenotypic patterns and domestication of *Polaskia chichipe* (Cactaceae) in the Tehuacan valley, centeral Mexico. J. Arid Environ., 60: 115-132.

Eig, A.V., 1929. Monographisch-kritische Ubersicht der Gattung *Aegilops*. Verlag des Repertoriums, Dahlem Bei Berlin.

Everitt, B.S. and G. Dunn, 1992. Applied Multivariate Data Analysis. Oxford University Press, New York, NY.

Ghafoor, A., F.N. Gulbaaz, M. Afzal and M. Arshad, 2003. Inter-relationship between SDS-Page markers and agronomic characters in chickpea (*Cicer arietinum* L.). Pak. J. Bot., 35: 613-624.

Ghaphor, A., A. Sharif, Z. Ahmad, M.A. Zahid and M.A. Rabbani, 2001. Genetic diversity in blackgram (*Vigna mungo* L. Hepper). Field Crop Res., 69: 183-190.

Grenier, C., P.J. Bramel-Cox and P. Hamon, 2001. Core collection of sorghum: I stratification based on eco-geographical data. Crop Sci., 41: 234-240.

Hair, J.F., R.E. Anderson, R.L. Tatham and W.C. Black, 1992. Multivariate Data Analysis. Macmillan Publishing Company, New York, USA.

Hegde, S.G., J. Valkoun and J.G. Waines, 2002. Genetic diversity in wild and weedy *Aegilops*, *Amblyopyrum* and *Secale* species-a preliminary survey. Crop Sci., 42: 608-614.

IBPGR, 1981. Revised Descriptors for Wheat. IBPGR, Rome, Italy.

Johnson, R.A. and D.W. Wichern, 1988. Applied Multivariate Statistical Analysis. Prentice-Hall, Englewood Cliffs, NJ.

Kaiser, H.F., 1960. The application of electronic computers to factor analysis. Educ. Psychol. Meas, 20: 141-151.

Kihara, H., K. Yamashita, M. Tanaka and S. Sakamoto, 1957. Geographical distribution of 4x and 6x forms of *Aegilops crassa*. Wheat Inform. Service, 5: 11-12.

Knaggs, P., M.J. Ambrose, S.M. Reader and T.E. Miller, 2000. Morphological characterisation and evaluation of the subdivision of *Aegilops tauschii* Coss. Wheat Inform. Service, 91: 15-19.

Leilah, A.A. and S.A. Al-Khateeb, 2005. Statistical analysis of wheat yield under drought conditions. J. Arid Environ., 61: 483-496.

Naghavi, M.R. and R. Amirian, 2005. Morphological characterization of accessions of *Aegilops tauschii*. Intl. J. Agric. Biol., 7: 392-394.

Naghavi, M.R. and M.R. Jahansouz, 2005. Variation in agronomic and morphological traits among Iranian chickpea accessions. Integ. Plant Bio., 47: 65-73.

Ortiz, R., E.N. Ruiz-Tapia and A. Mujica-Sanchez, 1998. Sampling strategy for a core collection of Peruvian quinoa germplasm. Theor. Applied Genet., 96: 475-783.

Smith, J.S.C. and O.S. Smith, 1992. Fingerprinting crop varieties. Adv. Agron., 47: 85-140.

Sneath, P.H.A. and R.R. Sokal, 1973. Numerical taxonnomy. W.H. Freeman and Co., San Francisco, USA.

Tie, P.Y.P. and J.D. Miller, 2002. Germplasm diversity among four sugarcane species for sugar composition. Crop Sci., 42: 958-964.

Upadhyaya, H.D. and R. Ortiz, 2001. A mini core subset for capturing and promoting utilization of chickpea genetic resource in crop improvement. Theor. Applied Genet., 102: 1292-1298.

Van Slageren, M.W., 1994. Wild Wheats: A monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. and Spach) *Eig* (Poaceae). Wageningen Agricultural University. Wageningen, the Netherlands, pp: 94-107.

Zaharieva, M., A. Dimov, P. Stankova, J. David and P. Monneveux, 2003. Morphological diversity and potential interest for wheat improvement of three *Aegilops* L. species from Bulgaria. Genet. Resour. Crop Evol., 50: 507-517.