http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (20): 3713-3717, 2007
ISSN 1028-8880

Evaluation of Genetic Diversity in *Aegilops tauschii* Accessions Using Morphological and AFLP Markers

Rasoul Amirian, Mohammad Reza Naghavi, Ali Akbar Shahnejat Busheri and Mansour Omidi
Department of Agronomy and Plant Breeding, Agricultural College, University of Tehran, Karaj, Iran

Abstract: Thirty two accessions of *Aegilops tauschii* were used to assess its genetic diversity by morphological and AFLP data and to evaluate relationship between morphological and AFLP markers. Thirty AFLP primer combinations led to the amplification of fragments ranging from 50 to 500 bp of which, 97 were polymorphic across the 32 accessions. Although both AFLP and morphological data classified accessions in two groups, one possessing subsp. *tauschii* accessions and the other contained all accessions of subsp. *strangulata* with some accessions from the subsp. *tauschii*. This may be explained by intermediate and hybrid forms between these two subspecies. Comparison of UPGMA dendrograms of morphological and AFLP markers using the cophenetic correlation indicated a non significant correlation (r = 0.37). Nevertheless, AFLP and selected morphological characters appear as useful and complementary techniques for evaluation of genetic diversity in subspecies of *A. tauschii*.

Key words: *Aegilops tauschii*, AFLP, cluster analysis, genetic diversity, morphological traits

INTRODUCTION

The diploid goat grass *Aegilops tauschii* Coss. [syn. *Triticum tauschii* (Coss.) Schmal., *Aegilops squarrosa* auct. Non L., 2n = 14] is the D-genome donor to bread wheat (*Triticum aestivum* L., 2n = 42, AABBDD) (Van Slageren, 1994). The level of genetic variation in D genome of bread wheat is reported to be low (Dvorak *et al*., 1998). In contrast, the level of genetic variation in *A. tauschii* is broaden particularly for resistance to biotic and abiotic stresses than the D genome of *T. aestivum* (Lubbers *et al*., 1991), therefore represents a potential source for the improvement of bread wheat. Either via direct hybridization or using appropriate synthetic hexaploid wheats, many agronomically beneficial genes have been introgressed from *A. tauschii* into common wheat (Gill and Raupp, 1987; Hussien *et al*., 1997).

Based on the morphphological traits, *A. tauschii* is divided into two subspecies named *tauschii* and *strangulata* (Van Slageren, 1994). The D genome of *T. aestivum* was shown to be genetically most closely affiliated with the subsp. *strangulata* gene pool in Transcaucasia, Armenia in particular and southwestern shore of Caspian Sea, Iran, where *T. aestivum* was likely originated (Dvorak *et al*., 1998). Accessions of subsp. *strangulata* from Armenia and Western Caspian Iran were suggested to be genetically similar to the D genome of wheat (Dvorak *et al*., 1998). Lagudah and Halloran (1988) found that subsp. *strangulata* occurring only along Southern part of Caspian Sea, Iran.

DNA-based markers have been applied successfully for the study of the *A. tauschii* gene pool (Lubbers *et al*., 1991; Dvorak *et al*., 1998; Pestsova *et al*., 2000; Monte *et al*., 2001; Katherine *et al*., 2004; Sasanuma *et al*., 2004). Also, Genetic diversity of *A. tauschii* subspecies has been evaluated using morphological data (Knaggs *et al*., 2000; Naghavi and Amirian, 2005). But it is important to determine whether different diversity estimation methods provide similar information concerning the degree of variation among subspecies. However, the goals of this study were to evaluate the genetic diversity of *A. tauschii* subspecies using morphological and AFLP markers, to assess the relationship between the morphological and AFLP loci employed in this study and consequently the genetic diversity study of genetic resources of such species including in wheat evolution may provide significant information regarding their potential for breeding purposes.

MATERIALS AND METHODS

Plant materials: Thirty two accessions of *A. tauschii* were provided by the collection of college of Agriculture, University of Tehran, Iran. Twenty accessions belong to

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

subspecies of *tauschii* and 12 accessions belong to subspecies *strangulata* were used. The accessions originated from Iran, Turkey, Azerbaijan, Tajikistan, Turkmenistan and Afghanistan and the origin of 2 accessions was unknown (Table 1).

AFLP analysis: Total genomic DNA was isolated from young leaves of greenhouse-grown plants using the modified Dellaporta method (Dellaporta *et al*., 1983). The AFLP analysis was performed as described by Vos *et al*. (1995) with minor modifications. Approximately 250 ng of the isolated genomic DNA per sample was double digested with two restriction enzymes *Eco*RI and *Mse*I and ligated with 5 pmol of *Eco*RI adapter and 50 pmol of *Mse*I adapter. The ligated DNA was preamplified using two primers containing with one selective nucleotide. Selective amplification was conducted in a total volume of 20 μL reaction mixture containing 50 ng of template DNA, 1X buffer, 200 μM of each of the four dNTPs, 1 unit Taq DNA polymerase, 2.5 mM $MgCl_2$ and 0.4 μM of each primer with 3 selective nucleotides. Thirteen primer combinations were selected for the analysis of genetic similarity (Table 2). The amplified DNA product was separated in a 6% denaturing polyacrylamide gel and detected by the silver staining method. Polymorphic amplified fragments were coded by 1 and 0 for presence or absence of band, respectively.

Table 1: *Aegilops tauschii* accessions used in this study

Geographic origin	Total accessions	No. of subsp. *tauschii*	No. of subsp. *strangulata*
Iran	17	12	5
Afghanistan	2	1	1
Azerbaijan	4	1	3
Tajikistan	2	1	1
Turkmenistan	2	1	1
Turkey	3	3	0
Unknown	2	1	1
Total	32	20	12

Table 2: Primer combinations and number of polymorphic bands

Primer combination	Total polymorph bands	Polymorphic bands in subsp. *tauschii*	Polymorphic bands in subsp. *strangulata*
E-ACG/M-CAA	6	6	4
E-ACG/M-CTA	5	5	4
E-ACG/M-CTT	10	9	9
E-ACC/M-CAA	11	11	8
E-ACC/M-ACA	7	5	7
E-ACC/M-CCT	6	6	5
E-ACC/M-CTG	7	6	6
E-ACC/M-ACG	11	11	11
E-AAC/M-CAA	5	5	5
E-AAC/M-CCA	9	9	8
E-AAG/M-CAA	6	4	5
E-AAG/M-CTG	7	6	5
E-AAG/M-CCA	7	7	5
Average	7.46	6.84	6.38

Table 3: Morphological traits and scoring pattern

Trait	Trait character	Scoring code
Spike firmness	Frail	0
	Firm	1
Stem color	Brown	0
	Yellow-brown	1
Spike color	Brown-purple	0
	Yellow-brown	1
Awn color	Brown-purple	0
	Yellow-brown	1
Stem state	Horizontal	0
	Straight	1
Spike thickness	Thin	0
	Thick	1
Seed shape	Short	0
	Tall	1
Awn state	Short	0
	Tall	1
No. of seeds in spikelet	1	0
	2	1

Table 4: Similarity of *Aegilops tauschii* accessions and its subspecies

Similarity	All accessions	Subsp. *tauschii*	Subsp. *strangulata*
Max	0.9800	0.9800	0.904
Min	0.2710	0.3020	0.500
Variance	0.0241	0.0295	0.007
Mean	0.5780	0.6090	0.693

Morphological traits: Each accession was planted in 1 m long rows with 0.5 m row spacing in experimental station of College of Agriculture, University of Tehran, Iran, during 2003-2004. Morphological data were recorded following descriptors established for *Aegilops* (IBPGR, 1981) with some modifications (Table 3).

Data analysis: For both morphology and AFLP data, genetic similarities were estimated from binary matrices (Table 4) using Dice similarity index as described by Nei and Li (1979). The cluster analysis was performed using the statistical software package, NTSYS-pc 2.02 (Rohlf, 1998) with the unweighted pair-group method of arithmetic means (UPGMA). Cophenetic correlation between similarity matrices based on morphological and AFLP data was calculated according to Mantel (1967).

RESULTS

AFLP markers: Thirteen AFLP primer combinations generated fragments ranging from 50 bp to 500 bp of which 97 fragments were polymorphic across the 32 accessions. The AFLP primer combinations E-ACC/M-CAA and E-ACC/M-CTA generated the highest (11 fragments) number of polymorphic bands and the lowest (5 fragments) were generated by E-ACG/M-CTT and E-AAG/M-CTT primer combinations.

A summary of the estimated genetic similarity for all accessions and also each subspecies is shown in Table 2.

The highest and the lowest level of genetic similarity between 32 accessions were 0.980 and 0.271, respectively, with an average of 0.578. The estimates revealed by the subsp. *tauschii* showed the lowest average value (0.609) and also the widest range of genetic similarity (from 0.980 up to 0.029) than what found by the subsp. *strangulata*. The highest range of genetic similarity revealed within unknown accessions and this was followed by Iranian accessions. On the other hand, the lowest range of genetic similarity was obtained within Afghanistan accessions.

The dendrogram obtained using UPGMA approach revealed two main clusters (Fig. 1a). The first, cluster A, included only subsp. tauschii accessions. The second, cluster B, contained all accessions of subsp. *strangulata* with six accessions from the subsp. *tauschii*.

Morphology analysis: The UPGMA dendrogram obtained using morphological characters clearly separated the accessions in two main clusters (A and B) (Fig. 1b). Cluster A, included only subsp. *tauschii* accessions, but in cluster B, both subspecies, *strangulata* and *tauschii* was found.

Comparison between AFLP and morphology: To provide an objective comparison, matrices of cophenetic values, generated from AFLP and morphological data, were compared using the Mantel test. Not significant and quite low correlation between the dendrograms was obtained ($r = 0.37$, $p = 0.97$) with the Mxcomp procedure from NTSYS program.

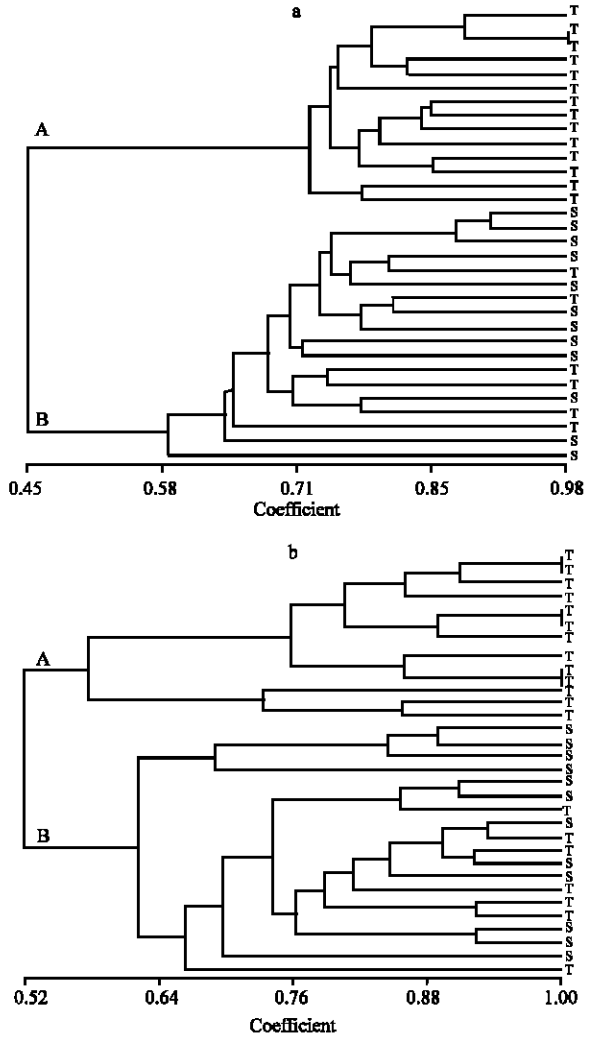

Fig. 1: Dendrogram of accessions of *Aegilops tauschii* based on AFLP markers (a) and Morphological traits (b). T: subsp. *tauschii*, S: subsp. *strangulata*

DISCUSSION

The estimation of the genetic similarities between genotypes gives useful information to address breeding program and germplasm resource management (Roldan-Ruiz *et al*., 2001). In this study, morphological data analyses of thirty two accessions of *A. tauschii* were coupled to molecular analysis (AFLP) to investigate the genetic diversity in the subspecies of *A. tauschii*. There has been an interest in differentiating between subsp. *tauschii* and subsp. *strangulata* accessions and several studies have been performed to assess the genetic diversity and differentiation of these two subspecies (Tsunewaki *et al*., 1991; Lubbers *et al*., 1991; Kim *et al*., 1992; Dvorak *et al*., 1998; Pestsova *et al*., 2000; Naghavi and Amirian, 2005).

The genetic similarity between accession pairs within the subspecies were used to evaluate the genetic diversity of different sub-species. Although the average of genetic similarity was greater in subsp. *strangulata* (0.693), the range of similarity was higher in subsp. *tauschii* (0.980-0.302). Therefore, subsp. *tauschii* has a higher level of genetic variation than sp. *strangulata*. This result is in agreement with that of Jaaska (1993) and Lagudah and Halloran (1988). In both morphological and AFLP data the maximum level of polymorphism was observed in Iranian and unknown accessions confirm a high genetic diversity found by Lelley *et al*. (2000) in this main origin area.

The UPGMA dendrogram showed two major clusters for both AFLP and morphological data which the second group, contained all accessions of subsp. *strangulata* with some accessions from the subsp. *tauschii* that may be explained by intermediate and hybrid forms between

these subspecies. Kihara *et al.* (1965) found intermediate and hybrid forms between subsp. *tauschii* and subsp. *strangulata* and this may explain the difficulty in distinguishing the two subsp. accessions. RFLP studies by Tsunewaki *et al.* (1991) and Lubbers *et al.* (1991) found close similarities between subsp. *tauschii* var. *meyeri* and subsp. *strangulata* and a recent molecular study by Dvorak *et al.* (1998) found evidence of gene migration between the different divisions in accessions from the southwest Caspian area of Iran. Pestsova *et al.* (2000) in analysis of subspecies of *Ae. tauschii* using microsatellite found two accessions that classified in different subspecies but differ only at two loci. These accessions were collected in the neighboring Caucasian countries of Armenia and Azerbaijan. Ribosomal DNA repeat unit polymorphism in *Aegilops* species also revealed a *strangulata* genotype that was similar to genotypes of *tauschii* group (Kim *et al.*, 1992).

Representative dendograms for the accessions based on AFLP markers (Fig. 1a) and morphological traits (Fig. 1b) showed that although there are apparent similarities between the groupings of particular genotypes, the overall correspondence between the similarity matrices appears to be rather low and the correlation between these two dendograms is not significant. Several studies have compared the use of phenotypic and molecular markers to examine genetic relatedness and most of these showed that relationships between two methods was low (Steiner and Garcia de los Santos, 2001; Semagn, 2002; Kjar *et al.*, 2004; Martínez *et al.*, 2005; Vollmann *et al.*, 2005). Two reasons have been mentioned by Semagn (2002) for these relationships molecular markers cover a larger proportion of the genome, including coding and noncoding regions, than the morphology and molecular markers are not subjected to artificial selection compared to morphology. For this reason, the AFLP and phenotypic data approaches will not necessarily yield closely matching results. The correlation between them could be improved if there was more morphological markers analyzed or more primer combinations of AFLP were used.

In conclusion any one of these methods could be used to study diversity and group genotypes, but none would be fully interchangeable in use. The choice of genetic diversity estimate will depend largely upon the tools available to the researcher and how they fit into the breeding scheme. There is much environmental influence accounting for the phenotypic variability observed. Therefore, when compared with DNA techniques, phenotypic traits are relatively less reliable and inefficient for precise discrimination of closely related genotypes and analysis of their genetic similarities. However, phenotypic traits, are useful for preliminary, fast, simple and inexpensive varietal identifications and can be used as a general approach for assessing genetic diversity among phenotipically distinguishable cultivars, although they are inefficient on account of the time and cost involved (Martinez *et al.*, 2005).

The utility of combining genetic (AFLP) and morphologic characteristics reveals combinations of variation among the *A. tauschii* accessions that would not be apparent with any single measurement and could provide a more complete understanding of the germplasm collections diversity.

ACKNOWLEDGMENTS

The authors would like to thank the Iran National Science Foundation (INSF) for funding this research. In addition, the authors wish to thank Dr. Zalie and Dr. V. Mohammadi for providing the *Aegilops* accessions.

REFERENCES

Dellaporta, S.L., J. Wood and J.B. Hicks, 1983. A plant DNA minipreparation: Version II. Plant. Mol. Biol. Rep., 1: 19-22.

Dvorak, J., M.C. Luo, Z.L. Yang and H.B. Zhang, 1998. The structure of the *Aegilops tauschii* genepool and the evolution of hexaploid wheat. Theor. Applied Genet., 97: 657-670.

Gill, B.S. and W.J. Raupp, 1987. Direct genetic transfers form *Aegilops squarrosa* L. to hexaploid wheat. Crop. Sci., 27: 445-450.

Hussien, T., R.L. Bowden, B.S. Gill, T.S. Cox and D.S. Marshall, 1997. Performance of four new leaf rust resistance genes transferred to common wheat from *Aegilops tauschii* and *Triticum monococcum*. Plant Dis., 81: 582-586.

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

Jaaska, V., 1993. Isoenzymes in the Evaluation of Germplasm Diversity in Wild Diploid Relatives of Cultivated Wheat. In: Biodiversity and Wheat Improvement. Damiania, A.B. (Ed.), John Wiley and Sons, Chichester, UK., pp: 247-257.

Katherine, S.C., J. Dvorak, E.S. Lagudah, E. Akhunov, M.C. Luo, P. Wolters and W. Powell, 2004. Sequence polymorphism in polyploid wheat and their d-genome diploid ancestor. Genetic, 167: 941-947.

Kihara, H., K. Yamashita and M. Tanaka, 1965. Morphological, Physiological, Genetical and Cytological Studies in *Aegilops* and *Triticum* Collected in Pakistan, Afghanistan and Iran. In: Cultivated Plants and their Relatives. Yamashita, K. (Ed.), Results of the Kyoto University Scientific Expedition to the Korakoram and Hindukush, 1955. Vol. 1. Kyoto University, Kyoto, Japan, pp: 4-41.

Kim, W.K., R.L. Innes and E.R. Kerber, 1992. Ribosomal DNA repeat unit polymorphism in six *Aegilops* species. Genome, 35: 510-514.

Kjar, A., S.A. Barfod, C.B. Asmussen and O. Seberg, 2004. Investigation of genetic and morphological variation in the sago palm (*Metroxylon sagu*; Arecaceae) in Papua New Guinea. Ann. Bot., 94: 109-117.

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

Lagudah, E.S. and G.M. Halloran, 1988. Phylogenetic relationships of *Triticum tauschii* the D genome donor to hexaploid wheat, variation in HMW subunits of glutenin and gliadins. Theor. Applied Genet., 75: 592-598.

Lelley, T., M. Stachel, H. Grausgruber and J. Vollmann, 2000. Analysis of relationships between *Aegilops tauschii* and the D genome of wheat utilizing microsatellites. Genome, 43: 661-668.

Lubbers, E.L., K.S. Gill, T.S. Cox and B.S. Gill, 1991. Variation of molecular markers among geographically diverse accessions of *Triticum tauschii*. Genome, 34: 354-361.

Mantel, M., 1967. The detection of disease clustering and a generalized regression approach. Cancer Res., 27: 209-220.

Martinez, L., P. Cavagnaro and R. Masuelli, 2005. Evaluation of diversity among Argentine grapevine (*Vitis vinifera* L.) varieties using morphological data and AFLP markers. Elect. J. Biotechnol., 6: 37-45.

Monte, J.V., P.J.G. De nova and C. Soler, 2001. AFLP-based analysis to study genetic variability and relationships in the Spanish species of the genus *Aegilops*. Hereditas, 135: 233-238.

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

Nei, M. and W.H. Li, 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA., 76: 5269-5273.

Pestsova, E., V. Korzun, N.P. Goncharov, K. Hammer, M.W. Ganal and M.S. Roder, 2000. Microsatellite analysis of *Aegilops tauschii* germplasm. Theor. Applied Genet., 101: 100-106.

Rohlf, F.J., 1998. NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System. Version 2.02. Exeter Software, New York.

Roldan-Ruiz, I., F.A. Van Eeuwijk, T.J. Gilliland, P. Dubreuil, C. Dillmann, J. Lallemand, M. De Loose and C.P. Baril, 2001. A comparative study of molecular and morphological methods of describing relationships between perennial ryegrass (*Lolium perenne* L.) varieties. Theor. Applied Genet., 103: 1138-1150.

Sasanuma, T., K. Chabane, T.R. Endo and J. Valkoun, 2004. Characterization of genetic variation in and phylogenetic relationships among diploid *Aegilops* species by AFLP: Incongruity of chloroplast and nuclear data. Theor. Applied Genet., 108: 612-618.

Semagn, K., 2002. Genetic relationships among ten endod types as revealed by a combination of morphological, RAPD and AFLP markers. Hereditas., 137: 149-156.

Steiner, J.J. and G. Garcia de los Santos, 2001. Adaptive ecology of *Lotus corniculatus* L. genotypes: I. Plant morphology and RAPD marker characterizations. Crop. Sci., 41: 552-563.

Tsunewaki, K., S. Takumi, N. Mori, T. Achiwa and Y.G. Liu, 1991. Origin of Polyploid Wheats Revealed by RFLP Analysis. In: Nuclear and Organellar Genomes of Wheat Species. Sasakuma, T. and T. Kinoshita (Eds.), Kihara Memo Found, Yokohoma, pp: 31-39.

Van Slageren, M.W., 1994. Wild Wheats: A Monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. and Spach) Eig (Poaceae). Wageningen Agricultural University, Wageningen, pp: 94-97.

Vollmann, J., H. Grausgruber, G. Stift, V. Dryzhyruk and T. Lelley, 2005. Genetic diversity in camelina germplasm as revealed by seed quality characteristics and RAPD polymorphism. Plant Breed., 124: 446-453.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, Theo van de Hee, M. Hornes. A. Frijers, J. pote, J. Peleman, M. Hornes, A. Frijers, J. Pot, J. Peleman, M. Kuiper and M. Zabeau, 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res., 23: 4407-4414.