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Chemotaxonomic Studies on *Aegilops* L. (Poaceae) In Iran

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Abstract: This study, using seed storage protein electrophoresis, evaluates the taxonomic status of the *Aegilops* L. species and the variation between the species. Therefore, with the seeds of seven species and 46 accessions of *Aegilops*, the prolamin was separated. On the base of the banding patterns, the cluster analysis among these species was studied. The results of this study show that most of the tetraploid species ($2n = 4x = 28$; $x = 7$) and the diploid species ($2n = 2x = 14$; $x = 7$) were grouped with each other. This document illustrates that a high gene flow exists among the diploid species. Further, high variability was observed among the accessions of *Aegilops* species. Morphologically; some of the species, which are very similar (*Ae. umbellulata* Zhuk., *Ae. columnaris* Zhuk.), have exactly been distinguished in this method. Chemo taxonomically, the status of these species was partially illustrated. Finally, Prolamin is an appropriate marker for identifying the taxonomic position of *Aegilops* species.

Key words: SDS-PAGE, Prolamin, *Aegilops*, chemotaxonomy, Iran

INTRODUCTION

Aegilops L. is one of the genuses of Poaceae and Triticeae having 13 species in Iran (Bor, 1968, 1970). This genus has an annual life with mainly out crossing breeding system (Waines and Barnhart, 1992). *Aegilops* has three ploidy levels including diploid ($2n = 2x = 14$), tetraploid ($2n = 4x = 28$) and hexaploid ($2n = 6x = 42$) with $x = 7$ (Bor, 1968). Due to the fact that *Aegilops* is a pastoral plant and also some of the *Aegilops* species are considered as the progenitors for polyploidy *Triticum* L. species and cause the evolution of *Triticum* genus, it is used as a source of resistance, salt and drought tolerance in wheat breeding (Baum and Apples, 1992).

The complexes occurred among the species were due to the high similarity and the hybridization between the *Aegilops* species (Lange and Jochemsen, 1992a), these evidences have obscured the morphological limits of the species and caused taxonomic confusions in the number of species in the genus *Aegilops* (Morrison, 1993a, b). High levels of variations have been observed in this genus, as well (Waines and Barnhart, 1992). Therefore the genus *Aegilops* has been of considerable interest among plant taxonomists (Keshavarzi and Rahiminejad, 2003).

Chemo taxonomically, in Triticeae several studies have been done on seed proteins (Waines and Johnson, 1971; Baum, 1977; Esen and Hilu, 1991; Morel, 1994; Chen *et al.*, 1997). Controversially, in *Aegilops* genus a few studies were done on prolamin (Masci *et al.*, 1992; Waines and Barnhart, 1992). Seed proteins are good markers for assessing taxonomic and phylogenetic relationships at various levels of species through subfamilies (Masci *et al.*, 1992; Chen *et al.*, 1997). Prolamin marker occurring in seed of cereals was applied in Tribe and Family and solved the complexity of taxonomy and grass phylogeny (Waines and Barnhart, 1992). Oda (1994) reported that friabilin is one of the seed proteins in *Aegilops*, which can be employed in detecting taxonomic position of the genus and species. Among the polyploidy species, the width of each protein bands is controlled by specific genome (Masci *et al.*, 1992). In addition, Holubec and Dvoracek (2005) emphasized the variability among *Aegilops tauschii* Coss. and *Ae. triuncialis* L., showing the relationships in different ploidy levels and their variability. Another finding is concerned with populations of *Ae. cylindrica* Host. in Hungary, where they have showed a high genetic diversity in gliadin patterns (Vorosvary *et al.*, 2000).

Due to the wide range of variation among the *Aegilops* species and insufficient information about the prolamin in the genus, it seems necessary to be evaluated taxa of the genus *Aegilops*. Regarding incomplete prolamin pattern studies among *Aegilops* species in Iran, the necessity for biosystematic investigations seems to be very important in this country. So the purposes of this study are: 1) identifying the variation of prolamin patterns among *Aegilops* species, 2) Displaying the taxonomic position via prolamin among *Aegilops* species and 3) Determining the relationships between tetraploid and diploid species in Iran.

MATERIALS AND METHODS

The *Aegilops* species were collected (seven species, 46 accessions) from natural habitats of Iran in July and August 1995 and 1996 (Fig. 1, Table 1). All the accessions were grown in the research field at Isfahan University in October 1996. The seed prolamin of each accession of *Aegilops* species was extracted by 60% isopropanol, 5% 2-mercaptoethanol and 10% Sodium Dodecyle Sulphate (SDS) (Esen and Hilu, 1991; Shewry, 1993; Morel, 1994; Wang *et al.*, 1994). Then the SDS-PAGE (12%) and Molecular Weight Marker 10 μL (11-B-064-03A) were applied. In order to evaluate the taxonomic position of *Aegilops* species, each prolamin bands of seven species were studied among the
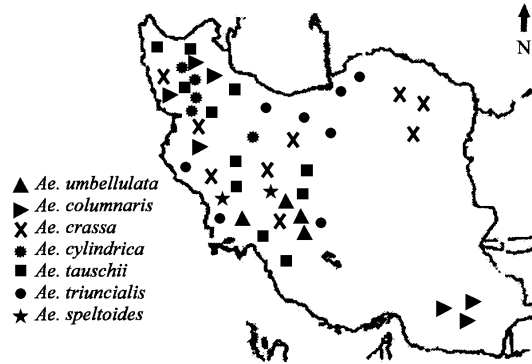

▲ *Ae. umbellulata*
▶ *Ae. columnaris*
✗ *Ae. crassa*
✳ *Ae. cylindrica*
■ *Ae. tauschii*
● *Ae. triuncialis*
★ *Ae. speltoides*

Fig. 1: Distribution of *Aegilops* species in natural habitat of Iran

Table 1: Locations of the *Aegilops* species in natural habitats of Iran

Species	Locality	Alt. (m)	Date
Ae. speltoides Tausch. (2n = 14, BB)	Chaharmahal va bakhtiyari, Cheshme Ali	2080	1997
Ae. speltoides	Lurestan, Yasouj	2050	1996,1997
Ae. speltoides	Lurestan, Yasouj	1850	1996
Ae. speltoides	Kermanshah	1720	1996
Ae. tauschii Coss. (2n = 14, DD)	Tehran, Delijan	1200	1997
Ae. tauschii	Tehran, Saveh	1280	1997
Ae. tauschii	Lurestan, Khoram abad	1820	1997
Ae. tauschii	Azerbayijan, Oroumieh, daryacheh Oroumieh	1300	1997
Ae. tauschii	West of Azerbayijan, Oroumieh, Kaboudan	1580	1997
Ae. tauschii	East of Azerbayijan, Ardabil, Moghan	1200	1997
Ae. umbellulata Zhuk. (2n = 14, UU)	Lurestan, Khoram abad	1820	1997
Ae. umbellulata	Kohkiloye va Boyer Ahmad, Yasouj	2050	1997
Ae. umbellulata	Kokiloye va Boyer Ahmad, Ysouj, Meymand	1750	1997
Ae. umbellulata	Kohkiloye va Boyer Ahmad, Dogonbadan	930	1997
Ae. umbellulata	Kohkiloyeh va Boyer Ahmad, Yasouj- Semirom	1600	1997
Ae. umnellulata	Chaharmahal va Bakhtiari, Shahrekord	2000	1997
Ae. columnaris Zhuk (2n = 28, UUMM).	West of Azerbayijan, Oroumieh	1995	1996
Ae. columnaris	East of Azerbayijan, Ardabil	1500	1997
Ae. columnaris	West of Azerbayijan, Oroumieh	1600	1997
Ae. columnaris	West of Azerbayijan, Baneh, Marivan	1640	1997
Ae. columnaris	Systan va Balouchestan, Zabol	1250	1996
Ae. crassa Boiss. (2n = 28, DDMM)	West of Azerbayijan, Mahaabaad	1650	1996
Ae. crassa	Khorasan, East of Bojnoord	1200-1800	1997
Ae. crassa	Markazi, Tafresh	2020	1997
Ae. crassa	Kohkiloye va Boyer Ahmad, Yasouj	2050	1997
Ae. crassa	Lurestan, Aligoudarz	2090	1997
Ae. crassa	Lurestan, Khoram abad	1140	1996, 1997
Ae. crassa	Chaharmahal va Bakhtiari, Lordegan	1750	1997
Ae. crassa	Azerbayijan, Khalkhal	1580	1997
Ae. crassa	West of Azerbayijan, Oroumieh	1332	1997
Ae. cylindrica Host. (2n = 28, DDCC)	West of Azerbayijan, Khoy-Marand	1200-1700	1997
Ae. cylindrica	West of Azerbayijan, Sardasht	1650	1997
Ae. cylindrica	Kermanshah	1720	1997
Ae. cylindrica	Azerbayijan, Khalkhal	1400	1997
Ae. cylindrica	West of Azerbayijan, Oshnouieh	1500	2000
Ae. cylindrica	West of Azerbayijan, Oroumieh	1400	1997
Ae. triuncialis L. (2n = 28, UUCC)	West of Azerbayijan, Sardasht	1650	1997
Ae. triuncialis	Tehran, Damavand-Gilavand	1950	1997
Ae. triuncialis	Golestan, Gonbad	1460	1996
Ae. triuncialis	Golestan, Tagh	1500	1996
Ae. triuncialis	Semnan	1935	1996
Ae. triuncialis	Tehran, Delijan	1850	1997
Ae. triuncialis	Lurestan, Boroujerd	1140	1997
Ae. triuncialis	West of Azerbayijan, Khoy-Marand	1200-1700	1997
Ae. triuncialis	15 km of Tehran	1950	1997
Ae. triuncialis	Khorasan, West of Bojnourd	1200-1800	1997

accessions, using Rf. value (the migration distance of the band/distance of solvent front) and Molecular Weight (MW) of prolamin bands. The MW of the protein band in each accession has been calculated by diagram of MW of marker (kDa)/migration distance of marker (cm) (Apaydin and Bilgener, 2000; Sharifnia and Assadi, 2003; Gulen and Eris, 2004). Four SDS-PAGE among different accessions of each species were performed (Fig. 2a, b). The statistical procedures such as Cluster analysis (between grouped linkage method and squared Euclidean Distance) and descriptive analysis were run using the SPSS V.14.

RESULTS

The electrogram of prolamin patterns showed 29, 41 and 30 bands which were observed among the accessions of *Aegilops* species (Fig. 2a-d). According to Rf. values, the highest amount of Rf.1 (run a1) is 1.4 (*Ae. cylindrica*) and 1.3 (*Ae. tauschii*), the lowest Rf.1 is 0.33 and is observed in *Ae. cylindrica* (Fig. 2a, Table 2). The highest Rf.2 (run b1) is 1.4 (*Ae. cylindrica*) (Fig. 2b, Table 2). The lowest Rf.2 is 0.52 and was observed in *Ae. speltoides* (Fig. 2b, Table 2). The highest Rf.3 (run a2)

is 1.64 (*Ae. cylindrica*), 1.58 (*Ae. tauschii*) and 1.54 (*Ae. speltoides*) (Fig. 2c, Table 2). The lowest Rf.3 is 0.64 which was found in *Ae. speltoides* (Fig. 2c, Table 2). The highest Rf.4 (run b2) is 2.22 (*Ae. triuncialis*), 1.28 (*Ae. umbellulata*), 1.25 (*Ae. cylindrica*) (Fig. 2d, Table 2). The lowest Rf.4 was observed in *Ae. cylindrica* (0.17) and *Ae. speltoides* (0.22) (Fig. 2d, Table 2).

In Fig. 2a the lowest M.W is 49 kDa and the highest M.W is 145 KDa that are related to *Ae. cylindrica*. In Fig. 2c, the highest MW is related to *Ae. triuncialis* (110 kDa). The lowest MW is related to *Ae. cylindrica* (35 kDa). In Fig. 2b, the highest MW is in *Ae. speltoides* (115 kDa) and *Ae. crassa* (114 kDa). The lowest MW is related to *Ae. crassa* (47 kDa). Most of the weight in this electrogram has Low MW. As Fig. 2d displays, the highest MW can be found in *Ae. cylindrica* (213 kDa) and *Ae. speltoides* (175 and 165 kDa). The lowest MW can be found in *Ae. triuncialis* (39 kDa) (Table 3).

The results of cluster analysis show that there were two groups comprised based on Rf. data (Fig. 3b); 1) *Ae. triuncialis, Ae. columnaris, Ae. umbellulata Ae. crassa* and *Ae. tauschii*, 2) *Ae. tauschii, Ae. speltoides* and *Ae. cylindrica*. Noticeably, two accessions of *Ae. tauschii* were separated from each other (Fig. 3b).
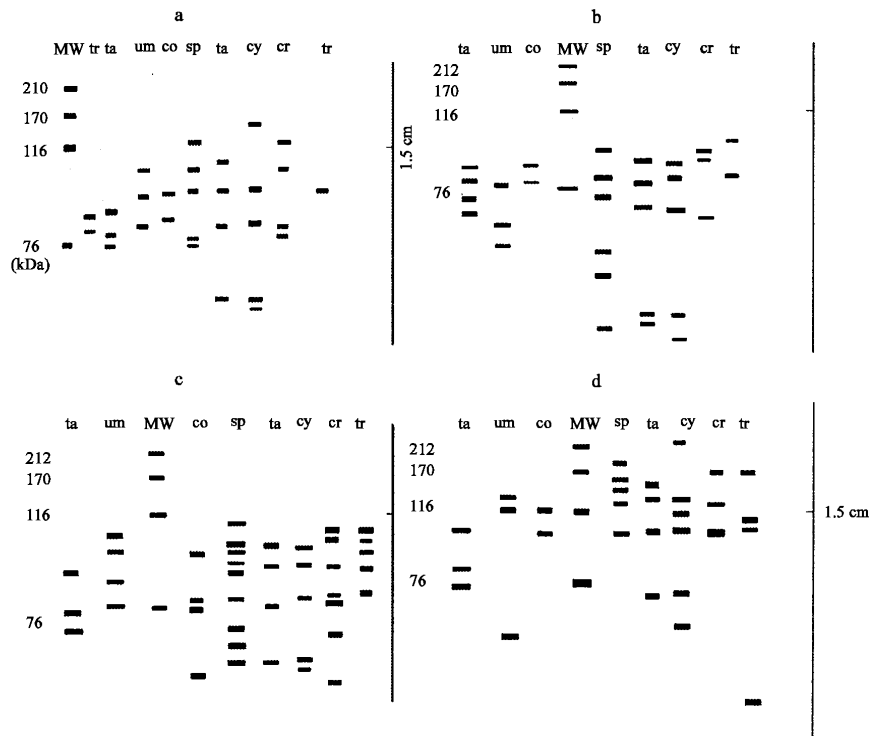

Fig. 2a-d: Diagram of prolamin bands among the accessions of *Aegilops* species. a: first run, b: second run, c: third run, d: fourth run. ta: *tauschii*, um: *umbellulata*, co: *columnaris*, sp: *speltoides*, cy: *cylindrica*, tr: *triuncialis*, cr: *crassa*

Table 2: Rf. values among the accessions of *Aegilops* species in Iran

| Run a1	Rf	Taxa	Run b1	Rf	Taxa	Run a2	Rf	Taxa	Run b2	Rf	Taxa
Band 1	0.80	*Ae. triuncialis*	Band 1	0.70	*Ae. triuncialis*	Band 1	0.96	*Ae. triuncialis*	Band 1	0.48	*Ae. triuncialis*
Band 2	0.83	*Ae. triuncialis*	Band 2	0.84	*Ae. triuncialis*	Band 2	0.80	*Ae. triuncialis*	Band 2	0.71	*Ae. triuncialis*
Band 1	0.66	*Ae. umbellulata*	Band 3	0.87	*Ae. triuncialis*	Band 1	0.98	*Ae. umbellulata*	Band 3	0.74	*Ae. triuncialis*
Band 2	0.75	*Ae. umbellulata*	Band 4	0.89	*Ae. triuncialis*	Band 2	1.16	*Ae. umbellulata*	Band 4	2.22	*Ae. triuncialis*
Band 3	0.83	*Ae. umbellulata*	Band 5	0.91	*Ae. triuncialis*	Band 3	1.22	*Ae. umbellulata*	Band 1	0.60	*Ae. umbellulata*
Band 1	0.78	*Ae. columnaris*	Band 1	0.71	*Ae. umbellulata*	Band 1	0.87	*Ae. columnaris*	Band 2	0.65	*Ae. umbellulata*
Band 2	0.90	*Ae. columnaris*	Band 2	0.71	*Ae. umbellulata*	Band 2	0.96	*Ae. columnaris*	Band 3	1.28	*Ae. umbellulata*
Band 1	0.46	*Ae. speltoides*	Band 3	0.91	*Ae. umbellulata*	Band 1	0.64	*Ae. speltoides*	Band 1	0.71	*Ae. columnaris*
Band 2	0.68	*Ae. speltoides*	Band 4	1.00	*Ae. umbellulata*	Band 2	0.93	*Ae. speltoides*	Band 2	0.82	*Ae. columnaris*
Band 3	0.75	*Ae. speltoides*	Band 1	0.87	*Ae. columnaris*	Band 3	1.04	*Ae. speltoides*	Band 1	0.22	*Ae. speltoides*
Band 4	1.00	*Ae. speltoides*	Band 2	0.96	*Ae. columnaris*	Band 4	1.29	*Ae. speltoides*	Band 2	0.34	*Ae. speltoides*
Band 5	1.10	*Ae. speltoides*	Band 3	1.00	*Ae. columnaris*	Band 5	1.38	*Ae. speltoides*	Band 3	0.54	*Ae. speltoides*
Band 1	0.68	*Ae. tauschii*	Band 4	1.38	*Ae. columnaris*	Band 6	1.54	*Ae. speltoides*	Band 4	0.65	*Ae. speltoides*
Band 2	0.75	*Ae. tauschii*	Band 1	0.52	*Ae. speltoides*	Band 1	0.82	*Ae. tauschii*	Band 5	0.85	*Ae. speltoides*
Band 3	0.93	*Ae. tauschii*	Band 2	0.77	*Ae. speltoides*	Band 2	0.95	*Ae. tauschii*	Band 1	0.77	*Ae. tauschii*
Band 4	1.30	*Ae. tauschii*	Band 3	0.78	*Ae. speltoides*	Band 3	1.00	*Ae. tauschii*	Band 2	0.91	*Ae. tauschii*
Band 1	0.33	*Ae. cylindrica*	Band 4	0.84	*Ae. speltoides*	Band 4	1.08	*Ae. tauschii*	Band 3	1.00	*Ae. tauschii*
Band 2	0.76	*Ae. cylindrica*	Band 5	0.89	*Ae. speltoides*	Band 1	0.88	*Ae. cylindrica*	Band 1	0.17	*Ae. cylindrica*
Band 3	0.93	*Ae. cylindrica*	Band 6	0.94	*Ae. speltoides*	Band 2	0.96	*Ae. cylindrica*	Band 2	0.60	*Ae. cylindrica*
Band 4	1.40	*Ae. cylindrica*	Band 7	1.10	*Ae. speltoides*	Band 3	1.14	*Ae. cylindrica*	Band 3	0.65	*Ae. cylindrica*
Band 5	1.40	*Ae. cylindrica*	Band 8	1.20	*Ae. speltoides*	Band 4	1.54	*Ae. cylindrica*	Band 4	0.77	*Ae. cylindrica*
Band 1	0.63	*Ae. crassa*	Band 9	1.35	*Ae. speltoides*	Band 5	1.64	*Ae. cylindrica*	Band 5	1.17	*Ae. cylindrica*
Band 2	0.70	*Ae. crassa*	Band 1	0.87	*Ae. tauschii*	Band 1	0.82	*Ae. crassa*	Band 6	1.25	*Ae. cylindrica*
Band 3	0.93	*Ae. crassa*	Band 2	1.01	*Ae. tauschii*	Band 2	0.85	*Ae. crassa*	Band 1	0.48	*Ae. crassa*
Band 4	0.96	*Ae. crassa*	Band 3	1.08	*Ae. tauschii*	Band 3	1.17	*Ae. crassa*	Band 2	0.54	*Ae. crassa*
Band 1	0.78	*Ae. triuncialis*	Band 1	0.78	*Ae. cylindrica*	Band 1	0.88	*Ae. tauschii*	Band 3	0.77	*Ae. crassa*
Band 1	0.80	*Ae. tauschii*	Band 2	0.85	*Ae. cylindrica*	Band 2	0.98	*Ae. tauschii*	Band 1	0.51	*Ae. tauschii*
Band 2	0.93	*Ae. tauschii*	Band 3	0.98	*Ae. cylindrica*	Band 3	1.14	*Ae. tauschii*	Band 2	0.60	*Ae. tauschii*
Band 3	1.00	*Ae. tauschii*	Band 4	1.30	*Ae. cylindrica*	Band 4	1.53	*Ae. tauschii*	Band 3	0.77	*Ae. tauschii*
			Band 5	1.40	*Ae. cylindrica*	Band 5	1.58	*Ae. tauschii*	Band 4	1.17	*Ae. tauschii*
			Band 1	0.70	*Ae. crassa*						
			Band 2	0.73	*Ae. crassa*						
			Band 3	0.87	*Ae. crassa*						
			Band 4	0.94	*Ae. crassa*						
			Band 5	0.98	*Ae. crassa*						
			Band 6	1.14	*Ae. crassa*						
			Band 7	1.36	*Ae. crassa*						
			Band 1	0.77	*Ae. tauschii*						
			Band 2	0.85	*Ae. tauschii*						
			Band 3	1.00	*Ae. tauschii*						
			Band 4	1.30	*Ae. tauschii*						
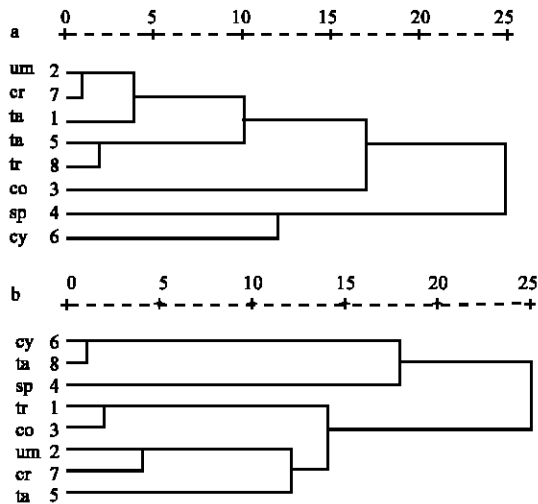
Fig. 3a-b: Dendrogram (cluster analysis) among the *Aegilops* species using MW (a) and Rf. (b) Values. ta: *tauschii,* um: *umbellulata,* co: *columnaris,* sp: *speltoides,* cy: *cylindrica,* tr: *triuncialis,* cr: *crassa*

Based on MW marker (Table 3), the results of clustering showed that two groups were comprised 1) *Ae. umbellulata, Ae. crassa, Ae. tauschii* and *Ae. tauschii, Ae. triuncialis, Ae. columnaris,* 2) *Ae. speltoides, Ae. cylindrica* (Fig. 3a).

In Table 4 and 5, the highest C.V was observed in *Ae. crassa* and *Ae. triuncialis* and the lowest was in *Ae. triuncialis.*

DISCUSSION

Based on the results of this study, among the diploid and tetraploid *Aegilops* species, there would be observed a similar origin (Fig. 2a-d). The sub-units of prolamin depict two regions based on the fast and slow movement which is observed among the accessions of *Aegilops* species. In this study, all of the electromorphic patterns of prolamin have LMW from 35-76 kDa (Table 3). Similarly, Zhang *et al.* (2006) observed that in the wild *Aegilops* species high molecular mass storage proteins' (glutenin) sub-unit possess a mobility which is slow and fast. In comparison to the other *Aegilops*

Table 3: MW values among the accessions of *Aegilops* species in Iran

Run a1	MW	Taxa	Run b1	MW	Taxa	Run a2	MW	Taxa	Run b2	MW	Taxa
Band 1	90	*Ae. triuncialis*	Band 1	98	*Ae. triuncialis*	Band 1	110	*Ae. triuncialis*	Band 1	173	*Ae. triuncialis*
Band 2	87	*Ae. triuncialis*	Band 2	87	*Ae. triuncialis*	Band 2	75	*Ae. triuncialis*	Band 2	100	*Ae. triuncialis*
Band 1	98	*Ae. umbellulata*	Band 3	85	*Ae. triuncialis*	Band 1	73	*Ae. umbellulata*	Band 3	113	*Ae. triuncialis*
Band 2	90	*Ae. umbellulata*	Band 4	84	*Ae. triuncialis*	Band 2	64	*Ae. umbellulata*	Band 4	39	*Ae. triuncialis*
Band 3	79	*Ae. umbellulata*	Band 5	76	*Ae. triuncialis*	Band 3	57	*Ae. umbellulata*	Band 1	120	*Ae. umbellulata*
Band 1	90	*Ae. columnaris*	Band 1	97	*Ae. umbellulata*	Band 1	85	*Ae. columnaris*	Band 2	116	*Ae. umbellulata*
Band 2	80	*Ae. columnaris*	Band 2	90	*Ae. umbellulata*	Band 2	75	*Ae. columnaris*	Band 3	65	*Ae. umbellulata*
Band 1	120	*Ae. speltoides*	Band 3	85	*Ae. umbellulata*	Band 1	95	*Ae. speltoides*	Band 1	117	*Ae. columnaris*
Band 2	90	*Ae. speltoides*	Band 4	77	*Ae. umbellulata*	Band 2	73	*Ae. speltoides*	Band 2	110	*Ae. columnaris*
Band 3	85	*Ae. speltoides*	Band 1	85	*Ae. columnaris*	Band 3	70	*Ae. speltoides*	Band 1	175	*Ae. speltoides*
Band 4	79	*Ae. speltoides*	Band 2	75	*Ae. columnaris*	Band 4	55	*Ae. speltoides*	Band 2	165	*Ae. speltoides*
Band 5	73	*Ae. speltoides*	Band 3	76	*Ae. columnaris*	Band 5	43	*Ae. speltoides*	Band 3	135	*Ae. speltoides*
Band 1	101	*Ae. tauschii*	Band 4	59	*Ae. columnaris*	Band 6	38	*Ae. speltoides*	Band 4	120	*Ae. speltoides*
Band 2	85	*Ae. tauschii*	Band 1	115	*Ae. speltoides*	Band 1	82	*Ae. tauschii*	Band 5	110	*Ae. speltoides*
Band 3	80	*Ae. tauschii*	Band 2	91	*Ae. speltoides*	Band 2	78	*Ae. tauschii*	Band 1	110	*Ae. tauschii*
Band 4	55	*Ae. tauschii*	Band 3	90	*Ae. speltoides*	Band 3	68	*Ae. tauschii*	Band 2	78	*Ae. tauschii*
Band 1	145	*Ae. cylindrica*	Band 4	85	*Ae. speltoides*	Band 4	67	*Ae. tauschii*	Band 3	70	*Ae. tauschii*
Band 2	87	*Ae. cylindrica*	Band 5	85	*Ae. speltoides*	Band 1	80	*Ae. cylindrica*	Band 1	213	*Ae. cylindrica*
Band 3	76	*Ae. cylindrica*	Band 6	78	*Ae. speltoides*	Band 2	75	*Ae. cylindrica*	Band 2	124	*Ae. cylindrica*
Band 4	54	*Ae. cylindrica*	Band 7	73	*Ae. speltoides*	Band 3	64	*Ae. cylindrica*	Band 3	115	*Ae. cylindrica*
Band 5	49	*Ae. cylindrica*	Band 8	63	*Ae. speltoides*	Band 4	43	*Ae. cylindrica*	Band 4	110	*Ae. cylindrica*
Band 1	120	*Ae. crassa*	Band 9	58	*Ae. speltoides*	Band 5	35	*Ae. cylindrica*	Band 5	72	*Ae. cylindrica*
Band 2	98	*Ae. crassa*	Band 1	85	*Ae. tauschii*	Band 1	95	*Ae. crassa*	Band 6	67	*Ae. cylindrica*
Band 3	77	*Ae. crassa*	Band 2	76	*Ae. tauschii*	Band 2	86	*Ae. crassa*	Band 1	173	*Ae. crassa*
Band 4	73	*Ae. crassa*	Band 3	73	*Ae. tauschii*	Band 3	62	*Ae. crassa*	Band 2	122	*Ae. crassa*
Band 1	86	*Ae. triuncialis*	Band 1	92	*Ae. cylindrica*	Band 1	83	*Ae. tauschii*	Band 3	115	*Ae. crassa*
Band 1	94	*Ae. tauschii*	Band 2	85	*Ae. cylindrica*	Band 2	74	*Ae. tauschii*	Band 1	147	*Ae. tauschii*
Band 2	79	*Ae. tauschii*	Band 3	82	*Ae. cylindrica*	Band 3	65	*Ae. tauschii*	Band 2	124	*Ae. tauschii*
Band 3	73	*Ae. tauschii*	Band 4	60	*Ae. cylindrica*	Band 4	45	*Ae. tauschii*	Band 3	110	*Ae. tauschii*
			Band 5	55	*Ae. cylindrica*	Band 5	43	*Ae. tauschii*	Band 4	70	*Ae. tauschii*
			Band 1	114	*Ae. crassa*						
			Band 2	111	*Ae. crassa*						
			Band 3	84	*Ae. crassa*						
			Band 4	79	*Ae. crassa*						
			Band 5	74	*Ae. crassa*						
			Band 6	65	*Ae. crassa*						
			Band 7	47	*Ae. crassa*						
			Band 1	92	*Ae. tauschii*						
			Band 2	85	*Ae. tauschii*						
			Band 3	80	*Ae. tauschii*						
			Band 4	58	*Ae. tauschii*						

species, *Ae. cylindrica* and *Ae. speltoides* accessions have the highest MW of prolamin which are 213-145 kDa, 175- 135 kDa, respectively (Table 3). Wan *et al.* (2002), using SDS-PAGE of total protein fractions from single seed of *Ae. cylindrica*, claimed the presence of three bands corresponding to HMW sub-units of glutenin. In accessions of *Ae. cylindrica* similar patterns of HMW sub-units with similar mobility were observed, which is in accordance with our results. In addition, the available studies have indicated that *Aegilops* is a rich source for novel variant of HMW seed protein sub-units. Lagudah and Halloran (1988) reported that all gliadin sub-units from *Ae. umbellulata* have HMW. The results of this study indicate that *Ae. umbellulata* have HMW prolamin sub-units (Table 3).

The variability among the diploid and polylploid levels showed that in estimating the CV among the accessions of *Ae. tauchii* and *Ae. triuncialis*, low CV is observed (Table 4, 5). Buren (2001) reported that the

Table 4: Descriptive analysis among the accessions of *Aegilops* species using Rf.

Species	Runa1	Runb1	Runa2	Runb2
Mean (*tauschii*)	0.71	0.76	0.91	0.63
SD*A	0.43	0.45	0.56	0.41
CV**B	60.50	59.20	61.50	65.00
Mean (*triuncialis*)	0.48	0.84	0.35	0.83
SD	0.44	0.08	0.48	0.83
CV	91.60	9.52	137.10	100.00
Mean (*crassa*)	0.46	0.96	0.40	0.25
SD	0.44	0.23	0.51	0.33
CV	95.60	23.90	127.50	132.00
Mean (*umbellulata*)	0.56	0.83	0.84	0.63
SD	0.37	0.14	0.56	0.52
CV	66.00	16.80	66.60	82.50
Mean (*columnaris*)	0.42	0.83	0.45	0.38
SD	0.48	0.58	0.52	0.44
CV	114.20	69.80	115.50	115.70
Mean (*speltoides*)	0.44	0.93	0.75	0.28
SD	0.45	0.25	0.62	0.32
CV	102.20	26.80	82.60	125.00
Mean (*cylindrica*)	0.80	0.88	1.02	0.76
SD	0.56	0.49	0.58	0.39
CV	70.00	55.60	56.80	51.30

A: Standard Deviation, B: Coefficient of Variation

Table 5: Descriptive analysis among the accessions of *Aegilops* species using MW

Species	Runa1	Runb1	Runa2	Runb2
Mean (*tauschii*)	63.00	61.00	67.20	78.70
SD*A	37.97	35.80	14.60	51.40
CV**B	60.20	58.60	21.70	65.30
Mean (*triuncialis*)	65.75	88.50	46.25	106.25
SD	43.86	6.45	55.28	54.96
CV	66.70	7.28	119.52	51.70
Mean (*crassa*)	46.00	71.62	30.37	51.25
SD	51.17	36.37	42.90	72.70
CV	111.20	50.70	141.20	141.80
Mean (*umbellulata*)	66.75	87.25	48.50	75.25
SD	45.17	8.42	32.98	56.00
CV	67.60	9.65	68.00	77.50
Mean (*columnaris*)	42.50	73.70	40.00	56.75
SD	49.24	10.81	46.36	65.59
CV	115.80	14.60	108.40	115.40
Mean (*speltoides*)	63.85	88.14	53.42	100.70
SD	46.00	13.40	30.52	72.50
CV	72.00	15.20	57.10	71.90
Mean (*cylindrica*)	68.50	62.33	49.50	116.83
SD	48.00	33.83	29.97	52.61
CV	70.00	54.27	60.50	45.00

A: Standard Deviation, B: Coefficient of Variation

alleles of seed storage proteins' (gliadin) genes are clustered in accessions of *Ae. tauschii* in Caspian region. Additionally, among the subspecies accessions of *Ae. taucchii* from Iran, these genes were of high similarity, which is due to the high gene flow present among them (Buren, 2001), thus classified together. Moreover, according to Kharazian (2007) *Ae. tauschii* (diploid species) have the lowest variation in different regions of Iran. Also, according to Keshavarzi and Rahiminejad (2003), some of the diploid species seemed to have a pure genome due to high gene flow resulting from the lower variation among the diploid species. Moreover, in some of the accessions of *Ae. triuncialis* and *Ae. crassa*, the CVs are higher than those of other species. The evidences demonstrate that some of the tetraploid species are more adapted and variable than the diploid species, confirming the evaluations of Kaminski *et al.* (1990), Salomon and Lu (1992) and Van Slageren (1994). Also Holubec and Dvoracek (2005) reported that tetraploid species have special bands which cause variability among them. Based on ploidy levels, Holubec and Dvoracek's (2005) research indicates that in tetraploid species, *Ae. triuncialis* have the lowest MW Among the tetraploid species of this study, some of the accessions of *Ae. triuncialis* and *Ae. crassa* have the lowest and highest MW In diploid species, *Ae. speltoides* has the highest MW, but *Ae. tauschii* has the lowest MW (Table 2, 3).

The cluster analysis indicated that in most of the data analyses such as Rf. values and MW, *Ae. triuncialis* and *Ae. tauschii* were grouped together (Fig. 3a-b), Yue *et al.* (2005) reported that between *Ae. tauschii* and *Ae. triuncialis* LMW-Td2 (glutenin subunit gene) is a

putative pseudo gene as it is proved to be extremely homologous due to the similarity between them, so it seems to be due to the relationships of prolamin bands observed in these two species. Wan *et al.* (2002) also reported that *Ae. tauschii* (diploid) is one of the parents of *Ae. cylindrica* (tetraploid) which have characteristic two-bands HMW-glutenin pattern, particularly, *Ae. cylindrica* has three band patterns. In this study, in the application of Rf. data, *Ae. tauschii* (DD) and *Ae. cylindrica* (DDCC) have similar genome which were grouped together (Jaaska, 1981, 1993) but in the MW are separated. These two species have HMW and LMW of prolamin (Table 2, 3). Noticeably, in this study prolamin patterns were similar among the *Ae. tauschii* (DD, $2n = 2x = 14$) and *Ae. crassa* (DDMM, $2n = 4x = 28$), in most of the cluster analyses, these two species were grouped together (Fig. 3a-b). Eig (1929), Masci *et al.* (1992), Lange and Jochemsen (1992a, b) and Van Slageren (1994) showed that *Ae. tauschii* is similar to *Ae. crassa*. In addition, some of the species can not be separate morphologically, in cluster analyses they can be grouped together but have exactly been distinguished, such as *Ae. columnaris* (UUMM, $2n = 4x = 28$) and *Ae. umbellulta* (UU, $2n = 2x = 14$) which in previous morphological studies, Eig (1929), Bor (1968, 1970) and Van Slageren (1994) considered them as similar species. Existing high genomic similarity between *Ae. columnaris* (UUMM) and *Ae. triuncialis* (UUCC, $2n = 4x = 28$), they are grouped together which is in accordance with the results of Eig (1929) and Van Slageren (1994).

The relationships among two ploidy levels such as tetraploid and diploid were observed, so *Ae. triuncialis* (UUCC, $2n = 4x = 28$), as a tetraploid species in the mainly procedure of data analyses, was grouped with *Ae. tauschii* (DD, $2n = 2x = 14$) and *Ae. umbellulata* (UU, $2n = 2x = 14$) (Fig. 3). Yue *et al.* (2005) and Holubec and Dvoracek (2005) showed that the sub-units of glutenin are extremely homologous in *Ae. tauschii* and *Ae. triuncialis*. In addition, *Ae. triuncialis* is a natural allopolyploid of *Ae. caudata* L. and *Ae. umbellulata*, but two new slow moving bands are present in this allopolyploid species, which are not observed in parents (Waines and Johnson, 1971). These bands may be caused by the act of tetraploidity. Using genome analysis and anatomical studies, Lange and Jochemsen (1992a, b) and Kharazian (2007) proved that *Ae. triuncialis* was related to *Ae. umbellulata* and some of the accessions of *Ae. tauschii* were fewer related to *Ae. speltoides* and these evidences have been observed in the results of the Cluster analysis in this study (Fig. 3a-b). Also, even the genomic formulae of *Ae. tauschii* (DD, $2n = 2x = 14$) and *Ae. crassa* (DDMM, $2n = 4x = 28$) and *Ae. triuncialis*

(UUCC, 2n = 4x = 28) and *Ae. umbellulata* (UU, 2n = 2x = 14) show the similarity between them (Fig. 3a-b) and support a close relationship between the two species (Eig, 1929; Van Slageren, 1994; Keshavarzi and Rahiminejad, 2003; Kharazian, 2007). In addition, the Cluster analysis shows that some of the diploid and tetraploid species have high gene flow, which is referred to as their 'taxonomic complexes'. Also, despite similar genomes among the tetraploid species such as *Ae. cylindrica* (DDCC) and *Ae. crassa* (DDMM, 2n = 4x = 28), these are separated. *Ae. columnaris* (UUMM, 2n = 4x = 28) and *Ae. crassa* (DDMM) have similar genome, in most of the clusters they are grouped together (Fig. 3a-b). According to Kharazian (2007), *Ae. triuncialis* (tetraploid species), *Ae. crassa* (tetraploid species) and *Ae. cylindrica* (tetraploid species) are widely distributed regarding the highest variation. These evidences show that *Aegilops* genomes have high variability, which is an excellent gene pool to plant breeding.

CONCLUSION

Finally, regarding the results of this study, *Aegilops* species are becoming increasingly important as a potential source of valuable traits that can be used in wheat (*T. aestivum*) breeding (Bultynck *et al.*, 2003). Also, prolamin patterns can be mentioned as an appropriate marker to display the chemotaxonomic position and the genomic relationships among them.

REFERENCES

Apaydin, Z. and M. Bilgener, 2000. The phenolic chemistry of *Luppula squarroza* (Retz.) Dumort., *L. barbata* (Bieb.) Gurke and *L. microcarpa* (Ledeb.) Gurke species. Turk. J. Bot., 24 (3): 169-176.

Baum, B.R., 1977. Taxonomy of the Tribe Triticeae using various numerical techniques. Can. J. Bot., 55 (13): 1712-1740.

Baum, B.R. and R. Apples, 1992. Evolutionary change at the 5s DNA loci of species in Triticeae. Plant Syst. Evol., 183 (3-4): 195-208.

Bor, N.L., 1968. Gramineae. In: Flora of Iraq, Towsend, C.C., Guest and A. Al-Rawi (Eds.). Ministry of Agriculture, Bagdad, pp: 73-87.

Bor, N.L., 1970. Gramineae. In: Flora Iranica, Rechinger, K.H. (Ed.). Graz, Austria, Wien: Akademische Druk-U. Verlagsanstalt, pp: 203-211.

Bultynck, L., F. Fiorani, E.V. Volkenburg and H. Lambers, 2003. Epidermal cell division and cell elongation in two *Aegilops* species with contrasting leaf elongation rates. Fun. Plant Biol., 30 (4): 425-432.

Buren, M., 2001. Polymorphisms in two homeologous a-gliadin and evolution of cultivated wheat. Genet. Res. Crop Evol., 48 (2): 205-220.

Chen, L., H. Fischer and U. Jensen, 1997. Accumulation of seed storage proteins and the taxonomy of Poaceae. Plant Syst. Evol., 206 (1-4): 243-257.

Eig, A., 1929. Monographisch-kritische Übersicht der Gattung *Aegilops*. Fed. Rep., 55 (52-54): 1-228.

Esen, A. and H.W. Hilu, 1991. Electrophoresis and immunological studies of prolamin in Poaceae. Taxon, 40 (1): 5-16.

Gulen, H. and A. Eris, 2004. Effect of heat stress on peroxidase activity and total protein content in Strawberry plants. Plant Sci., 166 (3): 739-744.

Holubec, V. and V. Dvoracek, 2005. Protein structure variability of *Aegilops* species. Czech. J. Genet. Plant Breed., 41 (Special Issue): 184-188.

Jaaska, V., 1981. Aspartate Aminotransferase and Alcohol Dehydrogenase isoenzymes: intraspecific differentiation in *Aegilops tauschii* and origin of the D genome polyploids in the wheat group. Plant Syst. Evol., 137 (4): 259-273.

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

Kaminski, A., R.B. Austin, M.A. Ford and C.L. Morgan, 1990. Flag leaf anatomy of *Triticum* and *Aegilops* species in relation to photosynthetic rate. Ann. Bot., 66 (3): 356-365.

Keshavarzi, M. and M.R. Rahiminejad, 2003. Anther length as a diagnostic character among Iranian *Aegilops* L. species. Iran. J. Sci. Technol., 27 (A1): 227-232.

Kharazian, N., 2007. The taxonomy and variation of leaf anatomical characters in the genus *Aegilops* L. in Iran. Turk. J. Bot., 31 (1): 1-9.

Lagudah, E.S. and G.M. Halloran, 1988. Phylogenetic relationships of *Triticum tauschii* the D genome donor hexaploid wheat. TAG., 75 (4): 592-598.

Lange, W. and G. Jochemsen, 1992a. Use gene pools of *Triticum turgidum* and *Aegilops squarossa* L. for the breeding of common wheat (*Triticum asetivum*) I. Trough chromosome doubled hybrids. Euphytica, 59 (1): 197-212.

Lange, W. and G. Jochemsen, 1992b. Use gene pools of *Triticum turgidum* and *Aegilops squarossa* L. for the breeding of common wheat (*Triticum asetivum*) II. Trough chromosome doubled hybrids. Euphytica, 59 (2-3): 213-220.

Masci, S.R.D., D. Lafiandra, O.A. Tanzarella and E. Proceddu, 1992. Electrophoresis and molecular analysis of alpha-gliadin in *Aegilops* species belonging to the genome cluster and in their putative progenitors. Plant Syst. Evol., 179: (1-2) 115-128.

Morel, M.H., 1994. Acid PAGE of wheat glutenins. Cereal Chem., 71 (3): 238-242.

Morrison, L.A., 1993a. *Triticum-Aegilops* systematics: Taking an Integrative Approach. In: Biodiversity and Wheat Improvement, Damania, A.B. (Ed.). John Wiley and Sons, New York, pp: 59-66.

Morrison, L.A., 1993b. Taxonomy of the Wheat: A Commentary. In: Biodiversity and Wheat Improvement, Damania, A.B. (Ed.). John Wiley and Sons, New York, pp: 65-70.

Oda, Sh., 1994. Two dimensional electrophoresis analysis of friabilin. Cereal Chem., 71 (4): 394-395.

Salomon, B. and B.R. Lu, 1992. Genomic groups, morphology and sectional delimitation in Eurasian *Elymus* (Poaceae). Plant Syst. Evol., 180 (1-2): 1-13.

Sharifnia, F. and M. Assadi, 2003. Seed protein analysis in relation to taxonomy of the Iranian *Linum* species. Iran. J. Bot., 10 (1): 49-54.

Shewry, P.R., 1993. Biological and Evolutionary Aspects of Cereal Seed Storage Proteins. In: Seed Storage Compounds: Biosynthesis, Interactions and Manipulation, Shewry, P.R. and A.K. Stobart (Eds.). Oxford University Press, UK., pp: 52-78.

Van Slageren, M.W., 1994. Wild Wheats. A monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. and Spach) Eig. (Poaceae). Wageningen Agriculture University.

Vorosvary, G., I. Marl, L. Hollyl and J. Kissimon, 2000. Analysis of genetic polymorphisms in jointed goatgrass (*Ae. cylindrica*) and annual wild rye (*Secale sylvestre*) populations from Hungary. Portugaliae Acta Biol., 19 (1-4): 137-147.

Waines, J.G. and B.L. Johnson, 1971. New protein bands in an amphiploid of *Aegilops caudata* and *Ae. umbellulata*. WIS., 32 (134): 22-24.

Waines, J.G. and D. Barnhart, 1992. Biosytematic research in *Aegilops* and *Triticum*. Hereditas, 116 (3): 207-212.

Wang, Ch., Z. Huaxiao, Z. Zhanming and W. Jian, 1994. Polyacrilamid gel electrophoresis of salt-solouble for *maize* variety identification and genetic purity assessment. Seed Sci. Technol., 21 (1): 51-57.

Wan, Y., D. Wang, P.R. Shewry and N.G. Halford, 2002. Isolation and characterization of five novel high molecular weight subunit genes from *Triticum timopheevi* and *Aegilops cylindrica*. TAG., 104 (5): 828-839.

Yue, Y.W., H. Long, Q. Liu, Y.M. Wei, Z.H. Yan and Y.L. Zheng, 2005. Isolation of low-molecular weight glutenin subunit genes from wild emmer wheat (*T. dicoccoides*). J. Applied Genet., 46 (4): 349-355.

Zhang, Y., Q. Li, Y. Yan, J. Zheng, X. An, Y. Xio, A. Wang, Y. Pei, H. Wang, S. Hsam and F. Zeller, 2006. Molecular characterization and phylogenetic analysis of novel glutenin gene (DY 10.1T) from *Aegilops tauschii*. Genome, 49 (7): 735-745.