Research and Full Length Article:

Investigation of Genetic Variations among Crested Wheatgrass Species Base of Agronomical Traits and Total Leaf Protein

Parvin Salehi Shanjani[A], Ali Ashraf Jafari[B], Razieh Jahanbaz[C]

[A]Assistant Professor, Research Institutes of Forests and Rangelands, Tehran, Iran
(Corresponding Author), Email: psalehi@rifr-ac.ir
[B]Professor, Research Institutes of Forests and Rangelands, Tehran, Iran
[C]Payam Noor University of Tehran, Tehran, Iran

Abstract. Genetic variations within the species of *Agropyron desertorum* and 2 varieties of *A. cristatum* subsp. *pectinatum* var. *imbricatum* and *A. cristatum* subsp. *pectinatum* var. *pectinatum* were studied using morphological traits and total protein profiles (with sodium dodecylsulphate polyacrylamide gel electrophoresis). An experiment was conducted in Research Institute of Forests and Rangelands (2012-2013) using a completely randomized block design with three replications. Means comparison of different agronomical traits among different wild populations of three taxa showed that *A. desertorum* had higher plant crown, plant height, panicle length, number of stems and plant dry matter weight; however, var. *pectinatum* had higher panicle length, grain yield and 1000-grain weight and got into the pollination and full flowering phases in a shorter period. The results of total protein profiles revealed that the genetic diversity of var. *imbricatum* samples was considerably higher than var. *pectinatum* and/or *A. desertorum*. Classification analysis of different populations of taxa using both markers showed that populations of var. *pectinatum* were closer to *A. desertorum* than var. *imbricatum*. The results demonstrated that the study of genetic diversity and the relationships among the crested wheatgrass species using agronomical traits and total protein profiles provides important information for the collection, genetic conservation and planning of future breeding programs.

Key words: *Agropyron* complex, Genetic diversity, Leaf total proteins, Morphology, Wild population

Introduction

Agropyrom is one of the most important grasses in the temperate regions of the world originated from the steps of central Asia (Asay *et al.*, 1992). This drought-resistant grass is an excellent source of forage and habitat for livestock and wildlife and it is valued for weed control, habitat use, soil stabilization and watershed management. *Agropyron* is generally adapted to the sub-humid to arid climatic conditions in steppe or desert regions (Rogler and Lorenz, 1983). *Agropyron* is one of the important perennial grasses that naturally grow in the arid to semiarid pastures and rangelands of Iran. It is used for grazing and producing hay as well as recovering the overgrazed sub-steppe rangelands (Jafari *et al.*, 2008).

The *Agropyron* Gaertn. genus was once thought to be one of the largest genera in the tribe Triticeae Dumortier comprising more than 100 species (Dewey, 1983). Nevski (1934) restricted *Agropyron* to perennial taxa with keeled glumes, a group of species referred in English as the crested wheatgrasses. Subsequent work showed that members of *Agropyron* sensu Nevski are diploids, tetraploids or hexaploids in which only the P genome is present (Dewey and Asay, 1975; Melderis, 1978; Dewey, 1983; Assadi, 1995; Jensen *et al.*, 2006). This narrow concept of *Agropyron* is now followed in most taxonomic works (e.g. Tzvelev, 1976; Melderis *et al.*, 1980; Melderis, 1985; Barkworth *et al.*, 2007). It was also supported by the inter-generic crossing experiments (Assadi and Runemark, 1995).

According to Tzvelev (1976), the genus *Agropyron* had 10 species, 9 subspecies of which were included in *Agropyron cristatum* (L.) Gaertn. and *A. desertorum*. *A. cristatum* is one of the well-known species of the "crested wheatgrasses" complex (Knowles, 1955). However, Dewey and Asay (1975) identified all Iranian crested wheatgrass collections as *A. cristatum*. Bor (1970) in

"Flora Iranica" did not cite any *A. cristatum* collections. He identified most specimens as *A. pectiniforme* Roem. & Schult. and the few remaining specimens as *A. imbricatum* (Bieb.) Roem. & Schult.

More recently, however, taxonomic realignment proposed that *A. cristatum* complex in Iran consists of 4 subspecies (subsp. *incanum*, subsp. *hamadanicum*, subsp. *puberulum* and subsp. *pectinatum*). Subsp. *pectinatum* was further divided into 3 varieties, var. *imbricatum* (= *A. imbricatum*), var. *pectinatum* (= *A. pectiniforme*), and var. *minor* (Asadi, 1995; Yousefi and Assadi, 2006).

The *A. cristatum* subsp. *pectinatum* var. *imbricatum* (with wide spikes and pilose spikekets), *A. cristatum* subsp. *pectinatum* var. *pectinatum* (with wide spikes and glabrous spikelets) and *A. desertorum* (with narrow spikes) are the important commercial crested wheatgrass species in Iran which makes them vulnerable to destruction (Asri, 2011). The destruction of natural habitats in central and southwestern Asia and the changes from traditional to modern methods of cultivation may cause genetic erosion (Hormaza *et al.*, 1998) and the loss of important germplasm. Therefore, it is important to preserve the genetic variability of natural crested wheatgrass populations.

The molecular approach for the identification of plant species or varieties seems to be more effective than traditional morphological markers because it allows the direct access to the hereditary material and makes it possible to understand the relationships between plants (Williams *et al.*, 1990; Paterson *et al.*, 1991). The introduction of biochemical and molecular techniques has been made to conduct a more accurate evaluation of genetic relationships of crested wheatgrass; allozyme gene markers (Refouf and Esnault, 2008), total protein profiles (Che and Li, 2007), Random Amplified

Polymorphic DNA (RAPD) (García *et al.*, 2002; Arghavani *et al.*, 2010) and the Amplified Fragment Length Polymorphism (AFLP) (Melish *et al.*, 2002). However, the protein profiling of germplasm and use of genetic marker have been widely and effectively used to determine the taxonomic and evolutionary aspects of several crops (Murphy *et al.,* 1990; Khan, 1990; Das and Mukarjee, 1995; Ghafoor *et al.,* 2002; Salehi Shanjani *et al.*, 2013). Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is the most economical, simple and extensively used biochemical technique for the analysis of genetic structure of germplasm. Total leaf proteins have been used as genetic markers in breeding programs (Hirano, 1982; Vries, 1996; Kamel and Hassan, 2001; Reddy and Munirajappa, 2005; Mohamed *et al.*, 2006) as well as basic studies on population genetics (Torkpo *et al.*, 2006) and reproductive biology (Cardeña *et al.,* 1998). To our knowledge, no studies have yet been made in Iran on the diversity of *Agropyron* complex germplasm based on total protein electrophoresis, and its association with agronomical traits. Therefore, in this study, we tried to I) estimate genetic variations and relationships within and between *Agropyron* Taxa; and II) analyze 11 quantitative traits in order to compare neutral and quantitative variations and thus, evaluate the role of natural selection in the maintenance of morphological integrity in wild populations.

Materials and methods
seed materials and experiment design

Seed materials of 15 wild populations of *A. cristatum* subsp. *pectinatum* var. *imbricatum* (five populations), *A. cristatum* subsp. *pectinatum* var. *pectinatum* (four populations) and *A. desertorum* (six populations) from different regions of Iran provided by the Iranian Natural Resources Gene Bank (INRGB) were used in the present study.

The research was conducted on the experimental field at the INRGB. A total of 30 seedlings of each population were grown in jiffy pots for forty days before transplanting into a field in March 2011. The field experiment was arranged in a completely randomized block with three replications. Each plot included 36 spaced plants (0.50 x 0.50 m) in a single row. Fertilizer application rates were 100 kg/h phosphorus (P) at sowing. The field was irrigated once a week during summer. No measurements were taken in the establishment year.

During the two-year investigation (2011 and 2012), nine phenotypic traits were observed in this research. The data were collected and analyzed for the following nine agronomical traits: day to flowering**,** day to pollination**,** plant height (cm), panicle length (cm), grain yield (kgh^{-1}), dry matter yield (kgh^{-1}), stem number, 1000 grain weight (g) and crown diameter (cm).

Total protein analysis

In this study, the extent of genetic variant was based on SDS-PAGE markers. Totally, 150 entries were selected from 15 wild populations (10 plants for each population). Preliminary experiments (data not shown) indicated that a larger sample (20 plants for each population) did not modify the results substantially regarding the amount or the structure of polymorphism. Total proteins were extracted from 14 day-old seedlings using protein extraction of 0.05M Tris-HCL pH=8, 0.2% SDS, 5M urea and 1% B-mercaptoethanol. Electrophoresis was carried out in the discontinuous Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) system of Laemmli (1970) using 12% (w/v) separating gel and 5% (w/v) stacking gel. The molecular weights of the dissociated protein were estimated using molecular weight standard proteins "MW-SDS-70

Kit". Gels were gently shaken until the background of gel became clear and polypeptide bands were clearly visible.

Data analysis

Analysis of variance was computed on the collected data for each trait for agronomical traits. The descriptive statistics and phenotypic correlation coefficients between traits were estimated using the SAS$_{9.1}$ software (SAS Institute Inc. 2003). Eleven classification variables had significant ($P \leq 0.01$) variations among populations and were subsequently used for multivariate analysis. The euclidean distances of populations were computed on the agronomical traits and then, they were used for the Principal Component Analysis (PCA) method using Minitab software version 14.

For protein profile data to avoid the taxonomic weighting, the intensity of bands was not taken into consideration; only the presence of bands was taken as an indicative. The scores were 1 for the presence and 0 for the absence of a band. The indices of genetic diversity such as the observed number of bands (Na), polymorphic loci percent (PPL) and expected heterozygosity (He) were calculated using POPGENE 32 software (Yeh *et al.*, 1999) on the basis of gene frequencies. At the same time, the genetic structures within and among populations were detected using the software AMOVA-PREP1.01 (Miller, 1997) and WINAMOVA (Excoffier, 1995) in order to partition the genetic variations among local and exotic groups, among the populations within groups and among the individuals within populations. The significance of each variance component was tested with permutation tests (Excoffier *et al.*, 1992). Genetic distances were estimated according to Nei (1978) and the resulting similarity matrix was subjected to Principal Coordinate Analysis (PCoA) and Neighbor-Joining (NJ) analysis using MEGA4 software

(Tamura *et al.,* 2007). Wright's Fst was used to estimate the population differentiation. The rate of gene flow (Nm) was estimated indirectly from the proportion of total diversity that was found among populations (Wright, 1931, 1951). A 999 random permutation Mantel test (Gower, 1966) was used to assess the correlation between the calculated distance matrices (using phenotypic and total protein profile data). The Pearson correlation between the genetic index within population, phenotypic traits and ecological factors was analyzed using the SPSS 11.0 software.

Results

Agronomical traits

Analysis of variance showed highly significant differences among species and populations in almost all the agronomical traits except of plant height. Low to moderate CV values were obtained for all the traits (Table 1). Means comparison of different agronomical traits among three species (Table 2) showed that *A. desertorum* had higher plant crown, plant height, panicle length, stem number and plant dry matter weight; however, var. *pectinatum* had higher panicle length, grain yield and 1000-grain weight; and got to pollination and full flowering phase in a shorter period (Table 2). The results of agronomical correlation showed a positive correlation between day to pollination and day to flowering while a negative value was obtained between day to flowering and grain yield. Dry matter yield was positively correlated with day to flowering, stem number and crown diameter. Number of stems was positively correlated with plant crown while a negative value was obtained between number of stems and 1000-grain weight (Table 3).

Genetic distance among 15 *Agropyron* entries was also estimated using data on nine agronomical traits using Euclidean distances which ranged from 1.01 (between population D-Shahrekord from *A. desertorum* and population Cpi-

Maravtappe from var. *imbricatum*) to 9.98 (between populations D-Bojnord and D-Boeenzahra both from *A. desertorum*) with an average value of 2.84 (Table 4). The agronomical data were used for conducting the Principal Component Analysis (PCA) to study further the genetic similarities among the 15 *Agropyron* populations (Fig. 1). The first three factors contributed to 76% of the total variance were observed. The first factor had high contributing factor loadings from day to flowering, day to pollination and dry matter yield contributing to 30.4% of the total variation. The second factor had high contributing loadings from grain yield, stem number and crown diameter contributing to 24.2% of the total variation. The third factor had high contributing loadings from dry matter yield and crown diameter contributing to 21.4% of total variation. The results of the PCA showed that the populations of three taxa were relatively separated from each other (Fig. 1). Moreover, the classification result of different populations of each taxa showed that populations of var. *pectinatum* were closer to *A. desertorum* than var. *imbricatum*.

Table 1. Results from the ANOVA on different studied characteristics

S.O.V.	df	MS								
		Day to Flowering	Day to Pollination	Plant Height	Panicle Length	Grain Yield	DM Yield	Stem Number	1000-Grain Weight	Crown Diameter
Species	2	198.5**	499**	23.86	3.63**	33.97**	27328**	1613.7**	0.29**	248.6**
Population	12	10.44**	18.0**	133.27	1.15**	24.29**	2108**	1674**	0.13**	158.1**
Error	30	3.2	1.61	21.89	0.1	1.99	249	64.62	0.02	16.06
CV		4.52	2.04	7.11	6.37	16.49	1.076	14.71	4.93	9.89

**, significant at 0.01 level

Table 2. Comparison of the mean values for the different traits in the wild populations of *A. cristatum* subsp. *pectinatum* var. *imbricatum* (with Cpi prefix), *A. cristatum* subsp. *pectinatum* var. *pectinatum* (with Cpp prefix) and *A. desertorum* (with D prefix)

Pop.	Day to Flowering	Day to Pollination	Plant Height	Panicle Length	Grain Yield	DM Yield	Stem Number	1000-Grain Weight	Crown Diameter
A.cristatum subsp. *pectinatum* var. *imbricatum imbricatum*									
Cpi-Mravtappe	45.53a	68.40b	51.37f	3.37g	120d-f	1230e	48.03b-d	2.43hg	33.87fg
Cpi-Esfahan1	41.43b-d	66.97b	66.27a-d	4.00f	160b-d	1977a	93.63a	2.70c-f	45.33b-d
Cpi-Esfahan2	41.57bc	67.57b	69.07a-d	5.07c-e	190ab	1867a-c	91.73a	2.63d-g	42.60b-e
Cpi-Chadgan	44.00ab	64.30c	64.37dc	4.63e	120d-f	1590dc	34.73d-f	2.40gh	31.50g
Cpi-Flaverjan	43.80ab	72.00a	73.67ab	5.23b-e	35 h	1540d	26.73f	2.80b-e	31.50g
A.cristatum subsp. *pectinatum* var. *pectinatum*									
Cpp-Gorgan1	39.57c-e	59.57d	55.20ef	5.10c-e	100fg	690f	31.53ef	2.97ab	35.30e-g
Cpp-Gorgan2	34.77f	54.77e	74.83a	5.27b-d	205a	1270e	41.60c-e	2.73b-f	48.47a-d
Cpp-Lordegan	33.97f	53.97e	62.37c-e	6.03a	170a-c	800f	58.00b	2.60d-g	36.37e-g
Cpp-Jiroft	34.23f	54.23e	67.40a-d	4.67ed	155b-e	752f	31.47ef	3.13a	35.97e-g
A. desertorum									
D-Shahrekord	39.27c-e	62.87c	62.10de	5.50a-c	75g	1247e	55.00cb	2.50f-h	35.13e-g
D-Boeenzahra	40.27c-e	62.33e	68.33a-d	5.77ab	125d-f	1817a-d	55.63bc	2.53f-h	48.80a-c
D-Fereidonshahr	38.03ed	59.33d	71.27a-c	5.07c-e	135c-f	1687b-d	45.50b-e	2.83b-d	35.73e-g
D-Asadabad	40.07c-e	63.70c	65.50b-d	5.97a	125d-f	1690b-d	102.63a	2.33h	54.70a
D-Bojnord	39.40c-e	63.10c	66.73a-d	5.07c-e	115d-f	1960a-d	50.43bc	2.90f-h	41.27a-c
D-Karj	36.83ef	59.10d	68.37a-d	4.67ed	95fg	1720a-d	53.20bc	2.57e-h	51.17ab
Cpi	43.27a	67.85a	64.95b	4.95b	125b	1641a	58.97a	2.59b	36.96b
Cpp	35.63c	55.63c	64.95b	5.27a	157a	880b	40.65b	2.86a	39.03b
A. desertorum	38.98a	61.74b	67.05a	5.34a	112b	1688a	60.40a	2.61b	44.47a

The values of columns with different letters are significant according to DMRT method (p<0.05)

Table 3. Pearson correlation analysis for the relationships between phenotypic parameters of the wild populations of *Agropyron* complex

	Day to Flowering	Day to Pollination	Plant Height	Panicle Length	Grain Yield	DM yield	Stem number	1000-grain weight
Day to pollination	**0.922****							
Plant height	-0.314	-0.095						
Panicle length	-0.45	-0.345	0.361					
Grain yield	-0.437	**-0.501***	0.165	-0.005				
Dry matter yield	0.417	**0.548***	0.442	-0.083	-0.032			
Stem number	0.096	0.249	0.025	0.118	0.372	**0.508***		
1000- grain weight	-0.42	-0.339	0.218	-0.068	0.058	-0.333	**-0.502***	
Crown Diameter	-0.275	-0.157	0.332	0.267	0.33	**0.503***	**0.626***	-0.281

*, **, significant at 0.05 and 0.01 level, respectively

Table 4. Pair-wise values for squared Euclidean distances (upper diagonal) and Nei's genetic distances (lower diagonal) of the wild populations of *A. cristatum* subsp. *pectinatum* var. *imbricatum* (with Cpi prefix), *A. cristatum* subsp. *pectinatum* var. *pectinatum* (with Cpp prefix) and *A. desertorum* (with D prefix)

| Populations	Cpi-mravtappe	Cpi-esfahan1	Cpi-esfahan2	Cpi-chadgan	Cpi-flaverjan	Cpp-gorgan1	Cpp-gorgan2	Cpp-lordegan	Cpp-jiroft	D-shahrekord	D-boeenzahra	D-fereidonshahr	D-asadabad	D-bojnord	D-karj
Cpi-mravtappe		2.789	2.663	1.235	2.197	2.579	1.529	1.544	1.734	1.015	1.986	1.552	2.359	2.646	1.999
Cpi-esfahan1	0.161		5.731	2.306	2.688	4.506	2.731	3.745	4.176	2.53	1.277	1.835	9.649	1.468	1.531
Cpi-esfahan2	0.184	0.287		2.303	2.331	4.098	2.403	3.477	3.914	2.225	1.305	1.842	9.63	1.314	1.319
Cpi-chadgan	0.177	0.260	0.055		1.895	3.387	1.737	2.573	2.607	1.569	1.224	5.916	2.274	1.904	1.482
Cpi-flaverjan	0.221	0.268	0.101	0.119		2.666	1.399	2.848	2.81	1.569	1.86	1.84	2.755	1.597	1.413
Cpp-gorgan1	0.136	0.271	0.037	0.078	0.089		2.083	1.739	1.579	2.096	3.797	3.555	4.013	3.944	3.346
Cpp-gorgan2	0.146	0.273	0.042	0.111	0.152	0.083		1.727	1.8	8.257	1.86	1.681	2.371	2.168	1.444
Cpp-lordegan	0.127	0.293	0.078	0.144	0.163	0.094	0.042		8.302	1.462	3.102	2.731	3.076	3.638	2.884
Cpp-jiroft	0.161	0.334	0.086	0.159	0.197	0.103	0.058	0.010		1.757	3.321	2.863	3.614	3.82	3.093
D-shahrekord	0.088	0.186	0.075	0.151	0.155	0.082	0.034	0.039	0.055		1.827	1.596	2.093	2.223	1.535
D-boeenzahra	0.087	0.262	0.129	0.214	0.194	0.117	0.077	0.074	0.099	0.045		7.467	1.479	9.983	6.632
D-fereidonshahr	0.093	0.112	0.171	0.158	0.184	0.135	0.136	0.136	0.170	0.101	0.131		1.874	1.501	1.078
D-asadabad	0.159	0.163	0.166	0.119	0.195	0.143	0.159	0.163	0.180	0.154	0.233	0.045		1.99	1.598
D-bojnord	0.146	0.112	0.203	0.159	0.209	0.226	0.174	0.172	0.231	0.139	0.214	0.094	0.113		8.6
D-karj	0.158	0.241	0.152	0.104	0.199	0.121	0.141	0.127	0.143	0.154	0.199	0.078	0.045	0.128	
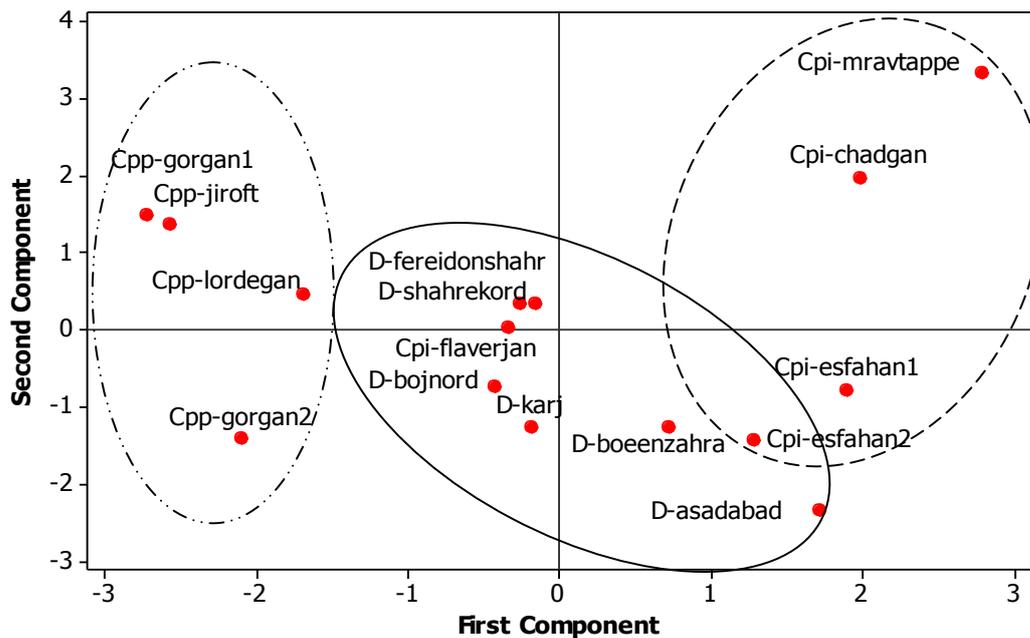
Fig. 1. Phenograms of the wild populations of *A. cristatum* subsp. *pectinatum* var. *imbricatum* (with Cpi prefix), *A. cristatum* subsp. *pectinatum* var. *pectinatum* (with Cpp prefix) and *A. desertorum* (with D prefix), based on phenotypic traits

Total protein polymorphism

On the basis of the relative mobility of total proteins on the gel, 40 polypeptide bands of different sizes ranging from 6.606 to 269.153 kDa from 15 populations of *Agropyron* were identified. The high proportion of bands was shared between three taxa (Fig. 2 and 3). The pooled values of *Na*, *PPL* and *He* were higher in the var. *imbricatum* samples than the *A. desertorum* and var. *pectinatum* samples (Table 5). The population of Cpi-Flavarjan (from var. *imbricatum*) had the highest level of variability (*Na*, *PPL* and *He* values: 38, 40% and 0.153, respectively) whereas population of Cpp-Jiroft (from var. *pectinatum*) had the lowest level of variability (*Na*, *PPL* and *He* values: 30, 2.5% and 0.012, respectively) (Table 5).

The pairwise values for Nei's genetic distances between the analyzed datasets of the *Agropyron* ranged from 0.010 (between the populations of Cpi-esfahan2 and Cpi-Flavarjan both from var.

imbricatum) to 0.334 (between the population of Cpi-esfahan1 from var. *imbricatum* and population of Cpp-Jiroft from var. *pectinatum*) with an average value given as 0.143 (Table 4).

Pairwise values for *Fst* (P≤0.01) ranged from 0.062 (between the populations of Cpp-Lordegan and Cpp-Jiroft both from var. *pectinatum*) to 0.907 (between the population of Cpi-Maravetape from var. *imbricatum* and population of Cpp-Jiroft from var. *pectinatum*) with an average value given as 0.541 (Table 6). The level of gene flow (*Nm*) between the populations of Cpp-Lordegan and Cpp-Jiroft both from var. *pectinatum* was 3.77 individuals per generation. However, *Nm* value between the population of Cpi-Maravetape from var. *imbricatum* and population of Cpp-Jiroft from var. *pectinatum* was 0.026 individuals per generation indicating that there was a low migration rate among populations (Table 6). This finding was consistent with the type of genetic

structure predicted by the expected heterozigosity analysis which suggested that 50% of the total variation was partitioned among populations. AMOVA analysis showed that the variations between the taxa, between the populations, and within the populations were accounted for 7.50 and 44% of the total variation, respectively (Table 7).

To elucidate the genetic relationships among different populations of three taxa, a PCoA biplot was produced using Nei's genetic distances. The results of the PCoA showed that the var. *imbricatum* populations were not separated from other taxa (Fig. 4). However, all the populations of var. *pectinatum* were located closely. Besides, the classification result of different populations of each taxa showed that populations of var. *pectinatum* were closer to *A. desertorum* than var. *imbricatum*. The first three principal coordinates were accounted for 62% of the total variation among the populations or taxa. Overall patterns of genetic differentiation were also examined using NJ analysis (Fig. 5). The resulting tree had long terminal branches which suggested that the populations and taxa were well differentiated. The dendrogram shows two major clusters. The first major cluster consisted of two minor clusters, one having different populations of var. *pectinatum* (three populations), var. *imbricatum* (one population) and *A. desertorum* (two populations); and the other one having three populations of var. *imbricatum* and one populations var. *pectinatum*. The second major cluster included four out of six populations of *A. desertorum*. The Mantel tests indicated that there was no significant associated relationships between genetic distance and geographic distance among the populations in *Agropyron* (*P*>0.05 for three taxa alone and all the combined populations).

Correlation coefficients among pairwise genetic and agronomical distance matrices were calculated using Mantel's test. Regression and correlation analyses between genetic and agronomical distances showed no significant correlations (*P*>0.05). Pearson correlative analysis showed that there was no significant correlation either between genetic diversity and latitude (*P*=0.94) or longitude (*P*=0.33).
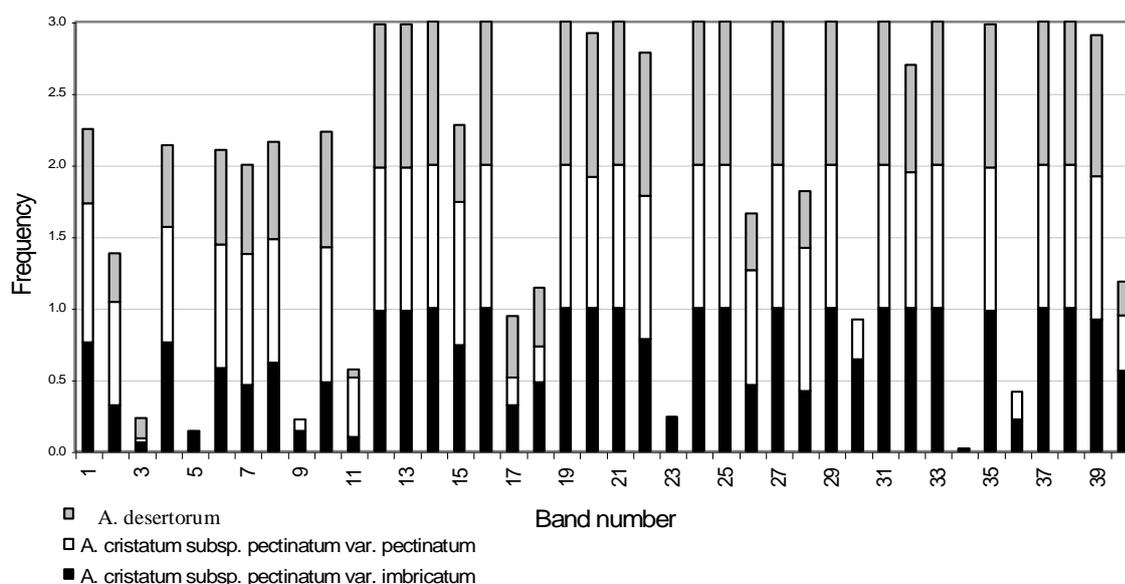

Fig. 2. Total protein band frequencies of the wild populations of *A. cristatum* subsp. *pectinatum* var. *imbricatum*, *A. cristatum* subsp. *pectinatum* var. *pectinatum* and *A. desertorum*
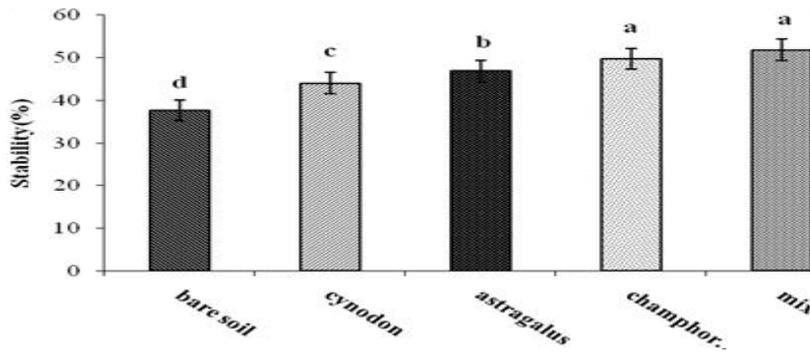
Fig. 3. Percent of stability in different situations

Table 5. Genetic diversity parameters of the wild populations of *A. cristatum* subsp. *pectinatum* var. *imbricatum* (with Cpi prefix), *A. cristatum* subsp. *pectinatum* var. *pectinatum* (with Cpp prefix) and *A. desertorum* (with D prefix).

Pop.	Na	PPL	He
A. cristatum subsp. *pectinatum* var. *imbricatum*			
Cpi-mravtappe	27	5	0.020
Cpi-esfahan1	22	10	0.034
Cpi-esfahan2	37	40	0.135
Cpi-chadgan	36	40	0.153
Cpi-flaverjan	38	40	0.152
Pooled	40	65	0.210
A. cristatum subsp. *pectinatum* var. *pectinatum*			
Cpp-gorgan1	36	23.5	0.132
Cpp-gorgan2	32	22.5	0.090
Cpp-lordegan	33	20	0.070
Cpp- jiroft	30	2.5	0.012
Pooled	37	45	0.148
A. desertorum			
D-shahrekord	32	32.5	0.133
D-boeenzahra	30	10	0.046
D-fereidonshahr	31	30	0.102
D-asadabad	28	15	0.057
D-bojnord	27	22.5	0.068
D-karj	28	7.5	0.025
Pooled	34	42	0.153

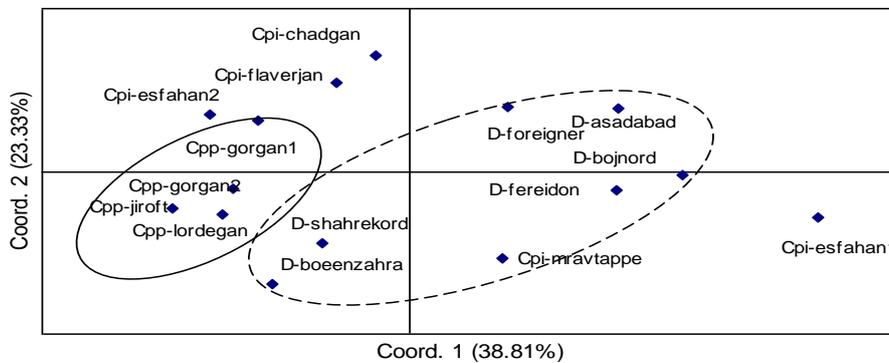Na = observed number of bands; PPL = Percentage of polymorphic loci; He = Nei's gene diversity

Fig. 4. Two-dimensional graph based on the ordination scores of the principal coordinate analysis of the wild populations of *A. cristatum* subsp. *pectinatum* var. *imbricatum* (with Cpi prefix), *A. cristatum* subsp. *pectinatum* var. *pectinatum* (with Cpp prefix) and *A. desertorum* (with D prefix) based on seed storage protein profiles

Table 6. Pair-wise values for Nm (upper diagonal) and Fst (lower diagonal) of the wild populations of *A. cristatum* subsp. *pectinatum* var. *imbricatum* (with Cpi prefix), *A. cristatum* subsp. *pectinatum* var. *pectinatum* (with Cpp prefix) and *A. desertorum* (with D prefix)

	Cpi-mravtappe	Cpi-esfahan1	Cpi-esfahan2	Cpi-chadgan	Cpi-flaverjan	Cpp-gorgan1	Cpp-gorgan2	Cpp-lordegan	Cpp-jiroft	D-shahrekord	D-boeenzahra	D-fereidonshahr	D-asadabad	D-bojnord	D-karj
Cpi-mravtappe		0.054	0.108	0.149	0.142	0.164	0.099	0.103	0.026	0.255	0.073	0.214	0.090	0.121	0.046
Cpi-esfahan1	0.821		0.087	0.122	0.137	0.103	0.071	0.061	0.028	0.146	0.041	0.218	0.113	0.197	0.052
Cpi-esfahan2	0.699	0.741		0.984	0.523	1.534	0.875	0.388	0.276	0.581	0.165	0.220	0.185	0.173	0.179
Cpi-chadgan	0.627	0.672	0.203		0.525	0.686	0.352	0.254	0.202	0.325	0.133	0.289	0.323	0.269	0.361
Cpi-flaverjan	0.638	0.646	0.323	0.323		0.666	0.288	0.256	0.195	0.356	0.170	0.278	0.223	0.230	0.218
Cpp-gorgan1	0.603	0.709	0.140	0.267	0.273		0.429	0.354	0.266	0.575	0.206	0.305	0.237	0.170	0.260
Cpp-gorgan2	0.716	0.779	0.222	0.415	0.465	0.368		0.622	0.294	1.323	0.208	0.230	0.157	0.166	0.142
Cpp-lordegan	0.709	0.803	0.392	0.496	0.494	0.414	0.287		3.773	1.016	0.198	0.219	0.143	0.158	0.142
Cpp-jiroft	0.907	0.899	0.476	0.553	0.561	0.485	0.459	0.062		0.543	0.058	0.139	0.089	0.090	0.046
D-shahrekord	0.495	0.632	0.301	0.434	0.413	0.303	0.159	0.198	0.315		0.578	0.420	0.220	0.277	0.203
D-boeenzahra	0.775	0.861	0.603	0.653	0.595	0.548	0.546	0.558	0.811	0.302		0.164	0.069	0.092	0.046
D-fereidonshahr	0.539	0.534	0.532	0.463	0.473	0.451	0.521	0.533	0.643	0.373	0.603		0.803	0.383	0.372
D-asadabad	0.736	0.689	0.574	0.436	0.529	0.513	0.615	0.636	0.738	0.531	0.783	0.237		0.253	0.491
D-bojnord	0.674	0.560	0.592	0.482	0.521	0.595	0.601	0.613	0.734	0.475	0.731	0.395	0.497		0.191
D-karj	0.845	0.828	0.582	0.409	0.535	0.490	0.638	0.638	0.844	0.552	0.845	0.402	0.337	0.567	

Table 7. Analysis of Molecular Variance (AMOVA) of *Agropyron* complex

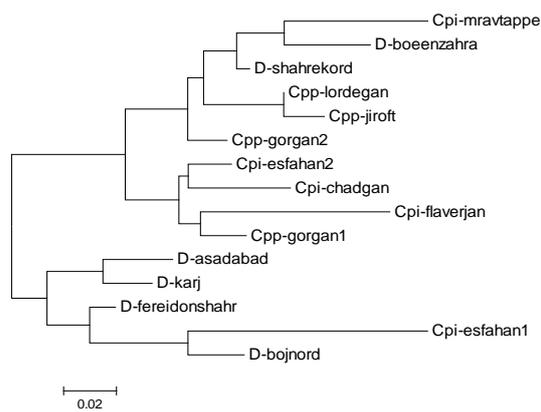| Source	df	SS	MS	%	Prob
Among species	2	68.865	34.432	7%	0.010
Among pops/species	12	254.066	21.172	50%	0.010
Within pops	127	228.267	1.797	44%	0.010
Total	141	551.197	57.402		

Fig. 5. Phenogram of the wild populations of *A. cristatum* subsp. *pectinatum* var. *imbricatum* (with Cpi prefix), *A. cristatum* subsp. *pectinatum* var. *pectinatum* (with Cpp prefix) and *A. desertorum* (with D prefix) based on seed storage protein profiles produced by the neighbor-joining clustering method

Discussion

The present survey examined 15 wild populations of *Agropyron* complex from Iran. Relatively high level of variation was observed for total agronomical traits and total protein profiles. As it was expected, the quantitative characters were more variable and the within and between taxa variations were more detectable. For almost all the characters with a quantitative genetic control, breeding for increasing or reducing a given phenotypic value would be possible. Parallel to our findings, a significant variation was observed with respect to morphological, phonological, biological and molecular properties between populations in previous studies (Mueller and Richards, 1986; Buhteeva *et al.*, 1990; Angell *et al.*, 1990; Huber-Sannwald *et al.*, 1996; Farshadfar and Farshadfar, 2004; Jafari *et al.*, 2008; Taghizadeh *et al.*, 2011; Salehi Shanjani *et al.*, 2013). The reason for this variation detected within populations may be related to a genetic structure which is probably due to the heterozygosis of cross-pollination of *Agropyron* species (Love, 1984). The cross-pollination mechanism, sexual reproduction, high seed ratio and incompatibility to produce the offspring of the *Agropyron* species could have resulted in the accumulation

of abundant genetic variations during the long evolutionary history (Asay *et al.*, 1992). This indicated that the improvement for these traits is possible through a simple selection. However, broadening the genetic base from diverse sources is recommended to include most of the genetic determinants of these traits (Laghetti *et al.,* 1998; Ghafoor *et al.*, 2002).

Protein profiles confirmed the presence of a much more pronounced and significant (P<0.01) differentiation among the populations within species (based on AMOVA, differences among species, among populations within species and within populations account for 7% 50% and 44% of the total protein profile variance, respectively). The coefficient of genetic differentiation (Fst = 0.541) of different populations of *Agropyron* in the present study was higher than that in the studies on wild *A. mongolium* populations using seed storage proteins (average Fst = 0.340) (Che and Li, 2007) and also other *Agropyron* species using RAPD (Fst = 0.47, Arghavani *et al.*, 2010; Fst = 0.37, Taghavizadeh *et al.,* 2011). In agreement with the other studies in *Agropyron* species (Che and Li, 2007; Arghavani *et al.,* 2010; Asghari *et al.,* 2011; Taghavizadeh *et al.,* 2011; Mellish *et al.,* 2002), it is suggested that different ecological conditions from which plants were obtained may have caused the observed variations. The results of this work implied that the genetic diversity of studied taxa was the result of the joint effects of one or several ecological factors, i.e., the ecological factors do not play an important role in influencing the protein profiles polymorphism of studied taxa. This study provides evidence that total protein marker polymorphisms are an informative and suitable approach to evaluate the polygenic relationships in wild population of *Agropyron* complex.

Mantel tests for the isolation by distance confirmed no correlations

between the pairwise genetic differentiation (Fst) and geographical distances among populations. This might have resulted from the combined effects including the exchange of pollen by wind, transplantation of wild individuals to cultivated populations, and using the seeds from wild progenitors to find new cultivated populations. The high gene flow may have prevented from genetic differentiation between *A. desertorum* populations. The neighbor-joining tree did not group the populations of different taxa into a discrete cluster. The high proportion of bands shared between the three taxa suggests either introgression or shared ancestral polymorphisms between these taxa.

Morphological and total protein profile data suggest that *A. cristatum* and *A. desertorum* are very recently diverged species. At the same time, the estimates of gene flow and estimates of interpopulation differentiation (Fst) indicate that gene flow is low. Thus, contemporary population structure has probably been shaped largely by the random genetic drift. This conclusion is consistent with the large number of bands shared between three taxa, and it further suggests that the measures chosen for distance tree construction are appropriate since they assume the evolution solely by the random genetic drift. Moreover, high proportion of bands shared between these taxa and the intermingling of taxa on the distance trees are consistent with the occurrence of interspecific introgression. On the other hand, these features would be expected for any two recently diverged species whether there is a current hybridization between them or not. Therefore, definitive documentation of hybridization between these species would require more thorough sampling of *Agropyron* populations, crossing experiments to establish inter-fertility and possibly the use of more rapidly evolving genetic markers.

Our results also provide a theory for further protection of germplasm resources. High levels of genetic diversity within a population and low gene flow among populations point to the possibility of the possession of unique genotypes in a single population that are not found in the other populations. It is therefore imperative for conservation planners when designing conservation strategies for wild populations of *Agropyron* in Iran to ensure that as many as possible separate populations are targeted for conservation rather than a few selected populations. *Ex situ* conservation may also be appropriate because the total genetic diversity in a population of *Agropyron* may be adequately captured in only a few plants from the wild which would not be the case for the species with high levels of genetic diversity within a population. It would be beneficial to find the ways to strengthen the gene flow between populations in order to maintain the natural genetic variation of *Agropyron* complex. Considering high genetic differentiation among the wild populations, preservation of only a few populations may not adequately protect the genetic variations within the species in Iran. Therefore, several populations throughout the entire range of the species in the country should be considered for conservation. Although *Agropyron* had not been listed as a species of conservation concern for Iran, it is an important economic forage species endemic to Iran. Therefore, the conservation and further reasonable utilization of the germplasm resources of this species is an urgent task.

Conclusion

The 15 *Agropyron* populations showed a wide range of agronomical variability. Means comparison of different agronomical traits shows that *A. desertorum* had higher plant crown, plant height, panicle length, stem number and plant dry matter weight; however, var.

pectinatum had higher panicle length, grain yield and 1000-grain weight and got to pollination and full flowering phase in a shorter period. Total variance (accumulated by three principal components ~ 80%) explains satisfactorily the variability manifested between the individuals. It is concluded that day to flowering, day to pollination and dry matter yield, and grain yield could be used as characters to distinguish the germplasm entries.

The polymorphism observed in total protein profiles among the *Agropyron* taxa in the present study demonstrated the effectiveness of this method in determining genetic variations. The study confirmed that genetic and morphological diversities work in different ways to determine the relationships among populations. To effectively exploit germplasms, we should utilize both methods in breeding work. In the present study, the lowest genetic similarity coefficients (i.e. highest genetic distances) were observed between *A. desertorum* populations D-bojnord and D-boeenzahra by phenotypic data and between populations Cpi-esfahan1 (from *A.* var. *imbricatum*) and Cpp-jiroft (var. *pectinatum*) with total protein profiles. Further studies are required to reveal whether there are other factors that cause genetic variations in *Agropyron* complex.

Acknowledgement

Literature Cited

Angell, R. F., Miller, R. F. and Haferkamp. M. R., 1990. Variability of crude protein in crested wheatgrass at defined stages of phenology. *Jour. Range Manage.* 43: 186–189.

Arghavani, A., Asghari, A., Shokrpour, M. and Chmanabad, M., 2010. Genetic diversity in ecotypes of two *Agropyron* species using RAPD markers. *Res. Jour. Environ.* Sci. 4: 50–56.

Asay, K. H., Jensen, K. B., Hsiao, C. and Dewey, D.R., 1992. Probable origin of standard crested wheatgrass, *Agropyron desertorum* Fisch. ex Link, Schultes. *Canadian Jour. Plant Sci.* 72: 763–772.

Asghari, A., Jafari, A. A., Shokrpour, M., Mohammaddoust, E. and Chamanabad, H.R., 2011. Genetic variation between and within populations of *Agropyron* Gaertn. using RAPD Markers. *Iranian Jour. Rangelands For. Plant Breed. Genetic Res.* 18: 143–153. (In Persian).

Asri, Y., 2011. Range plants of Iran, 1: monocotyledons. Publication of Research Institute of Forests and Rangelands, 573 p.

Assadi, M., 1995. Meiotic configuration and chromosome number in some Iranian species of *Elymus* L. and *Agropyron* Gaertner (Poaceae). *Bot. Jour. Linn. Society,* 117(2): 159-168.

Assadi, M. and Runemark, H., 1995. Hybridization, genomic constitution and generic delimitation in *Elymus* (Poaceae, Triticeae). *Plant Syst. Evol.* 194: 189-205.

Barkworth, M. E., Campbell, J. J. N. and Salomon, B., 2007. *Agropyron.* In: Barkworth, M.E., Capels, K.M., Long, S., Anderton, L.K. and Piep, M.B. (eds.) *Flora of North America*, Volume 24, Poaceae, part 1, pp. 277-279. New York: Oxford University Press.

Bor, N. L., 1970. Gramineae, in Rechinger, K. H. (ed.) Flora Iranica, no. 70. -Akademische Drueck-U, Verlagsanstalt, Graz, Austria.

Buhteeva, A. V., Malyshev, L. L. and Konarev, A.V., 1990. Wild-growing populations of wheat grass from Eastern European steppe province. Bull. VIR. Leningrad 198: 54–59.

Cardeña, R., Oropeza, C. and Zizumbo, D., 1998. Leaf proteins as markers useful in the genetic improvement of coconut palms. *Euphytica,* 102: 81–86.

Che, Y. H. and Li, L. H., 2007. Genetic diversity of prolamines in *Agropyron mongolicum* Keng indigenous to northern China. *Genet. Resour. Crop Evol.* 54: 1145–1151.

Das, S. and Mukharjee, K. K., 1995. Comparative study on seed proteins of *Ipomoea. Seed Sci. Tech.* 23: 501–509.

Dewey, D. R., 1983. Historical and current taxonomic perspectives of *Agropyron*, *Elymus* and related genera. *Crop Sci*, 23: 637-642.

Dewey, D. R. and Asay, K. H. 1975. The crested wheat grasses of Iran. *Crop Sci.* 15: 844-849.

Excoffier. L., 1995. AMOVA 1.55 (Analysis of Molecular Variance). University of Geneva, Switzerland, Genetics and Biometry Laboratory.

Excoffier, L., Smouse, P. and Quattro, J., 1992. Analysis of molecular Variances among DNA restriction data. *Genetics,* 131: 479–491.

Farshadfar, M. and Farshadfar, E., 2004. Genetic Variation Among Different *Agropyron* Species Based on Morphological and Chemical Indices. *Jour. Water Soil Sci. Isfahan Univ. Tech.* 8: 243–251.

Garcia, P., Monte, J. V. Casanova, C. and Soler, C., 2002. Genetic similarities among Spanisch populations of *Agropyron, Elymus* and *Thinopyrum,* using PCR-based markers. *Genet. Res. Crop Evol.* 49: 103-109.

Ghafoor, A., Ahmad, Z., Qureshi, A.S. and Bashir, M., 2002. Genetic relationship in *Vigna mungo* (L.) Hepper and *V. radiata* (L.) R. Wilczek based on morphological traits and SDS PAGE. *Euphytica,* 123: 367–378.

Gower, J. C., 1966. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika,* 53: 325–338.

Hirano, H., 1982. Varietal differences of leaf protein profiles in mulberry. *Phytochemistry,* 21: 1513–1518.

Hormaza, J. I., Pinney, K. and Polito, V. S., 1998. Genetic diversity of pistachio (*Pistacia vera,* Anacardiaceae) germplasm based on randomly amplified polymorphic DNA (RAPD) markers. *Econ. Bot.* 52:78–87.

Huber-Sannwald, E., Pyke, D. A., Caldwell, M. M., 1996. Morphological plasticity following species-specific recognition and competition between two perennial grasses. *American Jour. Bot.* 83: 919–931.

ISTA, 1993.International rules for seed testing. Seed Sci. Technol. 21: 288.

Jafari, A. A., Seydemohammadi, A. R., Abdi, N. and Areffi, H. M., 2008. Seed and hay production in 31 genotypes of desert wheatgrass (*Agropyron desertorum*) using drought tolerance indices. *Iranian Jour. Range Desert Res.* 15: 114–128. (In Persian).

Jensen, K. B., Larson, S. R., Waldron, B. L. and Asay, K.H., 2006. Cytogenetic and molecular characterization of hybrids between 6x, 4x, and 2x ploidy levels in crested wheatgrass. *Crop Sci,* 46: 105-112.

Kamel, E. A. and Hassan, L. M. A., 2001. The significance of cuticular features, petiole anatomy and SDS - PAGE in the Taxonomy of the Lauraceae. *Pakistan Jour. Biol. Sci.* 4: 1094–1100.

Khan, M. K., 1990. Production and utility of chickpea (*Cicer arietinum* L.) in Pakistan. *Progressive Farming,* 10: 28–33.

Knowles, R. P., 1955. A study of variability in crested wheatgrass. *Canad Jour. Bot.* 33: 534-546.

Laemmli, U. K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* 227: 680–685.

Laghetti, G., Pienaar, B. L., Pasdulosi, S. and Perrino, P., 1998. Ecogeographical distribution of *Vigna savi* in southern Africa and some areas of the Mediterranean basin. *Plant Genet. Resou. Newsletter,* 115: 6–12.

Love, A., 1984. Conspectus of the Triticeae. *Feddes Repertorium,* 95: 425–521.

Melderis, A., 1978. Taxonomic notes on the tribe Triticeae (Gramineae) with special reference to the genera *Elymus* L. and *Agropyron* Gaertner. *Bot. Jour. Linn. Soc.* 76: 369-384.

Melderis, A., 1985. *Agropyron* (Gaertner). In: Davis PH (ed.), *Flora of Turkey and the East Aegean Islands,* Vol. 9, pp. 204-206. Edinburgh: Edinburgh University Press.

Melderis, A., Humpries, C. J., Tutin, T. G. and Heathcote, S. A., 1980. Tribe Triticeae Dumort. In: Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M. and Webb, D. A. (eds.) Flora Europaea, Vol. 5, pp. 190-200. Cambridge University Press, Cambridge, England.

Mellish, A., Coulman, B. and Ferdinandez, Y., 2002. Genetic Relationships among Selected Crested Wheatgrass Cultivars and Species Determined on the Basis of AFLP Markers. *Crop Sci.* 42: 1662–1668.

Miller, M. P., 1997. AMOVA-PREP, a program for the preparation of AMOVA input files for use with WINAMOVA. Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ.

Mohamed, T. R., Khalifa, S. F. and Salah EL-Dine, R. M., 2006. Leaf Protein Electrophoretic Profiles and Chromosome Numbers of Some Araceae. Int. *Jour. Agri. Biol.* 8: 231–234.

Mueller, R. J. and Richards, J. H., 1986. Morphological Analysis of Tillering in *Agropyron spicatum* and *Agropyron desertorum. Annal. Bot.* 58: 911–921.

Murphy, R. W., Sites, J. W., Buth, D. G. and Haufler, C. H., 1990. Protein I: Isozyme Electrophoresis. In: Hillis DH, Moritz C eds. Molecular Systematic. Sinnauer Association, Sunderland, Massachusetts. pp. 45–126.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89: 583–590.

Nevski, S. A., 1934. Tribe XIV. Hordeae Benth. In: V. L. Komorov (ed.) Flora of the USSR., pp. 590-722. Leningrad: The Botanical Institute of the Academy of Sciences of the USSR.

Paterson, A. H., Tanksley, S. D. and Sorreis, M. E., 1991. DNA markers in plant improvement. *Adv. Agron.* 46: 39-90.

Reddy, P. M. Munirajappa, M., 2005. Electrophoretic studies in induced mutants of diploid mulberry genotype S13. *Indian Jour. Biotech.* 4: 422–423.

Refoufi, A. and Esnault, M. A., 2008. Population genetic diversity in the polyploid complex wheatgrass using isoenzym and RAPD data. *Biol. Plant.* 52: 543-547.

Rogler, G. A. and Lorenz, R. J., 1983. Crested wheatgrass: Early history in the United States. *Jour. Range Manage.* 36: 91–93.

Salehi Shanjani, P., Jafari, A. A. and Calagari, M., 2013. Genetic variation among wild and cultivated *Agropyron desertorum* populations based on total protein profiles and phenotypic traits. New Zealand *Jour. Crop Horticul. Sci.* 41: 117-135.

Salehi Shanjani, P., Jafari, A. A. and Hoseinzadeh, R., 2013. Effects of selection on genetic parameters of *Secale montanum* based on seed storage protein marker. *Jour. Rangeland Sci.* 3: 231-244.

Taghizadeh, R., Jafari, A. A., Imani, A. A., Asghari, A. and Choukan, R., 2011. Investigation of genetic variability in Iranian populations of desert wheatgrass (*Agropyron desertorum*) based on morphological and RAPD markers. *Iranian Jour. Rangelands For. Plant Breed. Genet. Res.* 19: 85–100. (In Persian).

Tamura, K., Dudley, J., Nei, M. and kumar, S., 2007. MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* 24: 1596–1599.

Torkpo, S., Danquah, E., Offei, S. and Blay, E., 2006. Esterase, total protein and Seed storage protein diversity in Okra (*Abelmoschus esculentus* (L.) Moench). *West* African *Jour.* Applied Ecol. 9: 7–18.

Tzvelev, N. N., 1976. Tribe 3. Triticeae Dumort. In: Fedorov, A.A. (ed.), Poaceae URSS. Navka Publishing House, Leningrad, pp. 147- 181.

Vries, I. M., 1996. Characterization and identification of *Lactuca sativa* cultivars and wild relatives with SDS–electrophoresis (*Lactuca* sect. *Lactuca*, Compositae). *Genet. Resour. Crop* Evol. 43: 193–202.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S.V., 1990. DNA polymorphisms amplified by arbitary primers are useful as genetic markers. *Nucl. Acids Res.* 18: 6531-6535.

Wright, S., 1931. Evolution in Mendelian populations. *Genetics*, 16: 97–159.

Wright, S., 1951. The genetical structure of populations. Annal. *Eugentics*, 15: 323–353.

Yeh, F. C., Yang, R. C. and Boyle, T., 1999. POPGENE version 1.32, Microsoft window base software for population genetic analysis: a quick user's guide. University of Alberta, Center for International Forestry Research, Alberta, Canada.

Yousofi, M. and Assadi, M., 2006. Tow New Taxa of *Agropyron cristatum* (Poaceae), for the flora of Iran. *Iranian Jour. Botany*, 12: 47-50. (In Persian).

بررسی تنوع ژنتیکی گونه‌های علــف‌گنــدمی *Agropyron* توسـط ویژگـی‌هـای مورفولوژیک و پروتئین‌های کل برگ

پروین صالحی شانجانی[الف]، علی اشرف جعفری[ب]، راضیه جهانباز[ج]

[الف]استادیار موسسه تحقیقات جنگلها و مراتع کشور، تهران، ایران (نگارنده مسئول)، پست الکترونیک: Psalehi@rifr-ac.ir
[ب]استاد موسسه تحقیقات جنگلها و مراتع کشور، تهران، ایران
[ج]فارغ‌التحصیل دانشگاه پیام نور، تهران، ایران

چکیده. تنـوع ژنتیکـی گونـه *Agropyron desertorum* و دو واریتـه *A.cristatum* subsp. *pectinatum* var. *imbricatum* و *A. cristatum* subsp. *pectinatum* var. *pectinatum* به وسیله ویژگیهای مورفولوژیک و الگو پروتئین‌های کل (با روش SDS-PGE) مطالعه شـدند. بـه ایـن منظـور آزمایشـی بـه‌صـورت طـرح بلوک‌های کامل تصادفی در سه تکرار (در سالهای ۱۳۹۲-۱۳۹۱) در موسسـه تحقیقـات جنگلهـا و مراتـع کشور اجرا شد. مقایسه مقادیر میانگین ویژگی‌های اگرونومیکی میان جمعیت های سه تاکسون نشان داد که بیشترین قطر و ارتفاع گیاه، طول خوشه، تعداد ساقه و وزن ماده خشک گیاه در گونـه *A. desertorum* بیشترین میزان تولید بذر، وزن هزاردانه و طول خوشه در واریتـه var. *pectinatum* مشـاهده شـد. بعـلاوه گیاهان واریته var. *pectinatum* در زمان کوتاه تری به گلدهی کامل رسیده و گرده‌افشانی مـی‌نماینـد. نتایج الگو پروتئین‌های کل سه تاکسون مورد بررسی حاکی از تنوع ژنتیکی بیشتر در جمعیت‌های واریتـه var. *imbricatum* نسبت به دو تاکسون دیگر است. گروه‌بندی جمعیت‌های مختلف سه تاکسون بـا هـر دو روش نشان داد که جمعیت‌های واریته var. *pectinatum* بـیش از واریتـه var. *imbricatum* بـه گونـه *A. desertorum* شباهت دارد. نتایج حاصل از مطالعه صفات اگرونومیکی و الگو پروتئین‌های کل اطلاعـات بـا ارزشی برای جمع‌آوری حفاظت ژنتیکی و برنامه‌های اصلاحی آینده بدست می‌دهند.

کلمات کلیدی: کمپلکس *Agropyron*، تنوع ژنتیکی، پروتئین‌های کل برگ ، مورفولوژی، جمعیت‌های وحشی