GENETIC VARIATION AMONG NATURAL POPULATIONS OF AGRPYRON CRISTAUM (POACEAE) BASED ON SDS-PAGE OF SEED PROTEINS

M. Yousofi, M. Esmaeili & M. Otroshy

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SDS-PAGE of seed proteins in 11 natural populations of *Agropyron cristatum* and one cultivated population of *Elymus junceus* was studied. In total 31 protein bands of different molecular weights were detected on the polyacrylamide gel indicating a high diversity. By performing Cluster Analysis and Principle Coordinate Analysis on these data, all studied populations were grouped in three major clusters. Clear correlation was seen between the population in three clusters with their geographical localities. This result suggests that seed protein markers between the natural populations of *Agropyron cristatum* are mainly correlated with eco-geographical factors and cannot be used alone for its taxonomy in the infra-specific levels.

Mehdi Yousofi (correspondence <myousefi@pnu.ac.ir>*), Department of Biology, Payam Noor University, Tehran, Iran. - Mehrnoosh Esmaeili, Department of Biology, Payam Noor University, Tehran, Iran. E-mail:* mehrnoosh_e2020@yahoo.com*. -Mahmood Otroshy, Agricultural Biotechnology Research Institute -Central region of Iran (ABRII), P.O. Box 85135-487, Isfahan, Najafabad, Iran. E-mail:* otroshy@yahoo.com.

Key words. Agropyron cristatum, cluster analysis, Iran, SDS PAGE, seed protein.

تنوع ژنتیکی بین جمعیت های خودروی Agropyron cristatum براساس الکتروفورز پروتئین‌های دانه

مهدی یوسفی، دانشیار گروه زیست‌شناسی، دانشکده علوم پایه، دانشگاه پیام نور، تهران.

مهرنوش اسماعیلی، دانشجوی کارشناسی ارشد گروه زیست‌شناسی دانشگاه پیام نور، تهران.

محمود اوتروشی، مؤسسه تحقیقات فناوری کشاورزی، منطقه مرکزی ایران، نجف آباد.

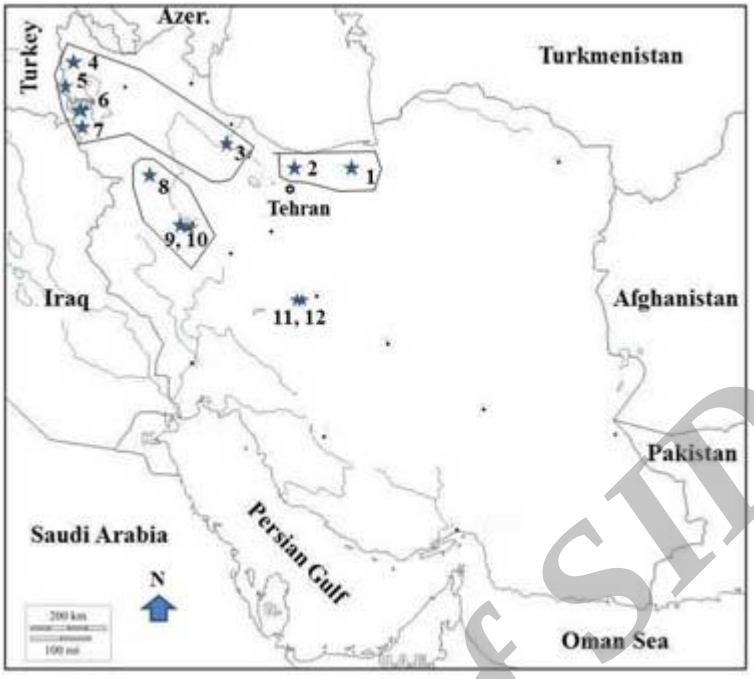
پروتئین‌های ذخیره‌ای دانه 11 جمعیت خودروی *Agropyron cristatum* و یک جمعیت کاشته شده*Elymus junceus* از طریق الکتروفورز روی ژل پلی آکریل آمید بررسی شدند. در مجموع 31 باند پروتئینی با وزنهای مولکولی متفاوت روی ژل ظاهر شد که نشانگر تنوع بالا است. با انجام آنالیز خوشه‌ای و آنالیز مولفه‌های اصلی روی داده‌های به دست آمده، تمام جمعیت‌های مورد مطالعه در سه خوشه گروه بندی شدند. ارتباط آشکاری بین تاکسونهای هر خوشه و مکانهای جغرافیائی آنها مشاهده شد. نتایج پیشنهاد می‌کند که مارکرهای پروتئینی دانه جمعیت های خودروی *Agropyron cristatum* عمدتا با عوامل اکوجغرافیائی همبستگی دارند و به تنهائی نمی‌توانند برای تاکسونومی این گونه در سطوح زیرگونه‌ای مفید واقع شوند.

INTRODUCTION

Agropyron Gaertner is a small genus in the tribe *Triticeae* Domurt. (*Poaceae*) with about 10 species, and approximately 20 infra-specific taxa (Dewey 1984; Love 1984). One of the important and widespread species of this genus is *A. cristatum* (L.) Gaertn. that is indigenous to Asia and Europe and is valuable forage grass known to be tolerant to drought, low temperature, and salinity and resistant to some pathogens (Tzvelev 1976; Dewey 1984; Love 1984). Bor (1970) did not mention this species from Iran, but later Dewey & Assay (1975) reported it from the country and stated that all Iranian *Agropyron* taxa must be considered as *A. cristatum*, but they avoided classifying them in the sub-specific levels. With time, more natural populations of this species in West and North West of Iran were studied by different authors (Assadi 1995; Yousofi & Aryavand 2004; Youosfi & Assadi 2006).

There are three ploidy levels in all populations and ecotypes of *Agropyron cristatum* in Iran; diploids (2n=2x=14), tetraploides (2n=4x=28) and hexaploides (2n=6x=42), but the diploids are very rare (Dewey & Asay 1975; Assadi 1995; Yousofi & Aryavand (2004).

A. cristatum populations show high level of



Map 1. Localities of *Agropyron cristatum* and *Elymus junceus* populations used for seed protein electrophoresis; 1) Firouzkooh, 2) Kandavan, 3) Rudbar, 4) Salmas, 5) Serou, 6) Urmieh, 7) Ghasemloo, 8) Bijar, 9 & 10) Assadabad-1 & Assadabad-2, 11 & 12) Fuzveh and Elymus.

morphological homogeneity and hence, identifying them merely by using phenotypic characteristics is inadequate. Therefore, researchers prefer to use new methods for discrimination their inter- and intra-population differences. SDS-PAGE of seed storage proteins is one of the valuable techniques that are widely used in plant studies. In the recent years many researchers applied this procedure onto closely related taxa of Poaceae (Chen & al. 1997; Aiken & al. 1998; Sheidai & al. 2008; Rafezi & al. 2008; Tamkoc & Arslan, 2011). In the present study seed protein analysis in 11 natural populations of *Agropyron cristatum* and one cultivated population of *Elymus junceus* is reported. *Elymus junceus* is a closely related species to *Agropyron* and was used in this work for comparison and better interpretation of the results.

MATERIAL AND METHODS

Plant material

The details of seed collections of 11 natural populations of *Agropyron cristatum* and one cultivated population of *Elymus junceus* used for electrophoretic analysis are shown in table 1 and map 1. The voucher materials are also kept in Isfahan Payam Noor University. All collected materials were determined according to Tzveleve (1976), Love (1984), Melderis (1985), Assadi, (1995) and Yousofi & Assadi (2006).

Protein electrophoresis

Mature seeds of five plants in each population were randomly collected. SDS-PAGE was conducted according to Saraswati & al. (1993) and Sheidai & al. (2008) with some modifications. For each sample 40 mg of seed flour was homogenized in Eppendorf tube with 300 µl of extraction buffer (1.5 M Tris-HCl buffer; pH 8.8, glycerol 78%, urea, SDS, bromonaphtol blue, 2-mercaptoethanol 5% and double distilled water), steered with vortex for 2 minutes and left in refrigerator (4ºC) over night and then, centrifuged at 10,000 rpm for 15 min. The supernatant of each sample was kept over ice until use for electrophoretic analysis. A volume of 30µl protein extract was added to equal volume of treatment buffer and boiled for five minutes in water bath before loading in the gel. About 10 µl of this mixture were loaded on the gels. Control wells were loaded with standard protein marker with the following molecular weights; 116, 66.2, 45, 35, 25, 18.4, and 14.4 KDa. In this work stacking gel 5% and resolving gel 12% were used. Electrophoresis was performed using AE-6220 Slab EP chamber in Agricultural Biotechnology Research Institute of Iran (ABRII), Isfahan branch. The run was performed at a constant rate of 30 mA for 16h. In order to protein bands detection, Coomassie Briliant blue G-250 was used for overnight gel staining followed by trichloroacetic acid as fixative (Hames & Rickwood

Table 1: *Agropyron cristatum* and *Elymus junceus* populations and their localities used for seed protein electrophoresis.

	Taxon	2n	Locality*	Abbreviation
Agropyron cristatum (L.) Gaertn.	subsp. *incanum* (Nabelek) Melderis	6x 42	West Azerbaijan, Urmieh, Serou, near Tukey borders, 1800 m, Yousofi, 3598.	Serou
	subsp. *pectinatum* (Bieb.) Tzvelev var. *minor* Yousofi	2x 14	Hamadan, 5 Km to Assadabad, Gallehbor region, 2250-2300 m, Yousofi & Rahiminejad, 3901.	Assadabad-1
	subsp. *pectinatum* (Bieb.) Tzvelev var. *pectinatum*	6x 42	West Azerbaijan, Urmieh, Ghasemloo valley, Silan region, 1700-2000 m, Yousofi, 3596.	Ghasemloo
		2x 28	Mazandaran, Tehran to Babolsar, Firouzkooh, 2400 m, Yousofi, 3672.	Firouzkooh
		6x 42	Gilan province, Rudbar, Jirandeh to Lushan, 1000 m, Yousofi & Esmaeili Sharif, 3962.	Rudbar
		6x 42	West Azerbaijan, Salmas, elevation of Tamar village, 1700-2000 m, Yousofi & Esmaeili Sharif, 3941.	Salmas
		2x 28	West Azerbaijan, Urmieh, Sheikh-Tappeh, 1500 m, Yousofi and Fallahi, 3614.	Urmieh
		2x 28	Kurdestan, Bijar, Nesa mountain, 2200 m, Yousofi & Esmaeili Sharif, 3911.	Bijar
		2x 28	Hamadan province, 5 Km to Assadabad, Gallehbor region, 2250-2300 m, Yousofi & Rahiminejad, 3904.	Assadabad-2
		2x 28	Isfahan province, Fuzveh Research Center, cultivated, 1560 m, Yousofi, 3832.	Fuzveh
	subsp. *pectinatum* (Bieb.) Tzvelev var. *imbricatum* (Roemer & Schultes) G. Beck	2x 28	Alborz province, Karaj to Chaloos, Kandavan, 2800-3000 m, Yousofi, 3621.	Kandavan
Elymus junceus Fisch.		2x 28	Isfahan province, Fuzveh Research Center, cultivated, 1560 m, Yousofi, 3833.	Elymus

*All herbarium numbers are belonging to the local herbarium of Isfahan Payam Noor University.

1990). Background color of the gel was removed by dipping it in distilled water and washed (replaced every 20 min) until the protein bands were seen as colored points on a transparent background and then was kept in acetic acid 7%. The gel was later photographed.

Data analysis of SDS-PAGE was conducted according to Sheidai & al. (2008). The protein bands were scored as 1 and 0 for present/absent, respectively. Then, the data were subjected to Custer Analysis, using Ward method and Simple Matching Similarity Coefficient (Sneath & Sokal 1973) and Principle Coordinate Analysis (PCOA). Statistical analyses were performed by STATISTICA software (StatSoft, Inc. 2007). Densitometry of protein bands was also performed using ImageJ software (Rasband 1997).

RESULTS AND DISCUSSION

Morphological homogeneity among subspecies, varieties and populations of *Agropyron cristatum* is usually high and this is one of the taxonomic complexities in this taxon (Dewey 1986). Therefore, in the present investigation SDS-PAGE of seed storage proteins was used for assessment of genetic diversity among 11 natural populations of *Agropyron cristatum.* In total 31 protein bands of different molecular weights, ranging from 116 KDa to 14.4 KDa, were detected in the tested populations (Fig. 1 and Table 2). *Elymus junceus* (a cultivated population) with 23 bands had the highest number of protein bands, but these bands for *Agropyron cristatum* populations ranged from maximum 19 (in Kandavan, Firouzkooh and Serou populations) to minimum 14 (in Urmieh and Rudbar populations). Some bands were identified as specific for one or more studied populations, whereas nine protein bands were common in all populations. For example, bands 12 and 16, in the range of 45 KDa proteins, occurred only in all *Agropyron cristatum* populations. The bands 10, 24 and 30 occurred in *Elymus junceus* population. These bands were located in the ranges of 66.2, 25 and 14.4 KDa proteins respectively. Some protein bands were also specified for only one population. For instance, bands number 2 and 3, with about 116 KDa weights, occurred in Serou population and band number 4, with about 66.2 KDa weight, occurred in Firouzkooh population (Fig. 1).

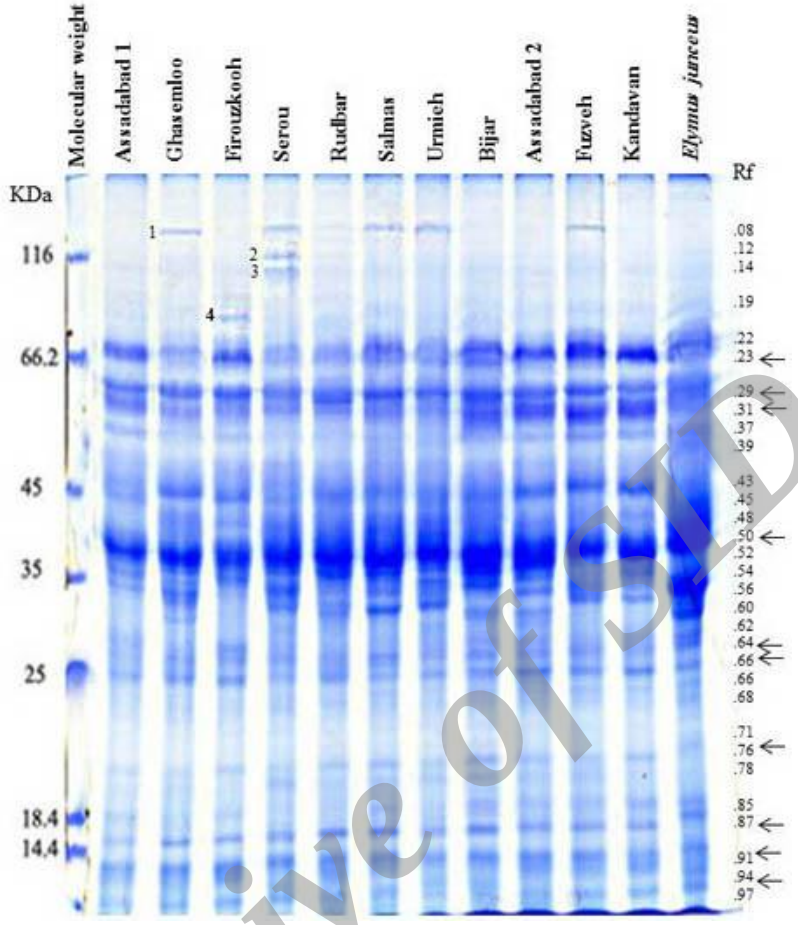


Fig. 1. The protein bands of 11 *Agropyron cristatum* and one *Elymus junceus* populations revealed on the electrophoretic gel in SDS-PAGE of seed storage proteins of the studied taxa; Rf is relative mobility of proteins on the gel. Arrows show the common bands in all studied populations and the numbers 1 to 4 show the specific bands. The left column shows standard protein markers with defined molecular weights.

Table 2. Presence (1) and absence (0) of the protein bands of 11 natural populations of *Agropyron cristatum* and one cultivated population of *Elymus junceus* revealed on the electrophoretic gel in SDS-PAGE of seed storage proteins.

Populations	Bands																												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Assadabad-1	0	0	0	0	0	1	1	1	1	0	0	1	0	0	1	1	1	0	0	1	1	1	0	0	1	0	1	1	1	0	1
Ghasemloo	1	0	0	0	0	1	1	1	0	0	0	1	0	0	1	1	1	0	1	0	1	1	0	0	1	0	0	1	1	0	1
Firouzkooh	0	0	0	1	0	1	1	1	1	0	1	1	1	1	1	1	0	0	0	1	1	1	0	0	1	0	1	1	1	0	1
Serou	1	1	1	0	0	1	1	1	1	0	0	1	0	0	1	1	1	0	1	0	1	1	0	0	1	1	0	1	1	0	1
Rudbar	0	0	0	0	0	1	1	1	0	0	0	1	0	0	1	1	1	0	1	0	1	1	0	0	1	0	0	1	1	0	1
Salmas	1	0	0	0	0	1	1	1	0	0	0	1	0	0	1	1	1	0	1	0	1	1	0	0	1	1	1	1	1	0	1
Urmieh	1	0	0	0	0	1	1	1	0	0	0	1	0	0	1	1	0	0	1	0	1	1	0	0	1	1	0	1	1	0	0
Bijar	0	0	0	0	1	1	1	1	1	0	0	1	0	0	1	1	0	0	1	1	1	1	0	0	1	1	1	1	1	0	0
Assadabad-2	0	0	0	0	0	1	1	1	1	0	0	1	0	0	1	1	1	0	1	1	1	1	0	0	1	1	0	1	1	0	1
Fuzveh	1	0	0	0	0	1	1	1	1	0	0	1	0	0	1	1	1	1	0	0	1	1	1	0	1	1	0	1	1	0	1
Kandovan	0	0	0	0	0	1	1	1	1	0	0	1	1	1	1	1	0	1	0	1	1	1	1	0	1	0	1	1	1	0	1
Elymus	0	0	0	0	1	1	1	1	1	1	0	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1

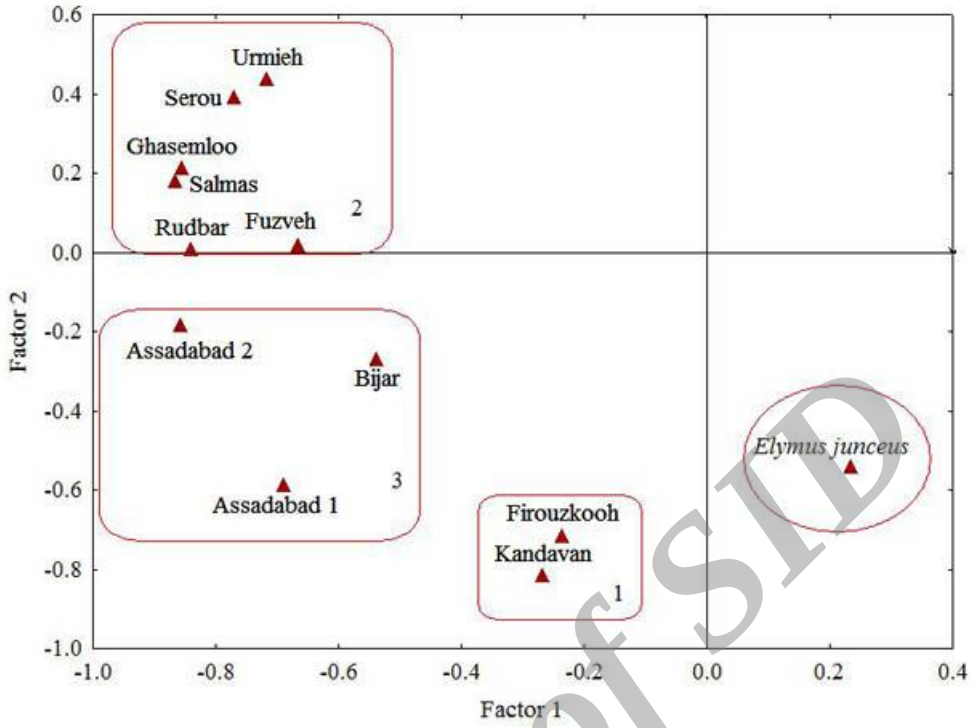


Fig. 2. PCO ordination of 11 natural populations of *Agropyron cristatum* and one cultivated population of *Elymus junceus* based on SDS-PAGE of seed storage proteins.

The most variations were observed among protein bands in the ranges of 35 to 66.2 KDa proteins. The densities of protein bands on the electrophoretic gel were not identical; the most density variations were seen in the protein bands ranging from 35 to 66.2 KDa weightes.

The results showed relatively high variations among the studied populations that were revealed by gel electrophoresis of seed storage proteins. According to Crawford (1990), seed storage proteins are stable products of genes and can reflect genome diversity of plants. Such variation is indicative of genome changes taken place during the species diversification. Chen & al. (1997) stated that these proteins are useful markers for assessing taxonomic and phylogenetic relationships in various levels of many plant families including grasses. In fact, the presence and absence of protein bands on the electrophoretic gel can be used as taxonomic markers. So far, these markers have widely been applied in classification of many closely related taxa of *Poaceae* (Jensen & Grumpe 1983; Moustakas & al. 1986; Gardiner & Forde, 1992; Sheidai & al. 2008; Tomkoc & Arslan 2011; Skrajna & al. 2012). Thus, the results of seed protein electrophoresis (Table 2) were subjected to cluster analysis. The validity of cluster analysis was also supported by ordination of data based on a PCOA (Fig. 2). Factor analysis of protein data indicated that the first 3 factors comprise 74.15% of total variances. The bands number 10, 12, 13, 14, 16 and 30 had higher loading (>70) on the first factor, numbers 4 and 11 on the second factor and number 31 on the third factor.

Fig. 3 shows the dendrogram resulted in cluster analysis. All studied populations were grouped in three major clusters as seen in the dendrogram. The first cluster is comprised of two samples of Firouzkooh and Kandavan populations (Table 1). These populations have already been identified as tetraploid cytotypes of *A. cristatum* (Yousofi & Aryavand 2004). The second cluster is comprised of the samples belonging to 6 populations of Rudbar, Ghasemloo, Urmieh, Serou, Salmas and Fuzveh (a cultivated tetraploid). Serou population is a hexaploid cytotype named *A. cristatum* subsp. *incanum* (Assadi 1995), but the others are *A. cristatum* subsp. *pectinatum* from which the Rudbar, Ghasemloo and Salmas populations are hexaploid whereas Urmieh and Fuzveh populations are tetraploid cytotypes (Yousofi & Aryavand 2004). The third cluster is comprised of three populations; Assadabad-1 that is a diploid cytotype of var. *minor* (Yousofi & Assadi 2006), Assadabad-2 and Bijar that are tetraploid cytotypes of subsp. *pectinatum* (Yousofi

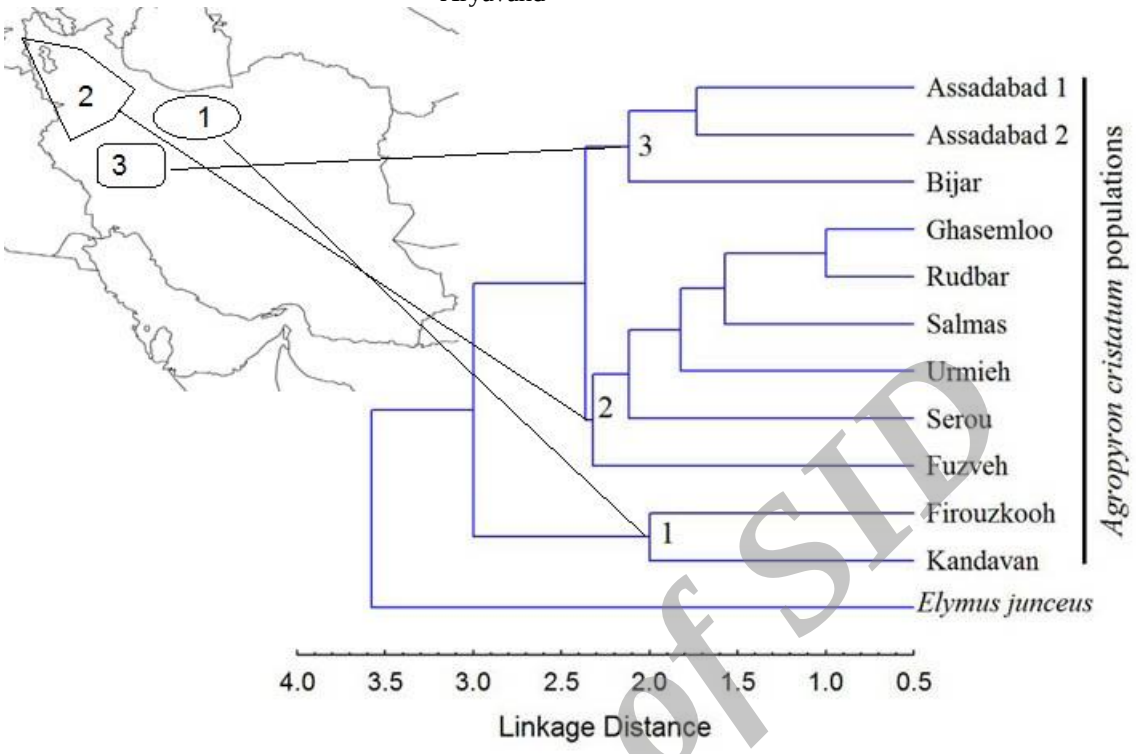


Fig. 3. Cluster analysis of seed protein bands of 11 natural populations of *Agropyron cristatum* and one cultivated population of *Elymus junceus* based on SDS-PAGE of seed storage proteins.

2004). The results showed that there were no clearly taxonomic relationships (at the intra-specific levels) as well as ploidy levels between the studied taxa in three clusters. Instead, the clusters were consistent with the geographical localities of the studied populations. The two populations of the first cluster both are located in the central parts of Alborz Mountains (Alborz and Mazandaran provinces) with an elevation of about 2400-3000 m above of the sea level (map 1). The populations in the second cluster, except Fuzveh (That it seeds were collected from a cultivated population with unknown geographical origin), are located in northwest of Iran (Gilan and Azerbaijan provinces) with an elevation ranged from 1000 to 2000 m above of the sea level. The populations in the third cluster are located in the west of Iran (Kurdestan and Hamadan provinces) with an elevation ranged from 2200 to 2300 m above of the sea level (map 1).

Our results are supported by Nevo & al. (1997), Sun & al. (1999) and Che & Li (2007) on the wild populations of grasses. Nevo & al. (1997) reviewed the protein variation within natural populations of wild barley in relation to their geographical origins and concluded that the patterns of protein diversity were often related to ecological factors. Che & Li (2007) revised the genetic diversity of prolamines in wild populations of *Agropyron mongolicum* Keng indigenous to northern China and concluded that the genetic distances among studied populations were related to the origin of those populations, and the populations with similar eco-geographical origin were clustered closely. However, it seems that few studies on the cultivated populations are inconsistent with our results (Wei-dong & al. 2006; Taghizadeh & al. 2009). This inconsistency can be interpreted so that such genetic diversities among wild populations are more dependent on the eco-geographical conditions than cultivated ones (Che & Li 2007). Indeed, this dependence among cultivated populations is none or less, because the cultivated plants are often grown in control and desirable conditions (Skrejna & al. 2012). Obviously, further studies are needed to confirm or disprove this conclusion

Agropyron cristatum is one of the important grasses in the west, northwest and north of Iran (Dewey & Assay 1975; Assadi 1995). Our results revealed considerable variations in the seed proteins of different

natural populations of this species and suggest that these markers alone cannot be used for taxonomic treatment of this species in the intra-specific levels and should be used along with the other taxonomic markers. Indeed, the seed protein diversity among the studied populations of *Agropyron cristatum* is mainly correlated with their eco-geographical and genetic factors.

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