Genetic Diversity of *Agropyron pectiniforme* using Morphological Traits and RAPD Markers

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Abstract

This study was conducted to analyze genetic diversity in 15 natural populations of Iranian *Agropyron pectiniforme* using morphological traits and RAPDs (Random Amplification of Polymorphic DNA) molecular markers. Five primers out of the 10 were highly polymorphic and produced 128 polymorphic bands ranging in length of 500 to 2200 bp. According to AMOVA (Analysis of Molecular Variance) results, there was higher genetic variation between populations (53%) than within them (47%). Cluster analysis based on RAPD data categorized the populations into five clusters. PCoA (Principal Coordinates Analysis) results showed the first four coordinates accounted for 95% of the total variation. The scatter of populations based on the first two components was in agreement with cluster analysis results. There was no significant mantel correlation coefficient between molecular and geographical data indicating the classification of *A. pectiniforme* based on RAPD marker was not in accordance with geographical distribution. With regard to morphological traits, characters such as plant height and the number of stems per plants were considered as suitable parameters for selection and breeding programs. The two marker systems gave different estimates of genetic variability among populations. Finally, our findings demonstrated the feasibility of the RAPD technique for quantifying genetic distances among *A. pectiniforme* populations. It was concluded that, there is sufficient genetic variation between Iranian populations of *Agropyron pectiniforme* making these populations potentially useful for breeding improved varieties.

Keywords: *Agropyron pectiniforme*, AMOVA, geographic data, molecular marker, morphological marker

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Introduction

Plants in the genus *Agropyron* Gaertn. grow in many countries, including Iran and they are the most important grass in the temperate regions of the world (Arghavani et al., 2010; Shirvani et al., 2014; Salehi Shanjani et al., 2015). *Agropyron* has many drought-resistant grasses that are used for weed control, soil stabilization, watershed management, and forage production for livestock grazing, and reclaiming overgrazed rangelands (Shirvani et al., 2014; Salehi Shanjani et al., 2015). Successful crossing between different species of *Agropyron* and *Triticum aestivum* has been reported (Sharma et al., 1987). In addition, *Agropyron* genus as an important wild relative of

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wheat, is a valuable resource of useful genes, which can be widely used for hybridization, especially to transfer new genes into cultivated wheat (Che and Li, 2007; Arghavani et al., 2010; Shirvani et al., 2014; Li et al., 2017). For example, resistance genes to WSMV (wheat streak mosaic virus) have been transferred from *Agropyron elongatum* to wheat (Friebe et al., 1996). Some species of *Agropyron* have genetic variation for salt tolerance, water stress, pathogen and disease resistance, and forage quality (Sharma et al., 1984; Sharma and Baezinger, 1986).

Knowledge of genetic diversity can be used to develop new and more productive crops that are resistant to biotic and abiotic stresses and adapted to changing environments (Shirvani et al., 2014). Moreover, this information can be applied to understand evolutionary processes, development of conservation strategies, the evaluation of cultivated or wild populations, and use of genetic resources in breeding programs (Che and Li, 2007; Rahimi Analoujeh et al., 2014).

In the last few years, many attempts have been made to understand genetic structure of natural populations of grass species (Che and Li, 2007). Morphological, agronomical, chemical, molecular (DNA, protein, isozymes), cytogenetic, and molecular cytogenetic markers were used for estimating genetic variability (Farshadfar, 2012). The information on morphological and agronomic aspects of the material is essential for the gene bank management (Padmaja et al., 2008). In the breeding programs, characterization of the genotypes is necessary to improve the breeding efficiency where morphological, biochemical, and molecular markers can be applied to identify the genotype (Varshney et al., 2005; Khan et al., 2016). Morphological traits are evaluated at the field or greenhouse and they are affected by environmental factors (Arghavani et al., 2010). It seems that application of molecular markers to identify plant species or varieties is more effective than traditional morphological markers (Salehi Shanjani et al., 2015). Analysis of genetic variability at the DNA level is the only means of assessing properly the diversity of any plant population without taking into account the complex relationships that exist between phenotypes, genotypes, and the environment (Szenejko and Rogalski, 2015). The use of molecular markers to detect genetic differences between the closest genotypes may be more expressive when compared with the use of morphological and agronomic descriptors (Rimoldi et al., 2010). It is shown that, RAPDs are faster and cheaper for showing genetic polymorphism and are an alternative to RFLPs for determining genetic variation at the molecular level. With respect to advantage and disadvantage of molecular and morphological markers, the combination of these markers could be useful in studying genetic diversity (Cortese et al. 2010). The aim of this study was to evaluate the relationships and genetic diversity of Iranian *A. pectiniforme* populations using morphological and RAPD molecular markers.

Material and Methods

In this study, 15 different populations of *A. pectiniforme* were used; names, origin, accession's code, and geographical coordinates of populations are shown in Table 1 and Fig. I. The experiment was conducted in Alborz Research Station in Karaj, Iran (latitude 35°42′ N and longitude 51°31′ E of the Greenwich meridian, and the altitude from sea level of 1291 m). For field experiment, a randomized complete block design with 4 replications was used. The studied morphological traits were days to heading, spike length, grain yield, 1000-grain weight, plant height, the number of stems per plant and forage dry matter yield. The collected data were analysis of variance and the genetic parameters were calculated using following formulas:

$$PCV\% = \frac{\sqrt{\sigma_p^2}}{\bar{X}} \times 100 \qquad \text{(Eq. 1)}$$

$$GCV\% = \frac{\sqrt{\sigma_g^2}}{\bar{X}} \times 100 \qquad \text{(Eq. 2)}$$

(Singh and Chaudhary, 1985)

$$h_b^2 = \frac{\sigma_g^2}{\sigma_{e+}^2 \sigma_g^2} = \frac{\sigma_g^2}{\sigma_p^2} \times 100 \qquad \text{(Eq. 3)}$$

$$\mathrm{GA} = h^2 \mathrm{K} \sqrt{\sigma_p^2} \qquad \text{(Eq. 4)}$$

(Dorri et al., 2014; Falconer, 1989)

Table 1
Accession number and origin of the studied populations of *Agropyron pectiniforme*

Population code	Accession No.	Origin	Province	1000 seeds Weight g	Altitude m	Longitude	Latitude
P1	1356	Urmia	West Azerbaijan	1.08	2100	45.0330° E	37.5330° N
P2	4052	Shahmirzad	Semnan	2.20	2250	53.4740° E	35.7790° N
P3	1569	Karaj	Alborz	2.50	1900	51.2460° E	35.6160° N
P4	8754	Lordegan	Choharmahal	4.71	2290	51.1330° E	31.4670° N
P5	1251	Meshginshahr	Ardebil	3.40	2300	47.6730° E	38.1670° N
P6	707	Tabriz	East Azerbaijan	2.60	1450	46.2910° E	38.2220° N
P7	62	Karaj	Alborz	1.70	1950	51.0520° E	35.8230° N
P8	101	Karaj	Alborz	2.50	1812	51.3460° E	35.7160° N
P9	8683	Borujerd	Lorestan	4.93	2400	48.8830° E	33.8500° N
P10	487	Zanjan	Zanjan	2.84	1800	48.2830° E	36.6670° N
P11	536	Damavand	Tehran	2.20	2200	51.6380° E	35.7240° N
P12	7794	Baft	Kerman	3.54	3000	56.5420° E	29.5000° N
P13	1712	Gorgan	Golestan	2.14	1800	54.5830° E	36.6670° N
P14	550	Damavand	Tehran	2.20	2150	51.9380° E	35.7240° N
P15	7769	Jiroft	Kerman	3.23	1600	57.7310° E	28.6690° N

where:
$\sigma_p^2 = \text{Phenotypic Variance}$
$\sigma_g^2 = \text{Genotypic Variance}$
$h_b^2 = \text{Broad sense heritability}$
PCV = Phenotypic coefficient of variation
GCV = Genotypic coefficient of variation
GA = Genetic advance
K = Constant = 2.06 at 5% selection intensity

For RAPDs molecular markers, name, sequence, and number of five polymorphic primers used are listed in Table 2. The seeds of populations sown in pots in the greenhouse and five individual plants were sampled from each population for DNA extraction. Total genomic DNA was extracted from young leaves using the CTAB procedure according to Saghai-Maroof et al. (1984). The quantity and quality of extracted DNA were measured using the spectrophotometer and 0.8% agarose gel electrophoresis. The mixture for the amplification reactions was performed in a volume of 15 µl containing 3.33 ng of DNA template, 2 mM $MgCl_2$, 0.2 mM each dNTP, 0.132 µM primer, 1U Taq DNA polymerase, and 1x PCR buffer.

The DNA fragments were amplified based on the following protocol. Each amplification cycle contained a denaturation step at 94 °C for 1 min, a primer annealing step at 37°C for 1 min, and an elongation step at 72 °C for 2 min. At the end of 40

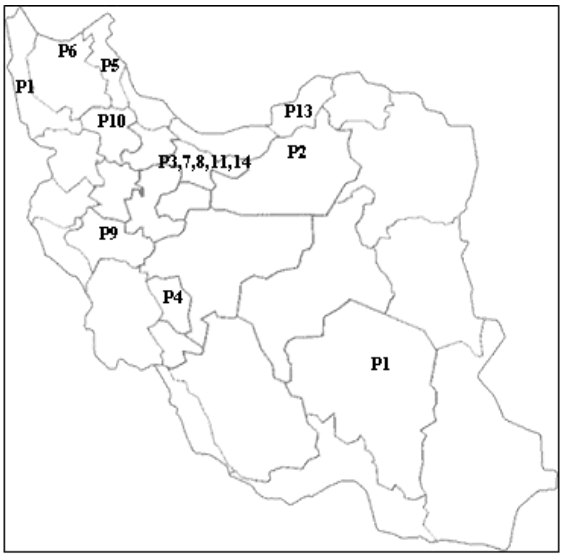

Fig. I. Geographical distribution of the studied *A. pectiniforme* populations in Iran

Table 2
Name, sequence, and number of polymorphic bands produced by studied primers

Primer	Sequence 5′⟶3′	Total bands	Polymorphic percentage
1	AGTCAGCCAC	34	30.59
2	ATTGCGATCC	30	30.00
3	’CCTGGGTCCA	25	42.67
4	GTCCACACGG	18	39.26
5	ACAGGGCTCA	21	35.24

Table 3
Mean squares (MS) of ANOVA for morphological traits in *A. pectiniforme*

S.O.V	Df	MS						
		Days to Heading	Stem number Per plant	Plant height (cm)	Forage yield (g)	Seed yield (g)	spike length (cm)	1000-seed weight (g)
Replication	2	14.58	1.60	13.28	47.79	0.18	0.02	0.008
Population	15	68.98**	270.9**	394.7**	1007.3**	2.19**	2.84**	0.041**
Error	30	5.16	27.56	30.69	86.98	0.091	0.081	0.0031

**=Significant at 1% probability level

Table 4
Estimation of genetic parameters for the studied traits in the *A. pectiniforme*

Trait	σ_p^2	σ_g^2	PCV%	GCV%	h_b^2	GA
Plant height(cm)	394.7	384.47	31.81	31.39	0.97	3986.53
1000-seed weight (g)	0.004	0.0039	2.28	2.25	0.97	12.7
(g) Seed yield	2.19	2.16	8.07	8.02	0.98	300.67
(g) Forage yield	1007.3	978.3	39.09	38.53	0.97	6349.84
Days to heading	68.98	67.26	22.83	22.55	0.97	1668.25
No. stem/plant	270.9	261.71	46.92	46.12	0.96	3275.58
Length of spike(cm)	2.84	2.81	37.81	37.64	0.99	343.89

$\sigma_p^2 =$ Phenotypic Variance, $\sigma_g^2 =$ Genotypic Variance, $h_b^2 =$ Broad sense heritability, PCV=Phenotypic coefficient of variation, GCV=Genotypic coefficient of variation, GA=Genetic advance

cycles, an additional elongation step was carried out at 72 °C for 5 min. Amplified products were scored on the basis of their presence or absence. Only data generated from the detection of clear and stable amplified fragments were analyzed. Genetic similarity values were obtained following Dice (1945). In this method, each pairwise comparison gives a value of 1 for a similarity and 0 for a difference and the genetic similarity is equal to the numerical mean of the set of observations. The molecular data were subjected to UPGMA cluster analysis using NTSYS software.

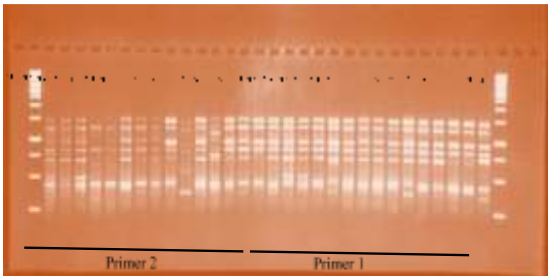

Fig. II. RAPD profile of 15 populations of *A. pectiniforme* with primer 1 (AGTCAGCCAC) and 2

Results

Estimation of genetic parameters

The results of ANOVA (Table 3) showed significant difference between populations for all the morphological traits under study (P<0.01) that is indicated high genetic variation between populations.

The genetic parameters such as the genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad sense heritability (h_b^2), and genetic advance (GA) were estimated using expected mean squares (EMS) from the analyses of variance all characters (Table 4). The highest genetic and phenotypic variance was observed for forage yield whereas the low genetic and phenotypic variance was detected for the1000-seed weight (Table 4). The highest PCV% and GCV% were recorded for plant height and in contrast, the lowest PCV% and GCV% belonged to 1000-seed weight. All traits, including forage and seed yield showed high heritability over 96%, which indicated that selection based on these characters would be more effective and is efficient for advanced generations in future breeding programs.

Table 5.
Mean of genetic variation within *A. pectiniforme* populations (using Nei's index and 5 RAPD Primers)

Population code	Accession No.	Origin	Total band number	Specific band number	Heterozygosity	Polymorphism percentage
P1	1356	Urmia	62	0	0.102	34.38%
P2	4052	Shahmirzad	58	1	0.078	28.13%
P3	1569	Karaj	52	0	0.104	29.69%
P4	8754	Lordegan	50	1	0.096	33.59%
P5	1251	Meshginshahr	52	1	0.090	29.69%
P6	707	Tabriz	58	1	0.108	37.50%
P7	62	Karaj	67	1	0.136	48.44%
P8	101	Karaj	61	0	0.108	36.72%
P9	8683	Borujerd	60	0	0.135	42.97%
P10	487	Zanjan	61	1	0.130	44.53%
P11	536	Damavand	62	0	0.122	42.19%
P12	7794	Baft	62	1	0.142	45.31%
P13	1712	Gorgan	32	0	0.047	20.31%
P14	550	Damavand	34	1	0.065	23.44%
P15	7769	Jiroft	35	1	0.059	25.00%

Table 6
Analysis of molecular variance (AMOVA) based of RAPD data for studied populations

Source of variation	DF	SS	MS	Variance components	Variance (%)
Between populations	14	1168.58	97.18**	8.95	53%
Within population	126	992.40	7.88**	7.88	47%
Total	139	22555.7	200.1	16.75	

**Significance at 0.01 probability

Table 7
Coefficient of similarity (below diagonal) and distance (above diagonal) between the studied populations based on Dice (1972) similarity and Nei genetic distance method, respectively

Population	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15
P1	***	0.03	0.04	0.2	0.25	0.23	0.24	0.25	0.22	0.27	0.28	0.28	0.27	0.26	0,25
P2	0.97	***	0.05	0.2	0.25	0.23	0.24	0.25	0.21	0.28	0.28	0.28	0.29	0.28	0.26
P3	0.95	0.94	***	0.17	0.22	0.19	0.22	0.21	0.2	0.24	0.25	0.26	0.23	0.21	0.21
P4	0.81	0.82	0.84	***	0.05	0.04	0.2	0.22	0.18	0.19	0.22	0.22	0.16	0.14	0.13
P5	0.77	0.77	0.80	0.94	***	0.03	0.24	0.24	0.21	0.22	0.27	0.25	0.21	0.21	0.20
P6	0.79	0.79	0.82	0.95	0.97	***	0.23	0.24	0.20	0.22	0.26	0.26	0.22	0.20	0.19
P7	0.78	0.78	0.80	0.82	0.78	0.79	***	0.05	0.05	0.20	0.23	0.21	0.19	0.19	0.17
P8	0.78	0.78	0.81	0.80	0.78	0.78	0.95	***	0.05	0.22	0.24	0.22	0.24	0.23	0.20
P9	0.80	0.80	0.81	0.83	0.80	0.82	0.95	0.95	***	0.19	0.22	0.20	0.21	0.20	0.18
P10	0.76	0.75	0.78	0.83	0.80	0.80	0.81	0.80	0.82	***	0.05	0.05	0.14	0.13	0.12
P11	0.75	0.75	0.77	0.80	0.76	0.76	0.79	0.79	0.80	0.95	***	0.03	0.18	0.16	0.16
P12	0.75	0.75	0.76	0.79	0.77	0.77	0.81	0.80	0.82	0.94	0.96	***	0.18	0.17	0.16
P13	0.76	0.74	0.79	0.85	0.80	0.80	0.82	0.78	0.81	0.87	0.83	0.83	***	0.02	0.04
P14	0.76	0.75	0.80	0.86	0.81	0.81	0.83	0.79	0.81	0.87	0.85	0.84	0.97	***	0.02
P15	0.78	0.76	0.81	0.87	0.82	0.82	0.84	0.81	0.83	0.88	0.85	0.85	0.95	0.98	***

Table 8
Correlation between geographic and molecular data in the *A. pectiniforme*

Code	Traits	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
X1	Min temperature	1													
X2	Max temperature	0.88**	1												
X3	Relative humidity	-0.14	-0.37	1											
X4	Rainfall	-0.13	-0.15	0.40	1										
X5	Altitude	-0.52*	-0.45	-0.26	-0.38	1									
X6	Total band no	-0.37	-0.40	-0.32	-0.22	0.38	1								
X7	Heterozygosity	-0.44	-0.38	-0.40	-0.09	0.51*	0.87**	1							
X8	Polymorphism	-0.40	-0.31	-0.39	-0.11	0.44	0.86**	0.99**	1						
X9	Days to heading	-0.09	0.04	-0.24	-0.11	0.11	-0.20	-0.08	-0.07	1					
X10	No. stem/plant	-0.39	-0.23	-0.06	0.12	0.01	-0.05	0.22	0.28	0.32	1				
X11	Plant height (cm)	-0.28	-0.12	-0.05	-0.18	0.02	-0.10	0.18	0.26	0.25	0.89**	1			
X12	Forage yield (g)	-0.46	-0.36	0.13	-0.06	0.22	0.36	0.56*	0.59*	-0.13	0.50*	0.63*	1		
X13	Seed yield (g)	0.10	0.18	-0.33	-0.41	-0.13	0.28	0.23	0.33	0.26	0.58*	0.57*	0.24	1	
X14	Length of spike	-0.13	0.13	-0.13	0.33	0.11	-0.43	-0.03	-0.06	0.35	0.47	0.36	0.30	-0.15	1
X15	1000-seed weight	0.57*	0.27	0.16	-0.10	-0.51*	-0.25	-0.38	-0.35	-0.39	-0.14	-0.01	-0.28	0.03	-0.4

* and **: significant at 5%,1%, and 0.1% levels of probability, respectively

Marker polymorphism

Results of this study showed that five out of 10 studied primers were highly polymorphic (Fig. II). The total number of bands produced by each of these five primers ranged from 18 to 34 (Table 5). A total of 128 bands with a size range of 500-2200 bp were produced. About 35% of the 128 bands showed polymorphism. Primer 3 produced the highest number of polymorphic bands (42.67%) and primer 2 produced the lowest number of polymorphic bands (30%). The highest and lowest number of amplified bands produced per *A. pectiniforme* population were obtained from P7 (67) and P13 (32), respectively (Table 5). With regard to Nei's index (Nei, 1972), the highest and lowest heterozygosity (0.136, 0.047) and polymorphism percentage (48.44, 20.31%) were observed in P7 and P13 populations, respectively. Among the studied populations of *A. pectiniforme*, the highest value of genetic diversity was observed in P7 based on polymorphic percentage, heterozygosity, and polymorphic bands (Table 5).

Cluster and PCoA analysis

Cluster analysis of RAPD data using UPGMA, classified the populations into five groups. As shown in Fig. (III) all groups had three populations. The results of Principal Coordinate Analysis based on Dice's similarity matrix **(**PCoA) showed 95% of total variation was explained by four principal components. The first four components justified 34%, 24%, 21%, and 16% of the total variation, respectively. As Fig. (IV) showed, two-dimensional display of populations based on the first two principal components supported the classification based on cluster analysis. According to Table 7, similarity coefficient values indicate that the Iranian populations of *A. pectiniforme* under study are genetically diverse.

Correlation between geographic, morphologic, and molecular data

Polymorphism percentage was positively correlated with total band number (r=0.86, p≤0.01) and heterozygosity (r=0.99, p≤0.01) (Table

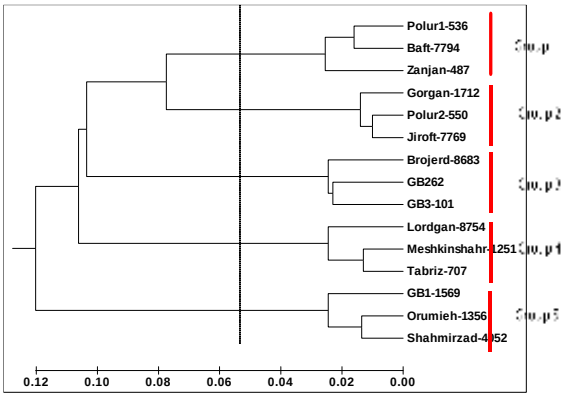

Fig. III. Dendrogram generated for 15 *A. pectiniforme* populations from RAPD markers by cluster (UPGMA) analysis

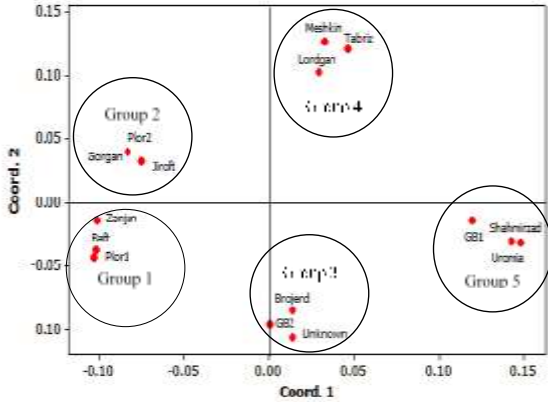

Fig. IV. Grouping of *A. pectiniforme* populations using the first and second coordinates

8). As shown in Fig. (V), according to Mantel test, there was no correlation between geographic and genetic distances based on RAPD markers (r= 0.003; p=0.28). Forage yield showed a significant correlation with heterozygosity (r=0.56, p≤0.05), polymorphism (r=0.59, p≤0.05), and plant height (r=0.63, p≤0.05). Correlations between seed yield and stem number and plant height were positive and significant. Morphological traits except 1000-seed weight showed no significant correlation with climate factors. The 1000-grain weight showed significant correlation with minimum temperature (Table 8). Finally, regression and correlation analyses between genetic and morphologic distances indicated non-significant correlation (Fig.VI).

Discussion

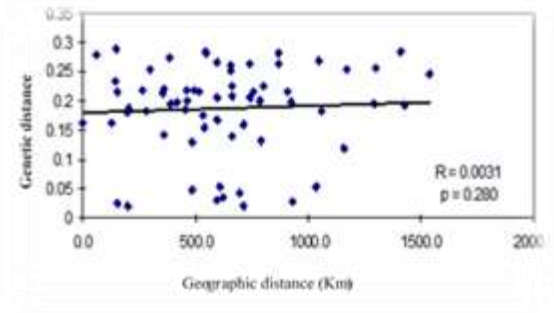

Fig. V. Correlation between geographical distance and genetic distance revealed by the Mantel test

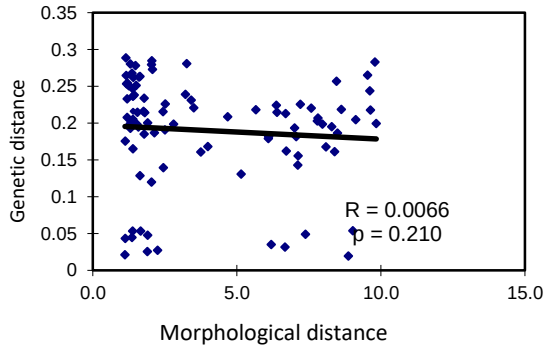

Fig. VI. Correlation between Morphological distance and genetic distance revealed by Mantel test

Morphological traits

Results of analysis of variance showed significant differences between populations for all of the recorded traits (p≤0.01). These results are in agreement with those published in the literature on *Agropyron trichophorum* (Shirvani et al., 2014; Mohsin et al., 2009) and *Agropyron elongatum* (Jafari et al., 2014), indicating that morphological traits provide useful information for the genetic conservation and utilization of future breeding programs in *A. pectiniforme*.

In this study, all of the traits expressed little differences between PCV and GCV, which indicated a low degree of environmental influence on the phenotypic expression of these characters. In this situation and based on the findings of Mazida et al. (2013), genetic variability could be successfully used through selection and hybridization among desirable lines for the development of superior genotypes. This means that selection based on these traits would be effective for future crossing programs. Estimates of heritability and genetic advance and variance

components depend on the population genetic and environmental factors (Majidi et al., 2009). All traits, including forage and seed yield showed more than 96% heritability, which indicated that selection based on these characters would be more effective and is efficient for advanced generations in future breeding programs. According to Tuhina-Khatun et al. (2015), these traits could be successfully transferred to offspring, if selection for these characters is performed in the hybridization program. On the basis of phenotypic performance, high heritability is a useful index to select superior genotypes (Maziida et al., 2013). The high heritability for dry weight and the number of stems per plant was observed in *Agropyron trichophorum* genotypes (Mohsin et al., 2009). Also, the highest heritability was reported for spike emergence time in *Agropyron trichophorum* (Mohsin et al., 2009) and *Dactylic glomerata* plants (Mohammadi et al., 2011). The highest heritability was found for plant height in the *Dactylic glomerata* (Mohammadi et al., 2011) and *Festuca arundinacea* (Ebrahimian et al., 2012) that indicated genetic variation could be more important than environmental variation. Moreover, the effect of the environment on expression of these traits is low. Our findings are in agreement with the results of these studies.

Johnson et al. (1955) suggested that heritability and genetic advance, when calculated together, would prove more useful in predicting the resultant effect of selection based on phenotypic expression. The high heritability and the low genetic advance were observed for the 1000-seed weight that revealed the non-additive gene action is effective in the expression of this trait. High heritability coupled with high genetic advance was observed for forage yield, plant height, and the number of stems per plant; therefore, the additive gene action controls the expression of these traits. With regard to genetic variation, heritability, and genetic advance it is deduced that forage yield, plant height, and the number of stems per plants are suitable parameters for selection of potential parents in the breeding programs.

Molecular Marker

With regard to the higher polymorphism of the five primers used in this study, it was suggested that these primers could be applied for detecting genetic variability of *A. pectiniforme* populations of Iran in future studies. The degree of polymorphism detected by RAPDs showed that this marker is efficient in detecting genetic variability between the studied populations. These results confirm the findings of studies by Vieira et al. (2004), Tuna et al. (2004), and Thaghizadeh et al. (2011) who reported the efficiency of RAPD markers in identifying populations of *Lolium multiflorum, Dactylis glomerata*, and *Agropyron desertorum*, respectively.

According to the AMOVA results, there was higher genetic variation between populations (53%) than within them (47%). This result is consistent with an outcrossing species with a different geographic distribution. Result of the AMOVA in a study of genetic diversity of switch grass populations using molecular marker indicated that most of the molecular variation (64%) exists among populations and lesser amounts were observed within populations (36%) (Cortese et al., 2010). Also in the study of genetic diversity in *Physaria bellii* using ISSR it was found that 76% of the total variation between populations and with lesser amounts within populations (24%) (Kothera et al., 2007), which are in agreement with these results. The high genetic variability among populations can help to improve the adaptation of plants. Low levels of intra-population differentiation could be contributed by unlimited gene flow (Behroozian et al., 2013) and geographic isolation (Nagl et al., 2011; Kamm et al., 2009). In general, it can be suggested for developing new varieties, the populations with high levels of genetic diversity could be used as a source for selection of desirable germplasm. In contrast to our results, genetic variation studies in various forage grasses using molecular markers indicated higher genetic variation within the populations than between populations (Che and Li, 2007; Refoufi and Esnault, 2008). The most important factors in the observed high degree of genetic diversity may be their open-pollination and different geographic distribution. P2 (Shahmirzad) and P13 (Gorgan) populations are recommended to be used for breeding improved varieties and heterosis in hybridization, because of higher genetic distance between them.

The proportion of variation explained by the first few PCoA was relatively low, indicating suitable genomic distribution for the markers under study. The results indicated that PCoA enables the recognition of different classes of forage plants. Thus, PCoA also confirms the results displayed by the Nei's UPGMA dendrogram. In other words, the genetic relationships delineated by cluster analysis were in agreement with the results obtained from PCoA. According to the results, it is suggested that RAPD markers are suitable for evaluating genetic variation within and among populations of *A. pectiniforme* germplasm. Information on genetic relationships obtained from this study could be useful for the selection of parents for future breeding programs or genetic studies.

Correlation between geographic, morphologic and molecular data

In this research, seed yield had significant and positive correlation with number of stems per plant and plant height. Considering the results of Mazid et al. (2013), seed yield is the quantitative trait that is influenced by genetic and interaction between genotype/environment. Moreover, this character is expressed late. Therefore, indirect selection using highly correlated characters is suggested for seed yield. The correlation between molecular, morphological traits and geographical data suggests that altitude was an important factor influencing genetic differentiation in *A. pectiniforme*. The higher genetic diversity among populations from high altitudes might be due to the reduced gene flow among these populations. A similar result was found by Byars et al. (2009) and Wang et al. (2006) for *Poa hiemata* and *Lolium perenne,* respectively. According to the correlation results, with increasing the heterozygosity and polymorphism forage, the yield is increased.

Populations of similar origin were grouped into various clusters thereby indicating a non-relationship between geographical and genetic diversity. In other words, the clustering of the populations under study in various groups was different from their geographical distribution. The geographic distribution and separation was not the only factor causing genetic diversity (Sihag et al., 2004).

Based on the regression and correlation analysis between genetic and morphologic distance it can be concluded that clustering of populations based on morphological characters was inconsistent with that derived from the RAPD markers analysis. The results suggested that the two marker systems gave different estimates of genetic relations among populations. Poor correlation of molecular marker diversity with phenotypic traits was detected in *Medicago* (Tucak et al., 2008), *Trifolium pratense* (Greene et al., 2004), and *Festuca arundinacea* (Amini et al., 2016).

The observed genetic differentiation between and within populations (Table 4) suggests that there was low gene flow among populations and it was in accordance with the geographic isolation of the populations. Similar to our finding, in the study of genetic variation in *Andropogon gerardii* using RAPD markers, Gustafson et al. (1999) demonstrated that genetic relationships among populations could not be predicted only with respect to the geographical vicinity. In contrast, in an evaluation of genetic diversity in Napier grass by RAPD and ISSR markers, Babu et al. (2009) showed that accessions from a common geographic origin grouped together in a cluster. Also, the Mantel test indicated that there was no relationship between RAPD marker and geographic distribution (Fig. V); therefore, populations were not grouped according to their geographical origin. This can also be seen in the UPGMA dendrogram which showed that some geographically close populations were clustered in different groups and far geographically distant populations were clustered in the same groups. In addition, the Mantel correlation coefficient is affected by sample size, number of alleles per locus, and the number of loci analyzed per individual (Landguth et al., 2012); the lack of relationship between the molecular marker and geographic data may be due to low sample size of the study. The high genetic variability observed in the present study indicates the RAPD markers are an efficient technique to study *A. pectiniforme* diversity. These results were expected because the populations under study were open-pollinated. On the other hand, RAPDs are a useful tool to identify appropriate populations for plant breeding programs in *Agropyron pectiniforme.*

Conclusions

This research showed that RAPD can be useful for detecting genetic diversity and quantifying genetic distances among Iranian populations of *A. pectiniforme.* RAPD markers will likely have a major impact on the conservation, management, and improvement of the studied populations. Considering morphological traits, it was found that the plant height and number of stems were appropriate indices for breeding programs. There was sufficient genetic variation among Iranian populations of *Agropyron pectiniforme* and these populations can be useful for germplasm improvement and cultivar development. Further studies are suggested to replicate the study using a larger sample to obtain a more accurate picture of the genetic diversity of *Agropyron pectiniforme.*

References

Amini, F., A.F., Mirlohi and **M.M., Majidi**. 2016. 'The possibility of use of AFLP molecular markers and phenotypic traits to increase forage yield in Tall Fescue (*Festuca arundinacea*) breeding'. *J Agri Sci Tech*, 18: 1419-1429.

Arghavani, A., A. Asghari, and **M., Shokrpour Chamanabad**. 2010. 'Genetic diversity in ecotypes of two *Agropyron* species using RAPD markers'. *Res J Environ Sci*, 4 (1): 50-56.

Babu, C., J., Sundaramoorthi, G., Vijayakumar and **S., Ganesh Ram**. 2009. 'Analysis of genetic diversity in Napier grass (*Pennisetum purpureum* Schum) as detected by RAPD and ISSR markers'. *J Plant Biochem Biot,* 18 (2): 181-187.

Behroozian, M., A., Jafari and **M., Farsi**. 2013. 'RAPD Analysis of Genetic Variation within and among natural populations of two species of *Dianthus* L. (Caryophyllaceae) in NE Iran'. *Iran J Bot,* 19 (2): 194-201.

Byars, S.G., Y. Parsons and **A.A., Hoffmann**. 2009. 'Effect of altitude on the genetic structure of an Alpine grass, *Poa hiemata.' Ann Bot,* 103: 885–899.

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

Cortese, L.M., J., Honig, C., Miller and **S.A., Bonos**. 2010. 'Genetic diversity of twelve swiss grass populations using molecular and morphological markers'. *Bioenergy Res,* 3: 262–271.

Dice, L.R. 1945. 'Measures of the amount of ecologic association between species'. *Ecology,* 26(3): 297-302.

Dorri, P., S. Khavari- Khorasani, M. Valizadeh and **P. Taheri.** 2014. 'The study of inheritance and gene effects on yield and agronomic traits of early generations of genetic maize Dehghan (KSC400)'. *Plant Gen Res.* 1. 2: 33-42. (In Persian)

Ebrahimian, M., M.M., Majidi and **A.F., Mirlohi**. 2012. 'Clonal evaluation and estimation of genetic similarity of tall fescue genotypes (*Festuca arundinaceae*)'. *J Plant Prod,* 19 (3): 91-108. (In Persian)

Falconer, D. S. 1989. 'Introduction to quantitative genetics'. Logman Scientific and technical logman house, Burnt Mill, Harlow, Essex, England.

Farshadfar, M. 2012. 'Genetic variability and Karyotype analysis of some *Agropyron* species'. *Ann Biol Res*, 3 (3):1515-1523.

Friebe, B., K.S., Gill, N.A., Tuleen and **B.S., Gill**. 1996.' Transfer of wheat streak mosaic virus resistance from *Agropyron intermedium* into wheat'. *Crop Sci,* 36:857-861.

Greene, S.L., M., Gritsenko and **G.,Vandemark**. 2004. 'Relating morphologic and RAPD marker variation to collection site environment in wild populations of red clover (*Trifolium pratense* L.).' *Genet Resour Crop Evol,* 51:643–653.

Grljusic, S., S., Bolaric, S., Popovic, T., Cupic, M., Tucak and **V., Kozumplik**. 2008. 'Comparison of morphological and RAPD markers in evaluation of red clover (*Trifolium pretense* L.) changes caused by natural selection'. *Period Biol,* 110 (3): 237-242.

Gustafson, D.J., D.J., Gibson and **D.L., Nickrent**. 1999. 'Random amplified polymorphic DNA variation among remnant big bluestem (*Andropogon geradii* Vitman) populations from Arkansas Grand Prairie'. *Mol Ecol,* 8: 1693-1701.

Jafari A. A., Elmi, A. and **Bakhtiari, M**. 2014.'Evaluation of yield and quality traits in 17 populations of tall wheatgrass *(Agropyron elongatum)* grown in rain fed area of Iran, under two cutting management'. *Romanian Agricultural Research* 31:49-58.

Johnson, H.W., H.F., Robinson and **R.E., Comstock.** 1955. 'Estimates of genetic and environmental variability in soybeans'. *Agron J,* 47: 314-318.

Kamm, U., P., Rotach, F., Gugerli, M., Siroky, P., Edwards and **R., Holderegger**. 2009.' Frequent longdistance gene flow in a rare temperate forest tree (*Sorbus domestica*) at the landscape scale'. *Heredity,* 103: 476–482.

Khan, S., K., Parveen and **S., Goyal**. 2011. 'Induced mutations in chickpea morphological mutants'. *Front Agr China,* 5: 35-39.

Kothera, L., C.M., Richards and **S.E., Carney**. 2007. 'Genetic diversity and structure in the rare Colorado endemic plant *Physaria bellii* Mulligan (Brassicaceae)'. *Conserv Genet,* 8: 1043–1050.

Landguth, E.L., B.C., Fedy, S.J., Oyler-Mccance, A.L., Garey, S.L., Emel, M., Mumma, H.H., Wagner, M.J., Fortin and **S.A., Cushman**. 2012. 'Effects of sample size, number of markers, and allelic richness on the detection of spatial genetic pattern'. *Mol Ecol Resour,* 12: 276-284.

Li, H., B., Jiang, J., Wang, Y., Lu, J., Zhang, C., Pan, X., Yang, X., Li, W., Liu and **L., Li.** 2017. 'Appling of novel powdery mildew resistance genes from *Agropyron cristatum* chromosome 2P'. *Theor Appl Genet,* 130 (1): 109-121.

Li, X., Y., Huang, J., Li and **H., Corke**. 2001. 'Characterization of genetic variation and relationships among Choix germplasm accessions using RAPD markers'. *Genet Resour Crop Evol*, 48: 189-194.

Majidi, M.M., A., Milohi and **F., Amini**. 2009. 'Genetic variation, heritability and correlations of agro-morphological in tall fescue (*Festuca arundinacea).' Euphytica*, 167: 323-331.

Mazid, M.S., M.Y., Rafii, M.M., Hanafi, H.A., Rahim and **M.A., Latif**. 2013. 'Genetic variation, heritability, divergence and biomass accumulation of rice genotypes resistant to bacterial blight revealed by quantitative traits and ISSR markers'. *Physiol Plant,* 149: 432-447.

Mohammadi, R., M.M., Khayyam-Nekouei, M.M., Majidi and **A.F., Mirlohi**. 2011. 'Estimation of yield potential and genetic variation of orchard grass genotypes (*Dactylic glomerata*)'. *J Crop Prod,* 3 (2): 139-158. (In Persian)

Mohsin, T., N., Khan and **F.N., Naqvi**. 2009. 'Heritability, phenotypic correlation and path coefficient studies for some agronomic characters in synthetic elite lines of wheat'. *J Food Agric Environ,* 7 (3&4): 278-282.

Nagl, N., K., Taski-Ajdukovic, A., Baburski, I., Seccareccia, D., Milic and **S., Katic**. 2011. 'Estimation of the genetic diversity in tetraploid alfalfa populations based on RAPD markers for breeding purposes'. *Int J Mol Sci,* 12: 5449-5460.

Nei, M. 1972. 'Genetic distance between populations'. *Am Nat,* 106: 283-292.

Padmaja, D., K., Radhika, S.L., Rao and **V., Padma**. 2008. 'Studies on variability, heritability and genetic advance for quantitative characters in rice (*Oryza sativa* L.)'. *J Genet Res,* 21(3):196-198.

Rahimi Analoujeh, N., A., Alizadeh and **A., Namayande.** 2014. 'RAPD molecular markers used to assess genetic and morphological diversity of some *Satureja hortensis* L.' masses. *JAEBS,* 4(1): 90-95.

Refoufi, A and **M.A., Esnault**. 2008. 'Population genetic diversity in the polyploidy complex of wheat grasses using isoenzyme and RAPD data'. *Biol Plant,* 52 (3): 543-547.

Rimoldi, F., P.S., Vidigal Filho, M.V., Kvitschal, M.C., Gonçalves-Vidigal, A.J., Prioli,S.M.A., Prioli and **T.R., Costa**. 2010. 'Genetic divergence in sweet cassava cultivars using morphological agronomic traits and RAPD molecular markers'. *Braz Arch Biol Technol,* 53(6): 1477-1486.

Saghai–Maroof, M.A., K.M., Soliman, R.A., Jorfenden and **R., Allars**. 1984. 'Ribosomal

DNA spacer-Length polymorphism in barley, Mendelian inheritance, chromosomal location, and population dynamics'. *PNAS,* 81: 8014-8018.

Salehi Shanjani, P., A.A., Jafari and **R., Jahanbaz**. 2015. 'Investigation of genetic variations among crested wheatgrass species base of agronomical traits and total leaf protein'. *Jour. Range Sci,* 5(3): 165-180.

Sharma, H.C., S.G., Aylward and **B.S., Gill**. 1987. 'Partial amphiploid from *Triticum aestivum* × *Agropyron scirpeum* cross'. *Bot Gaz,* 148 (2): 258-262.

Sharma, H.C. and **P.S., Baezinger**. 1986. 'Production, morphology and cytogenetic analysis of *Elymus caninus* (*Agropyron caninum*) × *Triticum aestivum* F1 hybrids and backcross-1 drrivatives'. *Theor Appl Genet,* 71: 750-756.

Sharma, H.C., B.S., Gill and **J.K., Uyemoto**. 1984. 'High levels of resistance in Agropyron species to Barley yellow dwarf and Wheat streak mosaic viruses'. *Phytopath,* 110: 143-147.

Shirvani, H., A.R., Etminan and **H., Safari.** 2014. 'Evaluation of variability and genetic parameters in morphological traits *Agropyron trichophorum* using multivariate statistical analysis'. *Int J Biol Sci,* 4 (2): 125-132.

Sihag, R., J.S., Hooda, R.D., Vashishtha and **B.P.S., Malik.** 2004. 'Genetic divergence in soybean (*Glycine max* L)'. *Ann Biol,* 20 (1): 17-21.

Singh, R.K., and **Chaudhary, B.D.** 1985. 'Biometrical methods in quantitative analysis'. Kalayani Publishers. New Delhi.

Szenejko, M. and **M., Rogalski**. 2015. 'Characterization of morphological triats and RAPD polymorphism in selected forms of Kentuchy bluegrass (*Poa pratensis*)'. *Biodiver Res Conserv,* 37: 1-10.

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

Tucak, M., S., Popovic, S., Grljusic, S., Bolaric and **V., Kozumplik**. 2008. 'Genetic diversity of alfalfa (Medicago spp) estimated by molecular markers and morphological characters'. *Period Biol*, 110 (3): 243-249.

Tuhina-Khatun, M., M.M., Hanafi, M.R., Yusop, M.Y., Wong, F.M., Salleh and **J., Ferdous**. 2015. 'Genetic Variation, Heritability, and Diversity Analysis of Upland Rice (*Oryza sativa* L.) Genotypes Based on Quantitative Traits'. *BioMed Res Int*: 1-7

Tun,a M., D.K., Khadka, M., Shrestha, K., Arumugahathan and **A., Golan-Goldhirsh**. 2004. 'Characterization of natural orchardgrass (*Dactulis glomerata* L.) Population of the Thraace Region of Turkey based on ploidy and DNA'. *Euphytica,* 135: 39-46.

Varshney, R.K., A., Garner and **M.E., Sorrells**. 2005. 'Genomics-assisted breeding for crop improvement'. *Trends Plant Sci,* 10: 621-630.

Vieira, E.A., C.M., Castro, A.C., Oliveira, F.I.F., Carvakho, P.D., Zimmer and **L.F., Martins**. 2004. 'Genetic structure of annual ryegrass (*Lolium multiflorum*) populations estimated by RAPD'. *Sci Agric,* 61 (4): 407-413.

Wang, J.L., Y.B., Gao, N.X., Zhao, A.Z., Ren, W.B., Ruah, L., Chen, J.L., Liu and **C.L., Li.** 2006. 'Morphological and RAPD analysis of the dominant species *Stipa krylovii* Roshev.' In inner Mongolia steppe. *Bot Stud,* 47: 23-35.