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Genetic variation among accessions of *Festuca arundinacea* Based on storage protein and soluble protein by SDS polyacrylamide gel electrophoresis

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Abstract

The genetic variation of seed storage protein and leaf soluble protein was assayed by SDS-PAGE for 14 accessions of *Festuca arundinacea*. Protein fragments of various molecular weights were separated in *Festuca* accessions. On the basis of the relative mobility of seed proteins on the gel, 9 bands and on the basis of the relative mobility of leaf proteins on the gel, 12 bands were detected in this study. The banding pattern revealed three regions. The greatest protein diversity was observed in area of 42-70 kDa. The majority of the similarity coefficients for seed protein and leaf protein between accessions were close to 0.78-0.82. This indicated the close relationships between the evaluated accessions, though they are collected from different origin. seed storage protein and leaf soluble protein based on UPGMA hierarchical clustering for grouping accessions based on Dice's coefficient were identified the three distinctive groups. Scatter plot for accessions based on first and second axis from principal coordinate analysis showed that genetic variation did not matching with the geographical distribution of accessions.

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Introduction

Tall fescue, a major forage and turf grass species in the temperate regions of the world, is a hexaploid (2n=6x=42) with a genome size of ≈5.27 to 5.83×106 kb (Rouf Mian *et al.,* 2002). Was reported a different genome size in 30 populations of tall fescue and mentioned that plant development related to DNA value (Behura, 2006). Knowledge of genetic variation is a useful tool in gene bank management, helping in the establishment of core collections, facilitating efficient sampling and utilization of germplasm (identifying and/or eliminating duplicates in the gene stock), and selecting of desirable genotypes to be used in breeding programs (Elham *et al.,* 2010; Vishwanath *et al.,* 2011). On the other hands, understanding genetic diversity of certain species is not only useful in addressing questions about evolutionary process and the development of conservation strategies, but also a prerequisite for efficient use of genetic resources in breeding programs. Interest in the genetic structure of natural populations of grass species has been increased in the last few years due to the necessity of broadening the knowledge of genetic variations in cultivated species (Che and Li, 2007). Characterization of germplasm using biochemical techniques (storage proteins and isozymes) has received a great attention in the last decades. This attention was attributed to the increased recognition of germplasm resources in croplands improvement. Genetic markers are useful for screening germplasm with the minimum cost in time and labour (Nakajima, 1994). The qualitative traits of the seed proteins obtained by electrophoresis have been successfully used to assess the genetic variation among the accessions of the wild species (Elham *et al.,* 2010; Vishwanath *et al.,* 2011). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS/PAGE) is among the biochemical technique that is widely used due to its simplicity and effectiveness for describing the genetic structure of the accessions of wild plant species. Seed storage proteins have been used as genetic markers in: (1) identifying variation among the taxa of each species; (2) screening the purity of the ever expanding umber of cultivars; (3) establishing genome relationships; (4) exploiting the important traits of landraces and wild relatives to provide increasing crop production and stabilizing yield (Sammour, 1991), and (5) using information on genetic diversity to make decisions regarding selection of superior genotypes for improvement yield of plants through breeding. Protein electrophoresis is considered a reliable, practical and reproducible method because seed storage proteins are the third hand copy of genomic DNA and largely independent of environmental fluctuations (Sammour, 1987; Javaid *et al.,* 2004; Iqbal *et al.,* 2005). Afkar *et al.,* (2010) revealed significant variability among Iranian *Festuca* accessions for seed storage proteins. Efficiency of seed storage protein and leaf soluble protein using Sodium dodecyl sulphate polyacrylamide gel electrophoresis was reported by other researchers to determine of genetic diversity between and within different plant species (Valizadeh M, 2001; Arsalan and Ertugrul, 2010; Rdawan *et al.,* 2013). The purpose of this study was to evaluate the genetic diversity 14 accessions of *Festuca arundinacea* using storage protein and soluble protein.

Materials and methods

Plant materials

In order to evaluation of genetic variation, 14 accessions of *F. arundinacea* were prepared from gene bank of Research Institute of Forests and Rangelands, Tehran, Iran (Table 1).

Protein extraction

Seed protein

Protein extraction was performed according to Saraswati *et al.,* (1993). Seeds were ground to fine powder with a pestle and mortar. Sample buffer was added to 0.5 g of seed flour as extraction liquid and mixed thoroughly in an Eppendorf tube by vortex. The extraction buffer contained the following final concentration: 0.5 M Tris-HCl (pH 6.8), 2.5% SDS, 5% urea, and 5% 2-merkaptoethanol. Before centrifugation at 12,000 ×g for 5 min (4°C), the sample buffer was boiled (95°C) for 4 min. A total volume of 40µl protein extract solution was loaded into each well and electrophoresis was carried out at

120V until the bromophenol blue dye reaches the bottom of the gel.

Leaf protein

Approximately 1 g of fresh leaf or root tissues was homogenized first in liquid nitrogen. To extract total proteins, leaf powder was added with protein extraction buffer (0. 5 M Tris (pH=7.5), 0.02 Na_2EDTa and 4% (V/V) 2-mercaptoethanol) centrifuged at 13,000 rpm for 10 minutes. For the electrophoretic separation, 40µL of the samples were applied in different lanes of the gel. The same volume of protein marker was applied in a separate lane of the gel, in order to allow the estimation of the molecular masses of the separated proteins.

Protein analysis

Seed proteins and leaf proteins were quantitatively estimated in each sample by the method of Bradford, (1976). The final concentration was adjusted to 40 µg/µl protein in sample buffer. The extracts were denaturated in 2X sample buffer (1M Tris-HCl pH = 6.8, 2% SDS, 20% glycerol, 0.02% BPB, 5% 2-mercaptoethanol), and heated at 100 ^oC for 4 minutes. Seed storage proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDSPAGE) in gel of 12.5% concentration (Laemmli, 1970). Leaf proteins were separated by carrying out electrophoresis in the discontinuous buffer system through vertical slab type SDS-PAGE using 10% polyacrylamide gel according to the method of Laemmli, (1970). After electrophoresis the proteins were stained with 50% methanol, 7% acetic acid and 1% Coomassie brilliant blue (CBB) R-250.

Data analysis

After staining the gels, the number of monomorphic and polymorphic protein bands was counted for each sample. The presence (1) or absence (0) of polypeptide bands was entered in a binary data matrix for use in cluster analysis. Similarity matrix, Cluster analysis and Principal coordinate analyses was also performed using the computer software NTSYS and DARwin 5.

Results

Seed storage protein

In this study SDS-PAGE of total seed storage proteins of 14 accessions was performed by extracting protein and running on the gel to investigate genetic diversity. On the basis of the relative mobility of seed proteins on the gel, 12 bands were detected in this study, which were used for examining the genetic diversity (Fig. 1). 9 major bands were recorded out of total 12 bands detected, while 66.66% of total were polymorphic. The banding pattern revealed large variations among accessions. Among which bands 3, 5 and 8 were common in most accessions but all the other bands showed diversity in most of the accessions. Electrophorograms which showing proteins banding pattern of different accessions are presented. The banding pattern revealed three regions. Region I comprised of 2 bands of above 70.0 kDa molecular weight (MW), Region II consisted of 4 bands lying between 42.0 and 70 kDa, while Region III had 3 bands between 14.0 kDa and 42.0 kDa. The bands below 14.0 kDa were diffused and not considered for analysis purpose. Accessions 10 and 8 had the most and accession 12 had the lowest band. Average percentage of polymorphism was equal to 66.66%, the lowest percentage of polymorphism was 44.44% and belonged to accession 14(Ardebil) and the highest was 100% and belonged to accessions 10(Kamyaran) and 8(Brojen) respectively.

Similarity Matrix

The average of similarity between accessions was 0.82. Similarity matrix based on Dice coefficient (Table 2), showed the highest genetic similarity between accessions 1(Baneh) and 4(Australia), 1(Baneh) and 7(Esfahan), 3(Ireland) and 9(Tavankesh), 4(Australia) and 7(Esfahan), 5(Gonabad) and 11(Tavankesh), 8(Brojen) and 10(Kamyaran) from group 2 with a similarity coefficient 1 and the lowest similarity belong to accession 12(Kamyaran) from group 1 with accession 6(Esfahan) from group 3 with similarity coefficient 0.50.

Grouping

UPGMA clustering for grouping accessions based on Dice's coefficient (Fig. 2) were identified the three distinctive groups. The first group consisted of accessions 2 (Brojen), 12 (Kamyaran), and 13 (Ireland), which the average similarity was 0.86 for this group. The second group included accessions of 1(Baneh), 3(Ireland), 4(Australia), 7(Esfahan), 9(Tavankesh) and 11(Tavankesh), which the average similarity coefficient was 0.94 for this group. The third group consisted of 6(Esfahan), 8(Brojen), 14(Ardebil) and 10(Kamyaran). The average of Dice's coefficient was 0.90 for this group. Scatter plot for accessions based on first and second axis from principal coordinate analysis (Fig. 3) showed that genetic variation did not matching with the geographical distribution of accessions. These results confirmed by cluster analysis and similarity matrix.

Table 1. Gen bank cod and origin of accessions of *F. arundinacea*.

Gen bank cod	Origin	Gen bank cod	Origin
1	Baneh	8	Brojen
2	Brojen	9	Tavankesh
3	Ireland	10	Kamyaran
4	Australia	11	Tavankesh
5	Gonabad	12	Kamyaran
6	Esfahan	13	Ireland
7	Esfahan	14	Ardebil

Table 2. Dice similarity coefficients between accessions of *F. arundinacea* based on seed storage protein.

accession	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1													
2	0.67	1												
3	0.92	0.73	1											
4	1.00	0.67	0.92	1										
5	0.93	0.77	0.86	0.93	1									
6	0.71	0.67	0.77	0.71	0.80	1								
7	1.00	0.67	0.92	1.00	0.93	0.71	1							
8	0.88	0.71	0.80	0.88	0.94	0.88	0.88	1						
9	0.92	0.73	1.00	0.92	0.86	0.77	0.92	0.80	1					
10	0.88	0.71	0.80	0.88	0.94	0.88	0.88	1.00	0.80	1				
11	0.93	0.77	0.86	0.93	1.00	0.80	0.93	0.94	0.86	0.94	1			
12	0.73	0.89	0.80	0.73	0.67	0.55	0.73	0.62	0.80	0.62	0.67	1		
13	0.83	0.80	0.73	0.83	0.77	0.50	0.83	0.71	0.73	0.71	0.77	0.89	1	
14	0.93	0.62	0.86	0.93	0.88	0.80	0.93	0.94	0.86	0.94	0.88	0.67	0.77	1

Leaf soluble protein

The Electrophoregram revealed 106 polypeptide bands of different insensitivity; most of them were polymorphic. The greatest protein diversity was observed in area of 42-70 kDa. On the basis of the relative mobility of leaf proteins on the gel, 15 bands were detected in this study (Fig. 4). 12 major bands were recorded out of total 15 bands detected, while 83.33% bands of total were polymorphic. The percentage of polymorphic bands observed for accessions of each origin/source was associated with the number of samples assayed. The banding pattern revealed three regions. Region I comprised of 3 bands of above 70.0 kDa molecular weight, Region II consisted of 6 bands lying between 42.0 and 70 kDa, while Region III had 3 bands between 14.0 kDa and

42.0 kDa. The bands below 14.0 kDa were diffused and not considered for analysis purpose. Among which bands 2 and 4 were common in most accessions but all the other bands showed diversity in most of the accessions. The most number of bands belonged to accessions 12(Kamyaran) and 4(Australia) and the least number related to accessions 2(Brojen) and 14(Ardebil), with 11 and 8 bands respectively.

Table 3. Dice similarity coefficients between accessions of *F. arundinacea* based on leaf soluble protein.

accession	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1													
2	0.78	1												
3	0.90	0.78	1											
4	0.95	0.84	0.95	1										
5	0.90	0.78	0.90	0.95	1									
6	0.84	0.82	0.84	0.90	0.84	1								
7	0.84	0.82	0.84	0.90	0.84	0.78	1							
8	0.90	0.89	0.90	0.95	0.90	0.95	0.84	1						
9	0.90	0.89	0.90	0.95	0.90	0.84	0.95	0.90	1					
10	0.84	0.94	0.84	0.90	0.84	0.78	0.89	0.84	0.95	1				
11	0.84	0.82	0.84	0.90	0.84	0.89	0.78	0.95	0.84	0.78	1			
12	0.86	0.74	0.86	0.91	0.86	0.90	0.80	0.86	0.86	0.80	0.80	1		
13	0.90	0.78	0.90	0.95	0.90	0.95	0.84	0.90	0.90	0.84	0.84	0.95	1	
14	0.89	0.75	0.78	0.84	0.78	0.94	0.71	0.89	0.78	0.71	0.82	0.84	0.89	1

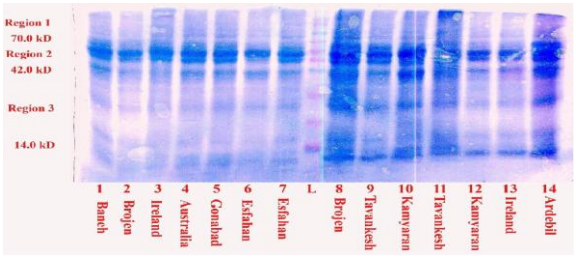

Fig. 1. Electrophorogram of 12.5% polyacrylamide gel banding pattern showing diversity in seed storage proteins of *F. arundinacea*.

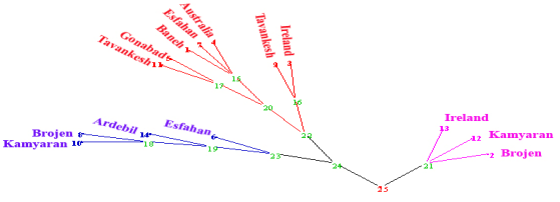

Fig. 2. Dandrogram based on SDS-PAGE of 14 accessions of *F. arundinacea* by UPGMA method.

Similarity Matrix

Dice's similarity coefficients were based on the data of SDS/PAGE profiles of the evaluated accessions (Table 3). It ranged from 0.95 {between accessions 1(Baneh) and 4(Australia), 4(Australia) and 5(Gonabad), 4(Australia) and 8(Brojen), 4(Australia) and 9(Tavankesh), 4(Australia) and 13(Ireland), 12(Kamyaran) and 13(Ireland)} to 0.70 {between accessions 14(Ardebil) and 7(Esfahan), 14(Ardebil) and 10(Kamyaran)}. It was noticed that the majority of the similarity coefficients between accessions was close to 0.78. This indicated the close relationships between the evaluated accessions, though they are collected from different origin.

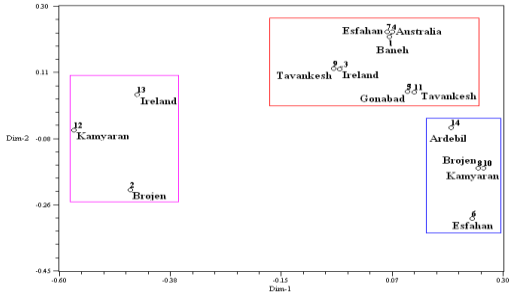

Fig. 3. Scatter diagram 14 accessions of *F. arundinacea* based on SDS-PAGE.

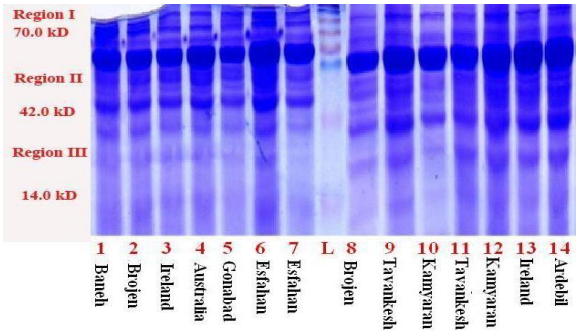

Fig. 4. Electrophorogram of 10% polyacrylamide gel banding pattern showing diversity in leaf soluble proteins of *F. arundinacea*.

Grouping

Based on cluster analysis by UPGMA method accessions were classified to three groups. The first group consisted of 6 accessions {1(Baneh), 3(Ireland), 4(Australia), 5(Gonabad), 12(Kamyaran) and 13(Ireland)}, and the second group included 4 accessions {6(Esfahan), 8(Brojen), 11(Tavankesh) and 14(Ardebil)} and the third group consisted of 4 accessions {2(Brojen), 7(Esfahan), 9(Tavankesh) and 10(Kamyaran)} (Fig. 5). The results of principal coordinate analysis for accessions confirmed the classification the cluster analysis (Fig. 6).

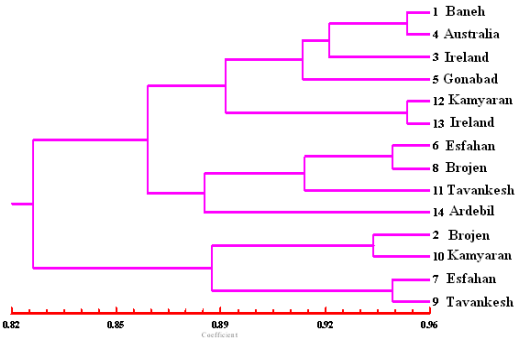

Fig. 5. Dandrogram based on SDS-PAGE of 14 accessions of *F. arundinacea* by UPGMA method.

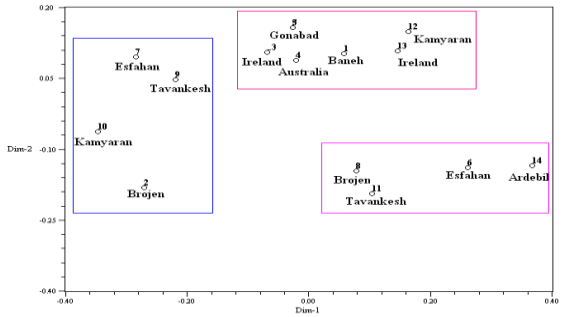

Fig. 6. Scatter diagram 14 accessions of *F. arundinacea* based on SDS-PAGE.

Discussion

The pool of genetic variation within accessions of this species is the basis for selection as well as for plant improvement. A better understanding of genetic diversity and its distribution in the accessions of the studied plant is essential for its conservation and use. It will help greatly in determining what to conserve as well as where to conserve, and will enhance our knowledge and understanding of the taxonomy, origin and evolution of *F.arundinacea*. SDS-PAGE is one of the most widely used techniques to separate and characterize the proteins and used to estimate the extent of genetic diversity in the present set of *Festuca* germplasm. We found low genetic diversity within available Iranian *Festuca* accessions which is contrary to the findings of Afkar *et al.,* (2010), who reported high level of genetic diversity within *Festuca* accessions. Polypeptides migrate according to molecular weight on SDS gels so the molecular weight of polypeptides may be easily and rapidly estimated. The analysis of the results reveals that some bands are characteristic and constant markers for each accession and allow the unequivocal identification of their electrophoregrams. Other bands are shared by more than one accession. The seed storage protein profiles could be used as a valuable marker to evaluate the genetic diversity and used to identify the quality accessions, improve and conserve the efficiency of those accessions. In the present study the results showed low variation among accessions because most of the proteins bands were common to all accessions. As revealed by the dendrogram, most of the accessions were in the same cluster showing the low diversity at genomic level for seed and leaf proteins. Similar findings were also reported by Ahmad *et al.,* (1996) who observed a low level of genetic variation in lentil. Singh *et al.,* (1996) reported little variation for protein bands in groundnut, which indicated that most of the accessions were the members of same conservative species. Cluster analysis and Principal component analysis is useful as it gives information about the groups where certain traits are more important allowing the breeders to conduct specific breeding programs. Studied accessions were clustered in tree

groups based on UPGMA clustering method. Grouping of accessions based on cluster analysis and principal coordinate analysis indicated that genetic variations do not in agreement with the geographical distribution of accessions. The dendrogram as a whole revealed very low genetic distance at protein level reflecting the similarity of genes responsible for the seed storage proteins and leaf soluble protein.

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