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# AFLP Analyses of Genetic Variation in Iranian Fescue Accessions

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**Abstract:** Fescues (*Festuca* sp.) are widely distributed in the temperate regions of the world representing a vast resource for genetic improvement of pasture and turf grass cultivars. In Iran despite wide geographical occurrence, no report is available on genetic diversity of fescue accessions and their similarity with accessions of other countries. In this study Amplified Fragment Length Polymorphism (AFLP) was used to detect the genetic diversity and relationships of 34 fescue accessions representing four species. Twelve primer combinations resulting in 421 polymorphic markers were used to differentiate this collection. Genetic Similarity Coefficient (SC) between accessions ranged from 0.19 to 0.88 showing high level of diversity. Both the Unweighted Pair Group Method with Arithmetic average (UPGMA) dendrogram and principal component analysis clearly separated accessions in distinguished groups. At the SC value of 0.28, AFLP markers could separate the coarse fescues (*F. arundinacea* and *F. pratensis*) from the fine fescues (*F. rubra* and *F. ovina*). At the SC value of 0.42, the accessions were grouped into four major clusters each corresponding to a separate species and accessions with same geographic region had larger SC value in each cluster. Tall fescue accessions were clustered in six subgroups that largely supported the known origins and some morphological characteristics of these accessions. Results indicated that AFLP marker system proved to be highly effective in discriminating a very diverse fescue collection. Iranian fescue accessions contains a high degree of genetic variability and very much diverged from accessions of other geographical regions. This broad genetic diversity can be exploited in breeding programs.

**Key words:** AFLPs, genetic diversity, similarity, *Festuca arundinaceae*

## INTRODUCTION

The *Festuca* sp. genus contains approximately 100 species that some are commonly used as forage and turf grasses. Based on leaf texture these are divided in two subgeneric types, including the coarse fescues e.g., *F. arundinacea* and *F. pratensis* and fine fescues e.g., *F. rubra* and *F. ovina* (Turgen, 1985). Tall fescue (*Festuca arundinacea* Schreb) is the most important perennial forage and turf grass species of the genus and is widely grown throughout the temperate regions of the world (Sleper, 1985; Saha *et al*., 2005). It is an allohexaploid (2n = 6x = 42) with the $PPG_1G_1G_2G_2$ genomic constitution. Meadow fescue (*F. pratensis* Huds) is believed to be the donor of the P genome and tetraploid fescue (*F. arundinace* var glaucescens Bioss) the donor of the G1G2 genome (Xu *et al*., 1994). Both species are prevalent in Iran and mainly grow in natural rangelands of central, west and north regions (Khayyam-Nekouei, 2001). In regions with lower precipitation they are found along irrigation ditches and farm levees. Understandings inter and interaspecific genetic diversity in wild germplasm collections of *Festuca* can greatly facilitate reliable classification of accessions and identification of subsets of core accessions with possible utility in breeding programs.

Among molecular techniques for genetic assessment, Amplified Fragment Length Polymorphisms (AFLP) is a DNA marker system based on combination of PCR and restriction enzyme analyses and reveals high level of polymorphism. AFLP is highly reproducible, less sensitive to reaction conditions and does not require DNA sequence information (Vos *et al*., 1995; Krauss and Peakall, 1998). The AFLP markers have been successfully

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used to determine genetic diversity and characterized cultivars and accessions of forage and turf grasses including outbreeding species (Sweeny and Danneberger, 1997; Guthridge *et al.*, 2001; Vergara and Bughrara, 2003; 2004; Wu *et al*., 2004).

Mian *et al*. (2002) used AFLP markers to determine genetic diversity and to distinguish 18 populations of tall fescue from USA, using DNA bulk strategy. In this study we used AFLP to evaluate the genetic diversity between 34 accessions comprising of four *Festuca* species. We compared the Iranian accessions to a subset of accessions obtained from Hungary and studied the genetic relationships among the species.

## MATERIALS AND METHODS

**Plant materials:** Twenty five *F. arundinacea*, four *F. pratensis*, four *F. rubra* and one *F. ovina* accessions were used in this experiment. Detailed information is shown in Table 1. Iranian accessions were collected from different geographical regions or obtained from Agricultural Biotechnology Research Institute of Central Regions of Iran (ABRICI). Accessions from Hungary, Poland and USA were kindly provided by Hungarian Institute For Agrobotany (HIFA), Tapioszele, Hungary. All accessions were germinated in a greenhouse at Isfahan University of Technology.

**DNA extraction and AFLP profiling:** For DNA extraction, young leaf tissue were equally sampled from 30 plants of each accession and bulked together. Genomic DNA was isolated according the procedure described by Dellaporta *et al.* (1983). DNA was quantified by spectrophotometer readings and its quality was checked by agarose gel electrophoresis.

For AFLP analysis, isolated genomic DNA (approximately 300 ng) was digested with *Eco*RI and *Mse*I restriction enzymes at 37°C for 3 h. The restricted DNA fragments were ligated to *Eco*RI and *Mse*I adaptors overnight at 37°C and the product was diluted (1:5). Pre-amplification reactions were performed with *Eco*RI+*C* and *Mse*I+*C* AFLP primers. The amplification products were diluted (1:5) and stored at -20°C until used for selective amplification. Selective amplification was down with 12 combinations of *Eco*RI+*3* and *Mse*I+*3* primers (Table 1). Selective amplifications were performed in a final volume of 20 μL containing 4 μL of the diluted pre-amplification product, 15 ng of the *Eco*RI and *Mse*I primers, 1X PCR buffer, 20 mM $MgCl_2$, 1.0 unit Taq polymerase and 0.2 mM dNTPs (deoxynucleotide triphosphates).

The selective amplification product was mixed with 10 μL of the loading buffer and the mixture was denatured at 95°C for 4 min and immediately placed on ice. Five microliter of the denatured samples was loaded on a 6% polyacrylamide gel containing 7M urea and electrophoresis was conducted with constant power (100 W) at a constant temperature of 50°C for 2.5 h in a Biometra S2 sequencing gel. After electrophoresis, gels were fixed for 30 min in 10% acetic acid and immediately afterwards, stained with silver nitrate (Pillay and Myers, 1999).

**Data analysis:** For data analysis, AFLP bands throughout the gel profile were scored as present (1), absent (0) or ambiguous (9) at least twice. The NTSYSpc v.2.02 software was used to generate genetic similarity matrixes, create dendrogram and corresponding cophenetic matrixes and calculate cophenetic correlation (Rohlf, 1997). Cophenetic matrix correlation values was calculated to measure goodness of fit of the tree matrix and were interpreted according to Rohlf (1997) as follows: less than 0.7, very poor fit; 0.7-0.8, poor fit; 0.8-0.9, good fit; and 0.9-1.0, very good fit. Genetic similarities were calculated based on the Jaccard's (1908) coefficients (Jaccard, 1908). Dendrogram were generated with the Unweighted Pair Group Method using Arithmetic average (UPGMA) clustering method. Principal Component Analysis (PCA) was also conducted to identify the number of groups based on Eigen vectors.

## RESULTS AND DISCUSSION

A total of 493 fragments were scored from 12-primer combinations with most bands ranging in size from 50 to 500 bp (Table 1). Of the 493 bands scored, 421 (85.4%) were polymorphic. The number of polymorphic bands for each primer combination varied from 10 to 75. The E-ATG/M-CCT primer combination produced the greatest number of polymorphic fragments (75 bands), while the E-AAG/M-CTC primer combination produced the lowest number of polymorphic fragments (10 bands). Most of the primer combinations tested in this study revealed workable patterns and can be used in future studies to estimate genetic variation between other fescue populations. For this set of primers none of the fescue accessions shared identical DNA marker profile indicating the collection doesn't contain duplications. The high level of polymorphism has facilitated analysis of the genetic diversity among accessions. Specific AFLP markers were also found for some species. Affirmation of these markers in other collections may assist in developing specific probes to effectively discriminate fescue species.

Table 1: Primer combinations for pre and selective-amplification, and bands scored in AFLP profiling

| No. | Primer combinations* | Total bands | Polymorphic bands | Monomorphic band | Polymorphic bands (%) |
|---|---|---|---|---|---|
| 1 | E-AAA , M-CGA | 47 | 43 | 4 | 91.4 |
| 2 | E-ATG , M-CCT | 78 | 75 | 3 | 96.1 |
| 3 | E-AAC , M-CGG | 20 | 17 | 3 | 85.0 |
| 4 | E-AAT , M-CGT | 66 | 61 | 5 | 92.4 |
| 5 | E-ATC , M-CGC | 56 | 50 | 6 | 89.2 |
| 6 | E-ATA , M-CTA | 48 | 42 | 6 | 87.5 |
| 7 | E-AGG , M-CCC | 17 | 13 | 4 | 76.4 |
| 8 | E-AAG , M-CTC | 31 | 10 | 21 | 32.2 |
| 9 | E-AAT , M-CTG | 35 | 30 | 5 | 85.7 |
| 10 | E-AAT , M-CTT | 30 | 27 | 3 | 90.0 |
| 11 | E-AGG , M-CAA | 45 | 41 | 4 | 91.1 |
| 12 | E-ACT , M-CAC | 20 | 12 | 8 | 60.0 |
| Total | | 493 | 421 | 72 | 85.4 |
| Mean | | 41 | 35 | 6 | 85.4 |

*E=pre-amplification primer of *EcoRI* (5'-GACTGCGTACCAATTC-3'); M=pre-amplification primer of *MseI* (5-'GATGAGTCCTGAGTAA-3').

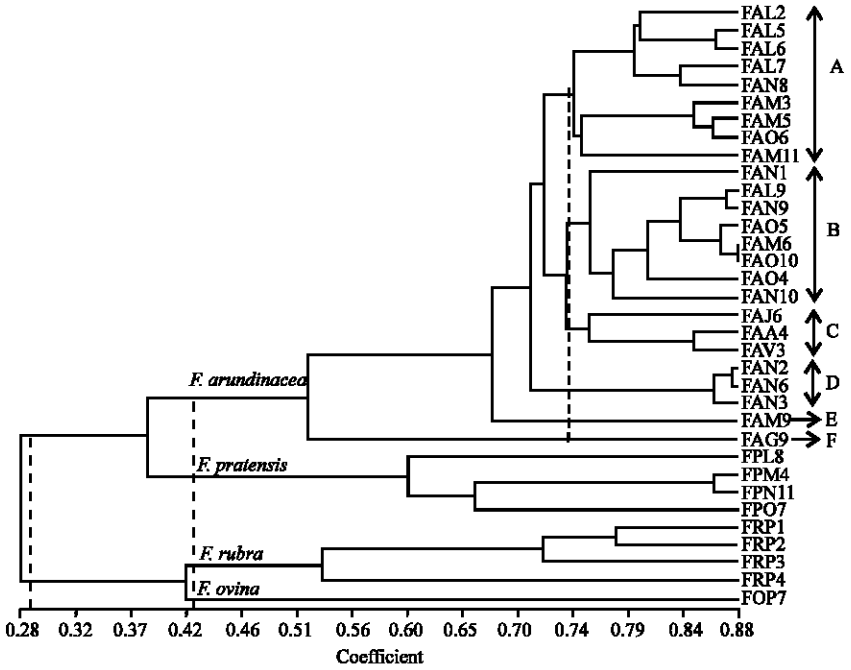


Fig. 1: UPGMA dendrogram of the 34 accessions of 4 *Festuca* sp. based on AFLP markers. For name and origin of the accessions (Table 1)

Genetic Similarity Coefficients (SC) based on AFLP markers ranged from 0.19 to 0.88 in these accessions (Table 3). The highest SC (0.88) for pair wise comparisons among the accessions was between two tall fescue accessions (FAM6 and FAO10) from Hungary. These two accessions were very similar for morphological and agronomical characteristics as well (Data not shown). The lowest SC value (0.19) was for the pair wise comparisons of FPM4 (a *F. pratensis* from Iran) and FRP4 (a *F. rubra* from Hungary). This was expected due to large variability in the genomic constitution of the two species and distinct geographical origin.

The UPGMA cluster tree generated by similarity coefficients matrix is depicted in Fig. 1. To test the dendrogram goodness of fit, the cophenetic correlation between the similarity matrix and the corresponding cophenetic matrix (computed from the tree matrix that generated the dendrogram) was calculated and interpreted according to Rohlf (1997). The results were plotted in a phenogram shown in Fig. 2. The coffenetic correlation was 0.97 indicating the high goodness of fit of the similarity indices. According to this interpretation the patterns evident in Fig. 1 were deemed significant because the correlation between the SC matrix and the cophenetic matrix was very good at 0.97.

At SC of 0.28, AFLP markers could separate the coarse fescues (*F. arundinacea* and *F. pratensis*) from the fine fescues (*F. rubra* and *F. ovina*) (Fig. 1). This

Table 2: Information of fescue accessions used for AFLP analysis of variation

| Num. | Accession name | Accession code | Species | Origin |
|---|---|---|---|---|
| 1 | FAL2 | 6000.39 | *F. arundinacea* | Yazdabad, Iran |
| 2 | FAL5 | 6000.13 | *F. arundinacea* | Semirom, Padena, Iran |
| 3 | FAL6 | 6000.78 | *F. arundinacea* | Yasooj, Iran |
| 4 | FAL7 | ANON1 | *F. arundinacea* | Semirom, Iran |
| 5 | FAL9 | RCAT064772 | *F. arundinacea* | Tyukod, Hungary |
| 6 | FAM3 | 6000.11 | *F. arundinacea* | Fozveh, Iran |
| 7 | FAM5 | 6000.30-1 | *F. arundinacea* | Fozveh, Iran |
| 8 | FAM6 | RCAT042281-1 | *F. arundinacea* | Pakozd, Hungary |
| 9 | FAM9 | 6000.112 | *F. arundinacea* | Daran, Iran |
| 10 | FAM11 | 10D2 | *F. arundinacea* | Mobarakeh, Iran |
| 11 | FAN1 | 14D-Rebel | *F. arundinacea* | NewJersey, USA |
| 12 | FAN2 | 6000.79 | *F. arundinacea* | Semirom, Iran |
| 13 | FAN3 | RCAT064767-1 | *F. arundinacea* | Pacin, Hungary |
| 14 | FAN6 | ANON3-3D | *F. arundinacea* | Gelogerd, Iran |
| 15 | FAN8 | ANON2 | *F. arundinacea* | Charmahal, Iran |
| 16 | FAN9 | RCAT042279-1 | *F. arundinacea* | Kecskemet-Solt, Hungary |
| 17 | FAN10 | 6477 | *F. arundinacea* | Anon, Hungary |
| 18 | FAO4 | RCAT042279-2 | *F. arundinacea* | Kecskemet-Solt, Hungary |
| 19 | FAO5 | RCAT064767-2 | *F. arundinacea* | Pacin, Hungary |
| 20 | FAO6 | 6000.38 | *F. arundinacea* | Yazdabad, Iran |
| 21 | FAO10 | RCAT041815-1 | *F. arundinacea* | Sarkad, Hungary |
| 22 | FAA4 | 1000.52 | *F. arundinacea* | Fozveh, Iran |
| 23 | FAG9 | 1000.247 | *F. arundinacea* | Fozveh, Iran |
| 24 | FAJ6 | 12000.26 | *F. arundinacea* | Anonymous, Poland |
| 25 | FAV3 | 4000.44 | *F. arundinacea* | Semnan, Shahrood, Iran |
| 26 | FPL8 | 6000.7 | *F. pratensis* | Fozveh, Iran |
| 27 | FPM4 | 6000.67 | *F. pratensis* | Borojen(Nasirabad), Iran |
| 28 | FPN11 | 2D | *F. pratensis* | Koohrang(Babaheidar), Iran |
| 29 | FPO7 | 6000.81 | *F. pratensis* | Gorgan, Iran |
| 30 | FRP1 | RCAT042391 | *F. rubra* | Tarhos, Hungary |
| 31 | FRP2 | RCAT042387 | *F. rubra* | Derekegyhaz, Hungary |
| 32 | FRP3 | 6000.21 | *F. rubra* | Fozveh, Iran |
| 33 | FRP4 | RCAT042393 | *F. rubra* | Veszto, Hungary |
| 34 | FOP7 | 6000.107 | *F. ovina* | Semirom, Iran |

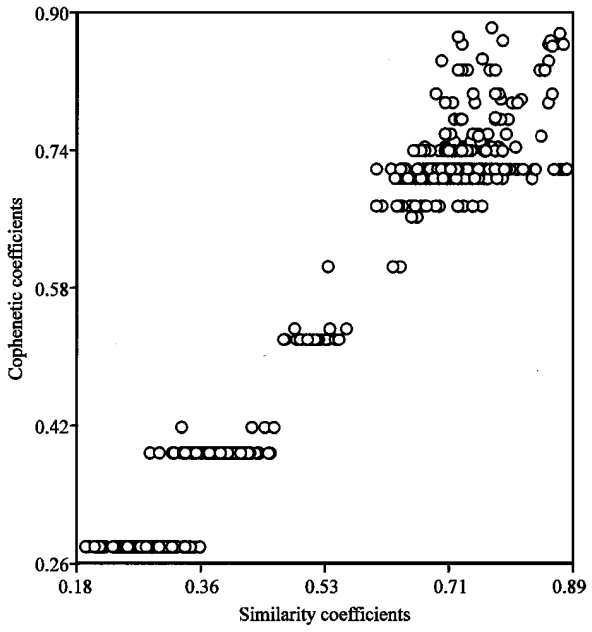


Fig. 2: Plot analysis of cophenetic coefficient correlations and similarity coefficients as a measure of goodness of fit of the similarity indices. r = 0.97 (= normalized Mantel statistic Z); Approximate Mantel t-test: t = 7.7371; p (Z < obs. Z: p = 1.0000)

shows how closely tall and meadow fescue are related and may further support the notion of meadow fescue being one of the tall fescue ancestors. Also, at this SC value, *F. rubra* and *F. ovina* were grouped in the same cluster showing more similarity between genomic constitutions of these two species. At 0.42 SC, the accessions were grouped into four major clusters each corresponding to a separate species (Fig. 1). The only one *F. ovina* accession in this study separated from other accessions in cluster 1. This clearly shows greater interspecific than interaspecific variation at the genomic level, even though accessions of one species may have originated from distinct geographical regions. Cluster 2 consisted of the four *F. rubra* accessions. The AFLP could separate the Iranian accession FRP3 from the other three Hungarian accessions in this cluster. This reflects the possible role of geographical region in creating interaspecific variability. Cluster 3 included all *F. pratensis* accessions. The FPM4 and FPN11 had highest similarity in this cluster. These two accessions were also related in terms of geographical locations (Table 2). Cluster 4, the largest in this grouping, included all 25 accessions of tall fescue. Genetic similarity coefficient in this cluster ranged from 0.47 to 0.88. With a few exceptions in this cluster, most accessions have fallen into sub clusters congruent with

Table 3: Jacard genetic similarity coefficients for 34 fescue accessions based on AFLP

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1.FAL2 | 1.00 | | | | | | | | | | | | | | | | |
| 2.FAL5 | 0.79 | 1.00 | | | | | | | | | | | | | | | |
| 3.FAL6 | 0.81 | 0.85 | 1.00 | | | | | | | | | | | | | | |
| 4.FAL7 | 0.80 | 0.81 | 0.85 | 1.00 | | | | | | | | | | | | | |
| 5.FAL9 | 0.72 | 0.70 | 0.75 | 0.77 | 1.00 | | | | | | | | | | | | |
| 6.FAM3 | 0.73 | 0.73 | 0.75 | 0.78 | 0.71 | 1.00 | | | | | | | | | | | |
| 7.FAM5 | 0.73 | 0.73 | 0.73 | 0.78 | 0.75 | 0.85 | 1.00 | | | | | | | | | | |
| 8.FAM6 | 0.74 | 0.68 | 0.75 | 0.77 | 0.81 | 0.70 | 0.73 | 1.00 | | | | | | | | | |
| 9.FAM9 | 0.68 | 0.64 | 0.66 | 0.68 | 0.66 | 0.73 | 0.76 | 0.70 | 1.00 | | | | | | | | |
| 10.FAM11 | 0.74 | 0.71 | 0.72 | 0.76 | 0.76 | 0.72 | 0.74 | 0.76 | 0.72 | 1.00 | | | | | | | |
| 11.FAN1 | 0.73 | 0.69 | 0.74 | 0.74 | 0.81 | 0.69 | 0.72 | 0.76 | 0.65 | 0.75 | 1.00 | | | | | | |
| 12.FAN2 | 0.68 | 0.72 | 0.74 | 0.72 | 0.76 | 0.64 | 0.70 | 0.71 | 0.61 | 0.67 | 0.72 | 1.00 | | | | | |
| 13.FAN3 | 0.70 | 0.77 | 0.77 | 0.74 | 0.83 | 0.70 | 0.74 | 0.74 | 0.64 | 0.71 | 0.75 | 0.87 | 1.00 | | | | |
| 14.FAN6 | 0.68 | 0.73 | 0.73 | 0.68 | 0.74 | 0.63 | 0.68 | 0.66 | 0.61 | 0.66 | 0.68 | 0.88 | 0.85 | 1.00 | | | |
| 15.FAN8 | 0.77 | 0.74 | 0.79 | 0.83 | 0.72 | 0.72 | 0.72 | 0.71 | 0.70 | 0.74 | 0.75 | 0.68 | 0.71 | 0.65 | 1.00 | | |
| 16.FAN9 | 0.73 | 0.70 | 0.76 | 0.77 | 0.87 | 0.70 | 0.75 | 0.86 | 0.67 | 0.74 | 0.79 | 0.74 | 0.79 | 0.70 | 0.72 | 1.00 | |
| 17.FAN10 | 0.73 | 0.64 | 0.69 | 0.74 | 0.81 | 0.65 | 0.69 | 0.76 | 0.66 | 0.73 | 0.71 | 0.70 | 0.71 | 0.66 | 0.72 | 0.78 | 1.00 |
| 18.FAO4 | 0.73 | 0.67 | 0.70 | 0.72 | 0.80 | 0.68 | 0.71 | 0.81 | 0.68 | 0.72 | 0.71 | 0.67 | 0.72 | 0.68 | 0.69 | 0.86 | 0.80 |
| 19.FAO5 | 0.75 | 0.71 | 0.78 | 0.81 | 0.82 | 0.72 | 0.76 | 0.87 | 0.69 | 0.75 | 0.75 | 0.72 | 0.74 | 0.69 | 0.74 | 0.84 | 0.78 |
| 20.FAO6 | 0.75 | 0.75 | 0.76 | 0.80 | 0.78 | 0.83 | 0.86 | 0.74 | 0.75 | 0.79 | 0.75 | 0.73 | 0.78 | 0.71 | 0.73 | 0.78 | 0.73 |
| 21.FAO10 | 0.74 | 0.71 | 0.77 | 0.80 | 0.82 | 0.70 | 0.73 | 0.88 | 0.68 | 0.75 | 0.77 | 0.70 | 0.75 | 0.69 | 0.72 | 0.85 | 0.73 |
| 22.FAA4 | 0.69 | 0.63 | 0.71 | 0.70 | 0.70 | 0.61 | 0.64 | 0.78 | 0.64 | 0.68 | 0.70 | 0.67 | 0.67 | 0.65 | 0.66 | 0.73 | 0.68 |
| 23.FAG9 | 0.53 | 0.51 | 0.51 | 0.50 | 0.53 | 0.51 | 0.49 | 0.54 | 0.50 | 0.53 | 0.52 | 0.52 | 0.53 | 0.51 | 0.52 | 0.55 | 0.48 |
| 24.FAJ6 | 0.68 | 0.67 | 0.72 | 0.71 | 0.73 | 0.67 | 0.70 | 0.79 | 0.66 | 0.70 | 0.72 | 0.69 | 0.72 | 0.63 | 0.71 | 0.76 | 0.67 |
| 25.FAV3 | 0.72 | 0.65 | 0.70 | 0.72 | 0.74 | 0.64 | 0.67 | 0.79 | 0.64 | 0.72 | 0.73 | 0.69 | 0.70 | 0.65 | 0.66 | 0.78 | 0.71 |
| 26.FPL8 | 0.36 | 0.38 | 0.37 | 0.38 | 0.37 | 0.33 | 0.35 | 0.37 | 0.33 | 0.37 | 0.36 | 0.35 | 0.37 | 0.36 | 0.36 | 0.37 | 0.35 |
| 27.FPM4 | 0.41 | 0.43 | 0.44 | 0.46 | 0.42 | 0.40 | 0.40 | 0.42 | 0.38 | 0.42 | 0.42 | 0.39 | 0.41 | 0.40 | 0.43 | 0.42 | 0.39 |
| 28.FPN11 | 0.41 | 0.44 | 0.44 | 0.45 | 0.41 | 0.40 | 0.41 | 0.42 | 0.38 | 0.40 | 0.42 | 0.40 | 0.41 | 0.40 | 0.43 | 0.42 | 0.37 |
| 29.FPO7 | 0.36 | 0.37 | 0.39 | 0.41 | 0.38 | 0.34 | 0.36 | 0.36 | 0.32 | 0.36 | 0.39 | 0.39 | 0.39 | 0.38 | 0.38 | 0.38 | 0.33 |
| 30.FRP1 | 0.25 | 0.27 | 0.28 | 0.29 | 0.26 | 0.27 | 0.27 | 0.29 | 0.26 | 0.28 | 0.27 | 0.28 | 0.27 | 0.24 | 0.28 | 0.29 | 0.24 |
| 31.FRP2 | 0.27 | 0.27 | 0.29 | 0.29 | 0.27 | 0.30 | 0.27 | 0.29 | 0.28 | 0.30 | 0.27 | 0.26 | 0.28 | 0.25 | 0.29 | 0.29 | 0.26 |
| 32.FRP3 | 0.29 | 0.31 | 0.31 | 0.32 | 0.31 | 0.31 | 0.29 | 0.32 | 0.31 | 0.32 | 0.31 | 0.29 | 0.30 | 0.29 | 0.32 | 0.32 | 0.30 |
| 33.FRP4 | 0.26 | 0.26 | 0.27 | 0.28 | 0.27 | 0.29 | 0.27 | 0.30 | 0.27 | 0.28 | 0.26 | 0.26 | 0.28 | 0.24 | 0.28 | 0.29 | 0.25 |
| 34.FOP7 | 0.29 | 0.29 | 0.31 | 0.34 | 0.32 | 0.31 | 0.28 | 0.35 | 0.30 | 0.32 | 0.31 | 0.31 | 0.32 | 0.29 | 0.31 | 0.36 | 0.30 |

Table 3: Continued

| | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18.FAO4 | 1.00 | | | | | | | | | | | | | | | | |
| 19.FAO5 | 0.78 | 1.00 | | | | | | | | | | | | | | | |
| 20.FAO6 | 0.74 | 0.79 | 1.00 | | | | | | | | | | | | | | |
| 21.FAO10 | 0.78 | 0.86 | 0.77 | 1.00 | | | | | | | | | | | | | |
| 22.FAA4 | 0.71 | 0.74 | 0.78 | 0.76 | 1.00 | | | | | | | | | | | | |
| 23.FAG9 | 0.55 | 0.52 | 0.52 | 0.52 | 0.47 | 1.00 | | | | | | | | | | | |
| 24.FAJ6 | 0.72 | 0.74 | 0.71 | 0.78 | 0.75 | 0.56 | 1.00 | | | | | | | | | | |
| 25.FAV3 | 0.71 | 0.76 | 0.71 | 0.79 | 0.84 | 0.51 | 0.76 | 1.00 | | | | | | | | | |
| 26.FPL8 | 0.37 | 0.38 | 0.34 | 0.38 | 0.34 | 0.28 | 0.34 | 0.37 | 1.00 | | | | | | | | |
| 27.FPM4 | 0.42 | 0.43 | 0.41 | 0.44 | 0.40 | 0.33 | 0.40 | 0.41 | 0.64 | 1.00 | | | | | | | |
| 28.FPN11 | 0.41 | 0.41 | 0.41 | 0.43 | 0.40 | 0.32 | 0.40 | 0.42 | 0.63 | 0.86 | 1.00 | | | | | | |
| 29.FPO7 | 0.34 | 0.37 | 0.37 | 0.38 | 0.34 | 0.30 | 0.33 | 0.35 | 0.54 | 0.67 | 0.66 | 1.00 | | | | | |
| 30.FRP1 | 0.23 | 0.25 | 0.26 | 0.26 | 0.23 | 0.28 | 0.26 | 0.25 | 0.22 | 0.22 | 0.22 | 0.22 | 1.00 | | | | |
| 31.FRP2 | 0.24 | 0.28 | 0.27 | 0.26 | 0.24 | 0.28 | 0.27 | 0.26 | 0.20 | 0.20 | 0.21 | 0.22 | 0.78 | 1.00 | | | |
| 32.FRP3 | 0.31 | 0.32 | 0.31 | 0.31 | 0.27 | 0.26 | 0.29 | 0.28 | 0.24 | 0.24 | 0.24 | 0.25 | 0.54 | 0.56 | 1.00 | | |
| 33.FRP4 | 0.24 | 0.26 | 0.28 | 0.26 | 0.23 | 0.25 | 0.26 | 0.27 | 0.21 | 0.19 | 0.21 | 0.20 | 0.72 | 0.71 | 0.49 | 1.00 | |
| 34.FOP7 | 0.30 | 0.33 | 0.32 | 0.34 | 0.28 | 0.31 | 0.29 | 0.33 | 0.25 | 0.25 | 0.27 | 0.28 | 0.43 | 0.45 | 0.33 | 0.46 | 1.00 |

their geographical origin. At SC value of 0.74, accessions of this cluster subdivided into six A, B, C, D, E and F subclusters (Fig. 1). Subcluster A comprised of nine accessions, all originated from Iran. The highest genetic SC value for accessions in subcluster A was between FAM5 and FAO6. Subcluster B consisted of eight accessions. At SC value of 0.77 the FAN1, a turf type cultivar from USA, stood apart form other seven accessions of Hungarian origin and had the most distance from other accessions in this group. The highest genetic SC value for accessions in subcluster B was between FAM6 and FAO10. Subcluster C contained accessions FAJ6, FAA4 and FAV3. The FAJ6, from Poland, had the most distance from other two accessions from Iran. In subcluster D, FAN2 and FAN6, from Iran, grouped with FAN3 from Hungary. In many molecular systems the lack of genetic differentiation between accessions of definite identity and distinct geographic origin is usually

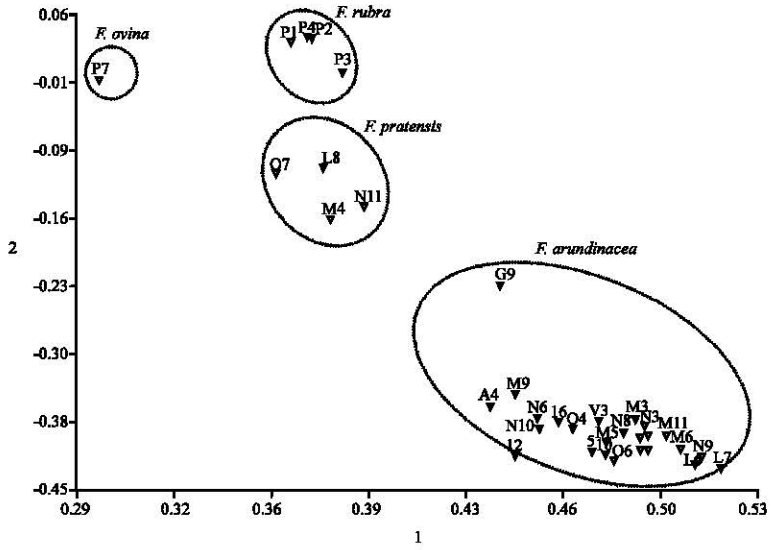


Fig. 3: Biplot of principle components analysis based on AFLP data for 34 fescue accessions. Names of accessions are briefed by deleting the first two letters from each name (e.g., FAG9 = G9)

attributed to random nature of genomic DNA amplification which is the case in AFLP (Roldan-Ruiz *et al.*, 2000). Two accessions, FA-M9 and FA-G9 (both from Iran), did not group with any other entry and consisted cluster E and F respectively. This may imply high interaspecific genetic variation in Iranian tall fescue accessions.

Principle component analysis (Fig. 3), in which PC1 accounted for 50.5% of total variation and PC2 accounted for 26.1% was generally consistent with results from the cluster analysis in groupings of the species and accessions. The PC2 values for *F. rubra* and *F. ovina* accessions were high. It was medium for accessions of *F. pratensis* and low for accessions of *F. arundinacea*. The values of PC1 for accessions of *F. ovina* were low, for accessions of *F. rubra* and *F. pratensis* medium and for accessions of *F. arundinacea* high. The FA-G9 and to a lesser extent FA-M9 were located far apart from other accessions (Fig. 3). This was very much in accordance with grouping of these two accession in clustering (Fig. 1), indicating their greater genetic divergence from other accessions. These accessions may be good candidates for breeding programs in constructing mapping populations or as parents of synthetic varieties.

The AFLP results in this study were in general agreement with available information regarding origins of these populations. Hungarian tall fescue accessions were grouped in a subcluster and separated from Iranian accessions. Accessions from USA and Poland also grouped separately within subclusters. This trend was also seen for accessions of other species. These results indicated that accessions are somewhat genetically diverse. Local environmental adaptation may play a significant role in Festuca diversity. Possibilities of genetic introduction may have occurred whit migration, selection and breeding among some accessions of Iran and Hungary but there is no evidence.

Results indicate that AFLP markers using DNA bulking strategy was able to assess variation among and between fescue species and distinguished accessions based geographical origins and some morphological traits. This strategy could be applied to assess diversity of accessions from outcrossing species in the breeding programs. Assessment of genetic diversity in germplasm collection from several geographic locations has been conducted for Bentgrass (Vergara and Bughrara, 2003) and Cynodon (Wu *et al.*, 2004) germplasem by means of AFLP markers. Important traits from other Festuca species can be introduced to cultivated fescues and AFLP analysis would be a useful tool to monitor introgression and molecular tagging. By means of specific amplified products, sequence characterized amplified primers may be developed to distinguish genetically the different fescue species in the future. Results showed that some accessions are genetically distinct from others indicating considerable potential for the improvement of new cultivars. Turfgrass breeders may develop superior cultivars either by crosses with germplasm accessions. AFLP analysis may also be used for identifying genotypes for constructing mapping populations, core collections and screening for duplicate or misclassified accessions in germplasm collections.

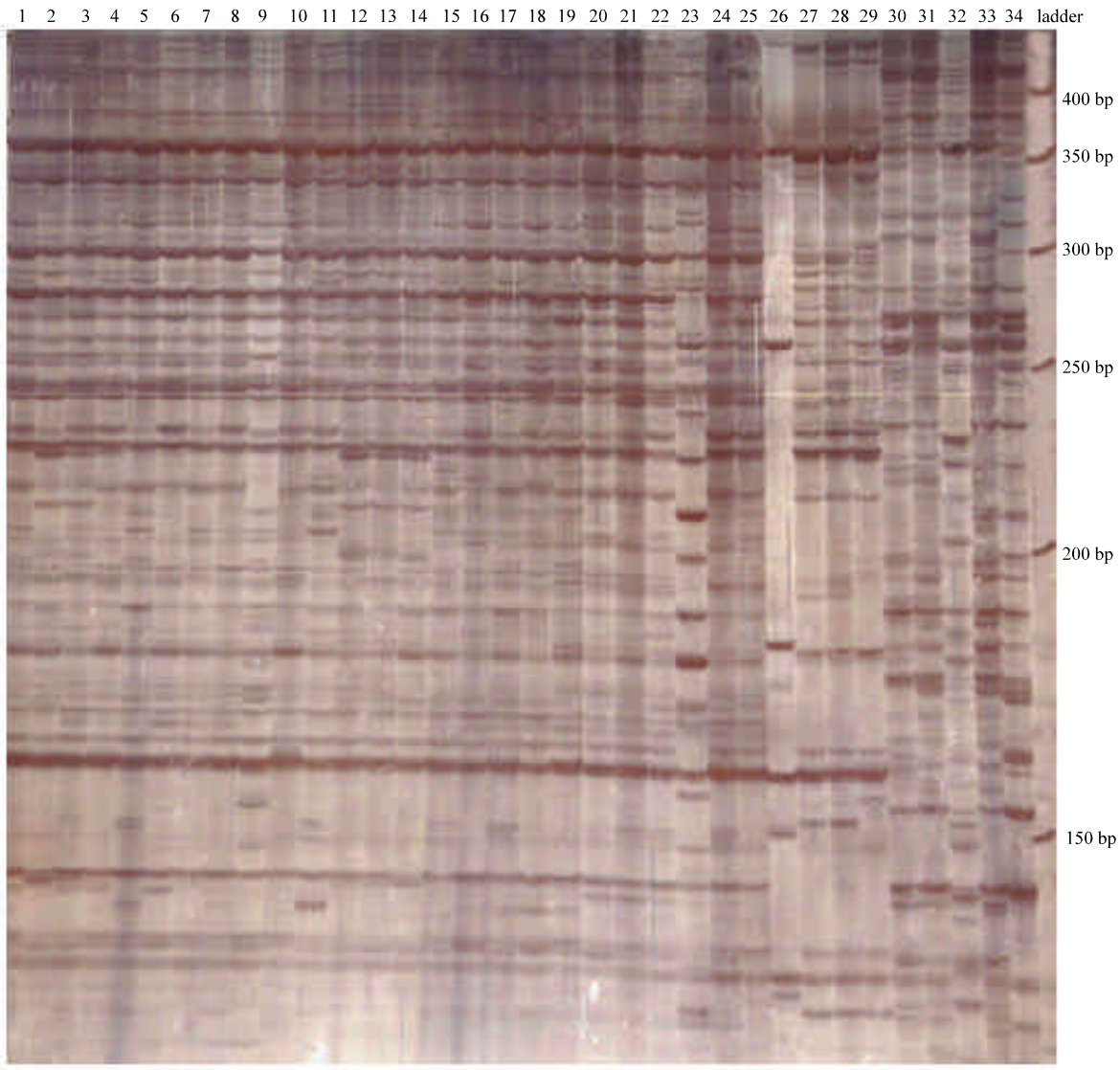


Fig. 4: AFLP profile of 34 fescue accessions using *EcoRI*-ATC and *MseI*-CGC primer combination

In conclusion, present results indicated that Iranian fescue accessions contains a high degree of genetic variability and very much diverged from accessions of other geographical regions, can be exploited in breeding programs. Further more using DNA bulking strategy, AFLP marker system proved to be highly effective in discriminating a very diverse fescue collection.

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