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# The Possibility of Use of AFLP Molecular Markers and Phenotypic Traits to Increase Forage Yield in Tall Fescue (*Festuca arundinacea* Schreb.) Breeding

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## ABSTRACT

**Tall fescue is an out-crossing allohexaploid grass species extensively used for forage and turf worldwide. Cultivars of outbreeding forage grasses such as tall fescue are usually synthetic populations derived from intercrossing several selected parents using the polycross method. In this study, the application of AFLP molecular markers to optimize genetic diversity in a polycross breeding program of tall fescue was evaluated. For both phenotypic characters and AFLP molecular markers, two polycrosses of six parental plants with contrasting levels of genetic diversity were composed. A fifth polycross population was composed using six genotypes with the highest general combining ability. The results of this study showed that marker assistant parental selection produced superior progenies, indicating that selection based on molecular marker diversity may be an appropriate means to improve first generation progenies of tall fescue. This may be mainly useful in large breeding programs because the identification of diversity based on phenotypic traits is time consuming and may be influenced by environmental effects.**

**Keywords:** AFLP, Phenotypic traits, Polycross, Tall fescue.

## INTRODUCTION

Tall fescue (*Festuca arundinacea* Schreb.= *Lolium arundinaceum* (Schreb) S. J. Darbysh.), is a major forage and turf grass species with the genomic constitution of PP G1G1 G2G2 (2n= 6x= 42). Tall fescue is an open pollinated species with a high level of self-incompatibility (Xu *et al.,* 1994). Most cultivars of this plant are usually produced through random mating of selected parental plants resulting in population- based synthetic cultivars (Barker and Kalton, 1989). These cultivars need a sufficient level of phenotypic homogeneity in order to pass variety trials and also at the same time a high level of heterozygosity should be attained in order to maximize adaptability and agronomic performance. Therefore finding the optimal level of diversity in synthetic cultivars is essential for successful breeding.

Previous studies (Bailey and Comstock, 1976; Burkhammer *et al.,* 1998) indicate that the probability of producing superior progeny is higher if parents are similar in performance but in the case of using diversity among parents to create a synthetic variety, the difference in gene frequencies enables the breeder to increase heterozygousity causing increased adaptability and fitness of populations (Reed and Frankham, 2003; Silvertown and Lovett Doust, 1993).

Polycross parental plants can be selected using phenotypic traits where individual plants are selected based on the visual scoring of desirable characters or based on their

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agronomic performance (Amini *et al.*, 2011). General Combining Ability (GCA), which is the average performance of the progeny of one genotype in a series of crosses, can also be used for parental selection (Wricke and Weber, 1986). In this approach, parents with high GCA are selected to produce progeny with better yield performance (Cox and Murphy, 1990, Diers *et al.,* 1996, Hartl and Seefelder, 1998, Tanaka *et al.,* 2013). Selection based on phenotypic traits may be greatly influenced by environmental effects and the presence of genotype × environment interactions may cover the true genotypic value (Amini *et al.,* 2013).

Molecular markers are powerful tools for the analysis of genetic diversity independent of the environment. AFLP (Amplification Fragment Length Polymorphism) is a DNA fingerprinting technique, based on selective PCR amplification of DNA restriction fragments under stringent conditions (Vos *et al.,* 1995). The advantages of this method are reproducibility, high level of polymorphism detection, genome wide distribution of markers, and no pre-requisite of knowledge of the genome being studied (Mueller and Wolfenbarger, 1999). AFLP has been shown to provide reliable estimates of genetic diversity in various forage crop species (e.g., Barcaccia *et al.,* 1999; Kolliker *et al.,* 2003; Skot *et al.,* 2002; Ubi *et al.,* 2003) and can be a valuable complement to phenotypic characters (Gilliland *et al.,* 2000; Rold´an-Ruiz *et al.,* 2000).

The aim of this study was to find the influence of genetic diversity among parental plants on progeny performance and genetic diversity. Phenotypic and molecular markers were used to select parents with contrasting levels of genetic diversity and the molecular genetic diversity as well as performance of the resulting progenies was evaluated.

## MATERIALS AND METHODS

### Plant Material and Field Experiment

The parental germplasm consisted of 25 tall fescue genotypes chosen from a large replicated nursery established in 2003 at Isfahan University of Technology and previously characterized for phenotypic traits (Majidi *et al.,* 2009) and molecular marker diversity (unpublished). The parental germplasm was selected to form two polycrosses of six parental plants each with contrasting levels of diversity based on phenotypic traits and two polycrosses of six parental plants with contrasting levels of molecular marker diversity as determined using AFLP markers (Table 1). A fifth polycross was composed from the six plants with the highest general combining ability (data not shown). The parents for each polycross were selected to be similar in date of pollen shedding which ranged from 62 to 69 days after March 30$^{th}$. Polycross nurseries were established in February 2010. Seeds of first generation Synthetic progenies (Syn1) were harvested on each parental genotype in June 2010. Equal amounts of Syn1 seed from each parent were pooled per polycross nursery and sown at the research farm of Isfahan, (32°32' N and 32°51' E, 1,630 m asl) in Autumn 2011. The plots were arranged as a randomized complete block design with three replications. Each plot consisted of 60 plants planted in six rows 50 cm apart with inter-row plant distance of 40 cm. Plots were fertilized with 200 kg N ha$^{-1}$ and 200 kg P ha$^{-1}$ prior to sowing and 100 kg N ha$^{-1}$ was applied to the trial.

### Phenotypic Analysis

Agronomic practices were the same as for the parental genotypes. Seed yield, forage yield and seven morphological characteristics were recorded individually on 60 plants of each plot during 2012 and 2013. Detailed information on morphological characters is as follows:

Number of Days to Anthesis (NDA): Number of days from March 1$^{st}$ until onset of pollen shedding in three panicles of each plant.

Plant Height (PH): The distance (cm) from the plant base to the top of the highest panicle after full anthesis.





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**Table 1.** Parental genotypes used for the generation of 5 *Festuca arundinacea* Syn1 populations with contrasting diversity.

| Parental genotype | Origin | Name and description of Syn1 |
|---|---|---|
| 4 | Iran, Isfahan—Mobarake | HPD (parents selected for High |
| 13 | Hungary—Csesznek | Diversity based on Phenotypic traits) |
| 17 | Iran, Isfahan—Fozve | |
| 19 | Iran, Semnan—Shahrood | |
| 23 | Poland | |
| 24 | Iran, Semnan—Shahrood | |
| 2 | Iran, Kohkiluye—Yasuj | LPD (parents selected for Low |
| 3 | Iran, Kohkiluye—Yasuj | Diversity based on Phenotypic traits) |
| 7 | Iran, Isfahan—Daran | |
| 11 | Hungary | |
| 12 | Hungary | |
| 14 | Hungary—Csesznek | |
| 2 | Iran, Kohkiluye—Yasuj | HMD (parents selected for High |
| 5 | Iran, Isfahan—Mobarake | Diversity based on Molecular genetic |
| 13 | Hungary—Csesznek | markers) |
| 20 | Iran, Isfahan—Fozve | |
| 24 | Iran, Semnan—Shahrood | |
| 25 | IranSemnan—Shahrood | |
| 3 | Iran, Kohkiluye—Yasuj | LMD (parents selected for Low |
| 4 | Iran, Isfahan—Mobarake | Diversity based on Molecular genetic |
| 8 | Iran, Isfahan—Fozve | markers) |
| 14 | Hungary—Csesznek | |
| 17 | Iran, Isfahan—Fozve | |
| 18 | Iran, Semnan—Shahrood | |
| 15 | Iran, Isfahan—Yazdabad | HGCA (parents selected for High |
| 18 | Iran, Semnan—Shahrood | General Combining Ability) |
| 19 | Iran, Semnan—Shahrood | |
| 20 | Iran, Isfahan—Fozve | |
| 23 | Poland | |
| 24 | Iran, Semnan—Shahrood | |

Number of Fertile Tillers (NFT): Number of fertile tillers per plant before threshing.

Plant Seed Weight (PSW): The weight of seeds per plant (g) after threshing, cleaning and drying.

Panicle Length (PL): The average length (cm) from first node to the tip of three randomly chosen panicles of each plant.

Flag Leaf Length (FLL): The average length (cm) from the leaf base to the tip of three flag leaves selected randomly from three tillers at the stage of full anthesis.

Flag Leaf Width (FLW): The average width (mm) at the middle of the leaf from the same three flag leaves that were used for measuring FLL.

Dry Matter Yield (DMY): The weight (g) of foliage per plant after drying at 72°C for 48 hours.

Stubble Surface (SS): The width (cm) of foliage remaining for each plant after the first cut.

## AFLP Analysis

Young leaves of 60 plants (10 per parental genotype) were collected from each Syn1 population and DNA was extracted using the





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DNeasy 96 Plant Kit (Qiagen, Hilden, Germany) and quantified using PicoGreen® (Molecular Probes, Eugene, OR, USA) and a fluorimeter (LS-30; Perkin Elmer Instruments, Shelton, CT, USA).

AFLP analysis was performed following the method of Vos *et al.,* (1995). AFLP templates were prepared by restriction digestion (EcoRI and MseI) and adaptor ligation of 1µg genomic DNA. Adaptors and primers were synthesized by Microsynth, Balgach, Switzerland. EcoRI+A and MseI+C primers were used to amplify double digested, adaptor ligated DNA (20 ng) in a 20µl reaction containing 1X PCR buffer, 5 pmol of each primer, 1.5 mM MgCl2, 0.2 mM dNTP and 0.5U Taq DNA polymerase (Promega GoTaq). PCR was performed in an MJ PT-200 (MJ Research, Waltham, MA, USA) thermocycler using an initial denaturation step of 2 minutes at 94°C, 26 cycles of 1 minute at 94°C, 1 minute at 56°C and 1 minute at 72°C, followed by a final extension of 5 minutes at 72°C. Amplification products were diluted by adding 100 µl $H_2O$. For subsequent selective amplification, five EcoRI/MseI primer pairs with three additional nucleotides were used each (E+AGA/ M+CAC, E+AGC/M+CAC, E+AGA/M+CTG, E+AGC/M+CTG, E+AGT/M+CTG). Selective amplification reactions contained 1 µl of diluted pre-amplified AFLP template, 5 pmol 6-FAM, HEX or NED labeled EcoRI+ANN primer, 6 pmol MseI+CNN primer, 1.5mM MgCl2, 0.2 mM dNTP and 0.4U Taq DNA polymerase in a total volume of 20 µl. PCR was performed using a touchdown PCR protocol with an initial denaturation of 2 minutes at 94°C, 12 cycles of 1 minute at 94°C, 30 seconds at 65°C (−0.7°C per cycle) and 1 minute at 72°C, followed by 23 cycles of 30 seconds at 94°C, 30 seconds at 56°C and 1 minute at 72°C with a final extension of 5 minutes at 72°C. PCR products were analyzed on an ABI Prism 3130 XL Genetic Analyzer using POP-7 polymer and 36 cm capillaries (Applied Biosystems, Foster City, CA, USA).

## Data Analysis

### Phenotypic Traits

Normal distribution and homogeneity of variance within the populations were tested for data of single plants. Split-plot in time model was used for analysis of variance of data for phenotypic traits using the General Linear Model of SAS software version 8.02 (SAS, 1999).

Significant differences of population means for each trait were identified by Duncan's multiple range test (Falconer and Mackay, 1996). Phenotypic correlations were obtained as described by Falconer and Mackay (1996).

Genetic diversity within populations was calculated using average Euclidean squared distance based on data standardized by subtracting the variable mean and dividing by the standard deviation using NTSYS-pc (Rohlf, 2000). Cluster analysis was performed using the Unweighted Pair Group Method with an Arithmetic mean (UPGMA).

### AFLP Markers

AFLP patterns were analyzed using GeneMarker ® 1.51 (SoftGenetics, LLC, State College, PA, USA) software and markers were visually scored for presence (1) or absence (0) and entered into a binary matrix containing the profile of each population.

Analysis Of Molecular Variance (AMOVA) was computed using Arlequin 3.1 software (Excoffier *et al.,* 1992) to partition the variation into, within and among population variance.

The number of polymorphic markers and mean frequency of markers were calculated for each population. Polymorphic information content for each allele was estimated following the procedure of Roldan-Ruiz *et al.* (2000) and averaged for each primer. Genetic diversity within populations was calculated based on Jaccard similarity coefficients. Genetic





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relationships among populations were investigated using cluster analysis in Mega 5 software (Kumar *et al.,* 2008).

## RESULTS

### Phenotypic Traits

Results from the analysis of variance revealed significant effects of population for all the traits with the exception of FLL (Table 2). The highest means and the lowest means of NDA were observed in HPD and LPD, respectively. Among the 5 populations, HMD (high molecular diversity) had the highest seed yield in 2013 while the differences between other populations were not statistically significant (Table 3). The highest and the lowest means of PH belonged to HMD and LPD, respectively. For plant dry matter yield, HPD and LMD and for stubble surface, HPD had the lowest values in 2012 while the highest means of DMY and SS were observed in HMD (Table 3).

Diversity within populations expressed as Euclidean squared distances ranged from 8.05 for LPD to 16.24 for HPD and clearly reflected the level of diversity among parental genotypes which ranged from 8.20 for LPD to 16.39 for HPD (data not shown). Cluster analysis based on average Euclidean squared distance of Syn1 populations clearly separated LPD from the remaining four populations but did not reveal any further separation of populations (Figure 1).

Association of DMY with PH, NFT and

**Table 2**. Split-plot (time) analysis of variance for 9 morphological traits in Syn1 populations of tall fescue evaluated in 2011 and 2012.

| Traits | Mean squares | | | | | |
|---|---|---|---|---|---|---|
| | Replication (Rep) | Population (Pop) | Rep×Pop | Year | Pop×Year | Error |
| NDA[a] | 23.17 ns | 228.22** | 22.26** | 310191.40** | 100.21* | 40.11 ns |
| PH (cm) [b] | 21590.30** | 4242.18** | 1235.64** | 3362044** | 2289.66** | 305.72** |
| NFT [c] | 75627.00** | 16411.83** | 9065.10** | 841021.00** | 17667.34** | 1311.10** |
| PSW (g) [d] | 31.83** | 25.21** | 2.93 ns | 24.45** | 17.99** | 1.57 ns |
| PL(cm) [e] | 74.27 ns | 393.38** | 63.20** | 6865.50** | 10.55 ns | 18.73 ns |
| FLL(cm) [f] | 324.08** | 35.16 ns | 70.67** | 3168.64** | 81.86* | 17.99 ns |
| FLW(mm) [g] | 8.12 ns | 17.86** | 8.29** | 196.95** | 54.58** | 3.77 ns |
| DMY(g) [h] | 46.83** | 63.10** | 53.89** | 5518.81** | 10.49 ns | 7.44 ns |
| SS (cm) [i] | 165.67* | 167.25** | 155.27** | 835.23** | 619.89** | 43.00 ns |

[a] Number of Days to Anthesis, [b] Plant Height, [c] Number of Fertile Tiller, [d] Plant Seed Weight, [e] Panicle Length, [f] Flag Leaf Length, [g] Flag Leaf Width, [h] Dry Matter Yield, and [i] Stubble Surface. ns, * and **: Not significant, significant at $P< 0.05$ and $P< 0.01$, respectively.

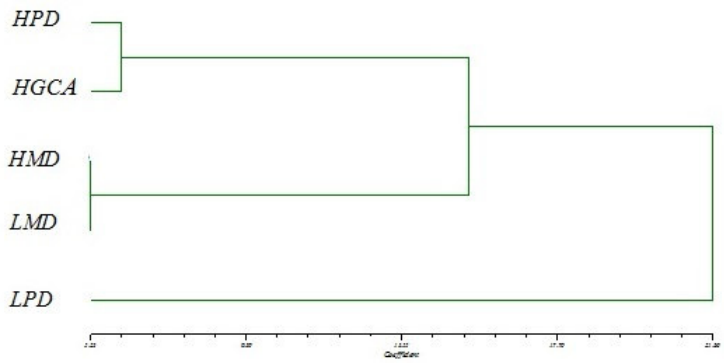


**Figure 1.** Cluster analysis of five populations of tall fescue based on eight phenotypic traits. Genotypes were derived from Syn1 progenies with contrasting levels of diversity: HPD (High Phenotypic Diversity); LPD (Low Phenotypic Diversity); HMD (High Molecular Genetic); LMD (Low Molecular Genetic), and HGCA (High General Combining Ability).





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**Table 3.** Comparison of mean values for eight phenotypic traits of five *Festuca arundinacea* Syn1 populations with contrasting levels of diversity. Traits were evaluated in the field using 180 genotypes per population arranged in three replicates.

| | 2012 | | | | | 2013 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Traits | HPD | LPD | HMD | LMD | HGCA | HPD | LPD | HMD | LMD | HGCA |
| NDA[a] | 62.48 [a](3.83) | 60.44[b] (6.72) | 60.66[b] (3.44) | 60.88[b] (7.89) | 60.68[b] (4.54) | 43.92[a] (4.27) | 38.41[b](6.76) | 40.50[ab] (8.11) | 43.38[ab] (9.26) | 40.28[ab] (8.63) |
| PH (cm)[b] | 55.93[a] (6.98) | 66.65[a] (7.98) | 71.23[a] (7.67) | 68.86[a] (7.66) | 77.50[a] (6.63) | 77.08[ab] (8.42) | 76.19[b] (10.21) | 90.08[a] (6.81) | 83.85[ab] (8.41) | 77.36[ab] (8.46) |
| NFT[c] | 35.26[b] (4.24) | 37.62[ab] (2.99) | 45.04[a] (4.40) | 41.93[ab] (8.16) | 38.68[ab] (6.91) | 84.78[a] (5.66) | 128.72[a] (6.68) | 104.83[a] (5.67) | 103.29[a] (6.15) | 136.73[a] (6.86) |
| PSW (g)[d] | 6.55[a] (5.76) | 9.49[a] (6.42) | 10.50[a] (5.81) | 8.01[a] (5.13) | 9.49[a] (5.65) | 5.76[b] (5.61) | 7.45[b] (5.21) | 13.14[a] (3.22) | 5.35[b] (5.85) | 4.93[b] (4.48) |
| PL(cm)[e] | 18.92[a] (7.33) | 19.13[a] (7.48) | 21.38[a] (7.59) | 21.20[a] (7.33) | 20.20[a] (7.14) | 12.96[a] (12.25) | 13.16[a] (11.57) | 14.32[a] (11.90) | 15.24[a] (11.76) | 16.88[a] (12.61) |
| FLL(cm)[f] | 6.73[a] (1.62) | 5.17[ab] (1.24) | 4.77[ab] (1.27) | 4.69[b] (1.11) | 4.25[b] (1.43) | 3.44[a] (0.66) | 4.17[a] (0.58) | 4.81[a](0.53) | 4.05[a] (0.80) | 3.78[a] (0.68) |
| FLW(mm)[g] | 110.87[a] (39.54) | 114.21[a] (40.71) | 130.91[a] (41.76) | 117.77[a] (39.51) | 119.70[a] (40.62) | 92.16[b] (25.40) | 204.34[ab] (51.60) | 280.23[a] (16.65) | 110.25[b] (27.70) | 228.92[ab] (36.10) |
| DMY(g)[h] | 21.00[a] (10.66) | 18.94[a] (9.07) | 19.68[a] (9.03) | 15.89[a] (10.62) | 15.22[a] (9.63) | 15.73[b] (11.84) | 17.93[ab] (10.55) | 19.66[a] (7.43) | 17.79[ab] (10.36) | 17.30[ab] (8.48) |

[a] Number of Days to Anthesis, [b] Plant Height, [c] Number of Fertile Tiller, [d] Plant Seed Weight, [e] Panicle Length, [f] Flag Leaf Length, [g] Flag Leaf Width, [h] Dry Matter Yield, and [i] Stubble Surface. For description of populations see Table 1. Values in columns followed by the same letter are not statistically significant according to Duncan's multiple range test (P= 0.05). Values in parentheses are Mean Square Error (MSE) for each trait, each polycross and each year.

PSW was positive and significant (Table 4). Stubble Surface (SS) was also positively associated with PH, NFT and DMY. Plant height, PSW, PL was significantly and positively correlated. The correlations between PSW, PL with NDA were significant and negative. There were no significant associations among SS and NDA, PL and FLW (Table 4).

## AFLP Markers

Genetic diversity of 60 Syn1 progeny from each of the five polycrosses (10 per parental plant) was assessed using the same AFLP markers scored in the parental germplasm. Diversity within populations expressed as Jaccard similarity coefficients ranged from 0.34 for LMD to 0.64 for HMD (Table 5). Molecular genetic diversity was higher within Syn1 progenies derived from polycross populations consisting of highly diverse parental genotypes. This difference was most pronounced in the HMD population which was based on parents selected for high molecular genetic diversity (Table 5). However, molecular genetic diversity in Syn1 progeny of population LMD, consisting of parents with low genetic diversity, was lower when compared to all the other populations analyzed (Table 5). Marker frequencies between parental plants and their respective Syn1 progenies were highly correlated with a correlation coefficient of $r$= 0.84 (P< 0.05) across all five polycrosses (data not shown). The genetic diversity within Syn1 progenies of HPD and HMD polycrosses was higher when compared to Syn1 progenies from LPD and LMD polycrosses (Table 5).

Analysis of molecular variance showed 31.21% of the variation to be due to variation among populations and 68.79% of it was due to variation among individuals within population. Cluster analysis based on molecular marker could mainly separate the individuals to the respect populations (Figure 2).

**Table 4.** Correlation coefficient between traits in first generation of synthetic variety.[a]

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1-NDA [a] | 1 | | | | | | | |
| 2-PH [b] | 0.69** | 1 | | | | | | |
| 3-NFT [c] | 0.85** | 0.25 | 1 | | | | | |
| 4-PSW [d] | -0.75** | 0.58* | 0.56* | 1 | | | | |
| 5-PL [e] | -0.76** | 0.78** | 0.70** | 0.82** | 1 | | | |
| 6-FLW [f] | 0.71** | 0.48* | 0.57* | 0.76** | 0.87** | 1 | | |
| 7-DMY [g] | 0.27 | 0.69** | 0.54* | 0.41* | -0.67** | 0.31 | 1 | |
| 8-SS [h] | 0.30 | 0.54* | 0.62** | 0.37* | 0.32 | 0.32 | 0.32 | 1 |

[a] Number of Days to Anthesis, [b] Plant Height, [c] Number of Fertile Tiller, [d] Plant Seed Weight; [e] Panicle Length, [f] Flag Leaf Width, [g] Dry Matter Yield, and [h] Stubble Surface. * and **: Significant at $P< 0.05$ and $P< 0.01$, respectively.

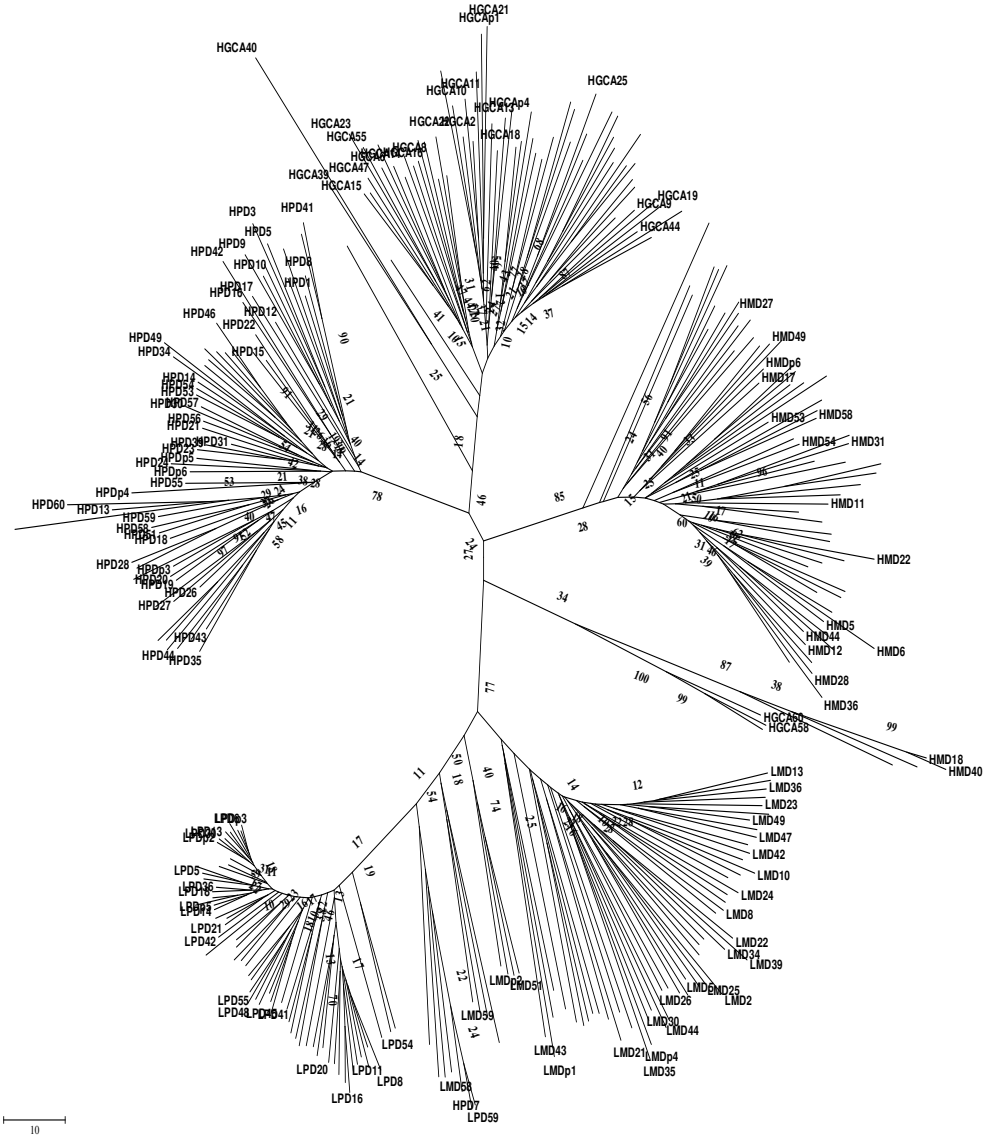


**Figure 2.** Cluster analysis of *Festuca arundinacea* genotypes based on AFLP markers. Genotypes represent parents (p1-p6) and five Syn1 progenies with contrasting levels of diversity: HPD (High Phenotypic Diversity); LPD (Low Phenotypic Diversity); HMD (High Molecular Genetic); LMD (Low Molecular Genetic), and HGCA (High General Combining Ability).





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**Table 5.** Average Jaccard similarity coefficient within Syn1 progeny, number of polymorphic markers, mean frequency of markers and means of Polymorphism Information Content (PIC) among Syn1 progenies of five *F. arundinacea* populations based on AFLP markers.

| Population | Jaccard similarities within Syn1 progeny | Number of polymorphic markers | Mean frequency of marker | Means of PIC |
|---|---|---|---|---|
| HPD [a] | 0.62 | 222 | 0.59 | 0.65 |
| LPD [b] | 0.43 | 105 | 0.34 | 0.88 |
| HMD [c] | 0.64 | 232 | 0.56 | 0.69 |
| LMD [d] | 0.34 | 194 | 0.33 | 0.89 |
| HGCA [e] | 0.63 | 175 | 0.54 | 0.71 |

[a] High Phenotypic Diversity; [b] Low Phenotypic Diversity, [c] High Molecular Genetic, [d] Low Molecular Genetic, and [e] High General Combining Ability.

The relationship between the genetic distances was estimated based on morphological traits and molecular markers were not statistically significant (r= 0.21).

## DISCUSSION

In this study DMY was significantly lower for the progeny from parents selected for high phenotypic diversity, which may be partly explained by the fact that DMY and DMY related traits were used to characterize phenotypic diversity of the parents, leading to the insertion of agronomical inferior genotypes. The significantly increased DMY in Syn1 progeny from parents selected for high molecular marker diversity is in agreement with the findings in perennial ryegrass (Kölliker *et al.,* 2005), timothy (Tanaka *et al.,* 2013) and tall fescue (Amini *et al.,* 2011). Since HMD progeny showed values comparable to or higher than the other progenies for all traits investigated, selecting parents for high molecular genetic diversity may provide an efficient means to improve first generation progeny in tall fescue.

The assessment of phenotypic and molecular genetic diversity of the five Syn1 populations showed high congruence with the diversity observed among the respective parental plants. The highest genetic diversity was observed in Syn1 progenies derived from parents with high molecular genetic diversity when compared to Syn1 progenies from other polycrosses. Analysis of molecular variance based on AFLP markers showed that most of the observed variation was due to differences within populations partly due to most parental genotypes being used for several polycrosses (Table 1). In general, the interpopulation variance observed in this study was in agreement with other studies in tall fescue (Amini *et al.,* 2011) and other forage grasses (Peter-Schmid *et al.,* 2008; De-Araujo and Coulman, 2002; Kölliker *et al.,* 1999). The high interpopulation variation is probably caused by the outcrossing nature of forage grass and also the small number of parental genotypes (Kölliker *et al.,* 2005).

From the positive and significant association of DMY with PH, NFT and SS, it appears that indirect selection would be effective to improve forage yield. Majidi *et al.* (2009) in tall fescue and Wilkins (1985) in perennial ryegrass reported significant correlation between DMY with PH and NFS. In the present study, the correlation between DMY and NDA was not significant, but NDA was positively correlated with components of forage yield (PH, and SS). The positive and significant correlation between PSW and DMY revealed the possibility of simultaneous improvement of these traits.

Cluster analysis based on polymorphic bands mainly separated the 300 individual genotypes into the respective groups (Figure 1). The main cause for the incomplete separation may lie in the high variability





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observed within populations which was confirmed by analysis of molecular variance. Limited separation of genotypes into the respective populations is often observed in outbreeding species (Amini *et al.,* 2011; Kölliker *et al.,* 2003; Semagn *et al.,* 2000). The comparison of using AFLP molecular marker to classify the population members in this study with the previous study using EST-SSR markers in tall fescue (Amini *et al.,* 2011) showed that AFLP molecular markers had better classification which may be due to the higher number of polymorphic locus and more separation of AFLP alleles through the genomes. However, in the present study, the separation of genotypes based on molecular markers was much more pronounced when compared to separation based on phenotypic traits. This may be due to large amounts of independent marker loci used for AFLP analysis. Cluster analysis based on molecular markers revealed little similarity to cluster analysis based on phenotypic characters which indicated poor correlation of molecular marker diversity with phenotypic traits (r= 0.21). The low correlation between molecular markers and phenotypic traits was often observed in forage crop species (Fernando *et al.,* 1997 and Kölliker *et al.,* 1999) and may be explained by the different genomic regions investigated with both markers. However, this correlation was lower than previous studies with EST-SSR in tall fescue (Amini *et al.,* 2011) and AFLP in perennial ryegrass (Kolliker *et al.,* 2005).

In conclusion, this study showed that the higher genetic diversity detected among parental plants using phenotypic traits or molecular genetic markers are transmitted to their Syn1 progeny. Marker assisted parental selection produced superior progeny indicating that selection based on molecular marker diversity may be an appropriate means to improve first generation progenies of tall fescue. This may be mainly useful in large breeding programs because the identification of diversity based on phenotypic traits is time consuming and may be influenced by environmental effects or epistatic interactions.

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## بررسی امکان استفاده از نشانگر مولکولی AFLP و صفات فنوتیپی برای افزایش عملکرد علوفه در پلی کراس فسکیوی بلند (*Festuca arundinacea* Schreb.)

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### چکیده

فسکیوی بلند گونه ای علفی دگرگشن، آلوهگزا پلوئید می‌باشد که در سطح جهان به طور عمده برای تولید علوفه و فضای سبز کاربرد دارد. ارقام گیاهان علوفه ای دگرگشن مثل فسکیوی بلند عمدتا واریته-های ساختگی هستند که از تلاقی چندین ژنوتیپ منتخب طی پلی کراس تولید می‌شوند. در این مطالعه امکان استفاده از نشانگر مولکولیAFLP برای بهینه کردن تنوع ژنتیکی در برنامه اصلاحی فسکیوی بلند ارزیابی شد. بدین منظور برای صفات فنوتیپی و نشانگر مولکولی AFLP هر کدام دو خزانه پلی کراس شش والدی با سطوح تنوع ژنتیکی متضاد تشکیل شد. خزانه ژنتیکی پنجم هم از تلاقی شش ژنوتیپ والدی دارای بیشترین قدرت ترکیب پذیری عمومی حاصل شد. نتایج این مطالعه نشان داد انتخاب با استفاده از نشانگر مولکولی برای افزایش عملکرد نتاج نسل اول موثر بوده است. این روش اصلاحی در برنامه های به‌نژادی بزرگ که تعیین تنوع ژنتیکی با استفاده از صفات فنوتیپی زمان بر و تحت تاثیر اثرات محیطی هستند مفید است.





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