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Genetic diversity among tall fescue ecotypes using agro-morphological traits, nutritional values and AFLP molecular markers

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Abstract

This study was carried out to compare agro-morphological traits and nutritive value of 25 tall fescue (*Festuca arundinacea*) ecotypes grown in Tehran, Iran. The experiments were carried out in a split plot design during 2016-2018 growing seasons. The results showed that there was a significant genetic variation in existing germplasm. The highest heritability was related to dry forage yield and collar diameter (88%). The low heritability levels of forage quality related traits indicated that environmental effects play a greater role in controlling this trait. The mean comparison showed that Isfahan ecotype 11 had the highest number of fertile shoots, dry forage yield and diameter of collar while ecotypes of Shahrood ecotype 9 had the highest percentage of dry mater and Dauphine genotype with 16.05% had the highest protein percentage. The number of fertile shoots (NFS) had a positive and significant correlation with dry forage yield (DFY) and plant height (PH). DFY was positively correlated with NDF (0.71) and negatively correlated with CP (-0.62). In AFLP analysis out of the 463 scored bands, 339 (68%) were polymorphic. PIC values ranged from 0.34 (EcoACA-MseCTA) to 0.10 (EcoAGC-MseCAC). The results showed that genetic distances between ecotypes based on agro-morphologic characters and nutritional values were correlated based on AFLP ($r=0.41$, $P=0.05$) results. Based on cluster analysis all genotypes were classified

into 4 genotypic groups. Considerable genetic variation and high heritability estimates indicate that direct selection for increasing forage yield could be promising. However, breeding nutritional values of forage quality might be more difficult due to environmental effects.

Key words: Cluster analysis, Forage yield, Forage quality, Heritability.

INTRODUCTION

The most important challenge facing agriculture is supplying enough water to produce food, especially in arid and semi-arid regions of the world (Diouf, 2003). Lack of forage is one of the main problems of animal husbandry in Iran (Eshgizadeh, 2008). Despite the prominence of the location of forage plants in livestock training, in Iran, the production and management of these plants have been neglected in comparison with the other crops, which have led to a shortage of animal protein and a deficiency in their quality in the country (Diouf, 2003). Feeding and grazing studies have shown that minor changes in forage digestibility can have a significant impact on animal performance, i.e. beef and milk production (Casler and Vogel, 1999).

Tall fescue (*Festuca arundinacea*), is a major cool-season grass widely distributed in temperate regions throughout the world (Huiying, 2017). This plant is not only more drought tolerant than perennial ryegrass but it also has a higher yield potential. Its nutritional value is due to the phonological stages, the frequency and the

degree of evaporation, and secondary factors such as temperature, germplasm and the availability of water and nutrients, as in other forage species (Scheneiter *et al.*, 2014). Tall fescue is grown primarily for pasture, silage, and hay. To obtain high-quality preserved forage (hay or silage), it is better to harvest at the boot stage. Under grazing and mowing, tall fescue develops a mature sod in 3 to 4 years (Hannaway, 1999). Tall fescue has a lower digestibility and sugar content and a higher fiber and protein content than perennial ryegrass.

The evaluation of the genetic diversity in crop species is useful for the expansion of the genetic base, conservation of genetic resources and practical applications in breeding programs. Estimates of variance components are used to determine the proportion of phenotypic variance due to genetic effects and the proportion of total genetic variance due to additive genetic effects. This information is helpful to estimate heritability and predict genetic gain from a selection. Mohajer *et al.* (2013) studied the forage yield and forage quality in different millet varieties. Analysis of variance showed that in all varieties, all factors were significant at 1% level. The highest average value was in foxtail (14.37).

The heritability of various agronomic traits has been estimated in many forage grass species (De-Araujo and Coulman, 2002). For tall fescue, the magnitude of these estimates has varied among the traits measured, with lower estimates for characteristics under complex genetic control like forage yield (Schiller and Lazenby, 1975) than for more simply inherited characteristics such as plant height (Jauhar, 1975).

Correlations between traits are of interest to determine whether selection for one trait will have an effect on another and also selection could be practiced on a highly heritable trait which correlates with a more complex trait such as yield. Regarding the polygenesis of the forage yield and the low heritability of this trait in most of the crops, one of the methods for increasing yield is the use of traits associated with high heritability. Considering the positive and significant correlations between yield and plant height, any attempt to select tall cultivars increases the yield of forage (Imani *et al.*, 2008). Correlations among traits have been estimated in many perennial forage kinds of grass (De-Araujo and Coulman, 2002). Schiller and Lazenby (1975) reported that forage yield was correlated with dry matter yield, and seed yield was positively correlated with fertility index, harvest index and plant height

in tall fescue. Combined phenotypic correlation between dry matter yield with both water soluble carbohydrates and crude protein were negatively significant, whereas its relationship with crude fiber was negative (Mohajer *et al.*, 2013).

Jafari and Javaresineh (2005) reported that heritability average was high in *Festuca arundinacea*, and stated that among the qualitative traits, the inheritance rate for the digestibility and soluble sugars in water was low and for other qualitative traits the inheritance rate was moderate to high. This indicates the importance of non-additive genes in controlling digestibility and soluble sugars and the role of incremental genes in controlling other qualitative traits.

To study genetic variation, characterize accessions and assess genetic similarities between ecotypes of grasses molecular markers can also be used. Amplified fragment length polymorphisms (AFLP) is a DNA marker based on both restriction enzyme and PCR analyses and usually shows high levels of polymorphism. AFLP is highly reproducible, less sensitive to reaction conditions and does not require DNA sequence information (Vos *et al.*, 1995). Amini *et al.* (2016) showed that AFLP marker assisted parental selection produced superior progenies in tall fescue.

This study was conducted to know the information on the extent and nature of genetic variation, heritability and trait correlations in tall fescue. The information could be used to identify superior genetic materials for future tall fescue breeding programmes.

MATERIALS AND METHODS

Plant materials and experimental design

Twenty five ecotypes of tall fescue (Table 1) were planted according to a Randomized Complete Block Design with 3 replications in 2016. Each plot consisted of two 5 m rows containing 20 plants, 50 cm apart. The soil was a Typic Haplargid, silty clay loam soil on the Research Station of University of Tehran, college of Abouraihan, Iran. The soil was fertilized with 200 kg N/ha (Urea) and 200 kg P/ha (Single Super Phosphate) prior to sowing and 100 kg N/ha was applied to the trial each September based on the previous study (Amini *et al.*, 2016). Iranian tall fescue populations were ecotypes from wide geographical areas grown in Iran. Foreign accessions were kindly provided by Hungarian Institute for Agrobotany (HIFA), Taposzele, Hungary and Agroscope Reckenholz Tanikon research Station (ART) Zurich, Switzerland.

Table 1. Gene bank code and origin of accessions of *F. arundinacea*.

Name	Origin
Ecotype 1	IranSemnan—Shahrood
Ecotype 2	Iran, Semnan—Shahrood
Ecotype 3	Hungary—Csesznek
Ecotype 4	Iran, Isfahan—Mobarake
Ecotype 5	Iran, Isfahan—Fozve
Ecotype 6	Hungary
Ecotype 7	Iran, Isfahan—Fozve
Ecotype 8	Iran, Isfahan—Mobarake
Ecotype 9	Iran, Semnan—Shahrood
Ecotype 10	Poland
Ecotype 11	Iran, Isfahan
Ecotype 12	Hungary
Ecotype 13	Iran, Kohkiluye—Yasuj
Ecotype 14	Hungary—Csesznek
Ecotype 15	Iran, Isfahan—Daran
Ecotype 16	Iran, Kohkiluye—Yasuj
Ecotype 17	Iran, Parsabad, Ardebil
Ecotype 18	Iran, Golestan, Gorgan
Ecotype 19	Iran, ChaharMahal, Boroujen
Ecotype 20	Iran, Isfahan, Semirrom
Ecotype 21	Iran, Hamedan, Hamedan
Dauphine	DSP/ART, Switzerland
Otaria	DSP/ART, Switzerland
Molva	DSP/ART, Switzerland
Belfine	DSP/ART, Switzerland

Data collection

Data were collected during the 2016-2018 growing seasons. Agro-morphologic and nutritional data were collected from March to October at different developmental stages of tall fescue. The phenotypic traits such as number of fertile shoot (NFS), dry forage yield (DFY), plant height (PH) and collar diameter (CD) were evaluated on 10 plant as follows:

Number of Fertile Shoot (NFS): Number of fertile tillers per plant before threshing.

Dry forage yield (DFY): The weight (g) of foliage per plant after drying at 72 °C for 48 hours.

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

Collar diameter (CD): The width (cm) of foliage remaining for each plant after the first cut.

In order to compare the ecotypes for nutritional value such as crude protein (CP), ash and neutral detergent fiber (NDF) the samples harvested in two steps. The first stage was the time of clustering (five plants) and the next stage was complete flowering (five plants). In order to determine the percentage of

dry matter, the fresh weight of the plant was recorded and then transferred to the oven at 72 °C for 48 hours. All dried samples were ground using a mill to pass a 1-mm screen and analyzed crude protein (CP), ash and neutral detergent fiber (NDF).

Crude protein (CP) was determined using the Kjeldahl method (Kjeldahl, 1883). The chemical composition of fodder samples including ash based on AOAC (2012) and the insoluble fiber in neutral detergents (NDF) was measured based on Van Suste's *et al.* (1991).

Data analysis

Raw data obtained from single plants were tested for the normal distribution. Analysis of variance was performed to examine differences between ecotypes, and to estimate the variance components, using the general linear model (GLM) of SAS Institute (1988). The variance components, correlation, genetic coefficient of variation and broad-sense heritability were computed as suggested by Burton (1989).

The analysis of variance on traits was carried out in a randomized complete block design using split-plot. The effect of ecotype was used as a main plot and the samples from two harvest times from different phonological levels was considered as the subplot. The Duncan's significant multiple range test was used to compare the treatment values at a probability level of 0.05%. Genetic variance was calculated based on the expected mean squares and used to determine genetic coefficients of variation according to Falconer and Mackay (1996). The genotypic coefficient of variation (CV_g) and phenotypic coefficient of variation (CV_p) were calculated as equation 1, 2:

$$(1) \quad CV_g = (\sigma_g / \bar{x}) 100$$

$$(2) \quad CV_p = (\sigma_p / \bar{x}) 100$$

Where σ_g , σ_p and \bar{x} are the standard deviation of the genotypic effect, standard deviation of the phenotypic effect, and the phenotypic mean, respectively.

Broad-sense heritability was estimated as equation 3:

$$(3) \quad h_b^2 = (\sigma_g^2 / \sigma_p^2) \times 100$$

Heritability between 80-100% was considered high, heritability between 60-80% was considered moderately high, heritability between 40-60% was considered moderate and heritability <40% was considered low (Falconer and Mackay, 1996).

The means of each trait were used for cluster analysis. Euclidean distance was used for cluster analysis with the Ward method by using SPSS software version 16. The number of groups in dendrogram was recognized

based on F-bill test by SAS software (analysis of variance based on completely randomized design is unbalanced and considers the groups as treatments and genotypes within each group are repeated with a discriminate function (by SPSS software).

AFLP analysis

A total of 25 tall fescue accessions were used for DNA extraction. Young leaves of 10 plants of each accession were collected and DNA was extracted using the DNeasy 96 Plant Kit (Qiagen, Hilden, Germany). The DNA content of the samples was quantified using a NanoDrop spectrophotometer (LS-30; Perkin Elmer Instruments, Shelton, CT, USA). Evaluation of genetic diversity based on AFLP molecular marker was performed. PCR products were evaluated on an ABI Prism 3130 XL (Applied Biosystems, Foster City, CA, USA).

AFLP data analysis

AFLP patterns were analyzed using GeneMarker 1.51 software (SoftGenetics, LLC, State College, PA, USA). The data were interpreted according to the presence and absence of bands, generating a binary matrix containing the profile of each ecotype. Euclidean distance among ecotypes was investigated and used for cluster analysis with the Ward method by SPSS software version 16.

RESULTS

Agro-morphological traits

Analysis of variance for forage yield and related traits such as NFS, DFY, PH and CD among 25 ecotypes of tall fescue (*Festuca arundinacea*) showed that the effect of year, replication, ecotype, harvest time and ecotype×harvest time was significant (Table 2).

Table 2. Analysis of variance for forage yield and related traits among 25 genotypes of tall fescue (up) and for forage quality and related traits among genotypes of tall fescue (down) in 2016 and 2017.

Source of variation	df	Mean of square			
		NFS	DFY	PH	CD
Year	1	202800 ^{**}	46875 ^{**}	10800 ^{**}	1875 ^{**}
Rep	2	28844.78 ^{**}	58583.38 ^{**}	21247.93 ^{**}	151.52 ^{**}
Rep×Year	2	211.45	442.36	88.25	1.48
Ecotype	24	394.89 ^{**}	152.47 ^{**}	70.43 ^{**}	2.68 ^{**}
Year×Ecotype	24	25.81 ^{ns}	21.58 ^{ns}	11.45 ^{ns}	0.12 ^{ns}
Rep×Ecotype	48	216.23 ^{ns}	146.61 ^{ns}	32.59 ^{ns}	1.48 ^{ns}
Rep×Ecotype×Year	48	212.24	154.23	85.26	3.25
Harvest	1	32910.56 ^{**}	3299.02 ^{**}	3282.99 ^{**}	66.19 ^{**}
Ecotype×Harvest	24	60.49 [*]	156.81 ^{**}	91.16 ^{**}	1.09 ^{**}
Year×Harvest	1	12.45 ^{ns}	8.25 ^{ns}	0.87 ^{ns}	0.01 ^{ns}
Year×Ecotype×Harvest	24	16.32 ^{ns}	12.23 ^{ns}	8.42 ^{ns}	0.05 ^{ns}
Error	100	33.43	32.18	13.45	0.13

Source of variation	df	Mean of square			
		DM	CP	NDF	ASH
Year	1	20164 ^{**}	49875 ^{**}	22800 ^{**}	2012 ^{**}
Rep	2	5432.23 ^{**}	4654.25 ^{**}	5245.41 ^{**}	45.63 ^{**}
Rep×Year	2	21.45	45.25	55.73	0.23
Ecotype	24	87.25 ^{**}	47.22 ^{**}	24.21 [*]	0.47 ^{**}
Year×Ecotype	24	6.04 ^{ns}	14.72 ^{ns}	10.47 ^{ns}	0.05 ^{ns}
Rep×Ecotype	48	4.41 ^{ns}	5.62 ^{ns}	14.03 ^{ns}	0.89 ^{ns}
Rep×Ecotype×Year	48	5.32	11.54	18.25	3.01
Harvest	1	283.04 ^{**}	452.41 ^{**}	325.03 ^{**}	28.32 ^{**}
Ecotype×Harvest	24	28.42 ^{**}	12.32 ^{ns}	41.21 ^{**}	32.14 ^{**}
Year×Harvest	1	8.14 ^{ns}	3.21 ^{ns}	1.87 ^{ns}	0.21 ^{ns}
Year×Ecotype×Harvest	24	6.01 ^{ns}	11.02 ^{ns}	6.25 ^{ns}	0.03 ^{ns}
Error	100	3.23	8.24	9.03	0.04

*, ** and ns: Significant at 5% and 1% and non-significant probability levels, respectively.

NFS: Number of fertile shoots, DFY: Dry forage yield, PH: plant height, CD: Collar diameter.

DM: Percentage of dry matter, CP: Percentage of crude protein, NDF: neutral detergent fiber and ASH: Ash percentage.

The highest genotypic and phenotypic coefficients of variation were 21.12 and 22.47, respectively, which were related to the CD. The lowest genotypic and phenotypic coefficients were 8.28 and 8.96 respectively, which were related to PH. The highest heritability was related to dry forage yield and collar diameter (0.88) (Table 3). Analysis of variance on forage quality related traits showed that the effect of year, replication, ecotype and harvest time was significant for all the evaluated traits ($p \leq 0.01$). The differences between ecotypes \times harvest time were significant for DM, NDF and Ash. The existence of this interaction effect shows that the response of ecotype were not the same in different harvest times (Table 2). In this study, coefficient of phenotypic variation and coefficient of genotypic variation for ash percentage was higher than other qualitative traits. A low heritability level was obtained for CP and ASH (31% and 38%) indicating that genetic enhancement has a small effect and environment has a greater effect in controlling this trait (Table 3).

The mean comparison table showed that Isfahan ecotype 11 and Dauphine genotype had the highest NFS (114 and 112, respectively) (Table 4). The lowest NFS (24) was related to ecotypes 15 and 21. Comparison of means for dry forage yield showed that ecotype from Isfahan (ecotype 11) with 72.01 gr and ecotype from Yasuj (ecotype 16) with 51.59 gr had the highest and lowest values, respectively. The ecotype of Isfahan (ecotype 11) with 6.23 had the highest diameter of collar and Shahrood ecotype (ecotype 9) with 4.72 lowest collar diameters (Table 4). The comparison of means showed that a Shahrood ecotype (9) had the highest percentage of DM with 49.23% and a Pars Abad ecotype (17) had the lowest percentage with 44.08%. However, the differences

between evaluated ecotypes were not significant (Table 4). The Dauphine genotype with 16.05% had the highest percentage of protein and Pars Abad ecotype with 12.66% had the lowest protein percentage. Comparison of means for ash percentage showed that ecotype Fozve (ecotype 4) and ecotype Mobarake (ecotype 5) with 15.13 and 15.03 had the highest values, respectively. The ecotype 9 from Shahrood had the lowest percentage of ash (12.00) (Table 4).

Cluster analysis was performed on the studied ecotypes (Figure 1) and 4 distinct groups were identified based on the F bill (Table 5) and discriminate analysis (data not shown). Analysis of variance showed a significant difference between groups in all traits, indicating a high diversity of ecotypes among the groups compared to the variation within the groups. Ecotypes of the third group had the highest values for FFY, DFY and CD. Ecotypes of the fourth group had the highest values for DM (Table 5). The differences between group 3 and 4 for CD, CP, NDF and Ash were not significant. The results of clustering indicated that the ecotypes collected from regions with different climates were grouped in the same group, showing a discrepancy between genetic variation and geographic diversity.

Association of NFS with DFY and PH was positive and significant (Table 6). Collar diameter (CD) was also positively correlated with NFS and DFY. There were no significant associations among yield quality related traits with the exception of DM and CP ($r=0.57$) (Table 7). Plant height and NFS were significantly and negatively correlated with CP. DFY was positively correlated to NDF (0.71) and negatively correlated to CP (-0.62). Correlation coefficient between PH and NDF was 0.68 (Table 6).

Table 3. Genotypic and phenotypic variances, heritability, genotypic and phenotypic coefficient of variation (CV) for forage yield and forage quality and related traits in tall fescue (*Festuca arundinacea*).

Traits	Estimation of variance components		Coefficient of variation (%)		h ² (%)
	Genotypic	Phenotypic	Genotypic	Phenotypic	
NFS	182.33	212.55	13.42	14.49	86
DFY	155.00	177.01	20.18	21.56	88
PH	25.99	30.43	8.28	8.96	85
CD	1.25	1.43	21.12	22.47	88
DM	3.8	6.53	4.17	5.47	58
CP	0.50	1.61	4.89	8.74	31
ASH	0.63	1.64	5.76	9.31	38

NFS: Number of fertile shoots, DFY: Dry forage yield, PH: Plant height, CD: Collar diameter.
DM: Percentage of dry matter, CP: Percentage of crude protein and ASH: Ash percentage.

Table 4. Comparison between means forage yield and forage quality and related traits among ecotypes of tall fescue (*Festuca arundinacea*) in two years.

Name	NFS	DFY	CD	PDFY	DM	CP	Ash
Ecotype 1	101.45 ^{bcdef}	63.18 ^{abcde}	5.09 ^{cdef}	63.10 ^{abcde}	46.29 ^{abcd}	13.79 ^{abcde}	13.98 ^{abcde}
Ecotype 2	109.66 ^{abc}	71.01 ^{ab}	5.86 ^{ab}	64.71 ^{ab}	42.23 ^{def}	10.45 [†]	11.23 ^{tgh}
Ecotype 3	98.30 ^{def}	62.57 ^{bcde}	5.15 ^{bcdef}	63.65 ^{abcde}	41.45 ^{ef}	11.32 [†]	10.24 ^h
Ecotype 4	100.58 ^{cdef}	64.23 ^{abcd}	5.44 ^{abcdef}	63.78 ^{abc}	48.38 ^{ab}	15.03 ^{ab}	15.03 ^{ab}
Ecotype 5	106.12 ^{abcd}	69.67 ^{abc}	5.56 ^{abcdef}	65.71 ^a	45.29 ^{bcde}	15.09 ^a	15.13 ^a
Ecotype 6	106.69 ^{abcd}	65.53 ^{abcd}	5.80 ^{abc}	61.37 ^{bcde}	41.23 ^{ef}	12.21 ^{def}	11.23 ^{tgh}
Ecotype 7	103.23 ^{abcde}	65.54 ^{abcd}	5.42 ^{bcdef}	63.29 ^{abcde}	47.25 ^{abc}	15.57 ^a	14.00 ^{abcde}
Ecotype 8	99.75 ^{cdef}	60.27 ^{de}	5.57 ^{abcde}	60.63 ^{bcde}	42.31 ^{de†}	12.14 ^{ef}	10.24 ^h
Ecotype 9	103.29 ^{abcde}	63.11 ^{bcde}	4.72 [†]	61.75 ^{abcde}	49.23 ^a	14.49 ^{abcd}	12.00 ^{tgh}
Ecotype 10	94.17 ^{ef}	55.58 ^e	5.47 ^{abcdef}	59.53 ^e	42.18 ^{def}	11.23 [†]	10.32 ^{gh}
Ecotype 11	113.56 ^a	72.01 ^a	6.23 ^a	63.46 ^{abcde}	46.18 ^{abcd}	13.76 ^{abcdef}	12.27 ^{efgh}
Ecotype 12	94.26 ^{ef}	57.47 ^e	6.12 ^a	61.53 ^{bcde}	42.17 ^{def}	12.25 [†]	10.25 ^{gh}
Ecotype 13	99.00 ^{def}	60.48 ^{cde}	5.61 ^{abcde}	61.36 ^{bcde}	46.01 ^{abcd}	14.81 ^{abc}	12.97 ^{cdef}
Ecotype 14	102.32 ^{bcde}	63.91 ^{abcde}	5.91 ^{ab}	62.86 ^{abcde}	41.23 ^{ef}	11.25 [†]	11.23 ^{gh}
Ecotype 15	94.21 ^{ef}	57.67 ^{de}	4.78 [†]	62.00 ^{abcde}	46.53 ^{abcd}	13.63 ^{bcdef}	14.35 ^{abc}
Ecotype 16	93.92 ^{ef}	51.59 ^e	4.79 ^{ef}	55.41 ^e	40.21 [†]	10.12 [†]	12.21 ^{efgh}
Ecotype 17	99.22 ^{cdef}	60.55 ^{cde}	5.48 ^{abcdef}	61.34 ^{bcde}	44.08 ^{ced}	12.66 [†]	14.32 ^{abcd}
Ecotype 18	92.65 [†]	57.46 ^e	4.98 ^{def}	62.57 ^{abcde}	40.21 [†]	11.24 [†]	11.25 ^{tgh}
Ecotype 19	102.80 ^{bcde}	63.14 ^{abcde}	6.15 ^a	61.14 ^{bcde}	41.32 ^{ef}	10.24 [†]	10.32 ^{gh}
Ecotype 20	97.64 ^{ef}	61.60 ^{cde}	5.77 ^{abc}	63.73 ^{abcd}	42.14 ^{ef}	11.25 [†]	11.24 ^{tgh}
Ecotype 21	100.79 ^{bcdef}	60.77 ^{cde}	5.76 ^{abcd}	60.50 ^{cde}	41.23 [†]	12.24 ^{def}	10.25 ^h
Dauphine	111.32 ^{ab}	67.26 ^{abcd}	5.92 ^{ab}	60.47 ^{de}	47.94 ^{ab}	16.05 ^a	13.69 ^{bcde}
Otaria	101.13 ^{bcdef}	60.57 ^{cde}	6.05 ^a	60.58 ^{bcde}	43.25 ^{def}	12.24 ^{def}	12.32 ^{efg}
Molva	97.82 ^{def}	55.69 ^e	6.19 ^a	56.72 ^e	41.32 ^{ef}	12.36 [†]	10.23 ^h
Belfine	91.81 [†]	52.01 ^e	4.93 ^{ef}	57.32 ^e	42.24 ^{def}	11.23 [†]	11.35 ^{tgh}

NFS: Number of fertile shoots, DFY: Dry forage yield, PH: Plant height; CD: Collar diameter.

DM: Percentage of dry matter, CP: Percentage of crude protein and ASH: Ash percentage.

Values in columns followed by the same letters are not statistically significant based on the Duncan's multiple rang test ($p=0.05$).

Table 5. Analysis of variance and mean comparison between groups of cluster analysis for agromorphologic and nutritional values.

Traits	Mean square between groups	Mean square inside the groups	Mean			
			Group 1	Group 2	Group 3	Group 4
NFS	185.70 ^{**}	100.63	101.38 ^b	92.86 ^c	112.44 ^a	100.89 ^b
DFY	167.28 ^{**}	61.71	62.11 ^b	51.82 ^c	69.63 ^a	63.05 ^b
PH	27.80 ^{**}	61.54	60.90 ^a	62.88 ^a	56.37 ^b	63.14 ^a
CD	1.31 ^{**}	5.56	5.81 ^a	4.86 ^b	6.07 ^a	5.88 ^a
DM	4.12 ^{**}	46.72	41.89 ^c	41.22 ^c	47.06 ^b	66.63 ^a
CP	1.91 [*]	14.49	11.89 ^b	10.67 ^c	14.90 ^a	14.38 ^a
NDF	6.27 [*]	57.39	57.43 ^b	56.77 ^b	60.24 ^a	60.54 ^a
ASH	2.23 [*]	13.77	11.08 ^b	11.78 ^b	12.98 ^a	13.97 ^a

* and ** : Significant at 5% and 1% probability levels, respectively.

NFS: Number of fertile shoots, DFY: Dry forage yield, PH: Plant height, CD: Collar diameter.

DM: Percentage of dry matter, CP: Percentage of crude protein, NDF: Neutral detergent fiber and ASH, Ash percentage.

Values in columns followed by the same letters are not statistically significant based on the Duncan's multiple rang test ($p=0.05$).

Table 6. Coefficient of correlation (r) between traits observed on 25 tall fescue accessions grown in 2016 and 2017.

Traits	NFS	DFY	PH	CD	DM	CP	NDF
DFY	0.921**						
PH	0.463*	0.774**					
CD	0.491*	0.409*	-0.71**				
DM	0.560*	0.684**	0.421*	0.440*			
CP	-0.710**	-0.622**	-0.681**	0.683**	0.570*		
NDF	0.452*	0.712**	0.682**	0.407*	0.742**	0.312 ^{ns}	
ASH	0.561*	0.622**	0.422*	0.461*	0.412*	0.085 ^{ns}	-0.359 ^{ns}

*, ** and ns: Significant at 5% and 1% and non-significant probability levels, respectively.

NFS: Number of fertile shoots, DFY: Dry forage yield, PH: Plant height, CD: Collar diameter.

DM: Percentage of dry matter, CP: Percentage of crude protein, NDF: Neutral detergent fiber and ASH: Ash percentage.

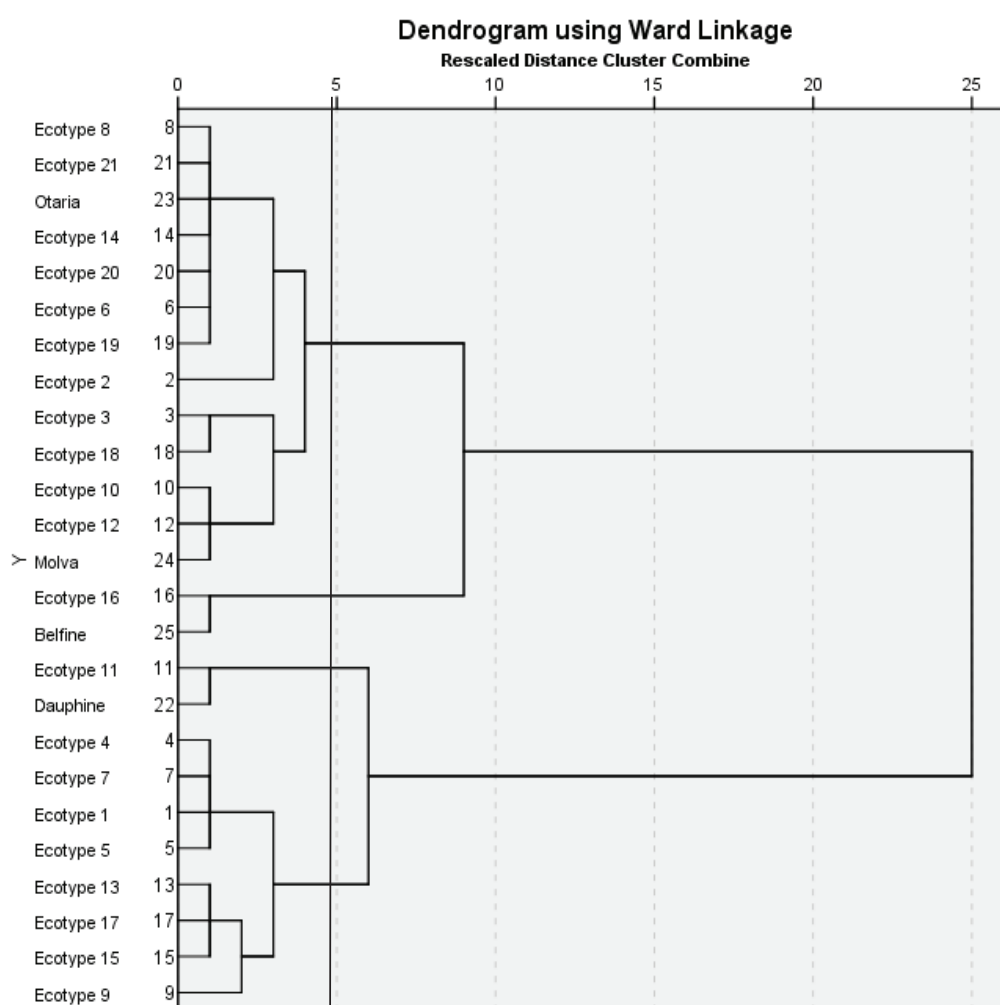


Figure 1. Ward-based dendrogram showing genetic relationship among 25 tall fescue ecotypes based on agro-morphological and nutritional values.

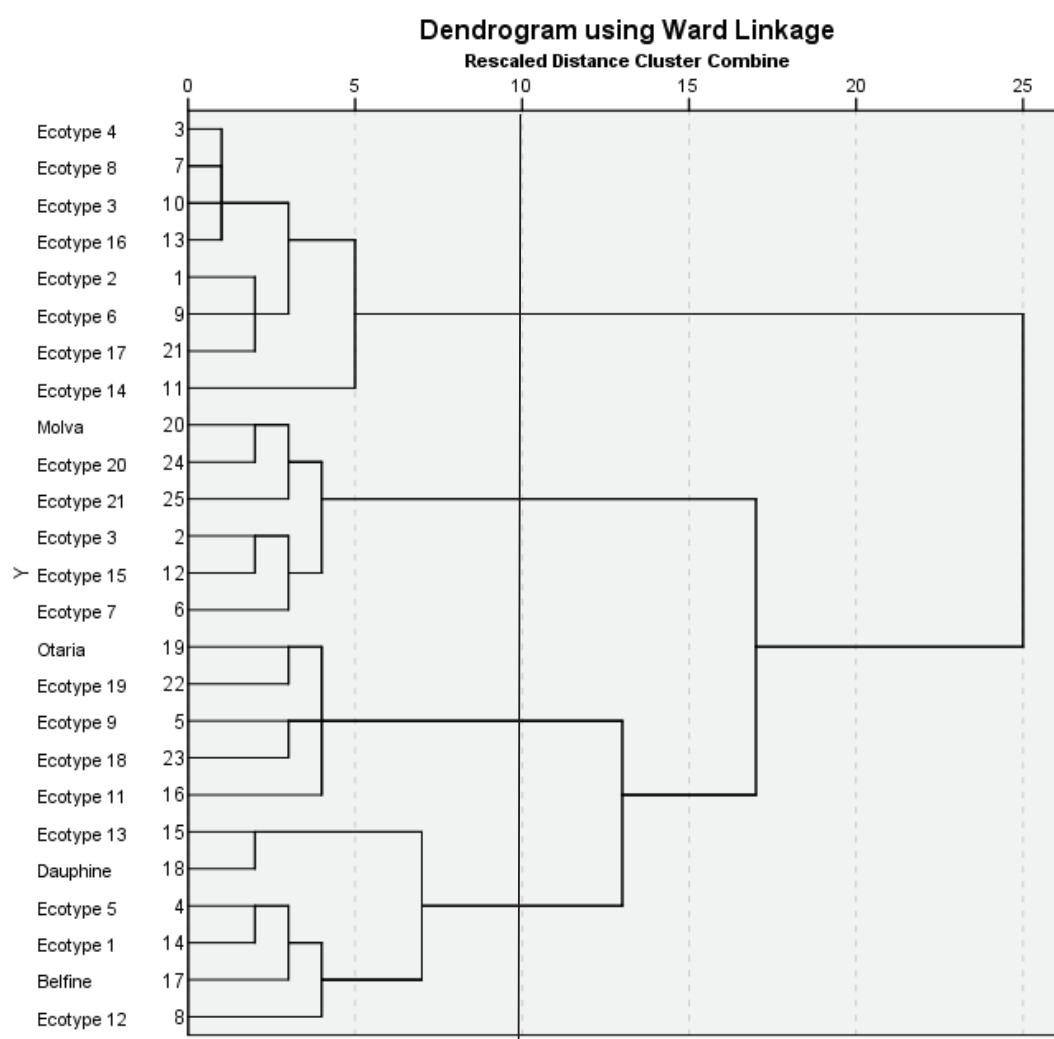
AFLP results

A total of 463 fragments were scored from 5-primer combinations (Table 7). Out of the 463 scored bands, 339 (68%) were polymorphic. The number of polymorphic bands for each primer combination

varied from 25 to 114. The EcoACA-MseCTA primer combination produced the greatest number of polymorphic bands, while the EEcoAGC-MseCAC primer combination produced the lowest number of polymorphic bands (Table 7).

Table 7. Primer combinations and the number of polymorphic AFLP bands.

Primer pair	Total number of bands	No. of selected polymorphic fragments	Polymorphism (%)	Average across germplasm
EcoACA-MseCTA	125	114	91.2	0.34
EcoAGA-MseCTA	104	94	90.4	0.25
EcoAGG-MseCAA	98	74	75.5	0.19
EcoAGA-MseCAC	72	32	44.4	0.12
EcoAGC-MseCAC	64	25	39.1	0.10
Total	463	339	-	-
Average	92.6	67.8	68.12	0.20

**Figure 2.** Ward-based dendrogram showing genetic relationship among 25 tall fescue ecotypes based on AFLP molecular markers.

PIC values ranged from 0.34 (EcoACA-MseCTA) to 0.10 (EcoAGC-MseCAC), and the mean PIC value among ecotypes was 0.20 for the complete set of AFLP markers. A large proportion of the markers presented high discrimination power (Table 7).

The pair-wise genetic distances of the accessions was calculated in order to cluster the tall fescue ecotypes that resulted in four distinct clusters (Figure 2). The first cluster contains 32 percent of ecotypes such as ecotypes number 4 and 8 from Isfahan, 3, 6 and 14 from Hungry,

2 from Semnan, 16 from Yasuj and 17 from Parsabad. Cluster 2 comprised of Molva and ecotypes 7, 15 and 20 from Isfahan and 21 from Hamedan. Cluster 3 contained Otaria and ecotypes 19 from Chaharmahal, 9 from Semnan, 18 from Gorgan and 11 from Isfahan. Cluster 3 included Daupin and Belfine ecotypes from Swizerland and ecotypes 13 from Yasuj, 5 from Isfahan, 1 from Semnan and 12 from Hungry.

The Mantel test showed that genetic distances between ecotypes based on agro-morphologic characters and nutritional values were correlated with genetic distances between ecotypes based on AFLP ($r=0.41$, $P=0.05$).

DISCUSSION

All breeding methods of plants and the production of high yield varieties are based on the use of genetic diversity. Genetic diversity is the basis of phenotypic selection, genotypic and qualitative and quantitative correction of plant species (Falconer and Mackay, 1996). The results showed that there is a significant genetic variation in existing germplasm and there is the possibility of effective utilization of this variation in improving the ecotypes. In the open-pollinated plant, the relatively high heritability estimates and low standard errors indicate that selection for yield is effective (Yoheand Poehlman, 1972). Estimates of forage yield heritability in this study is similar to, or higher than, estimates in the literature for perennial grasses such as *Festuca arundinacea* (Bean, 1972; Majidi *et al.*, 2009). In this study, the phenotypic variation coefficients for all traits were greater than the genetic variation coefficients (Table 3). The slight difference between these two coefficients indicates that the effect of the environment is low on the estimation of these traits. Considering the high heritability and low differences between phenotypic coefficient of variation and genotypic coefficient of variation for forage yield and related traits, it seems that the additive factors control these characteristics. Additive gene action has been the main factor responsible for variation in many agronomic traits in perennial forage grasses (Barker and Kalton, 1989; Burton, 1989; Aastiveit and Aastiveit, 1990). The low heritability for nutritional values showed that the environmental effects control these traits.

Generally in this study, the heritability of forage yield and its components were higher than forage quality traits (Table 3). There is considerably more information in the perennial grass literature about forage yield than forage quality. This supports the fact that, in

most cases, the main breeding objective is to improve the forage yielding capacity of the crop. However, in order to estimate heritability and genetic efficiency of yield and quality of tall fescue forage, the parents and their polycross progenies must be studied (Jaffari and Javarsineh, 2006). Heritability of forage yield was 55%, the date of cluster emergence was 92%, plant height was 50%, and diameter of the crown was 68%.

Estimates of heritability for DFY in the present study were generally higher than those previously reported for *F. arundinacea* (Hovin *et al.*, 1976; Majidi *et al.*, 2009), and *F. pratensis* (Aastiveit and Aastiveit, 1990; Kanapeckas *et al.*, 2005).

In this study, all tall fescue accessions in the second year (2017) produced higher DFY and were taller, denser and more vigorous than in 2016 because perennial forage crops undergo development over years resulting in increases in size and height (Jafari *et al.*, 2003). In this study, the year \times ecotype interaction was not significant for any traits showing performance ranking of all accessions did not change over years. In this study, ecotype of Isfahan ecotype (11) had the highest number of fertile shoots, dry forage yield and diameter of collar while the Shahrood ecotype (ecotype 9) had the highest percentage of dry mater and Dauphine genotype with 16.05% had the highest protein percentage. Therefore, ecotype 11, ecotype 2 and Dauphine were the best accessions for forage production over the two years.

Correlation is one of the important statistical parameters for investigating the relationship between traits. Recognition of the relationship between yield and quality of forage is very important for success in breeding programs is very important. Success in the cultivation and production of high quality cultivars is dependent on the genetic control of forage yield and its relation with other morphological traits and forage quality (Nakhichevan, 2016). Positive and significant correlations between dry forage yield, plant height and number of fertile shoots have been reported in this study. It can be concluded that by improving these related components, the forage yield may be increased. Jafari and Ziaaninasab (2001) reported a positive and significant correlation between forage yield and number of stems and crown diameter in *Folium perenne* from close relatives of *Festuca arundinaceae*. Positive correlation between dry mater yield and acid- and neutral-detergent fiber concentration has been reported in smooth brome grass (Falkner *et al.*, 1998). The relationship between dry matter yield and crude protein has usually been negative in perennial grasses (Berg and Hill, 1983).

Cluster analysis revealed that genetic and geographic distances were not related. For example the placement of the Poland ecotype in the fourth group with Iranian ecotypes indicates their kinship. These results were confirmed by the Mantel test showing a low coefficient of correlation between genetic and geographic distances. This can be due to the exchange of plant materials across the regions. However, the correlation between genetic distances of ecotypes based on agro-morphologic characters and nutritional values and genetic distance based on AFLP markers was positive and significant. Ecotype 2 and 6, ecotype 1 and 5, ecotype 19 and Otaria, ecotype 20, 21 and Molva clustered in the same group in both classifications revealing the most similarities between these accessions. So for cross pollination to produce synthetic varieties it can be helpful to choose one of the similar accessions.

Considerable genetic variation and high heritability estimates indicate that direct selection for increasing forage yield should be successful. Development of synthetic varieties is the most effective breeding method in out-crossing perennial forage grasses (Vogel and Pedersen, 1993). The high variation in tall fescue accessions is valuable for developing varieties with high dry forage yield and good nutritive value. In this study ecotype of Isfahan (ecotype 11) had the highest number of fertile shoots, dry forage yield and diameter of collar while ecotype of Shahrood (ecotype 9) had the highest percentage of dry mater and Dauphine genotype with 16.05% had the highest protein percentage. It can also be concluded that there are large resources of untapped genetic variation in collections of tall fescue, and most local tall fescue breeding pools have probably been through very few generations of selection. However, breeding of forage quality might be more difficult due to environmental effects.

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