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GENETIC VARIATION AND RELATIONSHIPS BETWEEN LOCAL AND EXOTIC GERMPLASM OF *DACTYLIS GLOMERATA*, BASED ON MORPHOLOGICAL AND TOTAL PROTEIN MARKERS

Parvin Salehi Shanjani[1*], Ali Ashraf Jafari[1] and Laleh Kohi[1]

[1]Research Institute of Forests and Rangelands, P O BOX 13185-116, Tehran, Iran
[2]Payam Noor University, Tehran, Iran
[1*]Corresponding author. E-mail: psalehi@rifr-ac.ir; psalehi1@gmail.com

ABSTRACT

Cocksfoot (*Dactylis glomerata* L.) is one of the main perennial grasses that naturally grow in arid to semiarid pastures and rangelands, with a typical Mediterranean climate, in northern and western Iran. Despite its importance, there is a lack of information on the genetic diversity within and among populations. Data on total protein profiles and 14 phenotypic traits, collected on 21 wild cocksfoot populations from seven different origins/sources (Iran, USA, Russia, Estonia, Kyrgyzstan, Netherlands and Spain) were utilized for estimations of genetic diversity. Dendrograms were prepared using cluster analysis based on Nei's similarity coefficients in case of total protein profiles and on squared Euclidean distances in case of phenotypic traits. Clustering pattern, made on the basis of protein analysis data, grouped the accessions differently and gave no clear indication of phenotypic performance or origin/source. Further examination of the different components of genetic variation by analysis of molecular variance indicated that larger proportions of variability existed within populations (88%). The results also showed that comprehensive germplasm collection in major geographic regions and exploitation of the existing variation are required to widen the genetic base and sample the full extent of the available variation in breeding strategies for cocksfoot.

Key words: cocksfoot, phenotypic diversity, genetic diversity, SDS-PAGE, wild population.

INTRODUCTION

Cocksfoot (*Dactylis glomerata* L.) is one of the main perennial grasses that naturally grow in arid to semiarid pastures and rangelands, with a typical Mediterranean climate, in northern and western Iran at altitudes of 500-2900 m. It is used for grazing and hay production (Jafari and Naseri, 2007). It is also an important intercrop in fruit orchards and under shading. Because of its dense network of roots, cocksfoot is recommended as a part of a seed mix for erosion control (Anderson and Brooks, 1975; Mclean and Clark, 1980). A few studies have been conducted on *D. glomerata* in different ecological conditions of Iran and revealed that there was considerable variation in herbage yield, seed yield and crude protein content (Jafari, 2004). Knowledge of genetic variability and relationships among traits is necessary for facilitating the transfer of useful genes and maximizing the use of available germplasm resources. The extent of genetic diversity in germplasm can be assessed through morphological characterization and genetic markers. The characterized material then helps the plant breeders to select the accessions to be utilized in hybridisation program (Ghafoor et al., 2002). The genetic structure of the Iranian cocksfoot populations, however, still remains unclear despite its usefulness as a genetic resource.

Variations in cocksfoot morphological features, distributional patterns, adaptive and agronomic characters, and allozymes are well documented (Lumaret et al., 1987; Volaire and Thomas, 1995; Volaire, 1995; Gautier and Lumaret, 1999; Sugiyama and Nakashima, 1999; Tosun et al., 2002). DNA profiling techniques that have been successfully used in assessing genetic diversity and relatedness of cocksfoot germplasm include randomly amplified polymorphic DNA (RAPD) markers (Zeng et al., 2006), inter-simple sequence repeat (ISSR) (Zeng et al., 2006), Sequence-

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related amplified polymorphism (SRAP) (Zeng et al., 2008) and AFLPs (Peng et al., 2008a, b). However, there is a lack of information on the genetic diversity of Iranian cocksfoot wild populations using biochemical markers.

Electrophoresis (SDS-PAGE) is widely used to describe seed protein diversity of crop germplasm (Das and Mukharjee, 1995). This method can also be used as a promising tool for distinguishing cultivars of particular crop species. However, a few studies indicated that SDS-PAGE method was not efficient method for cultivar identification (De-Vries, 1996). To our knowledge, no studies have yet been made in Iran on the diversity of cocksfoot germplasm based on protein electrophoresis. So the present study aimed to evaluate and to compare the genetic structure of 21 wild populations of cocksfoot from seven different origins/sources (Iran, USA, Russia, Estonia, Kyrgyzstan, Netherlands and Spain), using neutral and potentially selective markers. It is generally much easier to characterize differences between populations for molecular markers than for agronomical important traits. Because molecular markers are considered, a priori, neutral, while agronomic traits are most likely to be under selection even in natural populations, it is interesting to compare the inferences one can make from observations on these two kinds of traits.

MATERIAL AND METHODS

Seed material and Experiment layout

Seed material of 21 wild populations of cocksfoot from seven different origins/sources including Iran (11 populations), USA (two populations), Russia (three populations), Estonia (one population), Kyrgyzstan (one population), Netherlands (two populations) and Spain (one population) were obtained from National Natural Resources Gene Bank, Iran (Table 1).

Table 1. The environmental data of 11 Iranian wild populations of cocksfoot

Population	Annual average precipitation (mm)	Annual average maximum temperature (ºC)	Annual average minimum temperature (ºC)	Elevation (m from see Level)	Latitude (N)	Longitude (E)
Karaj1-4	208.00	21.87	9.38	1390	35º 49'	51º 00'
Zanjan	310.12	20.19	8.80	1650	36º 40'	48º 28'
Tabriz	243.77	19.29	8.11	1366	38º 05'	46º 17'
Ourmieh	260.28	18.40	5.63	1332	37º 32'	45º 20'
Ardebil	269.95	16.04	3.35	1311	38º 15'	48º 18'
Malayer	259.85	19.56	7.22	1750	33º 17'	48º 49'
Pasand1-2	425.38	20.92	8.98	40	36º 34'	53º 05'

For field experiment the 21 populations were evaluated under spaced planting, during 2008–2010 in Alborz Research Center, Karaj, Iran. From each population, 30 seedlings were established in compost. After growing in the glasshouse, the seedlings were transplanted to the field in October 2008. An experiment was established using a randomised complete block design with three replications. In each plot, ten genotypes of each population were allocated in 40 × 40 cm row spacing. The data were collected and analysed for following 8 phenotypic traits: day to heading, day to pollination, plant height (cm), forage dry matter yield (th^{-1}), stem number, panicle length (cm), 1000 grain weight (g) and grain yield ($kgha^{-1}$).

All populations were tested for germination characteristics. The normal ISTA laboratory germination test procedure was used with three replications. 150 seeds were sterilized with 70% ethyl alcohol for five minutes, and then washed with distilled water. Three replicates (50 seeds per replicate) of sterilized seed were placed in Petri dishes on double Whatman papers (TP). For protection

against moulds, the water used to moisten the seed samples and substrata contained 0.002% Benomil fungicide. The samples were immediately transferred into a germinator at (20±4°C) with 1000 lux light for 15 days. The percent and speed of germination were recorded at 3, 6, 9, 12 and 15 days. The length of roots and shoots of 10 randomly-selected seedlings from each replicate were measured in 15 days seedlings. After measuring shoot and root lengths, the caryopses were cut from the seedlings and fresh seedling weight of each replicate was recorded. The seedlings were then placed in an oven at 80°C for 24 hours, after which the dry weight of each replicate was recorded as a percentage of the fresh weight. The vigour index measures seedling performance, relating together the germination percentage and growth of seedlings produced after a given time (Abdul-Baki and Anderson, 1973).

Total protein analysis

For study the extent of genetic variation based on SDS-PAGE markers, a total of 210 entries were selected from 21 local and exotic populations. Total proteins were extracted from 14 day-old seedlings using protein extraction 0.05 M Tris-HCL, pH=8, 0.2% SDS, 5 M urea, 1% B-mercaptoethanol. Electrophoresis was carried out in the discontinuous Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) system of Laemmli (1970) using 12% (w/v) separating gel and 5% (w/v) stacking gel. The molecular weight of the dissociated protein were estimated by using molecular weight standard proteins "MW-SDS-70 Kit" Gels were shacked gently until the background of the gel became clear and polypeptide bands were clearly visible.

Data analysis

Analyses of variance (ANOVA) were conducted for morphological and seed germination traits using the SAS9 software (SAS Institute Inc). Euclidean distances from data on phenotypic traits were calculated and cluster analysis, was conducted based on Unweighted Pair Group Method with Arithmetic Averaging (UPGMA) method using NTSYS-PC software (Rholf, 1997).

For protein profile data, to avoid taxonomic weighing, the intensity of bands was not taken into consideration, only the presence of bands was taken as indicative. The scores were 1 for the presence and 0 for the absence of a band. Then, the indices of genetic diversity, such as the percentage of polymorphism and expected heterozigosity (*He*), were calculated using POPGENE 32 software (Yeh et al., 1999) on the basis of gene frequencies. At the same time, the genetic structure within and among populations were detected using the software AMOVA-PREP1.01 (Miller, 1997) and WINAMOVA (Excoffier, 1995) in order to partition the genetic variation among local and exotic groups, among populations within groups and among individuals within populations.

The significance of each variance component was tested with permutation tests (Excoffier et al., 1992). Genetic distances were estimated according to Nei (1978) and the resulting similarity matrix was subjected to principal component analysis (PCA) and algorithm of UPGMA using NTSYS-pc 2.01 (Rohlf, 1997). Mantel test (Gower, 1966) was used to assess correlation between the calculated distance matrices (using phenotypic and total protein profiles data) and the test statistic tested for significance against 999 random permutations. The Pearson correlation between the genetic index within population and morphological characteristics and ecological factors was analysed using the SPSS 11.0 software.

RESULTS

Phenotypic traits

ANOVA suggested significant differences among 21 local and exotic populations of cocksfoot for all the 14 traits. A relatively high CV was obtained for germination speed, seedling dry/fresh weight ratio, forage dry matter yield, stem number

and seed yield; moderate to low values of CV were obtained for the remaining nine traits (Table 2).

Genetic distance among 21 cocksfoot populations, estimated using data on 14 phenotypic traits using Euclidean distances among populations, ranged from 1.01 (between Karaj2 from Iran and Netherlands1) to 10.0 (between Zanjan from Iran and Estonia) with an average value of 4.08 (Table 3).

The Euclidean distances matrix was subjected to agglomerative hierarchical clustering utilizing UPGMA method to construct a dendrogram (Figure 1a). 21 populations of *D. glomerata* were classified into four groups. Cluster I consisted of populations Zanjan, Tabriz, Malayer, Passand2, Karaj2, Karaj4, Netherlands2 and Russia1; cluster II included populations Karaj1, Karaj3, Netherlands1, Estonia, Kyrgyzstan and Spain; cluster III consisted of populations Ardebil, Ourmieh and USA2; and cluster IV included populations Pasand1, USA1 and Russia2 (Figure 1a). Therefore, there was no relationship between phenotypic traits and the origin of these cocksfoot populations.

Table 2. Evaluation of data on 14 phenotypic traits in 21 local and exotic populations of cocksfoot

Pop	Speed of germination	Germination (%)	Seedling height (mm)	Vigor index	Root/ Shoot length ratio	Seedling dry/fresh weight ratio	Day to heading	Day to pollination	Plant height (cm)	Forage dry matter yield (t ha^{-1})	Stem number	Panicle length (cm)	1000 grain weight (g)	Grain yield ($kgha^{-1}$)
Karaj1[1]	50.8a-e	61.3ef	50.9d-h	31.2c-g	0.32cd	0.10a-d	40.7b	57.7b	49.2gh	4.76abc	26.55a-e	14.05abc	0.57e-j	103e
Karaj2[1]	64.9 ab	80.0a	67.1a	53.3a	0.53ab	0.14ab	40.1bc	49.5b-f	61.0ef	3.97b-f	14.28e	13.44a-d	0.70a-d	92e
Karaj3[1]	48.1e	58.7ef	45.7gh	27.2fg	0.51ab	0.09a-d	38.9bcd	56.4bc	61.3ef	3.44c-h	26.05a-e	9.88fgh	0.62b-h	314a-d
Karaj4[1]	55.4e	69.3a-e	49.9d-h	34.6b-g	0.53ab	0.10a-d	49.7a	65.1c	67.0cde	2.73e-h	24.58b-e	11.63c-h	0.53hij	159cde
Zanjan[1]	44.0cde	60.0def	54.9b-g	32.9c-g	0.27d	0.09bcd	24.9f	40.2gh	74.2bcd	2.11h	33.13a-d	10.83d-h	0.76a	255a-e
Tabriz [1]	65.7 a	74.7abc	52.3c-g	38.9bc	0.48ab	0.12a-d	36.4bcd	50.7b-e	64.6c-f	2.97d-h	28.78a-e	10.66e-h	0.58d-i	226a-e
Ardebil [1]	58.3a-d	68.0a-f	63.1ab	42.9b	0.44bc	0.13abc	30.5def	47.5d-g	57.3efg	2.55e-h	21.17cde	9.72fgh	0.65a-g	312a-d
Ourmieh[1]	52.8e	61.3def	58.8a-d	36.0b-f	0.57ab	0.08cd	25.2f	39.2h	80.2ab	3.29c-h	36.67abc	14.04abc	0.63b-h	408a
Malayer[1]	58.6a-d	69.3a-f	54.1b-g	37.4b-e	0.61a	0.12a-d	30.6def	46.2e-h	67.2cde	4.37bcd	35.73abc	11.80c-h	0.56e-j	318a-d
Pasand1[1]	55.9e	68.0f	42.8h	29.2d-g	0.52ab	0.07d	41.3b	56.3bc	61.1ef	3.83b-g	33.83a-d	9.20gh	0.46ij	201cde
Pasand2[1]	41.7e	58.7ef	52.7c-g	30.9c-g	0.57ab	0.07d	33.7b-e	50.8b-e	64.8c-f	2.86d-h	30.40a-e	12.49c-f	0.52hij	326a-d
USA1	46.9cde	60.0def	60.7abc	36.4b-e	0.47ab	0.11a-d	26.9ef	42.7gh	88.9a	6.13a	39.67ab	16.01a	0.74a-b	397ab
USA2	54.9e	65.3b-f	49.1d-h	32.0c-g	0.49ab	0.11a-d	38.5bcd	54.5bcd	75.6bc	4.59bc	42.03a	10.17fgh	0.56e-j	337abc
Russia1	64.8ab	76.0ab	48.9e-h	37.4b-e	0.31cd	0.14a	39.1bcd	54.1b-e	62.4ef	3.20c-h	22.13ade	10.14fgh	0.46j	169cde
Russia2	60.1abc	72.0a-d	55.7b-f	40.0bc	0.60a	0.12a-d	27.1ef	40.9gh	84.3ab	4.70abc	37.27abc	15.41ab	0.67a-f	349abc
Russia3	54.4e	70.7a-e	52.9c-g	37.4b-e	0.43bc	0.13abc	34.9b-e	51.2b-e	67.5cde	4.14b-e	21.77cde	13.67abc	0.67a-e	209b-e
Kyrgyzstan	47.9b-e	61.3def	46.3gh	28.5efh	0.57ab	0.09a-d	31.5cf	49.0c-f	67.1cde	2.46fh	18.05de	13.49a-d	0.71abc	217a-e
Estonia	40.2 e	57.3f	47.0gh	27.0g	0.50ab	0.10a-d	38.5bcd	53.7b-e	63.9def	3.56b-h	29.27a-e	10.07fgh	0.48ij	227a-e
Netherlands 1	54.4 a-e	66.7b-f	56.8b-e	37.9bcd	0.49ab	0.09a-d	49.1a	66.1a	54.1fgh	2.23gh	24.33b-e	11.93c-h	0.54g-j	109e
Netherlands 2	48.9e	64.0f	56.9b-e	36.5b-e	0.49ab	0.12abc	41.6b	55.7bcd	45.7h	5.07ab	23.88b-e	13.13b-e	0.61c-h	131de
Spain	58.2a-d	62.7c-f	53.4c-g	33.5c-g	0.52ab	0.10a-d	36.9bcd	54b-f	67.9cde	2.58e-h	23.37b-e	9.07h	0.55f-j	224a-e
Mean square	161.1*	119**	107**	106**	0.025**	0.127*	146**	157**	234.5**	3.41**	27478**	164.7**	12.69**	2.36**
CV	61.1	9.8	9.3	13.1	16.37	25.3	12.31	8.24	9.08	22.96	40.81	29.42	12.13	10.39

*, **significant at 0.05 and 0.01 levels, respectively.
[1]Cocksfoot wild populations from Iran.
Figures in the same column, followed by the same letter, are not significantly different at $p<0.05$.

Table 3. Pair-wise values for squared Euclidean distances (lower diagonal) and Nei's genetic distances (upper diagonal) of the 21 wild cocksfoot populations from seven different origins/sources

	Karaj1	Karaj2	Karaj3	Karaj4	Zanjan	Tabriz	Ourmieh	Ardebil	Malayer	Pasand1	Pasand2	USA1	USA2	Russia1	Russia2	Russia3	Kirghizia	Estonia	Netherland1	Netherland2	Spain
Karaj1[1]		0.045	0.052	0.047	0.036	0.033	0.064	0.017	0.035	0.034	0.093	0.058	0.116	0.069	0.103	**0.178**	0.048	0.093	0.052	0.080	0.038
Karaj2[1]	1.13		0.046	0.038	0.053	0.037	0.034	0.056	0.046	0.031	0.092	0.039	0.107	0.058	0.079	**0.129**	0.044	0.095	0.042	0.060	0.053
Karaj3[1]	5.65	6.06		0.026	0.056	0.063	0.065	0.051	0.076	0.064	0.132	0.067	0.143	0.075	0.055	**0.218**	0.059	0.165	0.077	0.099	0.058
Karaj4[1]	1.63	2.04	4.18		0.038	0.051	0.050	0.054	0.068	0.049	0.092	0.056	0.109	0.058	0.078	**0.170**	0.042	0.137	0.051	0.075	0.057
Zanjan[1]	4.18	4.54	1.75	2.78		0.018	0.059	0.018	0.054	0.038	0.087	0.065	0.134	0.076	0.099	**0.174**	0.028	0.078	0.045	0.072	0.020
Tabriz [1]	3.37	3.65	2.47	1.90	1.17		0.050	0.019	0.043	0.026	0.084	0.039	0.136	0.075	0.097	**0.151**	0.032	0.055	0.050	0.063	0.018
Ardebil [1]	**8.23**	**8.53**	2.68	6.75	4.10	4.93		0.079	0.042	0.047	0.073	0.066	0.062	0.053	0.076	**0.102**	0.035	0.110	0.037	0.047	0.068
Ourmieh[1]	5.63	5.91	**8.17**	4.18	1.73	2.36	2.70		0.057	0.036	0.107	0.064	0.158	0.091	0.103	**0.200**	0.044	0.082	0.065	0.095	0.019
Malayer[1]	5.80	6.11	7.17	4.32	1.78	2.49	2.46	5.78		0.022	0.102	0.042	0.093	0.037	0.091	**0.170**	0.050	0.108	0.030	0.073	0.056
Pasand1[1]	5.99	6.37	4.96	4.53	1.95	2.79	2.30	**8.37**	6.40		0.088	0.020	0.115	0.053	0.093	**0.169**	0.042	0.081	0.033	0.068	0.030
Pasand2[1]	2.67	3.13	3.05	1.23	1.69	**8.64**	5.62	3.08	3.18	3.40		0.108	0.059	0.097	0.180	**0.117**	0.103	0.103	0.059	0.025	0.100
USA1	**7.96**	8.27	2.47	6.47	3.83	4.69	4.42	2.52	2.24	2.06	5.36		0.146	0.056	0.076	**0.168**	0.057	0.091	0.061	0.088	0.047
USA2	6.31	6.66	**8.94**	4.81	2.30	3.04	2.01	1.15	7.02	6.81	3.67	1.76		0.081	0.159	**0.115**	0.113	0.187	0.053	0.040	0.163
Russia1	1.90	2.18	3.94	6.01	2.51	1.54	6.47	3.87	4.02	4.28	1.01	6.21	4.55		0.072	**0.157**	0.052	0.144	0.045	0.069	0.068
Russia2	6.69	6.96	1.43	5.19	2.60	3.36	1.63	1.34	**9.74**	1.11	4.08	1.39	7.39	4.90		**0.230**	0.061	0.184	0.091	0.131	0.087
Russia3	2.90	3.21	2.86	1.45	1.41	6.01	5.37	2.79	2.95	3.18	6.07	5.11	3.48	1.39	3.81		**0.127**	**0.084**	**0.135**	**0.104**	**0.207**
Kyrgyzstan	3.12	3.53	2.62	1.73	1.18	7.92	5.17	2.64	2.78	2.94	7.71	4.91	3.30	1.47	3.65	5.00		0.076	0.046	0.063	0.035
Estonia	3.36	3.85	2.34	1.96	**10.00**	**9.02**	4.92	2.45	2.55	2.66	**8.88**	4.65	3.01	1.79	3.44	**8.24**	5.33		0.098	0.097	0.080
Netherlands 1	4.92	1.01	5.52	1.40	4.07	3.21	**8.09**	5.48	5.65	5.86	2.54	7.82	6.16	1.73	6.54	2.76	3.02	3.25		0.038	0.062
Netherlands 2	**7.95**	1.41	4.93	1.04	3.47	2.66	7.50	4.88	5.07	5.25	1.99	7.24	5.59	1.26	5.97	2.19	2.42	2.66	7.41		0.082
Spain	3.29	3.64	2.45	1.81	1.08	4.54	4.97	2.41	2.56	2.78	7.91	4.71	3.07	1.54	3.44	5.05	4.89	5.98	3.14	2.58	

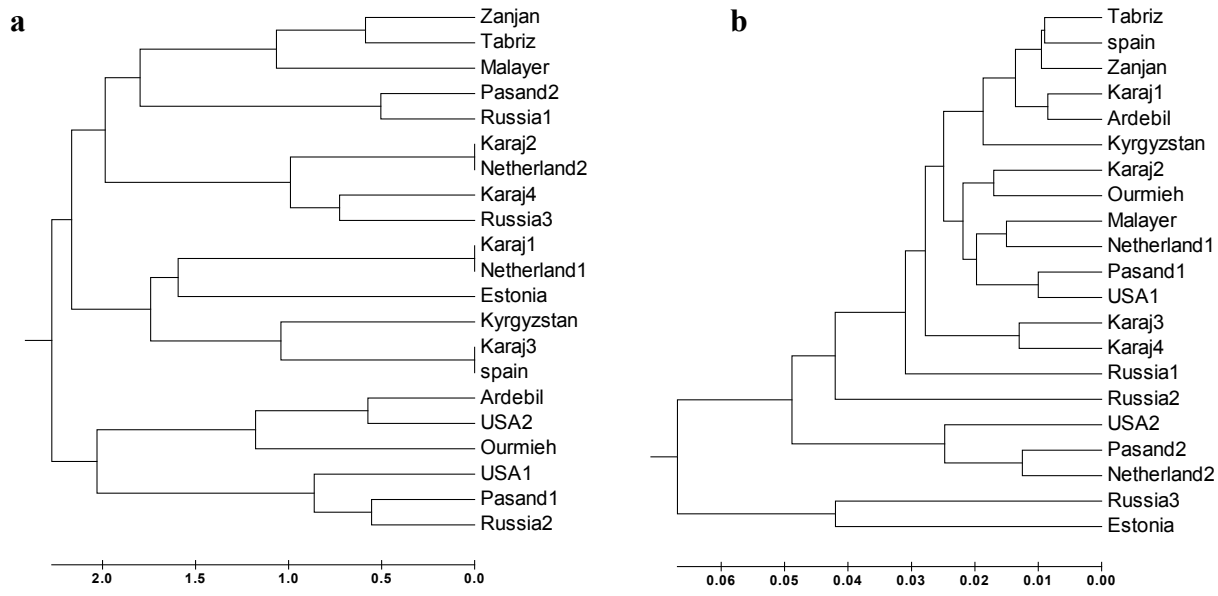

Figure 1. Dendrograms of the 21 wild cocksfoot populations based on phenotypic traits (a) and SDS-PAGE markers (b)

Total protein profiles

On the basis of the relative mobility of total proteins on the gel, 25 polypeptide bands of different sizes ranging from 7.63 to 276.88 kDa, from 21 local and exotic populations of cocksfoot, were identified. The percentages of polymorphic bands over the total bands detected ranged from 36% (Ardebil from Iran and Spain) to 92% (Ourmieh from Iran) with an average of 67% (Table 4).

Table 4. Genetic diversity parameters of 21 wild cocksfoot populations from seven different origins/sources

	No. Bands	Polymorphism %	*He*
Karaj1[1]	24	64	0.274
Karaj2[1]	25	84	0.387
Karaj3[1]	25	88	0.359
Karaj4[1]	24	80	0.354
Zanjan[1]	25	44	0.181
Tabriz [1]	25	60	0.268
Ardebil [1]	25	92	0.411
Ourmieh[1]	25	36	0.141
Malayer[1]	25	68	0.281
Pasand1[1]	25	64	0.273
Pasand2[1]	25	56	0.231
USA1	25	60	0.251
USA2	25	80	0.304
Russia1	25	84	0.361
Russia2	24	56	0.216
Russia3	25	84	0.369
Kyrgyzstan	25	88	0.384
Estonia	25	52	0.232
Netherlands1	25	56	0.229
Netherlands2	25	72	0.313
Spain	25	36	0.151

[1]: Cocksfoot wild populations from Iran

Assuming Hardy-Weinberg equilibrium, the values of Nei's genetic diversity (*He*) ranged from 0.141 (Ardebil from Iran) to 0.411 (Orumieh from Iran) (Table 4). High polymorphism was found within populations and the probability that two randomly sampled polypeptides in a given populations are different was 28.4% (H_e=0.284).

AMOVA using nuclear total protein profiles revealed that variation among local and exotic groups accounted for 1% of the total variance, among populations within each group and within populations for 11% and 88% of the total variation, respectively. This difference was statistically significant ($p< 0.001$) based on the permutation test.

The average genetic distance of the populations ranged from 0.017 (between Karaj1 and Ardebil, both from Iran) to 0.230 (Karaj3 from Iran and Russia3), with an average of 0.078 (Table 3). To elucidate the genetic relationships among local and exotic cocksfoot populations, an UPGMA dendrogram was produced using Nei's genetic distances (Figure 1b). The 21 populations were grouped into three clusters: cluster I having 16 populations, cluster II consisted of populations USA2, Pasand2 from Iran and Russia3, and cluster III having only 2 populations (Russia3 and Estonia). The total protein data were also used for conducting principal component analysis (PCA) to study further the genetic diversity among the 21 cocksfoot populations (Figure 2). The first three components of PCA accounted for 76.91% of the total variation. The 21 populations were separated into three groups, the smaller groups comprising 3 and 2 populations and the larger group comprising 16 populations. This clustering pattern of genotypes obtained on the basis of PCA largely resembled the clustering of populations in the dendrogram obtained through UPGMA analysis (Compare Figures 1b and 2). Besides, this clustering pattern, made on the basis of SDS-PAGE, grouped the accessions differently and gave no clear indication of phenotypic performance or origin/source.

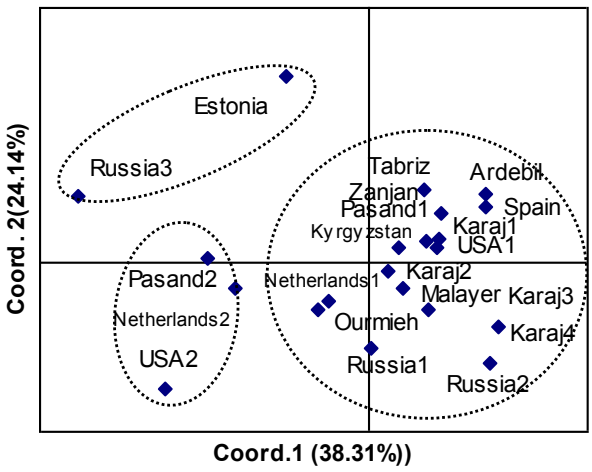

Figure 2. Scatter diagram of the 21 wild cocksfoot populations based on SDS-PAGE markers

Correlation between genetic and phenotypic traits and ecological factors

Correlation coefficients among pairwise genetic and phenotypic distance matrices were calculated using Mantel's test. Regression and correlation analysis between genetic distance and phenotypic distance showed no significant correlation (p>0.05).

The correlation analysis (Table 5) indicated that the diversity index of different Iranian populations had no significant correlation with elevation distance and climate factors. However, some phenotypic traits had significant correlation (p>0.05) with climate factors. The seedling dry/fresh weight ratio and 1000 grain weight had positive and negative correlation with annual average precipitation, respectively. Day to heading had positive correlation with annual average maximum temperature. There was also positive correlations between both seedling dry/fresh weight ratio and 1000-grain weight with elevation. There was no significant correlation between all phenotypic traits and the diversity parameter and annual average minimum temperature. The above correlations implied that the genetic diversity of *D. glomerata* was not the result from the joint effects of one or several ecological factors, i.e., the ecological factors have not played an important role in influencing the protein profiles polymorphism of *D. glomerata*.

Table 5. Pearson correlation analysis for the relationships between phenotypic and genetic parameters within Iranian populations of cocksfoot and ecological factors

Traits	Annual average precipitation	Annual average maximum temperature	Annual average minimum temperature	Elevation
Speed of germination	-0.482	-0.226	-1.221	0.289
Germination (%)	-0.302	-0.042	-0.019	0.179
Seedling height	-0.302	-0.042	-0.019	0.179
Vigor index	-0.423	-0.278	-0.305	0.347
Root/Shoot length ratio	0.111	-0.017	-0.104	0.277
Seedling dry/fresh weight ratio	0.679*	-0.268	-0.283	0.617*
Day to heading	0.063	0.645*	0.573	-0.217
Day to pollination	0.119	0.587	0.522	-0.258
Plant height	-0.047	-0.224	-0.174	0.159
Forage dry matter yield	-0.096	0.374	0.258	0.020
Stem number	0.273	-0.160	-0.084	-0.146
Panicle length	-0.239	0.229	0.124	0.189
1000 grain weight	-0.478*	-0.223	-0.200	0.635*
Grain yield	0.053	-0.587	-0.584	-0.038
Mean heterozygosity	0.206	-0.425	-0.336	-0.158

*, significant at 0.05 level

The estimates of Pearson correlation coefficients among all genetic and phenotypic traits on the 21 populations are shown in Table 6. The correlation coefficients between the *He* and all phenotypic traits were not significant (p>0.05).

Among phenotypic traits, there were significantly correlation coefficients within field as well as laboratory measured characters of germination traits, however between these two group characters, field and laboratory measured characters of germination traits; there was only a significantly positive correlation between the seedling height with the panicle length and the 1000-grain weight.

Table 6. Pearson correlation analysis for the relationships between phenotypic and genetic parameters of the 21 wild cocksfoot populations from seven different origins/sources

	Speed of germination	Germination (%)	Seedling height	Vigor index	Root/Shoot length ratio	Seedling dry/fresh weight ratio	Day to heading	Day to pollination	Plant height	Forage dry matter yield	Stem number	Panicle length	1000 grain weight	Grain yield
Speed of germination														
Germination (%)	0.902**													
Seedling height	0.252	0.291												
Vigor index	0.675**	0.765**	0.835**											
Root/Shoot length ratio	0.047	0.009	0.030	0.039										
Seedling dry/fresh weight ratio	0.617**	0.700**	0.448*	0.703**	-0.210									
Day to heading	0.165	0.225	-0.291	-0.048	-0.035	-0.007								
Day to pollination	0.048	0.063	-0.401	-0.219	-0.066	-0.115	0.968**							
Plant height	-0.042	-0.090	0.113	0.001	0.234	-0.069	-0.653**	-0.654**						
Forage dry matter yield	-0.018	0.027	0.147	0.106	0.087	0.307	-0.128	-0.188	0.203					
Stem number	-0.219	-0.317	-0.131	-0.308	0.150	-0.320	-0.422*	-0.388	0.631**	0.418*				
Panicle length	-0.123	0.001	0.438*	0.284	0.178	0.117	-0.332	-0.381	0.335	0.539*	0.123			
1000 grain weight	-0.103	-0.076	0.545*	0.329	-0.068	0.191	-0.603**	-619**	0.405	0.126	-0.054	0.548*		
Grain yield	-0.219	-0.360	0.071	-0.181	0.334	-0.202	-0.745**	-0.667**	0.750**	0.185	0.704**	0.133	0.253	
Mean Heterozygosity	0.056	0.154	-0.149	0.002	0.133	0.166	0.154	0.076	-0.004	0.142	-0.229	0.334	0.061	-0.116

*, **: significant at 0.05 and 0.01 level, respectively.

DISCUSSION

Evaluation of forage grass germplasm is essential to ensure its efficient and effective use. In the present investigation, high genetic variation was observed for total protein profiles and phenotypic traits. In agreement to our findings, important variations were observed with respect to morphological, phenological and biological properties between populations in previous studies (Sagsoz et al., 1996; Tosun et al., 2002; Jafari and Naseri, 2007). The reason for this variation detected within populations may be related to genetic structure, which is probably due to heterozygosis of cross-pollination of coocksfoot (Sagsoz et al., 1996). This indicated that improvement through simple selection for these traits is possible. However, broadening the genetic base from diverse sources is recommended to include most of the genetic determinants of these traits (Laghetti et al., 1998; Ghafoor et al., 2002). Protein profile variation revealed that *D. glomerata* populations had more genetic variation within rather than between populations (88% and 12%, respectively). According to Hamrick and Godt (1989), reproductive biology is the most important factor in determining the genetic structure of plant populations. They showed that out-crossing plant species tend to exhibit between 10% and 20% genetic variation among populations, while self-pollinated species exhibit on average 50% variation. Studies on the biology of flowering and pollination in *D. glomerata* indicate it as an out-crosser (Sagsoz et al., 1996). However, Hamrick and Godt (1996) pointed out that life history traits alone only explain a relatively low amount of the variation in genetic structure. The high intra-population variability and genetic homogeneity across populations could have arisen by high levels of gene flow.

In this study, relatively lower level of polymorphism (67%) and a higher level of similarity coefficient between populations (0.77-0.99) were detected in comparison with those earlier obtained with ISSRs (0.61-0.93; Zeng et al., 2006), AFLPs (0.43-0.99; Peng et al., 2008), RAPD (0.62-0.93, Zeng et al., 2008) and ISSR (0.61-0.93, Zeng et al., 2008)

for wild populations of cocksfoot with different ploidy levels (2X and 4X). Although the low percentage of variation (AMOVA: 12.02%) obtained by AFLP marker indicated a close genetic relationship between the wild Chinese diploid and tetraploid cocksfoot accessions, higher similarity in our germplasm might be explained by the fact that all studied cocksfoot populations in present work were tetraploid.

Results of this study showed that genetic diversity and geographic distribution in wild populations of *D. glomerata* were independent of each other and no definite relationship existed between genetic diversity and geographic diversity, which is contrary to other studies in *D. glomerata* using isozymes (Lumaret, 1984; Tosun et al., 2002), RAPD, ISSR, SRAP (Zeng et al., 2008) and AFLP (Peng et al., 2008).

Tosun et al. (2002) found major differences between populations with respect to the enzymes variation, suggesting that different ecological conditions from which plants were obtained may have caused the observed variations based on the investigated enzymes. Lumaret (1984), carrying out isozyme analysis to determine the variation in cocksfoot plants in different ecological conditions, stated that this variation was caused by environmental factors. Based on RAPD, ISSR and SRAP, Zeng et al. (2008) classified the accessions from the identical continent into the same group, indicating the geographical distribution of genetic diversity of *D. glomerata* based on AFLP analysis. Peng et al. (2010) also found the genetic variation of *D. glomerata* germplasms was closely associated with ploidy levels and geographical distributions. However, results of this work implied that the genetic diversity of *D. glomerata* was not the result from the joint effects of one or several ecological factors, i.e., the ecological factors had not played an important role in influencing the protein profiles polymorphism of *D. glomerata*. This is in accordance with the results of the studies on *Stipa grandis* (Zhao et al., 2004), *Lathyrus sativus* L. (Sammour et al., 2007) and *Triticum dicoccoides* (Fahima et al., 1999).

In the present study, the correlation of GS values based on total protein profiles with GS values based on phenotypic traits was poor. It may be noted that a small number or common sets of genes/QTLs may be controlling several correlated phenotypic traits evaluated in the present study. Therefore, the genome coverage represented by phenotypic traits is likely to be poor, and this may possibly be the reason for poor correlation between the GD values based on phenotypic traits and GS values based on molecular markers, which represent relatively better genome coverage.

This problem, however, may be overcome by using markers developed from expressed sequences that may be directly responsible for the traits related to fitness and adaptation of genotypes. For example, in a recent study on barley, in comparison to SSR markers, the RFLP analysis of stress responsive genes (SRG-RFLP) produced a better separation of barley cultivars according to eco-morphological characteristics (Maestri et al., 2002).

In the present study, the lowest genetic similarity coefficients (i.e. highest genetic distances) were observed between Karaj3 (from Iran) and Russia3 with total protein profiles and between Estonia and Zanjan (from Iran) with phenotypic data. This shows that the diversity ranks of different pairs of genotypes differ, when worked out on the basis of different marker systems and the phenotype. Under these circumstances, one may like to select a pair of most diverse genotypes on the basis of consistency in the diversity rank of a particular pair of genotypes. Among the above two pairs of genotypes (each of which was found to be diverse based on one of two different approaches), it is clear that crosses between the Iranian and Russia gene pools could create more genetic variability than crosses among Iranian or between Iranian and other exotic accessions. Therefore, this pair of genotypes (Karaj3 from Iran and Russia3) having the lowest GS value in protein profiles can be

selected as fairly diverse with a high level of confidence and used as parents in a hybridisation program.

CONCLUSIONS

From the present study it can be concluded that there was a close genetic relationship between local and exotic cocksfoot populations and that great variation exists among populations within local or exotic groups. Genetic diversity and relationships of wild cocksfoot can be used in the development of germplasm collection, breeding and conservation. The results clearly indicate that comprehensive germplasm collection in major geographic regions is required to broaden the genetic base and sample the full extent of the available variation. Breeding strategies need to exploit the existing variation within the wild cocksfoot germplasm. Besides, the study confirmed that genetic and morphological diversity work in different ways to determine the relationships among populations. To effectively exploit germplasm, we should utilize both methods in breeding work.

Further studies are required to reveal whether there are other factors to cause genetic variation in *D. glomerata*. Although *D. glomerata* had not been listed as a top conservation plant in Iran, it is an important economic forage species endemic to Iran. Therefore, the conservation and further reasonable utilization of the germplasm resources of this species is an urgent task. Our results demonstrate that the divergence of microenvironments had no obvious effect on the genetic diversity and genetic structure of *D. glomerata*. Consequently, major attention should be paid to the sustainable conservation of the wild populations of *D. glomerata* at different populations, when strategies for breeding and germplasm conservation are being implemented in future programs.

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