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A Study of Agronomic and Morphological Variations in Certain Alfalfa (*Medicago sativa* L.) Ecotypes of the Cold Region of Iran

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Abstract: The objective of this study was to characterize and classify the genetic diversity among alfalfa (*Medicago sativa* L.) ecotypes collected from the cold regions of Iran, based on some agro-morphological traits. Twenty one alfalfa ecotypes were collected and planted in a Randomized Complete Blocks Design (RCBD) with three replications in April 1998 at Nyshabour Agricultural and Natural Resource Research Station, Khorasan Razavi, Iran. Twenty three above ground agro-morphological characters were recorded during the growing seasons of 1999-2001. The variables were analyzed by descriptive statistics and multivariate statistical procedures to discriminate differences among genotypes and determine groups based on their similarities. Factor analysis was performed for all agro-morphological traits and reduced them down to 6 common factors which accounted for 80.45% of total variations among the genotypes studied. The twenty one ecotypes were classified in to 4 clusters by cluster analysis. Each group had at least one trait which made it different from the other groups (group 1: No. of pods per raceme and 100-seed weight; group 2: forage yield, dry matter yield, regrowth rate and stem dry matter yield; group 3: leaf-stem ratio, leaf dry matter yield and group 4: seed yield). These results suggest the presence of variation among alfalfa ecotypes available in cold regions in Iran, which could be considered for further breeding strategies and studies.

Key words: Alfalfa ecotypes, morphological traits, diversity, factor analysis, cluster analysis

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is the most important perennial forage crop plant in Iran. It is cultivated in about 600,000 ha of arable land in the country (Abbasi *et al*., 2006). Ecotypes and landraces of alfalfa are still widely used and transferred to new regions because they are known for their good persistence and productivity in their area of origin and adaptation and in each region, they are usually assigned with the name of that place. Recombination with local landraces has increased their diversity. It is said that alfalfa got transferred from the Hamadan Province to other parts of Iran. Hamadani alfalfa is considered as an Iranian alfalfa parent (Abbasi *et al*., 2007). Both of these factors stress the need of conservation of this germplasm resource. Some earlier efforts have been made to identify and describe populations of alfalfa in Iran based on some agro-morphological characteristics (Bahar *et al*., 2006; Farshadfar and Farshadfar, 2008).

Selection as a breeding program is practiced based on genetic diversity. With the increasing diversity of populations the selection range becomes more extended. Use of inbreed lines and using heterotic effect are the main steps in hybrid production. Successful hybrid production depends on genetic distance between parents (Falconer, 1983). For measuring genetic distance, varieties must be grouped based on similarities. Characterization and identification of the major traits of germplasms and grouping them make breeders able to avoid re-sampling from the populations (Sharma and Hore, 1993).

Agro-morphological traits have been used to classify and to study the genetic diversity in alfalfa germplasm collections, as well as other crops (Zaccardelli *et al*., 2003). Smith *et al.* (1995) classified 41 Middle Eastern alfalfa accessions collected from different elevations in Oman, Yemen and Southwestern Saudi Arabia based on morphological and agronomic relationships into separate classes with regard to their tolerance to low winter temperature. Fombellida (1998), analyzed 56 ecotypes of alfalfa collected from North Spain and classified them into four groups based on spring growth rate, regrowth rate after cutting, mortality, persistence and precocity rate. Bahar *et al*. (2006) classified Iranian local alfalfa populations into two main groups. Alfalfa populations Hamadani and Rahnani, which were adapted to cold climates, were grouped in one cluster and populations Bami, Yazdi and Nikshahri, which belonged to the tropical

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areas were placed in the next cluster. Abbasi *et al.* (2006, 2007) identified Iranian alfalfa gene pool landraces based on agro-morphological traits and divided them in 2 types of gene pool landraces, Northern province (cold temperate) and Southern province (sub-tropical) accessions, which were genetically different from each other.

The objective of the present study was to characterize and classify the genetic diversity among alfalfa (*Medicago sativa* L.) ecotypes, which were collected from the cold regions of Iran, based on some agro-morphological traits to help breeders in future breeding strategies and studies.

MATERIALS AND METHODS

Materials and data collection: This study was carried out during 1998-2001 growing seasons. Sample seeds of 21 alfalfa ecotypes (Table 1) were collected from different parts of the cold region of Iran (Fig. 1). They were planted at Nyshabour Agricultural and Natural Resource Research Station, Khorasan Razavi Province (36° 23' 146" N Latitude; 58° 84' 429" E longitude and 1252 m altitude), in the spring of 1998 in deep clay loam soil (fine,

Table 1: Names and sampling locations of 21 Iranian alfalfa ecotypes

Order	Ecotype name	Origin
1	Siyah-rood	West Azarbayjan
2	Ghara yonjeh	Hokmabad-Hamadan
3	Ghara yonjeh	West Azarbayjan
4	Hamadani	Kozareh-Hamadan
5	Hamadani	Mohajeran-Hamadan
6	Simbaz	Khoy-West Azarbayjan
7	Hamadani	Hamadan
8	Ghara yonjeh	Arzangodi-East Azarbayjan
9	Ghara yonjeh	Malekkandi-East Azarbayjan
10	Ghara yonjeh	Ghargolog-West Azarbayjan
11	Ghara yonjeh	Tazeh-kandim-West Azabayjan
12	Hamadani	Ghahavand-Hamadan
13	Ghara yonjeh	Ghareh aghaj-East Azarbayjan
14	Sedghyan	Salmas- West Azarbayjan
15	Ghara yonjeh	Ghareh Ghozlo-West Azarbayjan
16	Hamadani	Khorvandeh-Hamadan
17	Hamadani	Famaneain- Hamadan
18	Chalashter	Chaharmahal and Baghteyari
19	Rahnani	Esfahan
20	Ghara yonjeh	Sahand Ava-Tabriz, East Azarbayjan
21	Lordakan	Khanmirza- Charmahal and Baghteyari

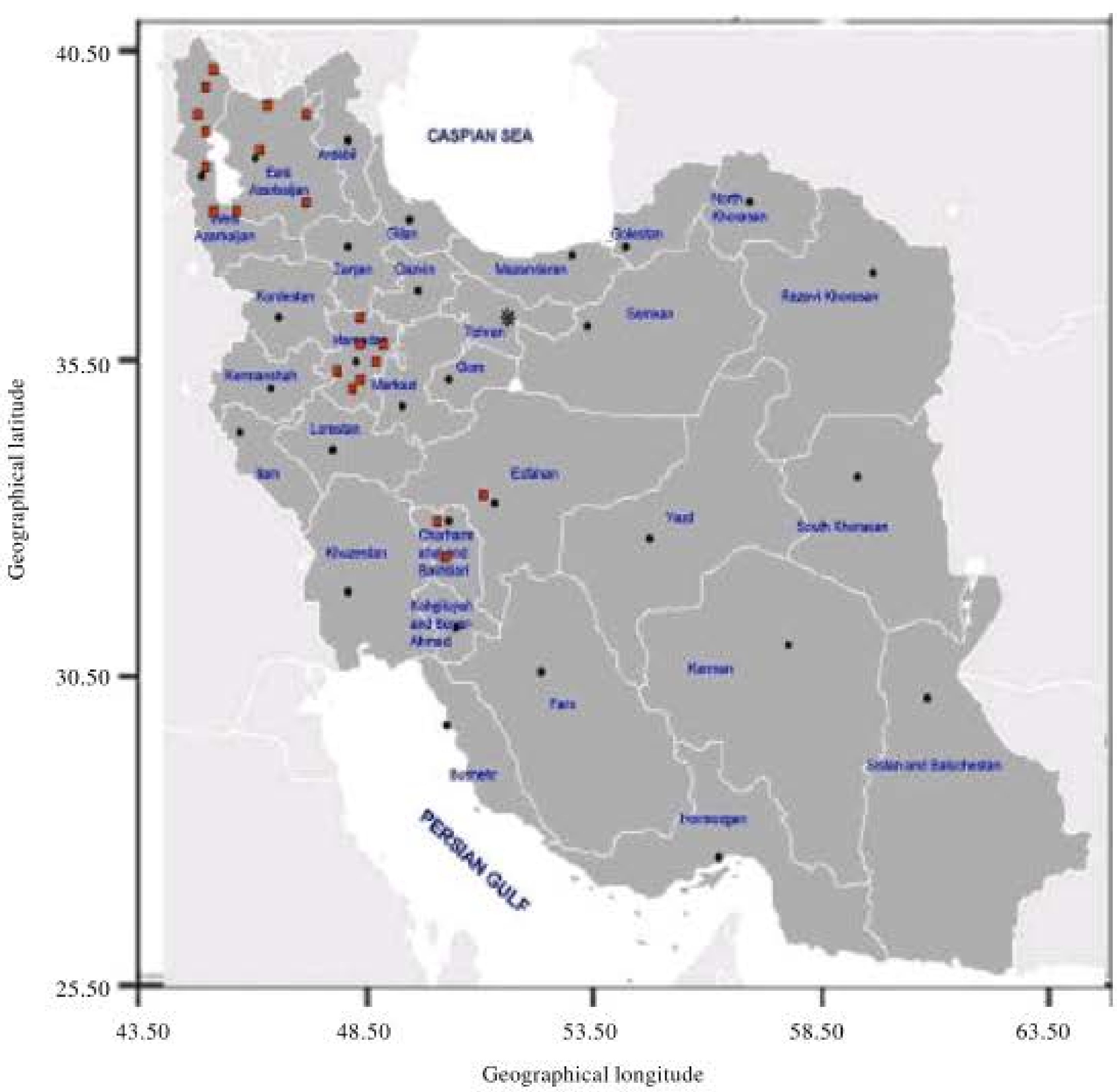

Fig. 1: Geographical distribution of studied Iranian cold region alfalfa ecotypes

Table 2: The evaluated agro-morphologic traits with their abbreviations

Traits	Abbreviations
Days to first flower (day)	DFF
Days to 10% flowering (day)	D10%F
First to 10% flowering intervals (day)	F10%FI
Plant height at 10% flowering (cm)	PH
Regrowth rate	RR
Fresh yield (t ha^{-1})	FY
Dry matter yield (t ha^{-1})	DMY
Leaf dry weight (g)	LD
Leaf fresh weight (g)	LF
Stem dry weight (g)	SD
Stem fresh weight (g)	SF
Leaf-stem ratio fresh	LSRF
Leaf-stem ratio dry	LSRD
Days to first ripen pod(day)	DFRP
Non violent flowers (%)	NVF
No. of flowers per raceme	NFPR
No. of pods per raceme	NPPR
No. of curls per pod	NCPP
No. of seeds per pod	NSPP
100-seed weight (g)	100SW
Pod yield (kg ha^{-1})	PY
Seed yield (kg ha^{-1})	SY
Raceme length	RL

loamy, mixed, thermic, typic haplocalcids, pH 8.1 and EC 0.64 dS m^{-1}) in Randomized Complete Blocks Design (RCBD) with three replications.

Field trial was under irrigated condition for four years. As to the procedure which was suggested by Corn and Forage Crop Department of Seed and Plant Improvement Institute (SPII), Iran, each plot consisted of four rows of 10 m length and 50 cm width and separated with one row as corridor for decreasing side effect errors and plant competitions. Seeding rate was adjusted as 52 g $plot^{-1}$ to represent a dense stand. Investigated traits are shown in Table 2. All data were recorded from the two central rows. First year was considered for uniformity. In years two and three, 6 consecutive harvests were made during each crop season to determine the forage yield and its related traits. The four highest yielding harvests were analyzed. The other harvests were discarded because not all ecotypes could be analyzed due to insect damage (Alfalfa weevil, hyper postica GYll) in early spring or low forage yield in early autumn. Regrowth rate and plant height were recorded at 2 weeks after each cutting and 10% flowering, respectively. In all harvests, 1000 g sample forage in each plot was analyzed for forage yield related traits. Information for seed yield and its components was recorded in the 4th year. For yield related traits, data were recorded on 10 randomly selected plants and sample means were characterized. Flowering date were scored on each plot when 50% of the stems had one flowering inflorescence. Prior to harvest, 5 well-podded inflorescence from each plant were sampled from each plot; then the number of pods per inflorescence and number of curls per pod were counted. The inflorescence were dried at 40°C until their weight was stable, the first two pods from these inflorescence were threshed and the seeds counted and weighed. From these data, the number of seeds per pod was calculated. For measuring pod yield, all plants in 0.5 m^2 from each plot were harvested and total pods weighed. After sampling, the remaining plants in the harvested area (8 m^2) from each plot were hand harvested, field dried and after primary threshing and cleaning, seeds and pods re-dried at 80°C for 72 h, threshed, cleaned, sieved and weighed and seed yield calculated.

Statistical method: Descriptive statistics such as minimum, maximum, mean, deviations and phenotypic coefficient of variation for all traits were computed to estimate diversity for all traits. Simple correlation coefficients were computed between trait pairs. Factor analysis was performed on means of traits according to varimax rotation method (Manly, 2005) using related correlation matrix to reduce the data of 21 ecotypes. Also, cluster analysis using ward method (Crochemore *et al*., 1998; Pecetti *et al*., 1999) was conducted and its dendrogram constructed for studying the genetic relationship of selected ecotypes and grouping them based on agro-morphological and quantitative traits. For identifying the superior ecotypes in each class, the deviation (%) for class mean from total mean was computed, respectively. All data obtained were subject to the SAS software (Version 7.0, SAS Institute Inc., 1989-95) and JMP software (Version 3.1.2, SAS Institute Inc., 1989-95).

RESULTS AND DISCUSSION

Descriptive statistics of traits: According to results in Table 3, pod yield ranged from 1361.3 to 2547.4 kg ha^{-1}, seed yield ranged from 609.17 to 1056.6 kg ha^{-1}, fresh yield ranged from 10.45 to 16.65 t ha^{-1} and dry matter yield ranged from 2.84 to 4.23 t ha^{-1}. Abbasi *et al*. (2006) reported large phenotypic variation for central leaflet area, days to 10% flowering after each cutting, plant height at 50% flowering and 1000 seed weight among alfalfa accessions of the National Plant Gene Bank of Iran.

The highest phenotypic coefficient of variation was obtained for non violet flowers (40%), pod yield (15%), seed yield (13%), forage yield (11%), dry matter yield (10%) and number of curls per pod (10%), respectively. For other traits, the coefficients were found to be smaller than 10% (Table 3). Abbasi *et al*. (2007) reported the highest and lowest diversity for petal color (CV = 56.9%) and forage moisture (CV = 4.8%), respectively. A range of 6.84 to 18.84% and 12.49 to 26.5% differences for genotypic and phenotypic coefficient of variation were reported among 18 Iranian cultivars by Farshadfar and Farshadfar (2008), which indicates the existence of genetic diversity in Iranian alfalfa cultivars.

Correlations between traits: There were significant correlations between some of the pairwise traits (Table 5). Fresh yield had positive correlation with plant height (r = 0.69, p = 0.01) and negative correlation with leaf-stem ratio (r = -0.46, p = 0.05). Leaf-stem ratio also had negative correlation with plant height (r = -0.60, p = 0.01). Plant height was different among four cuttings and was shorter in third cutting because of warm weather in July and it was different among ecotypes and years (data not presented). Growth period from cutting to next harvest (10% flowering) had negative correlation with regrowth rate (r = -0.60, p = 0.01) and leaf fresh weight (r = -0.55, p = 0.01). Growth period ranged from 30.58 to 37.49 days among ecotypes (Table 3). A range of 17 to 40 day differences for growth period was reported by Abbasi *et al*. (2006), but there were no significant correlations between this trait and plant height, leaf-stem ratio or seed yield components in their study. Lamb *et al*. (2003) and Sheaffer *et al*. (2000) reported that positive correlation for leaf and stem yield and negative correlation for leaf-stem ratio with time of harvesting at mid bud stage, early flower, late flower and green pod maturity, respectively.

Table 3: Descriptive statistics of agro-morphologic traits in alfalfa ecotypes of Iran

Traits	Min.	Max.	Mean	Deviations	Phenotypic coefficient of variation (%)
DFF	19.83	25.08	24.60	1.420	6
D10%F	30.58	37.49	34.40	1.810	5
F10%FI	8.50	10.67	9.70	0.530	5
PH	57.50	76.50	70.05	4.480	6
RR	5.08	7.16	6.20	0.560	9
FY	10.45	16.65	14.77	1.530	11
DMY	2.84	4.23	3.74	0.340	10
LD	22.63	31.63	26.90	1.910	7
LF	105.42	150.38	118.81	9.440	8
SD	29.82	38.75	33.56	2.490	7
SF	110.75	145.80	130.40	8.670	7
LSRF	0.87	1.16	0.95	0.072	8
LSRD	0.72	0.96	0.85	0.067	8
DFRP	35.30	40.30	38.23	1.410	4
NVF	17.00	84.00	50.50	19.800	40
NFPR	21.87	29.47	25.18	1.950	8
NPPR	11.23	13.60	13.18	0.870	7
NCPP	2.23	3.50	2.76	0.270	10
NSPP	4.00	5.60	4.82	0.480	6
100SW	0.19	0.25	0.22	0.020	9
PY	1361.30	2547.45	2100.90	314.770	15
SY	609.17	1056.67	830.20	110.620	13
RL	24.50	30.90	27.70	2.000	7

DFF: Days to first flower, D10% F: Days to 10% flowering, F10% FI: First to 10% flowering intervals, PH: Plant height, RR: Regrowth rate, FY: Fresh yield, DY: Dry matter yield, LD: Leaf dry weight, LF: Leaf fresh weight, SD: Stem dry weight, SF: Stem fresh weight, LSRF: Leaf-stem ratio fresh, LSRD: Leaf-stem ratio dry, DFVF: Days to first ripen pod, NVF: Non violent flowers, NFPR: No. of flowers per raceme, NPPR: No. of pods per raceme, NCPP: No. of curls per pod, NSPP: No. of seeds per pod, 100SW: 100-seed weight, PY: Pod yield, SY: Seed yield, RL: Racemes length

Seed yield had positive correlation with pod yield (r = 0.94, p>0.01) and raceme length (r = 0.56, p = 0.01). Agronomic practices and high degree of genetic impurity have caused large variations in seed yield among ecotypes. Some earlier studies have described the factors affecting seed yield in alfalfa (Steiner *et al*., 1992; Dordas, 2006; Zhang *et al*., 2008). Bolanos-Aguilar *et al*. (2000) reported that among-population variance and within population variance were accounted for 5 to 31% and 69 to 95% of total genetic diversity for seed yield components in alfalfa, respectively. Bolanos-Aguilar *et al*. (2002) also reported large variation for seed yield among cultivars and environments and high correlation between seed yield and lodging resistance (r = -0.89), harvest index (r = 0.99) and above ground phytomass (r = 0.94). Bodzon (2004) stated that seed yield per plant depended on the number of pods per raceme and seeds per pod and variability of these characters determined about 60% of the seed yield variability among alfalfa cultivars.

Factor analysis: To determine the relative values of traits in diversity, factor analysis with varimax rotation was performed for 23 agro-morphological traits and reduced them down to 6 common factors which were accounted for 80.45% of total variation among alfalfa ecotypes (Table 4). Factor 1 (vegetative period) accounted for 20.13% of total variations. Factor 2 (forage yield) included plant height,

Table 4: Results of factor analysis (Varimax rotated)

	Factors					
Variances	Vegetative period	Forage yield	Seed	Forage quality	Reproductive period	Fertilization factor
Eigen value	4.831	3.993	3.422	3.119	2.217	1.724
Proportional var.	0.2013	0.1664	0.1426	0.13	0.0924	0.0718
Cumulative pro. var.	0.2013	0.3677	0.5103	0.6403	0.7327	0.8045
Affected traits	D10% F	PH	PY	LF	DFRP	NCPP
	DFF	SD	SY	LD		NSPP
	F10% FI	SF	RL	LSRF		

D10% F: Days to 10% flowering, DFF: Days to first flower, F10% FI: First to 10% flowering intervals, PH: Plant height, SD: Stem dry weight, SF: Stem fresh weight, PY: Pod yield, SY: Seed yield, RL: Racemes length, LF: Leaf fresh weight, LD: Leaf dry weight, LSRF: Leaf-stem ratio fresh, DFRP: Days to first ripen pod, NCPP: No. of curls per pod, NSPP: No. of seeds per pod.

Table 5: Correlation coefficient between pairwise agro-morphological traits in alfalfa ecotypes of Iran

Traits	DFF	D10%F	F10%FI	PH	RR	FY	DMY	LD	LF	SD	SF	LSRF
DFF	1.00											
D10%F	0.80***	1.00										
F10%FI	0.34	0.83***	1.00									
PH	-0.33	-0.25	-0.10	1.00								
RR	-0.65**	-0.60**	-0.37	0.85****	1.00							
FY	-0.24	-0.34	-0.35	0.69***	0.65**	1.00						
DMY	0.32	0.17	-0.09	0.31	0.19	0.33	1.00					
LD	-0.19	-0.22	-0.15	-0.13	-0.01	-0.36	-0.01	1.00				
LF	-0.47*	-0.55**	-0.42	0.03	0.27	-0.10	0.06	0.80***	1.00			
SD	-0.16	-0.08	0.02	0.44*	0.38	-0.04	0.26	0.41	0.51*	1.00		
SF	-0.38	-0.30	-0.11	0.59**	0.53*	0.30	0.22	0.28	0.57**	0.78***	1.00	
LSRF	-0.19	-0.34	-0.36	-0.60**	-0.24	-0.46*	-0.24	0.63**	0.57**	-0.22	-0.34	1.00
LSRD	-0.12	-0.23	-0.22	-0.52*	-0.29	-0.15	-0.41	0.21	0.12	-0.70***	-0.43	0.63**
DFRP	0.10	-0.15	-0.36	0.42	0.34	-0.45*	0.52*	0.14	0.29	0.36	0.55**	-0.25
NVF	0.41	0.42	0.33	-0.20	-0.37	-0.20	0.07	-0.03	-0.14	-0.07	-0.09	-0.08
NFPR	-0.20	-0.05	0.10	-0.27	-0.17	-0.26	-0.24	-0.17	0.01	-0.16	-0.08	0.11
NPPR	0.16	0.36	0.41	-0.02	-0.13	-0.09	0.02	-0.28	-0.19	0.15	0.03	-0.29
NCPP	0.10	0.02	-0.10	-0.12	0.02	0.28	0.20	-0.11	-0.16	-0.26	-0.38	0.17
NSPP	-0.10	-0.18	-0.23	-0.18	0.04	0.18	-0.06	0.14	0.20	-0.02	-0.02	0.25
100SW	-0.15	-0.30	-0.34	0.14	0.28	0.33	-0.11	-0.30	-0.12	-0.43*	-0.24	0.08
PY	-0.46*	-0.34	-0.06	0.06	0.20	-0.25	-0.48*	0.16	0.18	0.25	0.24	0.05
SY	-0.48*	-0.35	-0.06	0.06	0.16	-0.26	-0.62**	0.19	0.15	0.20	0.20	0.06
RL	-0.57**	-0.37	-0.09	0.52*	0.56**	0.34	-0.09	0.20	0.24	0.38	0.45*	-0.19
		LSRD	DFRP	NVF	NFPR	NPPR	NCPP	NSPP	100SW	PY	SY	RL
LSRD		1.00										
DFRP		-0.27	1.00									
NVF		0.03	0.22	1.00								
NFPR		0.16	-0.51*	-0.10	1.00							
NPPR		-0.41	-0.01	0.18	-0.004	1.00						
NCPP		0.08	0.005	0.03	-0.05	0.20	1.00					
NSPP		0.24	0.07	-0.08	0.04	-0.03	0.35	1.00				
100SW		0.26	-0.08	-0.35	0.12	-0.15	-0.003	-0.20	1.00			
PY		-0.01	-0.09	0.11	0.03	-0.02	-0.38	0.12	-0.31	1.00		
SY		0.07	-0.17	0.03	0.06	-0.07	-0.33	0.02	-0.29	0.94***	1.00	
RL		-0.23	0.29	-0.01	-0.12	0.05	0.04	0.27	-0.29	0.56**	0.56**	1

*,**Significant at 5 and 1% level, respectively. DFF: Days to first flower, D10% F: Days to 10% flowering, F10% FI: First to 10% flowering intervals, PH: Plant height, RR: Regrowth rate, FY: Fresh yield, DY: Dry matter yield, LD: Leaf dry weight, LF: Leaf fresh weight, SD: Stem dry weight, SF: Stem fresh weight, LSRF: Leaf-stem ratio fresh, LSRD: Leaf-stem ratio dry, DFVF: Days to first ripen pod, NVF: Non violent flowers, NFPR: No. of flowers per raceme, NPPR: No. of pods per raceme, NCPP: No. of curls per pod, NSPP: No. of seeds per pod, 100SW: 100-seed weight, PY: Pod yield, GY: Grain yield, RL: Racemes length

stem fresh and dry weight and accounted for 16.64% of total variations. As to the results in Table 5, correlation between forage yield and plant height was significant ($r = 0.69$, $p = 0.01$). Factor 3 (seed factor) was more affected by pod yield, seed yield and raceme length and accounted for 14.26% of total variations. Correlation between raceme length with pod yield and seed yield were significant ($r = 0.56$, $p = 0.01$). Factor 4 (forage quality) was influenced by leaf-stem ratio and leaf weight and accounted for 13% of the total variations. Correlation between leaf-stem ratio with forage yield ($r = -0.46$, $p = 0.05$) and plant height ($r = -0.60$, $p = 0.01$) was negative and significant which indicated the importance of leaf-stem ratio on forage yield and quality. Factor 5 (reproductive period) and factor 6 (fertilization factor) revealed 9.24 and 7.18% of total variations, respectively.

The ratio of community variance to total variance was high for most traits. The values for plant height, stem fresh yield, leaf fresh yield, seed yield and raceme length were 0.84, 0.89, 0.84, 0.94 and 0.94, respectively, which were the most effective traits in factor 2, 3 and 4. High value of communality variance for most traits indicated that the six selected factors could well explain the major part of variation for forage yield, seed yield and their related traits. Abbasi *et al*. (2007) showed that the major part of community variation (43.2%) was impressed by fall dormancy score (0.965), regrowth rate (0.864), percent of light-blue violet (0.950) and red violet of petal colors (0.788) which were effective characters in the first and second factors, respectively. Farshadfar and Farshadfar (2008) concluded that 94% of the variance among 18 alfalfa cultivars were explained by two PCA. The plant height, tiller numbers, biomass and dry weight had the highest values 0.926, 0.707, 0.934 and 0.934, respectively.

Classifying the genotypes using cluster analysis: Cluster analysis was done to measure genetic distance between 21 different ecotypes applied based on 23 agro-morphological traits. All the 21 ecotypes could be classified into four clusters. Cluster 1 consisted of

Table 6: Mean and deviation values (%) from total mean for different traits of Iranian alfalfa ecotypes based on the cluster groups

Clusters	Ecotype No.	DFF	D10%F	F10%FI	PH	RR	FY	DMY	LD	LF	SD	LSRF
1	1, 3, 5, 6, 8, 11,	25.13[a]	35.39	10.11	69.12	5.96	14.43	3.66	25.97	113.40	32.80	0.93
	12, 15, 16, 17	2.15[b]	2.88	4.12	-1.32	-3.79	-2.29	-2.14	-3.46	-4.55	-2.26	-2.10
2	2, 4, 7, 10,	24.13	33.73	9.46	72.93	6.59	15.70	3.90	27.59	124.47	35.00	0.94
	13, 14, 19, 20	-1.91	-1.95	-2.47	4.11	6.29	6.30	4.28	2.57	4.76	4.29	-1.05
3	9	25.50	35.60	10.15	57.50	5.10	10.40	2.80	31.60	127.20	33.30	1.16
		3.66	3.49	4.64	-17.92	-17.74	-29.59	20.01	17.47	7.06	-1.37	22.11
4	18, 21	23.15	32.57	9.40	69.75	6.45	14.95	2.40	26.35	119.05	31.45	0.99
		-5.89	2.75	-3.09	-0.43	4.03	1.22	-35.83	-2.04	0.20	-6.14	4.21
Total mean		24.60	34.40	9.70	70.05	6.20	14.77	3.74	26.90	118.81	33.56	0.95
	Ecotype No.	LSRD	DFRP	NVF	NFPR	NPPR	NCPP	NSPP	100SW	PY	SY	RL
1	1, 3, 5, 6, 8,11,	0.84	37.70	0.48	25.53	13.48	2.75	4.50	0.227	1900.90	766.41	26.31
	12, 15, 16, 17	-1.18	-1.41	-4.00	1.39	2.28	-0.36	-6.64	3.180	-9.52	-7.68	-5.02
2	2, 4, 7, 10,	0.82	39.45	0.52	24.29	12.98	2.72	4.93	0.221	2216.98	852.93	29.18
	13, 14, 19, 20	-3.53	3.16	4.00	-3.68	-1.52	-1.45	2.28	0.450	5.53	2.74	5.34
3	9	0.97	37.30	0.77	23.40	12.60	2.80	5.20	0.204	2357.05	933.70	26.50
		14.12	-2.46	54.00	-7.07	-4.40	1.45	7.88	-7.270	12.19	12.47	-4.33
4	18, 21	0.95	36.50	0.34	28.00	12.75	2.70	5.45	0.227	2508.20	1006.45	29.55
		11.76	-4.55	-32.00	11.20	-3.26	-2.17	13.07	3.180	19.39	21.23	6.68
Total mean		0.85	38.24	0.50	25.18	13.18	2.76	4.82	0.220	2100.90	830.20	27.70

a: Mean, b: Deviation % from total mean. DFF: Days to first flower, D10%F: Days to 10% flowering, F10%FI: First to 10% flowering intervals, PH: Plant height, RR: Regrowth rate, FY: Fresh yield, DY: Dry matter yield, LD: Leaf dry weight, LF: Leaf fresh weight, SD: Stem dry weight, SF: Stem fresh weight, LSRF: Leaf-stem ratio fresh, LSRD: Leaf-stem ratio dry, DFVF: Days to first ripen pod, NVF: Non violent flowers, NFPR: No. of flowers per raceme, NPPR: No. of pods per raceme, NCPP: No. of curls per pod, NSPP: No. of seeds per pod, 100SW: 100-seed weight, PY: Pod yield, GY: Grain yield, RL: Racemes length

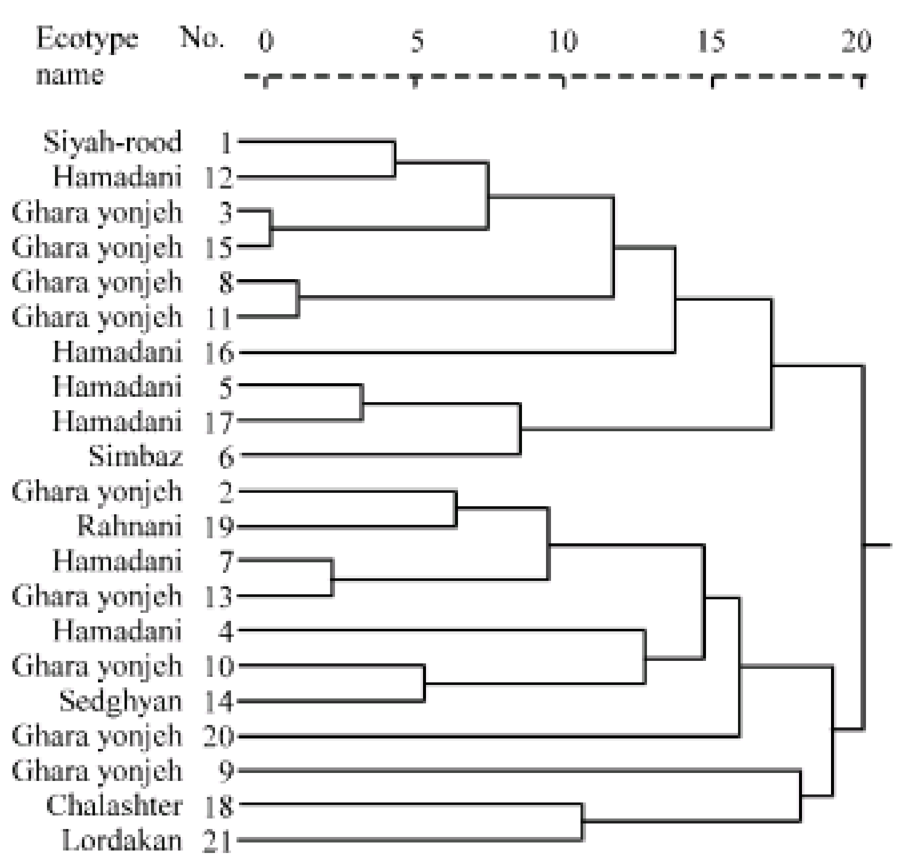

Fig. 2: Hierarchical dendrogram of agro-morphological traits of Iranian alfalfa ecotypes

ecotypes 1, 3, 5, 6, 8, 11, 12, 15, 16 and 17; cluster 2 included ecotypes 2, 4, 7, 10, 13, 14, 19 and 20; cluster 3 consisted of ecotype 9 and cluster 4 consisted of ecotypes 18 and 21 (Fig. 2). For showing the value of each cluster from the viewpoint of measured traits, the cluster's mean deviation (%) from total mean was calculated for each trait (Table 6). These deviations can partially explain the variation between the alfalfa ecotypes used. Since, the ecotypes in each group had more similar genetic relationship than ecotypes in different groups, it is possible using the ecotypes in each class for hybridization programs with regarding to the mean value of their desirable characters.

The average values of ecotypes in the first class were above the total mean of these traits: No. of days from cutting to first visible violet petal, No. of days from cutting to 10% flowering, No. of flowers per raceme, No. of pods per raceme and 100-seed weight. In this class, the mean values for No. of pods per raceme and 100-seed weight were realized much more than the means in other groups. Therefore, this class is suitable for improving these two traits.

In the second class (consist of 8 ecotypes), average values of the traits: plant height, leaf and stem dry matter yield, regrowth rate, forage fresh weight and dry matter yield, reproductive period, No. of seeds per pod, 100-seed weight, seed yield, pod yield and raceme length were found to be above the total mean. In this class, the mean value for traits: plant height, regrowth rate, forage fresh weight and dry matter yield, stem dry matter yield and reproductive period were higher than the means of other classes. Therefore, the ecotypes in this group are suitable for improving forage yield, dry matter yield, regrowth rate and stem dry matter yield.

In the third class, there was only the ecotype No. 9 (Malekkandi from East Azarbayjan). Average values of the traits: No. of days from cutting to first visible violet petal, No. of days from cutting to 10% flowering, No. of days from first visible violet petal to 10% flowering, leaf fresh weight and dry matter yield, leaf-stem ratio, percent of non violet flowers, No. of seeds and curls per pod, seed and pod yield were calculated above the total mean.

In this class, the mean values of traits: number of days from cutting to 10% flowering, number of days from first visible violet petal to 10% flowering, leaf fresh weight and dry matter yield, leaf-stem ratio, percent of non violet flowers and number of curls per pod were higher than the means of other classes. With regarding to the characteristics of this ecotype, it is suitable for improving forage quality (leaf-stem ratio and leaf dry matter yield). Also, the percent of non violet flowers in this ecotype was higher than the other classes which indicated high non genetic purity. Longer time between the first visible violet petals to 10% flowering which had a positive correlation with the percent of non violet flowers ($r = 0.33$, $p \leq 0.05$) was due to this non genetic purity (Table 5).

The fourth class included 2 ecotypes which had been sampled from the province of Charmaha-and-Bakhteyari and formed a single group. The cluster mean values for traits: regrowth rate, forage yield, leaf fresh weight, leaf-stem ratio, number of flowers per raceme, number of seeds per pod, 100-seed weight, raceme length, seed and pod yield were higher than the total means. Also, the averages for traits: vegetative period, intervals between first visible violet petals to 10% flowering, reproductive period and percent of non violet flowers in this group were lower than means in other classes. Lower non violet flowers in this group, indicates more genetic purity and more uniformity that caused the least intervals between first visible violet petals to 10% flowering in comparison to other ecotypes. Also, the shorter vegetative and reproductive periods had caused precocity for these ecotypes in comparison to other classes. Therefore, for increasing seed yield in breeding programs, these ecotypes are suitable, particularly since the correlation between raceme length with pod and seed yield was positive and highly significant ($r = 0.56, 0.94$, $p = 0.01$).

Marquez *et al*. (1998) in Mexico classified 41 alfalfa genotypes in to 5 groups by using quantitative traits with at least one specific trait in each group. Touil *et al*. (2008) divided 35 Mediterranean populations of cultivated alfalfa based on 9 morphological traits into 3 groups which were characterized by significant differences in poor yield, plant height, leaflet characteristics (length, width, surface), number of inflorescence, number of pods per raceme and stem dry matter yield.

The results of cluster analysis showed accordance with factor analysis results. Clusters two, three and four confirmed the results of second factor (forage yield), fourth factor (forage quality) and third factor (seed factor), respectively.

Smith *et al*. (1991) and Warburton and Smith (1993) evaluated 34 accessions from India and the Middle East and analyzed them by cluster analysis and PCA method. Both analytical approaches were in accordance with each other and showed that Indian accessions were phenotypically distinct from Arabian accessions. Results of their study showed that at least 6 regional germplasm groups existed among North African, Arabian and Indian nondormant alfalfa germplasm.

CONCLUSION

Results of factor analysis showed that the major part of community variance (43.9%) was contributed by plant height, stem fresh yield, leaf fresh yield, seed yield and raceme length which were the effective traits in factors 2-4. These characters had major effects on classifying alfalfa ecotypes.

According to cluster analysis, 21 ecotypes were classified in to four groups based on their agro-morphological similarities. The 10 ecotypes in cluster I were from West Azarbayjan (5 ecotypes), Hamadan (4 ecotypes) and East Azarbayjan (1 ecotype) Provinces. They were suitable for improving number of pods per raceme and 100-seed weight because their mean values for these traits were much higher than the means in other groups.

The 8 ecotypes in cluster II were from Hamadan (3 ecotypes), West Azarbayjan (2 ecotypes), East Azarbayjan (2 ecotypes) and Esphahan (1 ecotype) Provinces. These ecotypes were realized as suitable for improving forage yield, dry matter yield, regrowth rate after cutting and stem dry matter yield because their mean values were higher than the means of other classes.

Clusters III and IV distinguished from other groups with one and two ecotypes, respectively. The ecotype number 9 (Malekkandi from East Azarbayjan), the member of cluster III was suitable for improving forage quality (leaf-stem ratio and leaf dry matter yield). Also, genetic purity in this ecotype was low because of high percent of non violet flowers and longer intervals between the first visible violet petals to 10% flowering, which had a positive correlation with the percent of non violet flowers ($r = 0.33$, $p \leq 0.05$), in comparison to other groups.

The 2 ecotypes in cluster IV which were from the Charmahal and Baghteyari Province and were the only ecotypes from this province, were realized for improving seed yield in breeding particularly since the correlation between raceme length with pod and seed yield was positive and highly significant ($r = 0.56, 0.94$, $p = 0.01$). The genetic purity in these ecotypes were realized higher than other ecotypes, because the percent of non violet flowers, days of interval between first visible violet petals to 10% flowering and reproductive period in this group were lower than means in other classes.

Cluster analysis also showed that ecotypes which were sampled from different provinces, especially ecotypes in East and West Azarbyjan with Hamadan ecotypes have agro-morphological similarities which may be the result of transferring from their place of origin (Hamadan Province) to other cold regions of the country.

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