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GENETIC DIVERSITY OF 36 ALFALFA (MEDICAGO SATIVA L.) GENOTYPES USING MULTIVARIABLE STATISTICAL METHOD

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ABSTRACT

Alfalfa is the most important forage crop in the world for the improvement of which the presence of primary variation is the most substantial. In the present work, the situation of variability levels among 36 alfalfa genotypes was studied in a field experiment in the research farm of Bu-Ali Sina University with the evaluation of 9 traits in a completely randomized design in 2009-2010.Analysis of variances of data showed that the genotypes for all the studied traits were significantly different from each other. Genotypes of Lotus81, Feriken and Vernal were appeared to have the highest Forage dry matter yield. The results of the analysis of correlation of the studied traits indicated a positive and significant correlation between Forage dry matter yield and plant height and a negative and significant correlation with the ratio of leaf to stem dry weight at the probability level of 5%. In analysis to main components, about 79% of the total changes were confirmed by the first of the 4 main components. Based on cluster analysis, the genotypes were

clustered in five groups. The genotypes in the first cluster had appropriate forage quality and the genotypes in the second cluster were superior concerning forage quantity.

Keywords: Genetic variability, Alfalfa, Morphological traits, Cluster analysis

INTRODUCTION

Alfalfa (Medicago sativa L.) is the most important forage crops in the world that has special significance in terms of high protein content, nutritional composition, calcium, palatability, yield and low cellulose content. In addition, it has Long life, rapid regrowth after harvesting, high tolerance to environmental stresses and the effects on soil fertility [Mazaheri-Laghab 2008].

In order to maintain and improve agricultural productivity, determining the level of genetic variability is very useful for plant breeders [Heal et al. 2004]. Diversity and selection are two critical factors for any plant breeding program that selection process for each trait depends entirely on the presence of the desired diversity. Germplasm evaluation is necessary to investigate the diversity in any breeding program [Naroui Rad et al. 2006]. Due to the high degree of natural cross pollination (depending on the genotype and its interaction with local environmental conditions) and its open-pollination, alfalfa populations are highly heterozygous and heterogeneous and extensive genetic diversity have been considered among its varieties [Obsorn et al. 1997]. Genetic studies of this species are complicated because of wide genetic variation of individuals within a population. Therefore, access to useful information about variations of different varieties are critically important, especially when they are collected from various resources [Mengoni et al. 2000]. [Ba-Safa, and Taherian 2005] evaluated agronomic and morphological variations of 21 alfalfa ecotypes collected from cold regions in Iran, which included number of pods per inflorescence, 100-seed weight, forage yield, dry matter yield, regrowth rate and etc. Finally, these ecotypes were divided into four groups. [Li et al. 2009] reported that three of 12 populations had flowers with non-yellow color, so that this characteristic showed that their grain filling period began earlier than the other populations. They also reported that latitude, longitude and altitude of the area did not have any correlation with the genetic distances between populations. [Musial et al. 2002] studied genetic diversity between and within 19 alfalfa genotypes, which genetic variations between genotypes were higher than genetic variation within genotypes.

Due to the increasing importance of synthetic varieties in the form of commercial varieties of alfalfa and the existence of different populations with typical and distinctive characteristics [Yazdi-Samadi, and Abd-Mishani 1995], we analyzed genetic diversity and classification of 36 alfalfa genotypes on the basis of the morphological traits and the data with simple and multivariate statistical methods (variance, cluster and principal component analysis).

MATERIALS AND METHODS

Plant material

36 Iranian and foreign alfalfa genotypes provided by the gene bank of Bu-Ali Sina University and University of Tehran, Iran (Table 1). Field experiments were conducted in a completely randomized design (CRD) with two repetitions at the agricultural research station, Bu-Ali Sina University, Hamedan province, Iran during 2009-10. Autumn ploughing and disc were accomplished for seed bed preparation. Seeds were planted in the autumn, 2009. Each row was two meters in length and 50 cm apart. The studied traits included forage dry matter yield (g), stem dry weight (g), leaf dry weight (g), dry weight per plant (g), plant height (cm), ratio of the leaf to stem dry weight, ratio of the leaf to plant dry weight, number of stems per plant and number of leaves per plant. Sampling was carried out on a regular basis at %10 flowering time and was randomly taken from three plants of the internal rows. After that, the stems were separated into leaves and stem and then, separately oven dried at 75 C for 72 h to obtain dry weight. The mean of three plants were considered to analyze all traits. Statistical analysis, after ensuring normal distribution of data in the 95% confidence (Table 2)., was conducted using Duncan's multiple range test (Table 3) and Pearson's simple correlation coefficient were used for the continuous variables (Table 4). In order to reduce the amount of data, Principal component analysis (PCA) was performed using the correlation matrix (Table 5) and for grouping genotypes, cluster analysis was Ward's method (Figure 1) and the trait mean in each group was determined (Table 6). MINITAB, SAS and SPSS were applied to analyze the statistical data.

Table 1 Name and origin of 36 studied alfalfa genotypes

Genotype No.	Name of genotype	Origin	Genotype No.	Name of genotype	Origin
1	Baghdadi 110	-	19	Califordi	-
2	Bami 86	-	20	Tafresh 42	-
3	Yazdi105	-	21	Moopa Fc33626	-
4	Yazdi 35	-	22	Local marandi	-
5	Yazdi 39	-	23	Bam Polycross 13	-
6	Lotuse 81	France	24	Local bami	Seed breeding
7	One plant 23	-	25	Feriken	-
8	Topozabad rezaieh 9	Rezaieh	26	Shiraz polycross	-
9	Mahalat 25	-	27	Hamedan polycross	-
10	Khomein 11	-	28	Ardebil	-
11	Faiz 46	-	29	Rezaieh	-
12	Faiz 43	-	30	Vernal	-
13	Faiz 45	-	31	Reynani	-
14	Karimabad rezaieh 24	Rezaieh	32	Diopoy 34630	-
15	Hamedan 27	Tehran	33	Diopoy	-
16	Mohajeran hamedan 33	Hamedan	34	cody 91	-
17	Hamedani 54	Hamedan	35	Hamedani 32	Hamedan
18	Sarab 16	-	36	Asadabad 2	-

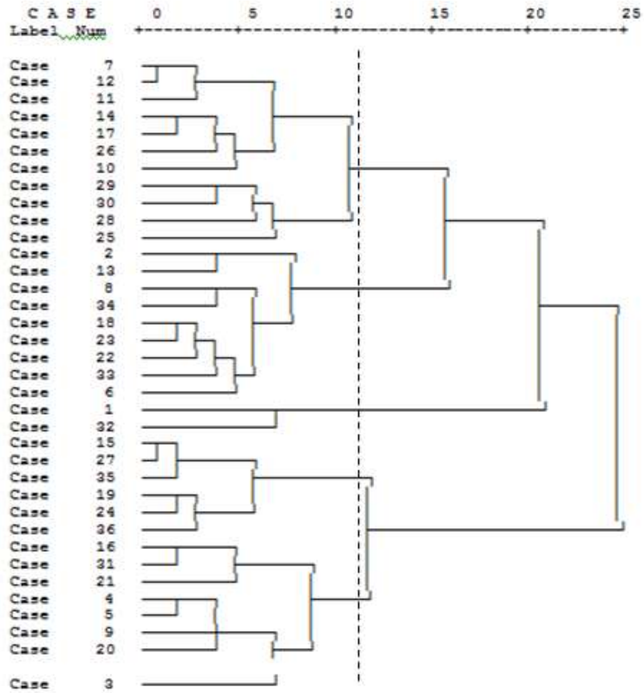

Figure 1: Dendrogram of alfalfa genotypes based on different traits (Ward method)

3. RESULTS AND DISCUSSION

3.1. Variance Analysis and Mean Comparison Test results

As shown in table 2, there were significant differences between the genotypes and different cutting treatment. The interpretation of the results lead to a wide variation among genotypes for all traits. The genotypes had significant differences at the probability level of 1% for the important agronomic traits, like forage dry matter yield. This diversity is very important for selection. [Zamanian 2003] reported that there were differences among the varieties for forage dry matter yield at the probability level of 1% and optimum temperatures under optimum ecological conditions had a significant influence on fresh and dry forage matter yields. The effects of cutting treatments and the interaction effect of genotypes × cutting treatments were significant on the most traits, so that, the genotypes had different responses to each cutting. However, [Jafari, and Noori 2001] evaluated seven alfalfa varieties under rainfed condition. Jafari et al. 2003 studied 18 alfalfa varieties under irrigated and rainfed conditions. These two groups reported different results about the non-significant interaction effect of dry forage matter yields. The non-significant interaction effect of genotype × year might be caused by the low number of varieties and the number of years could be effective on the interaction effects of genotype and year. [Jafari, and Gudarzi 2006] studied genetic diversity of 72 perennial alfalfa populations. They found that the interaction effect of genotype × year was significant for all traits. According to the mentioned above results, we suggest that evaluation of perennial forage crops like alfalfa will be performed for several years.

The mean comparison showed that the maximum forage dry matter yield were related to Lotus 81, Feriken, Vernal, Bam Polycross 13, respectively (Table 3). Therefore, it could be said that if the purpose is only increasing forage, these genotypes have the higher forage matter yields than the others. A report based on the comparison of qualitative and quantitative traits was presented for 64 common alfalfa varieties that the six traits were evaluated in several different cutting treatments during seven years, So that the analysis of the results showed a large amount of genetic variation for all traits and foreign cultivars had better forage wet matter and plant height than Iranian cultivars [Fazli, and Yazdisamadi 1992].

We suggest Feriken, Rezaieh, Karimabad rezaieh 24 and Rezaieh 24 genotypes to be used as parents of breeding programs that had the highest amount of leaf/stem dry ratio (quality forage). Feriken had high-quality forage as well as a high forage production potential. [Zamanian 2001] stated that forage quality of alfalfa varieties are varied depending on the cutting time which affected by environmental condition and plant growth stage during cutting time. [Crochmore et al. 1998] reported that forage quality is related to leafy stems and forage should be free of foreign objects. The highest height plant was Bami 86 with an average of 72.77 cm. [Farshadfar et al. 2008] evaluated genetic diversity for plant height among 18 alfalfa cultivars which Hamedani was the highest genotype. Lotus 81 with respect to the positive correlation between forage matter yield and plant height was also high compared to the others. Bami 86, Faiz 45 and Baghdadi 110 were high plants and had the highest number of leaves per plant. Tilling is one of the important factors which determine the amount of forage harvested. Cultivars with more tilling always deserve the attention of plant breeders [Karimi 1988]. In the present study, Khomeini 11, Moopa 33626Fc, Ardabil and Rezaieh produced the highest number of main stems per plant.

Table 2 :Combined analysis of variance for morphological traits in 36 genotypes of Medicago sativaL. Mean of squares. *, ** & ns significant at 5% and 1% levels, and nonsignificant, respectively

		Mean of squares (MS)								
S.O.V	DF	Forage dry matter yield	Stem dry weight	leaf dry weight	dry weight per plant	Plant height	Leaf to stem dry weight	Leaf to plant dry weight	Number of stems	Number of leaves
Genotype	35	0.257^{**}	0.389^{*}	0.708^{**}	1.021^{*}	119.441^{**}	0.134^{**}	0.271^{*}	1.892^{**}	3820.397^{**}
Replica × Genotype	36	0.116	0.396	0.405	0.478	69.627	0.058	0.297	0.759	45.628
Cutting	2	271.032^{**}	12.769^{**}	5.555^{**}	158.11^{**}	1204.514^{**}	0.585^{**}	195.864^{**}	386.501^{**}	942296.657^{**}
Genotype × Cutting	70	0.228^{**}	0.52^{*}	0.343^{*}	0.794^{*}	85.546^{**}	0.166^{**}	0.273	1.55^{**}	3768.478^{**}
Cutting × Replica	2	0.542	1.125	0.106	2.215	328.617	0.052	0.014	0.884	0.370

Table 3: Comparison of the mean of different agronomic traits in combined analysis of 36 alfalfa genotypes

No.	Genotype	Forage dry matter yield	Stem dry weight	leaf dry weight	dry weight per plant	Plant height	Leaf to stem dry weight	Leaf to plant dry weight	Number of stems	Number of leaves
1	Baghdadi 110	2.96^{abcde}	2.02^{ab}	2.46^{ab}	3.00^{abc}	60.33^{bcdef}	1.19^{abcdef}	1.69^{abc}	3.98^{abcd}	108.09^{b}
2	Bami 86	3.02^{abcde}	1.70^{abc}	1.73^{bcde}	3.05^{ab}	72.77^{a}	$1.02^{bcdefghi}$	1.45^{abc}	$3.10^{abcdefghi}$	128.19^{a}
3	Yazdi105	2.64^{ef}	2.11^{ab}	1.42^{cde}	2.27^{abcdef}	60.32^{bcdef}	0.82^{hi}	1.06^{bc}	$3.28^{abcdefghi}$	97.11^{c}
4	Yazdi 35	3.07^{abcde}	1.60^{abc}	1.42^{cde}	1.99^{def}	60.50^{bcdef}	$0.92^{cdefghi}$	1.29^{bc}	2.76^{defghi}	73.40^{ghi}
5	Yazdi 39	3.18^{abc}	1.54^{abc}	1.48^{cde}	1.79^{ef}	59.33^{cdef}	$1.00^{bcdefghi}$	1.29^{bc}	2.54^{fghi}	70.88^{hij}
6	Lotuse 81	3.38^{a}	1.72^{abc}	1.37^{cde}	2.02^{def}	70.72^{abc}	$0.90^{cdefghi}$	1.15^{bc}	2.34^{i}	79.54^{efg}
7	One plant 23	3.09^{abcde}	1.53^{abc}	1.45^{cde}	2.48^{abcde}	67.27^{abcde}	$1.04^{bcdefghi}$	1.48^{abc}	2.69^{efghi}	62.50^{jklm}
8	Topozabad rezaieh 9	3.09^{abcde}	2.14^{ab}	2.02^{abcd}	3.17^{a}	67.83^{abcde}	$0.93^{bcdefghi}$	1.43^{abc}	$2.98^{bcdefghi}$	82.96^{ef}
9	Mahalat 25	2.71^{cdef}	1.71^{abc}	1.46^{cde}	2.02^{cdef}	55.05^{f}	$1.03^{bcdefghi}$	1.19^{bc}	$2.95^{bcdefghi}$	49.84^{nop}
10	Khomein 11	2.97^{abcde}	1.59^{abc}	1.64^{bcde}	2.22^{abcdef}	58.22^{def}	$1.00^{bcdefghi}$	1.46^{abc}	4.25^{a}	96.51^{c}
11	Faiz 46	2.88^{bcde}	1.54^{abc}	1.67^{bcde}	2.38^{abcdef}	65.77^{abcdef}	$1.12^{abcdefghi}$	1.33^{abc}	2.43^{hi}	58.77^{klmn}
12	Faiz 43	2.98^{abcde}	1.60^{abc}	1.41^{cde}	2.59^{abcde}	63.77^{abcdef}	$1.14^{abcdefghi}$	1.46^{abc}	$2.98^{bcdefghi}$	53.91^{mnop}
13	Faiz 45	3.20^{ab}	1.45^{abc}	1.43^{cde}	2.32^{abcdef}	66.33^{abcdef}	$0.98^{bcdefghi}$	1.57^{abc}	2.51^{ghi}	113.70^{b}
14	Karimabad rezaieh 24	3.00^{abcde}	1.64^{abc}	2.02^{abcd}	2.57^{abcde}	59.50^{cdef}	1.23^{abc}	1.68^{abc}	$3.26^{abcdefghi}$	86.97^{de}
15	Hamedan 27	3.12^{abcd}	1.65^{abc}	1.38^{cde}	2.34^{abcdef}	66.61^{abcdef}	$0.93^{bcdefghi}$	1.38^{abc}	$2.96^{bcdefghi}$	79.89^{efg}
16	Mohajeran hamedan 33	2.89^{bcde}	1.38^{abc}	1.15^{de}	1.87^{def}	58.39^{def}	0.84^{ghi}	1.29^{bc}	$3.12^{abcdefghi}$	54.49^{mnop}
17	Hamedani 54	3.01^{abcde}	1.49^{abc}	1.74^{bcde}	2.30^{abcdef}	60.66^{bcdef}	$1.14^{abcdefghi}$	1.64^{abc}	$3.05^{abcdefghi}$	64.94^{ijk}
18	Sarab 16	3.10^{abcde}	1.84^{abc}	1.63^{bcde}	2.35^{abcdef}	64.22^{abcdef}	0.86^{fghi}	1.49^{abc}	$2.81^{cdefghi}$	68.42^{ij}
19	Califordi	2.98^{abcde}	1.40^{abc}	1.33^{cde}	1.89^{def}	66.88^{abcdef}	$0.99^{bcdefghi}$	1.33^{abc}	$3.39^{abcdefghi}$	53.13^{nop}

20	Tafresh 42	3.10^{abcde}	2.01^{ab}	1.53^{cde}	1.97^{def}	57.33^{def}	0.84^{fghi}	1.29^{bc}	3.76^{abcdef}	56.09^{lmno}
21	Moopa Fc33626	2.98^{abcde}	1.40^{abc}	0.94^{e}	1.46^{f}	57.83^{def}	0.80^{i}	0.99^{c}	4.14^{ab}	32.29^{s}
22	Local marandi	3.15^{abcd}	1.85^{abc}	1.60^{bcde}	2.82^{abcd}	63.16^{abcdef}	0.89^{defghi}	1.27^{bc}	$3.61^{abcdefgh}$	95.74^{c}
23	Bam Polycross 13	3.23^{ab}	1.81^{abc}	1.77^{abcde}	2.45^{abcde}	60.94^{abcdef}	1.21^{abcde}	1.46^{abc}	2.54^{fghi}	46.75^{pq}
24	Local bami	3.03^{abcde}	1.66^{abc}	1.43^{cde}	1.76^{ef}	64.83^{abcdef}	$0.99^{bcdefghi}$	1.26^{bc}	3.85^{abcde}	65.01^{ijk}
25	Feriken	3.33^{ab}	1.69^{abc}	2.19^{abc}	2.10^{bcdef}	66.38^{abcdef}	1.43^{a}	1.78^{ab}	4.00^{abcd}	47.90^{opq}
26	Shiraz polycross	2.69^{def}	1.71^{abc}	1.82^{abcde}	2.32^{abcdef}	62.42^{abcdef}	1.21^{abcd}	1.49^{abc}	$2.87^{cdefghi}$	82.36^{ef}
27	Hamedan polycross	3.05^{abcde}	1.72^{abc}	1.48^{cde}	2.37^{abcdef}	62.94^{abcdef}	$0.93^{bcdefghi}$	1.39^{abc}	$3.00^{bcdefghi}$	77.26^{fgh}
28	Ardebil	2.98^{abcde}	1.07^{c}	1.26^{de}	2.00^{def}	56.16^{ef}	$1.16^{abcdefg}$	1.72^{abc}	4.01^{abc}	50.15^{nop}
29	Rezaieh	3.07^{abcde}	1.51^{abc}	1.80^{abcde}	2.46^{abcde}	58.27^{def}	1.26^{ab}	1.33^{abc}	4.00^{abcd}	52.10^{nop}
30	Vernal	3.28^{ab}	1.72^{abc}	1.51^{cde}	1.84^{def}	59.58^{cdef}	$0.92^{cdefghi}$	1.40^{abc}	$3.71^{abcdefg}$	97.15^{c}
31	Reynani	2.89^{bcde}	1.32^{bc}	1.13^{de}	1.79^{ef}	62.27^{abcdef}	$0.93^{bcdefghi}$	1.29^{bc}	$2.90^{cdefghi}$	35.42^{rs}
32	Diopoy 34630	2.30^{f}	2.26^{a}	2.62^{a}	3.11^{a}	57.88^{def}	$1.15^{abcdefgh}$	2.08^{a}	$3.00^{bcdefghi}$	134.96^{a}
33	Diopoy	3.12^{abcde}	2.07^{ab}	1.72^{bcde}	1.98^{def}	62.88^{abcdef}	0.87^{defghi}	1.47^{abc}	2.35^{i}	93.94^{cd}
34	cody 91	3.29^{ab}	2.03^{ab}	1.67^{bcde}	2.63^{abcde}	68.27^{abcd}	$0.91^{cdefghi}$	1.18^{bc}	2.51^{ghi}	50.84^{nop}
35	Hamedani 32	3.17^{abcd}	1.56^{abc}	1.57^{bcde}	2.69^{abcde}	64.82^{abcdef}	$1.10^{abcdefghi}$	1.33^{abc}	$3.64^{abcdefgh}$	63.35^{jkl}
36	Asadabad 2	2.99^{abcde}	1.69^{abc}	1.35^{cde}	2.47^{abcde}	71.72^{ab}	0.86^{efghi}	1.18^{bc}	$2.85^{cdefghi}$	40.74^{qr}

Table 5: Eigenvalues, variance and Coefficients vectors for traits in PCA

Traits	PC1	PC2	PC3	PC3
Forage dry matter yield	-0.097	-0.274	0.811	-0.061
Stem dry weight	0.276	0.852	-0.037	0.053
leaf dry weight	0.856	0.337	-0.071	-0.042
dry weight per plant	0.721	0.469	0.191	0.039
Plant height	-0.079	0.235	0.822	0.210
Leaf to stem dry weight	0.418	-0.321	0.140	0.743
Leaf to plant dry weight	0.886	-0.148	-0.225	-0.051
Number of stems	0.198	-0.283	-0.047	-0.840
Number of leaves	0.610	0.382	-0.171	0.243
Eigenvalue	3.206	1.702	1.122	1.075
proportion	0.356	0.189	0.123	0.120
Cumulative variance	0.356	0.545	0.67	0.790

Table 6: Means of Medicago sativa L. traits of groups in cluster analysis

cluster	Forage dry matter yield	Stem dry weight	leaf dry weight	dry weight per plant	Plant height	Leaf to stem dry weight	Leaf to plant dry weight	Number of stems	Number of leaves
1	3.048	1.655	1.672	2.034	51.590	12.582	1.628	2.504	69.083
2	3.190	1.962	1.646	2.231	57.774	11.528	1.497	2.039	84.888
3	2.662	2.274	2.481	2.825	50.175	10.364	2.027	2.493	122.259
4	3.077	1.759	1.380	2.023	55.075	9.765	1.437	2.432	63.808
5	2.955	1.779	1.295	1.694	50.825	9.168	1.327	2.303	59.325

3.3. Principal component analysis results

In order to reduce the amount of data of the 36 genotypes in the three cutting treatments, principal component analysis was carried out based on the studied traits and the correlation matrix, which ultimately 79% of the variance was explained by the first four principal components. According to Table 5, the 35.6% of the total variance of the traits was explained by the first principal component (PC1), and with respect to the variables influenced (ratio of leaf to dry weight per plant , ratio of leaf to stem dry weight, leaf dry weight and number of leaves), PC1 was recognized as the high-quality forage component. The second principal component (PC2) justified 18.9% of the variation. Stem dry weight had the greatest impact on PC2 and the effect of dry weight per plant and number of leaves was modest. The third principal component (PC3) explained the 12.3% of the variation, and with respect to the variables influenced (forage dry yield and plant height), PC1 was recognized as the quantitative component of forage production. The fourth principal component (PC4) justified 12% of the remaining variation. Basafa and Taherian, (2005) [1] recognized six components that explained 80.45% of the total variance. PC1, PC2, PC3, PC4, PC5 and PC6 justified 20.13, 16.64, 14.26, 13, 9.24 and 7.18% of the total variations, respectively. Moreover, With respect to the variables influenced, PC1, PC2, PC3, PC4, PC5 and PC6 were identified as growth period, forage production, seed production, forage quality, reproductive period and fertility components, respectively.

3.4. Cluster analysis results

Based on all the traits, grouping can be a reliable method to determine similarities, differences or relative distances of genotypes. According to the dendrogram of cluster analysis by Euclidean distances and Ward's method and with respect to the cutting line at a distance of 11 units, alfalfa genotypes can be clustered into five groups (Figure 1).

The first cluster included genotypes number 7, 10, 11, 12, 14, 17, 25, 26, 28 and 29. These genotypes had high levels of stems number, leaf to stem dry weight ratio and leaf to dry weight per plant ratio (Table 6). These traits were positively correlated with increasing forage quality. In other words, this cluster in terms of forage quality was better than the other clusters. Feriken and Rezaieh had the highest rates of leaf to stem ratio which were located in this cluster as high quality genotypes.

The genotypes number 2, 6, 8, 13, 18, 22, 23, 33 and 34 were in the second cluster. They were higher than the others for forage dry matter yield and plant height. Thus, the genotypes of this cluster could be considered as superior genotypes in terms of forage quantity (genotypes with high forage yield). Lotus 81, Faiz 45, Bam Polycross 13, cody 91 were in the second cluster with the highest yield. Moreover, Bami 86 was in the same cluster as the highest height genotype. The third cluster was composed of two genotypes, 10 and 32 that had higher rates of leaf dry weight, stem dry weight, dry weight per plant, leaf to dry weight per plant ratio, and number of leaves than the other genotypes. The fourth cluster included genotypes number 15, 19, 24, 27, 35 and 36 that were in the middle status in terms of the most traits. The fifth cluster included genotypes number 3, 4, 5, 9, 16, 20, 21 and 31 that had the lowest rates of leaf dry weight, dry weight per plant , leaf to stem dry weight ratio, leaf to dry weight per plant ratio and number of leaves. On the other hand, they were in the middle status in terms of stem dry weight and forage dry yield. By calculating the Euclidean distance among the genotypes, it had been found that there was the maximum genetic distance between Baghdadi 110 (1) and Yazdi 105 (3) which can be used in the cross breeding programs.

On the basis of these results, genotypes with high forage quality were located in the same cluster, while genotypes with high forage yield were together in a cluster. Since the genotypes in each cluster had some specific traits and more genetic similarities than Genotypes of different clusters. When hybridization is necessary, phenomena such as heterosis and aggressive segregation can be used due to the genotypes in different clusters and the mean value of traits.

CONCLUSION

The results of this study show that high variability of the important forage traits were identified from the alfalfa genotypes. It is obvious that such diversities can be useful for maintenance of alfalfa germplasms and plant breeding programs.

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