Assessment of Relationships among Yield and Quality Traits in Alfalfa (*Medicago sativa*) under Dryland Farming System, Hamadan, Iran

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Abstract. Seeds of 200 accessions of alfalfa (*Medicago sativa* L.) were provided from Iranian natural resource gene bank and were sown as drilled plot using alpha designs/unreplicated with 2 repeated entries within all of 10 blocks under dry land farming system in agricultural research center of Hamadan, Iran during 2009 to 2010. The data were collected and analyzed for plant height, stem number, leaf stem ratio (LSR), node number, vegetation score, forage dry matter yield (DM yield), crude protein (CP), dry matter digestibility (DMD), water soluble carbohydrates (WSC), crude fiber (CF), acid detergent fiber (ADF), and total ash. DM yield was positively correlated with node number, plant height, stem number, vegetation score and negatively correlated with leaf/stem ratio, DMD(%), CP(%)and WSC(%). The factorial analysis was based on the principal component extraction and varimax rotation method. Results of factor analysis were accounted for 81% of total variance for first six factors. Factor 1 which was accounted for 19% of variation was associated with DMD, WSC and ADF. This factor was regarded as quality factors. Factor 2 accounted for 17% of variation was named as the productivity factor since it included DM yield, vegetation score and leaf/stem ratio. Since the third (CP and CF) and forth factors (plant height and node number) were important. Based on ward cluster analysis, 200 entries were divided into 7 groups. Accessions in 4 clusters were calculated well above the overall mean for DMD, CP, WSC and total ash. The accessions in cluster 7 had higher values for DM yield and morphological traits. The results of this study indicate that selection of variables in productivity factor (factor2) could enable breeders to release the desirable increment in forage yield of alfalfa.

Key words: Alfalfa (*Medicago sativa*), Yield, Quality, Factor analysis, Cluster Analysis.

Introduction

Alfalfa (*Medicago sativa*) is originated from Iran and it is one of the most important forage species in this country. Breeding for improving yield and quality traits are important objectives in herbage breeding programs. Smith *et al.* (1997) ranked forage traits in terms of their nutritional value for dairy production. Improved digestibility was the most important criteria and high crude protein and low fiber content was ranked as moderate priority in terms of quality objectives. High leaf to stem ratio (LSR) is also desirable because leaves are more palatable and retain higher digestibility much time than stems. There are positive correlations between LSR ratio and digestibility in alfalfa Julier *et al.* (1999). Alfalfa (*Medicago sativa*) is originated from Iran and it is one of the most important forage species with cultivated area 600.000 ha with average annual 7200 Kg ha-1 DM yield. Improved quality traits are an important goal in alfalfa breeding programs. Data from animal nutrition studies show the need to focus more attention on nutritive value to improve new varieties. Improved DMD, WSC and CP couple with low fiber content had higher priority in terms of forage quality for live weight gain and dairy production Smith *et al.* (1997). Genetic correlation between digestibility and fibre content is negative and correlation with crude protein is positively high and significant (Julier *et al.* 1999; Riday and Brummer, 2004). Some morphological characteristics have been related to forage quality. (Hanna 1993) reviewed literature showing that reducing the plant height reduces total dry matter but produces more leaves and increases forage quality. High leaf to stem ratio (LSR) is usually desirable because leaves are more palatable and retain higher digestibility over time than stems. LSR can be manipulated by management regimes such as genetical or defoliation frequencies. There are positive correlations between LSR and Forage quality in alfalfa (Julier *et al.* 1999, Rotilli *et al.* 2001; Hayek *et al.* 2008).

Assessment of genetic divergence between populations is vital to the success of plant breeding programs designed to exploit gene recombination. Strong positive relationships have been found between genetic distance and heterosis in broad range of crop species (Humphreys 1991). Measurement of genetic distance should be very important for breeding when it is based on a broad range of traits relevant to breeding objectives. In alfalfa breeding programs for improved DM yield, the knowledge of new variety's quality and its relationships with forage yield are very important. The objectives of this research were to determine the pattern of variation for DM yield and quality traits in Iranian 200 alfalfa germplasm and to identify the groups of accessions through a multivariate approach.

Materials and Methods

A total of 200 alfalfa accessions (*Medicago sativa* L.) from Natural resource gene bank of Iran were beeng examined. Due to a great number of evaluated accessions, the alpha design was used (Patterson and Williams 1976). Seeds were sown as drilled plot under dryland farming system using alpha designs/unreplicated with 2 repeated entries within all of 10 blocks in Hamadan agricultural research center, Hamadan, Iran during 2009 to 2010.

From total accessions, 155 accessions were domestic and 45 foreign accessions originated from Italy, Hungary, Turkey, France, USA, Kasakestan and Australia. In the establishment year, no measurement was taken. The evaluation was carried out for only 1 cut in 2009. The data were collected for the below items:

1) Plant height (cm) was averagely five measurements taken on random plants as they stood naturally in the field.
2) Stem number was measured as the number of tillers in a 25×25 cm frame.
3) Vegetation score in each plot was recorded visually from 1 to 5 while 1 and 5 were considered as very week green cover and the highest growth rate during growth seasons.
4) DM yield was determined by cutting each plot. Each plot sample was taken and dried at 70°C and DM yield was expressed as Kg ha^{-1}.
5) Estimating the leaf stem ratio was based on separating and weighing the leaf and stem in the sub samples.
6) To estimate quality traits, crude protein (CP), dry matter digestibility (DMD), water soluble carbohydrates (WSC), crude fiber (CF), acid detergent fiber (ADF), and total ash, the samples were ground to pass through a 1mm screen using a Retsch Impeller-type mill. Quality traits were estimated in the first and second cuts for each year using near infrared spectroscopy (NIR). Details of methodology and calibrations of NIR are given by Jafari *et al.* (2003a).

All of 200 accessions (unreplicated design with 2 control entries) were adjusted using the mean yield of neighboring checks using Agrobase Software (Mulitze 2004). Phenotypic correlation was determined between traits. Estimation of factor loading was based on data average over replications on 12 characteristics of 200 genotypes using MINITAB 16. The numbers of factors were estimated using the principal components extraction and varimax rotation method. The factor loading of rotated matrix, the percentage variability explained by each factor and the commonalties for each variable were determined.

Results and Discussion

In order to control the soil fertility gradients, an analysis of variance was made among 10 incomplete blocks (two control varieties). The results showed no significant differences among blocks for all of traits except for DM yield and vegetation score characteristics (Table 1). It indicated that there were similar soil fertility slopes for all of 200 entries. However, all of 200 accessions were adjusted using the mean yield of neighboring checks using Agrobase Software (Mulitze 2004). All of multivariate analyses were made on the adjusted data. The results showed large heterogeneity among population during seasonal growing under dryland faming system for the majority of examined characters. Remarkable variation between populations was observed in most of the traits. The success of plant breeding operations relies heavily on the extent of genetic variabilities existing in a crop species for a particular trait. In fact, plant breeders use the selection for improving the architecture of a crop by managing the available genetic variabilities. In present study, the coefficient of variation (CV %) was high for vegetation score followed by plant height, node number and DM yield (Table 1). It was found that DM yield variation was ranged from 431 to 2094 Kg ha^{-1} with average values of 1052 Kg ha^{-1} (Table 1). LSR variation ranged from 1.11 to 2.57 (Table 1). For DMD, WSC and CP,the variations were 40.3 to 54.4, 10.8 to 22.2, 11.8 to 16.9, respectively. These values indicate a good variation of quality traits in alfalfa genetic resources.

Phenotypic correlations were positive and significant among DM yield with those for plant height, stem number, node number and vegetation score. Similar results have been reported for alfalfa by Jafari *et al.* (2003b), Riday and Brummer (2004). It was suggested that these yield components may be good selection criteria to improve DM yield of alfalfa. In contrast, DM yield was negatively correlated with WSC, DMD, CP and LSR. The similar negative relationships have been reported in alfalfa (among yield and quality traits) (Mueller and Orloff 1994; Julier *et al.* 2000; Rotilli *et al.* 2001). The same trends as DM yield were observed for relationships between stem number and plant height with the other traits. Both traits were positively correlated with vegetation score, ADF, DM yield and were negatively correlated with LSR, WSC and DMD. DMD had positive correlations with LSR, CP and WSC, total ash and negative correlations with CF, ADF and DM yield. These correlations are similar to those reported in other studies (Riday and Brummer 2004; Fonseca *et al.* 1999).

The factor analysis was based on the principal components extraction and varimax rotation method. Table 4 shows the contribution of different significant characteristics to each factor. The axes represented 81% of total variance for first six factors. Factor 1 accounted for 19% of variation was associated with DMD, WSC and ADF. This factor was regarded as quality factors. Factor 2 which was accounted for 17% of variation was named as productivity factor since it included DM yield, vegetation score and leaf/stem ratio. Third (CP and CF) and forth factors (plant height and node number) were important elements.

Based on ward cluster analysis, 200 entries were divided into 7 groups (Figs. 1 and 2). The average of accessions in 4 clusters was well above the overall mean for DMD, CP, WSC and total ash. The accessions in cluster 7 had higher values for DM yield and morphological traits. This cluster was ranked as moderate for quality traits. The results of this study indicated that selection of variables in productivity factor (factor 2) could enable breeders to release the desirable increment in forage yield of alfalfa.

Table 1. Results of Variance Analysis among Check Genotypes to Control Block Soil Fertility Gradients

Traits	MS
Stem No.	278.5 ns
Plant Height (cm)	104.4 ns
Node No.	3.00 ns
DM yield (Kg ha^{-1})	4465 *
Vegetation score	4.23 **
LSR	0.07 ns
DMD%	10.71 ns
CP%	7.09 ns
CF%	8.53 ns
WSC%	0.76 ns
ADF%	9.87 ns
ASH%	0.29 ns

Table 2. Parametric Statistics Results for Evaluated Traits of Alfalfa

Traits	Mean	SE mean	SD	Minimum	Maximum	Range	CV%
Stem No.	79.38	0.77	11.93	46.03	110.66	64.62	15.04
Plant Height (cm)	30.93	0.52	8.14	17.07	54.27	37.19	26.32
Node No.	8.11	0.14	2.24	3.87	14.70	10.83	27.68
DM yield (Kg ha^{-1})	1052	22.9	355.2	431.3	2094.0	1663	33.76
LSR	1.76	0.017	0.26	1.11	2.57	1.45	15.04
Vegetation score	1.73	0.06	1.02	0.24	4.9	4.68	59.41
DMD%	47.81	0.17	2.64	40.3	54.4	14.16	5.53
CP%	16.26	0.14	2.16	10.8	22.2	11.32	13.3
WSC%	14.30	0.06	0.96	11.8	16.9	5.09	6.77
CF%	34.42	0.15	2.44	28.7	40.6	11.90	7.11
ADF%	45.78	0.17	2.69	38.7	53.0	14.33	5.87
ASH%	6.32	0.02	0.38	5.2	7.4	2.23	6.05

Table 4. Factor Matrix Eigin Values after Varimax Rotation and Total Variance Explained for each Factor on 12 Traits of 200 Alfalfa Genotypes

Traits	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
DMD%	**-0.89**	0.12	0.38	-0.11	0.12	0.05
WSC%	**-0.48**	0.08	0.53	-0.14	-0.01	0.06
ADF %	**0.93**	-0.12	-0.26	0.12	-0.10	-0.04
Vegetation score	0.11	**-0.96**	-0.03	0.15	-0.03	-0.14
DM yield (Kg ha^{-1})	0.13	**-0.81**	-0.04	0.17	-0.04	-0.21
Leaf/stem ratio	-0.16	**0.50**	0.04	-0.18	0.02	0.24
CP%	-0.30	0.03	**0.94**	-0.05	-0.03	0.04
CF%	0.38	-0.01	**-0.80**	0.01	-0.27	0.01
Plant Height (cm)	0.10	-0.35	-0.04	**0.52**	0.05	-0.07
Node No.	0.16	-0.20	-0.05	**0.94**	-0.01	0.02
Ash%	-0.14	0.04	0.09	0.00	**0.99**	-0.02
Stem No.	0.05	-0.24	-0.04	-0.02	0.02	**-0.96**
Variance	2.23	2.07	2.04	1.29	1.08	1.05
% Of variance	0.19	0.17	0.17	0.11	0.09	0.09
Commutative %	0.19	0.36	0.53	0.64	0.73	0.81

The bold underlined values are the most impotent traits for each factor axes

Table 5. Trait Means used in 7 Cluster Classification

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
	n=35	n=35	n=36	n=43	n=32	n=22	**n=13**
Stem No.	82.8 b	77.8 bc	74.2 c	78.9 bc	73.0 c	81.0 b	**89.9 a**
Plant Height (cm)	33.3 c	25.8 d	31.3 c	26.2 d	22.4 e	36.8 b	**41.3 a**
DM yield (Kg ha[1])	1205 c	876 d	848 d	94 d			**174.7 a**
Vegetation score	2.00 c	1.09 d	1.24 d	1.31 d	1.05 d	2.45 b	**4.12 a**
DMD%	47.7 c	46.1 d	47.4 c	**50.9 a**	49.3 b	43.7 e	47.6 c
CP%	16.3 b	14.3 c	16.4 b	**18.6 a**	16.6 b	13.7 c	16.7 b
WSC%	14.4 bc	13.6 d	14.3 c	**15.4 a**	14.7 b	13.0 e	14.3 c
ASH%	6.35 ab	6.21 bc	6.41 ab	**6.44 a**	6.37 ab	6.03 c	6.44 a
Leaf/stem ratio	1.59 d	1.85 bc	1.91 b	1.81 c	2.11 a	1	1.45 e
Node No.	8.9 b	6.7 c	8.8 b	6.6 c	6.0 c	10.0 a	9.6 ab
CF%	34.1 bc	36.9 a	34.1 bc	31.5 d	34.2 b	37.6 a	33.3 c
ADF %	46.0 c	47.3 b	46.3c	42.9 e	44.1 d	49.7 a	46.1 c

The means of each row with the same letters had no differences based on DMRT ($P \leq 0.05$)
The bold underlined values had higher values in the relevant clusters.

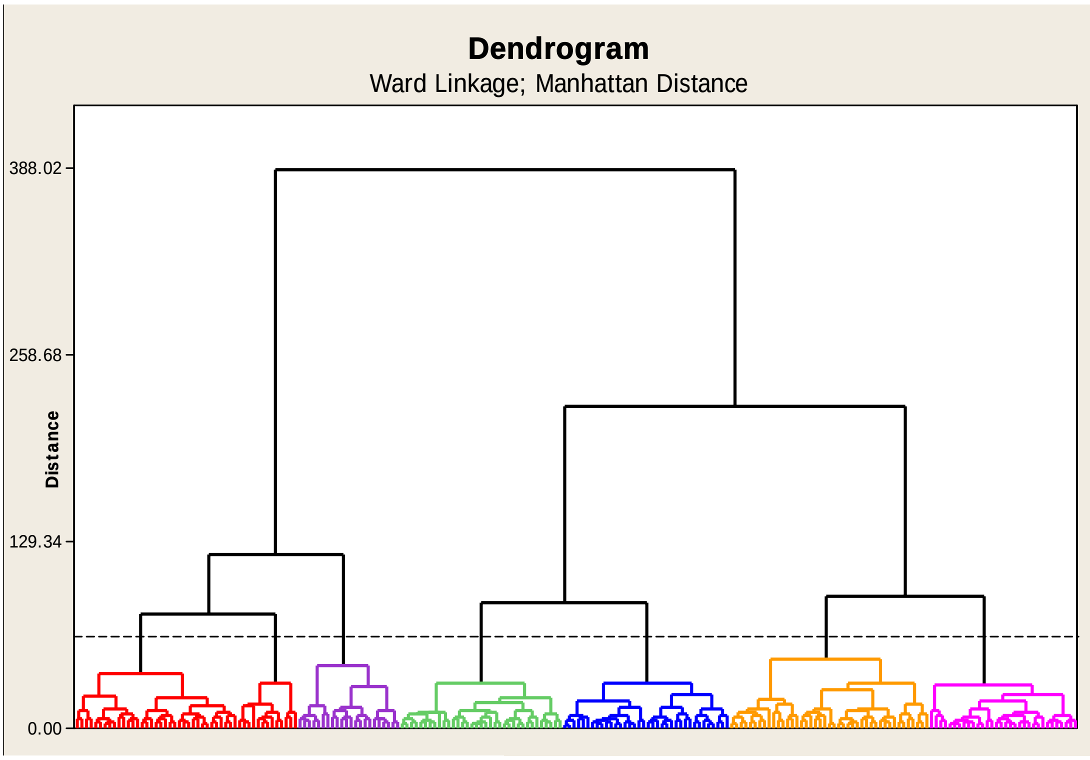

Fig. 1. Dendrogram of 200 Alfalfa Accessions by Analyzing 14 Traits using Ward Cluster Analysis Method.

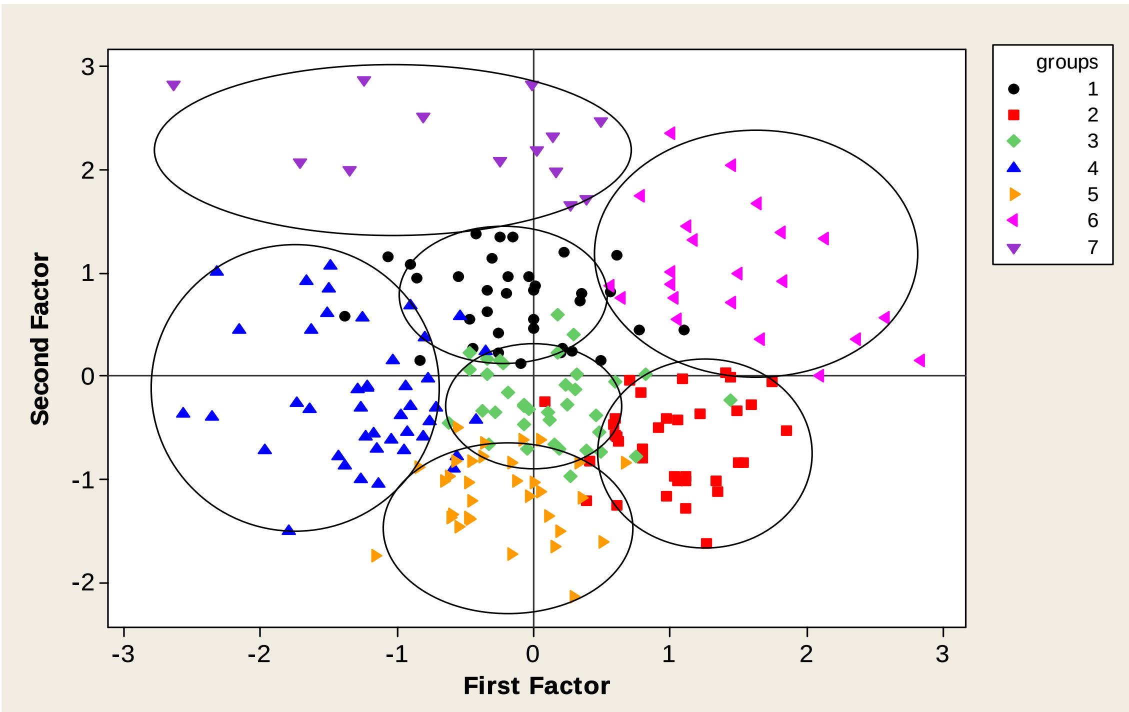

Fig. 2. Scatter Plot of 200 Alfalfa Accessions for First Two Factor Axes

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