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Genetic Variability among Lucerne Cultivars Based on Biochemical (SDS-PAGE) and Morphological Markers

[1]M. Farshadfar and [2]E. Farshadfar
[1]Kermanshah Research Centre of Agriculture and Natural Resources, Payame Noor University, Iran
[2]College of Agriculture, Razi University of Kermanshah, Iran

Abstract: The present research was conducted to determine the genetic variability of 18 Lucerne cultivars, based on morphological and biochemical markers. The traits studied were plant height, tiller number, biomass, dry yield, dry yield/biomass, dry leaf/dry yield, macro and micro elements, crude protein, dry matter, crude fiber and ash percentage and SDS- PAGE in seed and leaf samples. Field experiments included 18 plots of two meter rows. Data based on morphological, chemical and SDS-PAGE markers were analyzed using SPSSWIN soft ware and the multivariate statistical procedures: cluster analysis (UPGMA), principal component. Analysis of analysis of variance and mean comparison for morphological traits reflected significant differences among genotypes. Genotype 13 and 15 had the greatest values for most traits. The Genotypic Coefficient of Variation (GCV), Phenotypic Coefficient of Variation (PCV) and Heritability (Hb) parameters for different characters raged from 12.49 to 26.58% for PCV, hence the GCV ranged from 6.84 to 18.84%. The greatest value of Hb was 0.94 for stem number. Lucerne genotypes could be classified, based on morphological traits, into four clusters and 94% of the variance among the genotypes was explained by two PCAs: Based on chemical traits they were classified into five groups and 73.492% of variance was explained by four principal components: Dry matter, protein, fiber, P, K, Na, Mg and Zn had higher variance. Genotypes based on the SDS-PAGE patterns all genotypes were classified into three clusters. The greatest genetic distance was between cultivar 10 and others, therefore they would be suitable parent in a breeding program.

Key words: Cluster analysis, lucerne, SDS-PAGE, variability

INTRODUCTION

Plant genetic resources for food and agriculture are the basis of global food security. They comprise diversity of genetic material contained in traditional varieties, modern cultivars, crop wild relatives and other wild species. To meet the need for more food, it will be necessary to make better use of a broader range of the world's plant genetic diversity. The concept of germplasm conservation demands that collection methods initially capture maximum variation and subsequently, conservation and regeneration techniques minimize losses and genetic erosion through time (Astly, 1992; Rao *et al*., 2003; Kimbeng and Bingham, 1998). Genetic variability has been estimated based on morphological, agronomical, chemical, molecular (DNA, protein, isozymes), cytogenetic and molecular cytogenetic markers (Singh, 1993; Dehghan-Shoar *et al*., 1997). Traditionally, diversity is assessed by measuring variation in phenotypic traits, which are of direct interest to users. The genetic information provided by morphological characters is often limited and expression of quantitative traits is subjected to strong environmental influence. In the 1960s, biochemical methods based on seed protein and enzyme electrophoresis were introduced, which proved particularly useful in analysis of genetic diversity as they reveal differences between seed storage proteins or enzymes encoded by different alleles at one (allozymes) or more gene loci (isozymes). Use of biochemical methods eliminates the environmental influence; however, their usefulness is limited due to their inability to detect low levels of variation. DNA-based techniques are being used as complementary strategies to traditional approaches for assessment of genetic diversity, the major advantage being that they analyzed the variation at the DNA level itself, excluding all environmental influences. The analysis can be performed at any growth stage using any plant part and it requires only small amounts of material (Hodgkin *et al*., 2001; Krishnan and Sleper, 1997; Xiong *et al*., 2007; Przybylska, 1995; Robins *et al*., 2007).

Lucerne (*Medicago sativa* L.) with high protein content has excellent stock acceptance and produces impressively yield of high quality feed. It is a multi-purpose plant. *Medicago* genus has high variation for

Corresponding Author: Dr. Mohsen Farshadfar, Payame Noor University, Shahid Beheshti Bol. Keramanshah, Iran

different quality and quantity traits. Gene pool of medics includes land races, mutants, cultivars and wild types. It includes about 34 annul and 51 perennial species. This variability helps breeders to release improved varieties (Small and Jomphe, 1988; Tecle *et al*., 2006).

Alfalfa plant parts have different nutrient concentrations. Differences of exist among leaves according to their position on the plant. The variation in environmental conditions will influence nutrient concentrations in forage, because of changes in rate of dry matter production, ion movement in soil, root activity and the uptake of nutrient by the plant (Grabber, 2005). Variation in yielding and chemical composition: (crude protein crude cellulose, P, K, Ca and Mg rate and total non structural carbohydrates content has been reported be many authors (Jung and Engles, 2002; Lamb *et al*., 2007). Improvement in forage quality has been accomplishes mostly by selecting for higher crude protein (Pillips *et al*., 1982; Teuber and Pillips, 1988) or lower fiber concentrations (Coors *et al*., 1986; Sumberg *et al*., 1983). Breeding for NDSF has been considered an alternative strategy for developing alfalfa with superior quality characteristics (Jung and Lamb, 2006).

Current selection procedures often include feeding value characters (digestibility and fiber content) to improve the energy value of alfalfa forage. Genetic variation among cultivars for digestibility or fiber contents has been described (Buxton *et al*., 1987; Lenssen *et al*., 1991; Julier *et al*., 1996; Julier and Huyghe, 1997). Hebert *et al*. (1994) observed increasing environmental stresses revealed more variability among annual medic. They showed that biotic stresses exploited more diversity of different traits in annual medics. Martiniello *et al*. (1994) analyzed phenotypic variation among 54 lucerne population of *Medicago arborea*. They estimated high variability for dry weight, plant height and leaf to stem ratio. Vitale *et al*. (1998) analyzed genetic variability and rate outcrossing in *Medicago polymorpha* L. accessions by RAPD markers.

This investigation was carried out to determine the genetic variability of Lucerne varieties based on different agro-morphological, chemical and biochemical traits.

MATERIALS AND METHODS

To study genetic variability among 18 Lucerne cultivars selected from the gene bank of Research Institute of Forest and Rangelands of Iran. This research was carried out in Kermanshah Research Centre of Agriculture and Natural Resources in 2005. Field experiments included 18 plots of two meter rows. The traits studied were plant height, tiller number, biomass, dry yield, dry yield/biomass, dry leaf/dry yield, macro and micro elements, crude protein, dry matter, crude fiber and ash percentage and SDS-PAGE in seed and leaf samples. Data based on morphological, chemical and SDS-PAGE markers were analyzed using SPSSWIN soft ware and the multivariate statistical procedures: cluster analysis (UPGMA), principal component. The Genotypic Coefficient of Variation (GCV), Phenotypic Coefficient of Variation (PCV) and Heritability (Hb) parameters were estimated for different characters.

Cultivar No.	Cultivar name
1	Anzal-OROMIE
2	Yazdi22057
3	Garayonje-Ahar
4	Nushin-Oromie
5	Razan-Hamadan
6	Alborz
7	Nushin2-Oromie
8	Bonab
9	Turkeyun2122
10	Turkey
11	Garayonje2-Korest
12	Alborzun320
13	Hamadani
14	Simerchenska-21-Kazak
15	Simerchenska-Kazak
16	Nazlo-Oromie
17	Nikshahri
18	Hamadani-Khoram

RESULTS

The results of analysis of variance for morphological traits are shown in Table 1. There are significant differences between cultivars for plant height, biomass, tiller numbers, dry matter, leaf/biomass and leaf/stem. Mean comparison based on Duncan Multiple Range Test (DMRT) was done (Table 2). Cultivar 13 and 15 were greatest values for most traits, thereby indicating the existence of genetic diversity between the genotypes.

The Genotypic Coefficient of Variation (GCV), Phenotypic Coefficient of Variation (PCV) and Heritability (Hb) raged from 12.49 26.58% for PCV, hence the GCV ranged from 6.84 to 18.84%. The greatest value of Hb was 0.94 for stem number (Table 3).

Table 1: Analysis of variance of morphological characters

		Mean square					
SOV	df	Leaf/ stem	Leaf/ biomass	Dry weight	Fresh weight	Stem No.	Height
Genotype	17	0.181*	0.005*	235.127*	4062.338*	408.145**	83.927**
Error	17	0.073	0.002	76.373	1382.941	199.124	25.756

*, **Significant differences at 0.05 and 0.01 probability level, respectively

Table 2: Mean comparison among Lucerne genotypes based on Duncan Multiple Range Test (DMRT)

Genotype	Height	Stem No.	Fresh weight
Hamadani	75.76A	104.50A	286.3A
Turkey un2122	68.07AB	77.83ABCDE	241.8ABC
Simercheska	68.01AB	104.0A	280.3A
Nikshahri	66.88AB	55.00DE	170.6CD
Yazdi2	64.16BC	88.17ABCD	228.5ABC
Nazlu	63.65BC	84.33ABCDE	229.5ABC
Anzal	61.70BCD	80.67ABCDE	215.2ABCD
Garayonjeh	59.40BCD	91.17ABC	207.8ABCD
Nushin	59.42BCD	92.40ABC	210.8ABC
Alborz unknown	57.33BCD	92.50ABC	196.1ABCD
Gharaah youn2	62.23BCD	70.00ABCDE	216.8ABCD
Alborz un320	60.74BC	94.50AB	273.7AB
Simercheska2	57.22BCD	79.67ABCDE	186.3BCD
Hamadani korama	60.35BCD	70.50ABCDE	179.0CD
Bonab	53.53CD	59.50CDE	155.8CD
Razan	52.22D	52.33E	132.1D
Nushin2	51.76D	68.17BCDE	154.8CD
Turkey	51.39D	74.67ABCDE	157.5CD
	Dry weight	**Leaf/biomass**	**Leaf/stem**
Anzal	57.28ABCDE	0.595ABCD	1.565ABCD
Yazdi2	59.49ABCD	0.565ABCDE	1.355BCDE
Garayonjeh	56.15ABCDE	0.620AB	1.725ABC
Nushin	58.42ABCDE	0.600ABCD	1.550ABCDE
Razan	37.26E	0.615ABC	1.740ABC
Alborz unknown	55.73ABCDE	0.560ABCDE	1.365ABCDE
Nushin2	46.56CDE	0.590ABCD	1.545ABCDE
Bonab	42.14DE	0.615ABC	1.895AB
Turkey un2122	65.18ABC	0.460E	0.895E
Turkey	43.58DE	0.665A	2.015A
Gharaah youn2	57.02ABCDE	0.540BCDE	1.250BCDE
Alborz un320	68.40AB	0.600ABCD	1.555ABCDE
Hamadani	76.95A	0.510CDE	1.095CDE
Simercheska2	49.49BCDE	0.565ABCDE	1.320BCDE
Simercheska	73.48A	0.565ABCDE	1.350BCDE
Nazlu	60.15ABCD	0.505DE	1.025DE
Nikshahri	46.17CDE	0.520BCDE	1.105CDE
Hamadani korama	48.63BCDE	0.560ABCDE	1.325BCDE

The values with at least a similar word has no significant differences at 5% of probability level

Table 3: Genetic parameters (genotypic coefficient of variation, phenotypic coefficient of variation and heritability) for morphological traits

Genetic parameters	Height	Stem No.	Fresh weight	Dry weight	Leaf/ biomass	Leaf/ stem
PCV (%)	14.25	21.82	26.58	23.71	12.49	28.88
GCV (%)	9.67	18.84	18.52	18.34	6.84	18.51
Hb	0.81	0.94	0.82	0.89	0.68	0.80

Genetic distance: Cluster analysis was done to measure genetic distance between 18 different Lucerne cultivars applied based on agro-morphological traits. All 18 Lucerne cultivars could be classified into four clusters. Cluster one consisted of cultivars (3, 4, 1, 2, 6, 14, 18 and 11), cluster two included cultivars (9, 16 and 17), cluster three consisted of cultivars (12, 15 and 13) and cluster four included cultivars 5, 7, 8 and 10 (Fig. 1). To determine the relative values of traits in diversity, the Principle Component Analysis (PCA) was used. The 94% of variance among the cultivars were explained by two PCAs (Table 4). The plant height, tiller numbers, biomass and dry weight had highest value with 0.926, 0.707, 0.934 and 0.934, respectively (Table 5).

Based on the chemical component traits, 18 Lucerne cultivars were classified into five groups. First cluster contained 14 cultivars and other clusters included one cultivar (Fig. 2). The greatest genetic distance was between cultivar 10 and others. The principle component analysis exploited that 73.492% of variation were explained by four principle components (Table 6). Dry

Table 4: Principal component analysis based on morphological traits

Principal component	Eigen value	Variance (%)	Cumulative variance (%)
PCA1	4.242	70.692	70.692
PCA2	1.452	24.204	94.896

Table 5: Relative values of morphological traits explained by two principle components

Genetic parameters	Height	Stem No.	Fresh weight	Dry weight	Leaf/ biomass	Leaf/ stem
PCA1	0.926	0.708	0.921	0.934	-0.740	-0.784
PCA2	-0.152	0.649	0.341	0.337	0.656	0.590

Table 6: Principal component analysis based on chemical traits

Principal component	Eigen value	Variance (%)	Cumulative variance (%)
PCA1	3.678	28.294	28.294
PCA2	2.801	21.548	49.842
PCA3	1.817	13.977	63.819
PCA4	1.257	9.674	73.492

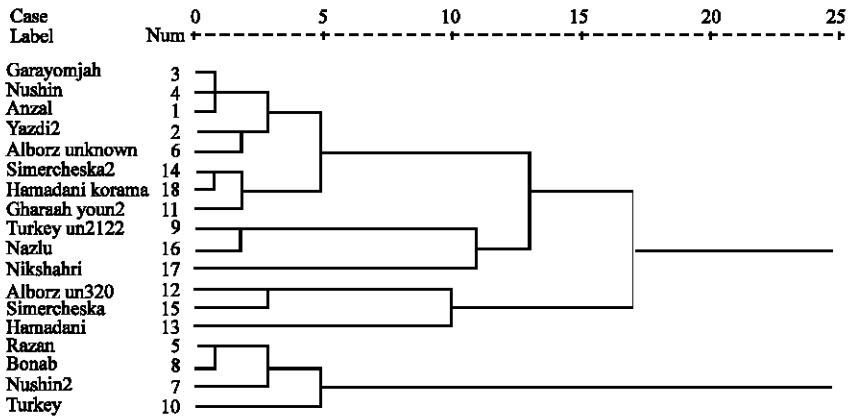

Fig. 1: Hierarchical dendrogram of morphological traits

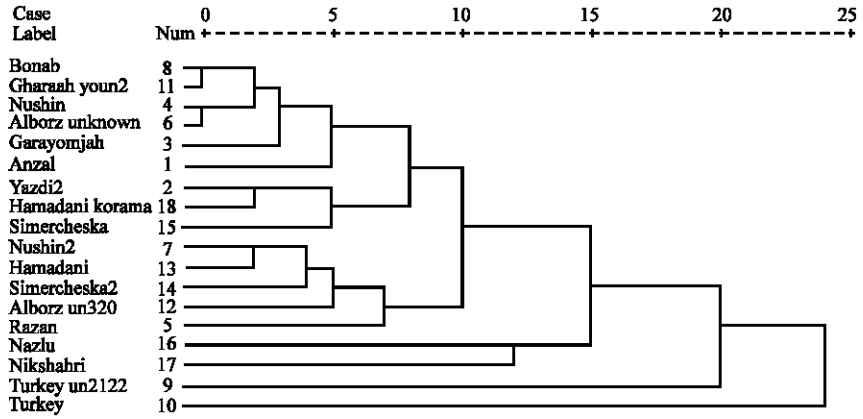

Fig. 2: Hierarchical dendrogram of chemical components

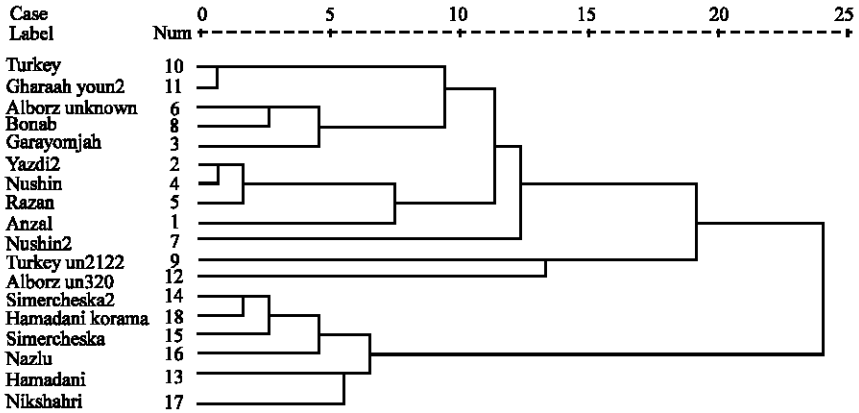

Fig. 3: Hierarchical dendrogram of seed protein in alcohol

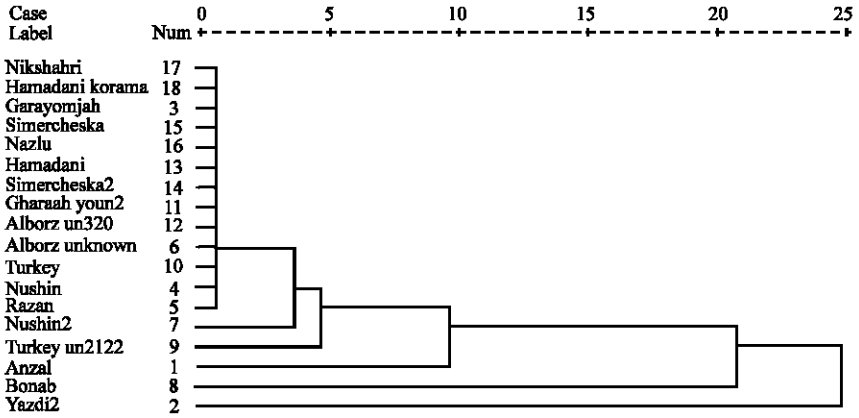

Fig. 4: Hierarchical dendrogram of leaf protein in salt

matter, protein, fiber, P, K, Na, Mg and Zn had higher variance value (Table 7).

Genetic diversity based on SDS-PAGE markers was measured. JACCARD method was used based on running bands obtained by protein electrophoresis. Classification results derived from seed protein patterns (soluble in alcohol) were shown in Fig. 6. All cultivars were classified into 3 groups. Cluster one contained 10 cultivars, cluster two consisted of two and cluster three included six cultivars (Fig. 3).

Table 7: Relative values of chemical traits based on principal component analysis

Principal component	Dry matter (%)	Protein (%)	Fiber (%)	Ash	Ca	P	K	Na	Mg	Cu	Mn	Zn	Fe
PCA1	-0.112	0.805	0.753	0.00	-0.16	0.75	0.86	0.53	0.462	0.19	0.21	0.65	0.34
PCA2	-0.708	0.000	0.370	0.93	0.51	-0.39	-0.11	0.12	-0.28	0.49	0.58	0.12	0.43
PCA3	-0.401	-0.170	0.215	0.00	-0.38	0.00	-0.26	-0.61	0.36	0.52	0.47	0.31	0.53
PCA4	-0.174	-0.301	0.368	0.17	0.53	0.31	0.16	0.11	-0.33	0.43	0.00	0.46	0.22

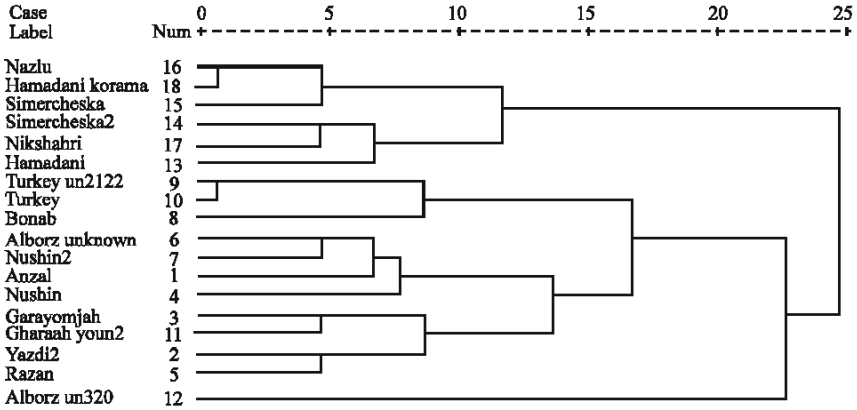

Fig. 5: Hierarchical dendrogram of seed protein in salt

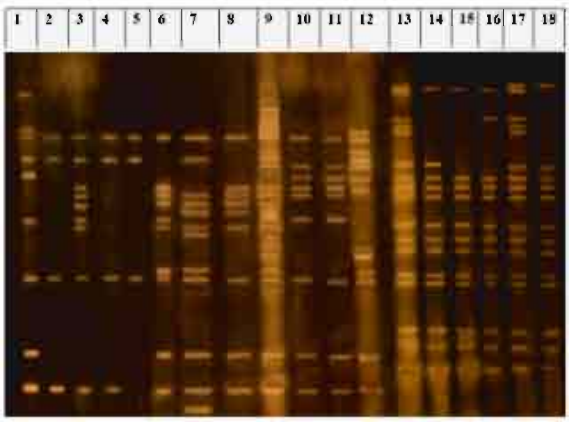

Fig. 6: Seed protein in alcohol SDS-PAGE

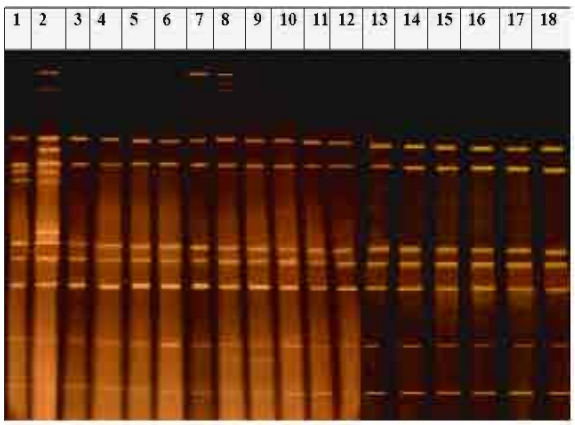

Fig. 7: Seed protein in salt SDS-PAGE

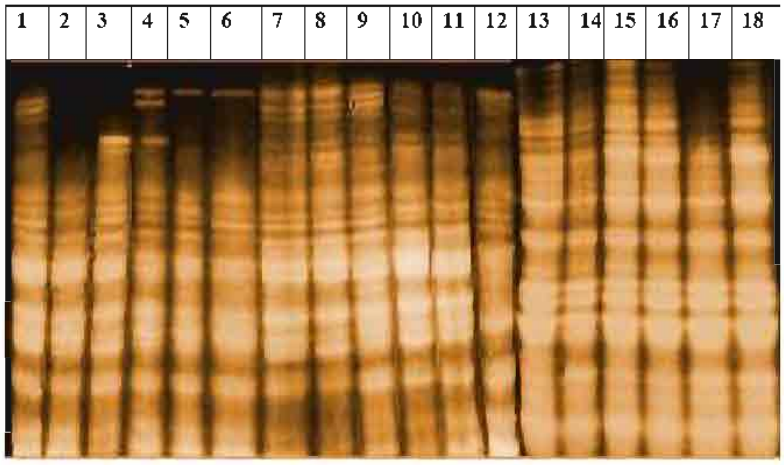

Fig. 8: Seed protein in salt SDS-PAGE

Based on leaf protein (glutamine soluble in salt) electrophoresis (Fig. 7), all cultivars were placed into three clusters (Fig. 4). The cultivars had more similar patterns by this method. SDS-PAGE for glutamine protein of seed sample was done (Fig. 8). The cultivars were grouped into three clusters (Fig. 5). Group one contained of six cultivars, group two included 11 cultivars and cluster three has one member. Based on these SDS-PAGE markers, cultivar 12 was separated cluster and cultivars 13, 14, 15, 16, 17 and 18 were located in same cluster. These cultivars with high variation are desirable parents in a breeding program.

DISCUSSION

Distance analysis, a multivariate statistical technique based on the measurement of several metric characters, is an efficient method for evaluating genetic divergence or distance. Several reports on the application of distance analysis to the selection of parents in different crops have been published. In recent years, much attention has been devoted to this activity, which has usually been referred to as classification or cluster analysis and the methodology has been applied in many diverse disciplines (Farshadfar, 1997; Rao, 1983; Riday *et al*., 2003).

The aim of Lucerne breeding is cultivar released with high forage yield and high protein content (Riday and Brummer, 2002; Marquiez Ortiz *et al*., 1996, Fonseca *et al*., 1999). The cultivar 13 and 15 had high yield and protein content. These cultivars are rich gene pool for breeders. They can be introduced as new varieties or were applied in crossing program. One hundred and twenty two Lucerne genotypes selected from American gene bank were evaluated by Bauchan *et al*. (1993). They classified the samples and finally core collection was selected to use in breeding programs.

Genetic parameters estimation is the basic analysis in a breeding program. Simple, phenotypic and additive genetic correlation coefficient were estimated from Half-Sib (HS) progeny tests of two alfalfa populations. In both populations, NDSF was negatively correlated with Acid Detergent Fiber (ADF), lignin and Neutral Detergent Fiber (NDF) and positively correlated with true *in vitro* Dry Matter Digestibility (IVDMD). Vigor was positively correlated with ADF, lignin and NDF and negatively correlated with IVDMD and Crude Protein (CP) only in NY9515 (Smith, 1990). He suggested that Middle Eastern alfalfa ecotypes had better productivity than elite nondormant cultivars, partly because the Middle Eastern ecotypes produced a higher proportion of crown shoots than leaf auxiliary shoots. Hebert *et al*. (1994) found great PCV for dry weight and height in 54 *Medicago arborea*. Suleyman (2002) studied yield component, morphology and forage quality of native alfalfa ecotype. Schnurr *et al*. (2007) studied different Morphology, Chemical Composition traits of Alfalfa and *Medicago truncatula*. They explained that younger stem internodes (top third of the stem) of both species had a higher protein concentration and greater cell wall polysaccharide digestibility and lower cell wall concentration than older internodes (bottom third of stem). Based on the data presented, it appears that *M. truncatula* is a suitable model for stem development, composition and digestibility of alfalfa.

Within-cultivar variance accounted for 31 to 70% of the genetic variance for LSR and quality traits and 57 to 100% for morphological traits and dry matter yield. Large within-cultivar variation for yield-related traits could impart yield stability across environments, as a result of competition in alfalfa canopies (Julier *et al*., 2000; Julier and Huyghe, 1997). Jung and Lamb (2006) compared

alfalfa clones identified as either low or high rapid (16 h), or low or high potential (96 h) stem *in vitro* Neutral Detergent Fiber Digestibility (IVNDFD) for stem detergent fiber, cell wall and morphology traits. They concluded, that the low rapid and high potential IVNDFD groups had longer stem and inter node lengths than their corresponding groups. Ghanavati *et al.* (2005) studied genetic diversity of 54 population from 22 species *Medicago* based on RAPD-PCR markers. They analyzed phylogenetic tree with Maximum Parsimony method with 11 UBC random primers. Result of that study showed that RAPD marker are applicable as a complementary tool in taxonomic identification of genus Medicago at both species and population levels. Vitale *et al.* (1998) studied RAPD markers to reveal the rate of out crossing in burr medic.

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