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Genetic divergence across the cutting management in Alfalfa (*Medicago sativa* L.)

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Alfalfa (*Medicago sativa* L.), a forage legume, commonly known as Lucerne is a member of family Leguminosae, tribe Trigonelleae, sub- family Papilionaceae. The species by and large is tetraploid (2n=4x=32) and insect pollinated in nature. This is multicut perennial forage legume. Alfalfa is the third important forage crop in India after Sorghum (*Sorghum bicolor* L.) and Berseem (*Trifolium alexandrinum L.*). It produces more protein per ha than grain or oilseed crops. The average protein yield of alfalfa is more than twice to that of soybean (Tyagi, 1997; Barnes *et al*., 1988). The variability accomplished with significant genetic distance among different accessions of a species is known as genetic diversity or genetic divergence. Genetic diversity plays an important role in plant breeding because hybrids between lines of diverse origin generally display a greater heterosis than those between closely related accessions (Ram and Panwar, 1970). The maximum heterosis generally, however, occurs at an optimal or intermediate level of diversity. The D^2 technique (Mahalanobis, 1936) has been used in assessing the variability present in crops, like maize, sorghum, pearl millet, wheat, linseed, cotton, tobacco, alfalfa and brassica (Moll and Stuber, 1974). In addition to it in the selection of divergent parents for hybridization, D^2 statistics measures the degree of diversification and relative proportion of each component character to the total divergence. The present investigation was carried out to ascertain magnitude of genetic divergence present in the alfalfa accessions with the objectives to develop high fodder yielding materials.

Materials used in present study consisted of eighty one accessions of alfalfa (*Medicago sativa* L.) collected from different parts of Rajasthan and Gujarat including one standard check, RL88. The materials were grown in 9x 9 double lattice designs in November 2003 in a plot size of 4m X 0.5m accommodating one row of one strain of 4m length at 50 cm distance. The plots were in a continuous fashion. One border row as a non experimental row was planted at the beginning and at the end of the each block in order to minimize uncontrolled variation. A plant- to- plant distance of 15-20 cm was kept. Standard agronomical practices were followed as per recommendations to raise the crop. The data on twelve quantitative traits *viz*, plant height (cm), number of primary basal branches, stem girth at crown region, number of nodes, internode length of main shoot, plant spread, leaf weight, stem weight, leaf stem ratio, green forage yield per plant, dry matter yield per plant and protein content were recorded regularly on five selected plants per plot. The total cuts were seven at about one month interval. The data pooled over the cuts were then subjected to Mahalanobis D^2 analysis using SPAR 2.0.

Results for diversity analysis indicated that eighty one accessions were grouped into 12 clusters based on pooled analysis of seven cuts. The distribution pattern indicated that number of accessions varied from cluster containing 03 to 11 accessions in pooled analysis. Similarly, accessions belonging to one location were also grouped in different clusters. Out of the twelve clusters formed of which eleven accessions were grouped into cluster IV followed by cluster X which had ten accessions. While cluster I,II III,V,VI,VII,VIII and IX comprised of seven, six, five, seven, eight, eight, six, six accessions respectively. The smallest clusters XI and XII had three and four accessions, respectively, (Table-1). Genetic diversity is generally associated with geographical diversity but in the present study former is not directly related to geographic distribution. The accessions within the same clusters were originated from different geographical region, which indicated that geographical distribution and genetic divergence did not follow the same trend. Similar results was reported by Cruz *et al*., (1994) in alfalfa. Thus geographical diversity although important was not the only factor responsible in determining the genetic diversity (Shukla and Singh, 2002). The grouping of accessions originating from different eco-geographical regions into

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Table 1: Cluster pattern of eighty one genotypes of alfalfa.

Cluster	No. of accessions	Accessions
I	7	IL-03-03, IL-03-04, IL-03-06, IL-03-07, IL-03-42, V54, IL-2000-176
II	6	IL-2000-128, IL-2000-62, IL-2000-60, IL-2000-77, IL2000-172, IL-2000-117
III	5	IL-03-13, IL-03-22, IL-03-25, IL-03-28, IL-2000-154
IV	11	IL-03-14, IL-03-19, IL-03-24, IL-03-31, IL-03-40, IL-03-41, IL-03-44, IL-03-48, IL-03-50, IL-03-52, IL-03-53
V	7	IL-03-02, IL-03-08, IL-03-15, IL-03-34, IL-03-35, IL-03-43, IL-2000-66
VI	8	IL-03-01, IL-03-117, IL-03-18, IL-03-45, IL-03-55, IL-2000-98, IL-2000-96, IL-2000-87
VII	8	IL-03-20, IL-03-21, IL-03-30, IL-03-38, IL-03-49, IL-03-51, IL-2000-141, IL-2000-102
VIII	6	IL-03-26, IL-03-27, IL-03-32, IL-03-33, IL-03-03-37, IL-03-47
IX	6	IL-03-05, IL-03-09, IL-200-147, IL-2000-157, IL-2000-68, IL-2000-118
X	10	IL-03-10, IL-03-16, IL-03-23, IL-03-29, IL-03-36, IL-03-46, IL-2000-126, IL-2000-174, IL-2000-105, IL-2000-149
XI	3	IL-03-11, IL-03-12, IL-03-49
XII	4	IL-2000-113, IL-2000-155, IL-2000-76, RL-88

Table 2 : Average intra and inter cluster D^2 values among eighty- one accessions of alfalfa based on mean of seven cutting environments.

Cluster	Plant height (cm)	No. of primary branches / plants	Number of nodes / plant	Inter-node length (cm)	Stem girth (mm)	Plant spread (cm)	GFY/ plant (gm)	L.S. ratio/ plant (gm)	Leaf weight /plant (gm)	Stem weight plant (gm)	DMY/ plant (gm)	Protein content (gm)
I	57.48	7.60	12.94	5.97	0.39	22.07	32.56	1.70	20.22	12.44	9.85	28.41
II	60.61	7.26	13.36	6.01	0.42	22.40	39.98	1.74	24.69	15.07	11.69	26.03
III	57.83	6.94	13.47	5.91	0.44	21.49	36.33	1.70	22.40	13.72	10.68	23.36
IV	58.01	6.92	13.31	5.89	0.40	21.49	32.49	1.68	20.16	12.38	9.99	25.28
V	60.38	7.26	13.29	6.29	0.40	22.34	34.16	1.76	21.39	12.84	10.24	22.46
VI	59.09	7.27	12.98	5.91	0.40	21.90	34.80	1.58	21.01	13.83	10.46	23.41
VII	59.49	6.79	13.57	6.23	0.39	21.52	32.38	1.69	20.15	12.38	9.79	23.91
VIII	60.38	6.84	13.17	6.09	0.38	21.18	34.58	1.67	21.34	13.25	10.77	25.14
IX	56.94	7.17	12.73	5.64	0.41	22.04	35.45	1.77	22.18	13.22	10.75	22.02
X	59.34	7.08	13.13	5.99	0.39	21.48	36.18	1.78	22.63	13.49	10.72	23.76
XI	58.19	7.22	13.55	6.02	0.43	21.04	31.05	1.72	19.48	11.55	9.41	21.08
XII	60.15	7.50	13.36	5.82	0.42	21.84	37.80	1.75	23.78	14.03	11.06	29.42

GFY= green fodder yield, DMY= dry matter yield

one cluster could be attributed to frequent exchange of genes and due to operation of similar force of natural and artificial selections resulting in perpetuation and stabilization of similar accessions (Murty and Arunachalam, 1966; Shukla and Singh, 2002). The average intra and inter cluster distance are presented in Table- 2. The intra-cluster distance was relatively smaller than inter-cluster distance indicating homogeneous nature of the groups and presence of narrow genetic variation with in a cluster in all the cuttings. Somayajulu *et al*. (1970) reported that the clustering based on lesser divergence has revealed instability, whereas the widely diverged clusters remained distinct in different environment. The main objective of forming clusters and to find out the intra and inter-cluster distance is to provide relevant information for selection of diverse parents for hybridization programme involved in the crosses (Reday and Brummer, 2002). Large inter-cluster distances signify that the accessions grouped in a cluster are different from the accessions of other clusters for one or more characters, which made them so divergent from others. Based on pooled D^2 analysis, the average intra-cluster value varied from 1.88(XII) to 2.54(V), whereas inter cluster values ranged between 2.52 (II and XII) to 7.8 (II and XI) indicating considerable diversity between the clusters. Apart from distribution of entire materials into 12 clusters based on mean of observations recorded over seven cuttings for 12 quantitative characters, the clusters were regrouped into high distant, medium and less distant cluster pair groups. In total 66 cluster pairs were constructed out of 12 clusters. In the highly distant group wherein magnitude of Inter-cluster distance was recorded to be 5, ten cluster pairs have been noticed namely cluster pairs I&II, I&VIII, I&XII, II&IV, II&VII, II&XI, VI&XI, VIII&XI, X&XI

and XI&XII. Selection of parents for hybridization is advocated from this group. The use of accessions in hybridization from these clusters having most of the desirable characters is likely to produce more transgressive segregants. The D^2 analysis further indicated that high variation for various yield contributing traits had maximum contribution towards genetic divergence.

Results obtained from this study indicated that the actual D^2 values among the accessions rather than inter-cluster D^2 values should also be considered because the clustering pattern on the basis of D^2 statistics is arbitrary. The hybridization among diverse parents is likely to produce heterotic hybrids and desirable transgressive segregants in further generations and hence the accessions with better mean values should be used for still better success in the breeding programmes. Rana and Murthy (1971), Sindhu and Singh (1975) and Singh and Gupta (1979) reported the classification according to D^2 analysis as subjective, firstly because of the cluster formation methods, secondly sometimes genetically related accessions may be grouped into different cluster and vice- versa and thirdly, the number and composition of clusters varies greatly under the influence of cutting environments.

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