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Research article

Genetic diversity studies in local collections and advanced generations of lucerne (*Medicago sativa* L.) genotypes for fodder yield and yield-attributing traits

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Abstract

Precise information about genetic divergence is vital for a productive breeding program, as genetically diverse parents produce high heterotic effects, producing desirable segregants with higher yield levels. This study assessed seventy genotypes and four checks to estimate the extent of genetic diversity based on ten morphological traits attributed to yield. The genotypes showed significant differences for all the traits considered. Based on the Mahalanobis D^2 statistics, the genotypes were grouped into ten clusters, with cluster I having the maximum number of genotypes, whereas cluster IX had a single genotype. The average inter-cluster distances revealed that the genotypes in clusters VI and VII were more diverse. In contrast, clusters I and III genotypes had the shortest inter-cluster distances, indicating genetic closeness. The study revealed a broad genotypic diversity within and between the alfalfa germplasm collections. The genotypes in clusters I, III, and V provide excellent genetic material for alfalfa yield improvement. They offer a possible way to exploit the existing variability to develop superior populations or composites.

Keywords: Composites, Diversity, Multivariate analysis, Variability

Introduction

Cultivated lucerne (*Medicago sativa* L.), also called alfalfa, is an important leguminous fodder crop and a natural autotetraploid (2n = 4x = 32). It is grown all over the world due to its high nutrient content (protein 16–25% and fiber 20–30%), yield potential (90–110 q ha^{-1} $year^{-1}$), ability to grow in a variety of soil conditions, and can withstand adverse climatic conditions. The lucerne crop is characterized by high cross-pollination, high inbreeding depression, self-incompatibility, and complex inheritance patterns. For the above reasons, commercial alfalfa cultivars are most commonly used to develop composite populations by inter-mating superior selected genotypes over several generations. In order to optimize the utilization of germplasm resources, it is essential to gather information regarding the extent and distribution of genetic diversity, as well as the interrelationship among materials within a breeding program (Musial *et al*., 2002; Karuri *et al*., 2010; Tucak *et al*., 2010). Therefore, the selection of genetically diverse material is critical for improving yield and quality in lucerne. Several multivariate techniques, *viz*., clustering methods, principal component analysis, and canonical variables, are commonly used to determine genetic diversity in different crops (Alom *et al*., 2003; Mohan and Seetharam, 2005; Naghavi and Amirian, 2005; Dias *et al*., 2009; Thul *et al*., 2009). The selection of methods should be based on desired precision, data collection methodology, and ease of analysis (Rangel *et al*., 1991).

Cluster analysis is a multivariate method that aims to classify the genotypes into clusters based on the similarity or dissimilarity between the characters (variables), so the magnitude of the association will be substantial between genotypes of the same cluster and weak between genotypes of different clusters. The cluster analysis can be performed using a measure of similarity levels and Euclidean distance (Mecha *et al*., 2017). The objective of this study was to use multivariate analysis to assess genetic diversity in alfalfa germplasm collections to determine the extent of genetic diversity and the contribution of selected characters to total diversity, as well as to identify the most promising genotypes that

could contribute to the improvement of yield and quality of alfalfa in further breeding programs.

Materials and Methods

Study site and experimental design: The experiment was conducted during *rabi* 2020-21 at SRRS, ICAR-IGFRI, Dharwad, which is situated at 15º26′ N latitude and 75 º07′ E longitude at an altitude of 678 m above mean sea level. It comes under the northern transitional tract (zone 8), between the western heavy rainfall and eastern low rainfall areas. The soil of the experimental site contained organic carbon (0.48%), available N, P and K around 235.2, 7.42 and 369.4 kg/ha, respectively, with soil pH 7.81 and EC 0.007. The experiment was conducted in randomized complete block design (RCBD), where each line was accommodated in a 4 x 4 sq. m plot size containing 13 rows of 4 m length with spacing of 30 x 10 cm with two replications. A recommended package of practices was followed to raise a good crop stand.

Experimental material and observations recorded: The experimental material comprised 70 genotypes of which 20 are local populations and 50 are F_3 progenies from eleven different crosses, along with four checks *viz*., Anand-2, Alamdar-55, RL-88 and CO-4 (Table 1). The checks were selected based on their agronomic performances and suitability to the region's agro-climatic conditions. The plants were harvested twice in the year of establishment (February and April 2021). The data obtained from both harvests was pooled and analyzed. Observations were recorded on five randomly selected plants from each line for ten morphological traits *viz*., days to first flowering (DFF), days to 50% flowering

Table 1. List of local populations and F3 progenies of lucerne

S. No	Genotypes	Pedigree	Source	Code
1	05 lines	Local collections	Maharashtra	G1, G2, G3, G4, G5
2	15 lines	Local collections	Rajasthan	G6, G7, G8, G9, G10, G11, G12, G13, G14, G15,G16,G17,G18,G19,G20
3	F3 progeny	RL-88 x Weevil check	IGFRI, SRRS, Dharwad	G23
4	F3 progeny	Anand-2 x Weevil check (2 lines)	IGFRI, SRRS, Dharwad	G36, G55
5	F3 progeny	Crau x RL-88 (2 lines)	IGFRI, SRRS, Dharwad	G69, G26
6	F3 progeny	Dharwar-1x Crau (2 lines)	IGFRI, SRRS, Dharwad	G31, G68
7	F3 progeny	RL-88 x Maris Kabul (2 lines)	IGFRI, SRRS, Dharwad	G59, G48
8	F3 progeny	RL-88 x Dry Lander Alfalfa (3 lines)	IGFRI, SRRS, Dharwad	G67, G37, G34
9	F3 progeny	Anand-2 x Vernal (4 lines)	IGFRI, SRRS, Dharwad	G44, G54, G61, G66
10	F3 progeny	Crau x Anand-2 (6 lines)	IGFRI, SRRS, Dharwad	G21, G28, G33, G50, G58, G70
11	F3 progeny	RL-88 x Crau (6 lines)	IGFRI, SRRS, Dharwad	G22, G30, G35, G45, G52, G60
12	F3 progeny	Crau x Dharwar-1 (8 lines)	IGFRI, SRRS, Dharwad	G24, G29, G42, G46, G49, G51, G56, G65
13	F3 progeny	Anand-2 x Ohoho (10 lines)	IGFRI, SRRS, Dharwad	G25, G32, G39, G40, G41, G43, G47, G53, G64, G67
14	DWR-1	Accession	IGFRI, SRRS, Dharwad	G62
15	Moopa	Accession	IGFRI, SRRS, Dharwad	G27
16	Polish Ecotype	Accession	IGFRI, SRRS, Dharwad	G38
17	Crau (OP)	Accession	IGFRI, SRRS, Dharwad	G53
Checks				
18	Anand-2	Selection from perennial type lucerne (from Bhuj area of Kutch district)	IGFRI, SRRS, Dharwad	G71
19	Alamdar-51	Selection from Kutchi lucerne	IGFRI, SRRS, Dharwad	G72
20	CO-4	Polycross derivative involving CO-1 as one of the parents	IGFRI, SRRS, Dharwad	G73
21	RL-88	Selection from local Ahmednagar lucerne	IGFRI, SRRS, Dharwad	G74

(D50%F), days to maturity (DM), plant height (PH, cm), number of branches per plant (NB), leaf to stem ratio (L/S ratio), regeneration ability (RA, days), dry matter percent (DMP), dry fodder yield (DFY, g/m row length) and green fodder yield (GFY, g/m row length). GFY was obtained by harvesting plants approximately 5 cm above the ground for a one-meter length, weighed on an electric balance, and expressed in grams. Fresh samples (100 g each) of randomly chosen plants from each line were weighed and dried at 105°C for 24 hours in the oven to assess the average dry matter percent (DMP). Dry Fodder Yield (DFY) was calculated by multiplying GFY with DMP.

Statistical analysis: The genetic divergence was analyzed using Mahalanobis generalized distance (D^2) to measure the degree of divergence between genotypes. They were grouped into clusters using Tocher's method (Mahalanobis, 1936; Rao, 1952). The analysis of canonical variables was used to evaluate the relative contribution of each character to the total diversity. All statistical analyses were performed using Window Stat software.

Table 2. Distribution of lucerne genotypes into different clusters

Cluster number	Number of genotypes	Genotype number
I	19	G6, G8, G9, G26, G28, G29, G30, G31, G39, G40, G42, G43, G45, G49, G52, G54, G57, G59, G64
II	10	G7, G18, G24, G32, G41, G47, G56, G61, G63, G66
III	08	G20, G36, G46, G51, G55, G58, G68, G69
IV	11	G1, G5, G10, G12, G16, G17, G19, G22, G23, G25, G33
V	02	G13, G14
VI	05	G2, G21, G38, G44, G50
VII	10	G3, G11, G15, G27, G34, G35, G53, G60, G65, G70
VIII	01	G62
IX	04	G4, G37, G48, G67
X	04	G71, G72, G73, G74

Results and Discussion

Genetic diversity: The analysis revealed that genotypes and checks were grouped into ten discrete clusters based on ten morphological traits. Cluster I comprised the maximum number of genotypes (19) among the clusters, followed by cluster IV with 11 genotypes. Clusters II and VII contained ten genotypes each, cluster III comprised eight genotypes, cluster VI five genotypes, clusters IX and X contained four genotypes each, cluster V comprised two genotypes, and cluster VI comprised a single genotype.

The local collections (Rajasthan and Maharashtra) and accessions, though not geographically diverse, were scattered in more than one cluster, indicating they are genetically diverse. Therefore, it is concluded that there is no parallelism between geographic diversity and genetic divergence (Yadav *et al.,* 2001; Veerabadhiran and Kennedy, 2001). All the checks did not scatter in different clusters but grouped into a single cluster (X) because they were extensively used in crossing programs to produce better populations, indicating their distinctness or similarity from the genotypes/accessions for the traits studied (Table 2).

The F_3 lines exhibited a random distribution pattern into various clusters, showing that they are heterogeneous and heterozygous in origin. The source and origin did not distinctly influence the clustering pattern of genotypes in the present study. Murty and Arunachalam (1966) and Bhatt (1970) suggested that genetic drift and natural selection forces operative under diverse environmental conditions within a country could cause more diversity than geographical isolation. Similar results were reported by Tucak *et al.* (2011) in lucerne and Deepthi *et al.* (2013) in hedge lucerne.

Relative contribution of each trait towards genetic divergence: Analysis of genetic divergence estimates the genetic distance among the selected genotypes in question and reveals the relative contribution of particular characters to the overall divergence. The genetic improvement through hybridization and selection depends upon the extent of genetic diversity between parents (Hailu *et al*., 2016). The study found that the regeneration ability was the primary contributing factor to the genetic divergence observed in the studied genotypes, followed by the leaf-to-stem ratio. These two traits, followed by dry fodder yield, days to maturity, dry matter percent, green fodder yield, days to first flowering, plant height (cm), and number of branches per plant, accounted for 99.3% of total genetic divergence in the materials studied. Similar findings were reported in cowpea earlier (Oo *et al*., 2023; Lal *et al*., 2017; Patel *et al*., 2017; Lal *et al*., 2018; Nguyen *et al*., 2019; Purohit *et al*., 2020). The trait days to 50% flowering was the most minor contributor to the divergence (Fig 1).

This suggested that regeneration ability, leaf-to-stem ratio and dry fodder yield are potent characters for genetic divergence. The yielding ability of the material, in general, was found to be coupled with high values for dry fodder yield, plant height, number of branches per plant, and leaf-to-stem ratio. Therefore, it is suggested that a crossing program involving germplasm selected for high dry fodder yield, number of branches per plant, plant height and leaf-to-stem ratio with quick re-growing

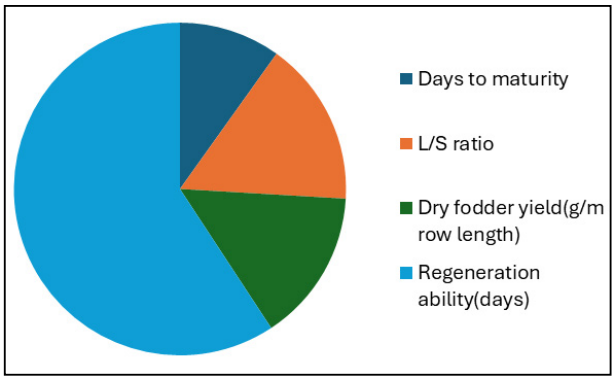

Fig 1. The relative contribution of selected characters towards divergence

ability might lead to an overall improvement in green fodder yield.

Inter and intra-cluster D² values: The inter-cluster distances (Table 3) revealed that clusters VI and VII (472.89) had the maximum distance, followed by clusters II and VI (437.91), cluster VII and X (282.96), cluster II and X (275.15) and cluster V and VII (256.68). So, crossing between the lines belonging to these clusters led to the formation of different superior segregants utilized to develop superior populations or composites. Similar findings were reported by Oo *et al.* (2023) in cowpea, Iqbal *et al.* (2017) in alfalfa and Poonia *et al.* (2020) in Oat genotypes. The distance between clusters I and III (32.28) was minimal, indicating that the genotypes belonging to these clusters were comparatively less diverse and that due care could be taken while attempting crosses among members of the clusters. Mean intra-cluster distances revealed that cluster IX, followed by cluster X, had the maximum intra-distance; in contrast, cluster V had minimum intra-distance, which showed the presence of high and low genetic variability within these clusters.

Cluster means: Cluster means for different morphological traits revealed that substantial variability existed for all the characters (Table 4). The highest mean values for plant height, number of branches per plant and green

Table 3. Cluster mean values for yield and yield attributing traits in lucerne genotypes

Clusters	DFF	D50%F	DM	PH	NB	L/S ratio	DFY	DMP	RA	GFY
Cluster 1	63.37	71.97	100.61	84.90	14.68	1.21	32.03**	23.13	2.00	138.11
Cluster 2	62.30	74.00	120.20	84.20	15.13	1.24	30.84	23.45	3.00**	131.20
Cluster 3	64.50	74.63	104.88	84.91	14.54	1.39**	21.88	21.88	2.00	117.06*
Cluster 4	62.64	73.18	99.36	87.26	15.90	1.14	22.82	22.82	2.00	221.73
Cluster 5	59.50*	70.75*	95.25*	93.05**	16.08**	1.28	21.00*	21.00*	2.00	356.00**
Cluster 6	64.10	76.50	104.70	86.30	14.94	1.24	23.70	23.70	1.10*	161.80
Cluster 7	65.65	75.80	101.75	85.87	13.93*	1.27	22.75	22.75	3.00**	163.00
Cluster 8	71.50	79.00	109.00	85.10	14.80	1.05*	23.00	23.00	2.00	121.00
Cluster 9	61.00	71.75	97.88	84.80	16.58**	1.31	23.00	23.00	2.00	138.50
Cluster 10	80.13**	87.88**	116.88**	83.63*	14.24	1.25	23.63	23.63**	2.00	183.63

* =Lowest value; **= Highest value

Table 4. Average intra-cluster (diagonal) and inter-cluster D^2 values in lucerne genotypes

Clusters	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8	Cluster 9	Cluster 10
Cluster 1	**18.86**									
Cluster 2	120.02	**20.68**								
Cluster 3	32.28	137.82	**27.65**							
Cluster 4	40.57	148.60	67.84	**26.89**						
Cluster 5	134.81	261.05	169.82	66.84	**5.80**					
Cluster 6	133.09	437.91	131.70	144.44	214.56	**29.57**				
Cluster 7	155.77	50.11	171.92	168.79	256.68	472.89	**76.81**			
Cluster 8	38.39	126.97	44.78	57.79	178.59	151.74	156.13	**0.00**		
Cluster 9	48.24	152.64	59.79	72.68	158.76	161.76	189.96	85.98	**89.77**	
Cluster 10	166.12	275.15	142.18	161.97	222.60	217.54	282.96	102.44	211.12	**79.79**

fodder yield were found in cluster V among all the clusters. Similarly, days to first flowering, days to 50% flowering, and days to maturity were observed in cluster V, thus indicating early genotypes in this group. Cluster I exhibited the highest cluster mean for dry fodder yield. Cluster III had a high mean value for the leaf-to-stem ratio. Except for G2, all the genotypes belonging to cluster VI had early regeneration ability. The most crucial yield attributing traits showed that the genotypes falling in cluster V exhibited better performance. Based on the observations, for the improvement of different characters *viz*. days to first flowering, days to 50% flowering, days to maturity, plant height, number of branches per plant, leaf to stem ratio, green fodder yield and dry fodder yield, under the present study, genotypes could be selected from clusters I, III and V. Use of multivariate analysis for identification of parents for hybridization program was used earlier in hedge lucerne by Deepthi *et al*. (2013) and in cowpea by Oo *et al*. (2023).

Conclusion

Lucerne, being a highly cross-pollinated crop with high inbreeding depression, makes it very difficult to breed for hybrid development. Hence, developing composites and synthetic populations could be an easier and more practical way out. So, choosing the superior parent genotype(s) is crucial for developing superior populations. Results of this study indicated that superior populations could be created by inter-mating genotypes belonging to Clusters VI and VII, followed by Clusters II and VI. The genotypes with high inter-cluster distances *viz*., G7, G18, G24, G32, G41, G47, G56, G61, G63, G66 (cluster II), G2, G21, G38, G44, G50 (cluster VI) and G3, G11, G15, G27, G34, G35, G53, G60, G65, G70 (cluster VII) could be utilized for further crop improvement.

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