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Research article

Association studies for yield and yield components in lucerne (*Medicago sativa* L.) genotypes

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Abstract

An investigation was carried out to evaluate seventy lucerne (*Medicago sativa* L.) genotypes during *rabi* 2020-21 at SRRS, IGFRI, Dharwad to determine the character association of ten morphological traits to improve the fodder yield. Analysis of variance revealed significant differences between the genotypes for all the traits studied. Correlation analysis revealed that dry fodder yield, plant height, number of branches per plant were significantly and positively associated at genotypic as well as phenotypic level with green fodder yield. Inter-correlation study revealed that a positive and significant association was found among plant height, number of branches per plant and dry fodder yield. The leaf to stem ratio, on the other hand, was negatively associated with green fodder yield. The results of path analysis revealed that dry fodder yield had the highest positive direct effect on green fodder yield, followed by plant height and number of branches per plant. While plant height and number of branches per plant contributed indirectly via dry fodder yield for green fodder yield. The study indicated that these characters would enhance the effectiveness of selection for higher fodder yield in lucerne.

Keywords: Association studies, Correlation, Fodder yield, Lucerne, Path analysis

Introduction

Lucerne or alfalfa (*Medicago sativa* L.) is an important leguminous and high biomass producing fodder crop. It has high crude protein (16-25%) and low fibre (20-30%) contents (Kapadia, 2019). Because of its adaptation to varied soil conditions and tolerance to drought, it is grown widely (Singh *et al.*, 2007; Kulkarni, 2016). Furthermore, it is perennial and provides forage continuously throughout the year with 8-10 cuts/annum and provides green fodder yield of 60-130 t/ha/year (Maurice *et al.*,1974; Kapadia, 2019). Alfalfa is known as the 'Queen of Forages' because it is rich in minerals (calcium, iron, magnesium, potassium) and vitamins (vitamins A, D, E, K, and a full family of B), and palatable to all the livestock (Singh and Garg, 2015). It is also an ideal crop for silage preparation. Due to its high vitamin-A and protein content, it is included in the feed component of piggery and poultry also (Adapa *et al.*, 2007; Kapadia, 2019).

Nowadays, the focus of lucerne research is to improve fodder yield, nutritional quality and tolerance to biotic and abiotic stresses (Kumar *et al.*, 2023). Knowledge about the interrelationship between yield and yield-attributing traits facilitates the choice of an efficient breeding method to be adopted for crop improvement. Hence, correlation coefficients were estimated at the phenotypic and genotypic levels in all possible combinations of yield components to estimate the association between two traits. Path analysis is a standardized partial regression coefficient that splits correlation into direct and indirect effects and measures the direct and indirect contribution of independent variables on the dependent variable. The path analysis was first suggested by Wright (1921) and later modified by Dewey and Lu (1959). It gives a clear picture of whether the independent variables are having a direct effect on green fodder yield or an indirect effect on green fodder yield via other independent variables and aids in the selection of important agronomic characters (Dewey and Lu, 1959). Hence, considering all the above aspects, an

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attempt was made to estimate the magnitude and direction of association among different traits, and direct and indirect effects of various yield components at genotypic as well as phenotypic levels.

Materials and Methods

Study site and experimental design: The experiment was conducted during *rabi* 2020-21 at SRRS, ICAR-IGFRI, Dharwad situated at 15º26´ N latitude, 75 º07´ E longitude and at an altitude of 678 m above mean sea level. It comes under the northern transitional tract (Zone 8), lying in between the western heavy rainfall and eastern low rainfall areas. The soil of the experimental site contained 0.48% organic carbon. The available N, P, and K were around 235.2 kg/ha, 7.42 kg/ha and 369.4 kg/ha, respectively with soil pH 7.81 and EC 0.007. The experiment was carried out in a randomized complete block design (RCBD), where each line was accommodated in a 4 x 4 m^2 plot containing 13 rows of 4 m length with 30 x 10 cm spacing in two replications. The recommended package of practices was followed to raise a good crop stand.

Plant material and observations recorded: The experimental material comprised of local populations and F_3 progenies of eleven different crosses along with four checks, *viz.* Anand-2, Alamdar-51, RL-88 and Co-4 (Table 1). The crop was harvested two times in the year of establishment (February 10 and April 4, 2021). The data obtained from the two cuttings was pooled and analysed. Observations were recorded on five randomly selected plants from each line on ten morphological traits *viz*., days to first flowering (DFF), days to 50 per cent flowering (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

Table 1. List of local populations and F_3 progenies of lucerne

SL. No.	Genotypes	Pedigree	Source
01	05 lines	Local collections	Maharashtra
02	15 lines	Local collections	Rajasthan
03	F_3 progeny	RL-88 x Weevil check	IGFRI, SRRS, Dharwad
04	F_3 progeny	Anand-2 x Weevl check (2 lines)	IGFRI, SRRS, Dharwad
05	F_3 progeny	Crau x RL-88 (2 lines)	IGFRI, SRRS, Dharwad
06	F_3 progeny	Dharwar-1x Crau (2 lines)	IGFRI, SRRS, Dharwad
07	F_3 progeny	Maris x Kabul (2 lines)	IGFRI, SRRS, Dharwad
08	F_3 progeny	RL-88 x Dry Lander Alfalfa (3 lines)	IGFRI, SRRS, Dharwad
09	F_3 progeny	Anand-2 x Vernal (4 lines)	IGFRI, SRRS, Dharwad
10	F_3 progeny	Crau x Anand-2 (6 lines)	IGFRI, SRRS, Dharwad
11	F_3 progeny	RL-88 x Crau (6 lines)	IGFRI, SRRS, Dharwad
12	F_3 progeny	Crau x Dharwar-1 (8 lines)	IGFRI, SRRS, Dharwad
13	F_3 progeny	Anand-2 x Ohoho (10 lines)	IGFRI, SRRS, Dharwad
14	DWR-1	Accession	IGFRI, SRRS, Dharwad
15	Moopa	Accession	IGFRI, SRRS, Dharwad
16	Polish Ecotype	Accession	IGFRI, SRRS, Dharwad
17	Crau (OP)	Accession	IGFRI, SRRS, Dharwad
Checks			
18	Anand-2	Selection from perennial type Lucerne (from Bhuj area of Kutch district)	IGFRI, SRRS, Dharwad
19	Alamdar-51	Selection from Kutchi lucerne	IGFRI, SRRS, Dharwad
20	Co-4	Polycross derivative involving Co-1 as one of the parents	IGFRI, SRRS, Dharwad
21	RL-88	Selection from local Ahmednagar lucerne	IGFRI, SRRS, Dharwad

in 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 at approximately 5 cm above the ground for a one-meter length and then weighed on an electric balance and expressed in grams. Fresh samples of randomly chosen plants were taken from each line and placed in paper bags. The samples (100 g each) were dried at 105°C for 24 h in an oven to assess the average dry matter percent (DMP). Dry fodder yield (DFY) was calibrated from green fodder yield as per the formula.

Statistical analysis: The correlation coefficient at the phenotypic and genotypic level was calculated from the variance and covariance according to Johnson *et al*. (1955). The direct and indirect effect of various traits contributing towards green fodder yield was calculated using the path coefficients analysis (Dewey and Lu, 1959).

Results and Discussion

Genetic variability: The mean squares for treatments were found to be significant for all traits indicating significant variations present among the genotypes for all the ten characters *viz*., days to first flowering, days to fifty percent flowering, days to maturity, plant height, number of branches, leaf to stem ratio, green fodder yield per running meter, dry fodder yield per running meter, dry matter percent and regeneration ability (Table 2). The traits like days to first flowering (58.0-74.5 days), days to 50 percent flowering (68.0-82.0 days), days to maturity (93.0-111.5 days), plant height (70.0-97.3 cm), number of branches per plant (9.25-20.8), leaf stem ratio (0.83-1.68), dry matter percent (19-27%), regeneration ability (1-3 days), dry fodder yield (13-77 g) and green fodder yield (58.5-369.0 g) showed a wide range of mean values showing substantial variability in the lucerne genotypes. Vinodkumar *et al*. (2022) reported that GCV and PCV values were high for traits like green fodder yield, dry fodder yield and regeneration ability suggesting that phenotypic selection would facilitate successful isolation of superior types. High heritability along with high GAM was recorded for regeneration ability, dry fodder and green fodder yields.

Two genotypes were with high GCA (White flower No. 3 and Fast-growing No. 4) and eight strains with moderate GCA (Fast-growing No. 15, Fast-growing No. 12, White flower No. 1, White flower No. 2 and Fast-growing No. 1 etc.). Strains with high yield and quality could be used as parents in the next crossing cycle. The estimated H^2 values for attributes affecting relative feeding value were in the order > hay yield > leaf-stem ratio ≥ crude protein > branch number > internode length > plant height > growth rate. Most H^2 values were >80%, which means they could be used as early-generation selection indicators in a breeding programme (Wang *et al*., 2016)

***Correlation studies**:* Correlation estimates at genotypic as well as phenotypic levels for the traits

Table 2. Analysis of variance for green fodder yield and yield attributing traits in genotypes of lucerne

Source	DFF	D50% F	DM	NB	PH	L/S ratio	GFY	DFY	DMP	RA
Replication	14.77	14.77	0.19	5.06	52.50	0.01	9.11	2.39	1.08	0.03
Treatments	83.45**	58.51**	58.59**	7.37**	63.65**	0.05*	7013.0**	349.40**	5.53**	0.63**
Error	3.38	4.73	8.67	1.65	16.09	0.012	311.55	21.57	1.99	0.03
Statistical parameters										
Mean	64.83	75.11	101.38	14.90	85.43	1.24	157.27	36.03	22.94	2.23
Range	58 - 74.5	68- 82	93- 111.5	9.25- 20.28	70- 97.3	0.83- 1.68	58.5- 369.0	13.0- 77.0	19.0- 27.0	1-3
SE	1.38	1.54	2.08	0.91	2.84	0.03	12.48	3.28	0.93	0.11
CD (P<0.05)	3.9	4.33	5.86	2.56	7.99	0.09	35.15	9.25	2.62	0.32
CV	3.02	2.9	4.7	8.63	4.7	3.56	11.22	12.89	5.73	7.18

*(P<0.050); **(P<0.01); DFF: Days to first flowering; D50%F: Days to 50 per cent flowering; DM: Days to maturity; PH: Plant height (cm); NB: Number of branches per plant; L/S ratio: Leaf to stem ratio; RA: Regeneration ability (days); DMP: Dry matter percent; DFY: Dry fodder yield (g/m row length); GFY: Green fodder yield (g/m row length)

Table 3. Genotypic (below diagonal) and phenotypic (above diagonal) correlation coefficients for yield and yield attributing traits in lucerne (*Medicago sativa* L.) genotypes

Traits	DFF	D50%F	DM	PH	NB	L/S ratio	DFY	DMP	RA	GFY
DFF	1	0.86**	0.60**	-0.14	-0.06	-0.01	-0.16	-0.02	-0.003	-0.17*
D50%F	0.91 **	1	0.57**	-0.15	0.03	0.002	-0.14	-0.06	0.09	-0.14
DM	0.71 **	0.70 **	1	-0.06	0.015	0.02	-0.24**	-0.04	0.08	-0.24**
PH	-0.17	-0.26*	-0.15	1	0.06	-0.02	0.39**	-0.02	-0.05	0.39**
NB	0.08	0.04	-0.02	0.11	1	-0.02	0.33**	-0.02	-0.07	0.34**
L/S ratio	-0.01	0.01	0.02	-0.01	-0.05	1	-0.18**	-0.15	0.03	-0.14*
DFY	-0.16	-0.14	-0.28*	0.53 **	0.43 **	-0.2	1	0.2	-0.06	0.97**
DMP	-0.04	-0.02	-0.09	0.11	0.13	-0.15	0.12	1	-0.03	-0.02
RA	0.01	0.09	-0.01	-0.03	0.1	0.12	-0.07	-0.01	1	-0.06
GFY	-0.17	-0.15	-0.27 *	0.52 **	0.40 **	-0.16*	0.98 **	-0.06	-0.06	1

*(P<0.050); **(P<0.01); DFF: Days to first flowering; D50%F: Days to 50 per cent flowering; DM: Days to maturity; PH: Plant height (cm); NB: Number of branches per plant; L/S ratio: Leaf to stem ratio; RA: Regeneration ability (days); DMP: Dry matter percent; DFY: Dry fodder yield (g/m row length); GFY: Green fodder yield (g/m row length)

analysed were recorded (Table 3). The study showed that green fodder yield was significantly and positively correlated with plant height, number of branches per plant and dry fodder yield. Similar results were observed by Tucak *et al*. (2008). Inter correlation study showed that plant height and number of branches per plant showed a significant positive correlation with dry matter yield. These findings agreed with Deepti *et al.* (2013). A negative association was observed between leaf-to-stem ratio and dry fodder yield, green fodder yield and plant height. Similar observations were reported by Tucak *et al*. (2008), and contrast findings were reported by Mary and Gopalan (2006) in fodder cowpea and Deepti *et al*. (2013) in hedge lucerne. When magnitude was considered, genotypic correlation coefficients were slightly higher than the phenotypic correlation coefficients for most of the traits studied. Similar findings were reported by Kumawat *et al*. (2020) in maize. This emphasized that inspite of the strong inherent association between various character pairs studied, the environment might modify the expression of traits. The traits which showed significant positive correlation with the green fodder yield could be simultaneously improved through indirect selection for those traits.

Path-coefficient studies: Since the correlation study does not indicate a cause-and-effect relationship, it was insufficient to explain the true association. As a result, the correlated traits were investigated further to record if independent variables had a direct or indirect effect on the dependent variable. Lucerne being a fodder crop, green fodder yield was taken as the dependent variable and other traits were taken as independent variables. The results of the path analysis at phenotypic as well as genotypic level were recorded (Table 4). The study revealed that dry fodder yield recorded the highest positive direct effect on green fodder yield followed by plant height and number of branches per plant. Similar observations were also made by Jain *et al*. (2010) in fodder sorghum. But plant height and number of branches per plant recorded positive indirect effects via dry fodder yield. Similar results were found by Chaudhary and Lodhi (1980) in forage guar and Deepti *et al*. (2013) in hedge lucerne. The residual effect at phenotypic and genotypic level (R= 0.033 and 0.072, respectively), indicated that most of the important traits were being considered during the path analysis. The correlation of leaf to stem ratio and green fodder yield was significantly negative. Similarly, the trait exerted negative direct effects on green fodder yield at both phenotypic and genotypic levels. Similar findings were found by Zhang *et al*. (2010) in bajra, Ramakrishnan *et al*. (2013) in guinea grass and Kapoor (2017) in fodder maize.

Table 4. Direct (on diagonal) and indirect (off diagonal) effects at phenotypic and genotypic level for yield and yield attributing traits in lucerne (*Medicago sativa* L.) genotypes

Traits		DFF	D50%F	DM	PH	NB	L/S ratio	DFY	DMP	RA	Correlation coefficient
DFF	P	-0.018	0.004	0.001	0.000	0.000	0.000	-0.16	0.005	0.000	-0.167*
	G	-0.018	-0.003	0.009	0.000	0.000	0.000	-0.164	0.007	0.000	-0.169
D50%F	P	-0.016	0.005	0.001	0.000	0.000	0.000	-0.147	0.015	0.000	-0.142
	G	-0.017	-0.003	0.009	0.000	0.000	0.000	-0.139	0.004	0.000	-0.145
DM	P	-0.011	0.003	0.002	0.000	0.000	0.000	-0.241	0.01	0.000	-0.238**
	G	-0.013	-0.002	0.012	0.000	0.000	0.000	-0.282	0.017	0.000	-0.268 *
PH	P	0.002	-0.001	0.000	0.003	0.000	0.000	0.402	-0.021	0.000	0.385**
	G	0.003	0.001	-0.002	0.032	0.000	0.000	0.535	-0.02	0.000	0.516 **
NB	P	-0.001	0.000	0.000	0.000	0.003	0.000	0.332	0.005	0.000	0.339**
	G	-0.001	0.000	0.000	0.000	0.003	-0.001	0.433	-0.024	0.000	0.403**
L/S ratio	P	0.000	0.000	0.000	0.000	0.000	-0.008	-0.187	0.034	0.000	-0.145*
	G	0.000	0.000	0.000	0.000	0.000	-0.012	-0.203	0.026	0.000	-0.165*
DFY	P	0.003	-0.001	0.000	0.001	0.001	-0.002	1.015	-0.05	0.000	0.988**
	G	-0.003	-0.001	-0.003	0.012	0.013	-0.002	1.001	-0.02	0.000	0.984**
DMP	P	0.000	0.000	0.000	0.000	0.000	-0.001	0.212	-0.237	0.000	-0.025
	G	0.001	0.000	-0.001.	0.000	0.000	-0.002	0.117	-0.176	0.000	-0.062
RA	P	0.000	0.000	0.000	0.000	0.000	0.001	-0.065	0.007	-0.003	-0.059
	G	0.000	0.000	0.000	0.000	0.000	0.001	-0.074	0.003	0.003	-0.066

*(P<0.050); **(P<0.01); DFF: Days to first flowering; D50%F: Days to 50 per cent flowering; DM: Days to maturity; PH: Plant height (cm); NB: Number of branches per plant; L/S ratio: Leaf to stem ratio; RA: Regeneration ability (days); DMP: Dry matter percent; DFY: Dry fodder yield (g/m row length); GFY: Green fodder yield (g/m row length)

Conclusion

The improvement of fodder yield from a single component is difficult hence there is a need to consider increasing the yield by indirectly selecting other highly correlated yield components. From the association study, it was observed that traits like dry fodder yield, plant height and number of branches per plant, and leaf-to-stem ratio are important traits to increase the fodder yield in lucerne. Hence, these traits might serve as selection indices and more emphasis should be given to these traits during the selection of the lucerne crop.

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