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Research Article

Studies on genetic variability, trait contribution for improved green fodder yield in lucerne (*Medicago sativa* L.)

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Abstract
The present study was carried out to evaluate 40 lucerne germpalsm including four checks for genetic variability and character associations for green fodder yield and attributing traits. The germplasm were evaluated for 16 quantitative and qualitative traits and subjected to pooled analysis for the successive three cuts (environment). The results clearly revealed significant differences among the genotypes for all the traits studied except leaf length. However, genotype x environment interaction indicated significant differences for all traits studied except for leaf length, leaf breadth, crude protein and neutral detergent fiber. Greater variation was recorded with the obtained values of PCV and GCV for all traits studied. High heritability and high genetic advance as a percent of the mean was noticed for leaf breadth, stem girth, dry matter yield, green fodder yield, crude protein and crude fiber. However, leaf length and leaf stem ratio exhibited high heritability with low GAM. The correlation coefficient for green fodder yield was significant and positively associated with plant height, stem girth, number of crowns per plant and dry matter yield. Positive direct effect was reported for plant height, stem girth, neutral detergent fiber, number of crowns per plant, leaf stem ratio and total ash on green fodder yield. Hence, these traits could be used to formulate a selection index for to improving the green fodder yield in lucerne.

Keywords: Lucerne, genetic variability, trait association, path coefficients

INTRODUCTION

Livestock and agriculture are intrinsically linked, each being dependent on the other, and both crucial for overall food security. India has the world's largest livestock population, which makes a significant contribution towards improving the sustainability of agriculture, farm revenue, rural livelihoods, human health and nutrition. The total livestock population in India is 535.78 million, showing an increase of 4.6% over the previous livestock census, which included 35.94 percent of cattle, 20.45 percent of buffaloes, 27.80 percent of goat and 13.87 percent of the sheep population (20th livestock census, 2019). India is still the largest producer of milk in the world. Milk production during the year 2021-22 was 221.06 million tonnes representing a growth rate of 5.29% per annum. In 2021-22 the per capita availability of milk was around 444 grams/day (DAHD-Annual Report 2022-23).

The livestock population is increasing and its feed requirements are also increasing day by day. The total green fodder availability is 734.2 million tonnes against the requirement of 827.19 million tonnes, with an overall green fodder deficit of 11.24% in the country (Roy *et al*., 2019). So, there is a need to meet the demand of an increasing number of livestock and also enhance their productivity by ensuring green fodder availability. High yielding fodder crops provide the bedrock for sustainable

agriculture. Among the fodders, lucerne is one of the high-yielding, nutritious legume fodder crops that supplies green fodder throughout the year.

Lucerne or Alfalfa (*Medicago sativa* L.) is one of the most important legume fodder crops and is also known as "Queen of fodder crops". Lucerne is a tetraploid species (2n=4x=32) and tetraploids are usually more vigorous than diploids. It possesses about 16-25% crude protein and 20-30% crude fiber with high palatability. The crop is cultivated as a fodder crop worldwide due to its yield potential, high nutritive value and quality. It is a perennial crop and it can supply green fodder for around 3-4 years from the same crop stand, so that 13 to 14 green fodder harvests can be made in a year at an interval of 25 days and able to yield upto 130 to 135 tonnes/ha/year.

Lucerne germplasm is one of the critical plant genetic resources for ensuring sustainable livestock production. Improvement of green fodder yield is one of the primary objectives in lucerne crop improvement programme. Selection of superior genotypes based on green fodder yield as such is difficult to the integrated structure of the plant in which most of the characters are interrelated. It necessitates a thorough knowledge on nature of relationships prevailed between contributory traits and green fodder yield. Therefore, the present investigation aims to access the variability together with the relative contribution of different yield-attributing traits to green fodder yield and their interrelationships in lucerne.

MATERIALS AND METHODS

The experimental materials consisted of 36 lucerne germplasm along with four checks *viz*., GETL1 to GETL 24, IVTLU 1 to IVTLU 6, Anand 2, RL 88, Krishna, LLC 5, AL 3, AL 4 and four checks (CO 1, CO 2, CO 3 and CO 4). The genotypes were evaluated in Augmented block design at Department of Forage crops, Tamil Nadu Agricultural University, Coimbatore during *rabi* 2022. Each germplasm was sown in two rows with spacing of 30 cm x 15 cm with a row length of 4m by replicating check varieties four times. The recommended agronomic practices were followed for proper growth and establishment. The biometrical observations were recorded in five randomly selected plants for 10 quantitative traits *viz.,* plant height (cm), days to fifty percent flowering, leaf length (cm), leaf breadth (cm), leaf/stem ratio, stem girth (cm), Number of crowns per plant, dry matter yield (g/plant), dry matter content (%), green fodder yield (g/plant) and six quality traits *viz*., crude protein (%), crude fat (%), crude fiber (%), total ash (%), acid detergent fiber (%) and neutral detergent fiber (%).

The data were collected for successive three cuts (environment), first cut was taken between 60-67 days after sowing and the subsequent cuts at an interval of 25 days. The data were subjected to pooled analysis over environments for analysis of variance (ANOVA) and to ascertain GCV and PCV (Burton and Devane, 1953), heritability (h^2) (Lush, 1940) and genetic advance as percent mean values (Johnson *et al*., 1955). Correlation coefficients were estimated as per the method suggested by Singh and Chaudhary (1979). Path coefficient was analyzed as per the prescribed procedure of Dewey and Lu (1959).

RESULTS AND DISCUSSION

Studies on genetic parameters: Pooled ANOVA over the environment for the studied traits showed significant difference among the genotypes for all traits except for leaf length (cm) (**Table 1**) whereas for the environment it showed significant difference for all the traits except for crude protein (%), crude fat (%), crude fiber (%), total ash (%) and acid detergent fiber (%). Similarly, the interaction between genotype x environment (g x e) revealed significant differences for plant height (cm), days to fifty percent flowering, leaf stem ratio, stem girth (cm), number of crowns per plant, dry matter yield (g/plant), dry matter content (%), green fodder yield (g/plant), crude fat (%), crude fiber (%), total ash (%) and acid detergent fiber (%).

Studies on variability (**Table 2**) revealed that GETL 4 was the tallest germplasm (56.74 cm) whereas AL 4 (80.27 cm) recorded short stature with a mean value of 69.52 cm. The check CO 1 recorded late flowering (38 days) and IVTLU 3 expressed early flowering (32 days). The overall mean for days to fifty percent flowering for the studied germplasm accessions was 35 days. The mean values for leaf length and leaf breadth were 2.68 cm and 1.24 cm with the range from 2.42 cm (GETL 6) to 3.13 cm (IVTLU 5) and 1.08 cm (GETL 3) to 1.75 cm (IVTLU 6) respectively. The trait leaf stem ratio ranged from 0.39 (GETL 6) to 0.46 (AL 4) with a mean ratio of 0.42. Stem girth was found to be diverse from 1.04 cm (GETL 7) to 1.43 cm (Anand 2) with 1.18 cm as a mean.

The genotype GETL 12 had the lowest number of crowns per plant (15 number of crowns) as compared to the check CO 3 recorded the highest number of crowns per plant (19 number of crowns) and the overall mean was 17.23. Dry matter yield exhibited a range from 16.73 g (IVTLU 2) to 32.27 g (LLC 5) with a mean of 24.50 g. However, the dry matter content varied from 21.43 % (GETL 17) to 24.34 % (GETL 21) with mean value of 22.91 %. Similarly, the germplasm IVTLU 2 had the lowest (77.20 g) green fodder yield per plant whereas the germplasm RL 88 registered the highest (135.47 g) yield per plant, while the overall mean was 106.74 g. Substantial variations were observed for crude protein, crude fat and crude fiber among the 40 lucerne germplasm accessions and it ranged from 17.83 % (GETL 24) to 24.75 % (GETL 19), 4.04 % (GETL 12) to 7.62 % (IVTLU 5) and from 16.17 % (GETL 1) to 30.17 % (IVTLU 5) with the mean values of 22.70 %, 5.76 % and 24.16 % respectively.

Table 1: Pooled analysis of variance for different traits among 40 Lucerne germplasm

Source of variation	Df	Mean sum of squares							
		PH	DFF	LL	LB	L/S	SG	CP	DMY
Environment	2	6951.10**	25385.30**	2.8095**	0.0763**	0.0052**	0.0293**	22.63**	3242.70**
Block	3	601.00**	3.20^{NS}	0.4682**	0.1245**	0.0005^{NS}	0.0936**	4.01**	90.50**
Check	3	31.20*	7.90**	0.0908^{NS}	0.0426*	0.0045^{NS}	0.0086^{NS}	0.59**	15.30**
Genotype	35	59.7**	4.10**	0.0531^{NS}	0.0387**	0.0005*	0.0206**	1.46**	26.20**
Genotype x Environment (g x e)	78	60.50**	7.00**	0.0480^{NS}	0.0165^{NS}	0.0009**	0.0143**	1.39**	24.10**
Residuals	33	7.90	1.10	0.0390	0.0121	0.0002	0.0032	0.04	2.00

Table 1.Continued..

Source of variation	Df	Mean sum of squares							
		DMC	GFY	CPR	CF	CFI	T.ASH	ADF	NDF
Environment	2	13.22**	58525.00**	0.68^{NS}	0.47^{NS}	0.05^{NS}	0.0079^{NS}	0.63^{NS}	21.86**
Block	3	1.99^{NS}	1459.00**	3.74*	3.28**	122.06**	0.1146^{NS}	8.78**	0.50^{NS}
Check	3	3.02^{NS}	120.00**	54.44**	0.89*	2.35^{NS}	0.0260^{NS}	1.07^{NS}	2.94*
Genotype	35	2.29*	357.00**	5.34**	1.70**	17.94**	0.1277**	4.21**	1.84*
Genotype x Environment (g x e)	78	3.00**	529.00**	0.91^{NS}	2.06**	16.12**	0.1013**	6.22**	1.57^{NS}
Residuals	33	1.24	3.00	1.10	0.24	1.27	0.0409	1.31	0.96

*, ** significant at 5% and 1% levels, respectively

[PH- Plant height (cm), DFF- Days to fifty percent flowering, LL- Leaf length (cm), LB- Leaf breadth (cm), L/S- Leaf stem ratio, SG- Stem girth (cm), CP- Number of crowns per plant, DMY- Dry matter yield (g/plant), DMC- Dry matter content (%), GFY- Green fodder yield (g/plant), CPR- Crude protein (%), CF- Crude fat (%), CFI- Crude fiber (%), T.ASH - Total ash (%), ADF- Acid detergent fiber (%), NDF- Neutral detergent fiber (%)]

Table 2. Pooled mean and variability parameters for 16 traits among 40 Lucerne germplasm

S.No.	Traits	Mean	Range		Coefficient of Variation		$h^2_{(b)}$	GA	GAM
			Min	Max	PCV (%)	GCV (%)			
1.	PH	69.52	56.74(GETL 4)	80.27(AL 4)	11.27	10.50	86.77	13.81	19.87
2.	DFF	35	32(IVTLU 3)	38(CO 1)	5.85	5.00	73.17	3.05	8.75
3.	LL	2.68	2.42(GETL 6)	3.13(IVTLU 5)	8.34	3.73	20.00	0.09	3.44
4.	LB	1.24	1.08(GETL 3)	1.75(IVTLU 6)	16.13	13.97	75.00	0.31	24.92
5.	L/S	0.42	0.39(GETL 6)	0.46(AL 4)	5.32	3.37	40.00	0.02	4.39
6.	SG	1.18	1.04(GETL 17)	1.43(Anand 2)	12.39	11.47	85.71	0.26	21.68
7.	CP	17.23	14.87(GETL 12)	18.82(CO 3)	7.08	6.98	97.26	2.42	14.05
8.	DMY	24.50	16.73(IVTLU 2)	32.27(LLC 5)	21.26	20.43	92.37	9.74	39.75
9.	DMC	22.91	21.43(GETL 17)	24.34(GETL 21)	6.60	4.47	45.85	1.43	6.24
10.	GFY	106.74	77.20(IVTLU 2)	135.47(RL 88)	82.98	82.63	99.16	38.60	90.39
11.	CPR	22.70	17.83(GETL 24)	24.75(GETL 19)	39.91	35.56	79.40	3.78	65.62
12.	CF	5.76	4.04(GETL 12)	7.62(IVTLU 5)	5.32	4.93	85.88	2.31	9.55
13.	CFI	24.16	16.17(GETL 1)	30.17(IVTLU 5)	42.10	40.59	92.92	8.11	80.67
14.	T.ASH	10.05	9.55(GETL 24)	10.39(LLC 5)	1.00	0.82	66.67	0.48	1.37
15.	ADF	34.64	32.00(GETL 9)	36.67(IVTLU 6)	3.58	2.97	68.88	2.91	5.08
16.	NDF	57.32	56.00(IVTLU 1)	59.00(GETL 24)	1.29	0.90	47.83	1.34	1.25

[PCV - Phenotypic coefficient of variation (%), GCV - Genotypic coefficient of variation (%), $h^2_{(b)}$-Heritability in broad sense, GA - Genetic advance, GAM - Genetic advance as percent of mean (%)]

The total ash content among the germplasm varied from 9.55 % (GETL 24) to 10.39 % (LLC 5) with overall mean of 10.05 %. Acid Detergent Fiber (ADF) was noticed to be highest in the germplasm IVTLU 6 (36.67 %) and lowest in GETL 9 (32.00 %), while the mean was 34.64 %. The neutral detergent fiber was found to be the highest in the accession GETL 24 (59.00 %) and lowest in IVTLU 1 (56.00 %) with an overall mean value of 57.32 %.

The magnitude of PCV ranged from 1.00 to 82.98 %, GCV ranged from 0.82 to 82.63%, heritability in broad sense varied from 20.00 to 99.16 % and genetic advance as percent of mean varied from 1.25 to 90.39 % (**Table 2**). The phenotypic coefficient of variation had a higher value than genotypic coefficient of variation indicating that these traits are influenced by the environmental factors. A similar report was given by Seiam and Mohamed (2020) in alfalfa; Abd EL-Naby *et al*. (2015) in berseem and by Bakheit, (1986) in Egyptian clover.

In the present study, high PCV and GCV were recorded for dry matter yield (g/plant), green fodder yield (g/plant), crude protein content (%) and crude fiber content (%). Therefore the selection for these traits will be effective for the improvement of green fodder yield in lucerne. Similar findings for dry fodder yield and green fodder yield were reported by Arvinth *et al*. (2021). Moderate PCV and GCV were observed for plant height (cm), leaf breadth (cm) and stem girth (cm) which depicts average chances for selection. Low PCV and GCV was observed for days to fifty percent flowering, leaf length (cm), leaf stem ratio, number of crowns per plant, dry matter content (%), crude fat (%), total ash (%), acid detergent fiber (%) and neutral detergent fiber (%). Arvinth *et al*. (2021) reported low PCV and GCV values for days to fifty percent flowering, suggesting that there is a need for creating variability followed by selection.

Heritability and genetic advance as percent of mean: Heritability and genetic advance are two important parameters. Their relative comparison gives clear idea about the nature of gene action governing a particular trait. Therefore, it is important to partition the variability into heritable and non-heritable components. In the present study, high heritability with high genetic advance as percent of mean was recorded for leaf breadth (cm), stem girth (cm), dry matter yield (g/plant), green fodder yield (g/pant), crude protein (%) and crude fiber (%) (**Table 2**), indicating that the heritability was due to additive gene effects and selection could be highly effective for improvement of both green fodder yield and quality traits in lucerne. Similar result was reported by Subbulakshmi *et al*. (2022). Arvinth *et al*. (2021) reported similar results for stem girth, dry fodder yield, green fodder yield and crude protein content.

High heritability coupled with low genetic advance as percent of mean was observed for days to fifty percent flowering, crude fat (%), total ash (%) and acid detergent fiber (%) which implies governance of non-additive gene action and selection for such traits may not be effective. Awaan *et al*. (2014) reported similar results for acid detergent fiber. However, the trait leaf length (cm) exhibited low heritability with low genetic advance as percent of mean indicating that the trait was highly influenced by the environment and direct selection might not be effective, so the indirect selection *via* component traits will be effective for improvement of such traits with less variability. To study the indirect selection of such traits, correlation coefficients are very much helpful.

Correlation coefficients of green fodder yield: Green fodder yield is one of the important traits. It is essential to note that when two traits are correlated, the selection for one trait ensures the improvement of the other trait. Therefore, the selection of these traits would help in selecting the germplasm with high green fodder yield and when it exhibits a strong correlation with yield implies simultaneous improvement of both the traits. In the present investigation, correlation coefficient between 16 traits was worked out based on the pooled data (**Table 3**). Pooled analysis over environments revealed that the green fodder yield was significant and positively associated with plant height (cm) (0.409), stem girth (cm) (0.503), number of crowns per plant (0.397) and dry matter yield (g/plant) (0.959). Positive association for plant height, number of crowns per plant and dry matter yield was noticed by Abdel-Galil *et al*. (2008), Iyanar *et al*. (2010) and Arvinth *et al*. (2021). It is also in accordance with the findings of Alla *et al*. (2013).

Regarding, inter correlations between different traits, plant height (cm) had a positive correlation with leaf length (cm), leaf breadth (cm), leaf stem ratio, stem girth (cm), number of crowns per plant and dry matter yield (g/plant). Similar findings were reported by Iyanar *et al*. (2010). Days to fifty percent flowering had positive correlation with stem girth (cm). Leaf length (cm) had a significant positive association with leaf breadth (cm), crude fiber (%) and the trait leaf breadth (cm) was significantly correlated with crude fiber (%) and acid detergent fiber (%). Similarly, leaf stem ratio was positively associated with acid detergent fiber (%). Stem girth (cm) had a positive correlation with dry matter yield (g/plant) and dry matter content (%). Number of crowns per plant and dry matter yield (g/plant) was significant and positively associated with dry matter yield (g/plant) and dry matter content (%) respectively.

Path coefficient analysis of different traits with green fodder yield per plant: Path analysis helps in partitioning the correlation coefficient into direct and indirect effects and also measures the importance of causal factors individually (Dewey & Lu, 1959). Green fodder yield was considered as dependent trait whereas other traits were considered as independent traits (**Table 4**). The residual effect of path coefficient in the pooled analysis was 0.446. Plant height (cm) contributed

Table 3. Simple correlation coefficients of green fodder yield in pooled over three cuts

Characters	PH	DFF	LL	LB	L/S	SG	CP	DMY	DMC	CPR	CF	CFI	T. ASH	ADF	NDF	GFY
PH	1.0000															
DFF	0.282	1.0000														
LL	0.514**	0.087	1.0000													
LB	0.472**	0.028	0.684**	1.0000												
L/S	0.414**	0.164	0.280	0.255	1.0000											
SG	0.344*	0.351*	0.085	-0.186	0.149	1.0000										
CP	0.394*	0.307	0.298	0.113	0.196	0.294	1.0000									
DMY	0.402*	0.260	-0.040	-0.104	0.205	0.581**	0.362*	1.0000								
DMC	0.114	0.157	-0.088	0.047	-0.222	0.337*	-0.037	0.475**	1.0000							
CPR	-0.173	-0.122	-0.052	0.124	0.130	-0.224	-0.338*	-0.251	0.073	1.0000						
CF	0.163	-0.123	0.280	0.137	0.114	0.133	0.119	0.186	0.092	-0.204	1.0000					
CFI	0.307	-0.177	0.509**	0.562**	0.122	-0.192	-0.048	-0.182	-0.133	-0.026	0.156	1.0000				
T.ASH	-0.192	0.062	-0.052	-0.112	0.034	0.134	-0.061	0.101	0.099	-0.036	0.238	-0.011	1.0000			
ADF	0.175	0.194	0.280	0.333*	0.375*	-0.067	0.008	0.097	0.029	0.044	0.198	0.235	-0.165	1.0000		
NDF	-0.102	0.028	-0.173	-0.102	0.026	0.098	-0.109	-0.048	0.110	-0.181	-0.090	-0.112	0.167	-0.031	1.0000	
GFY	0.409**	0.261	-0.021	-0.127	0.294	0.503**	0.397*	0.959**	0.224	-0.268	0.154	-0.178	0.080	0.125	-0.105	1.0000

*, ** significant at 5% and 1% levels, respectively

Table 4. Path coefficient analysis of different characters with green fodder yield in pooled over three cuts

Characters	PH	DFF	LL	LB	L/S	SG	CP	CPR	CF	CFI	T.ASH	ADF	NDF	GFY (r)
PH	**0.379**	-0.033	-0.139	-0.037	0.077	0.094	0.076	0.031	-0.006	-0.052	-0.034	0.035	0.021	0.409**
DFF	0.102	**-0.123**	-0.027	-0.005	0.017	0.102	0.058	0.024	0.005	0.025	0.020	0.033	-0.010	0.261
LL	0.193	-0.012	**-0.273**	-0.054	0.050	0.022	0.058	0.009	-0.011	-0.086	-0.009	0.057	0.035	-0.021
LB	0.178	-0.007	-0.185	**-0.079**	0.050	-0.052	0.023	-0.024	-0.005	-0.096	-0.020	0.068	0.021	-0.127
L/S	0.159	-0.011	-0.074	-0.021	**0.184**	0.044	0.025	-0.033	-0.006	-0.032	0.009	0.078	-0.002	0.294
SG	0.129	-0.045	-0.022	0.015	0.029	**0.276**	0.058	0.042	-0.005	0.034	0.025	-0.014	-0.021	0.503**
CP	0.148	-0.037	-0.082	-0.009	0.024	0.083	**0.194**	0.062	-0.005	0.008	-0.011	0.002	0.023	0.397*
CPR	-0.064	0.016	0.014	-0.010	0.033	-0.063	-0.066	**-0.182**	0.008	0.005	-0.007	0.010	0.037	-0.268
CF	0.061	0.015	-0.076	-0.011	0.028	0.039	0.023	0.036	**-0.039**	-0.027	0.043	0.041	0.019	0.154
CFI	0.117	0.018	-0.139	-0.045	0.035	-0.055	-0.010	0.005	-0.006	**-0.169**	-0.002	0.047	0.023	-0.178
T.ASH	-0.072	-0.013	0.014	0.009	0.009	0.039	-0.012	0.007	-0.009	0.002	**0.177**	-0.035	-0.035	0.080
ADF	0.064	-0.020	-0.076	-0.026	0.070	-0.019	0.002	-0.009	-0.008	-0.039	-0.030	**0.205**	0.006	0.125
NDF	-0.038	-0.006	0.046	0.008	0.002	0.028	-0.021	0.033	0.003	0.019	0.030	-0.006	**-0.207**	-0.105

Residual = 0.4464, Diagonal **bold** values are direct effect, above and below diagonal values are indirect effect;
*,** significant at 5% and 1% levels, respectively

maximum positive direct effect to green fodder yield (g/plant)(0.379 cm) , followed by stem girth (cm) (0.276), acid detergent fiber (cm) (0.205), number of crowns per plant (0.194), leaf stem ratio (0.184) and total ash (%) (0.177). These results are in line with those observed by Iyanar et *al*. (2010) and Arvinth *et al*. (2021). The traits such as days to fifty percent flowering (-0.123), leaf length (cm) (-0.273), leaf breadth (cm) (-0.079), crude protein (%) (-0.182), crude fat (%) (-0.039), crude fiber (%) (-0.169), neutral detergent fiber (%) (-0.207) had negative direct effect on green fodder yield.

Among the traits, leaf length (cm) (0.193), leaf breadth (cm) (0.178), leaf stem ratio (0.159), stem girth (cm) (0.129), number of crowns per plant (0.148) and crude fiber (%) (0.117) had positive indirect effects via plant height (cm) on green fodder yield (g/plant). However, the trait plant height (cm) had low positive indirect effect via leaf stem ratio (0.077), stem girth (cm) (0.094), number of crowns per plant (0.076), crude protein (%) (0.031), acid detergent fiber (%) (0.035) and neutral detergent fiber (%) (0.021). A negative low indirect effect of plant height (cm) via leaf length (cm) (-0.139) was recorded. Similarly leaf breadth (cm) via leaf length (cm) recorded low negative indirect effect. The days to fifty percent flowering had low positive indirect effect *via* plant height (cm) (0.102) and stem girth (cm) (0.102) on green fodder yield (g/plant). Similar results were reported by Ramakrishnan *et al*. (2013) and Singh *et al*. (2019). The other traits like crude protein (%), crude fat (%), crude fiber (%), total ash (%), acid detergent fiber (%) and neutral detergent fiber (%) expressed low and negligible indirect effects through other traits.

Correlation and path analysis showed that due importance should be given for plant height (cm), stem girth (cm), number of crowns per plant and leaf stem ratio. Thus, the improvement of any of these traits would simultaneously improve green fodder yield because of correlated response of other traits. Hence, these traits may be considered while designing the selection index for improvement of green fodder yield in lucerne.

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