Indian J. Genet., **67**(2): 135-139 (2007)

Evaluation of Egyptian clover germplasm using multivariate analyses

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(Received: March 2006; Revised: April 2007; Accepted: August 2007)

Abstract

Principal factor and cluster analyses were carried out with 15 fodder and seed traits in 100 accessions of Egyptian clover (*Trifolium alexandrinum* L.). Principal factor analysis identified four Principal Components which explained about 69% variability. Varimax rotation enabled loading of similar type of variables on a common Principal Component permitting to designate them as fodder yield and seed yield factors. Genotypes GP 3, 19, 27, 28 and 29 were found to be better performers on the basis of principal factor scores with regard to fodder yield and seed traits when both the principal factors were considered together. These genotypes may further be utilized in breeding programmes for improving fodder and seed yield. Hierarchical cluster analysis resulted into ten clusters containing one to sixty two genotypes. The best clusters with regard to fodder and seed characters were C II, VII and IX. The results of cluster and principal factor analyses were in agreement.

Key words: Egyptian clover, germplasm, cluster, principal factor and variability

Egyptian clover (*Trifolium alexandrinum* L.), popularly known as berseem, is an important *rabi* fodder crop of northern India. It is widely accepted crop because of its multicut nature, high fodder yield and better nutritional quality. Quantum of breeding output in this crop is less due to certain inherent hindrances which primarily arise from the methods of pollination, seed setting, self sterility, maintenance of germplasm and presence of lesser extent of genetic variability. To break the yield plateau it is important to proliferate the genetic variability through hybridization, mutation and enriching the genetic stock by collecting more variable types. Moreover, whatever variation is available, it has not been thoroughly exploited due to its small and complex flowers which make artificial hybridization difficult. Therefore, broader range of variation is likely to be of more value in increasing the use of germplasm in breeding programmes of this crop as it will form the base material for further improvement. The importance of broad genetic base in evolving new cultivars by incorporating new genes in the existing ones is well recognized. Therefore, careful characterization and evaluation of germplasm for various characters using germane and precise methods like principal factor and cluster analyses is imperative. Hence, the present investigation was undertaken in Egyptian clover with the objectives of evaluation and classification of germplasm and to determine the degree of similarity among the genotypes and relative importance of the characters.

Materials and methods

The experimental material comprised a set of 100 accessions collected from different indigenous and exotic sources. This included 5 lines from Pakistan, 2 from Egypt, 1 from Chile, 4 from USA, 18 from Punjab, 38 from Haryana, 15 from Utter Pradesh and 17 from Madhya Pradesh. These lines were grown during *rabi* 2002-03 and 2003-04 in single rows of 4 m length at Forage Research Area, CCS HAU, Hisar. Recommended package of practices to raise a good crop was followed. Two cuts of the crop were taken and then the crop was left for seed production. First cut was taken 65 days after sowing and second cut was taken 35 days after the first cut. Observations were recorded on 5 random plants in each genotype on 15 quantitative variables *viz.*, plant height (cm), number of tillers/plant, number of leaves/plant, fresh leaf weight/plant (g), fresh stem weight/plant (g), leaf:stem ratio, green fodder yield/plant (g), per cent regeneration, dry leaf weight/plant (g), dry stem weight/plant (g), dry matter yield/plant (g), number of capsules/plant, number of seeds/capsule, seed yield/plant and 1000-seed weight (g). Averages of both the cuts were computed for the traits *viz.*, plant height, number of leaves/plant, leaf:stem ratio and per cent regeneration, whereas sum of both the cuts for rest of the traits except number of capsules/plant, number of seeds/capsule, seed yield/plant and 1000-seed weight were taken for further analysis. Data of per cent regeneration were angular transformed before going for analysis.

Data were pooled over the years after subjecting it to Bartlett's test and means of both the years were used for further analysis. Principal factor and cluster analyses were carried out using SPSS software package. Principal factor analysis was carried out using Principal Component method for factor extraction. The principal components with eigen roots more than one were retained. As the initial factors loading were not clearly interpretable, the factor axes were rotated using Varimax rotation. Principal factor scores were calculated using Anderson-Rubin method. Scatter plots were drawn using two main Principal factors in order to identify the most distinct and useful accessions in different clusters. UPGMA (Unweighted pair-group method using arithmetic averages) method of hierarchical cluster analysis was utilized with city block distances to classify all the 100 accessions and dendrogram was prepared using the rescaled distances. The dendrogram was cut to demarcate the clusters [1].

Results and discussion

Only the first four Principal Components (PC) showed eigen values more than one and cumulatively they explained 69.44 % variability. The first PC explained 35.16 % of the total variation and the second, third and fourth principal components explained 13.93, 11.23 and 9.13 % variation, respectively. The first one absorbed and accounted for maximum proportion of total variability in the set of all variables and the remaining ones accounted for progressively lesser and lesser amount of variation. Literature revealed no study of principal component or factor analysis in Egyptian clover. However, some work of similar type has been reported in white clover and red clover and similar trend was obtained and the most important variables were identified [2, 3, 4].

When the analysis without rotation of axes failed to load all the variables signifying that it could not offer much information regarding the idea of correlation between the variables and the Principal components, Varimax rotation was applied and this resulted in loading of all the variables on different Principal Components. The correlation values 0.6 between the traits and Principal components were considered for construeing the relationship between the traits and that principal factor. Factors' loadings of different variables thus obtained are presented in Table 1. The first principal factor (PF) indicated fodder yield related traits *i.e.*, fresh leaf weight/plant, fresh stem weight/plant, green fodder yield/plant, dry leaf weight/plant, dry stem weight/plant and dry matter yield/plant which were important contributors for variability and thus, it can be designated as fodder yield factor. The second PF was found to be having high loadings for number of capsules/plant, number of seed/capsule and seed yield/plant and hence, can be called seed factor. The third PF had plant height, number of tillers/plant and number of leaves/plant as the main variables and the fourth PF had the

Table 1. Factor loadings of different characters with respect to different Principal Factors (Varimax Rotation) in Egyptian clover

Sr. No.	Trait/Principal Factors	PF 1	PF 2	PF 3	PF 4
1	Fresh stem wt./plant	0.925*	0.065	0.002	–0.141
2	Dry matter yield/plant	0.911*	–0.033	0.167	–0.064
3	Fresh leaf wt./plant	0.863*	–0.052	–0.147	0.179
4	Dry stem wt./plant	0.855*	0.021	0.128	–0.130
5	Dry leaf wt./plant	0.787*	–0.158	0.170	0.102
6	Green fodder yield/plant	0.693*	0.245	0.284	–0.312
7	No. of capsules/plant	–0.023	0.970*	0.013	0.014
8	Seed yield/plant	–0.022	0.956*	–0.029	–0.009
9	No. of seeds/capsule	0.358	0.686*	–0.550	–0.447
10	No. of leaves/plant	0.495	0.140	0.735*	–0.115
11	No. of tillers/plant	0.297	0.138	0.728*	–0.035
12	Plant height	0.367	0.216	0.648*	0.3.20
13	Leaf:stem ratio	–0.027	0.027	0.085	0.726*
14	Per cent regeneration	0.063	0.037	–0.174	0.643*
15	1000-seed wt.	0.030	0.131	–0.268	0.603*

variables like leaf:stem ratio, per cent regeneration and 1000-seed weight. The analysis clearly indicated that fresh leaf weight/plant, fresh stem weight/plant, green fodder yield/plant, dry leaf weight/plant, dry stem weight/plant, dry matter yield/plant, number of capsules/plant, number of seeds/capsule and seed yield/plant were the main variability contributing traits which accounted for 49% variation out of the total variation (70%) explained.

The Principal Factor Scores (PF scores) obtained in the analysis, were used to plot all the genotypes using PF 1 and PF 2 (Fig. 1) which cumulatively explained 49% variability and accounted for the most important fodder and seed characters *viz.*, fresh leaf weight/plant, fresh stem weight/plant, green fodder yield/plant, dry leaf weight/plant, dry stem weight/plant, dry matter yield/plant, number of capsules/plant, number of seeds/capsule and seed yield/plant. The plot clearly separated the high fodder yielding genotypes *viz*,. GP 28 and 29 towards the positive plane of PF I axis, which is the factor for green and dry fodder yield. The genotypes, which were found to be high seed yielding, separated towards the positive portion of PF 2 (factor accounting for seed characters) axis in the plot and such genotypes were GP 41, 66, 85, 20, 42 and 84. The genotypes which found place towards the positive end of both the factors are supposed to be superior for these two factors and hence, superior for all the characters which both of these factors are defining and these were GP 19 and 64. From the foregoing discussion it can easily be concluded that these genotypes can be used as parents in hybridization/ polycross programme for the improvement of fodder and seed characters.

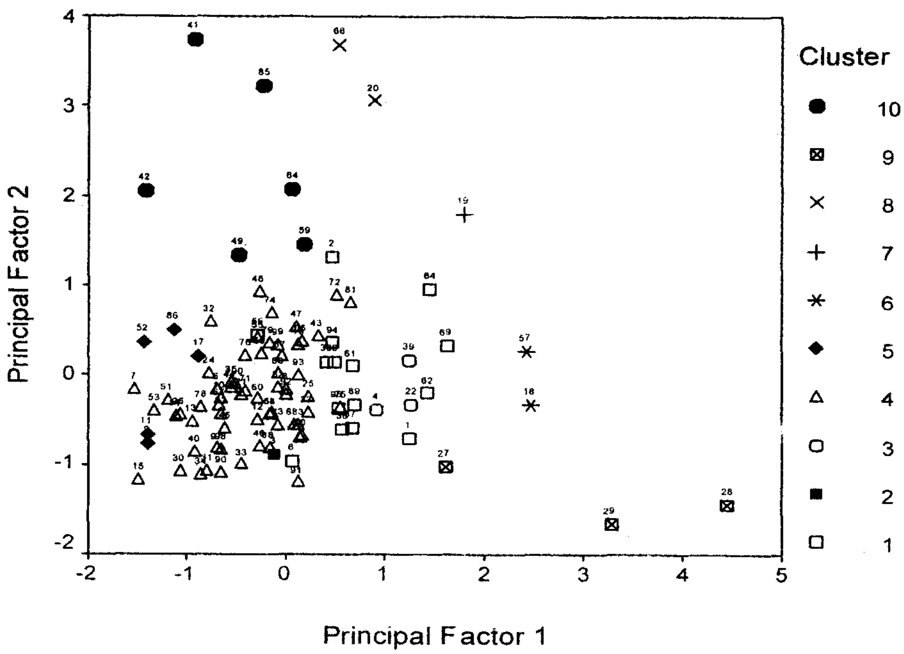

Fig. 1. Distribution of Egyptian clover genotypes based on Principal Factor 1 and 2

The UPGMA method with City Block distances in hierarchical cluster analysis divided the genotypes in 10 clusters (C). Cluster membership of different accessions is presented in Table 2. Maximum number of genotypes *i.e.*, 62 were grouped in Cluster IV (C IV) and minimum were grouped in C II and C VII which had only one genotype each. The clusters I, III, V, VI, VIII, IX and X comprised of 15, 3, 5, 2, 2, 3 and 6 genotypes, respectively. The relative association among the different genotypes is presented in the form of dendrogram (Fig. 2) which was prepared using the rescaled distances. The resemblance coefficient between the two genotypes is the value at which their branches join. The dendrogram elaborates the relative magnitude of resemblance among the genotypes as well as the clusters. No reference of cluster analysis could be found in Egyptian clover to corroborate the results, however, in red clover and white clover cluster analysis was used to group the accessions in different clusters [2, 5, 6].

No correspondence was observed between the geographical and genetic diversity in the present investigation. From clustering pattern it was found that genotypes from different geographic regions were grouped together in a cluster and vice-versa suggesting that geographical diversity does not necessarily represent genetic diversity and this may be due to free exchange of genetic material among different regions. This was in line with the results obtained earlier in Persian clover using D^2 analysis [7, 8]. Therefore, geographic diversity although important, was not the only factor responsible for determination of the genetic diversity.

The mean performance of different clusters calculated with regard to different characters revealed wide range of variation among the clusters. Cluster wise means and general mean for the characters studied are presented in Table 3. Perusal of this table revealed that C I, C IV, C V, C VIII and C X contained genotypes which exhibited poor to average performance for most of the fodder traits, however, C II and C VII, which contained only one genotype each, showed superiority for dry leaf weight, dry stem weight, dry matter yield, number of leaves and number of tillers per plant. Besides these C VII also performed well for seed characters like number of capsules/plant, number of seeds/capsule and seed yield/plant. Genotypes of C IX exhibited very high

Table 2. Cluster membership and number of genotypes in each cluster of Egyptian clover germplasm

Cluster	Genotype	No. of genotypes
C I	GP 1, GP 2, GP 6, GP 36, GP 37, GP 38, GP 56, GP 58, GP 61, GP 62, GP 64, GP 69, GP 89, GP 94 and GP 95	15
C II	GP 3	1
C III	GP 4, GP 22 and GP 39	3
C IV	GP 5, GP 7, GP 8, GP 10, GP 12, GP 13, GP 14, GP 15, GP 16, GP 21, GP 23, GP 24, GP 25, GP 26, GP 30, GP 31, GP 32, GP 33, GP 34, GP 35, GP 40, GP 43, GP 44, GP 45, GP 46, GP 47, GP 48, GP 50, GP 51, GP 53, GP 54, GP 55, GP 60, GP 63, GP 65, GP 67, GP 68, GP 70, GP 71, GP 72, GP 73, GP 74, GP 75, GP 76, GP 77, GP 78, GP 79, GP 80, GP 81, GP 82, GP 83, GP 87, GP 88, GP 90, GP 91, GP 92, GP 93, GP 96, GP 97, GP 98, GP 99 and GP 100	62
C V	GP 9, GP 11, GP 17, GP 52, GP 86	5
C VI	GP18 and GP 57	2
C VII	GP 19	1
C VIII	GP 20 and GP 66	2
C IX	GP 27, GP 28 and GP 29	3
C X	GP 41, GP 42, GP 49, GP 59, GP 84 and GP 85	6
Total		100

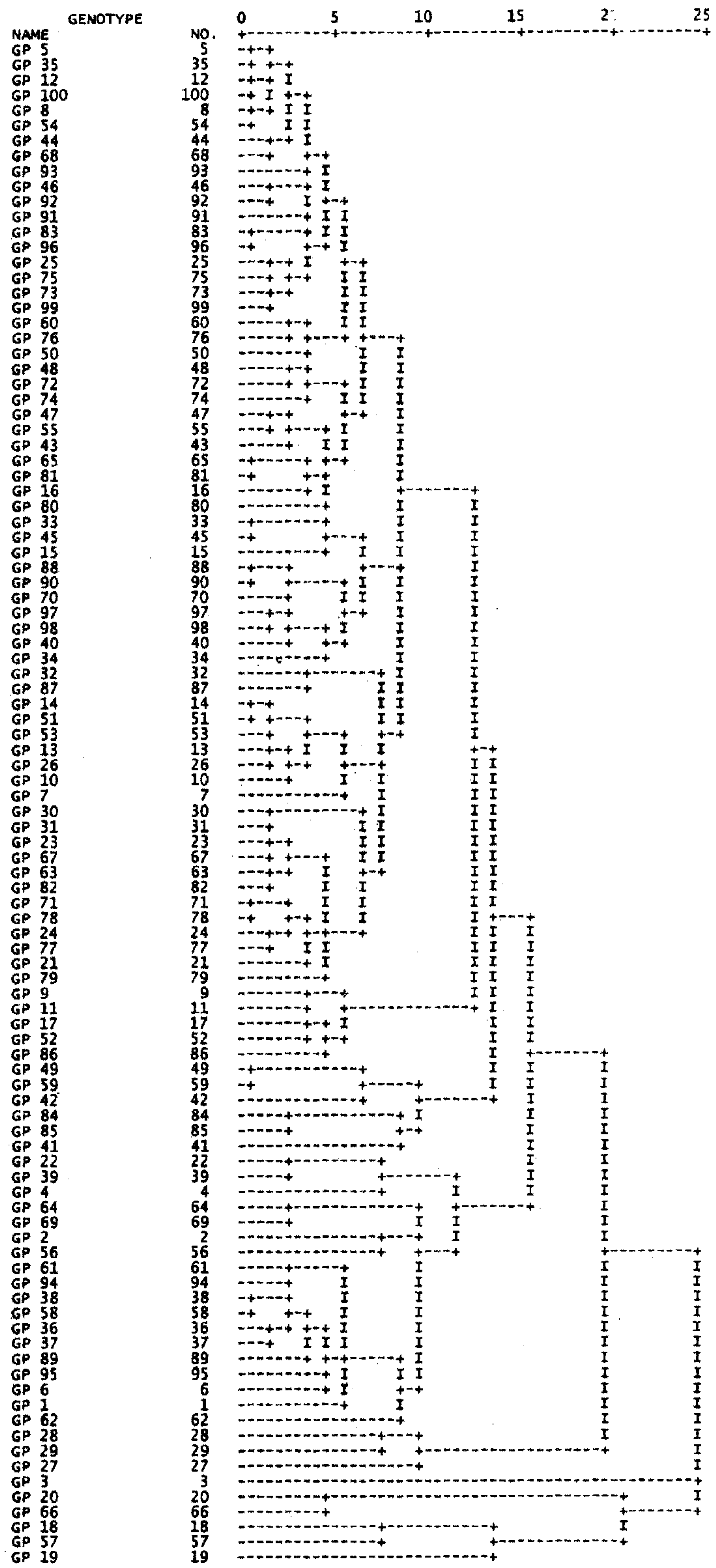

Fig. 2. Dendrogram showing the clustering pattern of different Egyptian clover genotypes

green and dry leaf and stem weights along with high dry matter yield and 1000-seed weight but poor green fodder yield which may be due to its lesser height, number of leaves and number of tillers. Plant height, number of leaves and number of tillers were more in C III and C VII which ultimately resulted in their high green fodder yield. Genotypes grouped in C VI also showed superior performance for plant height and fresh leaf and stem weight which got reflected in improved green and dry fodder yield. It also had more number of seeds/capsule but due to average 1000-seed weight its seed yield remained poor. Seed yield was maximum in the C VIII followed by C VII, both of which had more number of capsules/plant meaning thereby number of capsules/plant is more important trait than number of seeds/capsule as far as the seed yield is concerned.

The hybridization among diverse parents is likely to produce heterotic hybrids and desirable transgressive segregants in further generations. To assess the diversity inter and intra cluster distances were calculated which are presented in Table 4. The maximum inter cluster distance was found between C VII and C V followed by C VIII and C II, C V. and C VI and C VII and C II indicating that genotypes from these clusters can be usefully intercrossed. Based on the results of the present study, it is recommended to use GP 19 (C VII) and GP 3 (C II) as one of the parent for improving fodder yield and component traits.

The results of hierarchical cluster analysis and principal factor analysis confirmed the findings of each other. The plots of PF 1-PF 2 both accounting for 49 % cumulative variation (Fig. 1), revealed clear differentiation of genotypes according to their cluster membership. Genotypes grouped in a common cluster are denoted by same symbol. Genotypes belonging to a common cluster have fallen nearer to each other and *vice-versa* thereby, confirming the results of clustering. The genotypes like GP 3, 19, 27, 28 and 29, which were found superior using principal factor analysis were also

Table 3. Cluster means and general mean for different traits in Egyptian clover

S. No.	Trait/cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	General mean
1	Plant height	27.2	26.5	27.6	24.4	22.1	28.5	34.0	28.6	21.0	23.9	24.9
2	No. of tillers/plant	7.3	8.4	7.6	5.8	4.6	6.8	8.0	6.3	5.0	6.0	6.1
3	No. of leaves/plant	98.1	128.0	115.4	72.0	50.1	108.9	113.6	96.4	65.3	70.8	78.1
4	Fresh leaf wt./plant	12.3	3.4	15.3	9.3	6.7	17.5	15.0	15.0	29.0	9.7	10.7
5	Fresh stem wt./plant	28.0	5.7	34.2	19.7	11.5	51.2	44.3	34.1	45.9	18.1	22.7
6	Leaf:stem ratio	0.6	0.8	0.7	0.6	0.7	0.6	0.4	0.6	0.6	0.7	0.6
7	Green fodder yield/plant	40.2	10.3	49.7	29.1	18.0	68.5	59.3	49.2	26.8	28.0	32.0
8	Per cent regeneration	83.7	88.5	88.0	84.6	91.1	82.3	75.5	87.3	92.8	89.5	85.4
9	Dry leaf wt./plant	2.2	3.4	1.8	1.6	1.3	2.9	2.3	1.9	3.7	1.6	1.8
10	Dry stem wt./plant	4.9	5.7	5.3	3.3	2.0	5.2	6.7	3.7	6.9	2.9	3.7
11	Dry matter yield/plant	7.2	9.1	7.1	5.0	3.4	9.6	9.0	5.5	10.6	4.5	5.6
12	No. of capsules/plant	14.8	12.0	13.7	13.8	14.4	13.5	20.0	26.5	11.3	23.3	14.8
13	No. of seeds/capsule	54.0	30.7	48.0	52.3	46.4	60.2	61.3	57.4	58.7	48.7	52.2
14	Seed yield/plant	53.0	50.0	43.3	50.7	56.4	50.0	80.0	100.0	40.0	86.7	54.2
15	1000-seed wt.	2.5	2.3	2.5	2.6	2.6	2.6	2.5	2.6	2.8	2.7	2.6

Table 4. Inter and intra cluster distances (city block) in Egyptian clover

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X
I	54.70									
II	137.73	0.00								
III	74.85	146.45	47.93							
IV	86.61	144.52	125.14	55.76						
V	137.06	144.15	176.75	81.42	42.26					
VI	101.30	180.46	87.43	152.40	210.34	58.41				
VII	114.96	205.81	102.55	176.98	232.60	90.05	0.00			
VIII	104.36	215.29	119.81	148.29	193.40	136.77	95.71	37.48		
IX	130.67	202.34	138.79	109.88	135.04	147.61	193.47	184.59	63.98	
X	121.81	179.17	169.24	88.37	100.54	76.19	164.95	109.48	154.24	57.35

Diagonal values are intra-cluster distances

found to be members of the best performing clusters *i.e.*, C II, C VII and C IX. Such confirmatory results were also obtained in green gram and white clover [2, 9]. Therefore, it is suggested that in the absence of more precise methods, it is necessary to use more than one method to offset the limitations to a certain extent.

With the help of the present study the Egyptian clover germplasm accessions have been classified based on various fodder and seed characters and large number of variables have been reduced into four Principal Factors only. This multivariate analysis has enabled to identify various genotypes promising for different combinations of characters. The results of the present study will be useful in improving the understanding of diversity within a collection which can be put to a better use for evolving well defined approach in Egyptian clover.

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