Phenotypic Variability among the Germplasm Lines of Egyptian Clover (*Trifolium alexandrinum* L.)

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Egyptian clover or *Berseem* (*Trifolium alexandrinum*) is a widely adapted good nutritious annual fodder crop. To estimate the genetic variability, 598 lines of *berseem* were evaluated using augmented block design. The material included released varieties, advanced breeding lines, induced tetraploid lines and ecotypes. Although wide range of variation was observed for most of the traits, a closer look indicated that the majority of the accessions centered around the mean leaving little number of accessions on the extremes. The frequency distribution was observed to be symmetrical and unimodal for most of the traits indicating thereby a continuous variation among the population. Similar trend was observed for floral and seed characters. Marked differences were observed among the diploid and tetraploid genotypes for foliar traits such as leaflet shape, size, margin and surface hairiness. The study indicated that the germplasm had derived from narrow genetic base. Promising lines were identified which have high value for particular traits and can be exploited in genetic improvement programme.

Key Words: Genetic variability, *Berseem*, *Trifolium alexandrinum*, Germplasm evaluation

Plant Genetic Resource (PGR) which is the backbone of crop improvement programme has to be given greater importance with the changing scenario due to Intellectual Property Right (IPR) regime under the TRIPS and the National Biodiversity Act. In this context, it has become important to evaluate and document all the germplasm available in the country.

The genus *Trifolium* commonly called clovers comprises of 237 annual and perennial species (Zohary and Heller, 1984) out of which 25 species are agriculturally important as cultivated and pasture crops (Lange and Schifino-Wittmann, 2000). Egyptian clover, popularly known as *Berseem* (name derived from Arabic name 'Bersym' or 'Berzum') is believed to be indigenous to Egypt. Based on the branching behaviour and regeneration potential, three ecotypes of *Berseem* i.e., Mescavi, Fahli and Saidi are reported from Egypt. Out of the two ecotypes `Mescavi' and `Fahli' introduced in India during 1903, the former proved to be highly adaptable because of its multicut nature and is cultivated in approximately 2 million ha in India.

The present work was undertaken to estimate the extent of variability among the existing germplasm lines, maintained at Indian Grassland and Fodder Research Institute, Jhansi, which includes old introductions, its derivatives, selections and mutants.

Material and Methods

Seeds of 598 lines of Egyptian clover (*Trifolium alexandrinum*) including germplasm lines and advance breeding lines were grown in augmented block design in 3 m row plots with row to row distance of 50 cm and plant to plant distance of 5 cm. In total, there were 11 blocks consisting of 58 lines in each block. Wardan, JHB 146, BL 122 and HFB 155 were used as checks in each block and their position was randomized. Sowing was done in the third week of October and green fodder yield data recorded for three cuttings whereas morphological data was recorded on three randomly selected plants at 50 per cent flowering stage and for seed characters at maturity. Plant height was recorded in cm each before second cut, third cut and lastly at 50 per cent flowering stage. The other observations recorded were number of nodes per tiller, internode length between fourth and fifth node, petiole length, leaflet size, occurrence of multifoliate leaves, leaf colour, leaflet margin, leaf hairiness, stipule hairiness, stipule fused and free length and stipule total length, stipule width, total green fodder yield (GFY) per 3 m row recorded during second, third and fourth cut, date of flowering initiation, date of 50 per cent lowering, peduncle length, flower colour, number of flower whorls in a head from base to the top, number of flowers in the first whorl at maturity, date of 50 per cent pod maturity, length and width of the flower head at maturity, seeds/ inflorescence and 1000 seed weight. The mean values of meteorological data of the crop growing period is given in Table 1.

Results and Discussion

The data recorded on various morphological traits and yield are presented in the Tables 2-4 in the form of

Table 1. Mean Values of meteorological data during the years 1996-97

Months	Temperature °C		Relative humidity (%)		Rainfall (mm)	Bright sunshine hrs/day
	Max	Min	1st	2nd		
October	31.0	17.3	92	45	105.7	7.5
November	28.5	9.4	94	27	–	9.4
December	23.5	4.0	96	30	–	8.6
January	22.3	5.3	95	36	–	7.7
February	27.3	8.3	91	32	–	9.9
March	31.3	13.9	86	29	0.5	8.0
April	36.1	18.3	65	26	21.4	9.5
May	39.7	22.9	54	30	17.0	10.1

frequency distribution. For numerical traits the mean value and the maximum and minimum values, kurtosis, standard deviation, skewness and variance have also been presented in Tables 3 and 4. As observed on the basis of leaflet shape and particularly leaflet apex, out of 598 accessions, 329 showed homogeneous populations and 269 heterogeneous populations.

Plant Height and Node Character

The perusal of data revealed that the minimum plant height during three cutting stages ranged between 28 to 37.44 cm and the maximum height ranged from 71.68 to 123.93 cm. Most of the accessions during first and second cut grouped in 41 - 60 cm class whereas in third cut stage, the variation for plant height was observed to be narrowed down and most of the accessions were present in 55-75 cm class. Number of nodes and internodal length recorded during third cut stage ranged from 6.06 to 15.2 and 3.92 to 14.96 respectively but most of the accessions grouped in 6 - 11 nodes class and 6 - 12 cm internodal length.

The total green fodder yield per three meter row over three cuts ranged from 0.32 to 17.02 kg with an average value of 6.80 kg but most of the accessions yielded in the range of 0.32 to 10 kg. Twenty three accessions were identified as high yielders with forage yield of more than 23 kg (Table 5).

Leaf Characters

Petiole length varied from 1.02 cm to 4.54 cm and accessions were distributed more or less uniformly in different classes i.e., <2, 2.1 – 3.0, 3.1 - 4.0 cm with maximum number present in 2.1 - 3.0 cm class. Leaflet length varied from 2.33 to 5.45 cm and most of the accessions grouped in two middle classes. Accessions with too small and too large leaves were less in number. It was invariably observed that the tetraploid accessions possessed bigger leaflets. Similar trend was observed for leaflet width which ranged from 0.79 to 2.35 cm and most of them belonged to 1.0 to 2.0 cm group (Tables 3 and 4).

Leaflets of diploid accessions were entire whereas that of tetraploid accessions were serrate. On visual performance basis, 303 accessions were found to possess medium size leaves. Multifoliate nature of leaves was evident from the presence of pentafoliate, quadrifoliate, heptafoliate and trifoliate leaves in different combinations on the same branch of single plant. A total of 266 accessions were found to possess only trifoliate leaves whereas 234 accessions possessed around 10 per cent plants with multifoliate leaf. Some accessions were observed to have as high as 80 per cent plants multifoliate leaves. The findings are in conformity with an earlier report (Shukla and Malaviya, 1988) accompanying genetic explanation of pentafoliate leaves in greater frequency derived in nature. The leaf colour was recorded as dark, light and medium green. Most of the accessions were light green followed with dark green leaves. The hairiness on upper surface of the leaflets was also variable. A few accessions showed scant hairs whereas maximum accessions (406) were medium hairy and 173 accessions were marked for dense hairiness and most of them were tetraploid in nature. Thus, leaflet shape, size, margin and hairiness were distinguishing characters for diploid and tetraploid accessions.

Stipule length ranged from 1.29 to 3.12 cm and the width ranged from 4.2 to 10.08 mm. In most of the accessions, the stipule length varied from 1.5 to 2.5 cm and width ranged from 5.1 to 9 mm. Stipule hairiness was dense among 419 accessions, medium among 159 accessions and scant among 20 accessions.

Floral and Seed Characters

The initiation of flowering in 10 accessions belonging to Saidi group or its derivative was seen before 28 February. Sixty eight lines were early in flowering i.e. flower initiation noticed between March 1 to 15 and 488 lines

Table 2. Morphological traits observed in *Berseem* accessions

Character	Accessions (No.)
Population	
Homogeneous	329
Heterogeneous	269
Leaf size	
Bold	75
Medium	303
Small	146
Very bold	74
% Plants with multifoliate leaves	
All trifoliate	266
1-10	234
11-20	70
21-30	37
31-40	13
41-50	14
51-60	6
61-70	9
71-80	4
Date of 50% flowering	
Type of leaf	
Hepta/penta/tri	7
Penta/tri	204
Penta/hepta	5
Penta/hepta/tri	2
Fused	1
Penta/tri/hepta	3
Quadri/tri	4
Quadri/penta/tri	106
Trifoliate	266
Leaf colour	
Dark green	151
Medium green	118
Light green	329
Leaflet margin	
Entire	427
Serrate	171
Leaf hairiness	
Dense	173
Medium	406
Scant	19
Stipule hairiness	
Dense	419
Medium	159
Scant	20
Date of flowering initiation	
Before Feb 28	10
March 1-15	68
51-60	6
61-70	9
71-80	4
Date of 50% flowering	
Before Feb 28	6
March 1-15	8
March 16-31	156
April 1-15	427
April 16-30	1
Flower colour	
White	549
Pink/red	49
Trifoliate	266
Date of maturity	
Before March 31	7
April 1-15	434
April 16-30	157

between March 16 to 31. Only 32 accessions were late in flowering i.e. between April 1 to 15. Similarly 50 per cent flowering was noticed in 427 accessions between April 1 to 15 followed with 156 accessions showing 50 per cent flowering between March 16 to 31. Majority of the accessions showed 50 per cent pod maturity between April 16 to 30.

A total of 549 accessions were noticed for white flowers whereas 49 accessions were noticed for different shades of pink/red (Table 2). Beri (1983) in his study reported the purple flower be single gene governed character but in the present study different shades of pink flower indicated involvement of more than one gene. High degree of variation for peduncle length was seen which ranged from 1.64 to 9.92 cm but majority of them were ranging from 2.6 to 7.5cm.

Limited diversity was observed for the number of flowers in first whorl and the number of whorls. In majority of accessions, 6.6 to 8.5 flowers were seen in the first whorl and the number of flower whorls per head ranged from 7.1 to 11. Pod length also did not vary much and ranged between 1.29 to 2.98 cm. Most of the diploid accessions were grouped in 1.6 to 2.0 cm class and tetraploid accessions in 2.1 to 2.5 cm class. Seeds per head varied considerably. Although the maximum number of accessions were observed to have 51 to 75 seeds per head, good number of accessions showed less than 25 and more than 75 seeds per head also. 1000 seed weight ranged from 1.80 to 4.12 g with majority of them ranging between 2.1 to 3.5 g. For, this character too, the tetraploid accessions showed higher seed weight.

In the present study, the characters like plant height at third cutting stage, internode length, leaflet length, leaflet width, stipule fuse length, stipule free length, stipule total length, stipule width, peduncle length, number of whorls, flowers in last whorl, pod length and seeds/ inflorescence showed unimodal type of frequency distribution showing that the variation was of continuous nature and the majority of the accessions grouped near mean value of the population. Plant height recorded during the first two cuts and the total green fodder yield showed amodal type of frequency distribution (Tables 3, 4). The accessions having higher values for various traits are presented in Table 5.

Although the crop suffers from lack of genetic variability, still there are reports on occurrence of variability (Yadav *et al.*, 1974; Sidhu and Mehndiratta, 1976) but Shukla and Patil (1985) indicated that in such

Table 3. Variation in morphological traits observed in 598 accessions of *Berseem*

	Plant height 1 (cm)	Plant height 2 (cm)	Plant height 3 (cm)	Number of nodes	Internode length	Total Green Fodder Yield (kg)	Petiole length (cm)
Average	54.31	58.25	66.27	8.61	9.38	6.80	2.48
Max	87.98	71.68	123.93	15.20	14.96	17.02	4.54
Min	31.23	28.90	37.44	6.06	3.92	0.32	1.02
Kurtosis	0.86	2.59	15.50	3.80	1.56	1.06	-0.07
SD	8.66	5.35	7.56	0.99	1.45	2.07	0.67
Skewness	0.37	-0.76	2.69	1.08	-0.25	-0.32	0.49
CV%	15.94	9.10	11.40	11.55	15.48	30.42	27.04

	Leaflet length (cm)	Leaflet width (cm)	Stipule fuse length (cm)	Stipule free length (cm)	Stipule total length (cm)	Stipule width (mm)	Peduncle length (cm)
Average	3.83	1.34	1.07	0.93	2.00	6.34	4.90
Max	5.45	2.35	1.53	1.81	3.12	10.08	9.92
Min	2.33	0.79	0.54	0.22	1.29	4.20	1.64
Kurtosis	0.18	0.66	-0.11	0.74	0.22	1.22	0.40
SD	0.48	0.26	0.18	0.22	0.31	0.81	1.19
Skewness	0.17	0.97	0.15	0.21	0.57	0.67	0.35
CV%	12.52	19:74	16.18	24.04	15.81	12.71	24.31

	No. of whorls	Flowers in last whorl	Pod length (cm)	Pod width (cm)	Seeds/ inflorescence	1000 seed wt (g)
Average	8.10	7.42	1.94	1.44	55.10	2.76
Max	11.47	8.91	2.94	1.98	115.48	4.12
Min	5.65	6.06	1.29	1.07	5.63	1.80
Kurtosis	0.31	-0.29	0.41	0.52	0.13	-0.85
SD	0.90	0.50	0.26	0.15	19.19	0.45
Skewness	0.30	0.03	0.53	0.33	-0.62	0.61
CV%	11.11	6.73	13.63	9.82	34.82	16.60

Table 4. Frequency distribution for different traits in *Berseem*

Plant height 1		Plant height 2		Plant height 3		Total Green Fodder Yield	
Class	Accessions	Class	Accessions	Class	Accessions	Class	Accessions
<45	81	<41	4	<55	16	<5.0	107
46-60	367	41-50	32	55-75	553	5.1-10.0	368
61-75	140	51-60	335	76-95	19	10.1-15.0	22
>75	10	>60	214	96-105	3	>15.0	1
				>105	7		

Number of nodes		Internode length		Petiole length		Leaflet length		Leaflet width	
Class	Accessions	Class	Accessions	Class	Accessions	Class	Accessions	Class	Accessions
<8	165	<6	17	<2	147	<3	19	<1.0	18
8-11	419	6-9	199	2.1-3.0	331	3.1-4.0	367	1.0-1.5	446
12-14	13	10-12	364	3.1- 4.0	106	4.1-5.0	203	1.6-2.0	124
>14	1	>12	18	>4	14	>5	9	>2.0	10

Stipule fuse length		Stipule free length		Stipule total length		Stipule width		Peduncle length	
Class	Accessions	Class	Accessions	Class	Accessions	Class	Accessions	Class	Accessions
<0.75	19	<0.5	19	<1.5	21	<5.0	12	<2.5	10
0.75-1.0	212	0.5-1.0	360	1.5-2.0	316	5.1-7.0	470	2.6-5.0	323
1.1-1.25	275	1.1-1.5	212	2.1-2.5	222	7.1-9.0	113	5.1-7..5	254
1.26-1.5	86	>1.5	7	2.6-3.0	36	>9	3	>7.5	11
>1.5	6			>3.0	3				

No. of whorls		Flowers in last whorl		Pod length		Seeds/inflorescence		1000 seed weight	
Class	Accessions	Class	Accessions	Class	Accessions	Class	Accessions	Class	Accessions
<7.0	60	<6.5	18	<1.5	19	<25	72	<2.0	2
7.1-9.0	448	6.6-7.5	311	1.6-2.0	368	26-50	97	2.1-2.5	244
9.1-11.0	88	7.6-8.5	265	2.1-2.5	193	51-75	369	2.6-3.0	162
>11.0	2	>8.5	4	>2.5	18	76-100	55	3.1-3.5	156
						>100	5	3.6-4.0	32
								>4.0	2

Table 5. Selected genotypes possessing higher values for different traits

Character	Criteria	Accession number
Plant height at 50% flowering	>100 cm	IL2001-355, IL2001-356, IL2001-357, IL2001-358, IL2001-359, IL2001-361, IL2001-362, IL2001-364, IL2001-583
Node number	>12	IL2001-356, IL2001-358, IL2001-359, IL2001-360
Internode length	>12 cm	IL2001-1, IL2001-2, IL2001-3, IL2001-6, IL2001-8, IL2001-10, IL2001-11, IL2001-13, IL2001-19, IL2001-20, IL2001-22, IL2001-26, IL2001-28, IL2001-379, IL2001-459, IL2001-461, IL2001-463, IL2001-579
Total Green Fodder Yield	>10 kg/3m row	IL2001-311, IL2001-330, IL2001-332, IL2001-334, IL2001-335, IL2001-345, IL2001-346, IL2001-347, IL2001-354, IL2001-382, IL2001-384, IL2001-385, IL2001-386, IL2001-403, IL2001-427, IL2001-532, IL2001-533, IL2001-534, IL2001-535, IL2001-536, IL2001-559, IL2001-567, IL2001-573
Petiole length	>4 cm	IL2001-119, IL2001-198, IL2001-252, IL2001-286, IL2001-292, IL2001-294, IL2001-305, IL2001-313, IL2001-341, IL2001-362, IL2001-392, IL2001-437, IL2001-443, IL2001-450
Leaflet length	>5 cm	IL2001-183, IL2001-219, IL2001-221, IL2001-227, IL2001-281, IL2001-325, IL2001-327, IL2001-329, IL2001-376
Leaflet width	>2 cm	IL2001-219, IL2001-223, IL2001-232, IL2001-235, IL2001-239, IL2001-259, IL2001-290, IL2001-298, IL2001-299, IL2001-326
Stipule length total	>3 cm	IL2001-219, IL2001-246, IL2001-249
Stipule width	>9 mm	IL2001-333, IL2001-388, IL2001-474,
Peduncle length	>8.5 cm	IL2001-554, IL2001-570
No. of whorls	>10	IL2001-272, IL2001-284, IL2001-285, IL2001-287, IL2001-291, IL2001-295, IL2001-296, IL2001-308, IL2001-320, IL2001-377, IL2001-521
Flowers in first whorl	>8.5	IL2001-87, IL2001-179, IL2001-570, IL2001-584
Pod length	>2.5 cm	IL2001-26, IL2001-230, IL2001-272, IL2001-276, IL2001-284, IL2001-286, IL2001-287, IL2001-288, IL2001-289, IL2001-291, IL2001-295, IL2001-308, IL2001-310, IL2001-311, IL2001-315, IL2001-318, IL2001-322, IL2001-486
Pod width	>1.75 cm	IL2001-116, IL2001-196, IL2001-253, IL2001-273, IL2001-286, IL2001-299, IL2001-324, IL2001-342, IL2001-343, IL2001-366, IL2001-372, IL2001-375, IL2001-446, IL2001-450, IL2001-574, IL2001-584
No. of seeds/inflorescence	>100	IL2001-271, IL2001-298, IL2001-300, IL2001-321, IL2001-574
1000 seed weight	>3.75 g	IL2001-180, IL2001-280, IL2001-285, IL2001-289, IL2001-322, IL2001-323, IL2001-445, IL2001-449, IL2001-589
Seeds/inflorescence and 1000 seed weight	>80&>3 g	IL2001-59, IL2001-296, IL2001-298, IL2001-300, IL2001-307, IL2001-318, IL2001-321, IL2001-438, IL2001-563, IL2001-574, IL2001-584, IL2001-589
Pod length and Pod width	>2.5 cm&1.5 cm	IL2001-26, IL2001-272, IL2001-276, IL2001-284, IL2001-286, IL2001-289, IL2001-295, IL2001-308, IL2001-310, IL2001-311, IL2001-315, IL2001-318, IL2001-486
Leaflet length and Leaflet width	4.5 cm&1.75 cm	IL2001-219, IL2001-220, IL2001-221, IL2001-223, IL2001-224, IL2001-225, IL2001-226, IL2001-227, IL2001-239, IL2001-246, IL2001-252, IL2001-298, IL2001-299, IL2001-325, IL2001-329
Green fodder yield and plant height	>8 kg/3m row &>75 cm	IL2001-564, IL2001-577, IL2001-582, IL2001-586, IL2001-587

studies it was important to ascertain the substantial proportion of genetic variability in the gross variation, wider phenotypic range of variation and normal distribution of the data on one hand and the minimum G x E interaction on the other. The differential agronomic inputs and cutting management systems by and large may change the phenotypic performance.

Variability study conducted by Yadav *et al.,* (1974) indicated genotypic and phenotypic coefficient of variation to be moderate for number of secondary branches and low for others. Sindhu and Mehndiratta (1976) observed genetic differences for forage yield and its components viz., plant height, tiller number, leaf number, leaf length and leaf width. Beri (1983) also reported genetic variability for plant height, stem thickness, tiller number, secondary branches, leaflet length and breadth, green fodder yield, dry leaf weight, dry matter yield, leaf stem ratio, days to flowering, days to maturity, stem rot incidence and crude protein content.

The values of skewness were found to have a substantially low magnitude for the number of flowers in the last whorl, leaflet length and the stipule fused length, which indicated a marginal deviation of modal value from the mean. The respective low value of kurtosis revealed

a platycurtic curve. The values of kurtosis, predominately, were within the range of ± (<3) sketching platykurtic curve except for plant height and number of nodes where a high value of kurtosis was observed. This indicated that the plant height and number of nodes would show a leptokurtic curve meaning thereby less variability in the population besides a broad range of individual means. This is warranted with the respective low values of coefficients of variance (Table 3). The platycurtic curves within the limitations of skewness, otherwise, assure better distribution of individuals yielding a greater variance. The exploitable level of variability, however, may hardly be affirmed.

Most of the characters studied belonged to quantitative trait group. However, the characters like presence of multifoliate plants in population, heterogeneity for leaf shape and flower colour can be considered as qualitative traits and presence of intra-accession variation for these traits expectedly, appeared to be a resultant of out crossing in the population. The species is reported to be cross pollinated by Hossanein (1953), Latif *et al.* (1956) and Narayanan *et al.* (1961) whereas self pollinated by Bogdan (1966), Chowdhary *et al.* (1966) and Beri *et al.* (1985). However, in a recent study existence of different breeding populations in relation to mode of pollination has been reported (Roy *et al.*, 2005). In the present study, out of 598 accessions, 269 were recorded for heterogeneous nature *i.e.*, plants with varying leaflet shape particularly the leaflet tip, 332 accessions showed presence of different percentage of multifoliate plants and 49 accessions with varying shades of pink/red flowers in the population.

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