

GENETIC DIVERGENCE STUDIES IN FODDER COWPEA (*VIGNA UNGUICULATA*) USING D² STATISTICS

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SUMMARY

Thirty genotypes of fodder cowpea were evaluated in field during the kharif season of 2016 for fourteen characters at College of Agriculture, Kerala Agricultural University, Vellayani, Kerala, India. Based on D² analysis, these genotypes were grouped into eleven clusters. Cluster analysis revealed that cluster I (10 genotypes) and cluster II (5 genotypes) were the largest groups, followed by cluster III (4 genotypes) and cluster IV (4 genotypes). All other clusters from cluster V to cluster XI had only one genotype each. The maximum intra-cluster D² value was shown by cluster IV (146.57), followed by cluster I (127.52), cluster II (101.49) and cluster III (55.47). Highest inter-cluster D² values among genotypes existed between cluster VIII and cluster X (1559.98), followed by cluster VIII and cluster XI (1480.33), cluster VIII and cluster II (1367.65), and cluster VIII and cluster IV (1309.08). Minimum inter-cluster D² values among genotypes existed between cluster XI and cluster IX (160.10) followed by cluster I and cluster III (164.51), cluster XI and cluster IV (167.47). The analysis clearly shows the existence of significant difference between the 30 genotypes under study for the 14 characters. Hence selection of suitable parents for further breeding programmes based on the requirement can be done from the available gene pool.

Key words : Genetic divergence, fodder cowpea, *Vigna unguiculata*, D² statistics

Livestock sector plays a crucial role in rural economy and livelihood. As per 19th Livestock census, 2012 (GOI, 2014) India's livestock sector is one of the largest in the world with a holding of 11.6% of world livestock population. Global market for animal products is expanding fast, and it is an opportunity for India to improve its participation in global market. The nutritive value of feed and fodder has a significant bearing on productivity of livestock. Though the availability of feed and fodder has improved in the last decade, a lot is still required to bridge the gap between the demand and availability of fodder in the country, particularly during the lean periods and crisis situations. Straw from rice, barley, wheat, sorghum etc. are widely used in feeding ruminants. Their protein content is zero and their energy content low because of their largely lignified cell-walls. Rice or paddy straw has a high silica content in the cell walls which makes it difficult to digest. Legumes provide potential to enhance forage quality of grass (Pitman, 2011). Protein is required for growth, tissue repair and milk production among other things. Good sources of protein are leguminous forage, grain and oil-seed-cakes. Also for better health and yield of milk, livestock requires a balanced diet of three parts of

green grass and one part of leguminous fodder (Vendramini *et al.*, 2012; Sollenberger *et al.*, 2009). Hence the cultivation of fodder legumes is very important.

Cowpea (*Vigna unguiculata* L. Walp.) is a self-pollinating annual herbaceous legume belonging to the family Fabaceae which originated in West Africa (2n=22). It is grown for vegetable, grain, as fresh cut and carry forage, and for hay and silage (Roy *et al.*, 2016). It can be grown throughout the year and suitable for inter, mixed and relay cropping systems. It has a narrow genetic base (Asare *et al.*, 2010). This legume is well known for its inherent abilities like shade tolerance, drought tolerance, quick growth, rapid ground cover and protein content (Fatokun *et al.*, 2009). As a fodder crop, its short duration and multicut nature (KAU, 2015) makes it attractive to farmers. It requires very few inputs, as the plants root nodules are able to fix atmospheric nitrogen. The whole plant is used as an important nutritious legume for livestock (Singh and Tarawali, 1997). The nutritive value of cowpea leaves and haulms is very high. The crude protein content ranges from 22 to 30% in the grain and leaves (Bressani, 1985; Nielsen *et al.*, 1997) and from 13 to 17% in the haulms with high digestibility

(Tarawali *et al.*, 1997) while fiber content is about 6% (Bressani, 1985).

The evaluation of variability present in the available fodder cowpea germplasm is inevitable for classification and further improvement of the crop for fodder purpose. This study was undertaken for studying the available variability and divergence for selection of superior parents for hybridization for further improvement of yield and quality.

MATERIALS AND METHODS

The experimental material comprised of 30 genotypes of fodder cowpea (Table 1), collected from various parts of India. These genotypes were selected from available germplasm maintained in experimental fields of State Agricultural Universities, AICRPs and from local markets. The field experiment was carried out during *Kharif* season 2016 at College of Agriculture, Vellayani, Kerala Agricultural University, Kerala, India. Geographically Vellayani is situated in the tropics at 8.52°N latitude and 76.93°E longitude

with an altitude of 35 meters above the mean sea level.

The experiment was carried out in RBD with three replications. Each genotype was sown in raised beds of 4.5m² with 30cm x 15cm spacing in between rows and with in rows respectively. The recommended cultural and agronomic practices according to Package of Practices of KAU were followed to raise crop. During the experimental period weather parameters were also recorded.

For recording of observations five competitive plants of each genotype were randomly selected in each replication excluding the border plants. Observations were recorded on the characters, namely, plant height (cm), number of primary branches per plant, number of leaves per plant, days to first flowering, days to 50% flowering, leaf area index, green fodder yield per plant (g), dry matter yield per plant (g), leaf fresh weight per plant (g), stem fresh weight per plant (g), leaf dry weight per plant (g), stem dry weight per plant (g), crude protein content (mg/g) and crude fiber content (mg/g). The D² analysis was done by using the method as suggested by Mahalanobis

TABLE 1
List of genotypes evaluated during experiment

S. No.	Treatments	Accessions	Source/Origin
1.	T ₁	CO - 9	TNAU, Coimbatore
2.	T ₂	CO - 8	TNAU, Coimbatore
3.	T ₃	Vellayani-1	College of Agriculture, Vellayani
4.	T ₄	MFC - 09 - 1	AICRP on forage crops, Mandya
5.	T ₅	MFC - 08 - 14	AICRP on forage crops, Mandya
6.	T ₆	EC - 394779	AICRP on forage crops, Mandya
7.	T ₇	EC - 458489	AICRP on forage crops, Mandya
8.	T ₈	EC - 4216	AICRP on forage crops, Mandya
9.	T ₉	KBC - 2	UAS, Karnataka
10.	T ₁₀	IC - 1061	AICRP on forage crops, Mandya
11.	T ₁₁	IC - 1071	AICRP on forage crops, Mandya
12.	T ₁₂	IC - 9883	AICRP on forage crops, Mandya
13.	T ₁₃	IC - 25105	AICRP on forage crops, Mandya
14.	T ₁₄	IC - 39916	AICRP on forage crops, Mandya
15.	T ₁₅	IC- 97767	AICRP on forage crops, Mandya
16.	T ₁₆	IC - 201095	AICRP on forage crops, Mandya
17.	T ₁₇	IC - 202777	AICRP on forage crops, Mandya
18.	T ₁₈	IC - 202781	AICRP on forage crops, Mandya
19.	T ₁₉	IC - 202804	AICRP on forage crops, Mandya
20.	T ₂₀	IC - 253251	AICRP on forage crops, Mandya
21.	T ₂₁	IC - 402090	AICRP on forage crops, Mandya
22.	T ₂₂	IC - 402101	AICRP on forage crops, Mandya
23.	T ₂₃	IC- 402154	AICRP on forage crops, Mandya
24.	T ₂₄	IC - 402162	AICRP on forage crops, Mandya
25.	T ₂₅	IC - 458485	AICRP on forage crops, Mandya
26.	T ₂₆	IC - 394779	AICRP on forage crops, Mandya
27.	T ₂₇	IT - 38956-1	AICRP on forage crops, Mandya
28.	T ₂₈	IT - 37154999-38	AICRP on forage crops, Mandya
29.	T ₂₉	Pant Lobia - 2	College of Agriculture, Pantnagar
30.	T ₃₀	KBC - 5	UAS, Karnataka

458489, KBC-2, IC-39916, IT-37154999-38, Pant Lobia-2 and KBC-5 came in clusters V, VI, VII, VIII, IX, X and XI respectively.

(1936) and elaborated by Rao (1952); Murty and Arunachalam (1966). The character contribution towards genetic divergence was computed using the method given by Singh and Chaudhary (1977).

RESULTS AND DISCUSSION

Grouping of genotypes

The 30 fodder cowpea genotypes were grouped into 11 clusters (Table 2) using Toucher's method (Rao 1952) employed on the Mahalanobis's (D^2) values. The grouping of genotypes into different clusters revealed that cluster I was the largest group (10 genotypes) followed by cluster II (5 genotypes), cluster III and IV (4 genotypes), clusters V, VI, VII, VIII, IX, X and XI (1 genotype). The genotypes included in cluster I were CO - 8, EC - 394779, EC - 4216, IC - 9883, IC - 25105, IC- 97767, IC - 201095, IC - 202781, IC - 402101 and IC - 394779, where as in cluster II were Vellayani - 1, MFC - 09 - 1, IC - 1071, IC - 202804 and IC - 458485. The clusters III and IV had MFC - 08 - 14, IC - 202777, IC - 402090, IC - 402154 and IC - 1061, IC - 253251, IC- 402162, IT - 38956-1, respectively. Genotypes CO-9, EC-

The cluster analysis using Toucher's method grouped 30 fodder cowpea genotypes into six non-overlapping clusters. The grouping of germplasm lines into different clusters indicated presence of substantial amount of diversity in the material evaluated. Previous workers have also reported high degree of genetic diversity in fodder cowpea accessions (Lodhi *et al.*, 1990; Roquib and Patnaik, 1990; Sharma and Singhania, 1992; Sharawy and El-Fiky, 2002; Radhika, 2003; Omokanye *et al.*, 2003; Malarvizhi *et al.*, 2005; Sheela and Gopalan, 2006; Adeyanyu, 2009; Thaware *et al.*, 1991; Noubissié *et al.*, 2011). Presence of

substantial genetic divergence among the genotypes screened in present investigation suggested that this material might serve as good source for selecting the diverse parents for hybridization programme aimed at isolating desirable combination for green fodder yield as well as quality characters.

Intra and inter-cluster D^2 values

The intra and inter cluster D^2 values among six clusters are presented in Table 3 and Fig.1. The inter-cluster D^2 values were greater than the intra-cluster D^2 values, further indicating the considerable amount of diversity among the genotypes studied. The intra-cluster D^2 values ranged from 55.47 (cluster I) to 146.57 (cluster II). Moreover, the clusters V to XI were monogenic, as a result, its D^2 values were zero. The maximum inter-cluster D^2 values among genotypes existed between clusters VIII and X (1559.98), followed by clusters VIII and XI (1480.33), clusters II and VIII (1367.65), clusters IV and VIII (1309.08), clusters VI and VIII (1061.84), clusters V and VIII (1057.18), clusters V and IX (1050.25), clusters V and XI (1027.50), clusters V and IV (923.74), clusters VIII and IX (920.94), clusters I and VIII (855.99), clusters II and XI (809.27), clusters II and IX (809.11), clusters VI and IX (782.37), clusters III and VIII (781.10), clusters V and VI (754.32), clusters VI and XI (724.67), clusters V and VII (664.83) and clusters VII and X (661.53). Minimum inter cluster D^2 values were observed between clusters IX and XI (160.10), clusters I and III (164.51), clusters IV and XI (167.47), clusters II and III (170.55), clusters IV and IX (173.96), clusters I and VII (188.91), clusters II and VI (208.64), clusters I and II (239.10), clusters III and VII (246.44), clusters III and VI (255.93), clusters

TABLE 2
Grouping of genotypes into different clusters

Cluster number	Accessions in each cluster
I	CO - 8, EC - 394779, EC - 4216, IC - 9883, IC - 25105, IC- 97767, IC - 201095, IC - 202781, IC - 402101, IC - 394779
II	Vellayani - 1, MFC - 09 - 1, IC - 1071, IC - 202804, IC - 458485
III	MFC - 08 - 14, IC - 202777, IC - 402090, IC - 402154
IV	IC - 1061, IC - 253251, IC- 402162, IT - 38956-1
V	CO - 9
VI	EC - 458489
VII	KBC - 2
VIII	IC -39916
IX	IT - 37154999-38
X	Pant Lobe - 2
XI	KBC - 5

TABLE 3
Intra and inter cluster D² values

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	127.52										
II	239.10	101.49									
III	164.51	170.55	55.47								
IV	348.22	564.57	455.05	146.57							
V	430.68	408.82	345.12	923.74	0.00						
VI	351.13	208.64	255.93	574.73	754.32	0.00					
VII	188.91	518.31	246.44	399.71	664.83	513.24	0.00				
VIII	855.99	1367.65	781.10	1309.08	1057.18	1061.84	483.11	0.00			
IX	411.63	809.11	488.78	173.96	1050.25	782.37	268.69	920.94	0.00		
X	388.83	413.27	393.28	263.05	594.63	533.26	661.53	1559.98	459.41	0.00	
XI	563.02	809.27	573.44	167.47	1027.50	724.67	583.37	1480.33	160.10	283.89	0.00

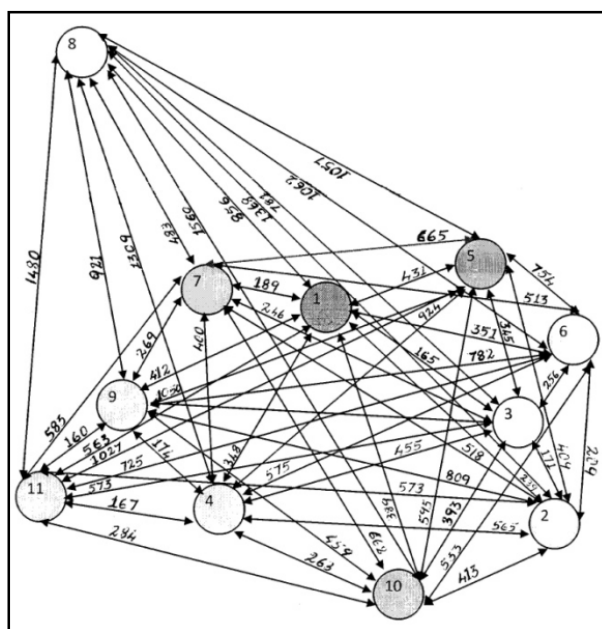


Fig. 1. Cluster Diagram

IV and X (263.05), clusters VII and IX (268.69), clusters X and XI (283.89), clusters III and V (345.12), clusters I and IV (348.22), clusters I and VI (351.13), clusters I and X (388.83), clusters III and X (393.28), clusters IV and VII (399.71), clusters II and V (408.82), clusters I and IX (411.63), clusters II and X (413.27), clusters III and IV (455.05), clusters IX and X (459.41), clusters VII and VIII (483.11), clusters III and IX (488.78), clusters VI and VII (513.24), clusters II and VII (518.31), clusters VI and X (533.26), clusters I and XI (563.02), clusters II and IV (564.57), clusters III and XI (573.44), clusters IV and VI (574.73), clusters VII and XI (583.37) and clusters V and X (594.63). The crossing between the genotypes of most divergent clusters could be adopted for transgressive segregants. Similar findings were also reported by Jain *et al.*, (2006) and Bhandari and Verma (2007).

Cluster mean and contribution of individual characters towards genetic divergence

The cluster mean for the 14 characters studied in the 30 fodder cowpea genotypes revealed that considerable difference among all the clusters exist (Table 4). From the data, it was evident that cluster I has highest mean value for days to 50% flowering (50.73days). Cluster II has minimum value for no. of primary branches (1.51) and stem dry weight per plant (4.17g). Cluster IV has minimum value for days to 50% flowering (46.75days). Cluster V has maximum value for plant height (247.83cm), green fodder yield per plant (274.07g), dry matter yield per plant (26.38g), leaf fresh weight per plant (168.56g), stem fresh weight per plant (105.51g) stem dry weight per plant (13.51g), minimum for days to first flowering (40.58days) and leaf are index (18.63). Cluster VI had highest mean for leaf dry weight per plant (12.97g), crude fiber content (401.67 mg/g) and minimum no. of leaves per plant (13.50). Cluster IX had maximum mean value for days to first flowering (44.30days) and leaf area index (54.59). Cluster X had minimum mean value for plant height per plant (43.00cm), dry matter yield per plant (9.46g), crude fiber content (95.00 mg/g) and maximum value for crude protein content (25.88 mg/g), no. of primary branches per plant (3.25). Cluster XI had maximum mean value for no. of leaves per plant (27.11) and minimum mean value for green fodder yield per plant (110.54g), leaf fresh weight per plant (71.78g), stem fresh weight per plant (38.76g), leaf dry weight per plant (5.74g), crude protein content (19.30 mg/g).

The results had shown high variations for mean values for all fourteen characters. Therefore, depending on the purpose of breeding for particular characters for example protein content, green fodder

TABLE 4
Mean value of different clusters for different characters along with per cent contribution

Characters	Clusters											% Contribution
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	
Plant height (cm)	178.64	157.65	182.23	161.06	247.83	177.17	183.42	187.58	171.33	43	191.39	-
No. of primary branches/plant	2.12	1.51	1.78	2.15	2.72	1.58	2.92	2.42	2.58	3.25	1.61	-
No. of leaves/plant	17.95	13.81	15.74	24.30	17.83	13.50	20.94	19.39	24.08	22.53	27.11	10.06
Days to first flowering	43.24	42.86	41.37	40.94	40.58	42.80	43.07	43.73	44.30	42.40	41.07	-
Days to 50% flowering	50.73	48.93	48.17	46.75	48.67	48.67	50.33	50.33	49.33	48.33	47.67	-
Leaf Area Index	25.12	19.83	24.55	31.87	18.63	21.96	21.06	25.42	54.59	40.85	30.97	12.10
Green fodder yield/plant (g)	179.39	132.91	119.78	167.20	274.07	161.55	184.05	248.23	183.94	179.41	110.54	0.50
Dry matter yield/plant (g)	15.49	10.98	10.44	17.77	26.38	24.33	17.69	16.72	15.32	9.46	10.53	10.06
Leaf fresh weight/plant (g)	101.63	84.17	74.38	101.20	168.56	89.57	97.61	151.65	101.16	113.19	71.78	24.72
Stem fresh weight/plant (g)	77.76	48.74	45.40	66.00	105.51	71.99	86.44	96.58	82.78	66.22	38.76	-
Leaf dry weight/plant (g)	8.12	6.80	5.92	9.72	12.86	12.97	9.35	10.81	7.60	7.50	5.74	-
Stem dry weight/plant (g)	7.37	4.17	4.55	7.14	13.51	11.36	8.32	5.90	7.72	5.93	4.79	16.48
Crude protein content (mg/g)	23.40	21.91	20.72	24.41	19.51	24.34	21.15	20.32	21.34	25.88	19.30	7.55
Crude fiber content (mg/g)	155.37	127.73	225.58	140.17	142.33	401.67	244.00	195.00	213.67	95.00	182.00	18.6

TABLE 5
Selection indices of genotypes and rank based on crude protein, green fodder yield and crude fiber content

S. No.	Genotype	Selection index	Rank	Cluster
1.	CO - 9	433.4756	8	V
2.	CO - 8*	625.8346	2	I
3.	Vellayani - 1	192.1024	29	II
4.	MFC - 09 - 1*	321.3372	19	II
5.	MFC - 08 - 14	326.5634	18	III
6.	EC - 394779	336.7769	17	I
7.	EC - 458489	616.8072	3	VI
8.	EC - 4216	316.3786	21	I
9.	KBC - 2	288.4645	23	VII
10.	IC - 1061*	511.6398	7	IV
11.	IC - 1071	316.797	20	II
12.	IC - 9883	307.0385	22	I
13.	IC - 25105	220.9049	27	I
14.	IC - 39916*	390.2878	11	VIII
15.	IC - 97767*	406.693	10	I
16.	IC - 201095	569.8944	5	III
17.	IC - 202777	377.7406	12	I
18.	IC - 202781	367.1625	15	II
19.	IC - 202804	282.9707	24	IV
20.	IC - 253251	177.1901	30	III
21.	IC - 402090	374.8028	13	I
22.	IC - 402101	373.4704	14	III
23.	IC - 402154	410.4742	9	IV
24.	IC - 402162	279.3483	26	II
25.	IC - 458485	352.3046	16	I
26.	IC - 394779	514.6545	6	I
27.	IT - 38956-1*	610.1032	4	IV
28.	IT - 37154999-38*	860.8776	1	IX
29.	Pant Lobe - 2*	282.0267	25	X
30.	KBC - 5	201.3062	28	XI

yield etc. the genotypes with high mean values and maximum inter cluster distances could be selected for crop improvement programmes.

Contribution of individual characters towards total divergence has been presented in Table 4. The maximum contribution to divergence was shown by leaf fresh weight per plant (24.72g) followed by crude fiber content (18.60 mg/g), stem dry weight (16.48g), leaf area index (12.10), dry matter yield per plant (10.06 g), no. of leaves per plant (10.06), crude protein content (7.55 mg/g), green fodder yield per plant (0.50 g). Plant height, no. of primary branches per plant, days to first flowering, days to 50% flowering, stem fresh weight per plant and leaf dry weight per plant did not contribute to genetic divergence. The above findings are broadly in agreement with previous workers (Lodhi *et al.*, 1990; Roquib and Patnaik, 1990; Sharma and Singhania, 1992; Sharawy and El-Fiky, 2002; Radhika, 2003; Omokanye *et al.*, 2003; Malarvizhi *et al.*, 2005; Sheela and Gopalan, 2006; Adeyanyu, 2009; Thaware *et al.*, 1991; Noubissié *et al.*, 2011).

The present study exhibited a high level of genetic diversity among 30 fodder cowpea genotypes which were grouped into eleven clusters based on D² statistics. The estimates of intra and inter-cluster D² values for eleven clusters revealed that the genotypes of the same cluster have little genetic divergence from each other with respect to aggregate effects of 14 characters under study. More genetic diversity was observed between the genotypes of different clusters. Since high or optimum genetic divergence is required between the parents for hybridization plan for obtaining high frequency of desirable recombinants, the chances of obtaining good segregants in the segregating generations is possible from the evaluated genotypes. It would be logical to attempt crosses between the diverse genotypes belonging to clusters separated by large inter-cluster D² values. The crossing between the genotypes which show high mean values coupled with relatively large inter cluster D² values would result in high heterotic expression.

Selection index calculated for the genotypes based on the desired characters was used in the present study for selection of parents for hybridization programme for yield and quality improvement. The genotypes were ranked for characters green fodder yield, crude protein content, crude fiber content and selection index. The average of the four ranks were calculated and again ranked accordingly. Based on this rank and maximum inter cluster distance eight genotypes, namely, CO-8, MFC-09-1, IC-1061, IC-

39916, IC-97767, IT-38956-1, IT-37154999-38 and Pant Lobia -2 were selected for further breeding programmes.

CONCLUSION

The evaluations of thirty genotypes of fodder cowpea, based on D² analysis, were grouped into eleven clusters. Cluster analysis revealed that cluster I was the largest cluster. Seven genotypes showed significant variation from main clusters and hence each existed as single cluster with only one genotype. The maximum intra-cluster D² value was shown by cluster IV. Highest inter-cluster D² values among genotypes existed between cluster VIII and cluster X. Minimum inter-cluster D² values among genotypes existed between cluster XI and cluster IX. The analysis clearly shows the existence of significant difference between the 30 genotypes under study for the 14 characters. The selected genotypes could be used for further hybridization for incorporating quality and yield to produce better varieties.

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