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GENETIC DIVERGENCE ANALYSIS IN COWPEA [*VIGNA UNGUICULATA* (L). WALP] GENOTYPES

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ABSTRACT
An investigation was undertaken with 196 cowpea genotypes during *kharif* 2009 to determine the genetic variation within cowpea populations collected from different part of India. Genetic divergence study using Mahalanobis D^2 statistic grouped 196 genotypes into 22 clusters. Cluster XXII was the largest comprising of 133 genotypes followed by cluster I with 23 genotypes, and all other clusters had only two genotypes each. Intra cluster distance was highest in the cluster XXII (2767.30), whereas, minimum in cluster II (4.19). The genotypes included are found to be very diverse in nature as they showed maximum inter cluster distance (D^2) between the clusters I and XIX (4343.52), the minimum D^2 value was between the clusters II and VI (34.66). With respect to the contribution of each trait to the total genetic divergence, the biometrical trait seed yield per plant contributed maximum (35.82) followed by test weight (26.99) and days to 50 % flowering (12.36).

INTRODUCTION

Cowpea [*Vignaunguiculata* (L). Walp] is a tropical grain legume widely distributed in sub-Saharan Africa, Asia, Central and South America as well as parts of southern Europe and the United States. Due to its high protein content (20-25%), cowpea plays a major role in human nutrition (Singh *et al*. 2002). It tolerates low fertility soil due to its high rate of nitrogen fixation (Eloward *et al.,* 1987). The loss of genetic diversity, in part due to the conventional breeding programs associated with modern agricultural practices, has been dramatic for many cultivated species. As better yielding crop varieties are adopted by farmers and they shift to other crops which give better returns, cowpea landraces and diversity may be lost. Consequently, the narrow genetic base of the elite germplasm has increased the potential of vulnerability to biotic and abiotic stress. Knowledge, access, and use of the available diversity in domesticated and wild relatives are essential in broadening the genetic base of cultivars to sustain improvement (Singh *et al*., 2002). Genetic diversity is the key to improvement and development of effective conservation strategies (Hodgkin, 1997). Genetic diversity present in the available germplasm has immense value in crop improvement for character of interest. From the point of selecting the parents for hybridization, which are divergent enough for the trait of interest, estimation of the genetic distance is most important.

Traditionally, diversity is estimated by measuring variation in phenotypic or qualitative traits such as flower colour, growth habit, or quantitative agronomic traits such as yield potential and stress tolerance (Li *et al*. 2001). Diversity has been used as a powerful tool in the classification of cultivars and also to study taxonomic status. Porter *et al*. 1974 reported that morphological variability in cowpeas abounds in the tropics suggesting adequate knowledge of the germplasm structure for the development of hybrids with specific ecology adaptation. However, this approach is often limited and expression of quantitative traits is subject to strong environmental influence (Kameswara, 2004). Knowledge of genetic variation and relationships among genotypes will assist breeders to develop appropriate breeding strategies to solve cowpea production constraints by providing an index of parental lines to be used in breeding programs.

The concept of genetic distance has been of vital utility in many contexts and more so in differentiating well defined populations. Quantification of genetic diversity existing within and between groups of germplasm is important and particularly useful in proper choice of parents for realizing higher heterosis and obtaining useful recombinants. Several methods have been advocated by various workers to estimate the genetic divergence in crop plants. Of the several methods available, Mahalanobis generalized distance estimated by D^2 statistic is a unique tool for discriminating populations considering a set of parameters together rather than inferring from indices based upon morphological similarities, eco-geographical diversity and phylogenetic relationships. Keeping all these in view, present study was conducted with the objective of assessment of genetic divergence in cowpea germplasm.

MATERIALS AND METHODS

Plant material

The present study comprised a set of 196 diverse cowpea genotypes including released varieties maintained at the AICRP on Arid Legumes, GKVK and Bangalore. These genotypes of cowpea (Table 1) were sown during *Kharif* 2009 in Simple Lattice Design, in three meter long rows with spacing of 60 cm x 30 cm and standard agronomic practices were followed. Five plants selected at random were tagged from each genotype and observations on nine quantitative traits (days 50 per cent flowering, days to physiological maturity, plant height, number of branches per plant, number of pods per plant, pod length, number of seeds per pod, test weight and seed yield per plant) were recorded on these plants.

Biometrical analysis

Mahalanobis's D2 - statistic (1936) was used for assessing the genetic divergence among the populations. All the n (n -1)/2 D^2 values were clustered using Tocher's method as described by Rao (1952). The intra, inter cluster distance and the character contribution towards diversity were calculated by the formulae given by Singh and Chaudhary (1977). Cluster analysis based on similarity matrices was carried out by using the un-weighted pair group method with arithmetic mean (UPGMA) to obtain a dendrogram with the help of Statistica 2 (Statsoft Inc. 1999) and Genres software's.

RESULTS AND DISCUSSION

In the present study on 196 cowpea genotypes, all the characters studied contributed to the total genetic divergence

Table 1: Relative contribution of nine characters towards divergence in cowpea genotypes

S. No.	Character	Percent contribution
1	Days to 50% flowering	12.36
2	Days to physiological maturity	8.76
3	Number of pods per plant	0.34
4	Number of seeds per pod	0.35
5	Pod length (cm)	7.01
6	Plant height (cm)	7.20
7	Number of branches per plant	1.13
8	Test weight (g)	26.99
9	Seed yield per plant (g)	35.82
	Total	100

Table 2: Clustering pattern of 196 cowpea germplasm lines

Clusters	No. of genotypes	Genotypes
I	23	EC 458489,IC 402101,NBC 14,IC 402166, V 24O,202804(83,V 585,C 325,EC 458506,IC 249593,IT 97K 499-38,IC 402180,202854(97),EC 472257,IC 249141,EC 170584,EC 472252,IC 202867(99),KM 5,IC 1071,EC 45841,NBC 29,EC 170584-1-1
II	2	NBC 33,EC 458417
III	2	IC 1071,EC 394779
IV	2	IC 249593,HC 9866
V	2	TC 201,CPD 15
VI	2	V 578-17,IC 402172
VII	2	GENOTYPE 36,EC 458418
VIII	2	NBC 51,NBC 10
IX	2	NBC 30,97767(10)
X	2	NBC 9,NBC 38
XI	2	27749(25),IC 402182
XII	2	IC 402175,EC 394779
XIII	2	EC 458430,IC 402098
XIV	2	NB 12,EC 458402
XV	2	EC 458505,NBC 43
XVI	2	EC 458402,EC 394708
XVII	2	IC 402159,TOME 77-4
XVIII	2	IC 402159,IC 10171
XIX	2	NBC 8,EC 458441
XX	2	CB 10,EC 394839
XXI	2	IC 249588,NBC 27
XXII	133	IC 402161,IC 2591054,IC 462099,IC 58905,EC 458473,IC 202777,IC 170574,CPD 31,EC 394779,NBC 4,IC 330996,IC 402166,IC 402164,EC 472250,IC 402114,V 585,EC 170584,NBC 32,NBC 39,EC 458442,EC 458470,IC 257428,IC 402048,IT 97499-38,CPD 19,NBC 6,202827(92),IC 198326(38),EC 170585(B9),EC 402159,IC 249793,C 131+CB-2,IC 201©,IC 402125,IC 402098,V 578 ©,NBC 7,IC 330996,EC 390287,V-16,EC 458440,IC 249593,EC 170578-1,NBC 40,IC 202779,EC 458402,202705(49),NBC 42,IC 402159,C 517,IC 402162,IC 202290,NBC 41,IC 402104,KBC 2,V 578,ETC 27,C 24-1,C 48475,198355(45),EC 458469,IC 202797(97),C 720,IC 202781,IC 402101,NBC 19,EC 472252,EC 458425,GC 3(C),IC 402104,NBC 38,IC 2591054,IC 202782,IC 402174,IC 4506,201095(52),EC 458480,EC 458483,C 1071,EC 458489,EC 458473,EC 394838,EC 458418,IC 1061,EC 458438,IC 402162,IC 249141,IC 206240,V 604-7-29-3,IC 25105,202827(93),EC 458490,IC 202711(58), C 458492,IC 49586,IC 402154,EC 458440,IC 202781,NBC 44,C 33,NBC 48,IC 198329(36),V 585©,NBC 36,IC 253251,EC 472250,EC 458402,NBC 18,EC 458480,EC 394839,NB 47,GC 3,IC 402090,EC 458469,C 457,NBC 7,EC 458453,IC 402161,IC 402106,EC 472271,EC 472267,CB 10,IC 402164,NBC 40,NBC24,C-152,TVX 944,KBC 2,km 5,HC 03-02,P 695,APC 243-1-865,TCM 44-1

Table 3: The nearest and farthest clusters from each cluster based on D^2 values in 196 cowpea genotypes

Cluster	Intra cluster	Nearest cluster	Farthest cluster
I	1689.36	III (852.01)	XIX (4343.52)
II	4.19	VI (34.66)	XXII (2710.87)
III	5.75	XXII (146.65)	XIX (3479.87)
IV	9.20	XVI (73.43)	XXII (2624.81)
V	17.00	XVI (85.17)	XXII (2400.23)
VI	19.40	XX (308.31)	XIX (3028.40)
VII	20.40	IX (148.13)	XXII (2223.36)
VIII	22.70	XVIII (154.82)	I (2491.62)
IX	26.60	XVIII (54.23)	I (2242.70)
X	30.80	VII (106.61)	I (2960.44)
XI	30.90	XX (250.02)	XXII (3324.46)
XII	34.10	III (146.65)	XIX (2454.56)
XIII	35.00	VIII (200.89)	XXII (2004.65)
XIV	35.50	XIII (248.37)	XX (2765.81)
XV	37.20	XIX (106.24)	I (3457.90)
XVI	38.20	XXI (157.27)	XXII (2389.66)
XVII	41.50	VIII (142.55)	XI (2590.42)
XVIII	44.10	IX (54.23)	XXII (2113.07)
XIX	45.70	XV (106.249)	XX (3470.99)
XX	48.80	XI (250.02)	XIX (3470.99)
XXI	52.40	X (164.93)	I (1626.56)
XXII	2767.30	XIII (2004.65)	I (3760.82)

Table 4: Cluster mean values for eight quantitative parameters in cowpea genotypes

Clusters	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	Overall score	Rank
I	52.82	18.83	12.02	26.36	7.00	14.59	33.08	87.63	96	12
II	54.00	23.88	10.70	47.51	7.68	12.35	31.48	88.00	80	10
III	53.00	19.12	12.22	17.43	6.57	13.95	32.64	88.00	108	17
IV	56.00	16.66	12.32	27.25	7.00	10.82	22.14	86.00	117	19
V	56.00	30.41	10.58	17.66	6.75	11.05	35.37	88.00	103	14
VI	54.00	21.00	10.68	48.65	6.76	13.52	29.93	88.00	89	11
VII	58.00	28.99	11.33	32.41	6.58	13.15	43.05	89.00	58	2
VIII	57.00	16.65	10.70	27.96	7.12	17.07	30.05	89.00	71	7
IX	57.00	17.74	13.00	24.66	6.97	12.67	29.39	89.00	77	8
X	58.00	28.29	11.99	22.70	6.41	12.45	42.16	88.00	80	9
XI	54.00	16.75	8.54	24.12	6.50	9.85	14.04	86.00	148	22
XII	54.00	13.66	11.20	23.12	4.91	14.85	22.76	88.50	123	20
XIII	56.00	21.08	13.25	28.00	6.17	15.82	44.50	87.75	70	6
XIV	57.00	18.25	14.08	45.41	7.24	14.77	38.21	88.00	56	1
XV	58.00	12.75	8.45	20.04	4.00	18.65	20.48	86.50	131	21
XVI	56.00	20.33	12.28	25.99	6.50	10.85	27.13	87.50	107	16
XVII	57.00	22.91	12.58	22.50	5.66	16.52	47.99	91.00	61	3
XVIII	57.00	28.73	12.07	21.74	7.40	12.70	43.52	88.75	64	5
XIX	59.00	15.62	8.75	22.55	4.87	18.50	24.95	86.50	116	18
XX	54.00	20.25	11.45	29.49	6.80	10.62	24.70	88.50	101	13
XXI	56.00	15.91	10.68	29.12	7.18	13.42	23.23	87.50	105	15
XXII	56.23	21.06	11.39	28.33	6.79	15.03	35.11	89.26	63	4

Where, X_1 - Days to 50% flowering; X_2 - Number of pods per plant; X_3 - Number of seeds per pod; X_4 - Plant height (cm); X_5 - Number of branches per plant; X_6 - Test weight (g); X_7 - seed yield per plant (g); X_8 - Days to physiological maturity

(Table 1). The highest contribution was made by seed yield per plant followed by test weight, days to 50 per cent flowering, days to physiological maturity, plant height, pod length, number of branches per plant, number of seeds per pod and the lowest contribution was from number of pods per plant. Rewale *et al*. (1996) reported maximum contribution towards the total diversity by days to 50% flowering and maturity, number of pods per plant, pod length, 100-seedweight and seed yield per plant. Similar results were also made by Backiyarani *et al*. (2000), Borah *et al*. (2002), Chikkdevaiah *et al*. (1985), Nigude *et al*. (2004), Narayankuttuy *et al*. (2003), Pandey *et al*. (2007), Santos *et al*. (1997) and Sulnathi *et al*. (2007). While Venkatesan, *et al*. (2007) reported clusters per plant, pods per cluster, pods per plant and seed yield per plant contributing maximum towards total divergence. Kumawat and Raje (2005) also reported that seed yield per plant contributed the highest towards the total genetic divergence, followed by days to 50 per cent flowering, seeds per pod and plant height.

In the present study based on D^2 values, the 196 cowpea

genotypes were meaningfully grouped into 22 clusters (Table 2). The maximum number of genotypes fell in the cluster XXII (133 genotypes) followed by the cluster I (23 genotypes), and remaining all clusters had two genotypes each. The genotypes falling in a particular cluster will have close genetic background with smaller intra-cluster distance between the genotypes within a cluster or the members of the same cluster are least divergent. The inter-cluster divergence expresses the diversification among clusters. The genotypes between the clusters have more D^2 value with more genetic distance. Dheeraj *et al.*, 2013 based on D^2 statistics grouped 46 sweet sorghum genotypes into 11 clusters, out of which cluster I shows the highest intra cluster value (13.79) followed by cluster II (13.64) while maximum inter cluster distance (*i.e.* 34.72) was observed between cluster V and cluster IX. Meenakshi *et al.*, 2013 divided 24 lentil genotypes into two clusters A and B at 41% similarity based on dendrogram generated by cluster analysis from ISSR markers. Further, genotypes belonging to more distanced clusters will serve as good sources of divergent genes which is very much required for breeding to exploit heterosis as reported by Gill *et al*. (1982).

The range of D^2 (4.19 – 4343.52) values indicates the degree of divergence among 196 genotypes of cowpea is high. Maximum inter-cluster distance was observed between the clusters I and XIX (4343.52, Table 3) indicating genotypes included in these clusters are highly divergent which indicated large differences between the cluster means for many characters like days to 50 per cent flowering, number of seeds per pod, number of branches per plant and test weight. For other characters like number of pods per plant, plant height, and seed yield per plant the differences were not substantial. Minimum inter-cluster distance observed between the clusters II and VI (34.66) and same was reflected in the cluster means for different characters showed small divergence between the clusters II and VI. Because, the group of genotypes resembling each other, hence with low intra-cluster divergence. Cluster XXII showed more D^2 distance with other clusters indicating that genotypes in this cluster are more divergent from genotypes of other clusters. Intra-cluster D^2 value was small in the cluster II (4.19) with only two genotypes whereas cluster XXII (2767.30) has recorded maximum intra-cluster D^2 value (Table 3) indicating that, 133 genotypes in the cluster XXII were not closely related compared to the genotypes in the cluster II. When we select the genotypes for hybridization it is desirable to select the genotypes from the clusters with maximum inter-cluster distance.

Cluster-means indicates average performance of all genotypes clubbed in a cluster. The uniqueness of members will be reflecting here. Hence, all the accessions spread over 22 clusters and means were scored across the clusters for eight quantitative characters (Table 4).The highest cluster mean was given the first rank and next cluster possessing next best means were given 2^{nd}, 3^{rd} and so on up to 22^{nd} rank for all the traits. Based on the overall score across eight traits, the clusters were ranked. The lowest scoring cluster was given first rank and next cluster possessing the score above the previous one were given 2^{nd}, 3^{rd} and so on up to 22^{nd} rank. Accordingly cluster XIV with overall score of 56 across eight quantitative characters received first rank indicating that cluster XIV possess the genotypes with high overall performance followed by cluster VIII, XVII and XXII, indicating presence of most promising genotypes in them and can be extensively used for further breeding program to generate new material. Choice of divergent parent(s) can be made for hybridization purposes on the basis of D^2 value between two genotypes or two clusters. Members from most divergent clusters can be chosen taking into account their desirable/complementary characters.

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REFERENCES

Backiyarani, S., Nadarajan, N., Rajendran, C. and Shanthi, S. 2000. Genetic divergence for physiological traits in cowpea (*Vigna unguiculata* L.). *Legume Res*. **23(2):** 114-117.

Borah, H. K. and Khan, A. K. F. 2002. Genetic divergence in fodder cowpea (*Vigna unguiculata* L.). *Madras Agric. J.* **88(10/12):** 625-628.

Chikkadevaiah 1985. Genetic divergence in cowpea (*Vigna unguiculata* L.). *Mysore J. Agric. Sci.* **19:** 131-132.

Dheeraj Shinde, Smitha Chavan and Jadav, B. D. 2013. Study os genetic divergence in sweet sorghum (*Sorghum bicolor*). *The Bioscan*. **8(1):**135-138.

Eloward, H. O. A. and Hall, A. E. 1987. Influence of early and late nitrogen fertilization on yield and nitrogen fixation of cowpea under well-watered and dry field conditions. *Field Crops Res*. **15:** 229-244.

Gill, J. S., Verma, M. M., Gumber, R. K. and Singh, B. 1995. Character association in mungbean lines derived from three intervarietal crosses in mungbean. *Crop Improv*. **22:** 255-260.

Hodgkin, T. 1997. Some current issues in conservation of genetic resources. IPGRI workshop 9 - 11 October 1995, Rome, Italy.

Kameswara, R. N. 2004. Biotechnology for Plant Resources conservation and use. *Principles of seed handling in Genebanks Training course*, Kampla, Uganda.

Kumawat, K. C. and Raje, R. S. 2005. Association analysis in cowpea [*Vigna unguiculata* (L.) Walp.]. *J. Arid Legumes*. **2(1):** 47-49.

Li, C. D., Fatokun, C. A., Benjamin, U., Singh, B. B. and Scoles, G. J. 2001. Determining Genetic Similarities and Relationships among Cowpea Breeding Lines and Cultivars by Microsatellite Markers. *Crop Sci*. **41:** 189-197.

Mahalanobis, P. C. 1936. On the generalized distance in statistics. *Proc. Nat. Acad. Sci.* (India). **12:** 49-55.

Meenakshi Joshi, S. K. Verma, J. P. Singh and Anupam, B. 2013. Genetic diversity assessment in lentil (*Lens culinaris* Medikus L.) genotypes through ISSR marker. *The Bioscan*. **8(4):** 1529-1532.

Narayanankutty, C., Mili, R. and Jaikumaran, U. 2003. Variability and genetic divergence in vegetable cowpea. *J. Maharashtra Agric. Univ*. **28(1):** 26-29.

Nigude, A. D., Dumbre, A. D., Lad, D. B. and Bangar, N. D. 2004. Genetic variability and correlation studies in cowpea. *J. Maharashtra Agric. Univ*. **29(1):** 30-33.

Porter, W. M., Rachie, K. O., Rawal, H. C., Wein, R. J. and Luse, R. A. 1974. *Cowpea germplasmcataloque Nob1*, IITA Ibadan, Nigerian, pp. 86-101.

Rao, C. R. 1952. *Advanced Statistical Methods in Biometric Research*. *J. Wiley Sons,* New York, p.390

Rewale, A. P., Birari, S. P. and Apte, U. B. 1996. Genetic divergence in cowpea (*Vigna unguiculata* (L.) Walp.). *Indian J. Agric. Res*. **30(2):**

73-79.

Santos, C. A. F., Menezes, E. A. and de Araujo, F. 1997. Genetic diversity in genotypes of cowpea under 2 different environments. *Revista Ceres*. **44(251):** 35-42.

Singh, M. K. and Verma, J. S. 2002. Variation and character association for certain quantitative traits in cowpea germplasm. *Forage Res*. **27:** 251-253.

Singh, R. K. and Choudhary, B. D. 1997. Biometrical methods in quantitative genetic analysis. *Kalyani Pub.*, New Delhi. pp. 204-252.

Stat Soft Inc. 1999. STATISTICA for windows (computer program manual).

Sulnathi, G., Prasanthi, L. and Sekhar, M. R. 2007. Character contribution to diversity in cowpea. *Legume Res*. **30(1):** 70-72.

Venkatesan, M., Prakash, M. and Ganeshan, J. 2007. Genetic variability, heritability and genetic advance analysis in cowpea (*Vigna unguiculata* L.) *Legume Res*. **26:** 155-156.