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Study of genetic divergence through D^2 analysis and molecular marker techniques in cowpea (*Vigna unguiculata* L. Walp)

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Abstract
The present experiment was conducted during summer 2016 for study of genetic diversity in 40 different genotypes of cowpea using D^2 statistics method of Mahalanobis andMolecular marker techniques. The fourty genotypes of cowpea were grouped into seven clusters following Tocher's method (Rao, 1952) which indicate the presence of diversity for different traits under study. The results indicate that a maximum number of genotypes appeared in cluster I (10 genotypes) followed by cluster V (9 genotypes), cluster III (7 genotypes), cluster VII (6 genotypes), cluster II and VI (3 genotypes) and cluster IV (2 genotypes). The maximum intra-cluster distance was recorded within cluster I (7.39) and the maximum inter-cluster distance between cluster VI and VII (D=19.83), indicating the existence of wide genetic variability. Based on mean performances, cluster IV and cluster II found superior for yield and yield contributing characters. Therefore genotypes selected for hybridization among the above said clusters would produce high heterosis and segregant for more than one economic character.The genomic DNA of 40 genotypes of cowpea was extracted by modified CTAB method for the molecular characterization through ISSR markers. Total 30 ISSR primers were screened across all the genotypes of cowpea. Out of 30 primers seven primers produce polymorphic band with an average of 1.13 bands per primer. The average polymorphism detected by the ISSR band in the present investigation was 97.14% with 0.756 average Polymorphic Information Content (PIC).The dendrogram, based on Jaccard's similarity co-efficient was able to classify all the genotypes of cowpea in two clusters. Among the cowpea genotypes GC-5 and Pusa-falguni evident maximum similarity; while the genotypes GP-761 and GP-452 shown minimum similarity.

Keywords: Genetic diversity, cowpea, D^2 statistics, cluster analysis

Introduction
Cowpea [*Vigna unguiculata* (L.) walp.] is self-pollinated crop which belong to family Fabaceae and sub family papillonaceae with chromosome number 2n=2x=22. Vavilov (1949) [15], recognized India and Africa as the centres of origin, while China is considered as secondary centre of origin of cowpea. Cowpea is multipurpose legume grown for seed as a pulse, green pod as a vegetable and whole plant as a fodder in tropical and subtropical region. Nutrition content of mature cowpea is protein (24.8%), fat (1.9%), fibre (6.3%) and carbohydrates (63.6%) but thiamine, riboflavin and niacin are present in trace, (Davis *et al.*, 2000) [5]. Because of its high protein content (20-25%) cowpea has been referred as "poor man's meat".

Genetic diversity is the basic requirement for a successful breeding programme. Collection and evaluation of genotype of any crop is a pre-requisite for any programme, which provides a greater scope for exploiting genetic diversity. A quantitative assessment of the genetic divergence among the collection of germplasm and their contribution of different traits towards the genetic divergence provide essential and effective information to breeder in this hybridization programme and thereby genetic improvement of yield. The necessity for finding out genetic divergence among the genotypes is more pronounced because of two reasons *i.e.* (I) the genetically diverse parent if included in the hybridization programme are likely to produce high heterotic effect, (II) a wide spectrum of variability could be expected in the segregating generation of crosses involving distantly related parents.

A method suggest by Mahalanobis (1936) [9] known as "Mahalanobis D^2 statistics" which is used to know genetic diversity in the available germplasm. This technique measures the force of differentiation at intra cluster and inters cluster levels and thus help in the selection of genetically divergent parents for their exploitation in hybridization programme. The D^2 statistics also measure the degree of diversification and determines the relative proportion of each component character to the total divergence.

Molecular techniques have been applied in the analysis of specific genes, as well as to increase understanding of gene action, generate genetic maps and assist in the development of gene transfer technologies.

Molecular techniques have also had critical roles in studies of phylogeny and species evolution. Molecular techniques have been applied to increase the understanding of the distribution as well as extent of genetic variation within and between species.

Materials and Methods

In the present investigation 40 genotypes of cowpea procured from the Pulses Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar were evaluated in Randomized Block Design (RBD) with three replications for different quantitative characters at Agronomy Instructional Farm, C. P. College of Agriculture, SDAU, Sardarkrushinagar during summer season of 2016. Each genotype sown in single row of 4 m length with spacing 45 cm between row and 15 cm between plants. Data were recorded for five selected competitive plants per genotype for characters Plant height (cm), Number of branches per plant, Number of pods per plant, Length of pod (cm), Number of seeds per pod, Grain yield per plant (g), Test weight (g) and Harvest index (%)in each replication and averages was worked out for statistical analysis. For the characters like days to 50 per cent flowering and days to maturity observation were recorded on per plot basis. The experimental data was analyzed statistically by the method of analysis ofvariance suggested by Panse and Sukhatme (1998) [12]. Multivariate analysis was done utilizing Mahalanobis D^2statistic (Mahalanobis, 1936) [9] and genotypes were grouped into different clusters following Tocher's method suggested by Rao, 1952 [14]. The inter and intra cluster distances were worked out as per method suggested by Murty and Arunachalam (1967) to find actual divergence within and between the clusters. The plant genomic DNA was isolated as per modified CTAB method of Doyle and Doyle (1990) [6] with minor modifications.

Protocol for DNA isolation

1. Juvenile leaves (1g) were grind to fine powder with liquid nitrogen in a mortar and pestle.
2. 1 ml of extraction buffer was added in powder with 50µl beta mercaptoethanol. The tubes were maintained in a water bath and incubate at 65 ºC for 30 min.
3. After incubation, equal volume of chloroform: Isoamylalcohol (24:1) was added and centrifuged with 10000 rpm for 15 minute at 37 ºC.
4. After centrifugation, the supernatant was transferred in fresh micro centrifuge tube and again add equal amount of C:I (24:1) was added and centrifuged with 10000 rpm for 15 minute at 37 ºC. 5. After centrifugation, the supernatant was transferred in fresh micro centrifuge tube and add 2-3 µlRNase in tubes and kept the tubes in hybridization oven at 37 ºC for 25 minutes.
5. Equal volume of Chloroform: Isoamylalcohol (24:1) was added after treatment of RNase
6. The tubes were centrifuged at 10000 rpm for 10 minutes at 37 ºC temperature.
7. After centrifugation, the supernatant was transferred in fresh micro centrifuge tube and 600µl of chilled isopropanol was add along with 150 µl of 5M NaCl and tubes were kept at -20 ºC over night for better precipitation of DNA.
8. Next day, the pellet of DNA was obtained and tube were centrifuged at 10000 rpm for 10 minutes at 4 ºC and the pellet was air dried and twice washed with 70% ethanol.
9. After wash with 70% ethanol the tubes were kept in hybridization oven for 15 minutes to remove of ethanol.
10. 100 µl of Tris-EDTA (TE) buffer was added and kept at room temperature.
11. The DNA was dissolved at room temperature and stored at -20 ºC for further use.

Results and Discussion

The analysis of variance revealed significant differences among the genotypes for all the characters under study *viz.*, days to 50 per cent flowering, days to maturity, number of seeds per pod, length of pod (cm), number of pods per plant, number of branches per plant, plant height (cm), grain yield per plant (g), harvest index (%), test weight (g) which indicated the existence of variability in the experimental material.

Based on D^2 value 40 genotypes of cowpea were grouped into seven clusters by Tocher's method as per suggested by Rao, 1952 [14] (Table 1). The results indicate that a maximum number of diverse genotypes appeared in cluster I(10 genotypes) followed by cluster V (9 genotypes), cluster III (7 genotypes), cluster VII (6 genotypes), cluster II and VI (3 genotypes) and cluster IV (2 genotypes). The pattern of grouping genotypes in different cluster proved the existence of significant amount of variability. These findings are confirmed by earlier reports of Badhe *et al*. (2015) [2] and Nobila *et al*. (2016) [11].

The intra and inter cluster distances D^2 between all possible pairs of seven clusters were computed and presented in Table 2 and depicted in Figure 1. The clustering pattern showed that varieties from different source were clubbed into one group and also varieties of same source forming different cluster indicated no relationship between geographical and genetic divergence. Murthy and Arunachalam (1966) [10] stated that genetic drift and selection in different environment could cause greater diversity than geographical distance. The maximum inter cluster distance was observed between VI and VII (D=19.83) followed by cluster V and VII (D=18.14), cluster I and VII (17.44), cluster III and cluster VII (17.20), cluster IV and cluster VI (D=16.86). The least inter cluster distance was observed between cluster III and V (D=8.27) followed by cluster I and V (D=8.78) and cluster II and IV (D=9.09). In the present study maximum intra cluster distance was observed for cluster I (D=7.39) followed by cluster II (D=7.35). In the present study more inter cluster distance was observed than the intra cluster which indicate presence of more diversity between the genotypes of inter cluster. Therefore, selection of genotypes for hybridization from between cluster possessing maximum genetic divergence is expected that more heterotic F1 and most promising segregant in segregating generations. In general, more inter cluster distance than intra-cluster distance suggested homogenous and heterogeneous nature of the genotypes within and between the clusters, respectively Pawar *et al.*, (2013) [13]. Similar findings were reported by Dalsaniya *et al*. (2009) [4] and Badhe *et al.* (2015) [2] for the cluster analysis in cowpea.

The utility of D^2 analysis is enhanced by its application to estimates the relative contribution of various characters to genetic divergence. The contribution of each character towards total genetic diversity is presented in Table 3. The present study revealed that, the characters harvest index (43.52%), length of pod (27.10%), number of pod per plant

(10.14%) and test weight (6.0%) contribute more genetic divergence. Therefore, selection for such traits may give more emphasis hybridization programme to generate large variability and will provide immense scope for the improvement of yield through selection. This type of result were also reported by Brahmaiah *et al.* (2014) [3] and Badhe *et al.* (2015) [2].

The mean performance of cluster values for all characters is presented in Table 4. Based on the mean value of days to 50% flowering and days to maturity genotypes of cluster I and cluster IV proved to be early while, for yield and yield contributing characters like Number of seeds per pod, Length of pod (cm), Number of pods per plant, Number of branches per plant, Harvest index (%) and Test weight (g) genotypes of cluster IV and cluster II found superior. It is suggested that parent selected for hybridization among the genotypes of above said clusters would produce high heterosis and segregant for more than one economic character. The potential lines are identified from different clusters and used as parents in a hybridization programme. The choice of genotype should be based on genetic distance and depending upon the objective of the breeding programme. Similar finding reported by Dalsaniya *et al*. (2009) [4] and Kiran and Krishna (2013) [8].

Many workers have observed that more diverse the parents within its overall limits of fitness, the greater are the chances of heterotic expression in F1's and a broad spectrum of variability in segregating generations (Arunachalam, 1981) [1]. Moreover, it will be effective to intercross genotypes belonging to more diverse clusters like cluster VI and II to create wide spectrum of variability and to produce transgressive segregates for cowpea.

Genetic variability through ISSR marker

The spectrophotometric analysis of DNA showed an average concentration of DNA was 373.45 (ng/µl). DNA concentration in 40 genotypes of cowpea ranged from 250.7 to 698.8 (ng/µl), Table 5. After Quality confirmation DNA was normalized and utilized for molecular characterization. The entire sample showed A260/A280 ratios 1.79-1.95 and quality and quantity of DNA was also estimated in agarose gel electrophoresis. Total 30 ISSR primers were used for screening of 40 cowpea genotypes. Out of 30, seven primers were found polymorphic were ISSR10, ISSR 9, ISSR 13, ISSR 26, ISSR 4, ISSR 11 and ISSR 16.

Pooled ISSR Results

The data collected from inter simple sequence repeat with seven ISSR primers produce a total 35 DNA fragments, among which 34 fragments were found to be polymorphic. As such the mean number of polymorphic bands per primer among 40 genotypes was found 1.13. The size of PCR amplified DNA fragments varied from 183 to 1811 bp. The maximum number of amplified band (8) was exhibited by primer ISSR 13 whereas, minimum number of amplified band (3) was exhibited by primer ISSR-11. All the primers showed 100% polymorphism except primer ISSR-10 which showed 80% polymorphism. The range of PIC value is 0.681 to 0.872. Percent polymorphism results are shown in Table 6.

Construction of dendrogram and classification

Jaccard's co-efficient were used to compare set of variables and to generate similarity matrix. Jaccard's co- efficient for all genotypes are shown in Fig 2. Similarity indices were estimated on the basis of seven primers ranged from 0.17 (between GP-792 and GP-761) to 1.00 (between PusaFalguni and GC-5). UPGMA (unweighted pair-group method with arithmetic mean) dendrogram was prepared by using Jaccard's similarity co-efficient through NYSYS-PC based software. These results indicate that the two genotypes, GP-792 and GP-761 were found to be the most diverse type. Cultivars showing the lowest genetic similarity are of great concern to plant breeders to be further selected as parents. Weising *et al*. (2005) [16] mentioned that it is mandatory that genetically divergent parents could be chosen which exhibit sufficient polymorphism, but are not so distant as to cause sterility of the progeny. In the present investigation, the results revealed that different types of profiles expressed different levels of genetic similarity among the 40 cultivars. This could be due to the different mechanisms of polymorphism detection by the different bands type. The accuracy of genetic similarity estimates based on molecular data depends on several variable factors such as the number of bands analyzed, their distribution over the genomes and the accuracy in scoring them Table 7.

Dendrogram clustered with the data generated by all the primers and their amplicons grouped the 40 genotypes into two clusters *i.e*Cluster A and Cluster B Fig: 2. The detailed distribution of genotypes based on the cluster from UPGMA dedrogram is showed in Table 8.

Dendrogram Fig: 2 showed two major clusters with co-efficient value 0.8691. The cluster A was dividing into only one cluster having GP-761 genotypes. The cluster B was further grouped in three group B1:1, B1:2 and B2. The cluster B1:1 contains 34 genotypes. The cluster B1:2 contain 4 genotypes while cluster B2 had only 1 genotype. Genotypes GC-5 and pusa-falguni had highest similarity.

In the present study total 30 ISSR markers have been employed out of which mean polymorphism 97.14% with mean PIC of 0.756. PIC value varied from 0.87 (ISSR-13) to 0.66 (ISSR-11) indicating that primers used in the present study are highly polymorphic and can be used to deciphering the molecular diversity analysis.

Based on 35 amplicondendrogram was constructed that grouped all 40 genotypes into 2 major cluster *i.e*A and B phylogenetic study indicates that GP-761 was diversely grouped then all other genotype under study as it showed bifuntional pattern of dendrogram, also validating previous analysis done by Gajera *et al.* (2014) [7] indicating strong allopatric evoluation pattern.

Table 1: Distribution of 40 genotypes of cowpea into different clusters.

Cluster No.	No. of genotypes	Genotypes included
I	10	GP-111, GP-763, GP-760, GP-761, GP-764, GP-785, GP-972, GP-1060, PGCP-14, PANT LOBIA-1
II	03	GP-793, GP-823, GP-1063
III	07	GP-330, GP-459, GP-585, GP-771, GP-951, GP-1052, GC-3
IV	02	GP-733, GP-802
V	09	GP-452, GP-458, GP-460, GP-779, GP-1068, PANT LOBIA-2, GC-5, GC-901, GC1008
VI	03	GP-792, GP-851, PGCP-12
VII	06	GP-282, GP-794, GP-1047, GC-4, PUSA FALGUNI, GC-521

Table 2: Intra (diagonal) and inter cluster D value of 40 genotypes of cowpea

Cluster	I	II	III	IV	V	VI	VII
I	07.39	10.99	09.60	15.37	08.78	09.55	17.44
II		07.35	09.40	09.09	10.13	14.23	15.53
III			00.00	11.44	08.27	11.72	17.20
IV				00.00	13.35	16.86	11.85
V					00.00	12.43	18.14
VI						00.00	19.83
VII							00.00

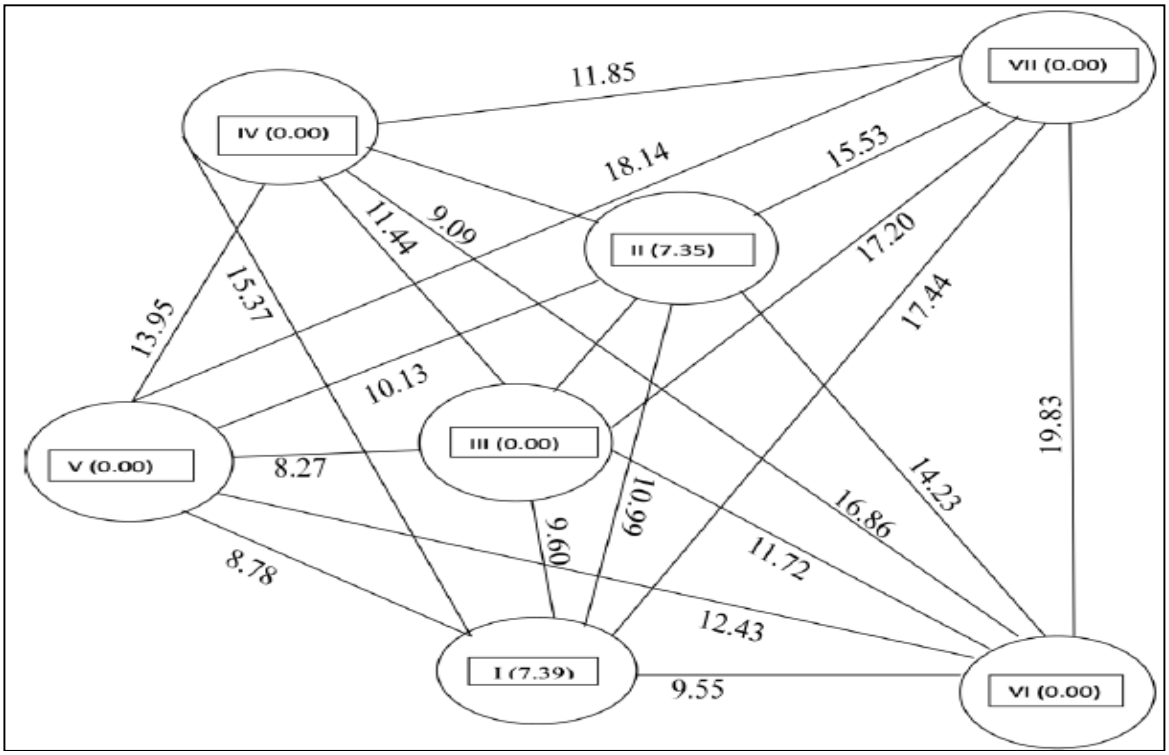

Fig 1: Cluster Diagrame

Table 3: The percent contribution of different quantitative characters to total genetic divergence in cowpea.

S. No.	Characters	Contribution %
1.	Days to 50% flowering	0.26
2.	Days to maturity	1.73
3.	Number of seeds per pod	0.40
4.	Length of pod (cm)	27.10
5.	Number of pods per plant	10.41
6.	Number of branches per plant	0.26
7.	Plant height (cm)	1.46
8.	Harvest index (%)	43.52
9.	Test weight (g)	6.00
10.	Grain yield per plant (g)	0.26

Table 4: Cluster mean for seed yield and its component in cowpea

Cluster	DF	DM	NS/P	LP	NP/P	NB/P	PH	GY/P	HI	TW
I	49.46	76.84	10.29	11.67	09.41	09.34	51.02	09.07	17.59	12.68
II	54.11	82.48	09.96	12.11	11.86	09.87	52.52	09.90	10.78	12.53
III	53.28	82.18	08.58	08.63	07.42	08.24	53.02	07.02	14.13	11.77
IV	50.33	75.73	11.53	12.02	12.04	09.97	48.37	10.73	33.41	14.49
V	52.07	81.14	09.76	09.97	10.91	08.31	51.44	09.09	22.26	11.22
VI	50.88	78.62	12.24	16.02	06.74	08.98	46.34	07.40	13.88	11.68
VII	51.16	79.51	09.47	09.80	08.24	09.12	51.21	08.21	27.61	11.60

DF: Days to 50% flowering, DM: Days to maturity, NS/P: Number of seeds per pod, LP: Length of pod (cm),NP/P: Number of pods per plant, NB/P: Number of branches per plant, PH: Plant height (cm), GY/P: Grain yield per plant (g), HI: Harvest index (%), TW: Test weight (g)

Table 5: Quantification of extracted DNA obtained from cowpea genotypes

S. No.	Germplasm	Nucleic acid concentration. (ng/µl)	A_{260}/A_{280}
1	GP-111	258.7	1.93
2	GP-282	281.9	1.95
3	GP-330	293.2	1.87
4	GP-452	610.4	1.89
5	GP-458	526.8	1.83
6	GP-459	354.1	1.81
7	GP-460	667.9	1.83
8	GP-585	629.7	1.87
9	GP-733	270.5	1.85
10	GP-763	698.8	1.80
11	GP-760	292.1	1.81
12	GP-761	256.6	1.86
13	GP-764	270.3	1.85
14	GP-771	680.3	1.82
15	GP-779	260.0	1.86
16	GP-785	491.4	1.94
17	GP-792	332.5	1.89
18	GP-793	312.1	1.89
19	GP-794	255.9	1.89
20	GP-802	347.0	1.89
21	GP-823	311.8	1.94
22	GP-851	304.0	1.94
23	GP-951	289.0	1.89
24	GP-972	443.7	1.91
25	GP-1047	312.9	1.91
26	GP-1052	262.7	1.92
27	GP-1060	358.4	1.79
28	GP-1063	654.1	1.93
29	GP-1068	623.8	1.88
30	PGCP-12	369.6	1.91
31	PGCP-14	427.0	1.87
32	PANTLOBIA-1	291.9	1.89
33	PANTLOBIA-2	275.7	1.91
34	GC-3	292.1	1.81
35	GC-4	250.7	1.87
36	GC-5	257.6	1.82
37	PUSA-FALGUNI	257.8	1.86
38	GC-521	263.3	1.86
39	GC-901	289.0	1.86
40	GC-1008	312.7	1.91
Average		374.45	
Range		250.7-698.8	1.79-1.95

Table 6: Percent polymorphisms revealed by ISSR analysis

S. No.	Primer Name	Total Band	Polymorphic Band	% polymorphism	PIC value	Molecular Weight Range (BP)
1	ISSR-10	5	4	80.0	0.793	236 to 1370
2	ISSR-9	6	6	100.00	0.812	270 to 1647
3	ISSR-13	8	8	100.00	0.872	203 to 884
4	ISSR-26	4	4	100.00	0.726	411 to 1811
5	ISSR-4	5	5	100.00	0.681	183 to 1266
6	ISSR-11	3	3	100.00	0.666	608 to 1346
7	ISSR-16	4	4	100.00	0.748	400 to 1780
	Total	35	34			
	Average	-	-	97.14	0.756	
	Range	-	-	80-100	0.681-0.872	183-1811

Table 7: Similarity Matrix from ISSR analysis

0	GP-111	GP-282	GP-330	GP-452	GP-458	GP-459	GP-460	GP-585	GP-733	GP-763	GP-760	GP-761	GP-764	GP-771	GP-779	GP-785	GP-792	GP-793	GP-794	GP-802	GP-823	GP-851	GP-951	GP-972	GP-1047	GP-1052	GP-1060	GP-1063	GP-1068	PGCP-12	PGCP-14	PANT-LO-1	PANT-LO-2	GC-3	GC-4	GC-5	PUSA-FAL	GC-521	GC-901	GC-1008
GP-111	1.00																																						
GP-282	0.85	1.00																																					
GP-330	0.79	0.85	1.00																																				
GP-452	0.48	0.48	0.53	1.00																																			
GP-458	0.70	0.75	0.75	0.68	1.00																																		
GP-459	0.69	0.81	0.69	0.45	0.77	1.00																																	
GP-460	0.52	0.57	0.62	0.59	0.71	0.59	1.00																																
GP-585	0.63	0.58	0.63	0.50	0.71	0.55	0.69	1.00																															
GP-733	0.50	0.55	0.55	0.32	0.59	0.52	0.45	0.57	1.00																														
GP-763	0.64	0.69	0.69	0.52	0.82	0.66	0.70	0.81	0.69	1.00																													
GP-760	0.68	0.68	0.68	0.45	0.76	0.65	0.58	0.74	0.62	0.87	1.00																												
GP-761	0.42	0.37	0.42	0.38	0.39	0.33	0.42	0.34	0.45	0.32	0.39	1.00																											
GP-764	0.65	0.76	0.65	0.42	0.73	0.72	0.60	0.66	0.53	0.83	0.77	0.27	1.00																										
GP-771	0.67	0.56	0.61	0.53	0.65	0.53	0.57	0.68	0.41	0.69	0.73	0.37	0.65	1.00																									
GP-779	0.53	0.48	0.48	0.27	0.53	0.45	0.39	0.55	0.78	0.61	0.60	0.43	0.52	0.48	1.00																								
GP-785	0.67	0.67	0.72	0.46	0.74	0.64	0.63	0.78	0.56	0.84	0.78	0.31	0.81	0.72	0.59	1.00																							
GP-792	0.52	0.52	0.42	0.43	0.51	0.48	0.42	0.58	0.35	0.59	0.58	0.17	0.71	0.62	0.39	0.68	1.00																						
GP-793	0.60	0.60	0.55	0.33	0.59	0.57	0.50	0.67	0.39	0.63	0.61	0.21	0.75	0.66	0.42	0.77	0.67	1.00																					
GP-794	0.71	0.66	0.66	0.48	0.74	0.63	0.67	0.77	0.55	0.78	0.72	0.38	0.74	0.77	0.53	0.87	0.67	0.76	1.00																				
GP-802	0.72	0.72	0.72	0.50	0.79	0.69	0.68	0.78	0.61	0.90	0.84	0.35	0.87	0.77	0.65	0.94	0.68	0.71	0.87	1.00																			
GP-823	0.76	0.70	0.70	0.47	0.73	0.67	0.60	0.77	0.59	0.77	0.77	0.36	0.73	0.70	0.62	0.81	0.60	0.69	0.80	0.87	1.00																		
GP-851	0.73	0.73	0.68	0.45	0.76	0.70	0.63	0.74	0.57	0.81	0.74	0.30	0.83	0.68	0.60	0.84	0.63	0.72	0.83	0.90	0.83	1.00																	
GP-951	0.48	0.48	0.48	0.22	0.48	0.50	0.38	0.45	0.73	0.57	0.61	0.43	0.52	0.43	0.86	0.55	0.33	0.37	0.48	0.60	0.57	0.55	1.00																
GP-972	0.65	0.65	0.65	0.42	0.73	0.67	0.66	0.71	0.53	0.83	0.83	0.31	0.79	0.70	0.62	0.81	0.55	0.63	0.74	0.87	0.79	0.83	0.69	1.00															
GP-1047	0.70	0.70	0.65	0.42	0.73	0.67	0.55	0.66	0.48	0.77	0.83	0.31	0.86	0.70	0.57	0.75	0.60	0.69	0.69	0.81	0.73	0.83	0.57	0.86	1.00														
GP-1052	0.64	0.59	0.53	0.56	0.68	0.61	0.48	0.55	0.37	0.61	0.66	0.27	0.68	0.70	0.35	0.59	0.65	0.63	0.63	0.65	0.68	0.66	0.34	0.62	0.74	1.00													
GP-1060	0.71	0.66	0.66	0.44	0.74	0.63	0.61	0.77	0.55	0.84	0.83	0.33	0.80	0.77	0.69	0.87	0.61	0.70	0.81	0.93	0.86	0.90	0.64	0.93	0.86	0.63	1.00												
GP-1063	0.68	0.73	0.73	0.50	0.81	0.70	0.69	0.74	0.62	0.87	0.80	0.34	0.83	0.68	0.60	0.84	0.58	0.67	0.83	0.90	0.83	0.93	0.61	0.89	0.83	0.66	0.90	1.00											
GP-1068	0.59	0.59	0.59	0.57	0.78	0.67	0.66	0.66	0.48	0.72	0.66	0.31	0.68	0.65	0.52	0.75	0.55	0.63	0.74	0.81	0.73	0.83	0.47	0.73	0.68	0.62	0.80	0.83	1.00										
PGCP-12	0.61	0.67	0.67	0.53	0.81	0.69	0.81	0.68	0.55	0.80	0.73	0.42	0.76	0.67	0.48	0.77	0.52	0.66	0.83	0.83	0.76	0.79	0.48	0.76	0.70	0.64	0.77	0.86	0.82	1.00									
PGCP-14	0.48	0.48	0.48	0.36	0.62	0.50	0.44	0.59	0.69	0.61	0.59	0.38	0.56	0.48	0.73	0.69	0.48	0.52	0.68	0.69	0.61	0.70	0.68	0.61	0.56	0.41	0.68	0.70	0.67	0.58	1.00								
PANT-LO-1	0.72	0.72	0.67	0.39	0.65	0.63	0.57	0.73	0.50	0.69	0.68	0.32	0.70	0.61	0.59	0.77	0.57	0.71	0.77	0.77	0.82	0.79	0.54	0.76	0.70	0.53	0.83	0.79	0.70	0.67	0.63	1.00							
PANT-LO-2	0.69	0.69	0.69	0.52	0.82	0.71	0.59	0.75	0.58	0.76	0.87	0.41	0.72	0.69	0.56	0.79	0.59	0.68	0.78	0.79	0.77	0.75	0.57	0.77	0.77	0.67	0.78	0.81	0.72	0.74	0.71	0.74	1.00						
GC-3	0.67	0.67	0.61	0.35	0.65	0.69	0.52	0.68	0.55	0.64	0.68	0.37	0.65	0.61	0.59	0.72	0.57	0.71	0.77	0.72	0.76	0.68	0.54	0.65	0.59	0.48	0.71	0.68	0.65	0.67	0.63	0.79	0.80	1.00					
GC-4	0.72	0.72	0.67	0.35	0.65	0.69	0.52	0.73	0.61	0.69	0.73	0.37	0.70	0.61	0.64	0.77	0.62	0.71	0.77	0.77	0.82	0.73	0.59	0.70	0.65	0.53	0.77	0.73	0.65	0.67	0.63	0.85	0.80	0.92	1.00				
GC-5	0.69	0.69	0.69	0.47	0.76	0.66	0.65	0.81	0.63	0.81	0.81	0.37	0.77	0.69	0.61	0.90	0.70	0.73	0.90	0.90	0.83	0.87	0.57	0.77	0.72	0.61	0.84	0.87	0.77	0.80	0.77	0.80	0.87	0.80	0.86	1.00			
PUSA-FAL	0.69	0.69	0.69	0.47	0.76	0.66	0.65	0.81	0.63	0.81	0.81	0.37	0.77	0.69	0.61	0.90	0.70	0.73	0.90	0.90	0.83	0.87	0.57	0.77	0.72	0.61	0.84	0.87	0.77	0.80	0.77	0.80	0.87	0.80	0.86	1.00	1.00		
GC-521	0.55	0.55	0.55	0.41	0.50	0.47	0.45	0.62	0.33	0.53	0.57	0.28	0.59	0.50	0.28	0.61	0.62	0.59	0.60	0.56	0.48	0.57	0.23	0.44	0.53	0.46	0.50	0.52	0.44	0.45	0.42	0.55	0.58	0.50	0.55	0.63	0.63	1.00	
GC-901	0.64	0.59	0.64	0.45	0.53	0.50	0.54	0.71	0.52	0.61	0.60	0.43	0.52	0.53	0.45	0.65	0.54	0.47	0.69	0.65	0.62	0.60	0.39	0.52	0.47	0.40	0.58	0.60	0.52	0.53	0.50	0.64	0.61	0.64	0.70	0.72	0.72	0.71	1.00
GC-1008	0.72	0.67	0.72	0.44	0.65	0.63	0.52	0.63	0.55	0.64	0.63	0.37	0.59	0.56	0.53	0.77	0.57	0.60	0.77	0.72	0.70	0.68	0.48	0.59	0.55	0.48	0.66	0.68	0.65	0.61	0.63	0.72	0.74	0.79	0.79	0.80	0.80	0.61	0.77	1.00

Table 8: Distribution of genotypes based on the clusters from UPGMA dendrogram from ISSR primer

Cluster	Sub-cluster	Genotypes
A		GP-761
B	B1:1	GP-111, GP-282, GP-330, GP-458, GP-459, GP-460, GP-585, GP-763, GP-760, GP-764, GP-771, GP-785, GP-792, GP-793, GP-794, GP-802, GP-823, GP-951, GP-972, GP-1047, GP-1052, GP-1060, GP-1063, GP-1068, PGCP-12, PANT-LOBIA-1, PANT-LOBIA-2, GC-3, GC-4, GC-5 PUSA-FALGUNI, GC-521, GC-901, GC-1008
	B1:2	GP-733, GP-779, GP-851, PGCP-14
	B2	GP-452

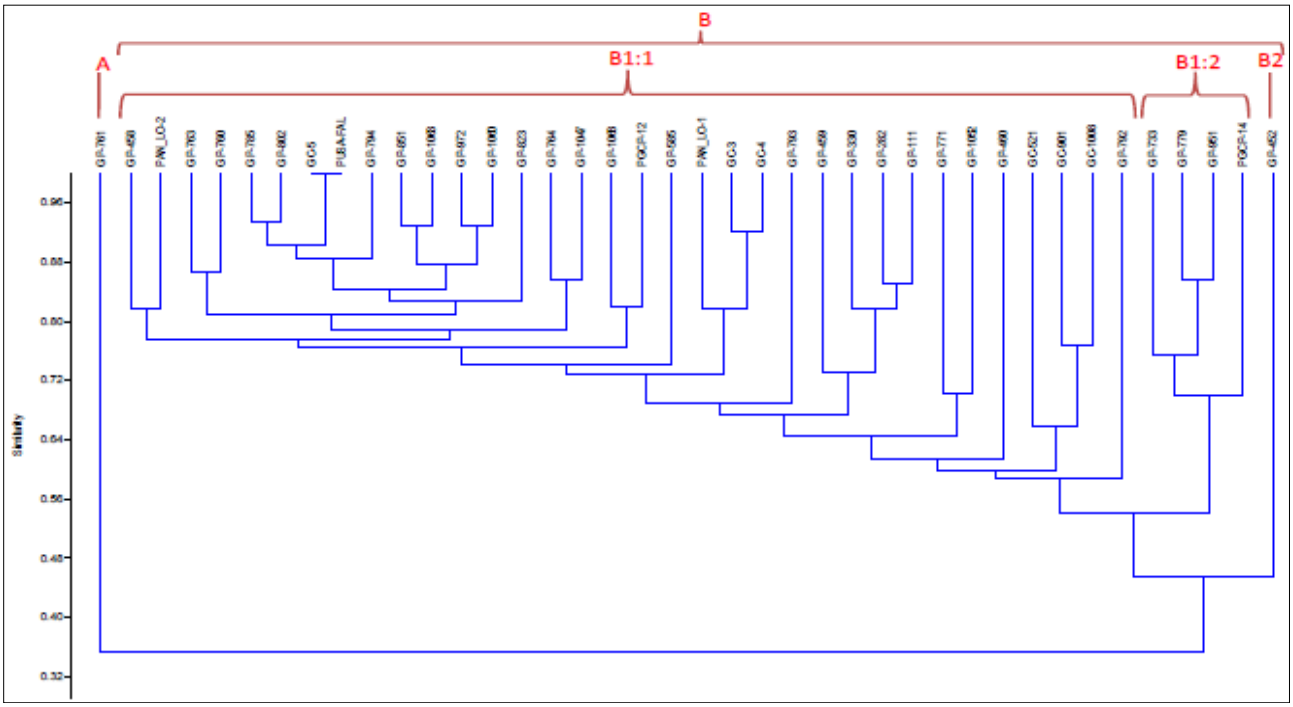

Fig 2: UPGMA dendogram of 40 genotypes of cowpea based on ISSR primers

References

1. Arunachalam V. Genetic distance in plant. Indian J of Genetics and Plant Breeding. 1981; 41:226-236.
2. Badhe PL, Magar NM, Snehal T, Sinde PY. Genetic divergence in cowpea (*Vigna unguiculata* (L.) Walp.) Journal of Food Legumes. 2015; 28(1):81-82.
3. Brahmaiah M, Jhansi RK, Sunil N, Keshavulu K. Genetic divergence in cowpea [*Vigna unguiculata* (L.) Walp.] For yield components and seed quality parameters. Electronic Journal of Plant Breeding. 2014; 5(1):107-111.
4. Dalsaniya SB, Poshiya VK, Savaliya JJ, Davada BK. Genetic divergence in cowpea (*Vigna unguiculata* L. Walp.). Legumes Research. 2009; 32(4):250-254.
5. Davis DW, Oelkae EA, Oplinger J, Doll JD, Hansan CV, Putnam DH. Alternative field crops manual, 2000.
6. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bulletin. 1990; 19:11-15.
7. Gajera HP, Domadiya RK, Patel SV, Golakiya BA. Appraisal of RAPD and SSR Markers for Genetic diversity analysis among Cowpea (*Vigna unguiculata* L. walp) genotypes. Journal of Crop Science Biotechnology. 2014; 17(2):79-88.
8. Kiran T, Krishna T. Genetic divergence for yield and quality components in cowpea [*Vigna unguiculata* (L.) Walp]. Trends in Biosciences. 2013; 6(5):555-557.
9. Mahalanobis PC. On generalized distance in statistics. Proceeding National institute of Science India. 1936; 2:49-55.
10. Murthy BR, Arunachalam V. The nature of divergence in relation to breeding system in crop plants. Indian J Genet. 1966; 26:188-189.
11. Nobila O, Elisabeth Mahama O, Nerbewende Adjima O, Mahamadi HO, Herve N, Mahamadou S. Evaluation of genetc diversity of cultivated and spontaneous accessions of cowpea (*Vigna unguiculata* Walp) in Burkina faso. International Journal of Research in Biosciences. 2016; 5(3):13-24.
12. Panse, Sukhatme. Statistical method for Agricultural workers. Indian council of Agricultural Research. New Delhi, 1998, 381.
13. Pawar RM, Prajapati RM, Sawant DM, Patil AH. Genetic divergence in Indian bean (*Lablab purpureus* L. Sweet), Elec. J Plant Breed. 2013; 4(2):1171-1174.
14. Rao CR. Advanced Statistical Methods in Biometrical Research. John Willey and Sons, Inc., New York, 1952, 357-363.
15. Vavilov NJ. The origin, variation, immunity and breeding of cultivated plants. Chronica Botanica. 1949; 13:364.
16. Weising K, Nyborn H, Wolff K, Kahl G. DNA fingerprinting in plants. Principles, methods, and Application, 2nd end. CRC Press, Boca Raton, florida, 2005.