

Studies on Crossability and Genetic Diversity in Cowpea (*Vigna unguiculata* L. Walp.)



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Abstract

Characterization of genetic diversity among cultivated cowpea (*Vigna unguiculata*) varieties using RAPD markers and assessment of crossability within the species was taken up. Three parameter i.e. pollen fertility, pollen germinability and pollen tube growth rate were measured on both selfing and crossing to assess the crossability. Pollen fertility exhibited less significant correlation with fruit set and high significant correlation was found in between the pollen germinability and pollen tube growth rate with the fruit set. In both selfing and crossing, pollen germination and pollen tube growth increased in a constant rate. The crossability level was found to be better on selfing when compared with crosses. A total of 30 RAPD primers were randomly selected to assess genetic diversity of 36 accessions of cowpea. Based on the PIC value, five primers (OPC 14, OPB 1, OPA 10, OPG 13 and OPA 4) were found to be more informative. The PIC value showed a ranged from 0.597 to 0.885 with the primer OPC 14 having the highest PIC value of 0.885. Base on the Euclidean similarity matrix, a clustered dendrogram was made by following ward's method (Ward, 1963), which indicated that PL-2 and CP-7 were found to be more distinct from IC-202826. Based on the PCA plot, the first component explained 18.56 % variation and the second and third component explained 16.85 % and 12.77 %, respectively among the 36 accessions of cowpea. The first three components explained 48.21 % of total variation.

Keywords: Crossability; Genetic diversity; RAPD; Dendrogram; PCA

Abbreviations: PG: Pollen Germination; PTG: Pollen Tube Growth0020

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) ($2n=2x=22$) is a self-pollinated dicotyledonous crop plant, belonging to the family Fabaceae and native to Central Africa. It is a climbing annual, warm season vegetable grown commonly throughout India as a summer and rainy season crop. According to Ng and Marechal [1], the cultivated cowpea separated into five group namely; *unguiculata*, *sesquipedalis*, *textiles*, *melanophthalmus*, and *biflora*. According to Blackhurst and Miller [2], cross-pollination is usually less than 1% in cowpea even though self-pollinated being morphologically. Hybridization is progressively more recognized as an important process in the evolution of plant populations and species (Kouam et al. [3]). Pollination is the important process in hybridization programme by which pollen is transferred from the anther to the female reproductive organs of a plant, thereby enabling fertilization to take place. So, for the successful fertilization to be occurred, a pollen grain produced by the anther (male reproductive part of a flower) must be viable, enabling to germinate on the stigma and ability to produce pollen tube that can penetrate and fertilize the ovule. Double fertilization and subsequent growth of embryo and endosperm must occur to provide the necessary stimulus

for fruit development (Thompson [4]). Pollen viability, pollen germinability and pollen tube share a great role in hybridization programme for improving the crop. According to Nascimento et al. [5], for a successful hybrid breeding programme, it is needed to assess the compatibility and direction of crossing within the species.

Characterization of genetic diversity among cultivated cowpea (*Vigna unguiculata*) varieties is important to improve the available genetic resources by the researchers through hybridization programme. According to Hegde and Mishra [6], the knowledge of the genetic diversity available within the local and regional germplasm collections can enhance the overall effectiveness of cowpea improvement programs. Cowpea breeding and genetic improvement programs around the world are mainly focused on combining desirable agronomic characteristics, e.g., time to maturity, photoperiod sensitivity, plant type, and seed quality with resistance to the major diseases, insect pests or parasites that agronomically afflict adapted cowpea cultivars (Timko et al. [7]; Timko and Singh [8]). The present study reports pollen germination and pollen tube behavior in relation to crossability between various accessions

of *Vigna unguiculata* and seed set and genetic diversity using RAPD markers.

Materials and Methods

The 36 accessions used were PL-3, Kashi Nidhi, IC-33922, Arka Garima, Kashi Unnati, PL-4, PL-1, PL-5, EC-472260, Sel-16, IC-559390, EC-9738, IC-3004, IC-202865, EC-9739, EC- 110598, Kashi Shyawal, IC-332198, EC-390221, EC-15296, EC-9736, EC-390211, Kashi Gowri, Kashi Kanchan, IC-202826, EC-30950 and PL-2. Some other cowpea accession were also collected from the different locations of North Eastern region of India and mention as N-1, PS-1, CP-7, HS, M-1, M-2, M-3, M-4 and M-5. Out of these accessions, only five were used for crossability studies. They were used for one way crossing and so the total number of selfing and crossing were 15 (Table 2). For each cross at least fifteen flowers were randomly selected from plants. Pollination was performed during June-May of 2016. Pollination was done in morning hours between 9-11 A.M. immediately after emasculation. After emasculation, the stigmatic surface was checked for the presence of pollen before cross- pollination was attempted.

For the pollen viability test, pollen grains from the five parents were taken and stained with 2 % acetocarmine solution and observed under the microscope. All reddish and dark stained pollen were scored as viable while transparent and irregularly shaped unstained pollen grains were scored as non-viable. Total number of viable and non-viable pollen was collected and expressed in percentage and compared with the percent fruit set. For pollen germination and pollen tube growth observations, the styles were collected after 2 hours, 4 hours and 6 hours of pollination and fixed immediately in 1:3 glacial acetic acid-ethyl alcohol for at least 24 hours and then preserved in 70 % alcohol till further use. The pollinated flowers were taken and gently rinsed in distilled water and pistils were separated from the flowers after which they were kept in a drop of 1N HCl for 10 minutes. They were again rinsed in distilled water and stained in 1 percent aniline blue. (Gerlach [9] and D'Souza [10]). The time required for staining was 10-20 seconds depending on the thickness of the style and the stage of penetration of the pollen tube in the stigma. After staining, the pistils were destined for 20-24 hours in a 1:1:1 mixture of 40 % acetic acid: orthophosphoric acid: distilled water. The pistils were then rinsed in distilled water and mounted in pure lactic acid and studied under the microscope. The pollen grains and pollen tubes stained deep blue. Pollen grains were considered to be germinated when pollen tube size were of the same size as or bigger than them (Ribeiro et al. [11] and Almeida et al. [12]). The germinated pollen grains were counted and expressed in percentage. The three longest pollen tube lengths were measured in terms of micrometer.

The genomic DNA was isolated from the 5-10 days old cowpea seedlings by using a modified cetyl trimethylammonium bromide (CTAB) method of Doyle and Doyle (1990). Young

actively growing leaves of different accessions were collected and used for DNA extraction. The quantification of DNA were done by staining DNA with ethidium bromide after electrophoresis in 0.8 % agarose gel at 80 V for 45 minutes in 0.5 X TBE buffer (0.04M Tris borate, 0.001M EDTA, pH 8.0) using known DNA concentration standard of 1Kb ladder (Gene Ruler, Fermentas). Molecular weight of bands was estimated by comparing with 1 Kb ladder for RAPD scoring. Each amplification product was considered a DNA marker and was scored across the 36 samples with 30 RAPD primers. The RAPD data was subjected to Paleontological Statistics, PAST v3.15 software (Hammer et al. [13]) to construct a dendrogram by hierarchical cluster analysis based on Ward's method (Ward [14]) using the Euclidean similarity matrix (Cruz and Regazzi [15]) as cited by Oliveira and Valls (2003). Based on the 'Eigen' vectors analysis, the principle components were extracted. The first three most important PCA were used to construct 2 D plot of the accessions. The PCA, hierarchical cluster, Euclidean similarity matrix, presented in this paper was computed using Paleontological Statistics, PAST v3.15 software (Hammer et al. [13]).

Results and Discussion

Pollen Fertility

The pollen fertility of the five parents under this study revealed that the maximum pollen fertility among the parents were observed in Arka Garima (96.79±0.24) whereas IC-33922 (90.35±0.25) had the least pollen fertility percentage (Table 1). However, the average percent pollen fertility did not show too much difference among the parents. According to Ribeiro et al. [16], the cowpea bean pollen can remain viable for about 42 hours after anthesis depending on air temperature and relative humidity. Ting et al. [17] stated that the success of hybridization includes the ability of the donor plant to produce viable pollen and the duration time of the pollen viability.

Table 1: Pollen fertility in the parents in cowpea.

Sl. No.	Parents	Pollen Fertility (%)	Fruit Set (%)
1	Arka Garima	96.79 ± 0.24	91.67
2	PL-3	93.57 ± 0.37	88.89
3	Kashi Unnati	92.32 ± 0.35	75.00
4	Kashi Nidhi	92.53±0.31	81.25
5	IC-33922	90.35±0.25	73.47

Pollen Germination

In case of selfing, maximum pollen germination after 2 hours of pollination was recorded in selfing of Arka Garima (66.95 %) and the minimum was recorded in selfing of Kashi Nidhi (58.61 %). After 2 hours of pollination, on selfing, maximum pollen germination was recorded in Arka Garima (66.95 %), followed by PL-3 (62.50 %), Kashi Unnati (61.18 %), IC-33922 (59.40 %) and Kashi Nidhi (58.61 %). After 6 hours of pollination, on selfing, maximum pollen germination was recorded in Arka Garima (71.11 %) and the minimum was recorded in Kashi Nidhi

(64.49 %) (Figure1). The results show that there was a constant increase in pollen germination from 2 hours to 6 hours in all the parents. In crosses between the parents, the maximum pollen germination was recorded in Kashi Nidhi X IC-33922 (57.59 %) after 2 hours of pollination and the minimum was found in PL-3 X Kashi Nidhi (50.92 %) after 2 hour of pollination. After 6 hours of pollination, maximum pollen germination was recorded in Arka Garima X IC-33922 (61.22 %) and the minimum was recorded in PL-3 X Kashi Nidhi (52.83 %) (Figures 2 & 3).

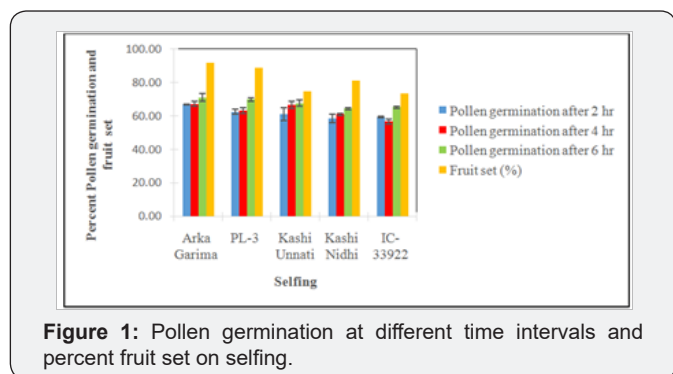


Figure 1: Pollen germination at different time intervals and percent fruit set on selfing.

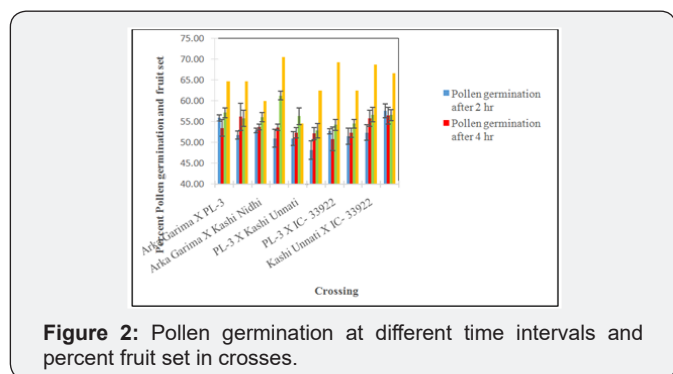


Figure 2: Pollen germination at different time intervals and percent fruit set in crosses.

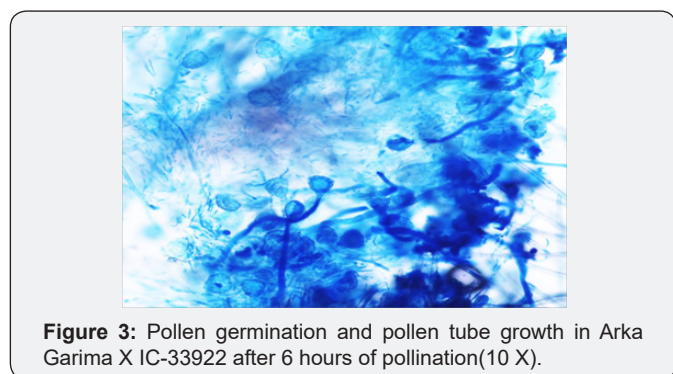


Figure 3: Pollen germination and pollen tube growth in Arka Garima X IC-33922 after 6 hours of pollination(10 X).

Table 2 shows that fruit set on selfing was maximum in Arka Garima (91.67 %) where pollen germination was also maximum i.e. 71.11 % and the least pollen germination after 6 hours of pollination was recorded in Kashi Nidhi (64.39 %) where the fruit set was quite high (81.25 %). In crosses, Arka Garima X IC-33922 showed the highest fruit set (70.59 %) which showed the maximum pollen germination (61.29 %) after 6 hours of pollination while PL-3 X Kashi Unnati which gave least fruit set (54.55 %) showed the pollen germination of 56.33 %, which is quite good. So, in general, pollen germination increased from 2

hour to 6 hours after pollination both in case of selfing as well as crossing.

Table 2: Pollen germination and pollen tube growth after 6 hours and percent fruit set on selfing and in crosses.

Sl. No.	Parents/Crosses	Pollen Germination After 6 Hours (%)	Pollen Tube Growth After 6 Hours (µm)	Fruit Set (%)
1	Arka Garima	71.11 ± 2.22	300.97 ± 1.63	91.67
2	PL-3	70.00±1.15	289.62 ± 1.11	88.89
3	Kashi Unnati	67.70±1.92	268.17 ± 1.32	75.00
4	Kashi Nidhi	64.39±0.55	271.76 ± 1.34	81.25
5	IC- 33922	65.28±0.59	264.87 ± 2.67	73.47
6	Arka Garima X PL-3	57.18±1.17	241.42 ± 1.77	64.71
7	Arka Garima X Kashi Unnati	55.79±1.97	238.98 ± 2.06	64.71
8	Arka Garima X Kashi Nidhi	56.11±1.11	249.34 ± 1.24	60.00
9	Arka Garima X IC-33922	61.29±1.07	250.25 ± 0.82	70.59
10	PL-3 X Kashi Unnati	56.33±2.03	224.49 ± 2.29	54.55
11	PL-3 X Kashi Nidhi	52.83±1.74	241.49 ± 2.14	62.50
12	PL-3 X IC-33922	54.21±1.28	228.48 ± 1.12	69.23
13	Kashi Unnati x Kashi Nidhi	54.49±1.03	236.69 ± 1.94	62.50
14	Kashi Unnati X IC-33922	56.69±1.79	246.19 ± 1.50	68.75
15	Kashi Nidhi X IC-33922	56.57±1.37	232.08 ± 2.17	66.67

In some cases, the pollen germination was reduced from 2 hours to 4 hours after pollination in the selfing of IC-33922 and the crossing of Kashi Nidhi X IC-33922 and Arka Garima X PL-3. But in general, pollen germination was more in the selfing as compared to the crossing and similar results have been observed in fruit set. Ribeiro et al. [16] while investigating the pollen properties of *Vigna unguiculata*, observed a downward linear effect in the regression analysis. This means that pollen which is genetically viable may have low rate of pollen germinability.

Pollen Tube Growth

After 2 hour of pollination, on selfing, the maximum pollen tube growth was observed in Arka Garima (93.69µm) and the minimum in IC-33922 (61.50µm) (Figure 4). In crosses, maximum pollen tube growth was observed in PL-3 X Kashi Unnati (60.01µm) and minimum was observed in Kashi Nidhi X IC-33922 (51.20µm). After 4 hours of pollination, there was a nearly double elongation in the pollen tubes whereas some had just started to elongate. After 6 hours of pollination, pollen tube growth became nearly three times that after 2

hours of pollination. On selfing, maximum pollen tube growth was observed in Arka Garima (300.97µm) and the minimum was observed in IC-33922 (264.87µm). In crosses, maximum pollen tube growth was observed in Arka Garima X IC-33922 (250.25µm) and the minimum pollen tube growth was observed in PL-3 X IC-33922 (228.48µm) (Figures 5 & 6). So, the results revealed that there was constant increase in the growth rate of pollen tubes after 2 hours to 6 hours of pollination.

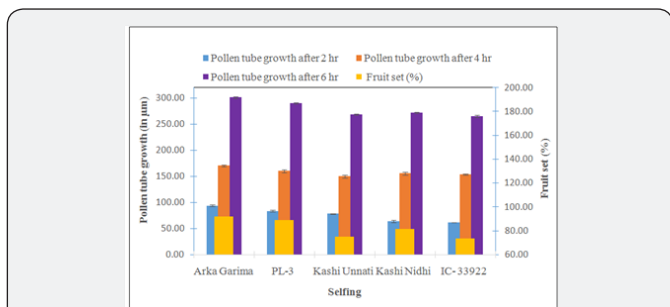


Figure 4: Pollen tube growth at different time intervals and percent fruit set in selfing.

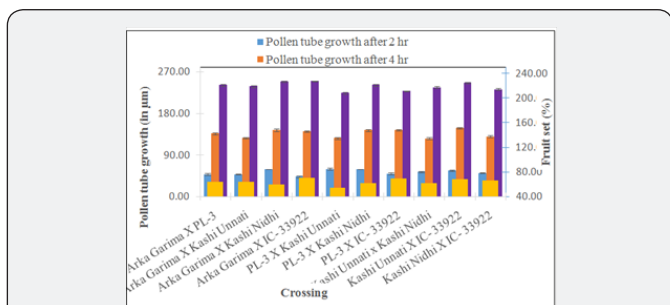


Figure 5: Pollen tube growth at different time intervals and percent fruit set in crosses.

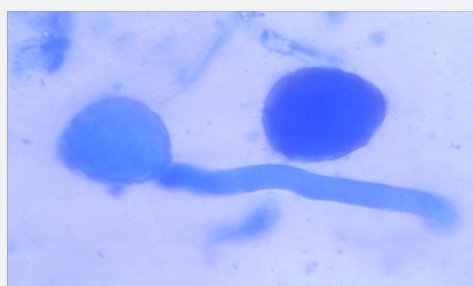


Figure 6: Pollen germination and pollen tube growth in PL-3 X IC-33922 after 6 hours of pollination (40 X).

On selfing, the fruit set was positively correlated with faster pollen tube growth whereas it was not so in the case of crosses. In case of selfing, the maximum pollen tube was recorded in the case of Arka Garima (300.95µm), where the fruit set was maximum (91.67 %), and the least pollen tube growth was in IC-33922 (264.87µm) where minimum fruit set (73.47 %) was obtained. But in the case of crossing, the maximum pollen tube was observed in Arka Garima X IC-33922 (250.25µm) where the fruit set (70.59 %) was maximum among the crosses. On the contrary, among the crosses, the minimum pollen tube was observed in PL-3 X IC-33922 (228.48µm) where the fruit set

percent (69.23 %) was quite high. But by observing as a whole, pollen tube growth rate of selfing was greater than that of crossing. In all the crosses including selfing and crossing, there was constant increment in pollen tube growth from 2 hours to 6 hours.

Fruit Set

Among the selfing, the maximum fruit set was obtained in Arka Garima (91.67 %) and the least in IC-33922 (73.47%) (Figures 7 & 8). When the parents were crossed, the maximum fruit set was obtained in Arka Garima X IC-33922 (70.59 %) and the least fruit set was obtained in PL-3 X Kashi Unnati (54.55 %) (Figures 9 & 10). In general, fruit set was higher in selfing as compared to crossing (Table 2).



Figure 7: Fruit set of Arka Garima after selfing.



Figure 8: Fruit set of IC-33922 after selfing.



Figure 9: Fruit set of Arka Garima X IC-33922.



Figure 10: Fruit set of PL-3 X Kashi Unnati.

Pollen germination was more in the selfing as compared to the crosses and similar results have been observed in fruit set. So, pollen germination found a positive correlation with the fruit set. On selfing, the fruit set was positively correlated with pollen tube growth whereas it was not so in the case of crosses. The maximum pollen tube was recorded in the case of Arka Garima (300.97µm), where the fruit set was maximum (91.67 %) and least was found in IC-33922 (73.47 %). But in case of crossing, the maximum pollen tube was observed in Arka Garima X IC-33922 (250.25µm) where the fruit set (70.59 %) was maximum among the crosses. On the contrary, among the

crosses, the smallest pollen tubes were observed in PL-3 X IC-33922 (228.48µm) where the fruit set percent (69.23%) was quite high (Table 2).

Correlation Studies in Crosses of *Vigna Unguiculata*

Correlation studies on pollen germination showed low significant values for percent pollen fertility except that pollen germination after 4 hours had a non-significant correlation with pollen fertility. However, pollen germination after 4 hours of pollination was highly correlated with pollen germination after 2 hours and 6 hours of pollination. Pollen germination after 6 hours of pollination had a highly significant correlation with pollen germination after 2 hours and 4 hours of pollination. Pollen tube growth recorded low significant correlation with percent pollen fertility except pollen tube growth after 6 hours which had a highly significant correlation with pollen fertility. Pollen tube growth at 2 hours, 4 hours and 6 hours was observed to be highly correlated with pollen germination after 2 hours, 4 hours and 6 hours of pollination. In the same way, pollen tube growth after 2 hours of pollination exhibited a high significant correlation with pollen tube growth after 4 hour and 6 hours of pollination. Pollen tube growth after 4 hours of pollination was observed to be highly correlated with pollen tube growth after 2 hours and 6 hours of pollination.

Table 3: Correlation studies for various characters in crosses of *Vigna unguiculata*.

Parameter		Pollen Fertility (%)	PG After 2 Hours (%)	PG After 4 Hours (%)	PG After 6 Hours (%)	PTG After 2Hours (µm)	PTG After 4 Hours (µm)	PTG After 6 Hours (µm)
PG after 2 hr (%)	(r)	0.908*						
	ρ	0.0165						
PG after 4 hr (%)	(r)	0.7631	0.886**					
	ρ	0.0667	0.000006					
PG after 6 hr (%)	(r)	0.809*	0.895**	0.895**				
	ρ	0.0485	0.000003	0.000003				
PTG after 2 hr (µm)	(r)	0.893*	0.800**	0.853**	0.806**			
	ρ	0.0207	0.0002	0.00003	0.0001			
PTG after 4 hr(µm)	(r)	0.890*	0.726**	0.701**	0.785**	0.765**		
	ρ	0.0214	0.0011	0.0018	0.0003	0.0004		
PTG after 6 hr (µm)	(r)	0.937**	0.842**	0.868**	0.927**	0.850**	0.888**	
	ρ	0.0094	0.00004	0.00001	0.0000003	0.00003	0.000005	
Fruit set(%)	(r)	0.891*	0.850**	0.826**	0.882**	0.742**	0.858**	0.916**
	ρ	0.0213	0.00003	0.0001	0.00001	0.0008	0.00002	0.000001

Note: *Correlation significant as $p < 0.05$ (5 % level of significance)

**Correlation significant as $p < 0.01$ (1 % level of significance)

PG= Pollen Germination; PTG= Pollen Tube Growth; r = Pearson's Correlation Coefficient; ρ = Population Correlation Coefficient

Fruit set had highly significant correlation with pollen germination (0.850, 0.826, and 0.882). A positive correlation with pollen tube growth was seen which was highly significant at 1 % level of significance (0.742, 0.858 and 0.916). But fruit set had a low significant correlation with pollen fertility (0.891) (Table 3). Kharkongar et al. [18] found a positive but

non-significant correlation of pollen fertility with the fruit set. However, Debbarama found a negative correlation between pollen fertility and fruit set.

In the present investigation, a total of 30 RAPD primers were randomly selected to assess the genetic diversity of 36

accessions of cowpea. These primers showed polymorphic percent in the range of 50-100 except OPE 18, which gave a very low polymorphic percent of 14.29 %. But an average total number of band of each primer (9.8 allele) with polymorphic band (8.4 allele) produced an average polymorphic percentage (85.37 %). These primers produced bands in the range of 100-3000 bp. Based on the Polymorphism Information Content (PIC) value, five primers; OPC 14 (0.885), OPB 1 (0.879), OPA 10 (0.868), OPG 13 (0.862) and OPA 4 (0.856) were found to be more

informative. The PIC value showed a range from 0.597 to 0.885 with the primer OPC 14 having the highest PIC value of 0.885 (Table 4). Udensi et al. [19] reported polymorphic percentage range from 75-100% while studying 20 cowpea accessions by 14 RAPD primers. Genetic diversity of the primer used ranged from 0.620-0.015, where OPB 07, OPT 15 as well as OPB 10 revealed the highest genetic diversity. Additionally, PIC showed a ranged of 0.5711-0.9087 with OPB 07 having the highest value of PIC (0.9087).

Table 4: Total no. of amplification bands (TNB), total no. of polymorphic bands (TPB), Percent polymorphism and polymorphism information content (PIC) of RAPD markers.

Primer	Sequence (5'-3')	Range of fragments (bp)	TNB	TPB	Percent polymorphism	PIC
OPA 4	AATCGGGCTG	300 - 2000	15	14	93.33	0.856
OPA 5	AGGGGTCTTG	425 - 1000	7	6	85.71	0.808
OPA 7	GAAACGGGTG	200 - 1600	11	10	90.91	0.869
OPA 9	GGGTAACGCC	250 - 1500	10	10	100.00	0.841
OPA 10	GTGATCGCAG	300 - 1600	12	11	91.67	0.868
OPA 13	CAGCACCCAC	300 - 2000	11	9	81.82	0.840
OPA 17	GACCGCTTGT	200 - 1400	11	10	90.91	0.851
OPB 1	GTTTCGCTCC	230 - 2000	12	11	91.67	0.879
OPB 5	TGCGCCCTTC	400 - 1300	6	5	83.33	0.806
OPB 7	GGTGACGCAG	300 - 1500	12	12	100.00	0.822
OPB 10	CTGCTGGGAC	250 - 1800	6	5	83.33	0.780
OPB 11	GTAGACCCGT	350 - 1600	8	7	87.50	0.830
OPB 12	CCTTGACGCA	250 - 1200	12	12	100.00	0.834
OPB 13	TTCCCCGCT	300 - 1200	4	3	75.00	0.597
OPB 17	AGGGAACGAG	200 - 1300	16	15	93.75	0.833
OPC 4	CCGCATCTAC	100 - 1400	11	8	72.73	0.807
OPC 5	GATGACCGCC	300 - 2200	15	10	66.67	0.807
OPC 6	GAACGGACTC	250 - 1500	8	6	75.00	0.798
OPC 11	AAAGCTGCGG	300 - 1600	9	7	77.78	0.788
OPC 14	AAGCCTCGTC	300 - 1500	12	11	91.67	0.885
OPC 15	CGTCTAGGTG	300 - 1200	10	10	100.00	0.856
OPD 18	GTGTGCCCA	300 - 1500	8	7	87.50	0.833
OPE 11	GGTACTGTG	275 - 1200	10	9	90.00	0.844
OPE 18	GGA CTGCAGA	600 - 2200	7	1	14.29	0.801
OPE 19	ACGGCGTATG	250 - 750	4	4	100.00	0.606
OPG 13	CTCTCCGCCA	200 - 3000	12	10	83.33	0.862
OPP 13	GGAGTGCCTC	300 - 1400	12	6	50.00	0.667
OPR 12	ACAGGTGCGT	450 - 1300	7	7	100.00	0.796
OPZ 3	CAGCACCGCA	400 - 1600	11	10	90.91	0.855
OPZ 13	GACTAAGCCC	250 - 1500	5	5	100.00	0.652

Based on the Euclidean similarity matrix, a cluster dendrogram (Figure 11) following the Ward's method generated 5 different clusters namely, A (5 accessions), B (9 accessions), C (11 accessions), D (6 accessions) and E (5 accessions). Base on the Euclidean similarity coefficient, PL-2 and CP-7 were found to be more distinct from IC-202826 (3.32) whereas (PL-3 and

IC-33922) and (EC-15296 and EC-30950) were found to be very less distinct with Euclidean value of 0.00. The value of Euclidean similarity coefficient ranged from 0.00 to 3.32. The dendrogram indicated that most of the accessions such as, M-1, M-4, HS, CP-7 and M-2 collected from North Eastern states belonged to the same cluster i.e. C cluster and the remaining accessions

were distributed in different clusters. Sarutayophat et al. [20] reported genetic relatedness among 36 yardlong bean/cowpea accessions. A total of 38 visible bands and 23 polymorphic bands were generated with the mean of 7.6 and 4.6 bands/primer, respectively. OPZ-03 gave the highest number of fragments (11 fragments) and 7 of these fragments were polymorphic. A dendrogram constructed from 23 polymorphic bands revealed fairly good separation of genetic groups between yardlong bean and cowpea.

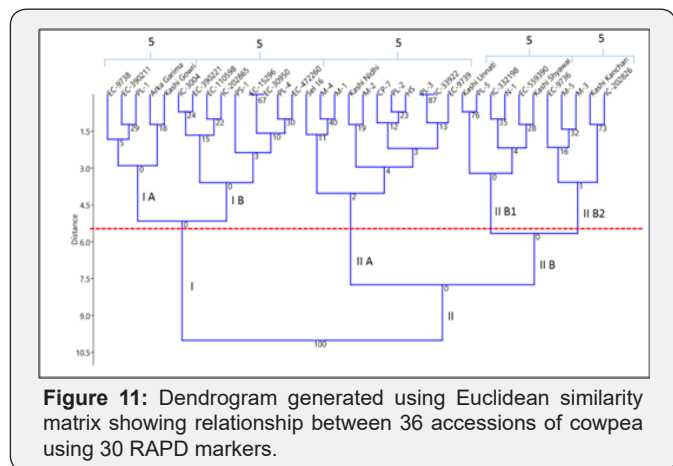


Figure 11: Dendrogram generated using Euclidean similarity matrix showing relationship between 36 accessions of cowpea using 30 RAPD markers.

In the principal component axes, the first component in PCA plot (Figure 12) explained 18.56 % variation and second and third component explained 16.85 % and 12.77 %, respectively among

the 36 accessions of cowpea. The grouping obtained through PCA was comparable to Euclidean similarity matrix cluster analysis. The Eigen values of the principal component axes was found maximum in PC 1 (0.53) followed by PC 2 (0.48) and PC 3 (0.37) (Table 5). The first three components explained 48.21 % of total variation. Adewale et al. [21] reported an assessment for genetic diversity of 9 breeding lines and a common cowpea cultivar by using eight phenotypic traits i.e. plant height at 4 weeks, number of pods per plant, pod length, number of days to first flower, number of days to first ripe pod, number of peduncles per plant, peduncle length and number of branches per plant. In this study, the mean Euclidean distance between the genotypes was 3.7479. The first three principal component axes (PCA) explained 80% of the total variation Figure 13 [22-24].

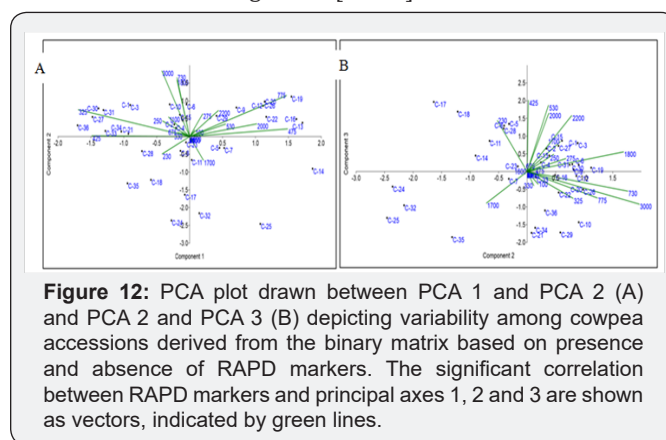


Figure 12: PCA plot drawn between PCA 1 and PCA 2 (A) and PCA 2 and PCA 3 (B) depicting variability among cowpea accessions derived from the binary matrix based on presence and absence of RAPD markers. The significant correlation between RAPD markers and principal axes 1, 2 and 3 are shown as vectors, indicated by green lines.

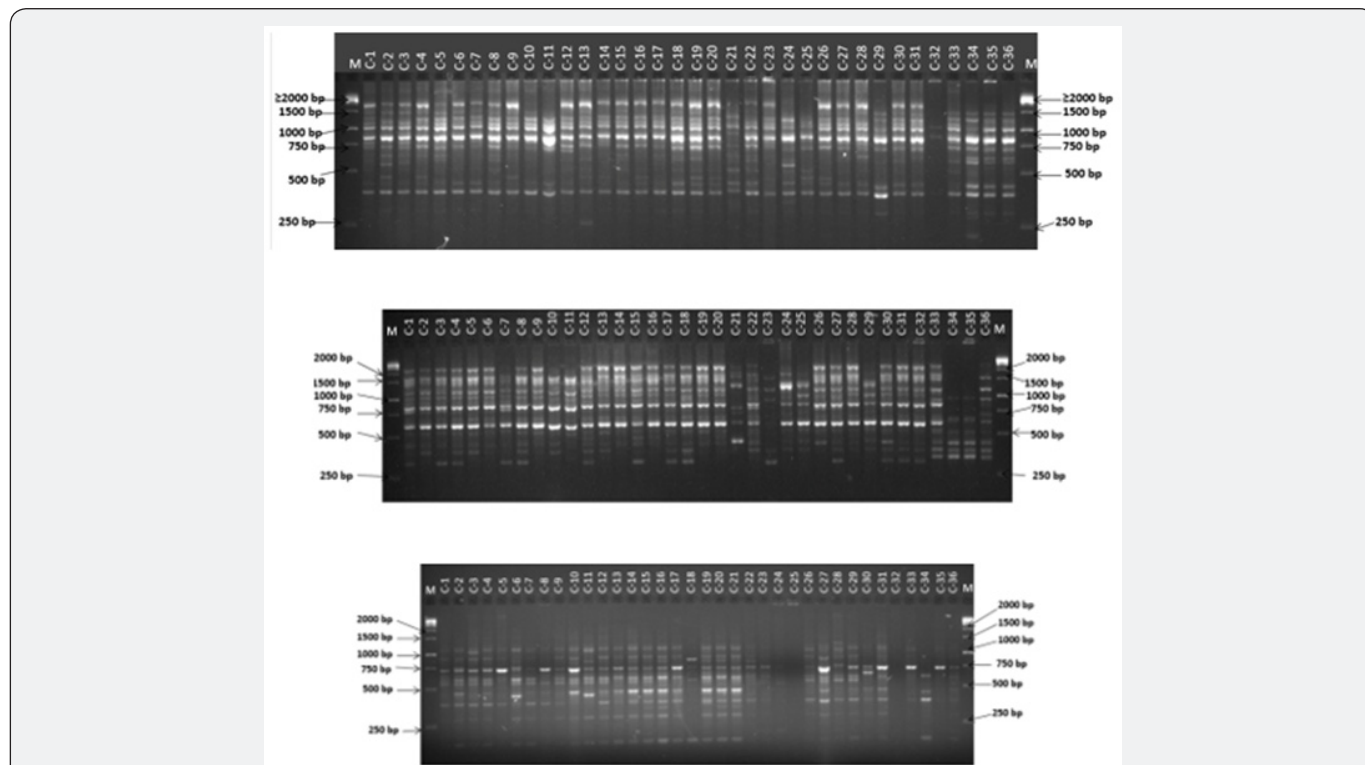


Figure 13: RAPD profiles generated by OPA 4, OPC 5 and OPB 17 primers, respectively on 36 accessions of *Vigna unguiculata*. Number C-1 to C-36 refer to accession numbers allotted to the 36 accessions. M indicates 1 Kb ladder.

Table 5: Eigen values of the principal component axes and the percent variations explained by the principal components on PCA plot generated based on the variations among the RAPD markers.

Principal Component (PC)	Eigen value	% variation	Cumulative % variation
1	0.53	18.56	18.56
2	0.48	16.87	35.44
3	0.37	12.77	48.21

Conclusion

Investigating properties of pollen grain and receptivity of the stigma of a particular crop species is essential for performing a successful hybridization programme, which is again a precious tool for crop improvement purposes. Pollen fertility, pollen germinability and pollen tube growth are the prerequisites for the development of a successful hybrid. Base on the Euclidean similarity coefficient, two pair of accessions, PL-2 and IC-202826 and CP-7 and IC-202826 was found to be more distinct from each other. These distinct pair of accessions could be used for breeding purpose for crop improvement.

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