Agrotechnology
ISSN: 2168-9881

Tondonba et al., Agrotechnology 2018, 7:2
DOI: 10.4172/2168-9881.1000179

Research Article Open Access

Crossability Studies and Genetic Diversity Analysis in Blackgram (*Vigna mungo* L. Hepper) Using Molecular Markers

Tondonba SP, Khanna VK* and Tejaswini VU

School of Crop Improvement, College of Post-Graduate Studies, Central Agricultural University, Barapani, Umiam-793103, Meghalaya, India

Abstract

Blackgram yield has remained low across subtropical and tropical Asia. It is important to estimate the genetic diversity in existing cultivars in order to see if the lack of genetic variability is a constraining factor. Crossability studies among cultivated Blackgram (*Vigna mungo*) varieties on the basis of pollen fertility, pollen germination and pollen tube growth along with genetic diversity analysis using SSR markers was undertaken. A positive non-significant correlation was seen between pollen fertility and pod set and high significant correlation was seen between pollen germinability, pollen tube growth rate with the pod set. A total of 30 SSR primers were screened for genetic diversity analysis in 35 accessions of Blackgram out of which a total of 11 primers were found to be polymorphic. A total of 357 amplification products were generated at an average of 32.45 per primer with an overall polymorphism of 32.97%. The extent of polymorphism was moderate to low. Simple matching coefficient values ranged from 0.14 to 0.82. Neighbor joining tree generated three clusters which revealed that there was greater homology between the accessions released from same source. The close genetic similarity between the cultivars could be explained due to the high degree of commonness in their pedigrees. The narrow genetic base of the Blackgram revealed in the present analysis emphasizes the need to exploit the large germplasm collections having diverse morphological traits in crop improvement programme.

Keywords: Crossability; Pollen; Polymorphism; Genetic diversity; SSR

Introduction

Blackgram [*Vigna mungo* (L.) Hepper], popularly known as urdbean or mash, is a self-pollinating grain legume belonging to the family Fabaceae. It is considered to have been domesticated in India from its wild ancestral form V. mungo var. silvestris [1]. Center of genetic diversity of Blackgram is found in India [2]. *Vigna mungo* (L.) Hepper (2n=22) is an essential grain legume crop in tropical and subtropical areas. It is cultivated as fallow crop after rice cultivation in India. It is grown in various agro-ecological conditions and cropping systems with diverse agricultural practices. In various parts of India, particularly the hilly regions, a number of traditional landraces of Blackgram are still being cultivated as intercrop between rice, sugarcane, cotton, groundnut and sorghum cultivating seasons.

Hybridization is the process of interbreeding between individuals of different species (interspecific hybridization) or genetically divergent individuals from the same species (intraspecific hybridization). Offspring produced by hybridization may be fertile, partially fertile, or sterile. Pollen sterility is one of the key factors limiting legume yield under high temperature [3]. Hybridization involves a series of events which includes pollen germination, pollen tube growth, fertilization, embryo and endosperm development, and seed formation. These events have been influenced by barriers which are well known as hybridization barriers. Hybridization barriers are divided into two broad groups, namely pre-fertilization and post-fertilization barriers [4]. The mechanism of pre-fertilization barriers was observed in wide crosses namely inhibition of pollen germination, delayed pollen tube growth and structural aberrations of pollen tubes in cotton [5] and in sesame [6].

Consideration of genetic diversity existing in a population is the basic requirement for effective improvement programme. Information on nature and degree of genetic divergence would help the plant breeder in choosing the right type of parents for purposeful hybridization. Molecular markers are effective and reliable tools for measuring genetic diversity in crop germplasm and studying evolutionary relationship. Molecular genetics techniques using DNA polymorphism is increasingly used to characterize and identify a novel germplasm for use in the crop breeding process [7]. Paterson [8] stated that evaluation of the genetic diversity would promote the efficient use of genetic variation in the breeding program. The present study reports crossability between different accessions of Blackgram on the basis of pollen development and pod set along with genetic diversity analysis using SSR markers.

Materials and Methods

The experimental material was collected from different parts of India which were kindly supplied by Dr. K Noren Singh, Professor, College of Post Graduate studies (CAU-Imphal), Indian Institute of Pulses Research, Kanpur, and some accessions were supplied by G. B. Pant University of Agriculture and Technology, Pantnagar. The 35 accessions that were used are given in Table 1. Out of these accessions five were used for crossability studies. They were used for one way crossing and so the total number of selfings and crossings were 15 (Table 2). For each cross at least fifteen flowers were randomly selected from plants. Pollinations were performed during October, 2017. Pollination was done in the morning hours between 4:30-5:00 A.M. immediately after emasculation. After emasculation, the stigmatic surface was checked for

***Corresponding author:** Khanna VK, School of Crop Improvement, College of Post-Graduate Studies, Central Agricultural University, Barapani, Umiam-793103, Meghalaya, India, Tel: 09436997876; E-mail: khannavk@rediff.com

Received August 24, 2018; **Accepted** September 28, 2018; **Published** October 08, 2018

Citation: Tondonba SP, Khanna VK, Tejaswini VU (2018) Crossability Studies and Genetic Diversity Analysis in Blackgram (*Vigna mungo* L. Hepper) Using Molecular Markers. 7:179. doi: 10.4172/2168-9881.1000179

S. No.	Accessions	Accession no.	Place of collection
1	KU-16-23	B-23	Manipur
2	KU-16-24	B-28	Manipur
3	KU-16-25	B-33	Manipur
4	KU-16-26	B-5	Manipur
5	KU-16-28	B-12	Manipur
6	KU-16-30	B-30	Manipur
7	KU-16-31	B-10	Manipur
8	KU-16-32	B-3	Manipur
9	KU-16-33	B-16	Manipur
10	KU-16-34	B-34	Manipur
11	KU-16-35	B-29	Manipur
12	KU-16-37	B-32	Manipur
13	KU-16-38	B-9	Manipur
14	KU-16-39	B-22	Manipur
15	KU-16-40	B-18	Manipur
16	KU-16-41	B-35	Manipur
17	GP-PKGU-1	B-13	Uttarakhand
18	GP-PGRU-9514	B-4	Uttarakhand
19	GP 11 I IPU 94(CR)	B-6	Uttarakhand
20	GP-19-TU-91-2	B-21	Uttarakhand
21	GP-41 IPU2-43	B-7	Uttarakhand
22	GP-43-U-218	B-8	Uttarakhand
23	GP(46)-IPU-99-213	B-1	Uttarakhand
24	GP 52. N0.5	B -11	Uttarakhand
25	GP-IPU-99-238	B-14	Uttarakhand
26	GP-106-JU-4	B-19	Uttarakhand
27	GP-115 IC 10703	B-15	Uttarakhand
28	GP-134.16.50 748	B-2	Uttarakhand
29	GP-148-PLU-250	B-27	Uttarakhand
30	PANT-U-6	B-20	Kanpur
31	PANT-U-19	B-17	Kanpur
32	PANT-U-30	B-25	Kanpur
33	PANT-U-31	B-24	Kanpur
34	PANT-U-40	B-26	Kanpur
35	PANT-U-35	B-31	Kanpur

Table 1: List of accessions collected for genetic diversity.

the presence of pollen before cross- pollination was attempted.

For the pollen viability test, pollen grains from 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 1 N HCl for 10 minutes. They were again rinsed in distilled water and stained in 1 percent aniline blue [9,10]. 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 destained for 20-24 hours in a 1:1:1 mixture of 40 % acetic acid: ortho-phosphoric 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 were of the same size as or bigger than them [11,12]. The germinated pollen grains were counted and expressed in percentage. The three longest pollen tube lengths were measured with a micrometer.

The genomic DNA was isolated from the 10-15 days old Blackgram seedlings by using a modified cetyl trimethylammonium bromide (CTAB) method of Doyle and Doyle [13]. Young actively growing leaves of different accessions were collected and used for DNA extraction. The quantification of DNA was 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 (0.04 M Tris borate, 0.001 M EDTA, pH 8.0) using known DNA concentration standard of 1 Kb ladder (Gene Ruler, Fermentas). Molecular weight of bands was estimated by comparing with 100 bp ladder for SSR scoring. Each amplification product was considered a DNA marker and was scored across the 35 samples with 30 SSR primers. PCR products were scored using binary number "0"

S. No.	Parents/Crosses	Pollen germination after 6 hours (%)	Pollen tube growth after 6 hours (µm)	Pod set (%)
1	KU-16-33	65.41 ± 2.54	263.48 ± 2.14	80.00
2	KU-16-26	69.07 ± 2.14	260.87 ± 1.72	76.92
3	GP-IPU-99-238	67.90 ± 2.16	243.30 ± 1.83	71.42
4	PANT-U-31	70.00 ± 1.15	284.47 ± 2.05	90.90
5	PANT-U-6	62 .66 ± 4.17	295.51 ± 2.54	91.66
6	KU-16-33 X KU-16-26	52.22 ± 1.21	244.11 ± 2.30	70.58
7	KU-16-33 X GP-IPU-99-238	51.33 ± 1.33	221.21 ± 2.64	60.00
8	KU-16-33 X PANT-U-31	57.32 ± 1.07	227.35 ± 2.09	62.50
9	KU-16-33 X PANT-U-6	53.44 ± 2.07	233.84 ± 2.24	64.28
10	KU-16-26 X GP-IPU-99-238	57.67 ± 1.70	217.11 ± 4.18	54.55
11	KU-16-26 X PANT-U-31	53.61 ± 1.20	238.18 ± 1.60	66.66
12	KU-16-26 X PANT-U-6	54.58 ± 0.97	225.68 ± 2.07	63.00
13	GP-IPU-99-238 X PANT-U-31	52.80 ± 0.65	217.10 ± 3.27	58.33
14	GP-IPU-99-238 XPANT-U-6	58.57 ± 2.78	225.31 ± 2.49	61.54
15	PANT-U-31 X PANT-U-6	56.99 ± 1.31	241.16 ± 0.98	68.75

Table 2: Pollen germination, pollen tube growth with pod set percent.

and "1" where "1" indicates presence of the allele while "0" indicates absence of the allele. The SSR data archived in the Microsoft excel sheet were used to generate cluster dendrogram according to simple matching coefficient using DARWIN 6.0.15 software. PIC was calculated using an online PIC calculator developed by University of Liverpool (www.liverpool.ac.uk/~kempsj/pic.html.).

Results and Discussion

Pollen fertility

The pollen fertility of all the accessions used as parents in the various crosses, under this study revealed that the maximum fertile stained pollen was recorded in KU-16-33 (94.06 ± 0.31) followed by PANT-U-31 (91.43 ± 1.73) whereas the lowest percentage of fertile pollen recorded was in GP-IPU-99238 (84.03 ± 1.66) (Table 3).

Pollen germination

In the current experiment on pollen germinability test it was found that there was an increase in percent pollen germination after 2 hours to 6 hours in both selfing and crossing (Table 2). In selfing, after 2 hours of pollination, maximum pollen germination was recorded in selfing of PANT-U-31 (66.67%) and the minimum was recorded in selfing of KU-16-26 (52.20%). In case of crosses, the maximum pollen germination was recorded in KU-16-26 X GP-IPU-99238 (56.46%) after 2 hours of pollination and the minimum was found in KU-16-33 X KU-16-26 (48.54%) after 2 hours of pollination. Highest pollen germination was recorded in selfing of KU-16-31 (70.00%) and lowest in crossing of KU-16-33 X GP-IPU-99-238 (51.33%) after 6 hours of pollination (Figures 1-4).

Studies on correlation showed out that there is a significant correlation between pollen germination and percent pod formation. Debbarama [14] and Kharkongar [15] also reported a highly significant correlation between pollen germination and fruit set in *Capsicum annuum* and *Solanum lycopersicum*, respectively. Adhikari [16] reported a non-significant correlation between pollen germination and fruit set in *Pisum sativum*. Rangkham [17] and Nameirakpam [18] reported that there was significant correlation between the pollen germination and pod formation percentage in *Vigna unguiculata*.

S. No.	Parents	Pollen fertility (%)	Pod set (%)
1	KU-16-33	94.06 ± 0.31	91.66
2	KU-16-26	86.65 ± 1.54	76.92
3	GP-IPU-99-238	84.03 ± 1.66	71.42
4	PANT-U-31	91.43 ± 1.73	90.80
5	PANT-U-6	86.65 ± 2.50	80.00

Table 3: Pollen fertility in the parents in blackgram.

Pollen tube growth rate

There was a steady increase in the pollen tube length from 2 hours to 6 hours. In case of selfing, after 2 hours of pollination, the maximum pollen tube growth was recorded in selfing of PANT-U-6 (83.48 µm) and the minimum was recorded in GP-IPU-99238 (69.85 µm) whereas in case of crosses the maximum pollen tube growth after 2 hours of pollination was recorded in KU-16-33 X KU-16-26 (68.50 µm) and minimum in KU-16-26 X GP-IPU-99238 (49.38 µm) (Figures 5 and 6).

After 4 hours of pollination, the length of pollen tube was almost doubled. Overall, the maximum pollen tube growth was still observed in selfing of PANT-U-6 (150.14 µm) and the least in crossing of KU-16-26 X GP-IPU-99238 (84.62 µm). After 6 hours of pollination, the length of pollen tubes was almost three times the initial length although some of the pollen tubes had just started to germinate. The maximum length was recorded in selfing of PANT-U-6 (295.51 µm) and minimum was in crossing of GP-IPU-99238 X PANT-U-31 (217.10). The results revealed that there was a constant increase in pollen tube length from 2 hours to 6 hours (Figures 7,8 and Table 2). There was highly significant correlation between pollen tube growth rate and pod set (Table 4).

Debbarama [14] and Kharkongar [15] also reported a highly significant positive correlation between the pollen tube growth rate and pod formation percent in case of *Capsicum annuum* and *Solanum lycopersicum*, respectively.

Pod set

From Table 2 it is seen that the maximum pod set was obtained in selfing compared to crosses. Between the selfings, the maximum pod formation was recorded in PANT-U-6 (91.66%) and the least in GP-IPU-99238 (71.42%). In case of crosses, the maximum pod formation was recorded in KU-16-33 X KU-16-26 (70.58%) and the least was in GP-IPU-99-238 X PANT-U-31 (58.33%) (Figures 9-12). Pod formation on selfing was highest in PANT-U-6 (91.66%) but pollen germination was highest in PANT-U-31 (70.00%). Also, the lowest pod set was recorded in GP-IPU-99238 (71.42%) but pollen germination was lowest in PANT-U-6 (62.66%). Moreover, in crosses the highest pollen germination was recorded in KU-16-26 X GP-IPU-99238 (57.67%) but

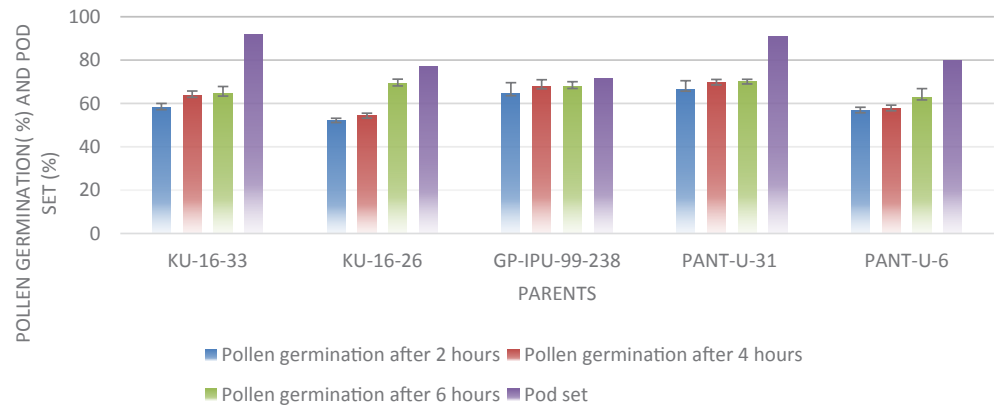

Figure 1: Pollen germination at different time intervals and percent pod set on selfing.

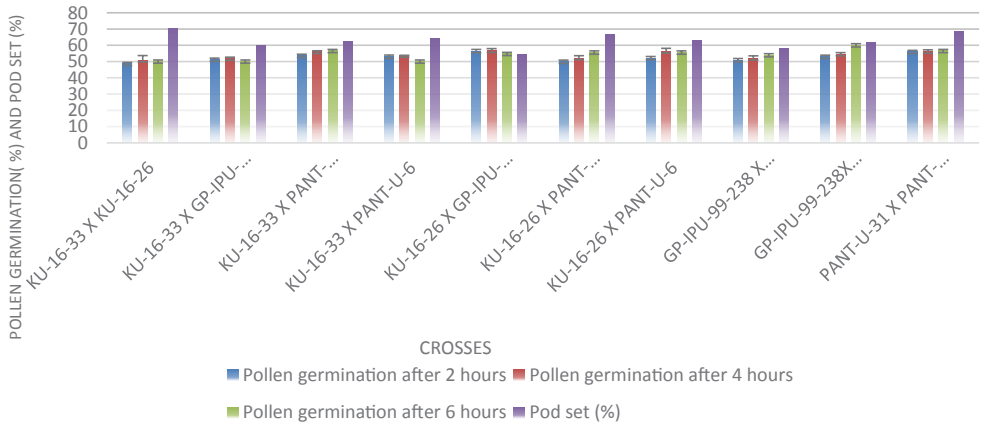

Figure 2: Pollen germination at different time intervals and percent pod set in crosses.

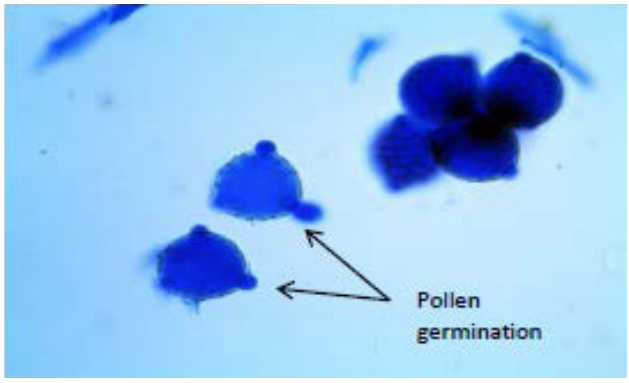

Figure 3: Pollen germination in KU-16-26 on selfing, after 2 hours of pollination (40X).

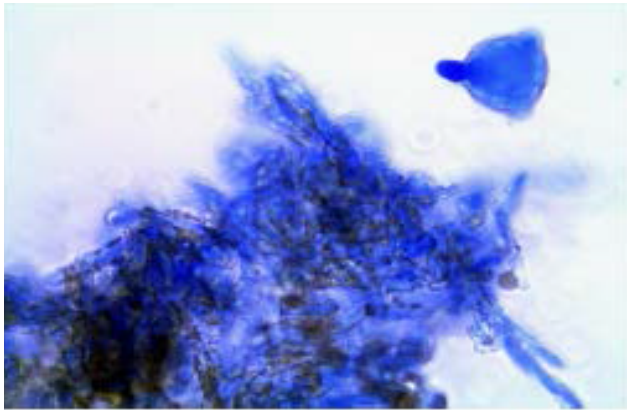

Figure 4: Pollen germination on crossing KU-16-26 X PANT-U-6 after 2 hours of pollination (40X).

it showed lowest pod set (54.55%).

The maximum pollen tube was recorded in the case of PANT-U-6 (295.51 µm) where pod set was 91.66% and least was found in GP-IPU-99-238 (243.30 µm) where pod set was 71.42% (Table 2). In case of crossing, the maximum pollen tube was observed in KU-16-33 X KU-16-26 (244.11 µm) where pod set (70.58%) was also maximum among the crosses. Also among the crosses, the smallest pollen tube growth was observed in GP-IPU-99-238 X PANT-U-31 (217.10 µm) where pod set percent was 58.33. Lowest pod set in crossing was observed in crosses of KU-16-26 X GP-IPU-99-238 (54.55%). Pod set was positively correlated with pollen tube in case of crosses whereas it was not so in the case of selfing (Table 4).

Correlation studies in crosses of *Vigna mungo*

Pollen fertility recorded a non-significant correlation with pollen germination, pollen tube growth and pod formation (Table 4). Pollen germination after 2 hours of pollination is highly significant with pollen germination after four hours and 6 hours. Pollen tube growth after 2 hours of pollination and 4 hours of pollination has a non-significant relationship with pollen germination after two hours of pollination. Pollen germination after 4 hours of pollination is highly significant with pollen germination after 6 hours and also observed to be significantly correlated with pollen tube growth after 2 hours, 4 hours and 6 hours of pollination. Pollen germination after 6 hours of pollination was highly significant with pollen tube growth. Pollen tube growth after 2 hours was recorded to be highly significant with pollen tube growth after 4 hours and 6 hours. Pollen tube growth after 6 hours of pollination was also recorded to be highly significant with pollen tube growth after 4 hours (Table 4).

Pod formation percent was recorded to be non-significantly correlated with pollen fertility. It was significantly correlated with pollen germination after 2 hours and 4 hours but was highly correlated with pollen germination after 6 hours of pollination. Moreover, a highly significant correlation was recorded between pod formation and pollen tube growth (Table 4).

Rangkham and Nameirakpam [17,18] also reported a highly significant correlation between the pollen germination, pollen tube growth and pod formation percentage in *Vigna unguiculata*. However, Adhikari [16] reported a non-significant correlation between pollen germination, pollen tube growth and pod formation.

Genetic diversity

In the present study, a total of 30 SSR primers were screened for genetic diversity analysis in 35 accessions of Blackgram out of which a total of 11 primers were found to be polymorphic and also in the remaining 19 primers, amplication was not observed with some primers, some being monomorphic and some having faint bands and

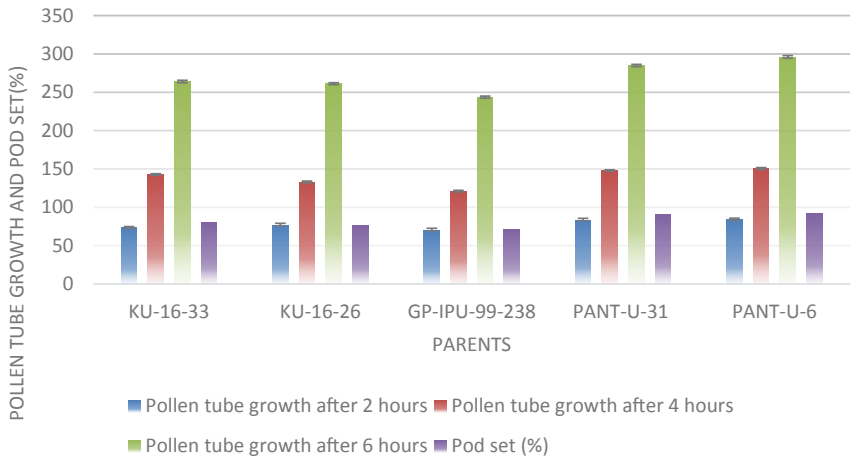

Figure 5: Pollen tube growth at different time intervals and percent pod set in selfing

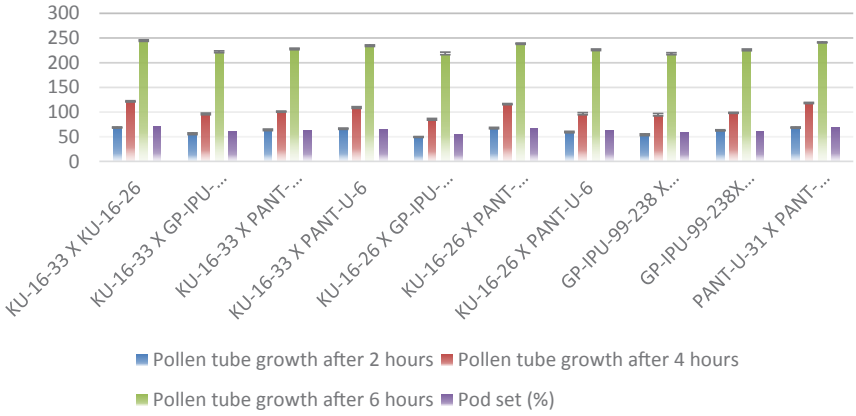

Figure 6: Pollen tube growth at different time intervals and percent pod set in crosses.

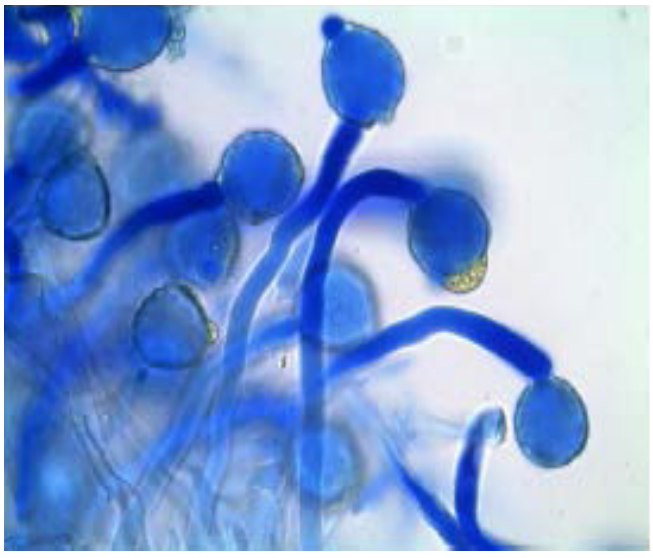

Figure 7: Pollen tube growth on selfing of PANT-U-6 after 6 hours of pollination (40X).

unclear fragments. These 11 primers generated a total of 27 alleles ranging in size between 150 to 300 bp in all the 35 accessions. A total of 357 bands were generated and 119 of these bands are polymorphic with 32.97% polymorphism and the rest 238 bands are monomorphic with 66.6% monomorphism. The highest percentage of polymorphism was recorded in CEDG 118 (48.48%), and lowest polymorphism was in VR 86 (5.17%). Based on the Polymorphism Information Content (PIC) value, two primers, CEDG 118 (0.537) and CDEG 279 (0.582) were found to be informative. The PIC value showed a range from 0.109 to 0.582. The details of polymorphism and PIC are given in Table 5 and the SSR pattern for the primer VR 198 is given in Figure 13.

Figure 14 shows the tree diagram for 35 accessions expressing the genetic distances based on the 11 SSR markers data. Clustering was done using Darwin 6.0.15. Cluster II with 19 genotypes is the largest Cluster. Cluster III is the second largest cluster with 12 genotypes and Cluster I consists of 4 genotypes. The observation recorded on the molecular data was subjected to simple matching coefficient. The genetic similarity between the 35 accessions of Blackgram assessed by simple matching coefficient ranged from 0.14 to 0.82. The least genetic similarity was observed between the genotypes KU-16-30 and KU-16-33 with a simple matching coefficient of 0.82. The greatest similarity was observed between the accessions GP-PGRU-9514 and GP-134.16.50

Table 4: Correlation studies for various characters in crosses of *Vigna mungo*.

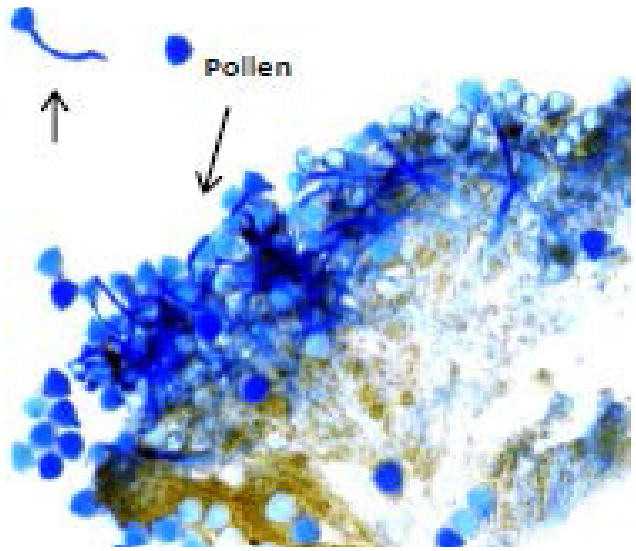

Figure 8: Pollen germination and pollen tube growth on crossing of PANT-U-31 X PANT-U-6 after 2 hours of pollination (4 X).

748 having a coefficient of 0.14. The close genetic similarity between the cultivars could be explained due to the high degree of commonness in their pedigrees. The narrow genetic base of the Blackgram revealed in the present analysis emphasizes the need to exploit the large germplasm collections having diverse morphological traits in crop improvement programme.

Priya [19] performed an experiment on genetic diversity of Blackgram using random amplified polymorphic deoxyribonucleic acid (RAPD) markers. They reported that forty decamer-primers could generate a total of 346 RAPD fragments in 8 Blackgram species, of which 338 or 97.68% were polymorphic.

Ghafoor [20] studied thirty-seven pure-lines of Blackgram through randomly amplified polymorphic DNA (RAPD) diversity analysis. The RAPD markers were found useful for studying genetic diversity but clustering did not give any indication for agronomic performance, whereas quantitative traits contributed more towards agronomic performance.

Summary and Conclusion

There were no significant differences in the average pollen fertility (84.03-94.06%) among the five parents but there were more differences

Character	Percent viable pollen	PG after 2 h	PG after 4 h	PG after 6 h	PTG after 2 h	PTG after 4 h	PTG after 6 h	Pod formation (%)
Percent viable pollen	1.000							
PG after 2 h of pollination	0.096	1.000						
PG after 4 h of pollination	0.268	0.966**	1.000					
PG after 6 h of pollination	0.284	0.776**	0.804**	1.000				
PTG after 2 h of pollination	0.209	0.479	0.514	0.701**	1.000			
PTG after 4 h of pollination	0.606	0.494	0.542	0.695**	0.964**	1.000		
PTG after 6 h of pollination	0.284	0.515	0.532	0.695**	0.950**	0.966**	1.000	
Percent Pod formation	0.372	0.549	0.549	0.710**	0.958**	0.973**	0.992**	1.000

Note: *Correlation significant as r > 0.05 (5% level of significance)
**Correlation significant as r > 0.01 (1% level of significance)
PG = Pollen germination; PTG = Pollen tube growth

Table 4: Correlation studies for various characters in crosses of *Vigna mungo*.

Primer	Range of fragments (bp)	TNB	TPB	(Tm°c)	Percent polymorphism	PIC
CDEG 143	130-300	35	7	57	20.00	0.268
CDEG 006	130-300	31	11	55	35.48	0.353
VR 198	200	32	14	53	43.75	0.498
CEDG 068	130-300	32	14	55	43.75	0.510
CEDG 008	130-300	33	10	55	30.30	0.408
CEDG 118	130-300	33	16	57	48.48	0.537
CEDG 282	130-300	34	8	52	23.52	0.295
VR 40	200	35	11	53	21.15	0.338
CEDG 056	130-300	28	12	55	42.85	0.369
V86	200	35	2	53	5.17	0.109
CEDG 279	130-300	29	14	55	48.27	0.582
Total		357	119		362	
Average		32.45	10.81		32.97	

Table 5: Total no. of amplification bands (TNB), total no. of polymorphic bands (TPB), percent polymorphism and polymorphism information content (PIC) of SSR markers.

Figure 9: Pod formation of KU-16-26 X PANT-U-31.

Figure 10: Pod formation of KU-16-26 X PANT-U-6.

Figure 11: Pod formation of GP-IPU-99-238 X PANT-U-31.

Figure 12: Pod formation of PANT-U-31 X PANT-U-6.

Figure 13: SSR pattern for the primer VR 198. L=molecular size ladder of 100 base pairs. Serial number of the genotypes corresponds to Table 1.

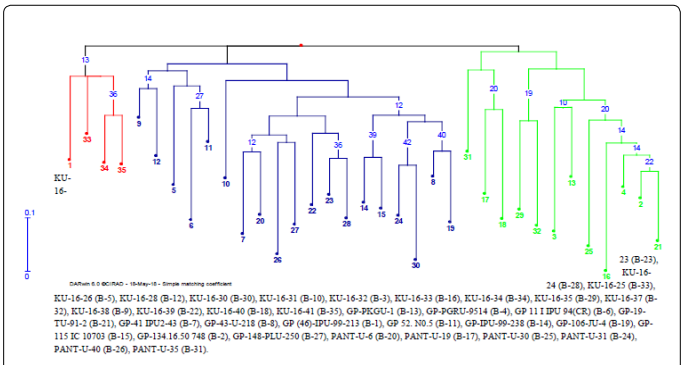

Figure 14: Unweighted Neighbor-Joining Tree (Hierachical view) between thirty-five accessions of blackgram generated from 11 polymorphic SSR markers.

in percent pod set (71.42-91.66%). Pollen fertility attained a non-significant positive correlation with pod set. The pollen germination in selfings and crosses were found to be in the range of 62.66-70.00% and 51.33-58.57%, respectively. Pollen germination gradually increased from 2 hours to 6 hours. Pollen germination after 6 hours had a highly significant positive correlation with pod set. The maximum pod set and pollen tube growth were obtained in selfing than in crossing. There was high significant positive correlation between pollen tube growth and pod set. No abnormal pollen and growth of pollen tubes were observed. A total of 30 SSR primers were selected to assess genetic diversity of 35 accessions of Blackgram. Based on the PIC values, three primers CEDG 068, CEDG 118 and CEDG 279 were found to be more informative. The PIC value showed a range from 0.109 to 0.537 with the primer CEDG 118 having the highest PIC value of 0.537. Based on the simple matching coefficient, KU-16-33 (B-16) and KU-16-30 (B-30) were found to be the most diverse genotypes whereas GP-134.16.50 748 (B2) and GP-PGRU-9514 (B4) were found to be very less distinct. The value of simple matching coefficient ranged from 0.14 to 0.82. Based on the simple matching coefficient, a cluster dendrogram was drawn based on Unweighted Neighbor-Joining method generating 3 different clusters namely, I (4 accessions), II (19 accessions) and III (12 accessions).

Investigating properties of pollen grains 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. Based on the simple matching coefficient, two pair of accessions, KU-16-30 (B-30) and KU-16-33 (B-16) were found to be most distinct from each other. These two accessions could be used for breeding purpose for crop improvement.

References

1. Lukoki L, Marechal R, Otoul E (1980) The wild ancestors of cultivated beans: *Vigna radiata* (L.) Wilczek and *V. mungo* (L.) Hepper. Bull Jard Bot Nat Belgium 50: 385-391.
2. Zeven AC, De Wet JMJ (1982) Dictionary of cultivated plants and their regions of diversity. Centre for Agricultural Publication and Documentation, Wageningen.
3. Porch TG, Jahn M (2001) Effects of high temperature stress on

microsporogenesis in heat sensitive and heat tolerant genotypes of *Phaseolus vulgaris*. Plant Cell Environ 24: 723-731.

4. Stebbins GL (1958) The inviability, weakness and sterility of interspecific hybrids. Adv Genet 9: 147-215.
5. Gunasekaran M (1997) Studies on palynological features as related to crossability barriers in some diploid and tetraploid species of genus Gossypium. Tamil Nadu Agricultural University.
6. Rajeshwari S, Ramaswamy NM (2004) Pollen tube growth and embryology of ovule abortion in S. alatum and S. indicum. J Genet Breed 58: 113-118.
7. O'Neill R, Snowdon R, Kohler W (2003) Population genetics: Aspects of biodiversity. Progress Bot 64: 115-137.
8. Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, et al. (1991) Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, environments. Genet 127: 179-97.
9. Gerlach D (1969) A rapid safranine-crystal violet-light green staining sequence for paraffin sections of plant materials. Stain Technol 44: 210-211.
10. D'Souza L (1972) Staining pollen tubes in styles of cereal with cotton blue: Fixation by ethanol-lactic acid for enhanced differentiation. Stain Technol 47: 107-108.
11. Ribeiro GS, Sao JAR, Reboucas TNH, Amaral CLF (2007) Floral biology aspects related to the production of sugar apple (*Annona squamosal* L.) fruits. Acta Sci Biol Sci 29: 369-373.
12. Almeida OS, Silva AHB, Silva AB, Amaral CLF (2004) Study of floral biology and reproductive mechanisms of *Ocimum officinalis* L. aimed at breeding. Acta Sci 26: 343-348.
13. Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissues. Focus 12: 13-15.
14. Debbarama C, Khanna VK, Tyagi W, Rai M, Meetei NT (2013) Wide hybridization and embryo-rescue for crop improvement in Capsicum. Agrotechnol S11: 003.
15. Kharkongar HP, Khanna VK, Tyagi W, Rai M, Meetei NT (2013) Wide hybridization and embryo-rescue for crop improvement in Solanum. Agrotechnol S11: 004.
16. Adhikari DN, Khanna VK, Noren KS (2018) Crossability and genetic diversity studies in pea (*Pisum sativum*). Curr Inves Agri Curr Res 3: 443-452.
17. Rangkham T (2017) Studies on crossability between various accessions and genetic diversity in cowpea (*Vigna unguiculata* L.Walp). Central Agricultural University 2: 1-10.
18. Nameirakpam B (2017) Studies on crossability between various accessions and genetic diversity in cowpea (*Vigna unguiculata* L. Walp). Central Agricultural University 13: 1-9.
19. Priya S, Anjana P, Prakash SD (2013) Genetic diversity analysis in different varieties of blackgram using RAPD markers. I J Biol Res 1: 43-48.
20. Ghafoor A, Sultana T, Zarrin FR (2012) Genetic diversity in blackgram [*Vigna mungo* (L.) Hepper] for randomly amplified polymorphic DNA (RAPD) markers. Pak J Bot 44: 473-478.