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Transferability of simple sequence repeat markers in blackgram (*Vigna mungo* L. Hepper)

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Abstract

Transferability of simple sequence repeat (SSR) markers was studied to increase the availability of molecular markers for germplasm evaluation, genetic analysis and new cultivar development in blackgram. Three hundred sixty one (361) simple sequence repeat markers developed for other food legumes were used to amplify genomic DNAs extracted from 24 diverse genotypes of blackgram. Out of these, 245 SSR markers (68%) amplified though only 39 (16%) were highly polymorphic among 24 diverse genotypes of blackgram. The polymorphism information content ranged between 0.08 to 0.80 with an average of 0.48. Cluster analysis based on UPGMA grouped the studied genotypes into three major clusters. The Jaccard's similarity coefficient was ranged from 0.13 to 0.90 indicating the extent of genetic variability within the test genotypes. It was observed that transferability of SSR markers with AG motif was more frequent in blackgram. A few SSRs also generated genotype specific allele that could be useful in maintaining the genetic purity of respective genotypes. These identified SSR markers may be useful for genetic analysis and marker assisted breeding in blackgram.

Keywords: *Vigna mungo*; blackgram genomics; marker transferability; simple sequence repeat.
Abbreviations: SSR-simple sequence repeat; PCR-polymerase chain reaction; PIC-polymorphic information content; PVP-plant variety protection; PCA-principal component analysis; EST-expressed sequence tagged.

Introduction

The genus *Vigna* comprises of ~150 species, of which *Vigna mungo* or blackgram is one of the most important food legume species. It has been reported to be originated in India with a secondary centre of origin in central Asia (Pratap and Kumar, 2011). This self-pollinated diploid (2n=2x=22) annual has a small genome size ~574Mbp (0.59 pg/1C) (Arumuganathan and Earle, 1991). It is extensively grown in south and south-east Asia. In India alone, this crop was grown on 3.26 million hectares in 2010-11, which produced 1.75 million tons grains with an average productivity of 535 kg/ha. Low productivity of this crop is mainly due to the use of low yielding cultivars by farmers, severity of various diseases, lack of genetic variability, poor harvest index and absence of suitable ideotypes for different cropping systems.

In the recent years, molecular markers have been reported to be used for accurate estimation of genetic diversity, identification of pure lines or cultivars for PVP and germplasm maintenance, selection of diverse parental combinations to generate segregating progenies with maximum genetic variability (Barrett and Kidwell, 1998) and introgression of desirable traits from diverse or wild germplasm into the available cultivars to broaden the genetic base (Thompson et al., 1998). Despite blackgram being an important crop of Asia, use of molecular markers in this crop is still limited due to slow development of genomic resources such as availability of polymorphic trait-specific markers. Among the different types of markers, simple sequence repeats (SSR) are easy to use, highly reproducible and locus specific (Tautz and Renz, 1984). These have been widely used for genetic mapping, marker assisted selection and genetic diversity analysis and also in population genetics study in different crops (Zeitkiewicz et al., 1994). In the past, SSR markers derived from related *Vigna* species were used to identify their transferability in black gram with the use of such SSR markers, two linkage maps were also developed in this crop (Chaitieng et al., 2006 and Gupta et al., 2008). However, use of transferable SSR markers in these linkage maps was limited and only 47 SSR loci were assigned to the 11 linkage groups (Chaitieng et al., 2006; Gupta et al., 2008). Therefore, efforts are urgently required to increase the availability of new polymorphic SSR markers in blackgram.

Development of SSR markers through genomic library construction involves enormous cost and time. However, identification of transferable markers from the related species has proved to be an important way to develop SSR markers and it was successfully achieved in the other food legume crops (Chandra 2011; Datta et al., 2011; Datta et al., 2010 a,b; Somta et al.; 2009, Guohao et al.; 2006, Gutierrez et al.; 2005, Choumane et al., 2004; Peakall et al., 1998) as well as in blackgram (Gupta and Gopalakrishna 2010). Therefore, in the present study, 361 SSR markers derived from adzukibean, commonbean, cowpea, mungbean, chickpea and pigeonpea were used to assess their transferability in blackgram and efficiency of transferable SSRs was tested in diverse genotypes of this species.

Results

Transferability of SSR markers

A total of 361 SSR markers derived from six legume species were used in the present study. Of the total 202 adzukibean SSR markers used in the study, 178 exhibited clear PCR products (91% transferability) in blackgram. Though

mungbean specific SSRs also showed almost similar transferability (46 out of 50, 92%), but 84% of them were monomorphic. SSR markers derived from cowpea, commonbean exhibited 86% and 20% transferability, respectively, were also observed monomorphic in present study. Of the total 30 chickpea and 20 pigeonpea specific SSR primer pairs used in this study, none could amplify DNA of blackgram. Transferable SSR markers produced collectively 297 alleles and each SSR marker produced 1 to 3 alleles with an average value of 1.20.

Polymorphism of the SSR markers

Out of the 245 primers showing amplification in blackgram, 39 markers were highly polymorphic (Table 2, Fig.1.a,b) and remaining were monomorphic (Supplemental data 1). PIC of these polymorphic SSR markers ranged between 0.08-0.8 with an average PIC value of 0.48 while number of alleles ranged between 2 to 3 with an average of 2.03 alleles per locus (Table 2). Highest frequency of PIC was observed between 0.41-0.5 (Fig. 2). Each SSR marker locus generated the expected size of allele in the blackgram and it was ranged from 125 to 400 bp (see Supplemental data 1).

Diversity analysis

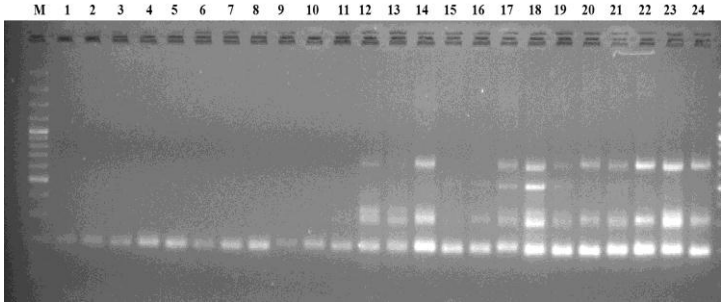
Thirty nine polymorphic SSR markers identified in present study yielded 79 alleles in 24 blackgram genotypes and the number of alleles per locus ranged from 2 to 3 with an average of 2.03 alleles per locus. The UPGMA analysis grouped these genotypes into three major clusters (Fig. 3). Jaccard similarity coefficient between the genotypes ranged from 0.13 to 0.9 (Table 3). Minimum similarity (13%) was observed between Shekhar-2 and PGRU95018 while nine genotype pairs showed similarity to the extent of 90 per cent (Table 3). Cluster I had 11 genotypes (IPU91-7, PLU1, IPU99-147, IPU99-79, UH80-26, NG2119, V3108, HPU180, PLU 328, IPU 99-22, PGRU95018) while cluster II comprised 5 genotypes (IPU99-232, GP215, U8-10, IPU99-168, AKU9904). Cluster III carried only 8 genotypes, which consisted mostly released cultivars (DPU88-31, Uttara, IPU-2-43, LBG20, T9, PDU1, Shekhar-2). Marker CEDG127 produced 231 bases long DNA amplicon specific to IPU99-232 and 218 bases long amplicon specific for IPU99-168. CEDG014 produced given amplicon of size 127 bases specific for the genotype PGRU95018 and CEDG044 produced amplicon of size 167 bases specific for AKU 9904. PCA analysis of the SSR data resulted in clustering of 24 genotypes into three groups and distinct position of each genotype was observed within each group (Fig. 4). First three most informative components in PCA analysis individually accounted 31%, 13% and 9% of total variation, respectively and collectively these three components explained 53% of total variability.

Discussion

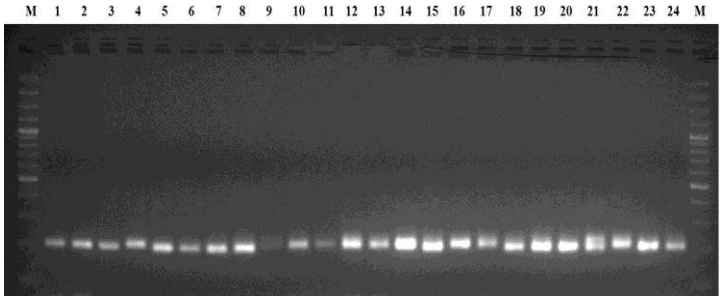
The extent of genomic similarity between two species determines the extent of transferability and use of molecular markers from one species to other related species. In case of SSR markers, it depends upon the extent of conserved primer binding sites flanking the SSR loci (Gupta and Gopalakrishna, 2010). It is a cost effective approach as compared to developing new SSR markers from genomic library. Earlier, cross-species transferability of SSR markers among legumes was studied by many workers (Datta et al., 2010 a,b; Choi et al., 2004; Choumane et al., 2004; Guohao et al., 2006; Pandian et al., 2000; Peakall et al., 1998). Transferability of adzuki bean primers was reported to be 100% in blackgram (Gupta and Gopalakrishana, 2009) and 72% in mungbean (Wang et al., 2009). In the present study also, high proportion of SSR markers (86-92%) were transferred in blackgram from closely related species belonging to *Vigna* genus such as mungbean (92%) adzukibean (91%) and cowpea (86%). However, transferability of SSR markers developed in other closely related genera (*Phaseolus*) was less (20%) while no transferability of SSRs in blackgram was observed from chickpea and pigeonpea which might be due to their phylogenetic distances (Cannon et al., 2011). These studies showed that there is further possibility to transfer SSR markers to blackgram from other closely related species.In this study, 39 highly polymorphic markers were obtained from adzukibean and mungbean. In order to demonstrate the potentiality of the transferable SSR markers, PIC values were used as parameter, which varied with a mean value of 0.47 and more than 38% SSR loci had greater than this average PIC value. It has been observed that markers should have many alleles to be considered useful for evaluation of genetic diversity (Ribeio-carvalho et al., 2004). The multiple locus amplification and high PIC values indicated the effectiveness of the transferable SSRs in germplasm characterization, evolution, breeding application and phylogenetic studies in blackgram. Several SSR markers also showed multiple banding patterns with very weak bands but these were not considered for analysis in present study. This poor banding pattern could be due the non-specific annealing of the SSR primers (Williams et al., 1990). A dendogram based on UPGMA analysis grouped 24 genotypes of blackgram in three major clusters with Jaccard's similarity coefficient ranging from 0.13 to 0.90. The clustering of the genotypes clearly showed the extent of genetic similarities among the test genotypes. In the first cluster, germplasm lines collected from one of the centers of diversity of blackgram i.e Western ghats in India were grouped together except for two lines, IPU99-232 and IPU99-168. Cluster II comprised of genotypes from different and even unknown origins while genotypes in Cluster III are mostly released varieties. The closeness in genetic similarities of genotypes in Cluster III is due to the similarities in the pedigree and except for PDU1 all others genotypes have T9 as a common parent. Of the polymorphic markers, a few produced genotype-specific alleles that could be promising tool for varietal identification and maintenance of genetic purity in blackgram. It could be particularly useful in those genotypes which are photo-insensitive and extra-early maturing (52-55 days maturity) such as PGRU95018 and AKU9904. Thus the results show that transferable markers are powerful enough to separate all 24 genotypes in distinct groups and genotype-specific SSR markers can aid in varietal identification and maintenance of such genotypes in the ongoing breeding programmes. Previously, genotype-specific alleles of SSR markers have also been identified for 15 blackgram genotypes (Gupta and Gopalakrishna, 2010) and use of SSR markers for varietal identification and maintenance has been reported in pigeonpea and chickpea (Datta et al., 2010 a,b). Of the total 39 polymorphic primer pairs, 20 contain AG dinucleotide repeat motif (7-37 repeats), which further indicated more genomic variability in flanking regions between the primer pairs among the test genotypes. From the remaining 19 primer pairs which bear different di-, tri- and or tetra-nucleotide repeat motif, 14 primer pairs bear AG dinucleotide repeat as well. This suggests that more SSR markers containing AG dinucleotide repeats from other food legumes

Table 1. Description of pedigree and other important features of blackgram genotypes used in the study.

Genotype	Pedigree	Reaction to MYMIV	Seed size	Duration	Remarks
IPU 91-7	Germplasm collection from Western Ghats	Resistant	Medium	Early	
PGRU 95018	Germplasm collection	Resistant	Small	Early	Extra early genotype (52-55 days)
PLU 1	Germplsam collection from North Western Plains	Resistant	Medium	Medium Late	
IPU 99-147	Germplasm collection from Western Ghats	Susceptible	Medium	Early	
IPU 99-79	Germplasm collection from Western Ghats	Resistant	Medium	Medium Late	
UH 80-26	Advanced breeding line; T9 x US 131	Resistant	Large	Medium Late	
NG 2119	Germplasm collection	Resistant	Medium	Medium Late	
V 3108	Germplsam collection from Northern Hills	Resistant	Medium	Late	
HPU 180	Germplsam collection from Northern Hills	Susceptible	Medium	Medium Late	
PLU 328	Germplsam collection from North Western Plains	Susceptible	Large	Medium Late	
IPU 99-22	Germplasm collection from Western Ghats	Susceptible	Medium	Medium Late	
IPU 99-232	Germplasm collection from Western Ghats	Susceptible	Medium	Medium Late	
GP 215	Germplasm collection	Susceptible	Medium	Medium Late	
IPU 99-168	Germplasm collection from Western Ghats	Susceptible	Medium	Medium Late	
PLU312	Germplsam collection from North Western Plains	Resistant	Medium	Medium Late	
U8-10	Germplasm collection from North Western Plains	Susceptible	Medium	Medium Late	
AKU 9904	Advanced breeding line	Susceptible	Medium	Early	Extra early genotype (52-55 days), developed by PDKV, Akola
DPU 88-31	Advanced breeding line; PLU 131 x T9	Resistant	Medium	Medium Late	Highly resistant to MYMIV but susceptible to powdery mildew, developed by DPR (now IIPR), Kanpur
Uttara	NP19 xT9	Resistant	Medium	Medium Late	The most popular cultivar in Northern India, resistant to both MYMIV and powdery mildew, developed by IIPR, Kanpur
IPU 02-43	DPU88-31x DUR-1	Resistant	Medium	Medium Late	Identified for release by CVRC in 2011, bred at IIPR, resistant to both North and South Indian strain of MYMIV and powdery mildew, developed by IIPR, Kanpur
PDU 1	Selection from IC8219	Susceptible	Medium	Early	Released variety, developed by DPR (now IIPR), Kanpur
LBG 20	T-9 x Netiminumu	Susceptible	Medium	Early	Released variety, developed by ARS, Lam, Andhra Pradesh, Tamilnadu
Shekhar 2	7378/2 x T9	Susceptible	Medium	Medium Late	Released variety, developed by CSAUAT, Kanpur
T9	Local selection from Bareilly, U.P.	Susceptible	Medium	Medium Late	Released variety in 1945, present in pedigree of many high yielding blackgram varieties, developed by CSAUAT, Kanpur



(A)



(B)

Fig 1. (**a)** Amplification profile of the SSR marker CEDG056 in the 24 genotypes of blackgram Lane M: 100bp ladder, Lanes 1 to 24: 1-24 blackgram genotypes Size 3.51 x 10 inch (**b)** Amplification profile of the SSR marker CEDG139 in the 24 genotypes of blackgram Lane M:100bp ladder, Lanes 1 to 24: 1-24 blackgram genotypes

can be tested for their successful transfer to *V. mungo*. In similar studies, Yu et al., (2000) reported prevalence of dinucleotide polymorphism in commonbean and Rongwen et al., (1995) observed trinucleotide polymorphism in Soybean, nevertheless many workers failed to report any correlation between the length of repeat motif and PIC of the markers (Bell and Ecker, 1994; Yu et al., 2000). In conclusion, this study identified 39 highly polymorphic SSR primers including CEDG180 (earlier reported by Gupta et al., 2008) from six food legume species *viz*., adzukibean, commonbean, cowpea, mungbean, chickpea and pigeonpea. These SSRs have been tested for their transferability to blackgram. These could be useful to discriminate blackgram germplasm of different origins, to study genetic diversity as well as for gene mapping and marker assisted breeding. Previously, transferability of SSR markers from other legumes resulted in development of only 47 SSR markers in blackgram which were also assigned to molecular linkage maps (Gupta et al., 2008; Chaitieng et al., 2006). Keeping this in view, there is an ample scope to develop/transfer more SSR markers in blackgram in order to saturate the present molecular linkage map in this important food legume. Therefore, sincere efforts are required to develop more number of polymorphic SSRs in blackgram, particularly genic SSRs by utilizing EST-databases available in public domain (Chandra, 2011).

Materials and methods

Plant materials

Twenty four (24) diverse blackgram (*Vigna mungo* L. Hepper) genotypes including released varieties, breeding lines and indigenous collections were used in present study to identify the polymorphic SSR markers. The germplasm lines were collected from different regions of India including Western Ghats (6 genotypes), North-West Plains (4 genotypes) and two genotypes from Northern Hills. Besides, three germplasm lines, PGRU95018, GP215 and NG2119, have unknown pedigree and these are being maintained at IIPR, Kanpur, India.

The released cultivars (6 genotypes) and three breeding lines were developed at IIPR, Kanpur; Agriculture Research Station, Lam, Andhra Pradesh; Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola and Chandra Shekhar Azad University of Agriculture and Technology, Kanpur (see Table 1 for details regarding pedigree and key characters).

DNA extraction

Total genomic DNA of the above mentioned genotypes of blackgram was extracted from 10 days old seedlings following Doyle and Doyle (1987). The quality of extracted DNA was checked by comparing with λ DNA while quantity was determined using spectrophotometer. Stocks were maintained at 25ng/µl in TE buffer. The working DNA sample was diluted to a standard concentration of 10ng/µl.

PCR amplification

PCR reactions were carried out in 20 µl volume containing 1x PCR reaction buffer (Fermantas, USA), 25ng template DNA, 0.6 U of Taq DNA polymerase (Fermantas, USA), 0.2mM dNTP mix (Fermantas, USA) and 20 picomoles of forward and reverse primer (IDT, USA and ILS, India). Amplification was performed using a thermocycler (G-Strom, UK) and it was programmed for an initial denaturation of 94°C for 3 min followed by 35 cycles each of denaturation at 94°C for 1min, annealing for 1min, elongation at 72°C for 2 min and final extension at 72°C for 7 min. The amplified products were separated on 3% agarose gel in 1X TAE buffer at 50-60 V. The gels were visualized and documented after staining with ethidium bromide (0.5µg/ml) under UV light using gel documentation system (UVITEC, UK).

SSR analysis, scoring and data analysis

Total 361 SSR primer pairs derived from adzuki bean (202 markers, Wang et al., 2004), mungbean (50 markers, Somta et al., 2009; Kumar et al., 2002a,b) cowpea (14 markers, Li et al., 2001), common bean (45 markers, Gaiten-Solis et al., 2002; Blair et al., 2003), chickpea (30 markers, Buhariwallla et al., 2005) and pigeonpea (20 markers, transferred to pigeonpea from chickpea, Datta et al., 2010a,b) were synthesized by IDT, USA & ILS, India and used in the present study. Genotyping data of each SSR marker was recorded in binary fashion (i.e. presence (1) and absence (0) of bands) using Firereader software 15.18. The polymorphism information content (PIC) values were calculated following Botstein et al. (1980) as follows:

$$PIC = 1 - \sum_{j=1}^{n} P_{ij}^{2}$$

where P_{ij} is the frequency of the j^{th} allele for the i^{th} marker, and summed over n alleles (Table 2 and supplementary file).
The binary data on all blackgram genotypes for 39 polymorphic markers were used to calculate the correlation matrix using similarity co-efficient analysis (Jaccard, 1908) based on which a dendogram was constructed using un-weighted pair group method with arithmetic average

Table 2. Primer Sequences and characteristics of 39 transferable Adzukibean and Mungbean specific SSR primer pairs.

Primer ID	Primer (5'-3')	Repeat Motif	T_m	No of alleles	PIC
	Adzukibean (Wang et al., 2009)				
CEDG204	CCTTGGTTGGAGCAGCAGC CACAGACACCCTCGCGATG	(AG)15	58.9	2	0.49
CEDG139	CAAACTTCCGATCGAAAGCGCTTG GTTTCTCCTCAATCTCAAGCTCCG	(AG)19	58.25	2	0.49
CEDG008	AGGCGAGGTTTCGTTTCAAG GCCCATATTTTTACGCCCAC	(AG)26	55.10	2	0.22
CEDG268	CATCTCCCTGAAACTTGTG GCTATCAATCGAGTGCAG	(AG)16	50.55	3	0.80
CEDG013	CGTTCGAGTTTCTTCGATCG ACCATCCATCCATTCGCATC	(AG)22	54.4	2	0.47
CEDG092	TCTTTTGGTTGTAGCAGGATGAAC TACAAGTGATATGCAACGGTTAGG	(AG)17	55.10	3	0.51
CEDG133	GCATACATAATGTGGTGAGATG GTCTCGTGCCTTTCACAC	(AT)3(AG)11	56.83	3	0.66
CEDG141	CCAGGCATCCATGATGACC GAAGTTGTTGGTAATGGTTGCCTC	(AT)6(AG)13	59.99	3	0.66
CEDG275	CACACTTCAAGGAACCTCAAG GTAGGCAACCTCCATTGAAC	(AG)14	57.90	2	0.44
CEDG284	GGTGCTAACGTTGGAAACTGAG CACTCCATTCTGAGGATCAATCC	(AT)19(AG)21 A(AG)5	60.12	2	0.22
CEDG127	GGTTAGCATCTGAGCTTCTTCGTC CTCCTCACTTGGTCTGAAACTC	(TG)3(AG)9	61.02	3	0.16
CEDG014	GCTTGCATCACCCATGATTC AAGTGATACGGTCTGGTTCC	(AT)12(AG)14	57.80	2	0.08
CEDG020	TATCCATACCCAGCTCAAGG GCCATACCAAGAAAGAGG	(AT)18(AG)20	56.41	2	0.44
CEDG264	GATTCCCTTCCTAGCTATGG CTGCTGGACATGAAGATTCAG	(AG)10 AT(AG)16	57.91	2	0.44
CEDG271	GCACTAAAGTTAGACGTGGTTC CACTCCCACTGCCAAACAAGG	(AT)12(AG)13 AA(AG)7	58.21	2	0.44
CEDG011	GTCCGACTTTATGTGTGGAG TTTCTAGTTCCAGCCCCGAC	(AC)9(AT)8 ...(AT)7(AC)4	60.07	2	0.44
CEDG056	TTCCATCTATAGGGGAAGGGAG GCTATGATGGAAGAGGGCATGG	(AG)14	61.00	3	0.60
CEDG180	GGTATGGAGCAAAACAATC GTGCGTGAAGTTGTCTTATC	(AG)11	54.50	3	0.51
CEDG044	TCAGCAACCTTGCATTGCAG TTTCCCGTCACTCTTCTAGG	(GT)10 AT(AG)18	57.80	3	0.66
CEDG018	AGCGTGTTTGTGGTGATAGC ACACAGGAACGAACAAACCC	(AG)32	55.65	2	0.44
CEDG016	TTAGTTCACTCCGCTTGGTC CACGTCATCCTCTGTTAGAC	(AG)26	53.50	2	0.44
CEDG030	TGAGGGAATGGGAGAGAGGC TCCGCAGATAGAGGCTCACG	(AG)45	58.70	3	0.60
CEDG022	AGGAATGTGAGATTTG AATCGCTTCAAGGTCAAGCC	(AG)27	49.05	2	0.50
CEDG024	CATCTTCCTCACCTGCATTC TTTGGTGAAGATGACAGCCC	(AG)18	54.30	2	0.49
CEDG198	CAAGGAAGATGGAGAGAATC CCTTCTAAGAACAGTGACATG	(AG)30	50.30	2	0.47
CEDG225	GAGGAAGTGTTGCAGCACC GTAGACTCTGCAGAGGGATG	(AG)8 TG(AG)3(TG)2(AG)4	59.79	2	0.46
CEDG077	ATCCCGTGACCCTTCTTCCT GCTCAAGCGAAAACCCAGCA	(AG)8	59.85	2	0.44
CEDG067	AGACTAAGTTACTTGGGCAACCAG TGACGGCCCGGCTCTCC	(AG)14	62.25	2	0.44
CEDG245	GATAGAGCTTAAACCCTC CTTTTGATGACAAATGCCC	(AC)10(AT)9(AG)14	53.00	2	0.44
CEDG059	AGAAAAGGGTGGCCTCGTTG GCAGGCATTTCCATCGCAG	(AG)8...(AG)18	59.79	2	0.44
CEDG099	TGGGTGAGCATGGATGTGGA GGTTCAAGGTGGAAGGCAGA	(AG)7	59.85	2	0.44

CEDG112	GCAATATTCGCATTATTCATTCA GTGTTTCAAAGCACTATACTTAA	(AT)18(AG)20	53.04	2	0.44
CEDG269	CTGTTACGGCACCTGGAAAG GCAGAGACACACCTTAACCTTG	(AG)14	59.96	2	0.44
CEDG104	TATGGCCCGAGCAAACCTTG CCGTTCGGTCTTCGGTTGAA Mungbean (Somta et al., 2009)	(AG)13	59.85	2	0.44
DMB-SSR182	TAGAGCCTTCTGGTTTTTCACA AGGAGGAGGATTTTGATGATGA	(TGA)3..(CT)3…(TCC)4	53.75	2	0.45
DMB-SSR186	GAGAGAGAAGGAGAGGGAGA ATTCTTTCTCCACCACAATG	(GGT)4…(GGT)3…(GGT)4	52.40	3	0.66
DMB-SSR217	TCCTTGCCTTATGATTCTGTGA TTTGGCCACTTCCAAACTTTA Mungbean BAC library (Zhang et al., 2008)	(TC)6…(ATAG)3	53.30	2	0.44
VrCS SSR3	GCAGACACAACCATAAATCC GGTCTTTGACGGCAATCTC Mungbean (Kumar et al., 2002a)	(AT)37	56.66	3	0.66
LR738A	CGCAAAGAGAGAGAGAGAG CCCCCATCTGAAAGAAAGAG	(AG)11…(GA)6	57.68	2	0.44

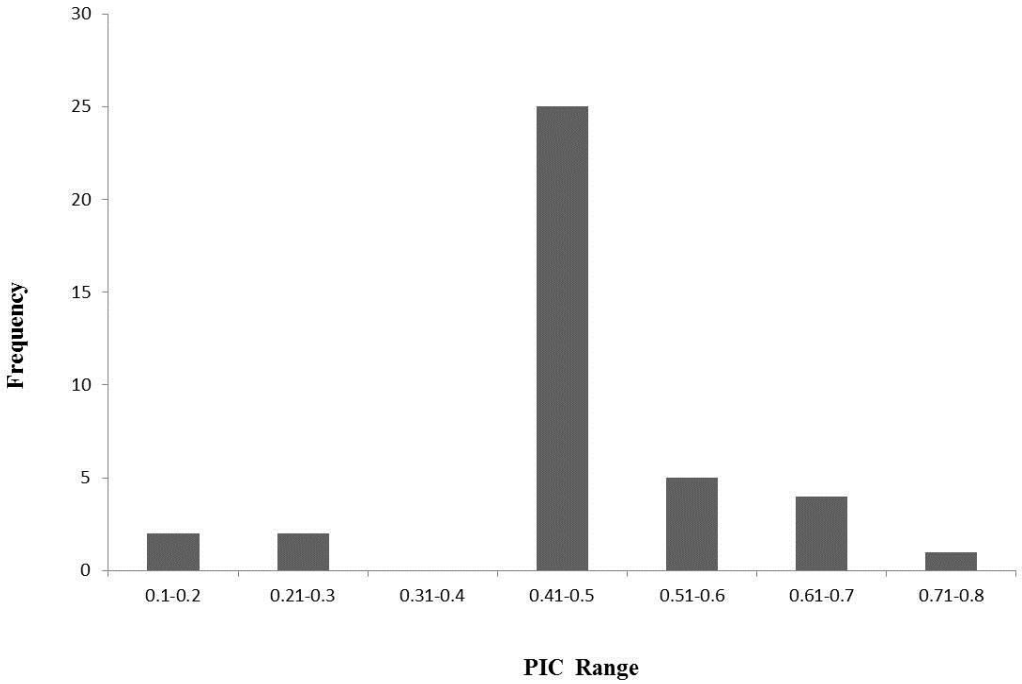


Fig 2. Frequency distribution of PIC range values of the polymorphic markers.

(UPGMA) using NTSYS pc- 2.11x (Rolf, 1998) software. The data were also subjected to Principal Coordinate Analysis (PCA) using NTSYS pc- 2.11x.

Conclusion

Blackgram (*V. mungo* L. Hepper) is an important food legume species. A major challenge for marker assisted breeding in this species is the lack of sufficient DNA markers. A set of 361 simple sequence repeat (SSR) markers developed from six other legume species *viz.* adzukibean, mungbean, cowpea, commonbean, chickpea and pigeonpea were evaluated for their transferability on 24 diverse genotypes of blackgram.

In total 245 (68%) SSR markers could produce amplicons and these were defined as transferable. Considerable transferability was observed with marker derived from related species belonging to genus *Vigna* such as mungbean (92%), adzukibean (91%), and cowpea (86%). Only 20% markers derived from commonbean were transferable to blackgram. However, no markers derived from chickpea and pigeonpea could amplify the DNA of blackgram. The polymorphism content of transferable SSR marker varied from 0.08 to 0.80 with average of 0.48. It was also observed that the SSR markers with dinucleotide repeat, AG, could be more frequent in transferability in blackgram. Thirty nine markers showed distinct polymorphism which were

Table 3. Jaccard Similarity matrix for 24 black gram genotypes based on SSR data.

Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Jacard Similarity Coefficient																								
IPU 91-07	1																							
PGRU 95018	0.5	1																						
PLU 01	0.9	0.57	1																					
IPU 99-147	0.8	0.64	0.9	1																				
IPU 99-79	0.8	0.5	0.9	0.8	1																			
UH 80-26	0.71	0.44	0.8	0.71	0.9	1																		
NG 2119	0.64	0.39	0.71	0.64	0.8	0.9	1																	
V 3108	0.71	0.44	0.8	0.71	0.9	0.8	0.9	1																
HPU 180	0.64	0.5	0.71	0.64	0.64	0.71	0.8	0.71	1															
PLU 328	0.71	0.44	0.8	0.71	0.71	0.64	0.71	0.8	0.71	1														
IPU 99-22	0.54	0.37	0.61	0.54	0.54	0.48	0.54	0.61	0.54	0.76	1													
IPU 99-232	0.33	0.29	0.39	0.39	0.33	0.29	0.33	0.39	0.33	0.5	0.61	1												
GP 215	0.48	0.28	0.54	0.48	0.48	0.54	0.48	0.42	0.48	0.54	0.65	0.54	1											
IPU 99-168	0.33	0.24	0.39	0.33	0.33	0.39	0.44	0.39	0.44	0.5	0.61	0.57	0.61	1										
PLU 312	0.24	0.13	0.29	0.29	0.33	0.29	0.33	0.39	0.24	0.39	0.42	0.44	0.37	0.33	1									
U 8-10	0.33	0.16	0.39	0.33	0.33	0.39	0.44	0.39	0.44	0.5	0.61	0.5	0.68	0.64	0.57	1								
AKU 9904	0.24	0.2	0.29	0.24	0.24	0.29	0.24	0.2	0.29	0.29	0.42	0.29	0.54	0.39	0.33	0.64	1							
DPU 88-31	0.29	0.16	0.33	0.33	0.39	0.33	0.29	0.33	0.2	0.33	0.42	0.44	0.48	0.29	0.71	0.5	0.39	1						
Uttara	0.24	0.2	0.29	0.29	0.33	0.29	0.24	0.29	0.24	0.29	0.37	0.39	0.42	0.24	0.64	0.44	0.39	0.9	1					
IPU 2-43	0.24	0.2	0.29	0.29	0.33	0.29	0.24	0.29	0.24	0.29	0.37	0.39	0.42	0.24	0.64	0.44	0.39	0.9	1	1				
PDU 1	0.29	0.16	0.29	0.24	0.24	0.29	0.24	0.2	0.33	0.29	0.37	0.29	0.54	0.33	0.39	0.57	0.5	0.57	0.64	0.64	1			
LBG 20	0.29	0.24	0.33	0.33	0.29	0.33	0.29	0.24	0.39	0.33	0.37	0.29	0.42	0.33	0.5	0.44	0.39	0.57	0.64	0.64	0.64	1		
Shekhar	0.29	0.13	0.29	0.24	0.33	0.39	0.33	0.29	0.29	0.29	0.37	0.2	0.42	0.33	0.39	0.44	0.44	0.57	0.57	0.57	0.71	0.57	1	
T 9	0.29	0.16	0.33	0.33	0.39	0.44	0.39	0.33	0.29	0.33	0.37	0.29	0.42	0.33	0.64	0.44	0.33	0.71	0.64	0.64	0.5	0.8	0.4	1

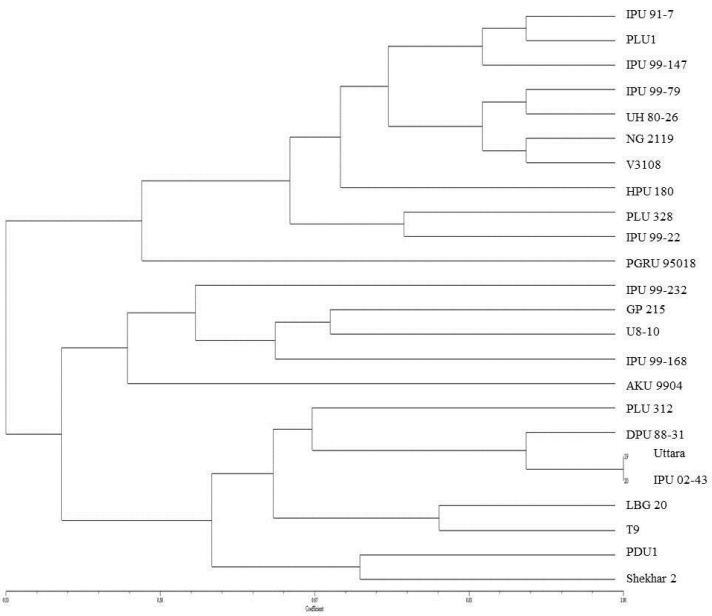


Fig 3. Dendogram based on Jaccard's similarity coefficient using UPGMA method of clustering.

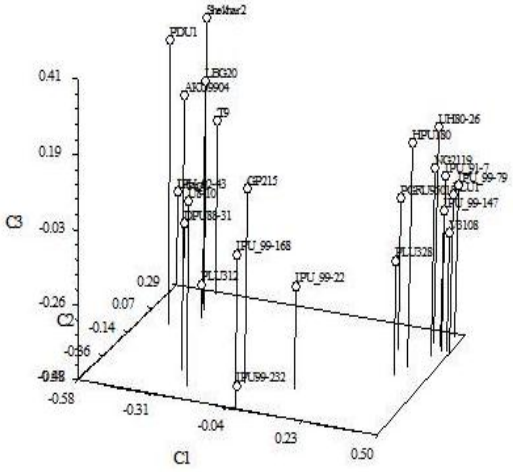


Fig 4. PCA analysis of the 24 test genotypes based on SSR genotyping data.

validated through diversity analysis and grouped 24 test genotypes into 3 major clusters indicating the efficiency of markers in germplasm distinctness. The markers identified by this comparative genomic approach could be useful for genetic analysis, germplasm evaluation and marker assisted breeding in blackgram.

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