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Full Length Research Paper

Genetic relationships among *Vigna mungo* (L.) Hepper and its close wild relatives using microsatellite markers

Kole, P. R.[1]*, Saxena, S.[1], Mathew, J.[2] and Bhat, K. V.[1]

[1]National Research Centre on DNA Fingerprinting, National Bureau of Plant Genetic Resources, New Delhi 110012, India.
[2]Department of Biotechnology, Bundelkhand University, Uttar Pradesh 284128, India.

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Genetic relationships of two wild *Vigna* species, *Vigna mungo* var. *silvestris* and *Vigna hainiana*, with accessions of a cultivated species, *V. mungo* known as urdbean or blackgram was studied with a view of using them as a donor of alien stress resistance genes in future breeding. POPGENE analysis conducted on a total of 375 individuals of 25 accessions using sequence tag microsatellite markers (STMS) revealed effective number of alleles varying from 3.0 to 1.7. The mean values of observed and expected heterozygosity were 0.084 and 0.335, respectively. Average Wright's fixation indices were: F_{IT} = 0.710, F_{ST} = 0.840 and F_{IS} = -0.84, indicating high level of outcrossing and genetic differentiation with limited gene flow between and within the three taxa studied. The *Nm* value for the accessions was found to be negligible, suggesting no gene flow within populations. For the groups, *Nm* value for *V. mungo* was 0.464, which was more than that of both *V. radiata* var. *silvestris* and *V. hainiana*. Since *V. mungo* is a cultivated species, it showed the highest within-species gene flow. Ewens-Watterson test for neutrality was done using 1000 simulated samples. All the loci were neutral to selection pressure as their observed F value was between lower and upper 95% limit.

Key words: Species relationships, *Vigna mungo*, microsatellite marker, blackgram.

INTRODUCTION

Blackgram also called as urdbean [*Vigna mungo* (L.) Hepper], a diploid species with 2n = 22, is one of the Asiatic species of the pantropical genus *Vigna*. It is a promising legume crop of South-East Asia and widely adapted to both semi-arid and sub-tropical areas. It is believed to have originated from the Indian subcontinent (Candolle, 1883; Vavilov, 1926) with maximum diversity in the Western Ghats, its secondary centre of origin being Central Asia. About 70% of the world's blackgram production comes from India. The area under traditional cultivation of blackgram is confined to South Asia and adjacent regions including India, Pakistan, Afghanistan, Bangladesh and Myanmar. These regions are characterized by farming systems that make limited use of inputs that compels breeding programme to concentrate on screening wild and cultivated germplasm for sources of disease and insect resistance as well as to identify other desirable traits, and combine multiple resistances. The desirable traits of wild sunflowers (*Helianthus* spp.) are worth an estimated sum of US $267 to US $384 million annually to the sunflower industry in the United States; one wild tomato variety has contributed to 2.4% increase in solids contents worth US$250 million; and three wild peanuts have provided resistance to the root knot nematode, which cost peanut growers around the world US $100 million each year (www.cropwildrelatives.org). The distribution of genetic diversity within and among the subspecies and the genetic relationship among the subspecies is to be considered under investigation. This information will help to provide an insight into the subspecies diver-

*Corresponding author. E-mail: prkole1979@gmail.com.

Table 1. Vigna species used for STMS analysis.

Vigna specie	Accession
	BBD-08-01B
	BBD-14-01B
	BB-03-09
	BB-03-10
V. mungo	Vamban 2
	PDU-1
	TMV-1
	PDU-1016
	UG-414
	IPU-99/10
	BBL-63-2K
	BBL-76-2000
	BBL-80-2K
	BBL-50-2K
	BBL-40-2K
V. mungo var. *silvestris*	BB-2638
	BB-2641
	BB-2642
	IC-251-407
	BBL-55-2K
	KP3-Rajgarh
	IC-251-377
V. hainiana	BB-2649
	BB-07-01A
	BBD-15-01A

gence and to exploit their genetic resources for breeding programs.

The dynamics of genetic diversity and population structure throughout the history of plant breeding for agricultural purposes has been a focus of attention for many crops. A plant breeder uses such information to help them better understand their germplasm, guide their breeding plans, and exploit genetic variation that is available to them. In recent years, a variety of molecular markers, based on microsatellites or simple sequence repeats (SSRs) have become the markers of choice, thus necessitating their development and use in a variety of plant systems (Gupta et al., 1996).

Most intraspecific relationships in the genus Vigna at molecular level have been done using allozymes (Panella and Gepts, 1992; Pasquet, 1993b; Vaillancourt et al., 1993), seed storage proteins (Panella et al., 1993; Fotso et al., 1994) and DNA markers like cpDNA (Vaillancourt and Weeden, 1993), amplified fragment length polymerphism (AFLP) (Coulibaly et al., 2002), RAPD (Ba et al., 2004) and sequence tag microsatellite markers (STMS) (Archana and Jawali, 2007). However, all these studies included a small number of wild accessions, where perennial subspecies were poorly represented.

The commercial value of crop wild relatives is impressive. In addition to their use in breeding, crop wild relatives are also used in their wild state. A number of wild cowpea species (Vigna spp.) in Africa contribute directly to food security through consumption of their tubers, fruit and seeds. Wild yams (Dioscorea spp.) are an important source of carbohydrate and income in Madagascar, and wild fruits such as apple, pistachio and sea buckthorn are harvested for food in Central Asia and the Caucuses. Crop wild relatives also provide other invaluable products such as animal fodder, building materials and medicines.

No systematic study has been done to investigate the extent of genetic variation and relationships between blackgram and its wild relatives, although variation within the cultivated and wild blackgram has been characterized with the use of various DNA-based markers (Souframainen and Gopalakrishna, 2004; Sivaprakash et al., 2004; Dikshit et al., 2007; Gupta, 2009). In order to develop high-yielding cultivars resistant to various stresses, exploitation of the gene pool is of paramount importance. The present study was designed to investigate the species diversity and relationship in blackgram germplasm and differentiation gene flow among V. mungo and its wild relatives using molecular marker analysis.

MATERIALS AND METHODS

In the present study, a total of 25 accessions of *V. mungo, V. mungo var. silvestris and Vigna hainiana* were analyzed for species relationships among them (Table 1). Total genomic DNA was extracted from fresh 15 day-old etiolated seedlings using a CTAB protocol modified from Saghai-Maroof et al. (1984). The DNA concentration was estimated with a DNA fluorometer (Hoeffer Scientific, San Francisco, USA) using Hoechest 33258 as the DNA intercalating dye and calf thymus DNA as standard (Brunk et al., 1979).

PCR and poly acrylamide gel electrophoresis (PAGE) PCR reactions were carried out in a DNA thermal cycler (Gene Amp 9600 PCR system, Perkin Elmer Cetus, Norwark, CT). Each 25 µl reaction mixture contained 1x reaction buffer (10 mM TrispH 8.3, 50 mM KCl), 3 mM MgCl2, 1 U of Taq DNA polymerase (Life technologies), 200 µM each of dATP, dGTP, dCTP and dTTP, 0.6 µM of primer and approximately 40 ng of template DNA. The PCR amplification conditions were as follows: Initial extended step of denaturation at 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at 32°C for 1 min and primer elongation at 72°C for 1 min, followed by extended elongation step at 72°C for 5 min. Reaction products were mixed with 2.5 µl of 10X loading dye (0.25% bromophenol blue, 0.25% xylene cyanol and 40% sucrose, w/v) and spun briefly in a microfuge before loading (Sambrook et al., 1989). The amplification products were electrophoresed on 6% PAGE at 100 V. Gels were stained with ethidium bromide and photographed on Polaroid 667 film under UV light.

Thirty (30) primer pairs were chosen for STMS analysis. Primer pairs were chosen on the basis of amplification and reproducibility. Out of 30 primer pairs, 10 were found to be polymorphic. List of primers used together with their sequences are given in Table 2.

Table 2. Forward and reverse primer sequences for STMS analysis and the number of alleles per locus and their allele size range.

Primer	Primer sequence (5'- 3')	Repeat unit	Tm (°C)	Total no of alleles	Allele size range (bp)
AB128093	CCCGATGAACGCTAATGC TG CGCCAAAGGAAACGCAGAAC	(AG)	57	2	180-200
MB122A	TGGTTGGTTGGTTCACAAGA CACGGGTTCTGTCTCCAATA	(TGGT)	48	3	180-250
AB128113	TCAGCAATCACTCATGTGGG TGGGACAAACCTCATGGTTG	(AG)	55	4	150-170
AB128135	ACTATTTCCAACCTGCTGGG AGGATTGTGGTTGGTGCATG	$(AG)_{46}$	48	3	150-210
MB91	GAGGCCAATCCCATAACTTT AGCACCACATCAGAGATTCC	(AG) (GA)	48	2	150-180
VM22	GCGGGTAGTGTATACAATTTG GTACTGTTCCATGGAAGATCT	(AG)	48	2	210-220
VM24	TCAACAACACCTAGGAGCCAA ATCGTGACCTAGTGCCCACC	(AG)	55	2	150-170
VM27	GTCCAAAGCAAATGABGTCAA TGAATGACAATGAGGGTGC	(AAT) (TC) (AC)	48	5	180-230
VM 31	GTGTTCTAGAGGGTGTGATGGTA CGCTCTTCGTTGATGGTTATG	$(CT)_{16}$	56	2	160-170
M323b	TGCTTCCTTTTGTCTGAGTTAGAA TGACGGAGAGAGAGAGAGAGAG	(GA)	57	4	300-320

RESULTS

Genetic structure of Vigna populations

The genotypes of 25 Vigna accessions were not identical (Figure 1) at 10 STMS loci studied. A total of 30 alleles were detected in 25 Vigna accessions by using 10 STMS markers. The number of alleles per locus ranged from two (AB128093, MB 91, VM 24 and VM 31) to five (VM 27), with an average of 3.0 alleles per locus. The overall size of amplified products ranged from 150 to 320 bp. Of the three *Vigna taxa*, for all the 10 loci, the maximum number of alleles was five in *V. mungo* var. silvestris. The mean number of alleles per locus was 3.0 and that for groups I (*V. mungo*), II (*V. mungo var. silvestris*) and III (*V. hainiana*) were 1.700, 2.900 and 2.200, respectively. Out of 30 alleles detected, eight were common (occurring with ≥0.05 frequency) and 22 were rare (occurring with <0.05 frequency).

The overall observed number of alleles and effective number of alleles were 3.0 and 1.7, respectively, while the overall genetic diversity in terms of Shannon's information index was 0.617 (Table 3). The overall observed heterozygosity and expected heterozygosity were 0.084 and 0.355, respectively. The observed (0.094) and expected heterozygosity (0.339) was highest for the *V. mungo var. silvestris*.

In three species groups (group I, *V. mungo*; group II, *V. mungo var. silvestris* and group III, *V. hainiana*), the observed number of alleles and effective number of alleles were higher in groups II (2.9 and 1.2, respectively) and III (2.2 and 1.7, respectively). The observed heterozygosity (0.094) and expected heterozygosity (0.339) were highest in Group II (Table 3). The Wright's F-statistics index F_{ST} and the gene flow *Nm* estimates were highest in Groups III (0.765) and I (0.464), respectively.

Evidence of differentiation and selection

Major genetic differentiation was found between the two wild and the cultivated species of *Vigna*. Using POPGENE

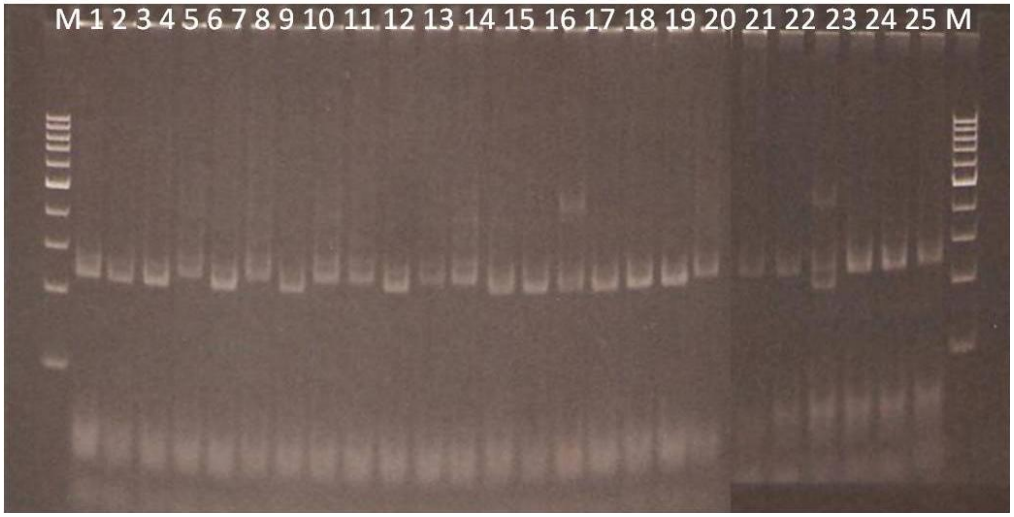

Figure 1. STMS profile of 25 accession of *Vigna* indicating the polymorphism of STMS markers, generated with the primer pairs AB128135. The lane marked M is the DNA molecular weight standard 100-base pair ladder of MBI. Fermentas (USA). Prominent fragments of approximately 190 to 250 bp were scored throughout all *Vigna* accessions investigated in the analysis.

Table 3. Administrative group-wise summary genetic variation statistics for all loci of *Vigna* populations.

Group	Population	Na	Ne	I	Ho	He	F_{ST}	Fis	Fit	*Nm*
I	*V. mungo*	1.700 ±0.948	1.190 ± 0.356	0.183±0.295	0.085 ±0.270	0.110±0.190	0.349	-0.198	0.221	0.464
II	*V. mungo* var. *silvestris*	2.900± 1.197	1.643± 0.488	0.574±0.328	0.094 ±0.294	0.339±0.205	0.508	0.430	0.720	0.242
III	*V. hainiana*	2.200±1.032	1.728 ±0.769	0.522±0.445	0.060 ± 0.135	0.318±0.274	0.765	0.192	0.810	0.076
--	Total	3.00± 1.054	1.738± 0.636	0.617±0.369	0.084 ±0.250	0.355±0.219	--	--	--	--

Na, Observed number of alleles; Ne, effective number of alleles; I, Shannon's information index; Ho, observe heterozygosity; He, expected heterozygosity; *Nm*, gene flow estimated from $F_{ST} = 0.25(1 - F_{ST})/ F_{ST}$.

software, Nei's original measure of genetic distance was calculated for all 25 accessions, on the basis of which the accessions were grouped into two unweighted pair group method with arithmetic mean (UPGMA)-based broad clusters (Figure 2). The first cluster (accessions BBD-08-01B, BBD-14-01B, BB-03-09, BB_03-10, IPU-99/10, Vamban 2, PDU-1, TMV-1, PDU-1016 and UG-414) comprised the cultivated varieties of *V. mungo* as well as 10 accessions of (BBL-63-2K, BBL-76-2000, BBL-80-2K, BBL-50-2K, BBL-40-2K, BBL-55-2K, IC-251-407, BB- 2638, BB-2641 and BB-2642) the wild relative *V. mungo* var. *silvestris*. Within this cluster, the cultivars formed an independent subcluster, closer to subcluster II consis-

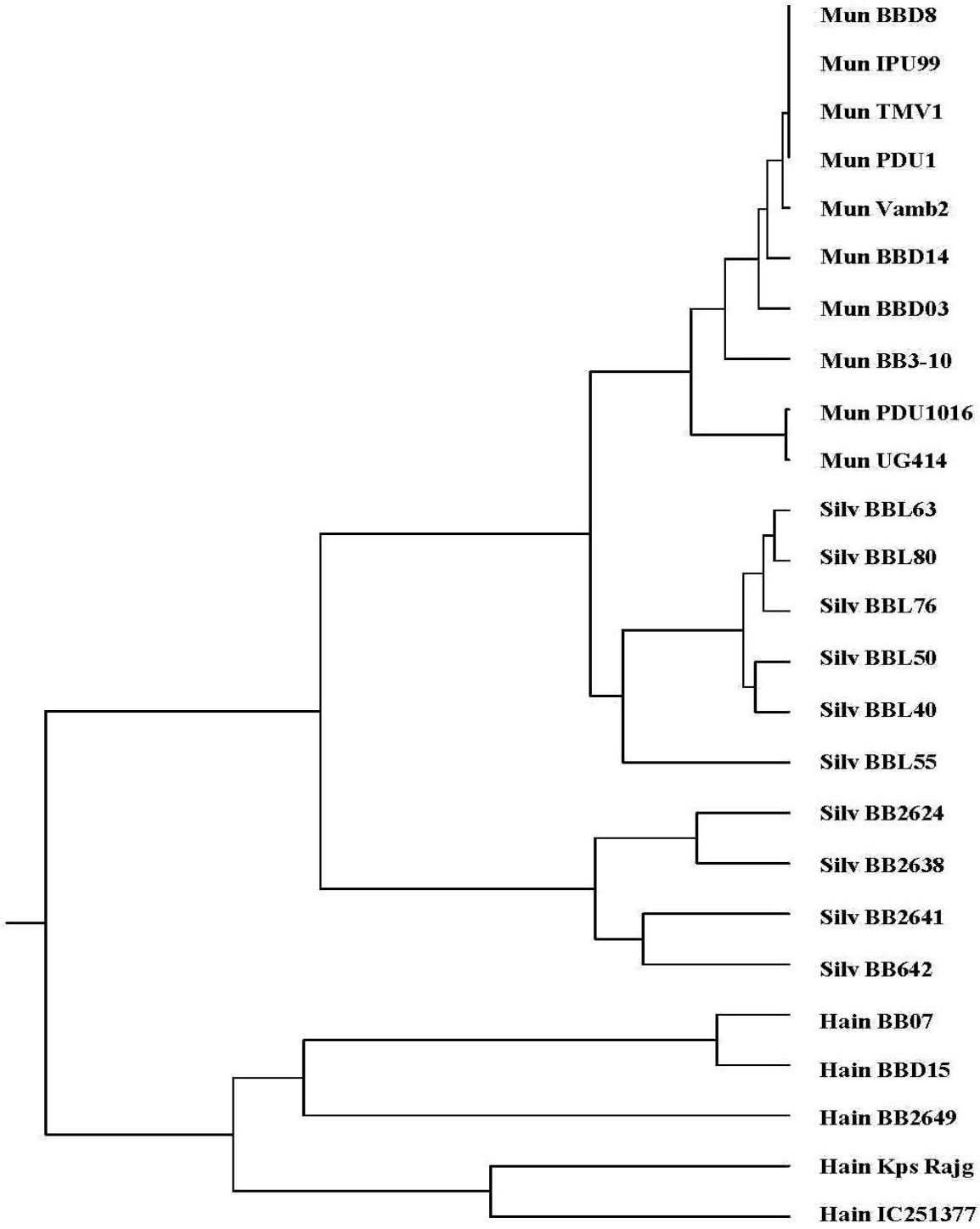

Figure 2. Dendrogram based on Nei's (1972) genetic distance: Method = UPGMA modified from NEIGHBOR procedure of PHYLIP Version 3.5.

ting of six accessions of *V. mungo* var. *silvestris*. Four accessions of *V. mungo* var. *silvestris* (BB-2624, BB-2638, BB-2641 and BB-2642) formed more differentiated subcluster III. Wild accession of *V. hainiana* (KP3-Rajgarh, IC-251-377, BB-2649, BB-07-01A and BBD-15-01A) formed a distant cluster.

In group I, the closest relation was found for the *V. mungo* accessions BBD08-1B, IPU99/10, TMV1 and PDU1. For group II, the closest relationship was found between *V. mungo* var. *silvestris* BBL63-2Kand BBL80-

2K. In Group III, accession BB07-2k and BBD15-2K were closest. Dendrogram was made by Nei's genetic distance for all the 25 accessions. Dendrogram were clearly clustered and no inter grouping was found in this dendrogram. Two main clusters were formed with three species. Cluster I consist of *V. mungo* and *V. mungo* var. *silvestris* accessions and cluster II was *V. hainiana* accessions.

Cluster I again divided into two, on which one contains *V. mungo* with some accessions of *V. mungo* var. *silvestris*. Another one consists of four *V. mungo* var. *silvestris* accessions which were closely related.

Fixation statistics were produced for individual SSRs and groups of germplasm. By using 1000 permutations, significance of the estimates were obtained. The percentage of variation within accessions was 16.03%, followed by 39.13% among accessions within groups and 44.84% among groups. Group differentiation is indicated by F_{ST} value, which measures the fixation of alleles in different accessions. F_{ST} values ranged from 0.35 to 0.76 which indicates that there is moderate to high genetic differentiation between accessions.

Ewens-Watterson test for neutrality was done using 1000 simulated samples. All the loci were neutral to selection pressure as their observed F value was between lower and upper 95% limit.

DISCUSSION

Wild relatives of crop species are sources of important genes for agriculture, and genetic diversity measured at the biochemical and DNA level is generally greater in wild species than in the related cultigen (Harlan, 1984; Xu et al., 2000). Intensive modern breeding has contributed to a narrowing of crop gene pools as few improved cultivars dominate large areas (Ladizinsky, 1985). Due to recent development of gene transfer technology, genes even from cross-incompatible wild species can be used for breeding (von Bothmer and Seberg, 1995). Therefore, evaluation of a broad array of wild species is an important approach to seeking genes which are rare or absent in the cultigens.

In the present study, POPGENE analysis of sequence-tagged microsatellite variation was done for the accession populations as well as for the species groups. Total number of alleles, effective number of alleles and Shannon's index were calculated for 25 accessions as well as for the three groups.

The effective number of alleles is low, but variable (overall 3.0 to 1.7 for cultivated blackgram), which suggest less variability among populations. Shannon's index was highest in *V. mungo* var. *silvestris,* which is a wild relative of *V. mungo*,supporting earlier findings that wild relatives of a crop species are more diverse than the cultivar (Harlan, 1984; Xu et al., 2000). Shannon's diversity index was maximal for *V. mungo* var. *silvestris* BB2641, so it can be a promising accession for enhancing genetic base of cultivars.

The mean values of observed and expected heterozygosity for 25 populations were 0.084 and 0.335, respectively. As observed, heterozygosity was less than expected heterozygosity; there was very low amount of cross-pollination in the studied populations, which is understandable due to the self-pollinating nature of *V. mungo*.

Accessions dendrogram made by Nei's genetic distance was clearly clustered and no inter-grouping was present. The clustering pattern suggests that *V. mungo* var. *sylvestris* is genetically less distant from *V. mungo* as compared to *V. hainiana,* which forms a separate cluster. The presence of sub-clustering in cluster I showed that subset of *V. mungo* var. *sylvestris* accessions is more closely related to *V. mungo* var. *mungo*, while another group of its populations is genetically more distant to *V. mungo.* This suggests that genetic diversity among accessions were more in *V. hainiana* as compared to other two species.

Average fixation indices over the three taxon groups, F_{IT} = 0.710, F_{ST} = 0.840 and F_{IS} = -0.84 is indicative of high level of outcrossing by high negative inbreeding coefficient and high differentiation among the groups with respective low gene flow between them. High levels of gene flow through germplasm exchange may have caused orchard stands in the northern plains and related regions to be genetically connected with little genetic differentiation (Table 4).

The population differentiation value (Fst) was used to determine the amount of gene flow (*Nm*). Fst value is inversely proportional to *Nm*. The *Nm* value for the accessions was found to be negligible, suggesting no gene flow within populations. For the species groups, *Nm* value for *V. mungo* was 0.4647 which was more than that for *V. mungo* var. *silvestris* (0.242) and *V. hainiana* (0.076, Table 3). Thus, cultivated *V. mungo* showed the highest gene flow among accessions, presumably through their use in the breeding programs (0.464) (Table 3).

In summary, the present study shows the utility of microsatellite markers in assessing genetic diversity and relationships among the cultivated blackgram and its wild relatives. Information generated from such relationship studies, can have a long-term impact on plant breeding, particularly for planning hybridization between intra-specific wild and cultivated taxa and for bridging related species by introgressing agriculturally important traits such as resistance, tolerance to biotic and abiotic stresses with the use of genetic maps between the microsatellite loci and the economically important quantitative trait loci (Table 5).

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Table 4. Nei's unbiased measures of genetic distance for all the *Vigna* populations.

	BBD-08-1B	BBD-1401B	BB-03-09	BB-07-01A	BBD-1501A	BB_03-10	BBL-63-2K	BBL-7-2000	BBL-80-2K	IPU-99/10	BBL-50-2K	BBL-40-2K	Vamban 2	PDU-1	TMV-1	PDU-1016	UG-414	BBL-55-2K	KP3-Rajgarh	IC-251-407	IC-251-377	BB-2638	BB-2641	BB-2641
BBD-08-1B																								
BBD-1401B	0.21																							
BB-03-09	0.03	0.02																						
BB-07-01A	0.58	0.58	0.52																					
BBD-1501A	0.38	0.37	0.33	0.07																				
BB_03-10	0.05	0.07	0.08	0.55	0.38																			
BBL-63-2K	0.12	0.15	0.14	0.32	0.3	0.11																		
BBL-7-2000	0.13	0.17	0.16	0.37	0.4	0.11	0.03																	
BBL-80-2K	0.13	0.14	0.15	0.33	0.3	0.09	0.02	0.02																
IPU-99/10	0	0.02	0.03	0.57	0.37	0.05	0.11	0.13	0.13															
BBL-50-2K	0.16	0.18	0.19	0.3	0.24	0.13	0.03	0.09	0.03	0.16														
BBL-40-2K	0.22	0.26	0.25	0.28	0.21	0.2	0.03	0.06	0.03	0.22	0.03													
Vamban 2	0.01	0.02	0.04	0.61	0.4	0.06	0.13	0.15	0.13	0.01	0.17	0.24												
PDU-1	0	0.02	0.03	0.59	0.38	0.05	0.12	0.14	0.13	0	0.16	0.23	0.01											
TMV-1	0	0.02	0.03	0.57	0.37	0.05	0.11	0.13	0.13	0	0.16	0.22	0.01	0										
PDU-1016	0.11	0.05	0.14	0.77	0.52	0.16	0.25	0.27	0.23	0.11	0.27	0.37	0.09	0.09	0.11									
UG-414	0.07	0.02	0.1	0.71	0.47	0.12	0.2	0.22	0.19	0.07	0.23	0.32	0.05	0.06	0.07	0								
BBL-55-2K	0.21	0.16	0.24	0.55	0.57	0.19	0.14	0.1	0.11	0.22	0.21	0.19	0.2	0.2	0.22	0.12	0.12							
KP3-Rajgarh	1.03	0.99	0.99	0.29	0.33	0.94	0.76	0.73	0.65	1.05	0.63	0.65	0.97	1.05	1.05	1.05	1.03	0.8						
IC-251-407	0.59	0.48	0.58	0.77	0.77	0.56	0.68	0.66	0.63	0.59	0.69	0.81	0.55	0.55	0.59	0.42	0.43	0.44	0.97					
IC-251-377	0.65	0.62	0.63	0.75	0.7	0.5	0.66	0.59	0.57	0.67	0.62	0.73	0.6	0.66	0.67	0.67	0.65	0.6	0.27	0.61				
BB-2638	0.38	0.28	0.37	0.6	0.57	0.35	0.44	0.43	0.4	0.38	0.46	0.53	0.35	0.36	0.38	0.23	0.24	0.24	0.85	0.08	0.51			
BB-2641	0.54	0.44	0.57	0.47	0.61	0.51	0.41	0.39	0.38	0.54	0.44	0.41	0.5	0.5	0.54	0.36	0.37	0.23	0.77	0.22	0.64	0.17		
BB-2642	0.31	0.25	0.32	0.48	0.54	0.3	0.31	0.26	0.27	0.32	0.35	0.39	0.27	0.3	0.32	0.23	0.23	0.15	0.6	0.2	0.36	0.11	0.14	
BB-2649	0.83	0.8	0.86	0.48	0.38	1.01	0.92	1.05	0.87	0.86	0.77	0.91	0.82	0.85	0.86	0.75	0.76	0.99	0.28	0.87	0.62	0.82	0.89	0.7

Table 5. Summary of diversity of 25 *Vigna* populations based on STMS primers.

Pop ID	Population name	Na	Ne	I	Ho	He	Nei's heterozygosity	Average heterozygosity
1	mungoBBD8	1.3000±0.4830	1.1284±0.3119	0.1183±0.2262	0.1000±0.3162	0.0775±0.1635	0.0749 ±0.1581	0.1098 ± 0.1171
2	mungoBBD8b	1.2000±0.4216	1.1565 ±0.3366	0.1253 ±0.2651	0.0533±0.1687	0.0901±0.1912	0.0871 ±0.1848	0.1098 ± 0.1171
3	mungoBB03	1.1000±0.3162	1.0142 ±0.0449	0.0245 ±0.0775	0.0000±0.0000	0.0129±0.0407	0.0124 ±0.0394	0.1098 ± 0.1171
4	hainBB07	1.0000±0.0000	1.0000 ±0.0000	0.0000 ±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000± 0.0000	0.1098 ± 0.1171
5	hainBBD15	1.4000±0.6992	1.2470 ±0.5148	0.1891 ±0.3538	0.0000±0.0000	0.1214±0.2289	0.1173 ±0.2213	0.1098 ±0.1171
6	mungoBB03_10	1.3000±0.4830	1.1942 ±0.3776	0.1575 ±0.2784	0.1000±0.3162	0.1106±0.2036	0.1069 ±0.1969	0.1098±0.1171
7	silvBBL63	1.5000±0.7071	1.3535 ±0.6400	0.2524 ±0.3846	0.1000±0.3162	0.1657±0.2543	0.1602 ±0.2458	0.1098 ±0.1171
8	silvBBL76	1.5000±0.7071	1.2502 ±0.3832	0.2248 ±0.3170	0.1000±0.3162	0.1483±0.2147	0.1433 ±0.2075	0.1098 ±0.1171
9	silvBBL80	1.7000±0.8233	1.3519 ±0.4228	0.3134 ±0.3426	0.1000±0.3162	0.2053±0.2303	0.1984 ±0.2226	0.1098 ±0.1171
10	mungoIPU99	1.1000±0.3162	1.1000 ±0.3162	0.0693 ±0.2192	0.1000±0.3162	0.0517±0.1636	0.0500 ±0.1581	0.1098 ±0.1171
11	silvBBL50	1.7000 ±0.8233	1.3651 ±0.5275	0.3076 ±0.3739	0.1000±0.3162	0.1952±0.2417	0.1887 ±0.2337	0.1098 ±0.1171
12	silvBBL40	1.4000±0.5164	1.2072 ±0.3288	0.1979 ±0.2683	0.1000±0.3162	0.1326±0.1872	0.1282 ±0.1810	0.1098 ±0.1171
13	mungoVamb2	1.4000±0.6992	1.2133 ±0.4147	0.1799 ±0.3262	0.1000±0.3162	0.1161±0.2146	0.1122 ±0.2075	0.1098 ±0.1171
14	mungoPDU1	1.4000±0.6992	1.1458 ±0.3175	0.1423 ±0.2524	0.1000±0.3162	0.0894±0.1718	0.0864 ±0.1661	0.1098 ±0.1171
15	mungoTMV1	1.1000±0.3162	1.1000 ±0.3162	0.0693 ±0.2192	0.1000±0.3162	0.0517±0.1636	0.0500 ±0.1581	0.1098 ±0.1171
16	mungoPDU1016	1.1000±0.3162	1.1000 ±0.3162	0.0693 ±0.2192	0.1000±0.3162	0.0517±0.1636	0.0500 ±0.1581	0.1098±0.1171
17	mungoUG414	1.2000±0.4216	1.1471 ±0.3342	0.1194 ±0.2557	0.1000±0.1811	0.0848±0.1841	0.0820±0.1780	0.1098 ±0.1171
18	silvBBL55	1.3000±0.4830	1.1771 ±0.3330	0.1586 ±0.2653	0.1000±0.3162	0.1087±0.1874	0.1051 ±0.1811	0.1098 ±0.1171
19	hainKPSRajg	1.2000±0.4216	1.1923 ±0.4058	0.1366 ±0.2881	0.1000±0.3162	0.1014±0.2138	0.0980 ±0.2067	0.1098 ±0.1171
20	silvIC251407	1.1000±0.3162	1.1000 ±0.3162	0.0693 ±0.2192	0.1000 ±0.3162	0.0517 ± 0.1636	0.0500 ±0.1581	0.1098 ±0.1171
21	hainIC251377	1.2000±0.4216	1.2000 ±0.4216	0.1386 ±0.2923	**0.2000 ±0.4216**	0.1034 ± 0.2181	0.1000 ±0.2108	0.1098 ±0.1171
22	silvBB2638	1.6000±0.6992	1.2691 ±0.3463	0.2623 ±0.3203	0.0667 ±0.1886	0.1687 ± 0.2075	0.1631 ±0.2006	0.1098 ±0.1171
23	silvBB2641	1.6000±0.5164	1.3994 ±0.4567	0.3194 ±0.3209	0.1067 ±0.3146	0.2257 ± 0.2389	0.2182 ±0.2309	0.1098±0.1171
24	silvBB2642	**1.9000±0.7379**	**1.5640 ±0.4306**	**0.4665 ±0.3382**	0.0733 ±0.2319	**0.3175 ± 0.2275**	**0.3069 ±0.2200**	0.1098±0.1171
25	hainBB2649	1.2000±0.6325	1.1273 ±0.4025	0.0892 ±0.2820	0.0000± 0.0000	0.0579 ± 0.1832	0.0560 ±0.1771	0.1098 ±0.1171
--	**Total**	3.0000 ±1.0541	1.7386 ± 0.6360	0.6174 ±0.3692	0.0840 ±0.2500	0.3559± 0.2190	0.3555 ±0.2187	0.1098±0.1171

Na, Observed number of alleles; Ne, effective number of alleles [Kimura and Crow (1964)]; I, Shannon's information index [Lewontin (1972)]; Ho, observe heterozygosity; He, expected heterozygosity; expected homozygosity and heterozygosity were computed using Levene (1949); **Nei's (1973) expected heterozygosity.

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