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Full Length Research Paper

Utility of adzuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi] simple sequence repeat (SSR) markers in genetic analysis of mungbean and related *Vigna* spp.

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In the present study, 78 mapped simple sequence repeat (SSR) markers representing 11 linkage groups of adzuki bean were evaluated for transferability to mungbean and related *Vigna* spp. 41 markers amplified characteristic bands in at least one *Vigna* species. The transferability percentage across the genotypes ranged from 60.97 to 92.6% with 87.8% in *Vigna radiata* and *Vigna mungo*, 62.2% in *Vigna unguiculata*, 91.8% in *Vigna umbellata*, 78% in *Vigna mungo* var. *sylvestris* and 80% in *Vigna trilobata*, respectively. Five major clusters were observed using unweighted pair group method with an arithmetic mean (UPGMA) with each cluster representing a particular *Vigna* species. We have successfully utilized adzuki bean SSRs in amplifying microsatellite sequences in *Vigna* species and inferring phylogenetic relationships by correlating the rate of transfer among them. The polymorphic SSR markers identified in this study would be useful in the analysis of genetic diversity, gene mapping and other aspects of genetic studies in mungbean and related species.

Key words: Adzuki bean, simple sequence repeat (SSR), inter-genomic marker transferability, genetic diversity, mungbean, *Vigna* spp.

INTRODUCTION

The genus *Vigna* is composed of more than 150 species originating mainly from Africa and Asia. The seven cultivated species of this genus are cowpea (*Vigna unguiculata* (L.) Walp.), bambara groundnuts (*Vigna subterranea* (L.) Verdc.), mungbean (*Vigna radiata* (L.) Wilczek), urdbean (*Vigna mungo* (L.) Hepper), adzuki bean (*Vigna angularis* (Willd.) Ohwi & Ohashi), moth bean (*Vigna aconitifolia* (Jacq.) Marechal), and rice bean (*Vigna umbellata* (Thunb.) Ohwi & Ohashi). The cowpea and bambara groundnuts originated from Africa and the five other species originated from Asia. Many of these species are used as pulse crops, fodder and cover crops in Asia and Africa. Mungbean is mainly grown in rain fed areas of India, Pakistan, Bangladesh, Sri Lanka, Laos, Cambodia, South China and Central Asia. Mungbean is a self pollinated diploid plant with 2n = 2x = 22 chromosomes and a genome size of 515 Mb/1C (Parida et al., 1990). Mungbean belongs to the Asian *Vigna* subgenus *Ceratotropis* with South Asia as its center of diversity (Tomooka et al., 2002). It has been estimated that 5.5 million hectares of mungbean is produced annually (Weinberger, 2003). The progenitor of mungbean, *V. radiata* var. *sublobata* is widely distributed from West Africa to Northern Australia and Papua New Guinea (Tomooka et al., 2002).

Adzuki bean *V. angularis* (Willd.) Ohwi & Ohashi is a popular crop in the Far East, mainly Japan, Korea and China. The early domestication started in Japan and later on, distributed in Nepal, India, etc. Adzuki bean is mainly cultivated in East Asian and Himalayan countries (Baudoin and Marechal, 1988). Its annual production in Japan and China has been estimated as 800000 metric tons (Vaughan et al., 2005). Two varieties are recognized within the species: *V. angularis var. angularis*, the

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cultivated form and *V. angularis var. nipponensis (Ohwi)*, the only known wild form. Adzuki bean includes mungbean [V. radiata (L.) Wilczek], known as the Asian Vigna (Tomooka et al., 2002) and is closely related to cowpea [V. unguiculata (L.) Walp.] and species of the genus *Phaseolus*.

Mungbean is consumed as "dhal", which is a soup porridge combined with cereal or other traditional cuisines, and provides a primary protein source for the vegetarian diet. Mungbean is a rich source of high protein, dietary fiber, vitamin C, riboflavin, foliate, copper, manganese, thiamin, niacin, vitamin B6, iron, phosphorus and potassium. Worldwide, mungbean is used for bean sprouts, starch noodles, mungbean soup and deep fried patties of different kinds. Mungbean is known for its easy digestibility and low flatus production as compared to other pulses. It is the third largest pulse crop in India occupying 3.72 million ha with 1.52 million tons produced during 2007 to 2008. Due to its earlier maturity, mungbean fits well in different cropping systems. However, the productivity of mungbean remains low due to biotic (mungbean yellow mosaic virus, powdery mildew and cercospora leaf spot) and abiotic stresses (drought, heat and preharvest sprouting). The genetic base of mungbean cultivars in India is narrow (Kumar et al., 2004). There is a need for improvement of mungbean utilizing both intraspecific and interspecific genetic diversity. Limited improvement has been achieved in mungbean using conventional breeding. The application of molecular markers will aid in directed improvement of mungbean cultivars.

Microsatellites or simple sequence repeats (SSRs) are clusters of short tandem repeated nucleotide bases distributed throughout the genome. SSR markers are a valuable tool for genetic mapping, genotyping and marker-assisted selection in breeding due to their characterization of co-dominant loci, high allelic variation and even distribution (Gupta and Varshney, 2000; Hernandez et al., 2002). The SSR markers are becoming the marker of choice because of their high level of polymorphism and occurrence throughout the genome. In recent years, efforts are devoted to the development of SSR markers in different crops. In mungbean, 35 polymorphic SSR markers were published by various researchers (Gwag et al., 2006; Somta et al., 2008; Kumar et al., 2002a, b). In a study by Somta et al. (2008), more than 200 primer pairs while amplifying SSRs were tested for polymorphism among 17 mungbean accessions, however only 12 (5.7%) primer pairs were polymorphic. Recently, Tangphatsornruang et al. (2009) reported 60 polymorphic SSR markers developed from genomic sequences. In mungbean, the number of SSRs reported is not sufficient for studying genetic diversity with high levels of polymorphism. There is a basic need to increase the number of genome-wide polymorphic SSR markers, for genetic mapping and marker-assisted improvement in mungbean.

Development of SSRs is a costly and time-consuming endeavor. The traditional method of SSR marker development involves construction of SSR-enriched libraries, cloning and sequencing, which is costly and labor intensive (Yu et al., 2009). Searching for transferable SSR markers developed from other legumes is a cost-effective way to increase DNA markers for mungbean and related *Vigna* species for genomic studies and genetic linkage map development. Transferability of DNA markers between the genomes of different species not only provides researchers with large pools of available markers, but also allows us to better understand the evolution and speciation of crops through comparative mapping. The objective of this study was to examine the transferability of adzuki bean SSR markers in mungbean and related *Vigna* spp. and study their validity in diversity analysis. This is the first attempt in identification of adzuki SSRs exhibiting polymorphism in Indian mungbean and related *Vigna* species.

MATERIALS AND METHODS

18 accessions of six *Vigna* species, namely *V. radiata* (L.) Wilczek, *V. mungo* (L.) Hepper, *V. unguiculata* (L.) Walp., *V. umbellata* (Thunb.) Ohwi & Ohashi, *Vigna mungo* var. *sylvestris* and *Vigna trilobata* were selected for the present study. The fragment size of adzuki bean accession HPU-51 was taken as the standard molecular weight reference for analysis of the band pattern of microsatellite markers. The sources of our studied species and cultivars/accessions are presented in Table 1. This study was conducted at the Division of Genetics, Indian Agricultural Research Institute, New Delhi 110012, India.

Genomic DNA isolation

Total genomic DNA from 5 g of fresh young leaf tissue, collected from five random plants per accession, was extracted following the cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980). The resulting DNA was precipitated with chilled iso-propanol and the DNA pellet was rinsed with 70% ethanol for 10 to 15 min to remove excess CTAB. The pellet was dried at room temperature overnight and suspended in Tris-EDTA (TE) buffer (pH = 8). The RNase treatment used was similar to the method by Murray and Thompson (1980). The purified DNA was quantified on 0.8% agarose gel with uncut lambda DNA (30 and 60 ng). The total genomic DNA was diluted to 20 ng/µl for polymerase chain reaction analysis.

Primer selection and PCR

78 mapped SSR primers representing 11 linkage groups of adzuki bean were evaluated for transferability to mungbean and related *Vigna* species. These adzuki bean (*V. angularis*) microsatellites were previously designed by Wang et al. (2004) and sequence information was utilized for custom oligo synthesis. The PCR reactions were performed in a 20 µl reaction mixture containing 50 ng template DNA, 200 µM dNTPs, 250 nM of each primer, 1.5 mM $MgCl_2$, 1x PCR buffer and 1 unit *Taq* DNA polymerase. The PCR amplification was performed with an initial denaturation at 94°C for 3 min followed by 35 cycles of 94°C for 1 min, 53 to 60°C (depending on the primer) for 1 min, 72°C for 2 min, and a final

Table 1. Source of studied species and cultivars/accessions.

S/N	Crop	Name of accession/cultivar	Species	Source
1	Mungbean	Pusa Ratna	*V. radiata (L.)* Wilczek	IARI, New Delhi
2		Pusa 9531	*V. radiata (L.)* Wilczek	IARI, New Delhi
3		Pant Mung 5	*V. radiata* (L.) Wilczek	GBPUAT, Pantnagar
4		AKM 9904	*V. radiata* (L.) Wilczek	PKV, Akola
5		Pant Mung 4	*V. radiata* (L.) Wilczek	GBPUAT, Pantnagar
6		Pusa Vishal	*V. radiata* (L.) Wilczek	IARI, New Delhi
7		COGG 912	*V. radiata* (L.) Wilczek	TNAU, Coimbatore
8	Urdbean	PU 06-20	*V. mungo* (L.) Hepper	GBPUAT, Pantnagar
9		PU 31	*V. mungo* (L.) Hepper	GBPUAT, Pantnagar
10		IPU 94-1	*V. mungo* (L.) Hepper	IIPR, Kanpur
11		Barabanki local	*V. mungo* (L.) Hepper	IARI, New Delhi
12	Cowpea	V 585	*V. unguiculata* (L.) Walp.	IARI, New Delhi
13		V 578	*V. unguiculata* (L.) Walp.	IARI, New Delhi
14	Ricebean	RBL 1	*V. umbellata* (Thunb.) Ohwi & Ohashi	PAU, Ludhiana
15		RBL 50	*V. umbellata* (Thunb.) Ohwi & Ohashi	PAU, Ludhiana
16		RBL 35	*V. umbellata* (Thunb.) Ohwi & Ohashi	PAU, Ludhiana
17	Wild spp.	IPUW 02-6	*Vigna mungo* var. *sylvestris*	NBPGR, New Delhi
18	Wild spp.	IC 251413	*Vigna trilobata*	NBPGR, New Delhi

IARI, Indian Agricultural Research Institute; GBPUAT, Govind Ballabh Pant University of Agriculture and Technology; PKV, Punjab Rao Krishi VishwaVidyalaya; TNAU, Tamil Nadu Agricultural University; PAU, Punjab Agricultural University; NBPGR, National Bureau of Plant Genetic Resources.

extension at 72°C for 8 min before cooling at 4°C. Amplification products were resolved on 3% agarose (metaphor) gel. A 100 bp ladder was used for approximate sizing of the products. The DNA samples were run on electrophoresis for 3 h at a constant voltage of 100 V in 1X Tris-Boric acid-EDTA (TBE) buffer stained with ethidium bromide and photographed with a Close Circuit Digital camera attached to a gel documentation system (Syngene Synoptics Ltd., USA).

Statistical analysis

The DNA fragments amplified by the primer sets were scored manually based on positions of the bands relative to the ladder sequentially from the smallest to the largest-sized bands. Diffused bands or bands revealing ambiguity in scoring were considered as missing data and designated as '9' in comparison with '1' for the presence of a band and '0' for the absence of a band in the data matrix. Unique alleles were defined as those detected in only one species. A binary matrix was then transformed to genetic similarity (GS) matrices using Jaccard's coefficient (Jaccard, 1908). A dendrogram based on similarity coefficients was prepared by using unweighted pair group method with an arithmetic mean (UPGMA) with the computer package NTSYS-pc 2.02 (Rohlf, 2000). Boot strap analysis was carried out to statistically support the cluster branches with 1000 replicates using the WIN BOOT software program (Yap and Nelson, 1996). The resolving power (Rp) for each primer was calculated as $Rp = \Sigma Ib$, where Ib [band informativeness) takes the value: $1 - (2 \times (0.5 - p))$], and p is the proportion of the genotype of different *Vigna* species containing that band (Prevost and Wilkinson, 1999). Polymorphism information content (PIC) values for each band was calculated according to the formula by Anderson et al. (1993).

RESULTS

Inter-genomic transferability of SSR markers

78 adzuki-specific SSRs were assessed. 41 amplified characteristic bands in at least one *Vigna* species. Table 2 shows sequence of these primers, repeat motif, annealing temperature, number of alleles, PIC value and resolving power. 20 primer pairs namely CEDG 006, CEDG 014, CEDG 15, CEDG 18, CEDG 020, CEDG 21, CEDG027, CEDG 50, CEDG 056, CEDG 73, CEDG 086, CEDG 091, CEDG 092, CEDG 127, CEDG 139, CEDG 154, CEDG 178, CEDG 204, CEDG 214 and CEDG 305 produced amplification in all *Vigna* genotypes studied indicating their absolute transferability. 31 markers showed overall transferability of more than 80% in the *Vigna* accessions and five amplified 61 to 80%. The additional five markers revealed transferability below 40%. The lowest transferability of 11.11% occurred by CEDG 294. Of the 41 transferred markers to *Vigna*, five markers including CEDC 036, CEDG 103, CEDG 141,

Table 2. Primer sequence, motif, Temperature (°C) and number of alleles of adzuki specific SSR markers transferable to other *Vigna* species.

Primer	Forward Primer	Reverse Primer	Motif	Linkage group	Temperature (°C)	Number of alleles	PIC value	Resolving power
CEDG006	AATTGCTCTCGAACCAGCTC	GGTGTACAAGTGTGTGCAAG	(AG)10AA(AG)18	2	57	2	0.44	1.80
CEDG010	TGGGCTACCAACTTTTCCTC	TGAGCGACATCTTCAACACG	(AG)21	3	57	3	0.57	1.70
CEDG013	CGTTCGAGTTTCTTCGATCG	ACCATCCATCCATTCGCATC	(AG)22	1	57	2	0.43	2.00
CEDG014	GCTTGCATCACCCATGATTC	AAGTGATACGGTCTGGTTCC	(AT)12(AG)14	5	57	2	0.50	2.00
CEDG015	CCCGATGAACGCTAATGCTG	CGCCAAAGGAAACGCAGAAC	(AG)27	6	60	2	0.28	2.00
CEDG018	AGCGTGTTTGTGGTGATAGC	ACACAGGAACGAACAAACCC	AG(32)	5	57	4	0.66	1.80
CEDG020	TATCCATACCCAGCTCAAGG	GCCATACCAAGAAAGAGG	(AT)18(AG)20	5	56	3	0.59	2.00
CEDG021	GCAGAATTTTAGCCACCGAG	AAAGGATGCGAGAGTGTAGC	(AG)26	10	57	2	0.48	1.80
CEDC027	ACTGGATGAGGGTTTAGTGCG	CTGTCTTGTCTTGTGGGTTCGTTC	(AC)8	1	60	3	0.60	2.00
CEDC036	GAAAAAGTAATCAAAGCTGGG	CTTTACTAACTCCAACTCCTAACTC	(AC)8	4	58	1	0.00	1.20
CEDG037	GAAGAAGAACCCTACCACAG	CACCAAAAACGTTCCCTCAG	(AG)16AC(AG)8	6	57	2	0.51	1.73
CEDG043	AGGATTGTGGTTGGTGCATG	ACTATTTCCAACCTGCTGGG	AG(14)	3	60	3	0.50	1.70
CEDG044	TCAGCAACCTTGCATTGCAG	TTTCCCGTCACTCTTCTAGG	(GT)10AT(AG)18	11	57	2	0.18	2.00
CEDG048	TCTCTTCCTCTATGGCTTGG	GCTCCTCTTTTTGCTGCATC	(AG)20(TG)3TC(TG)4(AG)12	1	57	3	0.48	1.80
CEDG050	GGCAGAATCGTACAAGTG	GTCAGATTCTCGCTTGCATG	AG(12)	2	53	5	0.71	2.10
CEDG056	TTCCATCTATAGGGGAAGGGAG	GCTATGATGGAAGAGGGCATGG	(AG)14	9	60	3	0.65	1.60
CEDG073	GGTTAGCATCTGAGCTTCTTCGTC	AACACCCGCCTCTTTCTCC	(AG)24	8	60	3	0.84	0.80
CEDG086	GAGTTTACAACAGATGGGGCTAA	AGGTCTTGATTGACTTTCTGGGT	(AG)11	4	60	4	0.56	1.90
CEDG088	TCTTGTCATTTAGCACTTAGCACG	TTGTTGTTTACTAAGAGCCCGTGT	(AG)7	4	60	2	0.60	1.40
CEDG091	CTGGTGGAACAAAGCAAAAGAGT	TGCGTCTTGGTGCAAAGAAGAAA	(AG)7	4	58	2	0.20	1.80
CEDG092	TCTTTTGGTTGTAGCAGGATGAAC	TACAAGTGATATGCAACGGTTAGG	(AG) 17	8	60	5	0.71	1.70
CEDG097	GTAAGCCGCATCCATAATTCCA	TGCGAAAGAGCCGTTAGTAGAA	(AG)7	10	60	2	0.60	1.80
CEDG103	CACCGCTGTCCATTGAAGTATTA	TCTTAGAGTGCCCTGTGAGATTG	(AG)37	4	60	2	0.92	0.60
CEDG104	TATGGCCCGAGCAAACCTTG	CCGTTCGGTCTTCGGTTGAA	AG(13)	11	60	2	0.03	2.40
CEDG111	TGGAAGTTTCCAAGAGGGTTTTC	TCTCACCACCTTTTACCTTCTCA	(AT)7(AG)14	7	58	2	0.55	1.30
CEDG115	GGCTCATTGTACCACTGGATAT	ATGCCTCCTTTCAGGTGATTGT	(AT)3(AG)11	5	58	1	0.00	1.40
CEDG127	GGTTAGCATCTGAGCTTCTTCGTC	CTCCTCACTTGGTCTGAAACTC	(TG)3(AG)9	4	58	2	0.50	2.00
CEDG133	GCATACATAATGTGGTGAGATG	GTCTCGTGCCTTTCACAC	(AT)3(AG)11	1	58	2	0.64	1.40
CEDG139	CAAACTTCCGATCGAAAGCGCTTG	GTTTCTCCTCAATCTCAAGCTCCG	(AG)19	4	58	2	0.40	2.00
CEDG141	CCAGGCATCCATGATGACC	GAAGTTGTTGGTAATGGTTGCCTC	(AT)6(AG)13	1	58	3	0.93	1.20
CEDG143	GATGAACTCGTCTCGCTCATCG	CTGGACGCGTCTACTCAGAC	(AG)12	7	58	2	0.10	1.90
CEDG149	GGCTGAAGGTGATGACAGAAG	GGCACTGGTTTTCTAAGGTTGTTG	(AT)12(AG)16	1	58	3	0.72	1.60
CEDG154	GTCCTTGTTTTCCTCTCCATGG	CATCAGCTGTTCAACACCCTGTG	(AG)14	4	58	3	0.59	1.80
CEDG178	CGGAAGAAGAACGCAGAGTG	GCATCAACAAGGACTTCTGC	(AG)10G(AG)5	1	58	2	0.35	1.80
CEDG204	CCTTGGTTGGAGCAGCAGC	CACAGACACCCTCGCGATG	AG(15)	1	58	2	0.79	1.00
CEDG214	CACTCACTGCAAAGAGCAAC	CTACCTATCTGAGGGACAC	(AG)4AA(AG)31	1	58	2	0.20	1.80
CEDG232	GATGACCAAGGTAACGTG	GGACAGATCCAAAACGTG	AG(16)	4	58	1	0.00	0.60
CEDG253	CACTTCCATGATGACTCACC	CACCCTTCTTTATCCTCTTCG	(AG)30	5	58	1	0.00	0.40
CEDG294	CACCTTCTTAATCTCTTCACC	GGGTTTCTCTTAATTCATTGAGTC	(AT)27(AG)15	3	58	1	0.00	0.20
CEDG305	GCAGCTTCACATGCATAGTAC	GAACTTAACTTGGGTTGTCTGC	(AG)22	3	58	4	0.71	1.60

CEDG 253 and CEDG 294 did not show amplification in mungbean. Primer CEDG 253 amplified only rice bean accessions, while CEDG 103 amplified both urdbean and rice bean. However, Primer CEDG 141 amplified cowpea and ricebean genotypes. 13 primers did not show amplification in cowpea. Primer CEDG 232 amplified only in mungbean and CEDC 036 amplified in all studied species except mungbean. The primer CEDG 104 did not show amplification in urdbean and cowpea but CEDG 37 and CEDG 115 exhibited amplification among the crossable mungbean, urdbean, *V. mungo* var. *sylvestris* and *V. trilobata*. Number of transferable SSRs and unique alleles represented by individual species is shown in Table 3.

Polymorphism of SSR markers

Of the 41 primers showing amplification, 36 primers revealed polymorphism, while 5 primers viz. CEDC 036, CEDG 115, CEDG 232, CEDG 253 and CEDG 294 produced monomorphic amplicons of 142, 98, 205, 253 and 197 bp, respectively. The transferable SSRs produced a total of 97 alleles in *Vigna* genotypes (Table 2). The PIC value of primers ranged from 0.00 to 0.93 (CEDG 141). The number of alleles produced by the 41 transferable markers in the *Vigna* genotypes ranged from 1 to 5. The primers CEDG 050 and CEDG 092 produced the maximum number of alleles (5). The amplification pattern of the SSR marker, CEDG 149 in different *Vigna* species is shown in Figure 1.

Phylogenetic relationship among *Vigna* species

Using 41 transferable adzuki bean primers, 97 SSR alleles were amplified in the *Vigna* species. Genetic distances among the *Vigna* species revealed five clusters (Figure 2). Cluster I comprised of seven cultivated *V. radiata* (L.) Wilczek genotypes. Pusa Ratna and Pusa 9531 were grouped in the first sub cluster while, Pant M 5, AKM 9904, Pant M 4, Pusa Vishal and COGG 912 were grouped in the second sub-cluster. Pusa Ratna and Pusa 9531 are early maturing mungbean varieties originating from Asian Vegetable Research Development Centre (AVRDC) germplasm lines. The studied SSR markers exhibited 100% similarity for Pant M 5 and AKM 9904 and Pant M 4 and Pusa Vishal. Cluster II included the *V. mungo* (L.) Hepper genotypes PU 06-20, PU 31, IPU 94-1 and Barabanki local. However, PU 06-20 was grouped into a separate sub-cluster. Genotypes PU31, IPU94-1 and Barabanki local were highly similar based on SSR allelic diversity data revealing that the marker was insufficient to discriminate among the urdbean genotypes. Cluster III comprised of three ricebean genotypes RBL 1, RBL 35 and RBL 50. Cluster IV included *V. mungo var. sylvestris* (IPUW02-6) and *V. trilobata* (IC556571) genotypes, sharing 44% similar alleles. Cluster V included the cowpea genotypes V 585 and V 578.

DISCUSSION

Limited availability of polymorphic DNA markers has been a major bottleneck in mungbean genomic research. Therefore, developing and/or identifying polymorphic SSR motifs of mungbean is the most important requirement. This comparative genetic study indicates that the gene content and order are conserved among the related species. The critical evaluation of sequence data from different crops indicates the existence of adequate homology between genomes in regions flanking the SSR loci. Hence, the primer pair designed for one species could be used to detect SSRs in related species. Conservation of SSR flanking regions have been reported in *Medicago sativa* and *Pisum sativum* (Kalo et al., 2004); *Medicago truncatula* and *P. sativum* (Aubert et al., 2006) and *M. truncatula* and *Medicago sativum* (Phan et al., 2007). Weeden et al. (1992) was the first researcher to report genetic relatedness in chickpea, lentil and pea using RFLP markers. Their study revealed that 40% of the linked loci in the lentil linkage map were conserved in pea (*P. sativum*). Since then, many works have reported transferability of sequence tagged microsatellite primers across major pulse crops. Across species amplification within *Glycine* genotypes (Peakall et al., 1998) and transferability of sequence tagged microsatellite (STMS) primers from field pea and chickpea in Vetch and lentil (Pandian et al., 2000) yielded an overall transferability of 3 to 62%. High interspecific transferability across the legume genera including *M. sativa*, *P. sativum*, *V. radiata*, *Glycine max* and *P. vulgaris* has also been reported (Choudhary et al., 2008). Wang et al. (2009) reported transferability of adzuki bean primers in mungbean and found that 70% of them generated clear and repeatable bands in mungbean. Successful transferability of genomic SSRs is reported to be low as compared to EST derived SSRs. We however, found 100% transferability in different species with adzuki bean primers. Our study implies that adzuki bean primers can successfully amplify intervening SSRs in other legume species.

Dinucleotide motifs $(AG)_n$ and $(AC)_n$ were reported to be abundant in adzuki bean SSRs which was in agreement with Wang et al. (2004). Perfect $(AG)_n$ repeats were reported to be more frequent than the compound $(AG)_n$ repeats. Most of the primers used in our study based on $(AG)_n$ repeat motifs were successful in amplifying the microsatellite regions in different species, however the size of amplicons produced in mungbean and related *Vigna* species was variable. A few amplification products were sequenced to confirm the presence of $(AG)_n$ repeats in *Vigna* accessions. Eight

Table 3. Number, percent and unique alleles of adzuki primer pairs amplified in mungbean and related *Vigna* species.

Species	Genotype	Number of marker amplified	Percentage transferability	Unique allele	
				Name	Size (bp)
V. radiata (L.) Wilczek	Pusa Ratna	36	87.80	-	-
	Pusa 9531	36	87.80	CEDG 111	95
	Pant M 5	36	87.80	-	-
	AKM 9904	36	87.80	-	-
	Pant M 4	36	87.80	-	-
	Pusa Vishal	36	87.80	-	-
	COGG 912	36	87.80	-	-
V. mungo (L.) Hepper	PU 06-20	36	87.80	CEDG 305 CEDG 092	135 162
	PU 31	36	87.80	-	-
	IPU 94-1	36	87.80	-	-
	Barabanki local	36	87.80	-	-
V. unguiculata (L.) Walp.	V 585	26	63.41	CEDG 305 CEDG 111	144 212
	V 578	25	60.97	CEDG 15 CEDG 305	163 103
V. umbellate (Thunb.) Ohwi & Ohashi	RBL 1	38	92.68	CEDG 15 CEDG 092	168 204
	RBL 50	38	92.68	-	-
	RBL 35	37	90.24	CEDG 18	146
Vigna mungo var. *sylvestris*	IPUW 02-6	32	78.04	-	-
Vigna trilobata	IC 556571	33	80.48	-	-

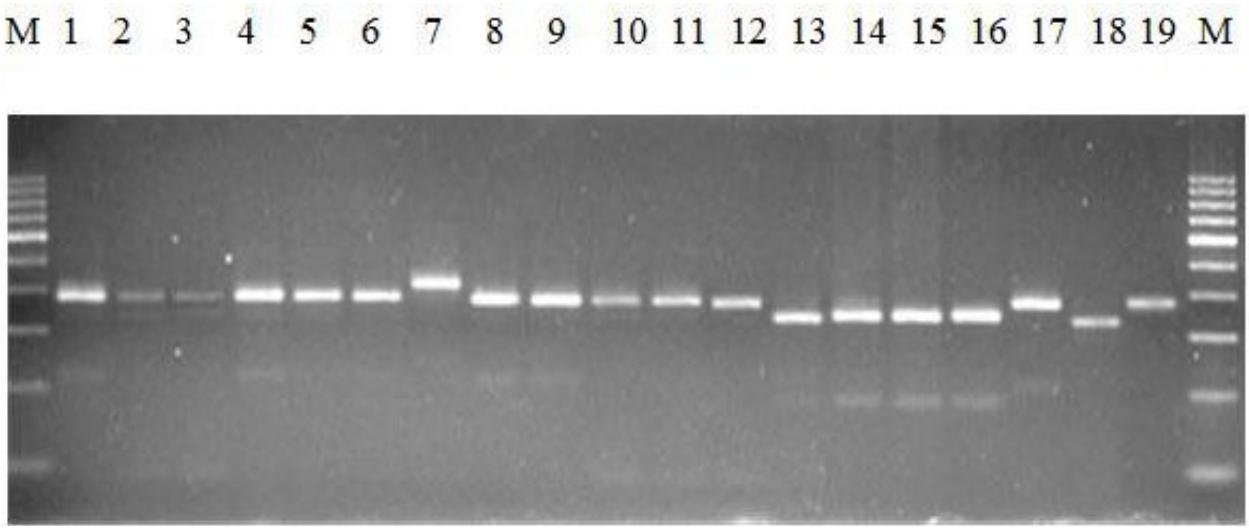

Figure 1. SSR profile of *Vigna* genotypes obtained with Adzuki bean SSR marker CEDG149. Lanes M, 100 bp ladder; 1, Pusa Ratna; 2, Pusa 9531; 3, Pant M-5; 4, AKM 9904; 5, Pant M-4; 6, Pusa Vishal; 7, COGG 912; 8, PU 06-20; 9, PU-3; 10, IPU 94-1; 11, barabanki local; 12, V-585; 13, V-578; 14: RBL-1; 15, RBL-50; 16, RBL-35; 17, *Vigna sylvestris*; 18, *Vigna trilobata*; 19, adzuki bean control; HPU-51.

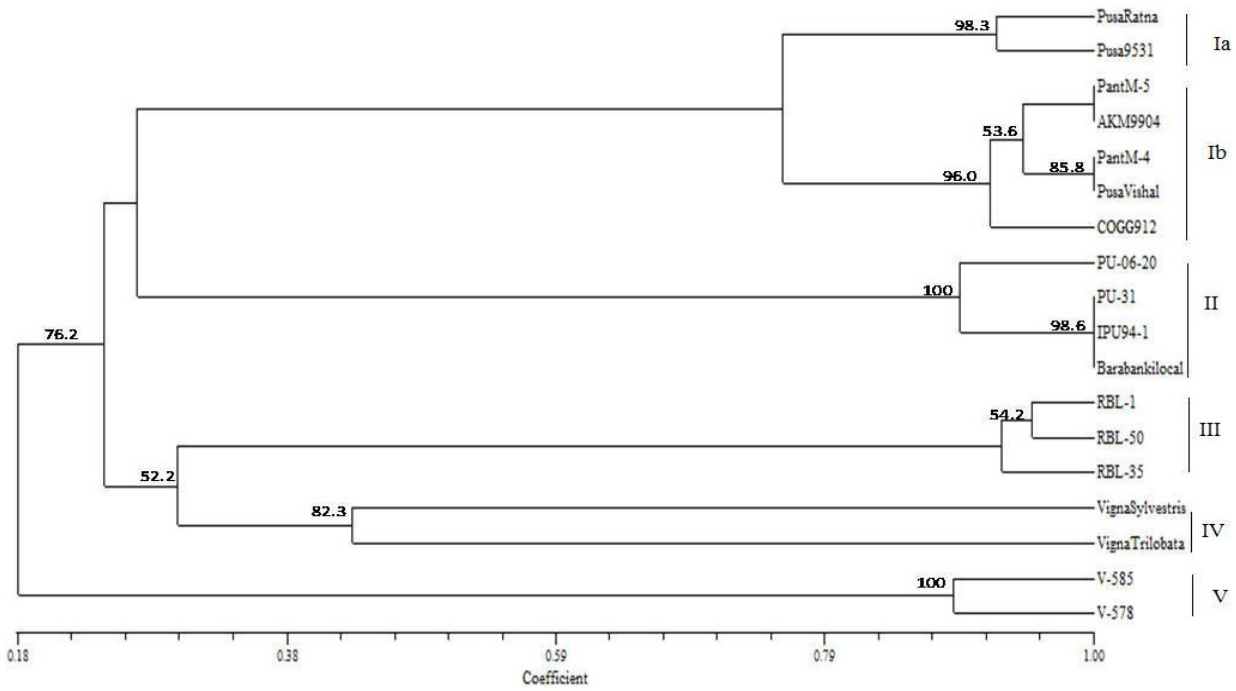

Figure 2. Clustering of mungbean and related *Vigna* species derived from an unweighted pair group mean average (UPGMA) cluster analysis using the Jaccard's similarity coefficient based on adzuki bean SSR primers.

primer pairs produced multiple bands for one or more accession. These multiple bands may be due to annealing at more than one locus or duplication of primer binding sites in the cross-species legumes. This indicates a high level of sequence conservation among the flanking regions of microsatellites. This sequence conservation formed the basis of cross-species utilization of SSR primers in assessing phylogenetic relationships across species and genera.

In conclusion, the transferability of SSRs due to homology of flanking regions between closely related species may reduce costs, and avoid the laborious cloning procedures involved in their development. Recent research suggests that successful cross-species amplification in plants is largely restricted to closely related genera. This may allow for the comparative map construction, molecular characterization and evaluation of crop species such as mungbean which are lacking sufficient DNA markers. Further success rates can be achieved by using primers based on expressed sequences which may be more conserved in genomic regions.

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