



## Research Article

# Cross species amplification of Adzuki Bean derived microsatellite markers in Asian *Vigna* species

M. Srimathy and P. Jayamani

### Abstract

The *Vigna* is one of the important genus of grain legumes which forms the source of dietary protein and seven species of this genus, are domesticated as food crops in Asia. In recent years, molecular marker technology has greatly accelerated breeding programs for the improvement of various crops. Among the different DNA markers, microsatellite or simple sequence repeats (SSRs) are the markers of choice for various genetic studies due to their co-dominant nature, loci specificity and high reproducibility. To date, only few reports are available on isolation and development of microsatellite markers in some of the *Vigna* species. Therefore, the available SSR markers from other *Vigna* species should be validated for their transferability and utility in those species in which they are unavailable. In the present study, a set of 40 microsatellite primers pairs derived from adzuki bean (*Vigna angularis*) were used to assess the transferability and tested for their ability to amplify microsatellite loci in different species of Asian *Vigna*. The materials for this study included eleven different genotypes belonging to seven species of Asian *Vigna* such as *V. mungo* var *silvestris*, *V. mungo*, *V.umbellata*, *V. trilobata*, *V. aconitifolia*, *V. radiata* var *sublobata* and *V. radiata*. All the 40 SSR primer pairs showed cross species amplification and produced a total of 158 alleles in the genotypes studied. The percentage of amplification varied for each species which ranged from 37.5% (*V.trilobata*-2) to 100% (*V. mungo* var *silvestris* and *V.mungo*), while others showed more than 50% amplification. Apart from amplification, sufficient levels of polymorphism were also observed between cultivated blackgram and greengram and their progenitors *V. mungo* var *silvestris* and *V. radiata* var *sublobata* respectively. These findings suggest that microsatellite markers from adzuki bean could be used in genomic studies of other *Vigna* species and thus aid in their improvement.

### Key words

Adzuki bean, microsatellite markers, cross-amplification, Asian *Vigna* species

### Introduction

Grain legumes are the most important crops in the world next to cereals, since they provide one-third of the dietary protein for human consumption. These crops belong to the family Fabaceae. The *Vigna* is one of the most important genus in the Fabaceae and contains 100 to 150 species mainly found in Asia and Africa. This genus is divided into seven subgenera (Verdcourt, 1970; Marechel *et al.*, 1978). Two of these subgenera viz *Vigna* and *Ceratotropis* contain the most important cultivated species. The sub genus *Vigna* includes two cultivated species viz *V.unguiculata* and *V.subterranea* and is widely distributed in Africa. The subgenus *Ceratotropis* includes seven domesticated crops viz *V.radiata*, *V.mungo*, *V.aconitifolia*, *V.umbellata*, *V.angularis*, *V.trilobata* and *V.reflexo-pilosa* which are mainly distributed in Asia. Hence the species of subgenus *Ceratotropis* is called as Asian *Vigna* species.

Productivity of the Asian *Vigna* has been poor owing to various reasons such as lack of genetic variability, susceptibility to various pests and diseases, poor harvest index and absence of suitable ideotypes for different cropping systems. The transfer of agronomically important traits to the cultivars by conventional breeding methods is quite laborious and time consuming, and almost impractical when the trait is governed by polygenes.

In recent years, molecular marker technology has greatly accelerated the breeding programs for the improvement of various crops. Different molecular markers have been used for the molecular analysis of grain legumes (Gupta and Gopalakrishna, 2008). DNA based markers such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), and Simple Sequence Repeats (SSRs) or Microsatellites are used in molecular analysis of *Vigna* species.

Among the different DNA markers, microsatellite or simple sequence repeats (SSRs) are the markers of choice for various genetic studies due to their co-dominant nature, loci specificity and high reproducibility (Tautz and Renz, 1984). These markers have been used in genome mapping and genetic diversity studies in many crop plants. To date, few reports are available on isolation and development of microsatellite markers in some of the *Vigna* species. Therefore, the available SSR markers from other *Vigna* species should be validated for their transferability and utility in those species in which they are unavailable. In the present study, a set of 40 microsatellite primers pairs derived from adzuki bean (*Vigna angularis*) were used to assess the transferability and tested for their ability to amplify microsatellite loci in different species of Asian *Vigna*.

#### Materials and methods

The materials for this study included eleven different genotypes belonging to seven species of Asian *Vigna* such as *V. mungo var silvestris* (2), *V. mungo* (2), *V. umbellata* (1), *V. trilobata* (2), *V. aconitifolia* (2), *V. radiata var sublobata* (1) and *V. radiata* (1). The materials were obtained from Department of Pulses, Centre for Plant Breeding and Genetics, TNAU, Coimbatore. The young leaves of the 10 days old plants were collected and the DNA was extracted by CTAB (mini-prep) method for black gram lines. For other *Vigna* species seeds were used for the extraction. The extracted DNA was purified for the RNA contamination by RNAase treatment. The extracted and the purified DNA were then quantified and quality assessed by agarose gel-electrophoresis. Based on intensity of bands produced the DNA were diluted to appropriate concentration for use in molecular analysis.

#### SSR amplification

Forty SSR primer pairs derived from adzuki bean were tested for their amplification in different *Vigna* sp. SSR primers published for adzuki bean (Han *et al.*, 2005) were synthesized from Agile Lifescience Technologies India Pvt. Ltd. SSR amplification reaction were carried out in a volume of 15  $\mu$ l containing 50ng of genomic DNA, 0.5 units of Taq DNA polymerase, 0.1mM each dNTP, 3mM Primer (Forward and Reverse) and 1.5 $\mu$ l Taq buffer. The amplification was performed in PTC thermal cycler (AB PCR). Amplification conditions were, initial denaturation at 94 $^{\circ}$ C for 3 minutes followed by 35 cycles of denaturation at 94 $^{\circ}$ C for 45 seconds, annealing at 45 $^{\circ}$ -60 $^{\circ}$ C for 1 minute and extension 72 $^{\circ}$ C for 1 minute and a final extension at 72 $^{\circ}$ C for 10 minutes.

PCR amplified products were subjected to gel electrophoresis in a 3% agarose gel in 1X TBE at 90 V for 3 hours using gel electrophoresis unit. The ethidium bromide stained gels were visualized under UV and documented using Alpha Imager 1200.

#### Results and discussion

Forty SSR primer pairs derived from adzuki bean (*V. angularis*) were tested for their ability to support amplification in the seven Asian *Vigna* species. All the 40 SSR primer pairs (Table 1) showed cross species amplification and produced a total of 158 alleles in the genotypes studied. The percentage of amplification varied for each species which ranged from 37.5% to 100%. The highest percentage of amplification was observed in both the genotypes of *V. mungo var silvestris* and *V. mungo* (100%). The lowest percentage of amplification was observed in *V. trilobata* 2 (37.5%). The genotypes that showed more than 50% amplification were: *V. radiata var sublobata* – 65%; *V. radiata* and *V. umbellata* – 80%; *V. trilobata*, *V. aconitifolia* and TMV (Mb) 1 – 87.5% (Table 2). Cross amplification of adzukibeans SSR was studied in *Vigna radiata* (Sangiri *et al.* 2007) and in *Vigna mungo* (Chaitieng *et al.* 2006; Gupta *et al.* 2008; Gupta and Gopalakrishna 2009; Souframanien and Gopalakrishna 2009).

Of the forty SSR markers of adzukibeans studied seven markers *viz.* CEDG 10, CEDG 27, CEDG 68, CEDG 86, CEDG 92, CEDG AG 001 and CEDG 181 showed amplification in all the species (Fig. 1). Three markers CEDG 91, CEDG 173 and CEDG 225 produced monomorphic bands in all the genotypes while all other markers produced polymorphic bands.

Sufficient levels of polymorphism were also observed between cultivated blackgram and greengram and their progenitors *V. mungo var silvestris* and *V. radiata var sublobata*, respectively. Seventeen out of forty SSR primer pairs (42.5%) revealed polymorphism between cultivated blackgram and its wild progenitor. Similarly 13 (32.5%) primer pairs showed polymorphism between greengram and its wild progenitor. Two primer pairs showed polymorphism between cultivated mothbean (TMV (Mb) 1) and its wild type *V. aconitifolia*.

The ability to use the same microsatellite markers in different plant species depends on the extent of sequence conservation in the primer binding sites flanking the microsatellite loci and the stability of the sequence during evolution (Decroocq *et al.* 2003). Microsatellite primer pairs used in the current study originated from adzukibeans and all the 40 microsatellite primer pairs were able to amplify SSR loci across all the Asian *Vigna* genotypes studied. This indicates the conservation of microsatellite

sequences among the related *Vigna* species during evolution. The transferability of microsatellite markers across species increase their utility and may potentially decrease the development cost. The microsatellite markers conserved between the species also serve as a valuable tool for comparative mapping studies (Dirlewanger *et al.* 2004; Yu *et al.* 2004; Gupta *et al.* 2008).

Of the 40 microsatellite markers that showed amplification, 37 markers showed polymorphism. Seventeen markers out of forty SSR markers revealed polymorphism between cultivated blackgram and its wild progenitor. Similarly 13 markers showed polymorphism between green gram and its wild progenitor. Two markers showed polymorphism between cultivated mothbean and its wild type *V. aconitifolia*. These markers could be used in mapping of economically important traits.

Variation in allele size, number and null alleles were observed among the genotypes studied. Null alleles were observed for most of the markers in *Vigna trilobata*-2. If a locus is not amplified at all, then either small mutation occurred at these few nucleotides, or the locus is not present at all. However, the amplification of a particular locus in one genome with primers designed for another species depends not only on the evolutionary distance between the two species, but also on the rate of evolution of genomic sequence where the primer sequences are located (Souframanien and Gopalakrishna, 2009).

Three of the microsatellite markers showed monomorphic banding pattern in the present study. Similarity in allele size was observed between *Glycine max* and *G. claudenstina* despite sequence differences which is due to size homoplasy, in which SSR alleles had the same size but different underlying sequences (Peakall *et al.* 1998). So it will be interesting to study any such sequence variation that would exist in Asian *Vigna* in spite of small allele size.

Amplification of all SSR markers derived from adzuki bean indicates their potential to be used in genome mapping and linkage analysis in other related Asian *Vigna* species. Cross-species amplification provides an additional source of markers along with those markers which are being published for the corresponding crop. The transferability of microsatellite markers across species increase their utility and may potentially decrease the development cost. The microsatellite markers conserved between the species also serve as a valuable tool for comparative mapping studies. The present study suggests that microsatellite markers

from adzuki bean could be used in genomic studies such as construction of linkage maps of other *Vigna* species and thus aid in their improvement.

## References

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**Table 1. List of Microsatellite markers used in the study**

S.No	Primer	Forward and Reverse sequence	Annealing Temperature (°C)	Number of alleles
1	CEDG 08	AGGCGAGGTTTCGTTTCAAG GCCCATATTTTTACGCCAC	60	5
2	CEDG 10	TGGGCTACCAACTTTTCCTC TGAGCGACATCTTCAACACG	60	4
3	CEDG 27	ACTGGATGAGGGTTTAGTGCG CTGTCTTGTCTTGTGGGTTTCGTTT	65	3
4	CEDG 43	AGGATTGTGGTTGGTGCATG ACTATTTCCAACCTGCTGGG	60	7
5	CEDG 44	TCAGCAACCTTGCATTGCAG TTTCCCGTCACTCTTCTAGG	60	4
6	CEDG 48	TCTCTTCCTCTATGGCTTGG GCTCCTCTTTTTGCTGCATC	60	4
7	CEDG 50	GGCAGAATCGTACAAGTG GTCAGATTCTCGCTTGCATG	60	4
8	CEDG 56	TTCCATCTATAGGGGAAGGGAG GCTATGATGGAAGAGGGCATGG	60	7
9	CEDG 68	TCTCCATAGGAACCCCTGAAAG TGGGATCAGTGAATTCGCCAG	60	6
10	CEDG 86	GAGTTTACAACAGATGGGGCTAA AGGTCTTGATTGACTTTCTGGGT	60	4
11	CEDG 91	CTGGTGGAAACAAAGCAAAGAGT TGCGTCTTGGTGCAAAGAAGAAA	60	1
12	CEDG 92	TCTTTTGGTTGTAGCAGGATGAAC TACAAGTGATATGCAACGGTTAGG	60	4
13	CEDG 97	GTAAGCCGCATCCATAATTCCA TGCGAAAGAGCCGTTAGTAGAA	60	3
14	CEDG 111	TGGAAGTTTCCAAGAGGGTTTTTC TCTCACCACCTTTTACCTTCTCA	55	4
15	CEDG 115	GGCTCATTGTACCACTGGATAT ATGCCTCCTTTTCAAGGTGATTGT	60	6
16	CEDG 118	AACCCAACCAACCCTTGTGGTAAG GCTGGAATCATAATACCGCCTTGT	60	5
17	CEDG 127	GGTTAGCATCTGAGCTTCTTGCTC CTCCTCACTTGGTCTGAAACTC	60	3
18	CEDG 41	CCAGGCATCCATGATGACC GAAGTTGTTGGTAATGGTTGCCTC	50	5
19	CEDG 143	GATGAACTCGTCTCGCTCATCG CTGGACGCGTCTACTCAGAC	60	3
20	CEDG 149	GGCTGAAGGTGATGACAGAAG GGCACTGGTTTTCTAAGGTTGTTG	60	3

**Table 1. contd...**

S.No	Primer	Forward and Reverse sequence	Annealing Temperature (°C)	Number of alleles
21	CEDG 151	GTAGAACAGTTATGACACATG TGTTAACTTCGTTGGGTACAC	50	4
22	CEDGAG 001	CTCATCAGGGACATCCTCCC GATCGTGATCGATCCAACGGTC	60	3
23	CEDAAG 002	GCAGCAACGCACAGTTTCATGG GCAAACTTTTCACCGGTACGACC	60	3
24	CEDG 015	CCCGATGAACGCTAATGCTG CGCCAAAGGAAACGCAGAAC	60	3
25	CEDG 037	GAAGAAGAACCCTACCACAG CACCAAAAACGTTCCCTCAG	60	4
26	CEDG 071	GGTCCATTGAGACGGATCGAG TCCCACCTCAGCGGAATCC	60	4
27	CEDG 090	ATAAGTAGAAATTGGTTCAAATG GGTTCGTTAAAGTAACTTTTAAT	50	3
28	CEDG 117	GTACACTTCCACTAATCCAAAATT TGGTACCTTCCTTATCTGAAATTA	50	5
29	CEDG 173	GATAAGAGATGCATCACTC CTTCTCTCCATCACATCTG	45	1
30	CEDG174	GAGGGATCTCCAAAGTTCAACGG GAAGGCTCCGAAGTTGAAGGTTG	60	2
31	CEDG 181	CGCGAGATCTGGATCGTTGATC GCAGTACGGTAACGTCCTTGAC	60	6
32	CEDG 186	GGATGGGAGAGTAAGAAG GCATGGCATGATGACTTG	55	2
33	CEDG 225	GAGGAAGTGTGCAGCACC GTAGACTCTGCAGAGGGATG	60	1
34	CEDG244	GCATATAAGAAAAGCTTATCC CTCTTGGAGTGATTTGATC	55	4
35	CEDG 245	GATAGAGCTTAAACCCTC CTTTTGATGACAAATGCC	55	4
36	CEDG254	CGATGTCTCTTGCTTCAAGG GTGAAGGACTAGCCAAGTTTG	60	5
37	CEDG 271	GCACTAAAGTTAGACGTGGTTC CACTCCCCTGCCAAACAAGG	60	5
38	CEDG 282	CAGCAACAAGACATGGAGTG GGTGACCACTTAGACAGAC	60	5
39	CEDG 304	ACCACTTCATAATCCCTGAG GTTGCATGCTATATTTTGGTTTAC	55	5
40	CEDG 133	GCATACATAATGTGGTGAGATG GTCTCGTGCCTTTCACAC	60	4
<b>Total</b>				<b>158</b>

**Table 2. Details of markers amplified and their percentage of amplification for each genotype**

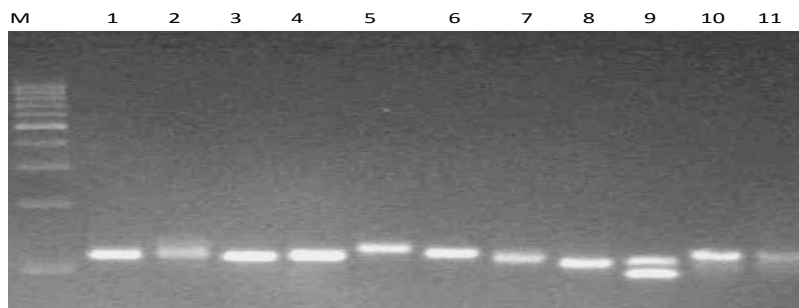
S.No	Species	Name of markers amplified	Percentage of amplification
1	<i>V. mungo var silvestris</i>	All markers* (40)	100
2	<i>V. mungo var silvestris</i>	All markers* (40)	100
3	<i>V. mungo</i> (VBN (Bg) 4)	All markers* (40)	100
4	<i>V. mungo</i> (VBN(Bg) 5)	All markers* (40)	100
5	<i>V.umbellata</i>	CEDG 08, CEDG 10, CEDG 27, CEDG 43, CEDG 48, CEDG 50, CEDG 68, CEDG 86, CEDG 91, CEDG 92, CEDG 97, CEDG 111, CEDG 115, CEDG 118, CEDG 127, CEDG 141, CEDG 143, CEDG 149, CEDG 151, CEDGAG 001, CEDG 015, CEDG 037, CEDG 071, CEDG 090, CEDG 173, CEDG174, CEDG 181, CEDG 186, CEDG 225, CEDG 282, CEDG 304, CEDG 133 (32)	80
6	<i>V. trilobata-1</i>	CEDG 08, CEDG 10, CEDG 27, CEDG 43, CEDG 44, CEDG 48, CEDG 50, CEDG 56, CEDG 68, CEDG 86, CEDG 91, CEDG 92, CEDG 97, CEDG 111, CEDG 115, CEDG 118, CEDG 127, CEDG 149, CEDG 151, CEDGAG 001, CEDG 015, CEDG 037, CEDG 071, CEDG 090, CEDG 117, CEDG 173, CEDG174, CEDG 181, CEDG 186, CEDG 225, CEDG244, CEDG 245, CEDG 271, CEDG 304, CEDG 133 (35)	87.5
7	<i>V. trilobata-2</i>	CEDG 08, CEDG 10, CEDG 27, CEDG 43, CEDG 44, CEDG 48, CEDG 56, CEDG 68, CEDG 86, CEDG 92, CEDG 115, CEDGAG 001, CEDG 181, CEDG254, CEDG 133 (15)	37.5
8	<i>V. aconitifolia</i>	CEDG 08, CEDG 10, CEDG 27, CEDG 43, CEDG 44, CEDG 48, CEDG 50, CEDG 56, CEDG 68, CEDG 86, CEDG 91, CEDG 92, CEDG 97, CEDG 111, CEDG 115, CEDG 118, CEDG 127, CEDG 141, CEDG 143, CEDG 149, CEDGAG 001, CEDAAG 002, CEDG 015, CEDG 037, CEDG 090, CEDG 117, CEDG 173, CEDG174, CEDG 181, CEDG 225, CEDG244, CEDG 271, CEDG 282, CEDG 304, CEDG 133 (35)	87.5

**Table 2. contd...**

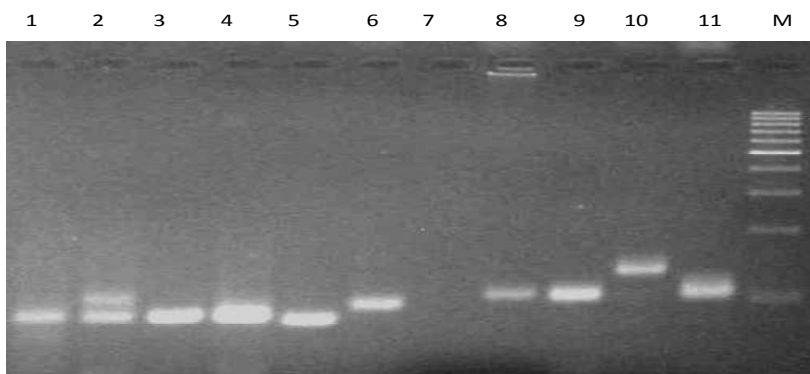
S.No	Species	Name of markers amplified	Percentage of amplification
9	<i>V. aconitifolia</i> (TMV (Mb) 1)	CEDG 10, CEDG 27, CEDG 43, CEDG 44, CEDG 48, CEDG 50, CEDG 68, CEDG 86, CEDG 92, CEDG 97, CEDG 111, CEDG 115, CEDG 118, CEDG 127, CEDG 141, CEDG 149, CEDG 151, CEDGAG 001, CEDAAG 002, CEDG 015, CEDG 037, CEDG 071, CEDG 090, CEDG 117, CEDG 173, CEDG174, CEDG 181, CEDG 186, CEDG 225, CEDG244, CEDG254, CEDG 271, CEDG 282, CEDG 304, CEDG 133 (35)	87.5
10	<i>V. radiata var sublobata</i>	CEDG 08, CEDG 10, CEDG 27, CEDG 50, CEDG 68, CEDG 86, CEDG 91, CEDG 92, CEDG 97, CEDG 115, CEDG 118, CEDG 127, CEDG 141, CEDG 149, CEDGAG 001, CEDAAG 002, CEDG 015, CEDG 71, CEDG 117, CEDG 173, CEDG174, CEDG 181, CEDG 271, CEDG 282, CEDG 304 (26)	65
11	<i>V. radiata</i> (Co (Gg) 912)	CEDG 08, CEDG 10, CEDG 27, CEDG 43, CEDG 44, CEDG 50, CEDG 56, CEDG 68, CEDG 86, CEDG 91, CEDG 92, CEDG 97, CEDG 111, CEDG 118, CEDG 127, CEDG 141, CEDG 149, CEDGAG 001, CEDAAG 002, CEDG 015, CEDG 037, CEDG 071, CEDG 117, CEDG 173, CEDG174, CEDG 181, CEDG 186, CEDG244, CEDG254, CEDG 271, CEDG 304, CEDG 133 (32)	80

\* Marker details refer Table 1.

a)



b)



**Fig. 1.** Polymorphism of the microsatellite amplified by adzuki bean SSR primer **a)** CEDG 68 and **b)** CEDG 304. Lane M: 100 bp marker. Lanes 1- 11 are the Asian *Vigna* genotypes listed in Table 2.