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Short Communication

Development of EST-derived microsatellite markers in mungbean [*Vigna radiata* (L.) Wilczek] and their transferability to other *Vigna* species

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Abstract

Twenty microsatellite markers were developed from mungbean [*Vigna radiata* (L.) Wilczek] EST sequences available in public database. Out of these, ten microsatellite markers (50%) were found to be polymorphic when screened on 22 genotypes. The number of alleles ranged from two to three with an average of 2.3 alleles per locus. Polymorphic information content of the microsatellite markers ranged from 0.09 to 0.59 with an average of 0.31. When these microsatellite markers were screened on eight other *Vigna* species, they showed high transferability (90%) to other *Vigna* species. These EST-derived microsatellite markers may be useful for linkage mapping, gene tagging and genetic diversity analysis in mungbean and other related *Vigna* species.

Key words : Microsatellite markers, polymorphism, mungbean, allele, *Vigna* species.

Mungbean [*Vigna radiata* (L.) Wilczek] also known as green gram belongs to the genus *Vigna* in the tribe Phaseoleae. It is an important grain legume which provides an important and inexpensive source of dietary protein to the people of Asia. Although a large mungbean germplasm is available, understanding the genetic variability and population structure in these collections is hampered by the lack of appropriate molecular markers. Microsatellite or simple sequence repeat (SSR) markers, which detect sequence variation in the hypervariable regions of tandem repeats of 2 to 6 base pairs, are a powerful tool for genome analysis because of their hypervariability, codominant nature, locus specificity and high reproducibility [1]. Although, microsatellite markers have been developed in mungbean [2-4], their number is still very less. Therefore, the current study was conducted with the aim of developing microsatellite markers in mungbean based on the EST sequences and to check their transferability to other *Vigna* species.

A total of 889 EST sequences of mungbean were downloaded from NCBI nucleotide database (http://www.ncbi.nlm.nih.gov) on 14th June 2010 and searched for the presence of dimeric to hexameric microsatellite repeats using SSR locator program [5]. For SSR identification, a minimum motif length was fixed at five repeats for di and trinucleotiode, four repeats for tetra, penta and hexanucleotides. PCR primer pairs were designed using the Primer3 software (http://frodo.wi.mit.edu/primer3/). Parameters for designing PCR primers were: optimum primer length 20 mer (range was 18-25 mer), optimum annealing temperature 60^{o}C (range was 55-62^{o}C), optimum GC content 50% (range was 40-80%) and rest of the parameters had the default value.

Total genomic DNA was extracted from 15 days old seedling using the modified CTAB Method. Microsatellite primer pairs were evaluated for the polymorphism on 22 mungbean genotypes. PCR reactions were performed in 25 µl volume containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 unit *Taq* DNA polymerase

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(Jonaki, Hyderabad, India), 50 ng template DNA, 20 ng each of forward and reverse primer. PCR amplifications were performed in an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) using the following thermal profile: 1 cycle of 95^{o}C for 2 min, followed by 39 cycles of 94^{o}C for 30 sec, 55-60^{o}C for 30 sec, 72^{o}C for 30 sec and a final extension of 72^{o}C for 7 min. About 5 µl of each reaction mixture was electrophoresed on a 6% denaturing polyacrylamide gel containing 7 M urea in 1X Tris-borate-EDTA (TBE) buffer. Electrophoresis

Table1. Details of 20 microsatellite markers developed from mungbean EST sequences

Marker name	Primer sequence (5'….3')	Repeat motif	Annealing temp. (^{o}C)	Gen Bank Acc. No.
VrSSR01	F:ACCTCTCTTCTCGACCCCAC R:GGGTTGCATGGTAAGACTGC	$(TC)_6$	55	AM696651
VrSSR03	F:AAGTTTTTGGTTGACCGCAG R:CCCTTGCATAGACAGGTGGT	$(TGG)_5$	60	AM696364
VrSSR04	F:CTGATTCAGCCTCAGGTTCC R:CACCGCTAAGATGCTCACAA	$(CT)_5$	60	AM696354
VrSSR05	F: GGGCCAGTGACAAATGAGAG R: TCTCGTTTGTGGTGGTTGAG	$(AGA)_6$	56	AM696320
VrSSR08	F:CGGTTCGTCCGTCTTACAAT R:TGGTTCTCGTCTTTCCAAGG	$(TTA)_6$	60	AM696004
VrSSR09	F:TCCATTTTAGCCAATGAGGC R:GTGTGAATGAGCAGAAGCCA	$(GAT)_5$	60	AM695991
VrSSR10	F:TTTTTCTTCCTGACCGATGG R:TCCATGGGCTATATGTGCAA	$(AT)_6$	58	AM695953
VrSSR11	F:TGATGAAGAAGGGGATCCTG R:TAGAAGGTGGCTGGGGAGTT	$(GAG)_5$	58	AM695924
VrSSR12	F:TCCCTCTCCCACCTTCTTCT R:GATGCAGATTGTTGCCTTGA	$(AT)_6$-$(AG)_8$	60	AM695923
VrSSR13	F:TTGATACGGCCACTTTCTCC R:CCATCAACGGTTTTTACGCT	$(TG)_7$	60	AM695922
VrSSR14	F:AGCGTCGTAGGGAGAAAATG R:GCTAGAGGGATGCTTCACCA	$(GT)_7$	58	AM695835
VrSSR15	F:CATGACCGAGAAGACAAGCA R:CCACAACAAATCCAAGAGCA	$(AT)_8$	58	AM695820
VrSSR16	F:TCTCCATCCCCATCTTCATC R:GGAGAGATCTGCGACCTTTG	$(TGTA)_4$	60	AM696191
VrSSR17	F:AACTTCGTCCTGCGCTTAAA R:AGCATGACCACACCAATCAA	$(TGTT)_5$	58	AM696190
VrSSR19	F:AAATGTTCGTGGAATCCTGC R:TTTCTTGTCCCTGAGTTCCAA	$(TA)_5$	58	AM696109
VrSSR20	F: CTGCCAAATCAAGAGGGGTA R: TCTCCTCGAGCATTGTGATG	$(GT)_5$	60	AM696097
VrSSR23	F:CCCTCCATGGTTATTAATTCT R:AAATGTTCTCCTCAGTGGTG	$(TC)_5$	55	AM696636
VrSSR27	F:AGGGAGCAGAATAAGAGG R:GTGAGAACTGAGAAGATTGG	$(GAA)_5$	58	AM695940
VrSSR28	F:CCAATTTACAAAGCCTAAAC R:CATTTTTGGTTACAGATTCA	$(TC)_5$	56	AM695782
VrSSR30	F: TCTACCTGGTTCCAGTCTTT R:GCCAATAGCAAATACAGACA	$(GA)_5$	58	AM696070

was performed at a constant power of 50 W for about 2 h in a Sequi-Gen GT Sequencing system (Bio-Rad, USA). Gels were stained using a modified silver staining protocol [6]. Allelic variation was calculated from the frequencies of genotypes at each locus as the polymorphic information content (PIC). PIC of each SSR marker was calculated by applying the formula of Anderson *et al*. [7]: PIC = $1 - \Sigma(P_{ij})^2$, where P_{ij} is the frequency of the j^{th} alleles for i^{th} loci. To check the transferability of microsatellite markers to other *Vigna* species, microsatellite markers were screened on other eight *Vigna* species, namely, *V. mungo*, *V. unguiculata*, *V. angularis*, *V. glabrescens*, *V. trilobata*, *V. umbellata* and *V. vexillata*. PCR products were separated on 2% agarose gel using 1X Tris-borate-EDTA (TBE) buffer, stained with ethidium bromide and photographed in a Gel documentation system (Syngene, UK).

Downloaded 889 mungbean ESTs were assembled into 799 singlets and 14 contigs using the CAP3 program [8]. Hence, a total of 813 unique mungbean EST sequences were subjected to SSR mining and 33 EST sequences (4%) with microsatellite repeats were identified. The most frequent repeat type found in mungbean ESTs were dinucleotides (72.7%) followed by trinucleotides (21.2%) and tetranucleotides (6.1%). The copy number varied from 5 to 17 for dinucleotides, 5 to 6 for trinucleotides and 4 to 5 for tetranucleotides repeat motifs. The most abundant repeat class was GA (41.6 %) among dinucleotides and TAA (28.5%) among trinucleotide. GA motif has been observed as the most predominant dinucleotide repeat in the ESTs of many other plants including cowpea [6] and soybean [9].

Out of the 33 ESTs carrying SSR, only 25 EST sequences were found to be suitable for primer designing. A total of 20 primer pairs produced clear amplification pattern (Table 1) and were screened on 22 mungbean genotypes to assess the polymorphism level. Ten microsatellite markers (50%) were found to be polymorphic. Each polymorphic microsatellite locus detected two to three alleles with an average of 2.3 alleles. PIC of the microsatellite markers ranged from 0.09 to 0.59 with an average of 0.31. The polymorphism level of the microsatellite loci in the present study was

Table2. Cross-species amplification of mungbean microsatellite markers in other eight *Vigna* species

Locus	*mungo*	*vexillata*	*glabrescens*	*aconitifolia*	*trilobata*	*angularis*	*umbellata*	*unguiculata*
VrSSR01	+	-	+	+	+	+	+	-
VrSSR03	+	+	+	+	+	+	+	+
VrSSR04	+	+	+	+	+	+	+	+
VrSSR05	+	+	+	+	-	-	-	-
VrSSR08	+	+	+	+	+	+	+	-
VrSSR09	+	+	+	+	+	-	-	+
VrSSR10	+	+	+	+	+	+	+	+
VrSSR11	+	+	+	+	+	+	+	+
VrSSR12	+	+	+	+	+	+	+	+
VrSSR13	+	+	+	+	+	+	+	+
VrSSR14	+	-	+	+	+	+	+	-
VrSSR15	+	+	+	+	+	+	+	+
VrSSR16	+	+	+	+	+	+	+	+
VrSSR17	+	+	+	+	+	+	+	+
VrSSR19	+	+	+	+	+	+	+	+
VrSSR20	-	-	-	-	-	-	-	-
VrSSR23	+	+	+	+	+	+	+	+
VrSSR27	+	+	+	+	+	+	+	+
VrSSR28	-	-	-	-	-	-	-	-
VrSSR30	+	+	+	+	+	+	+	+

similar to those previously reported in mungbean [3, 4].

To explore cross-species amplification, all the 20 microsatellite markers were evaluated in other eight *Vigna* species. A total of 18 microsatellite markers (90%) were found to be transferable with thirteen markers (65%) being transferable to all eight *Vigna* species, one marker (5%) to seven *Vigna* species, three markers (15%) to six *Vigna* species and one marker (5%) to only four *Vigna* species (Table 2). Two microsatellite markers (VrSSR20 and VrSSR28) did not amplify in any of the *Vigna* species except mungbean and were, therefore, mungbean specific. Most of the microsatellite markers produced length variation between the species. The transferability of microsatellite markers across species will increase their utility and potentially decrease the marker development cost.

The present study has increased the limited pool of mungbean microsatellite markers and their application will certainly expedite the future work of mungbean breeding. These microsatellite markers may be useful for genome mapping, genetic diversity and marker-assisted selection in mungbean and other related *Vigna* species.

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