Molecular Ecology Notes (2002) **2**, 96–98

PRIMER NOTE

Isolation of microsatellite markers in mungbean, *Vigna radiata*

S. V. KUMAR,* S. G. TAN,* S. C. QUAH‡ and K. YUSOFF†
*Department of Biology and †Department of Biochemistry and Microbiology, Universiti Putra Malaysia, 43400 Serdang, Malaysia, ‡School of Engineering and Science, Monash University Malaysia, Bandar Sunway, 46150 Petaling Jaya, Malaysia

Abstract

A simple and rapid method for isolating microsatellite loci in mungbean, *Vigna radiata*, based on the 5′-anchored polymerase chain reaction technique revealed 23 microsatellite loci and six cryptically simple sequence repeats. We report on the characterization of seven polymorphic microsatellite loci in *V. radiata*. The number of alleles per locus ranged from 2 to 5 while the observed heterozygosity ranged from 0 to 0.9048. These markers should prove useful as tools for detecting genetic variation in mungbean varieties for germplasm management and crossbreeding purposes.

Keywords: microsatellites, mungbean, polymorphism, *Vigna*

Received 4 September 2001; revision accepted 22 October 2001

The mungbean (Subgenus *Ceratotropis*) is an important food crop in South and Southeast Asia where 80% of the world's mungbean is grown. Although an important crop, little is known about its genetic structure. Despite the availability of restriction fragment length polymorphism and random amplified polymorphic DNA markers, there is hardly any information regarding microsatellite markers in the mungbean. Here we present seven *Vigna radiata* microsatellite loci and also evaluate their usefulness in closely related *Vigna* species.

Mungbean seeds were obtained from the Asian Vegetable Research and Development Center (AVRDC). Genomic DNA from a single seed was isolated using a plant DNA extraction kit (Clontech, USA) following the protocol of the manufacturer. The microsatellites were isolated using a library-enrichment protocol based on the procedure of Fisher *et al*. (1996). A single degenerate primer, LR7 (5′-KKVRVRVAGAGAGAGAGAGAGAGAG-3′) where K = G/T, V = G/C/A and R = G/A was used to amplify the mungbean genome. This 5′-anchored primer was specifically designed to include a longer tandem repeat motif (n = 10) to allow capturing of longer repeat microsatellites similar to Brachet *et al*. (1999).

Correspondence: Prof. Dr S. G. Tan. Fax: 603–86567454; E-mail: sgtan@fsas.upm.edu.my

A polymerase chain reaction (PCR) was carried out in a total volume of 10 µL containing 30 ng of genomic DNA, 2.5 mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton-X 100, 0.5 mM each of dATP, dCTP, dGTP and dTTP, 15 pmol of primer LR7 and 2.5 U of *Taq* DNA polymerase (Promega, USA). PCR amplification was performed as follows: predenaturation 96 °C (3 min), then followed by 40 cycles of 96 °C (10 s), 57 °C (10 s) and 72 °C (30 s). A final extension step of 72 °C for 10 min was included. The PCR products were then cloned into the TOPO TA cloning vector (Invitogen, USA). The recombinant clones obtained were then screened by hybridization to probes for (GA)n dinucleotide repeats. Eight clones from a total of 139 positive clones obtained were randomly selected for sequencing. Plasmid extraction was performed according to Sambrook *et al*. (1989) and the clones were sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) on the ABI PRISM 377 DNA sequencer. Primers were then designed to amplify regions containing the dinucleotide repeats using PRIMER3 (Rozen & Skaletsky 1997).

These primers were then used to screen for polymorphisms in mungbean. PCR amplifications were performed in a 10-µL final reaction volume containing 30 ng of genomic DNA, 1 mM of $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl and 0.1% Triton-X 100, 0.2 mM each of dNTPs, 0.15 µM of each forward and reverse primers, and 0.4 U *Taq* DNA polymerase

Table 1 Primer sequence and characteristics of seven *Vigna radiata* microsatellite loci. Number of alleles and observed and expected heterozygosities were calculated for 45 individuals. T_a, annealing temperature; H_O, observed heterozygosity; H_E, expected heterozygosity

Locus	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	Size of cloned allele (bp)	No. of alleles	H_O	H_E	GenBank Accession no.
LR7319B	$(TG)_5\ldots(CT)_7$	ctgctttttggggatttcag cacgcaaacagaaagcagag	47	257	3	0.0233	0.0679	AF322394
LR7322B	$(TC)_{10}$	tcagtcagtgtcgatagcatagc gacacagagagagagagagag	47	171	4	0.0444	0.5195	AF322395
LR7323A	$(GA)_{13}$	tgacggagagagagagagagag tgcttccttttgtctgagttagaa	47	201	5	0.5122	0.7701	AF322396
LR7323B	$(CT)_{10}$	gctatgctatcgacactgactga gcgcaaagagagagagagaga	50	285	4	0.8333	0.7327	AF322396
LR7315A	$(AG)_{12}$	gtagcgcagagagagagagag caaaacggctcattcagctt	47	204	3	0.9048	0.6120	AF322883
LR738A	$(AG)_{11}\ldots(GA)_6$	cgcaaagagagagagagag cccccatctgaaagaaagag	47	199	4	0.1000	0.6428	AF320012
LR733B	$(TC)_7$	gagagcaacgattgaaaaatg gttcgtagttacattgtccc	47	159	2	0	0.0868	AF322880

Table 2 Cross-species amplification using primers designed for *Vigna radiata*. One sample of each species was tested. Assays producing amplifications are indicated by ' +'; no amplification is indicated by '–'

	Locus						
Species	LR7319B	LR7322B	LR7323A	LR7323B	LR7315A	LR738A	LR733B
V. nakashimae	+	–	–	+	+	+	+
V. riukiuensis	+	–	–	+	+	+	+
V. glabrescens	–	+	–	+	+	+	+
V. trinervia	+	+	–	+	+	+	+
V. reflexo-pilosa	+	+	–	+	–	+	+
V. minima	+	+	+	–	+	+	+
V. umbellata	+	+	–	–	+	+	+
V. radiata var. *sublobata*	+	+	+	+	+	+	+

(Promega, USA). The loci were amplified using a PTC-200 Thermal Cycler (MJ Research Inc., USA). The PCR conditions were as follows: a predenaturation for 2 min at 94 °C followed by 40 cycles of 10 s denaturation at 94 °C, 10 s at an optimum annealing temperature, 30 s extension at 68 °C, and concluded with a 5-min final extension at 68 °C. This amplification protocol is similar to that used by Yu *et al*. (1999) to amplify the common bean (*Phaseolus vulgaris*) genome. The PCR products were ran on a 4% MetaPhor gel (BMA, USA) and visualized over UV through ethidium bromide staining. The population data were analysed using POPGENE (version 1.32) computer software (Yeh & Boyle 1997).

The eight clones sequenced revealed a total of 23 microsatellites and six cryptically simple sequence repeats (data not shown). Each sequenced clone contained terminal microsatellites at the 5′ and 3′ ends. Primers were designed for seven microsatellite loci. Between 2 and 5 alleles were detected at each locus. The observed heterozygosity ranged from 0 to 0.9048 (Table 1). The primer pairs were also used to amplify several other *Vigna* species using conditions optimized for *V. radiata* (Table 2). The results showed that they were successful in amplifying microsatellite loci in some if not all of the *Vigna* species screened here. However, it may be possible to improve the success of amplification in other species by optimizing the PCR conditions.

The primers described in this paper can provide useful markers to investigate levels of genetic variation in mungbean varieties with respect to germplasm management and genetic studies in wild plant populations for breeding and cross-breeding purposes.

Acknowledgements

This work was funded mainly by IRPA grant 09-02-04-0004: Ministry of Science, Technology and the Environment,

Malaysia. The authors would like to thank Monash University Malaysia for some financial support.

References

Brachet S, Jubier MF, Richard M, Jung-Muller B, Frascaria-Lacoste N (1999) Rapid identification of microsatellite loci using 5′-anchored PCR in the common ash *Fraxinus excelsior*. *Molecular Ecology*, **8**, 160–163.

Fisher PJ, Gardner RC, Richardson TE (1996) Single locus microsatellites isolated using 5′-anchored PCR. *Nucleic Acids Research*, **24**, 4369–4371.

Rozen S, Skaletsky HJ (1997) *Primer 3*. Code available at http://www-genome.wi.mit.edu/genome_software/other/primer3.html.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Yeh FC, Boyle TJB (1997) Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belgian Journal of Botany*, **129**, 157.

Yu K, Park SJ, Poysa V (1999) Abundance and variation of microsatellite DNA sequences in beans (*Phaseolus and Vigna*). *Genome*, **42**, 27–34.