Characterization of new microsatellite markers in mung bean, Vigna radiata (L.)

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PRIMER NOTE

Characterization of new microsatellite markers in mung bean, *Vigna radiata* (L.)

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Abstract

The present work reports the isolation and characterization of new polymorphic microsatellites in mung bean (*Vigna radiata* L.). Of 93 designed primer pairs, seven were found to amplify polymorphic microsatellite loci, which were then characterized using 34 mung bean accessions. The number of alleles ranged from two to five alleles per locus with an average of three alleles. Observed and expected heterozygosity values ranged from 0 to 0.088 and from 0.275 to 0.683, respectively. All seven loci showed significant deviations from Hardy–Weinberg equilibrium, whereas only one pairwise combination (GBssr-MB77 and GBssr-MB91) exhibited significant departure from linkage disequilibrium. These newly developed markers are currently being utilized for diversity assessment within the mung bean germplasm collection of the Korean Gene Bank.

Keywords: genetic diversity, microsatellite, mung bean (*Vigna radiata*)

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Mung bean (*Vigna radiata* L.), also known as 'green gram', belongs to the family *Fabaceae* and is an important pulse crop in many Asian countries where it is widely cultivated for its edible seeds and pods. Like soybean sprouts (Bae *et al*. 2004; Lee & Kim 2004), mung bean sprouts are important traditional food in Korea and are excellent source of protein, calcium and vitamin C. It has been commonly used as a vegetable accompaniment to a meal. Mung bean seed flour is also used for making soup and pizza-like 'Bindaeddeog' in Korea. Despite its usefulness, limited research efforts have been made to understand its genome structure and genetic diversity as compared to other legumes (Humphry *et al*. 2002). Accurate identification of genetic diversity within germplasm collections is essential for establishing and managing an appropriate breeding program. The Rural Development Administration (RDA) Gene Bank of Korea has a mung bean germplasm collection of about 1400 accessions. However, understanding the degree of genetic variability within this collection has been greatly limited due to a lack of appropriate molecular markers, which represents a great challenge for successful improvement of this crop. Although few reports are available on the use of molecular markers such as random amplified polymorphic DNA (RAPD) and microsatellite for estimation of genetic variability of mung bean cultivars (Lakhanpaul *et al*. 2000; Kumar *et al*. 2002; Betal *et al*. 2004) but their numbers are very limited. The present work reports the isolation and characterization of new polymorphic microsatellites in mung bean.

A modified biotin–streptavidin capture method (Dixit *et al*. 2005) was used to construct a microsatellite-enriched library. Briefly, total genomic DNA of mung bean was digested with blunt end-producing restriction enzymes *Eco*RV, *Dra*I, *Sma*I, *Pvu*I, *Alu*I, *Hae*III and *Rsa*I in separate reactions. The digested DNA samples from each reaction were pooled together and size-fractionated on 1.4% agarose gel. Fragments ranging from 300 bp to 1500 bp were eluted from the gel followed by purification with a gel extraction kit (QIAGEN). Approximately 1 μg of DNA fragments was ligated with 1 μg of double-stranded adaptor molecules AP11/AP12. The adaptor DNA was prepared by mixing equal molar amounts of oligonucleotides AP-11 (5′-CTCTTGCTTAGATCTGGACTA-3′) and AP-12

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Table 1 Characteristics of seven polymorphic loci analysed among 34 accessions of mung bean

Marker name	Primer sequence (5′–3′)	Repeat motif	Fluorescent dye*	T_a (°C)	Size range (bp)	N_A	H_O	H_E	GenBank Acc. no.
GBssr-MB7	F: CTTGCTTGCGAGGATGAC R: TCCAGTGCAGCAGATTGA	$(CT)_7$	FAM	55	288–356	5	0.088	0.552	DQ345299
GBssr-MB13	F: GCAGCAACAACAGCAACA R: GCAGGTTTTGTGGCTCAG	$(GAG)_4$	FAM	55	171–200	2	0.029	0.275	DQ345300
GBssr-MB14	F: TGGAATTTGGAAGGAAGGA R: GATGCAGGTGTTTGGGAG	$(AAGA)_4$	HEX	55	269–275	2	0.029	0.507	DQ345301
GBssr-MB17	F: ACCTGCAAGTTGGCAAGA R: TATGTGCACGCATGGAAG	$(AG)_{10}$	HEX	50	164–166	2	0.000	0.058	DQ345302
GBssr-MB77	F: GGAGAGGAAGGAACAGGG R: GGCAGAGCATAACATGGC	$(GTT)_5(GA)_5A(AG)_6$	HEX	55	319–331	3	0.000	0.536	DQ345303
GBssr-MB87	F: TCCCTTGTGGGAGATCCT R: CTTTGCCACACTCCTTGC	$(CAA)_4$	FAM	55	200–293	2	0.029	0.497	DQ345304
GBssr-MB91	F: GAGGCCAATCCCATAACTTT R: AGCACCACATCAGAGATTCC	$(AG)_{34}(GA)_{14}$	FAM	54	171–183	5	0.029	0.683	DQ345305
Mean						3	0.029	0.444	

T_a, annealing temperature; N_A, number of allele; H_O, observed heterozygosity; H_E, expected heterozygosity; *dye linked with forward primer in each case.

(5′-TAGTCCAGATCTAAGCAAGAGCACA-3′), heating to 94 °C, and cooling to 25 °C over a period of 4–5 h. The adaptor-ligated DNA was hybridized with a mixture of long (40–45 nucleotides) biotin-labelled SSR probes [$(GA)_{20}$, $(AGC)_{15}$, $(GGC)_{15}$, $(AAG)_{15}$, $(AAC)_{15}$, $(AGG)_{15}$]. The hybridized DNA fragments were captured with streptavidin-coated magnetic beads (Promega). After stringent washing, the captured DNA fragments were eluted in 50 µL of distilled water. Final elutes were amplified with AP11 primer and cloned into pGEM-T easy vector (Promega). The recombinant clones were selected on LB media containing ampicillin, X-gal and IPTG. Plasmid DNA from randomly selected white clones was isolated using QIAprep Spin Miniprep Kit (QIAGEN) and sequenced using an ABI PRISM 3100 DNA sequencer with a BigDye terminator kit (Applied Biosystems).

SSR identification within cloned sequences and primer design were carried out using the SSR MANAGER program (Kim 2004). A total of 671 clones were randomly picked from primary transformation plates and sequenced. Sequence analysis revealed the presence of 20 redundant clones. Of the remaining 651 unique clones, 215 clones were found to contain microsatellite sequences. Primer pairs could be designed only from 93 clones due to a lack of sufficient flanking sequences in the remaining clones. These primer pairs were evaluated for polymorphisms in a panel of 10 mung bean accessions using a procedure described earlier (Dixit *et al*. 2005). Of these 93 primer pairs, seven produced reproducible polymorphic bands. The variability at the loci defined by these seven primer pairs were further characterized in 34 accessions of mung bean. The M13-tail polymerase chain reaction (PCR) method of Schuelke (2000) was used to measure the size of PCR products, as described earlier (Dixit *et al*. 2005). Microsatellite alleles were resolved on an ABI PRISM 3100 DNA sequencer (Applied Biosystems) using GENESCAN 3.7 software and precisely sized using GeneScan-500 ROX (6-carbon-X-rhodamine) molecular size standards (35 bp-500 bp) with GENOTYPER 3.7 software (Applied Biosystems).

The number of alleles per microsatellite locus ranged from two to five with an average of three alleles (Table 1). The values for observed and expected heterozygosities ranged from 0 to 0.088 and from 0.275 to 0.68376, respectively. Tests for Hardy–Weinberg equilibrium (HWE) and pairwise linkage disequilibrium (LD) of seven polymorphic loci were performed with the help of POWERMARKER version 3.23 software (Liu & Muse 2005). All seven loci showed significant deviations from HWE ($P < 0.05$), whereas only one pairwise combination (GBssr-MB77 and GBssr-MB91) exhibited significant deviation from LD. Our data indicated that a narrow genetic base existed among the mung bean accessions used in this study. The narrow genetic base could be one of the reasons for the very low yield of polymorphic markers in this study. These newly developed microsatellite markers are currently being utilized for assessment of genetic diversity within the mung bean germplasm collection of the RDA Gene Bank of Korea.

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References

Bae K-G, Nam S-W, Kim K-N, Hwang Y-H (2004) Growth of soybean sprouts and concentration of CO_2 produced in culture vessel affected by watering methods. *Korean Journal of Crop Science*, **49**, 167–171.

Betal S, Chowdhury PR, Kundu S, Raychaudhuri SS (2004) Estimation of genetic variability of *Vigna radiata* cultivars by RAPD Analysis. *Biologia Plantarum*, **48**, 205–209.

Dixit A, Jin M-H, Chung J-W *et al.* (2005) Development of polymorphic microsatellite markers in sesame (*Sesamum indicum* L.). *Molecular Ecology Notes*, **5**, 736–738.

Humphry ME, Konduri V, Lambrides CJ *et al.* (2002) Development of a mung bean (*Vigna radiata*) RFLP linkage map and its comparison with lablab (*Lablab purpureus*) reveals a high level of colinearity between two genomes. *Theoretical and Applied Genetics*, **105**, 160–166.

Kim KW (2004) Developing one step program (SSR MANAGER) for rapid identification of clones with SSRs and primer designing. Thesis (MSc), Department of Plant Science, The Graduate School, Seoul National University, Korea.

Kumar SV, Tan G, Quah SC, Yusoff K (2002) Isolation of microsatellite markers in mung bean, *Vigna radiata*. *Molecular Ecology Notes*, **2**, 96–98.

Lakhanpaul S, Chadha S, Bhat KV (2000) Random amplified polymorphic DNA (RAPD) analysis in Indian mung bean [*Vigna radiata* (L.) Wilczek] cultivars. *Genetica*, **109**, 227–234.

Lee Y-S, Kim Y-H (2004) Changes in postharvest respiration, growth, and vitamin C content of soybean sprouts under different storage temperature conditions. *Korean Journal of Crop Science*, **49**, 410–414.

Liu K, Muse SV (2005) POWERMARKER: an integrated analysis environment for genetic marker analysis. *Bioinformatics*, **21** (9), 2128–2129.

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology*, **18**, 233–234.