Indian J. Genet., 79(1): 66-76 (2019)
DOI: https://doi.org/10.31742/IJGPB.79.1.9

Development and characterization of STMS molecular markers and genetic diversity analysis of mungbean [*Vigna radiata* (L.) Wilczek]

Sujan S. Bimal*, S. P. Chavan, A. B. Gaikwad and K. V. Bhat

Division of Plant Genomic Resources, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi 110 012

(Received: September 2018; Revised: January 2019; Accepted: February 2019)

Abstract

Mung bean is an important crop in Asia because of its high protein content and other economic uses. However, because of the unavailability of polymorphic DNA markers, genomic research of mung bean is lacking. In this study, we developed and characterised simple sequence repeat (SSR) molecular markers by screening SSR-enriched partial genomic libraries with SSR probes and used them to analyse the genetic diversity of mung bean. Thus, we isolated, cloned, sequenced a genomic library that contained microsatellite loci from the mung bean variety 'MCV-1'. The polymorphisms of microsatellite loci were evaluated using the unweighted pair group method of arithmetic means, and MDS cluster analysis showed genetic relationships in a panel of 96 mung bean core collection genotypes. Genetic diversity analysis results showed contrasted levels of variability within cultivated and wild accessions. A total of 98 alleles were detected using 19 polymorphic markers, with an average of 4.9 alleles per locus, whereas observed heterozygosity ranged from 0.1 to 0.5, with a mean of 0.42 per locus. The number of alleles and the high level of polymorphism make these new markers useful for gene tagging, diversity analyses and marker assisted selection in mung bean.

Keywords: Microsatellite marker, molecular characterisation, polymorphism, genetic variation

Introduction

Mung bean [*Vigna radiata* (L.) Wilczek] is an important crop in developing economies from Asia, Africa, and Latin America, where its dried seeds, fresh green pods, and leaves are consumed (Tangphatsornruang et al. 2009). Because it contains high amounts of proteins, vitamins, and minerals, mung bean is a beneficial crop for human and animal consumption (Dahiya et al. 2015). Several biotic and abiotic stresses, such as diseases, insects, drought, high temperature, salinity, and heavy metals, limit the yield of mung bean. Improved mung bean cultivars have a narrow genetic base that limits their yield potential and are poorly adapted to varying growth conditions in different agroecological zones (Pandey et al. 2007). Plant genetic resources, such as landraces, are a source of several novel genes and alleles that can be readily used in crop improvement programmes (Bisht et al. 2004). Therefore, the genetic potential of landraces should be exploited. Characterisation and genetic diversity analysis are prerequisites that can provide relevant information regarding germplasm, including the range of genetic variation. Molecular markers in mung bean should be used for characterisation, diversity profiling, and evolutionary history tracking.

Among different molecular markers, microsatellites are the most hypervariable, reproducible and cost effective. Because these markers are abundantly present in plant genomes, they are largely used in studies on population genetics, molecular characterisation, and genomic applications. Simple sequence repeats (SSRs) can potentially be applied in mung bean for evolutionary studies, germplasm characterisation, genetic diversity profiling, gene(s) and quantitative trait loci (QTL) mapping, map-based cloning, and marker-assisted breeding programmes. SSRs have tandemly repeated motifs of 1-6 bases and are present in both coding and noncoding regions and are characterised by a high degree of length polymorphism (Abdul-Muneer 2014). Di-, tri-, and tetra-nucleotide microsatellites are more widely used for

*Corresponding author's e-mail: Sujan.Bimal.2010@live.rhul.ac.uk
Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by www.isgpb.org; indianjournals.com

STMS analysis because they give a clear banding pattern (Hearne et al. 1992). A limited number of SSR markers were reported earlier for mung bean (Kumar et al. 2002a,b; Gwag et al. 2006; Somta et al. 2008; Seehalak et al. 2009). Hence, this study was made with an aim to isolate and characterise microsatellite loci and investigate their evolution in mung bean; and to demonstrate their polymorpohism in a core set of mung bean diversity.

Materials and methods

Mungbean [*V. radiata* (L.) Wilczek]; landrace labelled MCV-1 was used for the marker development and construction of enriched genomic libraries. A set of mung bean core collection genotypes (Table 1) were used in for characterization of the identified SSRs. Genomic DNA was extracted from young fresh leaves of 7-day-old seedlings by using the microprep procedure described by Fulton et al. (1995) and the cTAB protocol adapted by Shaghai-Maroof et al. (1984). The DNA concentration was quantified by comparing it to a DNA standard marker (Sigma) after ethidium bromide staining on 0.8% agarose gel. The concentration was adjusted to 100 ng/µL, and 5 µL of DNA was used for further digestion.

Shearing of genomic DNA

A genomic library was constructed using genomic DNA sheared by using the nebuliser method, followed by size selection through electrophoresis on 1% agarose gel. Sheared DNA was treated with exonuclease and T4 DNA polymerase to create blunt ends. DNA fragments, ranging from 400 to 750 bp in size, were isolated from agarose gels by using Geneclean II (Bio 101, La Jolla, California), and purified DNA fragments were ligated into the *Sma*I site of pBluescript (Stratagene, La, Jolla, California). The ligation mixture was diluted 10-fold with dH2O, and 5 ng/µL of DNA was used for PCR amplification with 10 mM of the *Tru*9I primer corresponding to the sequence of the *Tru*9I adapter in 50 µL of PCR reaction mixture containing 1× PCR buffer, 10 mM dNTP, 1.5 mM $MgCl_2$, 10 mM *Tru*9I primer, and 1 unit of *Taq* polymerase. The following PCR amplification conditions were used: 94°C for 30 s, 56°C for 1 min, and 72°C for 1 min for a total of 20 cycles.

Table 1. The set of mung bean core collection accessions and their source, used for SSR characterization

	Source	Accession name/number
Indian	Andhra Pradesh	IC-253932, IC-25789, IC-25133, IC-25997
	Bihar	IC-735336, PLM 11, PLM-16369-5, PLM 37-A, PLM 88-1, PLM 119, PLM-213
	Gujarat	IC-10184, IC-10451, IC-9923, IC-11895, IC-11379, 12434, IC-11488, IC-39486, PLM 748 A, PLM 957, PLM 953, PLM 962, PLM-973
	Haryana	PLM 427
	Himachal Pradesh	PLM 346, PLM-350
	India misc.	ET-52181-1, ET-52188-1, ET 52189, ET 52190 (BASANTI)
	Madhya Pradesh	IC 22427, IC-22463, IC-2056-2, PLM 528
	Maharashtra	IC-9886-32, STV-2469, STV-2663, STV 2669, STV-2743, STV 2761, STV 2762 STV 2766, STV-3765, TAP 7, WGG-37
	Punjab	IC-0114, IC-557, IC-6155 IC-78465, PLM 391-A, PLM-399, PLM 416, PLM-469 IPU-99228, IPU-99229, IPU-99-239, IPU-98126, IPU-99-16, V-5, PLU-59
	Rajasthan	IC-8961-3A, IC-8961-1, IC 8961-5-A, IC-618-5, IC-8917
	Uttar Pradesh	PLM 1057
Exotic	Bangladesh	ET 52185
	Europe	EC-5478, EC 8837-2, EC-130722, EC-245952, EC-251810, EC-16569-5, EC- 259174
	Pacific Islands	EC-261798-8
	Pakistan	EC-27853, EC-450-444, EC-450447, EC-450446, EC-450-450, ET 52193, ET 52196 ET-52197, ET-52198, ET-52197-A, ET-52199, ET-52200, ET-52201
	Taiwan	EC-251557, EC-10732, EC-13079-2, EC-13079-2

Capture of specific repetitive sequences with biotinylated oligonucleotides

Biotinylated SSR oligonucleotides with the sequence of $(GC)_{15}$, $(CT)_{15}$ (0.5 ng each) were used to capture DNA fragments containing repetitive sequences. Streptavidin-coated magnetic beads M280 (10 ng/µL; Dynal, Norway) were added to capture biotinylated oligonucleotide probes, which complementarily bound to SSR-containing DNA fragments. The bound complex was drawn to magnetic separation stands (Promega Inc., USA). Then, DNA fragments were washed seven times and cleaned using the Nucleo Spin® Extract (Macherey-Nagel) spin column. The elute was used for PCR amplification with 10 mM of the *Tru* 9I primer. The 50-µL PCR reaction mixture contained 1× PCR buffer, 10 mM dNTP, 1.5 mM $MgCl_2$, 10 mM *Tru*9I primer, and 1 unit of *Taq* polymerase. The following PCR conditions were used: 94°C for 30 s, 56°C for 1 min, and 72°C for 1 min for total of 35 cycles. Finally, PCR amplified products were used for constructing the DNA library.

DNA library construction

Enriched DNA fragments were ligated to the pGEM-T easy vector (Promega, USA) and transformed into competent *Escherichia coli* DH5α cells through electroporation. Transformed cells were grown on LB agar plates containing 0.1 g/mL of ampicillin with 0.2 M X-Gal and 0.1 M IPTG. Transformed clones were selected through blue/white colony screening.

DNA library screening through PCR amplification with repeat probes

White transformed colonies were picked using a toothpick and used as an inoculum in a single well of 96-well GM plates (for storage) and 96-well deep microtitre plates containing 2× LB medium (for plasmid isolation). The plates were covered with air pore tape sheets for proper aeration and kept overnight in an incubator cum shaker at 37°C at 300 rpm for culture growth. After overnight incubation, the plates were taken out from the incubator cum shaker for plasmid DNA extraction. Individual clones were cultured in 3 mL of LB liquid medium and incubated overnight at 37°C for plasmid DNA extraction by using an alkaline lysis (Sambrook et al. 1989) Millipore kit. Pooling of isolated plasmid DNA was performed to screen hybridised primers (repeat probes), namely, (GA)20, (CA)20, (GAA)15, (AGA)15, (ACA)15, (CAT)15, and (GATA)20, and one universal primer OG-1 repeat at all levels of pooling (primary, secondary, and tertiary screening) through PCR amplification with 10 mM of the *Tru* 9I primer. The 50-µL PCR reaction mixture contained 1× PCR buffer, 10 mM dNTP, 1.5 mM $MgCl_2$, 10 mM *Tru*9I primer, and 1 unit of *Taq* polymerase. The following PCR conditions were used: 94°C for 30 s, 56°C for 1 min, and 72°C for 1 min for a total of 35 cycles.

DNA sequencing of positive clones

DNA of SSR-containing clones was sent to the sequencing facility, Chromous Pvt. Ltd., India. Sequencing was performed using the M13F universal primer and Big Dye terminator chemistry. The sequencing reaction was stopped by adding of 10 µL of sequencing stop dye (98% formamide, 0.025% bromphenol blue, 0.025% xylene cyanol, and 10 mM 0.5 EDTA; pH8.0). The sequence results were used for primer designing and further analysis.

Primer designing, testing and optimisation

Forward and reverse primers were designed from sequences flanking microsatellite arrays identified in the insert. The primer designing was done using Tandem Repeat Finder (https://tandem.bu.edu/trf/trf.html) and Oligo software (https://www.oligo.net/) following the criteria: primer length of 20-28 nucleotides, annealing temperature of 55°C-65°C, G + C content of 45-65%, and putative amplicon size of 150-350 bp. Oligonucleotide primers were synthesised by the Bio-service Unit (Biotech, India) taking into consideration the primer design parameters provided by Hoelzel and Green (1992).

SSR primers were tested for optimum amplification, product size and presence of polymorphism using mungbean core collection. The PCR reaction was performed in a 20 µL reaction mixture containing 50 ng of DNA, 1× PCR buffer, 10 mM dNTP, 1.5-3 mM $MgCl_2$, 10 µM each of forward and reverse primers, and 1 unit of *Taq* polymerase (NEB). The following PCR conditions were used: 94°C for 1 min, 45°C-55°C for 30 s and 72°C for 1 min for a total of 35 cycles. Amplified products were separated on 1.4% agarose stained with ethidium bromide (1 mg/mL) by using a 100-bp DNA ladder (*Phi*X174/*Hinf*I) for estimation of the amplified fragment size loaded separately. Horizontal gel electrophoresis was performed at 100 V for 3-4 h in 1× TBE electrode buffer. Resolved bands were visualised under UV at a wavelength of 302 nm. The gel pattern was photographed for recording by using a Geldoc System from Syngene, India.

Data analysis

Amplified products were scored from photographs as the presence or absence of a band. Each band was treated as one marker and scored across all 96 samples. The homology of bands was based on the distance of migration in the gel. The presence of a band was scored as '1', and the absence of a band was scored as '0', whereas missed samples were designated as '9'. Pairwise similarities between accessions was evaluated by calculating Simple Matching Coefficient. Pairwise comparisons were based on the proportion of shared bands produced by primers.

A dendrogram was constructed based on sequential hierarchical agglomerative nonoverlapping clustering technique of the unweighted pair group method of arithmetic means (UPGMA) (Sneath and Sokal 1973). These computations were performed using the statistical analysis package NTSYS-pc v2.10t (Rohlf 1994). The standard error (SE) was calculated and inserted in graphs for all corresponding data. One-way analysis of variance was performed to evaluate significant differences in the means of at least triplicate samples, followed by Tukey's test.

Results and discussion

Isolation of microsatellite repeats and genomic library construction

An microsatellite enriched genomic library was constructed to isolate SSR loci from the *V. radiata* genome. However, the enriched library (Supplementary Fig. S1, www.isgpb.org) prepared in the present study resulted in a high percentage of microsatellites (7.4%), the redundancy of some microsatellite regions was common. A selected set of 288 positive clones were sequenced, which revealed the unexpectedly high prevalence of repeat regions. Although redundancy in enriched libraries has been reported to be useful in resolving ambiguities in a DNA sequence (Rallo et al. 2000; Mba et al. 2001; Burns et al. 2001), redundancy limits the number of microsatellite loci isolated effectively and increases the cost incurred per SSRs identified.

Screening for clones containing repeat regions

The isolated plasmid DNA of transformed clones (2304 colonies) was pooled in three stages and used as a template for PCR-based screening of clones. The PCR reaction was performed with one primer complementary to the adaptor sequence and the other primer complementary to the repeat region. Clones containing a plasmid DNA carrying microsatellite repeats amplified two or more fragments/bands, whereas clones not containing a microsatellite region

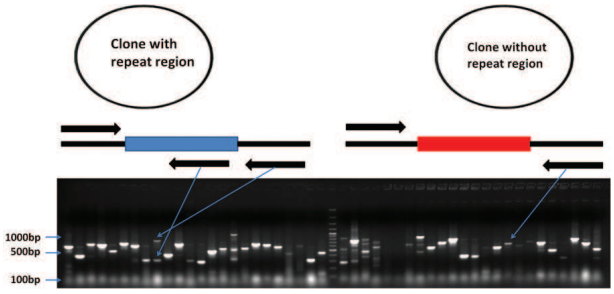


Fig. 1. Strategy for PCR-based screening of plasmid DNA containing insert microsatellite clones with GA from pooled samples in a single plate (tertiary screening)

amplified only a single band/product (Fig. 1). A total of 288 (12.5%) clones identified through PCR-based screening were selected for the sequencing of plasmid DNA. The efficiency of the protocol used in this study can be understood by comparing our results with those reported by Nunome et al. (2004); they used a traditional method for screening 72,000 plaques and could identify only 56 positive clones, whereas we screened only 2,304 recombinant clones and identified 288 positive clones for sequencing, of which 170 clones had the repeat region. We selected 124 sequences for primer designing depending upon the copy number of repeat motifs and the distance of repeat regions from the cloning site (Supplementary Table S1, www.isgpb.org).

The number of positive clones containing microsatellites obtained from the screening of conventional libraries usually ranges from 0.04-12%. Such isolation strategies can be effective only within taxa with a high frequency of microsatellites in the genome. Enrichment of genomic libraries can enhance the identification of microsatellites (Ostrander et al. 1992; Kandpapal et al. 1994; Paetkau 1999; Ueno et al. 1999).

Sequence analysis and characterisation of microsatellite repeats

The sequence information of all clones was analysed for the presence of a microsatellite region by using TRF software. Groups of repeats were categorised into three units, namely perfect, imperfect, and compound, based on the type of repeat regions identified. We found 38 (31%) repeats to be perfect

and 38 (47%) to be imperfect, whereas only 27 (22%) interspersed repeat regions in the sequencing data (Fig. 2A). A total of 288 (12.5%) clones were found with inserts carrying microsatellite repeat regions (Fig. 2B).

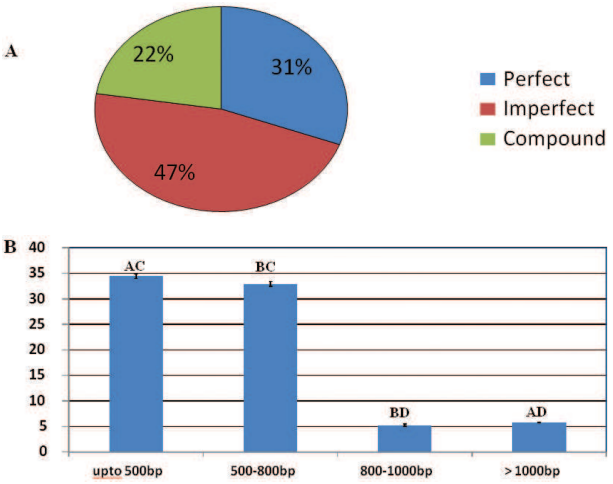


Fig. 2. Characterisation of insert sequences, A. Type of repeats produced, B. Insert number and size distribution, plotted based on average, error bars show SE, and one-way ANOVA test was performed (P value is significant), followed by Dunnett's test, where the mean of each sample was compared to the mean of other samples

The length of repeat motifs ranged from dinucleotides to more than heptanucleotides (Fig. 3). The frequency of a dinucleotide repeat motif was the maximum, followed by that of trinucleotide, octanucleotide, pentanucleotide, hexanucleotide, tetranucleotide, octanucleotide, and heptanucleotide repeat motifs (Fig. 3). The maximum primers were designed and amplified by a trinucleotide, followed by a dinucleotide, whereas more polymorphic primers were produced by a dinucleotide compared with a trinucleotide (Fig. 3).

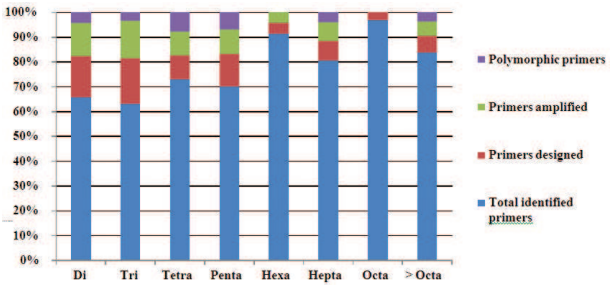


Fig. 3. Characterisation of primers designed in mung bean's genomic library

The number of microsatellite repeat motifs recovered ranged from 2 units to >15 units (Fig. 4A). Most of the SSRs had less than 5 repeat motifs, followed by 5-8, 8-12, 12-15 units and >15 (Fig. 4A). Among dinucleotide repeat motifs, the AG/CT repeat was more abundant compared with the GT/CA repeat. Among other repeat motifs, GA nucleotides were more abundant. The copy number of the repeat motif AG/CT varied from 7.5 to 23.5, with an average of 12.7 repeat motifs per clone (Supplementary Table S1, www.isgpb.org). The size of PCR amplified product of newly developed primers ranged from 150 to 450 bp (Fig. 4B).

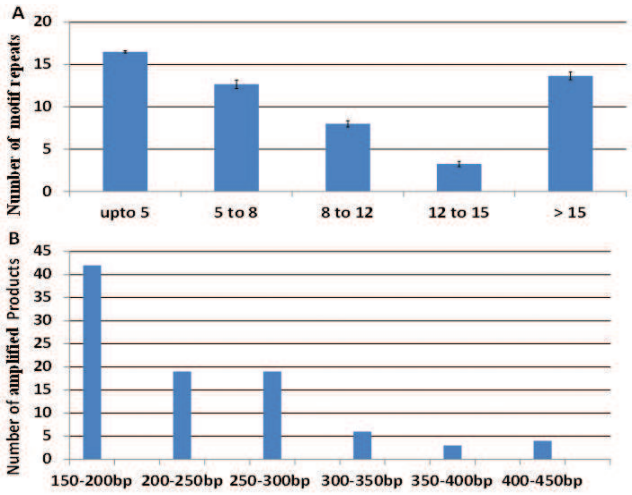


Fig. 4. Distribution of numbers of motif repeats and range of PCR amplified products, A. Size and number of repeat motifs was plotted against average with standard error bars , and one-way ANOVA was performed (P value is not significant), B. Plot of repeat number against size of amplified products

Approximately 28% of all primer pairs designed in this study failed to amplify repeat regions despite optimisation. This result is in line with the reports by Numone et al. (2004) who could not obtain amplification with 39% of designated primer pairs and attributed this to A- and T-rich sequences, which cause instability at the 32 end by hindering perfect complementarity to primers. Further, the short length of oligonucleotides used as a probe may result in a high proportion of false positives. The length of a probe applied for hybridisation can affect the type and length of captured microsatellites. Armour et al. (1994) found that a longer oligonucleotide probe not only favoured the isolation of relatively long arrays but also effectively eliminated

mismatches. Although the protocol used in this study required 10-15-bp-long oligonucleotides, other more successful studies (e.g. Stajner et al. 2005) have used much longer probes (200-550 bp).

The abundance of the AG/CT class of repeats in the enriched library and hence in the entire study was apparent. This result is consistent with those of previous studies on hop (*Humulus lupulus* L.) (Stajner et al. 2005), white clover (*Trifolium repens* L.) (Kolliker et al. 2001), and *Lolium temulentum* (Senda et al. 2004), all of which enrichment was employed. However, a study on sunflower (*Helianthus annus* L.) that involved enrichment of dinucleotide repeats also identified the AT repeat as the most abundant nontargeted SSR (Tang et al. 2002). The highly successful isolation after enrichment in these studies suggest that SSRs occur very frequently in the genome of most plants.

Primer designing for the microsatellite region identified

A total of 124 microsatellite sequences with core sequences being repeated from 4 times to more than 100 times were considered for primer designing by using Oligo software (Supplementary Table S1, www.isgpb.org). Some of the clones with ideally high number of repeats and copy numbers could not be used for primer designing because the core repeat region was close to either of the ends of the fragment or because of redundancy in the sequence. A total of 102 (126%) primer pairs provided satisfactory amplification within the expected size range after optimisation of amplification conditions as per the annealing temperature.

Allelic variation of SSRs among mungbean accessions

Of the 124 SSR loci, 37 (46%) showed polymorphism in the mung bean core collection (Fig. 5). These selected primers generated a total of 112 alleles. The number of alleles per microsatellite locus ranged from one to six, with an average of three alleles, whereas 52 (65%) alleles were monomorphic. All 37 (46%) alleles displayed reproducible polymorphic loci, and 12 (32%) of them were diallelic. In addition, some primers produced nonspecific double and multiple alleles (data not shown). Despite a large number of primer sets, poor amplification can be due to unsuitable primer sequences and improper PCR amplification conditions in addition to quality of DNA sequences (Akagi et al. 1996). The lack of amplification of an allele in the mung bean germplasm could also be due to divergence in sequences flanking SSRs, creating a null allele (Smulders et al. 1997). However, it could also result from the production of an undetectable amount of PCR product due to improper PCR conditions for the pair of primers (Lavi et al. 1994). In the latter case, more intensive optimisation of PCR conditions would be necessary for individual lines or cultivars that did not amplify any fragments. The use of PCR-based SSR markers may result in size homoplasy of PCR products of *V. mungo* (Garza et al. 1995). The same size allele of an SSR locus may contain different sequence/primer variants; thus,

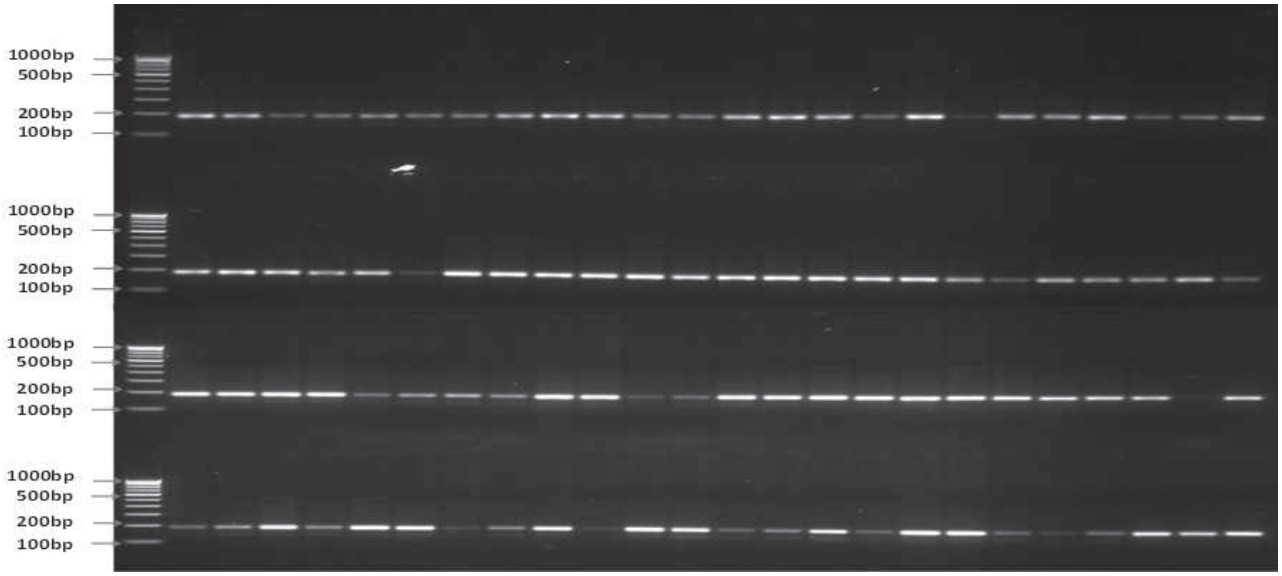


Fig. 5. Electrophoresis banding pattern of primer mgssr 15 in mungbean core collection resolved on 1.4% agarose gel

species sharing the same SSR allelic size that are identical by descent may have originated from convergent evolution.

The polymorphism information content (PIC) for the SSRs varied from 0.34 to 0.70, with an average of 0.52. The proportion of polymorphic products varied from 48.84% in exotic accessions to 54.56% in Indian accessions. The number of polymorphic loci reported in Indian and exotic accessions was 36 and 40, respectively. The mean of observed heterozygosity and expected heterozygosity was 0.09 and 0.24, respectively, in Indian accessions, and 0.08 and 0.17, respectively, in exotic accessions.

Our data indicated the presence of a narrow genetic base among mung bean accessions analysed in this study. The narrow genetic base is one of the reasons for the very low yield of polymorphic markers. A total of 124 microsatellite loci were utilised for the assessment of genetic diversity within the mung bean germplasm. However, frequent similarities in allele size despite different underlying SSRs were observed in soybean and chickpea (Peakall et al. 1998; Choumane et al. 2004). Similar results were reported in mung bean by Pandey et al. (2011). The PIC values of SSR

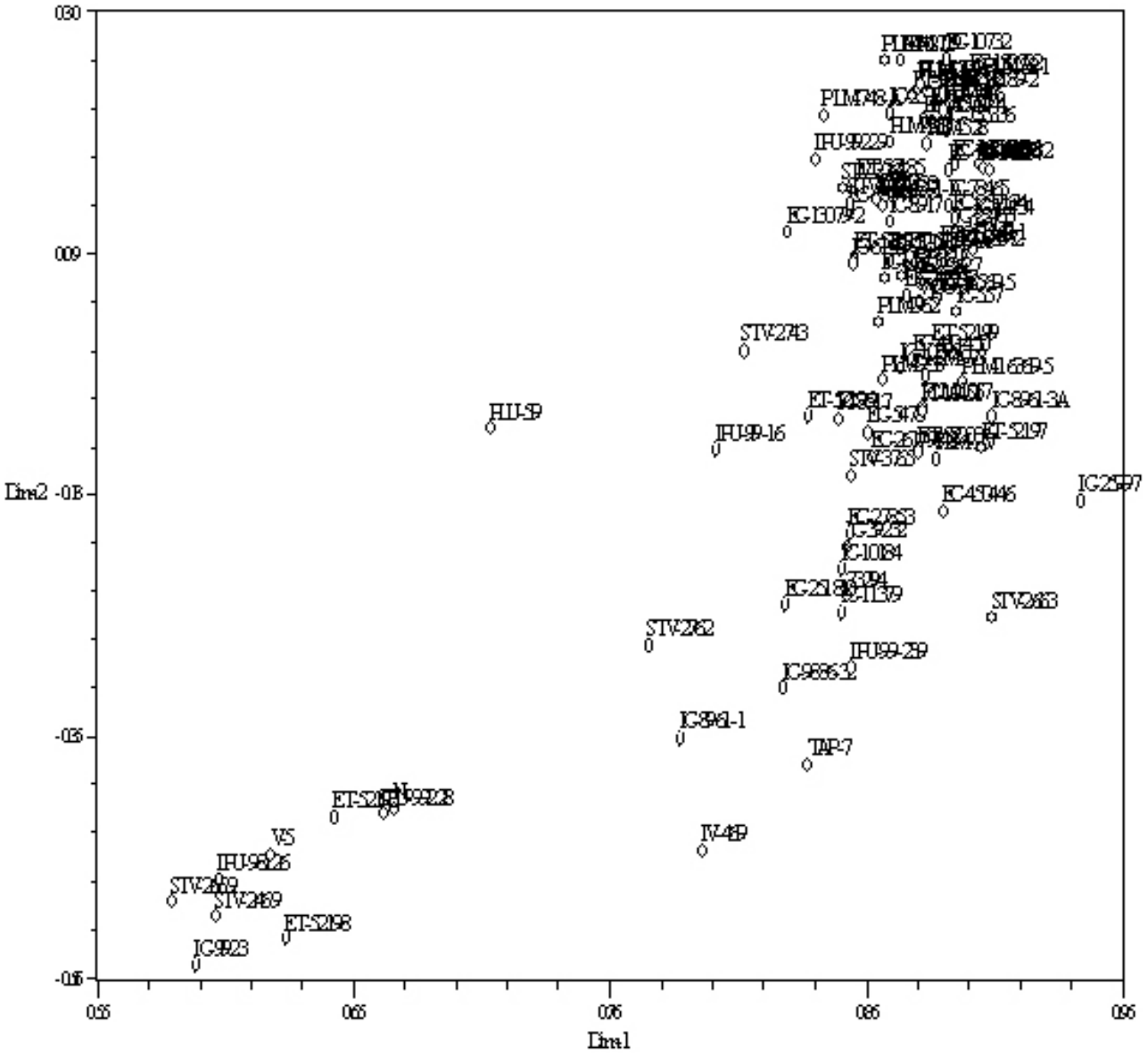


Fig. 6. Two dimensional plot of first two principal components for the mungbean core collection accessions based on polymorphism for SSR markers

microsatellite markers in a similar study by Li et al. (2001) ranged from 0.02 to 0.73. Comparable results have been reported in studies that used microsatellite markers to investigate genetic diversity in several crops, such as black gram (Soubramanien and Gopalakrishna 2009), soybean (Rongwen et al. 1995), groundnut (Mace 2007), and cowpea (Li et al. 2001). Similarly, microsatellite markers were used to investigate the genetic basis of cowpea yellow mosaic virus (CYMV) resistance (Gioi et al. 2010). Thus, the level of microsatellite polymorphism in mung bean is much lower than that in other crops. These findings suggest that the cross-species amplification of SSR markers from closely related species can be helpful in the large-scale development of locus-specific microsatellite markers in mungbean.

Analysis of genetic relationships and diversity in mung bean

An unrooted dendrogram was constructed using UPGMA procedure (Supplementary Fig. S2, www.isgpb.org). Cluster analysis results revealed that the mung bean core collection under study can be categorised into five major groups. The genetic similarity based on STMS microsatellite primers ranged from 0.1 to 0.5, indicating that the genetic distance in the mung bean core collection germplasm is narrow. In the dendrogram five major groups were identifiable; the maximum number of accessions of the exotic origin belonged to group I, Indian accessions belonged to groups II and III, and overlapped accessions of Indian and exotic collections belonged to groups IV and V. However, there was a minor overlap between the germplasm and groups (Fig. 6). The results are in agreement with those of previous studies that used noncoding sequences of trnT-F (Yano et al 2004; Ye and Yamaguchi 2007). By contrast, studies using AFLP (Tomooka et al. 2002), rDNA-ITS, and atpB-rbcL sequences (Doi et al. 2002) have recognised three groups within the Asian *Vigna*. Similar analyses reported earlier using morphological traits (Tomooka et al. 2002), rDNA and cpDNA sequences (Ye and Yamaguchi, 2007; Doi et al. 2002), CYMV resistant and susceptible groups distinguished by microsatellite makers and STMS markers had also revealed diverse relationships in mung bean accessions (Tangphatsornruang et al. 2009; Gioi et al. 2010; Pandey et al. 2011).

Principal component analysis

Principal component analysis performed using microsatellite loci was used to generated a two-dimensional plot of the mung bean core collection accessions. The analysis indicated presence of five major groups among the mung bean accessions which comprised exotics in group I, indigenous accessions in II and III, and the composite grouping of both type of accessions in groups IV and V.

It is notable that the germplasm of the Indian origin was placed together, whereas that of the exotic origin was well separated; however, a few of exotic source accessions were placed separetely at the extreme end in bi-plot. The results are similar to the reports for the resistant and susceptible groups soybean germplasm with CYMV and rhizoctonia aerial blight response in two- and three-dimensional principle coordinate analyses (Gioi et al. 2010; Bimal et al. 2013).

Authors' contribution

Conceptualization of research (KVB, ABG); Designing of the experiments (KVB, ABG); Contribution of experimental materials (KVB, ABG); Execution of field/lab experiments and data collection (CSP, SSB); Analysis of data and interpretation (ABG, KVB); Preparation of manuscript (SSB, CSP, ABG, KVB).

Declaration

The authors declare no conflict of interest.

Acknowledgements

The authors are thankful for the generous funding from Department of Biotechnology, Government of India, and the facilities provided by the Indian Council of Agricultural Research for conducting this work.

References

Abdul-Muneer P. M. 2014. Application of microsatellite markers in conservation genetics and fisheries management: Recent advances in population structure analysis and conservation strategies. Genet. Res. Int., Article ID 691759: 11.

Akagi H., Yokozeki Y., Inagaki A. and Fujimura T. 1996. Microsatellite DNA markers for rice chromosomes. Theor. Appl. Genet., **93**(7): 1071-1077.

Armour J. A., Neumann R., Gobert S. and Jeffreys A. J. 1994. Isolation of human simple repeat loci by hybridization selection. Hum. Mol. Genet., **3**(4): 599-565.

Bimal S. S., Aggarwal A. and Saini N. 2013. Molecular characterization and diversity analysis of soybean varieties against *Rhizoctonia aerial blight resistance*. Int. J. Bioinfo. Biol. Sci., **4**: 2319-5169.

Bisht I. S., Bhat K. V., Lakhanpaul S., Biswas B. K., Ram B. and Tanwar S. P. S. 2004. The potential of enhanced germplasm for mung bean (*Vigna radiata* (L.) Wilczek) improvement. Plant Genet. Resour., **2**(2): 73-80.

Burns J. M., Jansen H. D., Hajibabaei M., Hallwaschs W. and Hebert P. D. N. 2007. DNA barcodes of closely related (but morphologically and ecologically distinct) species of skipper butterflies (Hesperiidae) can differ by only one to three nucleotides. J. Lepid. Soc., **61**(3): 138-153.

Choumane W., Winter P., Baum M. and Kahl G. 2004. Conservation of microsatellite flanking sequences in different taxa of Leguminosae. Euphytica, **138**: 239-245.

Dahiya P. K., Linnemann A. R., Van Boekel M. A., Khetarpaul N., Grewal R. B. and Nout M. J. 2015. Mung bean: Technological and nutritional potential. Crit. Rev. Food Sci. Nutr., **55**(5): 670-688.

Dixit A., Jin M. H., Chung J. W., Yu J. W., Chung H. K., Ma K. H., Park Y. J. and Cho E. G. 2005. Development of polymorphic microsatellite markers in sesame (*Sesamum indicum* L.). Mol. Ecol. Notes., **5**(4): 736-738.

Doi K., Kaga A., Tomooka N. and Vaughan D. A. 2002. Molecular phylogeny of genus *Vigna* subgenus *Ceratotropis* based on rDNA ITS and atpB-rbcL intergenic spacer of cpDNA sequences. Genetica., **114**(2): 129-145.

Deininger P. L. 1983. Random subcloning of sonicated DNA: Application to shotgun DNA sequence analysis. Anal Biochem., **129**(1): 216-223.

Diwan N., Bouton J. H., Kochert G. and Cregan P. B. 2000. Mapping of simple sequence repeat (SSR) DNA markers in dipliod and tetraploid alfalfa. Theor. Appl. Genet., **101**(1-2): 165-172.

Eujayl I., Sledge M. K., Wang L., May G. D., Chekhovskiy K., Zwonitzer J. C. and Mian M. A. 2004. Medicago truncatula EST-SSRs reveal cross-species genetic markers for *Medicago* spp. Theor. Appl. Genet., **108**(3): 414-422

Finnegan E. J., Genger R. K., Peacock W. J. and Dennis E. S. 1998. DNA methylation in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol., **49**: 223-247.

Gwag J. G., Chung J. W., Chung H. K., Lee J. H., Ma K. H., Dixit A., Park Y. J., Cho E. G., Kim T. S. and Lee S. H. 2006. Characterization of new microsatellite markers in mung bean (*Vigna radiata*). Mol. Ecol. Notes., **6**(4): 1132-1134.

Gioi T. D., Boora K. S. and Chaudhary K. 2010. Investigation of genetic relationship among yellow mosaic virus resistant cowpea lines using microsatellite markers. Omonrice., **17**: 29-40.

Garza J. C., Slatkin M. and Freimer N. B. 1995. Microsatellite allele frequencies in humans and chimpanzees with implications for constraints on allele size. Mol. Biol. Evol., **12**(4): 594-603.

Hearne C. M., Ghosh S. and Todd J. A. 1992. Microsatellites for linkage analysis of genetic traits. Trends Genet., **8**(8): 288-294.

Hoelzel A. R. and Green A. 1992. Analysis of population-level variation by sequencing PCR-amplified DNA. *In*: Molecular genetic analysis of populations (ed A.R. Hoelzel). IRL Press, Oxford, UK:159-187.

Jarne P. and Lagoda P. J. 1969. Microsatellites from molecules to populations and back. Trends Ecol. Evol., **11**(10): 424-429.

Kandpapal R. P., Kandpal G. and Weissman S. M. 1994. Construction of libraries enriched for sequence repeats and jumping clones, and hybridization selection for region-specific markers. Proc. Natl. Acad. Sci. U. S. A., **91**(1): 88-92.

Kim K. W. 2004. Developing one-step program (SSR MANAGER) for rapid identification of clones with SSRs and primer designing. Unpublished Master Thesis. Seoul National University, Republic of Korea.

Koelliker R., Jones E. S., Drayton M. C., Dupal M. P. and Forster J. W. 2001. Development and characterization of simple sequence repeat (SSR) markers for white clover *(Trifolium repens* L.). Theor. Appl. Genet., **102**(2-4): 416-424.

Kumar S. V., Tan S. G., Quah S. C. and Yusoff K. 2002. Isolation of microsatellite markers in mung bean, *Vigna radiata.* Mol. Ecol. Notes., **2**: 96-98.

Kumar S. V., Tan S. G., Quah S. C. and Yusoff K. 2002. Isolation and characterization of seven tetranucleotide microsatellite loci in mung bean, *Vigna radiata*. Mol. Ecol. Notes., **2**: 293-295.

Levinson G. and Gutman G. A. 1986. Slipped-strand mispairing: A major mechanism for DNA sequence evolution. Mol. Biol. Evol., **4**(3): 203-221.

Li C. D., Fatokun C. A., Benjamin U., Singh B. B. and Scoles G. J. 2001. Determining genetic similarities and relationships among cowpea breeding lines and cultivars by microsatellite markers. Crop Sci., **41**(1): 189-197.

Lavi U., Akkaya M., Bhagwat A., Lahav E. and Cregan P. B. 1994. Methodology of generation and characteristic of simple sequences repeat DNA marker in avocado *(Persea Americana* M). Euphytica, **80**(3): 171-177.

Mace E. S., Varshney R. K., Mahalakshmi V., Seetha K., Gafoor A., Leeladevi Y. and Crouch J. H. 2007. In silico development of simple sequence repeat markers within the aeschynomenoid/dalbergoid and genistoid clades of the Leguminosae family and their transferability to *Arachis hypogaea*, groundnut. Plant Sci., **174**(1): 51-60.

Mba R. E. C., Stephenson P., Edwards K., Melzer S., Nkumbira J., Gullberg U., Apel K., Gale M., Tohme J. and Fregene M. 2001. Simple sequence repeat (SSR) markers survey of the cassava (*Manihot esculenta* Crantz) genome: Towards an SSR-based molecular genetic map of cassava. Theor. Appl. Genet., **102**(1): 21-31.

Nunome H., Asai T., Ikegami Y. and Sakurai S. 2002. Three-dimensional kinetic analysis of side-foot and instep soccer kicks. Med. Sci. Sports Exerc., **34**(12): 2028-2036.

Ostrander E. A., Jong P. M., Rine J. and Duyk G. 1992. Construction of small insert genomic DNA libraries highly enriched for microsatellite repeat sequences. Proc. Natl. Acad. Sci. U.S.A., **89**(8): 3419-3423.

Peakall R., Gilmore S., Key W., Morgante M. and Rafalski A. 1998. Cross-species amplification of soybean (*Glycine max*) simple sequence repeats (SSRs) within the genus and other legume genera: Implications for the transferability of SSRs in plants. Mol. Biol. Evol., **15**(10): 1275-1287.

Pandey A., Kumar A. and Ramya P. 2011. Genetic diversity in mung bean accessions as revealed by STMS markers. Afr. J. Biotechnol., **10**(75): 17081-17087.

Paetkau D. 1999. Microsatellites obtained using strand extension: An enrichment protocol. Biotechniques, **26**(4): 690-292,694-697.

Rallo P., Dorado G. and Martin A. 2000. Development of simple sequence repeats (SSRs) in olive tree (*Olea europaea* L.). Theor. Appl. Genet., **101**: 984-989.

Rohlf F. J. 2000. NTSYSpc (Numerical taxonomy and multivariate analysis system) version 2.1 user guide; State University of New York Stony Brook, New York, USA, 11794-5245.

Rohlf, F. J. 2005. NTSYSpc (Numerical Taxonomy & Multivariate Analysis System). Version 2.2, Exeter Software, Applied Biostatistics Inc., Exeter Publishing Ltd. New York, USA.

Rongwen J., Akkaya M. S., Bhagwat A. A., Lavi U. and Cregan P. B. 1995. The use of microsatellite DNA markers for soybean genotype identification. Theor. Appl. Genet., **90**(1): 43-48.

Saghai-Maroof M. A., Soliman K. M., Jorgensen R. A. and Allard R. W. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dymnamics. Proc. Natl. Acad. Sci. U.S.A., **81**(24): 8014-8018.

Sambrook J., Fritsch E. F. and Maniatis T. 1989. Molecular cloning. *In*: A Laboratory Manual, vol. III. Cold Spring Harbor Laboratory Press, New York, USA: 1626.

Sethy N. K., Shokeen B., Edwards K. J. and Bhatia S. 2006. Development of microsatellite markers and analysis of intraspecific genetic variability in chickpea (*Cicer arietinum* L.). Theor. Appl. Genet., **112**(8): 1416-1428.

Somta P., Musch W., Kongsamai B., Chanprame S., Nakasathien S., Toojinda T., Sorajjapinun W., Seehalak W., Tragoonrung S. and Srinives P. 2008. New microsatellite markers isolated from mung bean *(Vigna radiata* (L.) Wilczek). Mol. Ecol. Resour., **8**(5): 1155-1157.

Seehalak W., Somta P., Sommanas W. and Srinives P. 2009. Microsatellite markers for mungbean developed from sequence database. Mol. Ecol. Resour., **9**(3): 862-864.

Sneath P. H. A. and Sokal R. R. 1973. Numerical taxonomy. *In*: The principles and practice of numerical classification. San Francisco, W.H. Freeman and Company, USA: 515-573.

Smulders M. J. M., Bredemeijer G., Rus-Kortekaas W., Arens P. and Vosman B. 1997. Use of short microsatellites from database sequences to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessions of other *Lycopersicon* species. Theor. Appl. Genet., **97**: 264-272.

Souframanien J. and Gopalakrishna T. 2004. A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. Theor. Appl. Genet., **109**: 1687-93.

Stajner N., Jakse J., Kozjak P. and Jovornik B. 2005. The isolation and characterization of microsatellites in hop (*Humulus Lupcus* L.). Plant Sci., **168**(1): 213-221.

Senda T., Kubo N., Hirai M. and Tominaga T. 2004. Development of microsatellite markers and their effectiveness in *Lolium temulentum*. Weed Res., **44**(2): 136-141.

Seehalak W., Tomooka N., Waranyuwat A., Thipyapong P., Laosuwan P., Kaga A. and Vaughan D. A. 2006. Genetic diversity of the *vigna* germplasm from Thailand and neighboring regions revealed by AFLP analysis. Genet. Resour. Crop Ev., **53**(5): 1043-1059.

Su Y. A., Lee M. M., Hutter C. M. and Meltzer P. S. 1997. Characterization of a highly conserved gene (OS4) amplified with CDK4 in human sarcomas. Oncogene., **15**: 1289-1294.

Tautz D., Trick M. and Dover G. 1986. Cryptic simplicity in DNA is a major source of genetic variation. Nature., **322**: 652-656.

Tangphatsornruang S., Somta P., Uthaipaisanwong P., Chanprasert J., Sangsrakru D., Seehalak W., Sommanas W., Tragoonrung S. and Srinives P. 2009. Characterization of microsatellites and gene contents from genome shotgun sequences of mungbean (*Vigna radiata* (L.) Wilczek). BMC Plant Biol., **9:** 137.

Tang S., Yu J. K., Slabaugh M. B., Shintani D. K. and Knapp S. J. 2002. Simple sequence repeat map of the sunflower genome. Theor. Appl. Genet., **105**(8): 1124-1136.

Takundwa M., Chimwamurombe P. M., Kunert K. and Cullis C. A. 2010. Isolation and characterization of microsatellite repeats in marama bean (*Tylosema esculentum*). Afr. J. Agric. Res., **5**(7): 561-566.

Tomooka N., Yoon M. S., Doi K., Kaga A. and Vaughan D. 2002. AFLP analysis of diploid species in the genus *Vigna* subgenus *Ceratotropis*. Genet. Resour. Crop Ev., **49**(5): 521-530.

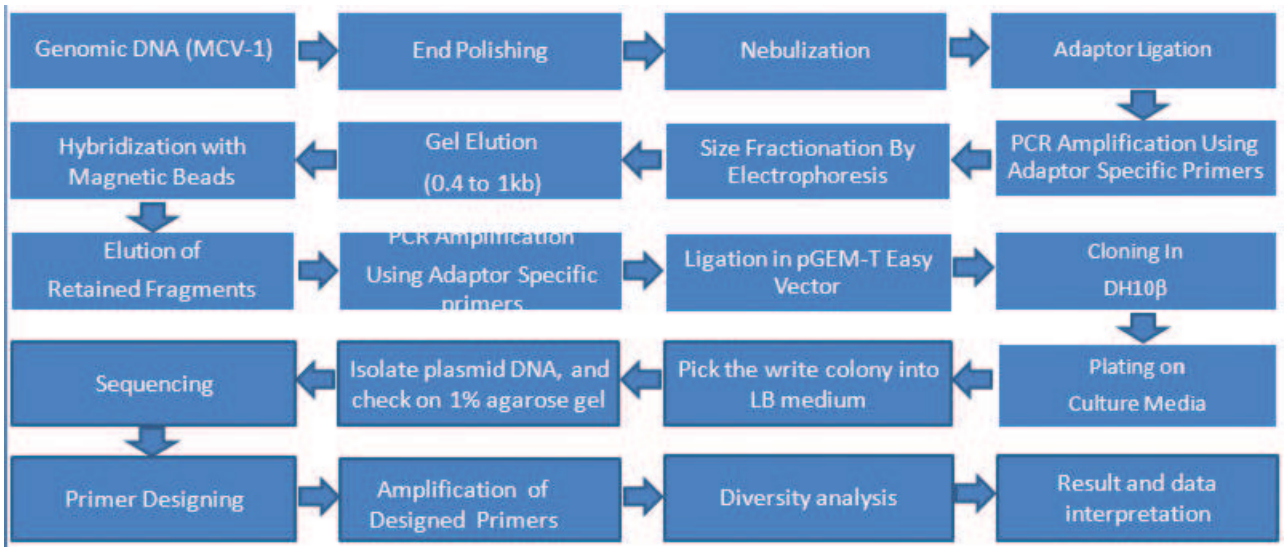
Tomooka N., Vaughan D. A., Moss H. and Maxted N. 2002. The Asian *Vigna*. *In*: Genus *Vigna* subgenus *Ceratotropis* genetic resources (ed. N.Tomooka, D.A. Vaughan, H. Moss and N. Maxted). Kluwer Academic Publishers, Dordrecht, Netherlands: 214-270.

Tun Y. T. and Yamaguchi H. 2007. Phylogenetic relationship of wild and cultivated *Vigna* (subgenus *Ceratotropis*, Fabaceae) from Myanmar based on sequence variations in non-coding regions of *trn*T-F. Breed. Sci., **57**: 271-280.

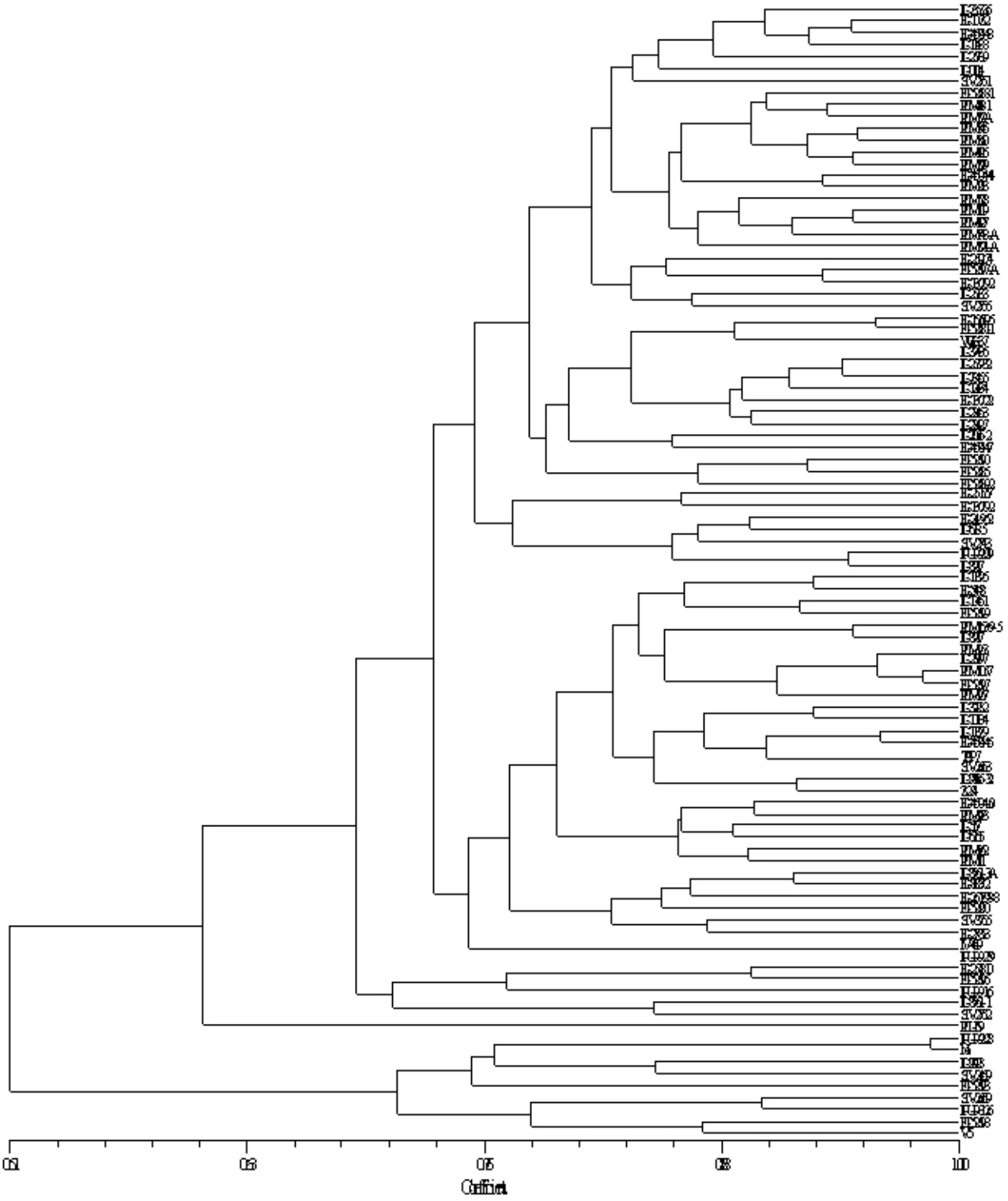
Ueno S., Yoshimaru H., Tomaru N. and Yamamoto S. 1999. Development and characterization of microsatellite markers in *Camellia japonica* L. Mol. Ecol., **8**(2): 335-336.

Winter P., Pfaff T., Udupa S. M., Huettel B., Sharma P. C., Sahi S., Arreguin-Espinoza R., Weigan F., Muehlbauer F. J. and Kahl G. 1999. Characterization and mapping of sequence-tagged microsatellite sites in the chickpea (*Cicer arietinum* L.) genome. Mol. Gen. Genet., **262**(1): 90-101.

Yano A., Yasuda K. and Yamaguchi H. 2004. A test for molecular identification of Japanese archaeological beans and phylogenetic relationship of wild and cultivated species of subgenus *Ceratotropis* (*Genus Vigna*, Papilionaceae) using sequence variation in two non-coding regions of the trnl and trnf genes. Econ. Bot., **58**: 135-146.



Supplementary Fig. S1. Work flow for the microsatellite enrichment, DNA sequencing, primer design and data analysis following for mung bean



Supplementary Fig. S2. Genetic relationships among the 96 mungbean core collection accessions constructed using microsatellite markers

Supplementary Table S1. SSR primer identified from the DNA sequence data from the mung bean microsatellite enriched library screening along with their characteristics

S.No.	Primer ID	Left primer (Forward)	Right primer (Revesrse)	Core repeat	Copy No.	Nature of repeat	Product size
1	mgssr1	5'-GATGAAGAAGAAGAAGAAGACGG	5'-AGGTGCTATTTTATGGAACAGG	GAT	6.7	simple	242
2	mgssr2	5'-TTTTGGGGCTAATGCTGAGG	5'-CCCTGCTTGTATTTTTCCTGC	AG	7.5	simple	300
3	mgssr3	5'-TTGCATGAGAAGAAAGTTTGTGC	5'-GGAAAAGATGGAAAATCTTCCC	AATT	4.2	simple	465
4	mgssr4	5'-GTAGGTGGTGAGCAAAAGGG	5'-CCACTCCTCTCTCCTTTCACC	TGA	4.7	simple	181
5	mgssr5	5'-GAGGACACTGTGTAGTTTAAGTGTGG	5'-TGGCTCAAATCTCACACAGGG	GTTAAATTTTTT	5	simple	308
6	mgssr6	5'-ATGAGAGAGCTTATTAGAGAAGGC	5'-CCATGGTCTAATTTTGAATGGC	AAGAA	5	simple	360
7	mgssr7	5'-GACACCAATTTCTTTTCTTCCC	5'-GGGAAGAGGTTACCTTAGCC	TTCTTCCTC	14.4	compound	464
8	mgssr8	5'-CGCTCACATCGTCCTTGTGC	5'-GATGCGAGCGATCACACACG	TTC	5.3	simple	187
9	mgssr9	5'-TGTTGGGGTATGCATATGGG	5'-CGTAATTGAAAGGATTTGGG	TC	16.5	simple	152
10	mgssr10	5'-AGTCCAGATCTAAGCAAGAGTGC	5'-AAGATGTAGAAGTATGGTAGTGG	TGT	55.7	simple	434
11	mgssr11	5'-CGATTCTCTTGCTTAGATCTGG	5'-CCCATCATGATCCTCATCCC	GT	5.5	simple	150
12	mgssr12	5'-AAGGAAAACGAATACCTCGG	5'-GTGGCTGAGATTGCTCCTGG	GAA	9	simple	316
13	mgssr13	5'-AGGGAAGAAATTACCTTAGCC	5'-GATTGTAGGAGGAGGTGGTGC	AG	58.5	simple	441
14	mgssr14	5'-CCTAAATGCAACACACAATGACG	5'-GCACATCAAATTTTGCATGGG	CACAA	7.4	simple	171
15	mgssr15	5'-AAATTGATGACAATGGAGGACC	5'-AGATCTCTTGGTCTAATTCCTCC	TTC	6.3	simple	180
16	mgssr16	5'-GAGGAAGATAAAGATGATGAGGG	5'-CACATGATCTTTTCTTTCTTCCC	AAG	9.3	simple	163
17	mgssr17	5'-GATATGTTTGGGCTTGGACC	5'-CCAAAATCCTCATCCAAGGG	AC	5	simple	130
18	mgssr18	5'-AGGATGGAATTAGAATTCAGAGC	5'-TTTCTTTTCTCTTCTTCTCTCGC	AG	10.5	simple	204
19	mgssr19	5'-CTTAATGCAAACAAACTCAGTGC	5'-TGCTTAGATCTGGACTATCTCTCC	GA	10	simple	329
20	mgssr20	5'-AGAAAAACGGCACAAAGACC	5'-TCTCTTTCTCTCATAGCTACCACC	AG	11.5	simple	279
21	mgssr21	5'-CGATTCTCTTGCTTAGATCTGG	5'-AGATTGGGTTCCATGATGGG	AGAGA	35	simple	365
22	mgssr22	5'-AGACCTAGAGAGAAAACGTAGGG	5'-CTACAAGTTCTTCCTTCTCTCGC	AG	25	simple	270
23	mgssr23	5'-TAGAAAGAGAGTCGTTATCGTGG	5'-CAGAAACTTGCAGTGGCAGG	GA	9.5	simple	245
24	mgssr24	5'-GATGAAGGTAGTGGTGGTGGC	5'-TTGCTTAGATCTGGACTAACCG	GAGGAAGAA	7.1	compound	306
25	mgssr25	5'-CCATCATTCTTGCAATTGCG	5'-AGCAACGAGACCTTGTTGCC	AACAC	6.2	simple	206
26	mgssr26	5'-AGAAGAAGAAGAGAGAAGAGAGGG	5'-CCTTCCCTTTTAAGATTGGG	AGAAA	9.4	simple	189
27	mgssr27	5'-AGGTAAGTATGAAGTGGGTGGC	5'-TTCCTTGAACAGCACACTCACG	GA	5	simple	221
28	mgssr28	5'-CTCTTCTTTCCGATGTTGTCG	5'-TGATTCTCTTGCTTAGATCTGG	TC	7.5	simple	175

29	mgssr29	5'-GAAGAGATGAAAATGGTGAGTGG	5'-ATTTTCCTTCTCTCTCCTCTGC	AG	14.5	simple	169
30	mgssr30	5'-ATTTAAAGCATTCGACTGCG	5'-AACCAAACAGTTTAAGCACGG	GT	14.5	simple	260
31	mgssr31	5'-ATTTAAAGCATTCGACTGCG	5'-AACCAAACAGTTTAAGCACGG	AT	8	simple	260
32	mgssr32	5'-TTGTTGTTGTTCCTGTTGCTGC	5'-GTATTTATACGCCTTTAGTTCCG	GTT	109.3	simple	129
33	mgssr33	5'-CGATTCTCTTGCTTAGATCTGG	5'-CACACACTCATACATACACAAACACG	AG	13	simple	266
34	mgssr34	5'-GAGGAGTGTAGGAGGAGGTCG	5'-TAAGTGGGTTCTCTCGTAGGG	CTTGTT	5.7	simple	250
35	mgssr35	5'-CCTTCCTCCAATCTTTACCC	5'-CACTCCTTGTCGATGAGGTGG	CGC	8.7	simple	143
36	mgssr36	5'-TGGCGATGATGTGATGGTGG	5'-GCTTGATCTGGACTATCATCGG	TGG	12.3	simple	169
37	mgssr37	5'-CGATTCTCTTGCTTAGATCTGG	5'-GTGAGAGTAGGGGATGTTGCC	GAGAGAAA	16.1	compound repeat	312
38	mgssr38	5'-GGAGTGTAGGAGGAGATGGTGC	5'-GCAAAGAAGAGAGAAAAGAGAGC	TTC	5.7	simple	152
39	mgssr39	5'-GGTTTTTGATTTGTGTTCGTCG	5'-GCAAAAACCTTGACTATGTCCG	TTGT	7	simple	209
40	mgssr40	5'-CCCTAAAGAAGAATGTCAAGCC	5'-CCTCTTTTTCTCAACCTTCTCC	AGA	5.3	simple	296
41	mgssr41	5'-GAGCTTATGGATAAGAAAAGGG	5'-CCATGGTAAGAGTAGGAGTTGCC	AGAAGAGAAAAAGAG	11.8	compound	283
42	mgssr42	5'-TCTCTCATATGGCTTCTTCGC	5'-GATACGCAAGCACAGATCGG	TC	14	simple	147
43	mgssr43	5'-TGGTTCTCATCATGCAAAGAGG	5'-CCACTTTTGAGATAGGCAGACC	CAT	6.7	simple	181
44	mgssr44	5'-GGAGACTGAAGCAGTTCCTCC	5'-CAACAAACCAAGGACAAGGG	TTC	7	simple	168
45	mgssr45	5'-GTGTTTAAATTATGCCACGAGC	5'-CTGAAACCACACACACTTCCACC	GT	11.5	simple	251
46	mgssr46	5'-TTCCGTTAAAGGCCAGCACC	5'-AGCTGACCGCCTGTTCGACC	GAG	4	simple	168
47	mgssr47	5'-TTGTGGATTGTGGTGGTGGC	5'-GGTCGTCACCATCACTGTCACC	GTG	17.3	simple	294
48	mgssr48	5'-AAGAGAGAAGAGAAGAAGAAGCC	5'-GGTTCTATGAGGAGTGTAGGAGG	AAGAAGAGAC	5.9	compound	205
49	mgssr49	5'-ATCTTCCTTTAACGACATGGC	5'-GCACCCAAACTACCTCCTCC	TTG	5.7	simple	218
50	mgssr50	5'-CTGGACTATGTCCTGCATCACC	5'-GTGTTCTTCATCAAGCTGCTCC	CAC	5.7	simple	261
51	mgssr51	5'-CTCATGAAGTTCTTGAATGAGGG	5'-TGAGAATAGGGGATGTTGCC	AGAGAGAAAA	8	compound repeat	363
52	mgssr52	5'-TGGAACCATTTTAAGTCCAGG	5'-TGTCAAGTATGCACTAAACATCACC	TG	20	simple	166
53	mgssr53	5'-AGGGAAGAAATTACCTTAGCC	5'-GATTGTAGGAGGAGGTGGTGC	AG	58.5	compound repeat	441
54	mgssr54	5'-CAAGCAAATATAGGATGAAGAGC	5'-GTCTTTCTTTTCAAGTTCTTCCC	AAG	65.3	simple	350
55	mgssr55	5'-CCCTGAAGAAGATTGTAAGGC	5'-TCTCTCCACTTGCTCACACCG	AAG	5.3	simple	219
56	mgssr56	5'-CTAAATGCAACAACACATGACACC	5'-ATTTGTATGGGTGCGACACC	CACAA	7.4	simple	159
57	mgssr57	5'-TGTGTCTATGTTTGTTTGGG	5'-CATACACAAACACATACTCG	GT	16.5	simple	223
58	mgssr58	5'-CATGCTTAGATCTGGACTATCCG	5'-TGAAGCTACGCTTGCTAGGG	AG	20.5	simple	290
59	mgssr59	5'-AAATGATAAGGTTGTCAAGGAGG	5'-TCTCTTCCTTTTCTTTTCTCTCC	AG	7.5	simple	220
60	mgssr60	5'-AAAAGAGAGAAGGAAAGAAGAGC	5'-TAAAGGATGGAAAACCTCCC	AAAG	13.5	simple	205

60	mgssr60	5'-AAAAGAGAGAAGGAAAGAAGAGC	5'-TAAAGGATGGAAAACCTCCC	AAAG	13.5	simple	205
61	mgssr61	5'-AGAATCAGGAGAAGAACAAGAGG	5'-TTAAACACTCACAGGTGTTTCTCC	AGA	7.3	simple	226
62	mgssr62	5'-GAATCGTACCAGTCTCTACTGGG	5'-CCATTTATCCCACTTTCCTCC	AT	6	simple	256
63	mgssr63	5'-TCAGGATATGCTCACCGTGG	5'-CCACCTCCTAGGGAGTGTCC	ATA	10	simple	216
64	mgssr64	5'-GGACTACTCTCAAGTAGCATCCG	5'-CTTCCTTTTCTACCTCTTTCTCC	AGA	7.3	simple	242
65	mgssr65	5'-GCTTAGATCTGGACTAGGGACG	5'-CTCCAATCCTTTGGTTTCCC	AC	5.5	simple	201
66	mgssr66	5'-TCAGCAAAAACTGACCCAGG	5'-CAAACAATCAGCATAAGAACACG	TGT	4.3	simple	179
67	mgssr67	5'-GAGAACTAGTGAGAAGAGAAGAGGG	5'-CGAGAGTAGGGGATGTTGCC	AAG	6.3	simple	198
68	mgssr68	5'-GGACGGTCTGAACCTTCTCC	5'-TTGAACGTATAACCACATTGTGC	ATC	53.3	simple	418
69	mgssr69	5'-TTGGTGTGTTTTGAAATGGAGG	5'-AACATTGGATTGCAACGTCC	AATAA	5.4	simple	167
70	mgssr70	5'-ATCTGGACTATGGGCTTGCG	5'-AAATTTGGGAGGTGCACTGG	GAA	5	simple	301
71	mgssr71	5'-GATCTGGACTATAGAGAGAAACCG	5'-GTGTGTGGAATAGCTCTGGACG	ATCT	4	simple	208
72	mgssr72	5'-TTGAGAGGGATGAGAGAAGTGG	5'-ATAACTCCCCTTCAAGCTGG	TA	14.5	simple	245
73	mgssr73	5'-AAAGAAAGGGAATAGGGAGG	5'-CTCACTTTTTCTCTTTCTCACACC	AG	9.5	simple	175
74	mgssr74	5'-GATGCACCAAACCCTTCACC	5'-ATGCCAACGACACAACCACC	TTC	6.7	simple	575
75	mgssr75	5'-GGAACTTGATTAGGACGTGTGG	5'-CCAACCAACCAAACAATGCC	ATG	6.3	simple	215
76	mgssr76	5'-TCTGGACTAAGGTGATCATGGG	5'-TGAGTTTCTTGTATTCCTAAGGG	AAGAG	40.4	simple	576
77	mgssr77	5'-GAAGAGACCGTGGGTGGTGC	5'-GGTGCCCCATCAGAAACTCC	GGC	6.3	simple	209
78	mgssr78	5'-TGTGTAGGTGTGTCTATGTTTGGG	5'-AAACCCCCACACATACCACC	TG	55.5	simple	205
79	mgssr79	5'-CAATGGCGATGAGACATGGG	5'-CCTGTCATAGTAGGTCCTAATCCC	GAA	6.3	simple	281
80	mgssr80	5'-TGTTCAAGCATTCACATAAGCG	5'-AAGGTTCTTTTGTACTGGGAGG	AATT	6	simple	224
81	mgssr81	5'-CCAAAAGGTTACGTCTTGCC	5'-CTCCTAAGGAATTTGCAGACC	AG	7.5	simple	200
82	mgssr82	5'-CAGAAGAGAGAGGAGGATGGG	5'-TAGATCTCTCTATATTGCCAGCC	AG	23.5	simple	248
83	mgssr83	5'-TGAGCAACGAAGAATGGAGG	5'-CAGCTTCCTCTTCTTCTCGG	GAA	6	simple	170
84	mgssr84	5'-GGGCTTAGATCTGGACTATGGG	5'-CGGATACATCGTGTTTCAACACC	TTA	9	simple	205
85	mgssr85	5'-GCAGATATTACGCACACACCG	5'-CTTCTTCTACTTCGTGTTCTCCC	AC	7.5	simple	433
86	mgssr86	5'-TTGGTGTGTTTTGAAATGGAGG	5'-AACATTGGATTGCAACGTCC	AATAA	5.4	simple	167
87	mgssr87	5'-GCCAAAACAGGTAGACATGGG	5'-CATCAAATTTGTATAGGCTGCG	AC	13.5	simple	199
88	mgssr88	5'-TCTGGACTACTCTGTCTGTGAGTCG	5'-GACACACACACACACACAGACACCC	TG	35	simple	163
89	mgssr89	5'-TGGACGATGGATGAGTTCACC	5'-TCCTCATCTCCAGCCTCAGC	GAT	8.7	simple	149
90	mgssr90	5'-TAGTGGTGGTTGCGACTGCG	5'-AACCACCACCATTGCTACCG	TGG	8.7	simple	424
91	mgssr91	5'-GTGAGCATGAACTTGAGTTGAGG	5'-GCAAACTTCTTTATCATGAGGC	GAT	6.7	simple	166

92	mgssr92	5'-CTTCAAGATTTACCAATGCTCC	5'-ATCAGTAGATGCCCTATCAGTCC	GAT	7	simple	190
93	mgssr93	5'-GCAGATATCCGCAGATACCC	5'-GACCATCTAACCAATTCAACCC	TAAAA	4.2	simple	172
94	mgssr94	5'-GAAGTGGTCATGAAAGGGTGC	5'-CTTGCTTAGATCTGGACTATCTCC	AGA	4.7	simple	148
95	mgssr95	5'-AGATGGAGTTTTTATTGGACAGG	5'-TCTTCATCTTCCCCCTCAGC	AGA	5	simple	183
96	mgssr96	5'-GAAGAGAACTAGAATTTGGAGCC	5'-AAGGAATTTGGAACATTCCC	AAGAGAG	42.3	compound	463
97	mgssr97	5'-TTTGCAATTGTGTGTAGTTGGG	5'-AACAGAAACACACACACATGTGTGC	TG	27	simple	282
98	mgssr98	5'-AGCTGGTTTTCTTGTGAACTGG	5'-CAAAACAATTAGCCATCACTCC	TC	6.5	simple	128
99	mgssr99	5'-GGGTTTGTAGCTATGGCTGC	5'-CTGGCAGAACCTCATGTGACG	TGC	4	simple	282
100	mgssr100	5'-GAAAAGTTTCTTGTTAGGAAGGG	5'-GGAGATGAAACTCGACACAGGG	GA	7	simple	146
101	mgssr101	5'-AGAGTGATTTGTGAAAGCAGAGG	5'-CATGAGGGTTGAACCCGTGG	AAATA	6.8	simple	164
102	mgssr102	5'-GAGGTCTTTGGACTCTCTAGCC	5'-AAATTGATGTGGCTAAGTCCC	AATTTT	5	simple	157
103	mgssr103	5'-AGAGAGAGAAGAAAGAATAGAGCG	5'-ATCTGGACTATGGAAAACCTCC	AAG	36.3	simple	301
104	mgssr104	5'-TGTTCAAGCATTCACATAAGCG	5'-AAGGTTCTTTTGTACTGGGAGG	AATT	6	simple	224
105	mgssr105	5'-CAGTATTTCTGAACAAGTTCCTGG	5'-GGCATCATATCCATAGTTAGCG	GATAGTGAC	6.4	compound	181
106	mgssr106	5'-AGGCGATGATGGTCTTACCG	5'-CCACCACCACATGTGCCACC	TGG	18.3	simple	226
107	mgssr107	5'-CTTCTTCGCTTTAGATCTCGG	5'-GGTAACCAGGACCGTAACCG	GGGTTTA	33.4	simple	289
108	mgssr108	5'-AAGCAGCTAAAAATCACCAGG	5'-ACAGTAGGATGCCTCCCAGC	AAG	18.3	simple	227
109	mgssr109	5'-AGCAAGCAAGGATAGAAGAGG	5'-TTCTTTTGTAGTCTCTCTTCCTGC	AGA	6	simple	169
110	mgssr110	5'-TTCGATTCTCTTGCTAGATCTGG	5'-CAAACACACATACACAAACATACGC	TG	14.5	simple	258
111	mgssr111	5'-AGGATAGAGAGATTAAAGAGAGAGC	5'-TTGTAAAAGTAGGAGATGTTGCC	AGAGA	6	simple	288
112	mgssr112	5'-CGAAAATGAAACAATGACTGCC	5'-CGTATAAGTATACGGCACGACG	AG	8.5	simple	270
113	mgssr113	5'-CCATGTTGTATCCGGTTACACG	5'-GCAAAGGCAGCACCTACAGC	TCA	3.3	simple	176
114	mgssr114	5'-GCATGAGAAGAAAGTTTGTGCC	5'-TGGGTTCTATGGTTGGAGGC	AGAGA	29.2	simple	405
115	mgssr115	5'-CGTTCAAACTATTGAAAGTGTGG	5'-AGGAGCTGCAACACTCACTGG	TGA	6	simple	172
116	mgssr116	5'-GGGAAATAAGTTCAAGTTGGC	5'-CTAATCAGTAGGTGTTGATACTCCC	AAG	6.7	simple	160
117	mgssr117	5'-GGAACCATCATTCCAGACTCC	5'-GAAACTTGAGACTGTTCCTCGG	CAA	5	simple	243
118	mgssr118	5'-TGGGCGTGAGTTAGTGTGTGC	5'-ACATATCACCCACATAAACACACG	TG	12	simple	197
119	mgssr119	5'-CTCATGAAGTTCTTGAATGAGGG	5'-GCTCTTTTTCCTTTTCCTCC	AG	28.5	simple	292
120	mgssr120	5'-CGTACCAACTCAAAAACAGGC	5'-TGAAAGAGGAGCTAAAGAGGG	TA	5.5	simple	345
121	mgssr121	5'-TTGGACTAGCTCACTTTTCCG	5'-TTAGCATCAATGATAGAAGTCGG	TA	5.5	simple	187
122	mgssr122	5'-GACTAATTGCCCTGTTTCCC	5'-CAGGATCCTCATCCTCCACC	GT	5.5	simple	190
123	mgssr123	5'-TTCGATCTCTTGCTTAGATCTGG	5'-TCCAAGGCAAAGACAAACGC	GT	35.5	simple	297
124	mgssr124	5'-GATTTTGTTCCTAGTGGCAGC	5'-ACTCCATTTACCTTCAAAGCC	TG	5	simple	246