

## Development of SSR markers in mung bean, *Vigna radiata* (L.) Wilczek using *in silico* methods

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### ABSTRACT

Nucleotide sequences available in public database provide a cost effective and valuable source for the development of molecular markers. In this study, the nucleotide sequence database available in National Centre for Biotechnology Information (NCBI) is utilized to identify and develop SSR markers in mungbean (*Vigna radiata*). A total of 803 genomic sequences, 829 EST sequences and 82 GSS sequences were downloaded from NCBI. Eight hundred and forty two SSRs from genomic sequences, 240 SSRs from EST sequences and 60 SSRs from GSS sequences were obtained using SSRIT tool. Primers pairs were successfully designed for 109 SSR motifs from genomic sequence, 110 SSR motifs from EST sequence and 25 SSR motifs from GSS sequences using Primer3 (<http://frodo.wi.mit.edu>) software. Fifteen SSR primers were finally characterized and validated in 24 mungbean and six urd bean accessions.

**Keywords:** EST, GSS, NCBI, SSR MARKER, SSRIT

Mungbean (*Vigna radiata* L. Wilczek) is an important pulse crop in developing countries of Asia, Africa and Latin America, where it is consumed as dry seeds, fresh green pods (Karuppanapandian *et al.*, 2006). Mungbean serves as vital source of vegetable protein (19.1-28.3%), mineral (0.18-0.21%) and vitamins. It is native of India-Burma and is cultivated extensively in Asia (Khattak *et al.*, 2007). India is the leading mungbean cultivator, covers up to 55% of the total world acreage and 45% of total production (Rishi, 2009). Molecular markers are indispensable for genomic study. Among various marker systems such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Sequence Tagged Sites (STSs) and Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSRs) have occupied a pivotal place because of their reproducibility, multiallelic nature, codominant inheritance, relative abundance and good genetic coverage. SSRs are clusters of short tandem repeated nucleotide bases distributed throughout the genome. Major features that made SSRs very popular are their abundant distribution in the genomes examined to date and their hyper variable nature (Toth *et al.*, 2000). Production of SSR markers can be achieved by methods such as database searching, cross-species amplification, screening genomic libraries and screening of RAPD amplicons. The traditional method of SSR marker development involves construction of SSR-enriched library, cloning and sequencing, which is costly and labour intensive (Kalia *et al.*, 2011).

With this background of knowledge, the present investigation was taken up with the aim to design primers for SSR markers isolated from *Vigna radiata* genomic, EST and GSS sequences using *in silico* techniques.

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### MATERIALS AND METHODS

Experiment was conducted in laboratory of Centre of Plant Molecular Biology (CPMB), Tamil Nadu Agricultural University (TNAU), Coimbatore.

#### Retrieval of nucleotide sequences from NCBI database

Nucleotide sequences of *Vigna radiata* variety *radiata* are freely available at NCBI website (<http://www.ncbi.nlm.nih.gov>). All genomic, EST and Genomic Survey Sequences of *Vigna radiata* available at NCBI database were obtained.

#### SSR mining with SSRIT tool

This tool finds all perfect possible SSR present in sequence submitted. Sequences obtained from the NCBI database were submitted in this software. Maximum repeat motif was given heptameric repeat and minimum repeat motif was given two.

#### Primer designing using PRIMER3 software

SSR primers were designed using primer 3 (<http://frodo.wi.mit.edu>) software. Parameters selected were GC content from 45 to 60 %, SSR repeats were marked as target region, product size ranges from 300 to 500bp, primer length from 18 to 25 nucleotides and melting temperature of (50 to 65)<sup>0</sup>C. A general rule followed by most primer design programs is to bracket the G/C content of primers to between 40- 50 %. A G-C pairing involves three hydrogen bonds versus two for an A-T pair, where an optimal balance of GC content enables stable specific binding, yet efficient melting at the same time. The primer melting temperature is a straightforward estimation of a DNA-DNA hybrid stability and critical in determining the annealing temperature. AT too high will result in insufficient primer template hybridization and therefore, low PCR product yield. non-specific products caused by a higher number of

base pair mis matches, where mismatch tolerance has been found to have the strongest influence on PCR specificity. Short 8-12mer oligo nucleotides, which have multiple annealing sites, are used in a Greedy algorithm to minimize the total number of primers needed for applications, where all the target sequences are known (Mann *et al.*, 2009).

**Fast PCR analysis**

FastPCR is freeware software. Primers designed were analysed in this software. To analyze pre designed primers click on the Primer Test option given in the software. Paste or type the primer or primers sequence (s) at any TAB Editors. The programme will immediately show primer characteristics its length in bases, melting temperature, CG% content, molecular weight, the extinction coefficient (e260), nmol per one OD, the mass - µg per one OD, linguistic complexity (%) and primer quality. If the primer is self-complementary, the program will show a picture of where this self-complementarity happens. A self-priming ability will also be detected and shown by the program (Kalendar *et al.*, 2011)

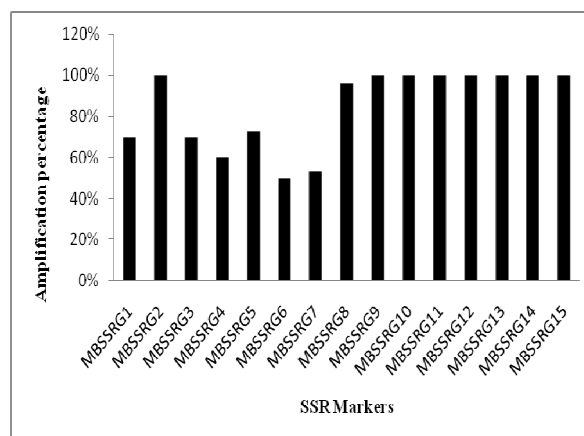
**PCR amplification of mungbean and urbean accessions**

SSR Primers designed using *in silico* methods were checked on mungbean and urbean accessions. Mungbean and urbean accessions were obtained from Department of Pulse at Tamil Nadu Agricultural University Coimbatore. Twenty four mungbean and six urbean accessions were sown in pots and genomic DNA isolated from 15 days old mungbean and urbean seedling following the modified protocol of Karupandiyan *et al.* (2006). The quality and quantity of DNA checked by agarose gel electrophoresis and nanodrop spectrophotometer. The final concentration to do PCR was adjusted to 25ng µl<sup>-1</sup>. PCR was taken as confirmatory tool to check it. About 50 to 100ng of DNA were used as a template. The reaction was carried in a total reaction volume of 15µl containing DNA 25 ng µL<sup>-1</sup>, 10X assay buffer, Primer (10µm), dNTPs (2.5 mM) (Bangalore Genei Ltd., India), Taq polymerase (3 units µL<sup>-1</sup>) (Bangalore Genei Ltd., India) and Sterile distilled H<sub>2</sub>O. The amplification was carried out in an Eppendorf master cycler. Agarose gel (3%) electrophoresis was performed to separate the amplified products.

**RESULTS AND DISCUSSION**

The present study was focused on the development of SSR markers specific for mungbean genotype. All genomic, EST and GSS sequences were obtained from NCBI database. It was found that there were 803 genomic sequences, 829 EST sequences and

82 GSS sequences present in *Vigna radiata* genome. All genomic, EST and GSS sequences were submitted in SSRIT tool. SSRIT tool scrutinizes all SSR presents in submitted sequences. Maximum motif length was given heptamers and minimum number of repeat was given two. Hence, this tool searched all di-nucleotide to hepta-nucleotide repeats which was at least two times repeated in a submitted sequence. SSRs which were more than or equal to ten nucleotides in length were selected for primer designing. 842 SSR repeats were obtained from genomic sequences. 242 SSR repeats were obtained from EST sequences. 60 SSR repeats motifs were present in GSS sequences. All repeat motifs do not function as SSR markers and primer designing for all repeats is not possible since primer designing depends



upon flanking sequences.

**Fig. 1: Amplification percentage of different SSR markers produced from mungbean genotype**

Thus, only selected repeats were taken to design primers. SSR primers were designed by Primer 3 (<http://frodo.wi.mit.edu>) software. 109 SSR primers were designed from genomic sequences, 110 SSR primers were designed from EST sequences and 25 SSR primers were designed from GSS sequences. SSR primers designed from genomic sequences, EST sequences and GSS sequences are respectively listed in table 1, 2 and 3. 15 primers from genomic sequences were checked on 24 mungbean and six urbean accessions. Primers details given in table 4. Amplification percentage of 15 primers is given in fig.1. Allelic variation was obtained from primers for seven SSR namely MBSSRG1, MBSSRG2, MBSSRG10, MBSSRG11, MBSSRG12, MBSSRG13 and MBSSRG14. Amplification by MBSSRG10 given in fig. 2.

**Table 1: SSR primers designed from genomic sequences**

Gen bank no.	Primer sequences (5'-3')	Tm (°C)	GC%	motif	No. of repeats	Product size(bp)
gii45331284 gb AY485988.1 -132	F:GGTGTGTGTCGTGTGGTTTT R:CATCGCTGAATCTACGACCA	61.00 59.82	50.00 50.00	caccga	2	327
gii38045974 gb AY437639.1 -6	F:CAGCTTCTTGTCTTGCCTCTT R:TTGACGAGGCAATAGCAGGT	60.19 60.80	45.45 50.00	ta	8	301
gii2502086 gb AF022926.1 -69	F:GTGGGGAAACCGGAATATCT R:ACAGGCAAGACCAGAGGAGA	60.02 59.99	50.00 55.00	tcaga	2	364
gii1478369 gb S81594.1 -39	F:GGGACTGTAAATGCGGTCACT R:GTCTCACTTGGCCATCCTC	60.00 60.48	55.00 55.00	gatga	2	355
gii1184120 gb U20808.1 VRU20808-87	F:TGATGGTGAATTTGCTGGAGA R:ATGCTGGAAGATCCAAAGTC	60.20 59.69	45.00 47.62	ctatttc	2	322
gii1141783 gb U31211.1 VRU31211-12	F:GTTGAGGCTCAGCAACACCT R:CGACACACATGACACCTTGA	60.45 59.10	55.00 50.00	ac	5	347
gii1006804 gb U34986.1 VRU34986-105	F:CATGAACGGTTGAAGACCT R:CCAAATGGATAGAGTGTCTCGT	59.97 58.58	50.00 45.45	tattac	2	333
gii967124 gb U08140.1 VRU08140-125	F:GGCCTAGACAACCAGGCATA R:TATAGTGGCCCTCTGGATG	60.10 59.91	55.00 55.00	gggaca	2	352
gii951322 gb U31467.1 VRU31467-127	F:ATTTCCGAGGAGCAACCTC R:CCTCCCAACACCTTTTCTT	60.58 60.33	50.00 50.00	taaac	2	304
gii849135 gb U26709.1 VRU26709-119	F:GTTCTCGCATCGGATCTC R:AGGGCTTGTGTCCGTAAC	59.78 60.04	50.00 50.00	atggc	2	306
gii506851 gb L20507.1 VIRCALMODU-36	F:TCGATCGAAGAACTCGAAC R:AATACCCGGAATGCCTCTTT	58.02 59.80	45.00 45.00	aagaa	2	344
gii506849 gb L20691.1 VIRCALMOD-40	F:CAACTGAGGCAGAGTTGCAG R:GTCTCACTTGGCCATCCTC	59.77 60.48	55.00 55.00	gatga	2	324
gii458337 gb U06046.1 VRU06046-75	F:CTGGGGTTTCTTTGAGTTGG R:GGTACCCTTCTCCAGTCCA	59.56 58.99	50.00 55.00	tcagt	2	338
gii295447 gb L07843.1 VIRNADPHP4-153	F:TAGCCCTCTCTCTCTCTCT R:TTCTCTCTCTCTCTCTCTCA	59.53 59.73	60.00 50.00	caacta	2	390
gii169324 gb L07634.1 PHVC4HYDRO-117	F:ACCGCAACCTCACTCAACTC R:TCTTCTGACGTCTCCAC	60.31 59.81	55.00 57.89	cccga	2	327
gii189169789 gb EU239689.2 -100	F:GGAATGGCACCTATCAATGG R:CCCAAACAACAATGTCGTAG	60.16 60.00	50.00 50.00	gtggg	2	314
gii9587210 gb AF279252.1 -118	F:CCCTGGAGATGGCAGAGTAA R:TTGATCTACGCTGAGCTTCC	60.21 58.20	55.00 50.00	agaca	2	315
gii9587204 gb AF279249.1 -45	F:TTCAAGGCTGGGTCTCAGAT R:CAGTGACAATGGCTTGAACG	59.80 60.30	50.00 50.00	ggtga	2	313
gii8954297 gb AF139470.2 -52	F:TGAACAAGGGTACCCAGGAG R:CGGTGGCTACATTAGAGTACTGA	59.96 58.49	55.00 47.83	caaat	2	355
gii8954296 gb AF139469.2 -45	F:TCTCCTCTCCAGCTTACGAA R:GCGTCTTATGGCTCAACTC	60.14 59.84	52.38 55.00	gtgccg	2	304
gii8954294 gb AF139468.2 -38	F:TCCCACCAATCTTCCAAGC R:CTTCGCTAGTTGTCGAACC	59.89 60.83	50.00 55.00	aca	5	367
gii8954288 gb AF139464.2 -85	F:TGGTGTGTGCTTGCTCAGAC R:GCACAACCTCAGCAAAAAGGTG	60.03 59.49	50.00 50.00	gcaaa	2	344
gii7682676 gb AF229794.1 -114	F:GAAAGCAGGCTCTATGTTTC R:AGACCAACAGCCATTTGAGC	59.98 60.26	55.00 50.00	tgcaa	2	312
gii6979535 gb AF195806.1 -95	F:GGTTTGGCTCTGTTCTGTC R:GCGTCTTATGGCTGAGGTTT	59.86 59.34	50.00 50.00	tccac	2	307
gii5305365 gb AF071550.1 -405	F:AGAAGACTGTGGGAACAGTGG R:ACGGCCACCAGAAATAGTCAC	59.21 60.00	52.38 55.00	tgtaaag	2	362
gii9587206 gb AF279250.1 -43	F:CGTGGAGGTTTACCGTATTG R:CGGTGGTAGTTTCCCACTGT	60.24 59.88	55.00 55.00	aaatt	2	325
gii8954291 gb AF139466.2 -61	F:CCAAGCACCAACTTCTCA R:TCTGCTCTGGTCCGATGAT	59.87 60.47	50.00 50.00	ttcggg	2	386
gii269980508 gb FJ857948.1 -37	F:CGTCTCTCTGCTCTCTCA R:GTCACTGAAGCGGTGATTT	60.95 60.12	55.00 50.00	ctt	4	358
gii16930801 gb AF441854.1 -18	F:GCTTGGCAATCTTGGTAGA R:AAAAGGTGCTAACGGCAGTG	60.21 60.30	50.00 50.00	actttt	2	303
gii13682803 gb AF126871.2 -83	F:CAGGTTGTGAGTATCCAAAGC R:AGGATTCATCGAGAGTAGTCA	60.71 55.64	52.38 40.91	tcttg	3	326
gii9587208 gb AF279251.1 -74	F:CCAAGCCTAACAAAATCAGG R:AAGGATTCATCGAGAGTAGTCA	57.37 55.64	45.00 40.91	tcttg	3	311
gii7682679 gb AF229795.1 -115	F:TGAAGGAGGTACGATCTGG R:TTGCAGCCAGTTTGTGTAG	60.07 59.90	55.00 50.00	tgcaa	2	338
gii7025484 gb AF229849.1 -53	F:GCTGCTGATTTGATCCCTGT R:GCCAGAGAAGAAATGGAATGC	60.23 59.78	50.00 50.00	tgtaa	3	330
gii158251952 gb EF990627.1 -90	F:CAACTCCGCAATATCACT R:AGAAGGAGGGTGTGGGTTT	57.70 59.83	45.00 50.00	aatac	2	327
gii158251950 gb EF990626.1 -87	F:AACCAACACCTCTCTCTT R:CCATGCTGCTGTGTCTCTC	59.83 59.58	50.00 55.00	aacgac	2	379
gii162296029 gb EU288914.1 -21	F:CGTGACCATCGAGTCTTGA R:GCTTAACTCAGCGGGTAGC	59.83 59.14	50.00 55.00	tcagg	2	337
gii90969278 gb DQ445950.1 -118	F:CCACGACTGATCCAGAAAAGG R:CGCTACCCCAAAATACCAAAA	60.65 59.83	55.00 45.00	ttctaa	2	367
gii90968745 gb DQ445738.1 -28	F:CAAACCAATCCGACTCAGC R:GCGTCAAAGACTCGATGGT	59.23 60.26	52.63 50.00	ggag	3	314
gii7211426 gb AF156667.1 -133	F:CTAGTCCGAGCTGGTGGAG R:TCTCCGTAGCCTGTCTTTC	60.01 59.43	60.00 55.00	agaag	2	371
gii6934187 gb AF143208.1 -83	F:GCAGCAACAACAATCTCTCAC R:GCCACAGAAAGCTATTGTA	59.30 59.87	50.00 50.00	tgga	2	327
gii259019991 gb GQ893027.1 -446	F:TTCTCACTCCACCCAGAAGC R:CCTCGTGTACCAGTTCAAA	60.09 59.72	55.00 50.00	ta	12	302
gii223886027 gb FJ591131.1 -6	F:CAGCTTCTTGTCTGTCTCTT R:AGTTGACGAGGCAATAGCAG	60.19 58.13	45.45 50.00	ta	9	301
gii251831253 gb GQ227550.1 -185	F:CTCAGGCAATGACGTTCC R:AGCTCTTCTGATCTGGGTTG	60.40 57.03	52.63 50.00	ccatt	2	391
gii238915390 gb FJ883469.1 -23	F:CCCTTCTGTCAAGGATCGAA R:AAGGATGCGGTTAAAGGGTTC	60.19 60.32	50.00 50.00	ggcaag	2	346
gii238915388 gb FJ883468.1 -26	F:CCCTTCTGTCAAGGATCGAA R:GGTGAAGGGTTCAAAGTCCA	60.19 59.94	50.00 50.00	ggcaag	2	338

Table 2: SSR primers designed from EST sequences

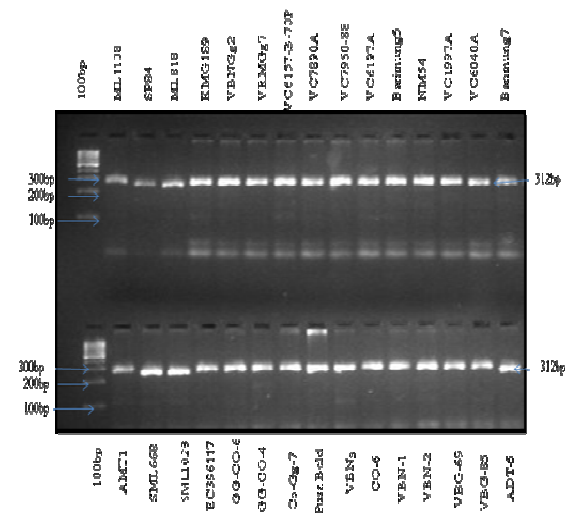
Gen bank no.	Primer sequences(5'-3')	Tm(°C)	GC%	Motif	No. of repeats	Product size (bp)
gi 213645856 gb AM910789.1 AM910789-39	F:CCAAGCCAACAGAGAGAAG R:CTCCTTCACATCACGGACAA	59.98 59.68	55.00	tgctct	2	308
gi 186877713 gb AM696683.1 AM696683-26	F:GACAGGAGCCAGCAAATGAT R:AAGGAAGGCTGCTCAGGAT	60.23 60.35	50.00 50.00	ccaaa	2	347
gi 186875963 gb AM696658.1 AM696658-17	F:GCACGTGTCAACAACCTTTGG R:AGAGGCTTGCTGAGCCTTTG	60.20 62.11	50.00 55.00	aaaat	2	348
gi 186835460 gb AM696644.1 AM696644-15	F:CCGTGGATTGGTTCCAGTAT R:TACTCGCCACGATGGTAAGG	59.67 61.04	50.00 55.00	Gccaag	2	376
gi 186835453 gb AM696637.1 AM696637-22	F:GGCTGGTTTCTTGAACCTGGA R:ACATGGGATGAGCCAGAAGT	60.23 59.54	50.00 50.00	ttaat	2	301
gi 186834740 gb AM696633.1 AM696633-22	F:TGCCTACGCTGGAGAGTAT R:CAGTCGAGACCCAGAGACAAA	59.86 60.02	55.00 55.00	aatcag	2	377
gi 186834002 gb AM696613.1 AM696613-19	F:TCAGAATGCGCTGGTAACAC R:TAGACCAGCTCGACAACAT	59.87 59.47	50.00 50.00	tgagg	2	323
gi 186833259 gb AM696592.1 AM696592-19	F:CGGTGAGGAAGTGAGGATA R:CCGCCATAAGGATATGGACT	60.07 58.88	55.00 50.00	atgaga	2	305
gi 186830309 gb AM696538.1 AM696538-18	F:GATCTCAAGGGTCAGCCAAA R:TCCACCCACAATGAGAAACA	60.20 59.94	50.00 45.00	cttgg	2	347
gi 186830308 gb AM696537.1 AM696537-14	F:TGAACCAACCAACCTACCA R:CAAAAAGGCATACAAGGAGACG	59.88 60.99	47.62 45.45	catcc	2	243
gi 186830306 gb AM696535.1 AM696535-32	F:GGGTCAAGTGCAGAGTCAAT R:GCGCCCAAAAATTTGTAAC	60.12 60.36	55.00 45.00	ttttac	2	361
gi 186830304 gb AM696533.1 AM696533-23	F:CTCAAGCGTTGATCAGATGG R:ATCATCTGGGTTGGGATCTG	59.39 59.74	50.00 50.00	gcct	2	367
gi 186795581 gb AM696516.1 AM696516-18	F:GGTTGCTTAATGCCACAGGA R:TATGCTTCCAGTCTTGCCAC	61.03 59.87	50.00 50.00	ctggtt	2	325
gi 186830308 gb AM696537.1 AM696537-14	F:CTGAACCAACCAACCTACCA R:CAAAAAGGCATACAAGGAGACG	59.88 60.99	47.62 45.45	catcc	2	243
gi 186830306 gb AM696535.1 AM696535-32	F:GGTGGTCAACCAACCCACAT R:CCCCCTCGACTCAATTTGT	59.08 59.91	50.00 52.63	ttttac	2	350
gi 186830304 gb AM696533.1 AM696533-23	F:CTCAAGCGTTGATCAGATGG R:ATCATCTGGGTTGGGATCTG	59.39 59.74	50.00 50.00	gcct	2	367
gi 186795581 gb AM696516.1 AM696516-18	F:GGTTGCTTAATGCCACAGGA R:GGGTACCCCTTTGTTTAGGG	61.03 59.62	50.00 52.38	ctggtt	2	209
gi 186794691 gb AM696508.1 AM696508-22	F:CTCTAATGGACCACAGAGCAGA R:GGATCTGGAATTTGGGAAAG	59.50 60.63	50.00 50.00	accaca	2	311
gi 186793793 gb AM696491.1 AM696491-15	F:AAACCTGCATGACCACCTT R:GCTTAGGCACTTGAGGATGG	60.43 59.84	50.00 55.00	ttgctg	2	228
gi 186793789 gb AM696487.1 AM696487-9	F:TCACCAAGCAGAGAGGGTTT R:GCCAGTTGAACAGGTTGCTT	59.84 60.30	50.00 50.00	accaa	2	215
gi 186791996 gb AM696457.1 AM696457-18	F:GCCATTAATCCCATGCTTA R:GCCTGAAAACCTAGAGAATATAAAGA	59.76 59.66	45.00 37.04	ggatg	2	304
gi 186791110 gb AM696453.1 AM696453-23	F:CACAGGGAGAGTGATGCTGA R:CCAATGGAAAGTTGACCAG	59.98 60.10	55.00 52.63	agtga	2	323
gi 186789281 gb AM696419.1 AM696419-17	F:CTCCCTGATGCTCTAGATTTC R:CACCAAGACAAAGCGTTCC	59.33 60.67	50.00 50.00	Aagaga gaaca	2 2	334 391
gi 186789273 gb AM696411.1 AM696411-29	F:TGGCACAGTCACTGCTTTCT R:CGCTGCTATGAAAGAGCTT	59.62 59.75	50.00 50.00	aagag	2	303
gi 186729655 gb AM696395.1 AM696395-12	F:GCTAAATGCGGCTTCTIACC R:GGCTATTCTCAACCTGTTTGC	58.99 62.17	50.00 50.00	tcctgc	2	343
gi 186728696 gb AM696364.1 AM696364-38	F:TGGTTGACCGCAGCATAGT R:TGTGCTGCGTGACCTTAGTT	60.28 59.51	52.63 50.00	atgagga	2	307
gi 186727742 gb AM696345.1 AM696345-25	F:CCTACAGCACCAGAACCTT R:TCTGATCTCTGGCCTGCTCT	60.17 60.25	55.00 55.00	cagag	2	310
gi 186727249 gb AM696322.1 AM696322-22	F:GTGGTTCAGAAAACCCAGAG R:CAGCCTTTGCCACCAAGTATT	59.55 60.13	55.00 50.00	aga	6	342
gi 186727247 gb AM696320.1 AM696320-22	F:GGGCCAGTGACAAATGAGAG R:CACGACAGTTCAACCAAGCAT	60.66 59.75	55.00 50.00	ca	2	313
gi 186726319 gb AM696315.1 AM696315-9	F:CTTGACCCCTCAAGCTATT R:GAGGACAACCAACAGCTGAAC	59.34 59.70	50.00 55.00	tttcc	2	311
gi 186726315 gb AM696311.1 AM696311-24	F:CGCTCTTGGTTGCTATGTCA R:GAGTGGTGTGATGGCAAATG	60.01 59.97	50.00 50.00	gtatg	2	357
gi 183206217 gb AM696051.1 AM696051-29	F:AAGTGGTAGGACCTGGTGGA R:TTGGAATCTCTCCCTGCTC	59.42 59.36	55.00 50.00	aaagga	2	397
gi 183206214 gb AM696048.1 AM696048-42	F:GGGCAAGAAGAGGATCTGA R:CCAAGGGTAGAATGGGACAA	59.36 59.78	50.00 50.00	gaaaa	2	304
gi 183206213 gb AM696047.1 AM696047-35	F:TAGGTGGTTGGTTGGAGAG R:TTCAGAGGTTCCGACTTTGG	59.96 60.22	55.00 50.00	gatgtg	2	325
gi 183206210 gb AM696044.1 AM696044-49	F:CAGAAAAGGGCTTCGCATAAG R:CGAGATGTCTTCCACACT	59.97 60.11	50.00 55.00	acttc	2	394
gi 183206208 gb AM696042.1 AM696042-34	F:AGGATCAGGGTTGAGCATGT R:GCTACATGCAGTGGCAAGAA	59.54 60.02	50.00 50.00	ttggg	2	352
gi 183206206 gb AM696040.1 AM696040-37	F:CTCTACTGCATCGGTTGG R:TCTCACACCCAGGCTCTCT	59.31 59.83	55.00 55.00	agaatc	2	317
gi 183206205 gb AM696039.1 AM696039-33	F:TCATCAATCTGCGTCTGACC R:AGAACCAGCAAACCCAGGAT	59.79 60.88	50.00 50.00	gggtg	2	308
gi 183206202 gb AM696036.1 AM696036-28	F:GAGGCAACATCACCTCCTA R:TCATGGACCACCACTGAAT	60.07 61.21	55.00 50.00	gcttt	2	321
gi 183206201 gb AM696035.1 AM696035-26	F:CTGAAGGGTAGCCAGCAAG R:CAGCTACTGCAGTTTCCAGT	60.01 59.55	50.00 50.00	cccttt	2	323
gi 183206199 gb AM696033.1 AM696033-32	F:TCCCAATGGTTCCGGTTA R:TCTGGATTACTGGGCTTGA	59.70 60.59	50.00 50.00	gatgaa	2	370
gi 183206198 gb AM696032.1 AM696032-40	F:CACCCCTGTCCCTAAGAA R:TTTCTTCCCTCCACACT	59.90 60.48	57.89 55.00	attgt	2	332
gi 183206197 gb AM696031.1 AM696031-45	F:GCTGCACAGGAGTATGCTGA R:CCGAAAGCTATTACGGTCCA	60.17 60.21	55.00 50.00	catcaa	2	376
gi 183206195 gb AM696029.1 AM696029-32	F:ATCCACGCTTACTAGGCAT R:TCACACTGAAGCATCACAC	60.69 60.91	50.00 50.00			

**Table 3: SSR primers designed from genomic survey sequences**

Gen bank no.	Primer sequences(5'-3')	Tm (°C)	GC%	Motif	No. of repeats	Product size (bp)
gi 257367024 gb GS377372.1 GS377372-23	F: AGCTTGGCGTAATCATGGTC R: ACCAGAAAGCAAGCCGATCT	60.10 61.29	50.00 50.00	ttgcg	2	308
gi 166709893 gb ET203890.1 ET203890-28	F: GTCCTCGCGAATGCATCTA R: TACGAACACTTTCGCCACTG	59.92 59.90	52.63 50.00	gggtt	2	400
gi 149939382 gb ER896028.1 ER896028-27	F: TGATTCGAGCTCGGTACCTC R: CGATTCAAACGTCCGTGAG	60.36 60.25	55.00 52.63	aaaat	2	543
gi 149939381 gb ER896027.1 ER896027-41	F: GTCCTCGCGAATGCATCTA R: GTTCTTTCGCGAGAGAGTT	59.92 59.76	52.63 50.00	aataaat	2	307
gi 149939380 gb ER896026.1 ER896026-39	F: AATAAAGGGGGACACATGC R: TGGGGAGAATAACTCTGACTGG	60.94 60.49	50.00 50.00	aaccc	2	381
gi 149939378 gb ER896024.1 ER896024-29	F: ATAATGGGGACACATGC R: GGGGGATAATTGGGAGAATAGG	60.42 61.82	52.63 50.00	aaccc	2	350
gi 144925907 gb EI522402.1 EI522402-29	F: TAACCGACGCCTAGGTGATT R: GAGGCAGCTAGCAAATGGAG	59.59 60.12	50.00 55.00	cattt	2	381
gi 149939378 gb ER896024.1 ER896024-29	F: ATAATGGGGACACATGC R: GGGGGATAATTGGGAGAATAGG	60.42 61.82	52.63 50.00	aaccc	2	350
gi 144925907 gb EI522402.1 EI522402-30	F: TAACCGACGCCTAGGTGATT R: GAGGCAGCTAGCAAATGGAG	59.59 60.12	50.00 55.00	ttgtg	2	381
gi 8602614 gb AZ254294.1 AZ254294-26	F: TGTAACCTTGGCAACAACGAG R: CTGTACAGGGGTGTTAGCTTC	59.76 57.95	50.00 50.00	agttt	2	319
gi 8602604 gb AZ254289.1 AZ254289-23	F: TGAGGGATCCAAGTCTTTGC R: CACTGGCTTCCCCAATAA	60.20 60.84	50.00 52.63	agaacc	2	303
gi 8602600 gb AZ254287.1 AZ254287-37	F: CGTCATACTACTCCCAAT R: GCTGGCACAAGGGTACTA	60.61 60.13	52.38 55.00	tgcaa	2	312
gi 8602580 gb AZ254277.1 AZ254277-29	F: AGTGGGAGCAGGCTAAATGA R: AGAGTGCTCCAGCAAGCAAT	59.84 60.16	50.00 50.00	cattt	2	351
gi 8602569 gb AZ254272.1 AZ254272-27	F: CTGGAGAACAAGACGGTGGT R: CACCTGCCACTACAGAGAGC	60.15 58.62	55.00 60.00	tgctga	2	325
gi 8602559 gb AZ254267.1 AZ254267-22	F: CTTGATCAAACCTGCCTGCAA R: GCCGGAGTTTGAAGTCAAT	59.99 60.12	45.00 50.00	aacct	2	331
gi 8602535 gb AZ254255.1 AZ254255-27	F: GGTGTCATTAAGGGCATCT R: TCGATTCTCCTTTGACCAC	59.93 60.05	50.00 50.00	aagaa	2	368
gi 8602533 gb AZ254254.1 AZ254254-25	F: GCCAAGGTGCCAGATATGAG R: GGCATGCTAGCGAAACATTC	60.62 60.75	55.00 50.00	ttcttg	2	354
gi 8602527 gb AZ254251.1 AZ254251-30	F: TCCTCTCCTTCACTCGTTG R: AACACAGGCTACAGCTCAACC	60.38 59.42	55.00 52.38	tgaaa	2	398
gi 8602510 gb AZ254243.1 AZ254243-9	F: ATGAGCAAGGGCAAGTATG R: TTCCCAACAGCTCAGGTGT	60.10 59.31	50.00 50.00	tcaaag	2	172
gi 8602504 gb AZ254240.1 AZ254240-18	F: GAGCGTAGGCTTGTCTTTGAG R: CACGGGAGGTAGTACAAT	60.29 59.84	55.00 55.00	Accca	2	333
gi 8602502 gb AZ254239.1 AZ254239-25	F: CCAGTGTGGTGAATCTGA R: CCTCCAATGGATCCTCGTTA	59.52 59.89	50.00 50.00	ggtgacg	2	328
gi 8602497 gb AZ254237.1 AZ254237-29	F: TTGCCCTATCACCTTTCAC R: GTAGACCCGGGTTCCGAAT	59.93 61.09	50.00 57.89	tacag	2	365
gi 8602493 gb AZ254235.1 AZ254235-30	F: GTGCCCAACACACTTCTTT R: CTTGCCGTACAACCTCTTGA	60.01 58.92	50.00 50.00	actga	2	304
gi 8602488 gb AZ254233.1 AZ254233-20	F: GCACCACAATGCATCAACAC R: GAAGCCTGTAGACCTTGACTC	61.03 59.39	50.00 54.55	tgtag	2	451
gi 8602484 gb AZ254231.1 AZ254231-26	F: GGTGTTCTTTGTGACGTGGA R: AGCGTAATAAAGCGCCACAG	59.57 60.42	50.00 50.00	gccct	2	396

**Table 4: Primers checked on mungbean and urdbean accessions**

Marker	Gene bank no.	Primer sequences(5'-3')	Repeat motif	Product size (bp)	Tm(°C)	GC%
MBSSRG1	HQ148143.1	F:AATTGCAGAATCCCGTGAAC R: AAGAGCGTCTTTGCCTGTTT	(CGG) <sub>4</sub>	308	58.4	45
MBSSRG2	HQ148143.1	F: GTCGATGACCCAATCCAAT R: TGCGTTCAAAGACTCGATG	(TCCTC) <sub>2</sub>	330	58.4	45
MBSSRG3	AY900122.1	F: ATCTGACGAGAGCATGTGGA R: CTCCTCTTTCAGCACAATCA	(TTGGTG) <sub>2</sub>	325	58.4	50
MBSSRG4	AY900122.1	F: GAAGCGCATTCGTAAGTACA R: TACAACCGAAGACACGCAAG	(GAACA) <sub>2</sub>	326	58.4	50
MBSSRG5	AY683030.1	F: TGATGTGTTCCTCCCGAGTT R: AACAAAGTACCCGTTGCCAAG	(TATTC) <sub>2</sub>	307	58.4	50
MBSSRG6	AY233257.1	F: ACCTTCAGGCTTCAACAACG R: CGACGTAGAAACACACGATCA	(TGA) <sub>4</sub>	209	58.4	48
MBSSRG7	HQ148143.1	F: GTCGATGACCCAATCCAAT R: TTGCGTTCAAAGACTCGATG	(ACGAA) <sub>2</sub>	330	58.4	45
MBSSRG8	HQ148144.1	F: AATTGCAGAATCCCGTGAAC R: AAGAGCGTCTTTGCCTGTTT	(CGG) <sub>4</sub>	308	58.4	45
MBSSRG9	HQ148144.1	F: CGTAATGCGTCCATACCACA R: CCGATGCTCTTTTCATGGT	(CTCCT) <sub>2</sub>	383	59.4	47
MBSSRG10	HQ148144.1	F: CGCCTCCTCCTCTTCAG R: CCGATGCTCTTTTCATGGT	(ACGAA) <sub>2</sub>	312	61.4	54.1
MBSSRG11	HQ148144.1	F: AATTGCAGAATCCCGTGAAC R: AAGAGCGTCTTTGCCTGTTT	(CAATC) <sub>2</sub>	308	58.4	45
MBSSRG12	HQ148145.1	F: TTGCAGAATCCTGTGAACCA R: AAGAGCGTCTTTGCCTGTTT	(CGG) <sub>4</sub>	306	58.4	45
MBSSRG13	HQ148145.1	F: ATCATTGTGATGCCCAAAC R: AGGATTCTGCAATTCACACCA	(CTCCT) <sub>2</sub>	301	58.4	45
MBSSRG14	HQ148145.1	F: TTGCAGAATCCTGTGAACCA R: AAGAGCGTCTTTGCCTGTTT	(CAATC) <sub>2</sub>	306	58.4	45
MBSSRG15	HQ148145.1	F: ATCATTGTGATGCCCAAAC R: TTGCGTTCAAAGACTCGATG	(GGAGGG) <sub>2</sub>	327	58.4	45



**Fig. 2: Amplification by SSR primer MBSSRG10**

Hence from this study, it is evident that development of SSR markers using database searching is more cost effective and cheap in compare to the isolation of the same from genomic libraries and cross- species amplification. Bioinformatics approach produces good and more informative microsatellite markers in a very short span of time. There is a plenty number of crops which are playing very important role to meet our food security but genetic study on the development of SSR marker is lagging in such crops. However, using database searching and bioinformatics methods we can obtain nucleotide sequence of information which can be utilized to carry out genetic study on such crops. Hence, these *in silico* methods are playing very important role in contributing to the development and progress in the field of science and agriculture.

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