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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)^{0}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 urdbean accessions

SSR Primers designed using *in silico* methods were checked on mungbean and urdbean accessions. Mungbean and urdbean accessions were obtained from Department of Pulse at Tamil Nadu Agricultural University Coimbatore. Twenty four mungbean and six urdbean accessions were sown in pots and genomic DNA isolated from 15 days old mungbean and urdbean seedling following the modified protocol of Karuppandiyan *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^{-1}. 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^{-1}**,** 10X assay buffer, Primer (10µm), dNTPs (2.5 mM) (Bangalore Genei Ltd., India), Taq polymerase (3 units μL^{-1}) (Bangalore Genei Ltd., India) and Sterile distilled H_20. 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.

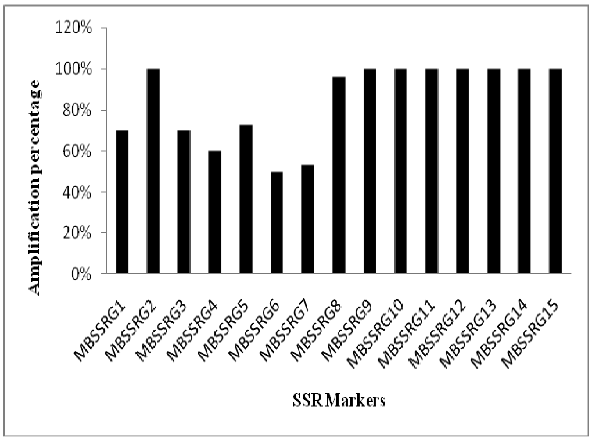

Fig. 1: Amplification percentage of different SSR markers produced from mungbean genotype

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 urdbean 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 (^{0}C)	GC%	motif	No. of repeats	Product size(bp)
gi|45331284|gb|AY485988.1|-132	F:GGTGTTGTCGCTGTGGTTTT R: CATCGCTGAATCTACGACCA	61.00 59.82	50.00 50.00	caccga	2	327
gi|38045974|gb|AY437639.1|-6	F: CAGCTTCTTGTTCTTGCTCCTT R: TTGACGAGGCAATAGCAGGT	60.19 60.80	45.45 50.00	ta	8	301
gi|2502086|gb|AF022926.1|-69	F: GTGGGGAAACCGGAATATCT R: ACAGGCAAGACCAGAGGAGA	60.02 59.99	50.00 55.00	tcaga	2	364
gi|1478369|gb|S81594.1|-39	F: GGGACTGTAATGCGGTCACT R: GTCCTCACTTGGCCATCATC	60.00 60.48	55.00 55.00	gatga	2	355
gi|1184120|gb|U20808.1|VRU20808-87	F: TGATGGTGATTTGCTGGAGA R: ATGCTGGAAGATCCAAAGTC	60.20 59.69	45.00 47.62	ctatttc	2	322
gi|1141783|gb|U31211.1|VRU31211-12	F: GTTGAGGCTCAGCAACACCT R: CGACACACATGACACCTTGA	60.45 59.10	55.00 50.00	ac	5	347
gi|1006804|gb|U34986.1|VRU34986-105	F: CATGAACGGGTTGAAGACCT R:CCAAATGGATAGAGTGTTCGTC	59.97 58.58	50.00 45.45	tattac	2	333
gi|967124|gb|U08140.1|VRU08140-125	F: GGCCTAGACAACCAGGCATA R: TATAGTGGCCCCTCTGGATG	60.10 59.91	55.00 55.00	gggaca	2	352
gi|951322|gb|U31467.1|VRU31467-127	F: ATTTCCGAAGGAGCAACCTC R: CCTTCCCAACACCCTTTCTT	60.58 60.33	50.00 50.00	taaaac	2	304
gi|849135|gb|U26709.1|VRU26709-119	F: GTTTCTCGCATCGGATCTTC R: AGGGCTTGTGTGTCCGTAAC	59.78 60.04	50.00 50.00	atggc	2	306
gi|506851|gb|L20507.1|VIRCALMODU-36	F: TCGATCGAAGAAACTCGAAC R: AATACCCGGAATGCCTCTTT	58.02 59.80	45.00 45.00	aagaa	2	344
gi|506849|gb|L20691.1|VIRCALMOD-40	F: CAACTGAGGCAGAGTTGCAG R: GTCCTCACTTGGCCATCATC	59.77 60.48	55.00 55.00	gatga	2	324
gi|458337|gb|U06046.1|VRU06046-75	F: CTGGGGTTTCTTTGAGTTGG R: GGTACCCTTTCTCCAGTCCA	59.56 58.99	50.00 55.00	tcagt	2	338
gi|295447|gb|L07843.1|VIRNADPHP4-153	F: TAGCCCCCTCTCTCTCCTCT R: TTCCTCTTCCTCCTCCATCA	59.53 59.73	60.00 50.00	caacta	2	390
gi|169324|gb|L07634.1|PHVC4HYDRO-117	F: ACCGCAACCTCACTCAACTC R: TCTTCCTGACGTCGTCCAC	60.31 59.81	55.00 57.89	cccgca	2	327
gi|189169789|gb|EU239689.2|-100	F: GGAATGGCACCTATCAATGG R: CCCAAACACAATGTCGTCAG	60.16 60.00	50.00 50.00	gtggg	2	314
gi|9587210|gb|AF279252.1|-118	F: CCCTGGAGATGGCAGAGTAA R: TTGATCTACGCTGAGCTTCC	60.21 58.20	55.00 50.00	agaca	2	315
gi|9587204|gb|AF279249.1|-45	F: TTCAAGGCTGGGTCTCAGAT R: CAGTGACAATGGCTTGAACG	59.80 60.30	50.00 50.00	ggtga	2	313
gi|8954297|gb|AF139470.2|-52	F: TGAACAAGGGTACCCAGGAG R:CGGTGGCTACATTAGAGTACTGA	59.96 58.49	55.00 47.83	caaatt	2	355
gi|8954296|gb|AF139469.2|-45	F: TCTCCTCTCCAGCTGTTACGA R: GCGTCCTTATGGCTCAACTC	60.14 59.84	52.38 55.00	gtgccg	2	304
gi|8954294|gb|AF139468.2|-38	F: TCCCACCAATCTATCCAAGC R: CTTCGCGTAGTTGTCGAACC	59.89 60.83	50.00 55.00	aca	5	367
gi|8954288|gb|AF139464.2|-85	F: TGGTGTTTGCTTGCTCAGAC R: GCACAACTCAGCAAAAGGTG	60.03 59.49	50.00 50.00	gcaaag	2	344
gi|7682676|gb|AF229794.1|-114	F: GCAAGCAGGCCTCTATGTTC R: AGACCAACAGCCATTTGAGC	59.98 60.26	55.00 50.00	tgcaa	2	312
gi|6979535|gb|AF195806.1|-95	F: GGTTTTGGCTCTGTTTCTGC R: GCGTCTTATGGCTGAGGTTT	59.86 59.34	50.00 50.00	tcccac	2	307
gi|5305365|gb|AF071550.1|-405	F:AGAAGACTGTGGGAACAGTGG R: ACGGCCACCAGAATAGTCAC	59.21 60.00	52.38 55.00	tgtaaag	2	362
gi|9587206|gb|AF279250.1|-43	F: CGTGGAGGGTTACCGTATTG R: CGGTGGTAGTTTCCCACTGT	60.24 59.88	55.00 55.00	aaatt	2	325
gi|8954291|gb|AF139466.2|-61	F:CCAAGCACCACAACTTCTCA R: TCTGTCCTGGTTCCGATGAT	59.87 60.47	50.00 50.00	ttcggg	2	386
gi|269980508|gb|FJ857948.1|-37	F: CGCTCCTTCTGCTTCTCTCA R: GTCACTGAAGGCGGTGATTT	60.95 60.12	55.00 50.00	ctt	4	358
gi|16930801|gb|AF441854.1|-18	F: GCTTGGCAATCCTTGGTAGA R: AAAAGGTGCTAACGGCAGTG	60.21 60.30	50.00 50.00	actttt	2	303
gi|13682803|gb|AF126871.2|-83	F: CAGGTTGTGAGTGATCCAAGC R: AGGATTCATCGAGAGTAGTCA	60.71 55.64	52.38 40.91	tcttg	3	326
gi|9587208|gb|AF279251.1|-74	F: CCAAGCCTAACAAAATCAGG R:AAGGATTCATCGAGAGTAGTCA	57.37 55.64	45.00 40.91	tcttg	3	311
gi|7682679|gb|AF229795.1|-115	F: TGAAGGGAGGTACGATCTGG R: TTGCAGCCCAGTTTGTGTAG	60.07 59.90	55.00 50.00	tgcaa	2	338
gi|7025484|gb|AF229849.1|-53	F: GCTGCTGATTTGATCCCTGT R: GCCAGAGAAGAATGGAATGC	60.23 59.78	50.00 50.00	tggaa	3	330
gi|158251952|gb|EF990627.1|-90	F: CAACTCCGCCAATATTCACT R: AGAAGGAGGGTGTTGGGTTT	57.70 59.83	45.00 50.00	aatac	2	327
gi|158251950|gb|EF990626.1|-87	F: AACCCAACACCCTCCTTCTT R: CCATGCTGCTGTTGTCTCTC	59.83 59.58	50.00 55.00	aacgac	2	379
gi|162296029|gb|EU288914.1|-21	F: CGTGACCATCGAGTCTTTGA R: GCTTAAACTCAGCGGGTAGC	59.83 59.14	50.00 55.00	tcagg	2	337
gi|90969278|gb|DQ445950.1|-118	F: CCACGACTGATCCAGAAAGG R: CGCTACCCCAAAATACCAAA	60.65 59.83	55.00 45.00	ttctaa	2	367
gi|90968745|gb|DQ445738.1|-28	F: CAAACCAATCCGACTCAGC R: GCGTTCAAAGACTCGATGGT	59.23 60.26	52.63 50.00	ggag	3	314
gi|7211426|gb|AF156667.1|-133	F: CTAGTTCCGAGCTGGTGGAG R: TCTCCCGTAGCCTGTCTTTC	60.01 59.43	60.00 55.00	agaag	2	371
gi|6934187|gb|AF143208.1|-83	F: GCAGCAACAAACATCCTCAC R: GCCACACGAAGCTCATTGTA	59.30 59.87	50.00 50.00	tggga	2	327
gi|259019991|gb|GQ893027.1|-446	F: TTCTCACTCCACCCCAGAAC R: CCTCGTGTCACCAGTTCAAA	60.09 59.72	55.00 50.00	ta	12	302
gi|223886027|gb|FJ591131.1|-6	F: CAGCTTCTTGTTCTTGCTCCTT R: AGTTGACGAGGCAATAGCAG	60.19 58.13	45.45 50.00	ta	9	301
gi|251831253|gb|GQ227550.1|-185	F: CTCAGGCAAATGACGTTCG R: AGCTCTTCTGATCTGGGTTG	60.40 57.03	52.63 50.00	cccatt	2	391
gi|238915390|gb|FJ883469.1|-23	F: CCCTTCTGTCAAGGATCGAA R: AAGGATGCGGTAAAGGGTTC	60.19 60.32	50.00 50.00	ggcaag	2	346
gi|238915388|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(^{0}C)	GC%	Motif	No. of repeats	Product size (bp)
gi|213645856|gb|AM910789.1|AM910789-39	F:CCAAGGCCAACAGAGAGAAG	59.98	55.00	tgtct	2	308
	R: CTCCTTCACATCACGGACAA	59.68	50.00			
gi|186877713|gb|AM696683.1|AM696683-26	F:GACAGGAGCCAGCAAATGAT	60.23	50.00	ccaaa	2	347
	R: AAGGAAGGCTGCTTCAGGAT	60.35	50.00			
gi|186875963|gb|AM696658.1|AM696658-17	F: GCACGTGTCAACAACTTTGG	60.20	50.00	aaaat	2	348
	R: AGAGGCTTGCTGAGCCTTTG	62.11	55.00			
gi|186835460|gb|AM696644.1|AM696644-15	F: CCGTGGATTGGTTCCAGTAT	59.67	50.00	Gccaag	2	376
	R: TACTCGCCACGATGGTAAGG	61.04	55.00			
gi|186835453|gb|AM696637.1|AM696637-22	F: GGCTGGTTTCTTGAACTGGA	60.23	50.00	ttaat	2	301
	R:ACATGGGATGAGCCAGAACT	59.54	50.00			
gi|186834740|gb|AM696633.1|AM696633-22	F: TGCCTACGCCTGGAGAGTAT	59.86	55.00	aatcag	2	377
	R:CAGTCGAGACCGAGACACAA	60.02	55.00			
gi|186834002|gb|AM696613.1|AM696613-19	F: TCAGAATGCGCTGGTAACAC	59.87	50.00	tgagg	2	323
	R: TAGACCAGCTCGCACAACAT	59.47	50.00			
gi|186833259|gb|AM696592.1|AM696592-19	F: CGGTGAGGAAGTGAGGATA	60.07	55.00	atgaga	2	305
	R: CCGCCATAAGGATATGGACT	58.88	50.00			
gi|186830309|gb|AM696538.1|AM696538-18	F: GATCTCAAGGGTCAGCCAAA	60.20	50.00	ctttg	2	347
	R: TCCACCCACAATGAGAAACA	59.94	45.00			
gi|186830308|gb|AM696537.1|AM696537-14	F: TGAACCAACCAAACCTACCA	59.88	47.62	catcc	2	243
	R:CAAAAAGGCATACAAGGAGACG	60.99	45.45			
gi|186830306|gb|AM696535.1|AM696535-32	F: GGGTCAGGTGCAGAGTCAAT	60.12	55.00	ttttac	2	361
	R: GCGCCCACAAAATTGTAAAC	60.36	45.00			
gi|186830304|gb|AM696533.1|AM696533-23	F: CTCAAGCGTTGATCAGATGG	59.39	50.00	gccct	2	367
	R: ATCATCTGGGTTGGGATCTG	59.74	50.00			
gi|186795581|gb|AM696516.1|AM696516-18	F: GGTTGCTTAATGCCACAGGA	61.03	50.00	ctggtt	2	325
	R: TATGCTTCCACGTCTTGCAC	59.87	50.00			
gi|186830308|gb|AM696537.1|AM696537-14	F:CTGAACCAACCAAACCTACCA	59.88	47.62	catcc		
	R:CAAAAAGGCATACAAGGAGACG	60.99	45.45		2	243
gi|186830306|gb|AM696535.1|AM696535-32	F: GGTGGTCATCACAACCACAT	59.08	50.00	ttttac	2	350
	R: CCCCCTCGACTCAATTTGT	59.91	52.63			
gi|186830304|gb|AM696533.1|AM696533-23	F: CTCAAGCGTTGATCAGATGG	59.39	50.00	gccct	2	367
	R: ATCATCTGGGTTGGGATCTG	59.74	50.00			
gi|186795581|gb|AM696516.1|AM696516-18	F: GGTTGCTTAATGCCACAGGA	61.03	50.00	ctggtt	2	209
	R:GGGTACCCTTTGTGTTTAGGG	59.62	52.38			
gi|186794691|gb|AM696508.1|AM696508-22	F:CTCTAATGGACCACAGAGCAGA	59.50	50.00	accaca	2	311
	R:GGATCTGGAATTGGGGAAAG	60.63	50.00			
gi|186793793|gb|AM696491.1|AM696491-15	F: AAACCTGCATGACCACACCT	60.43	50.00	ttgctg	2	228
	R: GCTTAGGCACTTGAGGATGG	59.84	55.00			
gi|186793789|gb|AM696487.1|AM696487-9	F: TCACCAAGCAGAGAGGGTTT	59.84	50.00	accaa	2	215
	R: GCCAGTTGAACAGGTTGCTT	60.30	50.00			
gi|186791996|gb|AM696457.1|AM696457-18	F: GCCATTAATCCCCATGCTTA	59.76	45.00	ggatg	2	304
	R:GCCTGAAAACCTAGAGAATATACAAGA	59.66	37.04			
gi|186791110|gb|AM696453.1|AM696453-23	F: CACAGGGAGAGTGATGCTGA	59.98	55.00	agtga	2	323
	R: CCAATGGAAGTTGCACCAG	60.10	52.63			
gi|186789281|gb|AM696419.1|AM696419-17	F:CTCCCCTGATGCTCTAGATTTC	59.33	50.00			
	R: CACCAAAGACAAAGCGTTCC	60.67	50.00	Aagaga	2	334
gi|186789273|gb|AM696411.1|AM696411-29	F: TGGCACAGTCACTGCTTTCT	59.62	50.00	gaaca	2	391
	R: CGCTGCTATGAAAGGAGCTT	59.75	50.00			
gi|186729655|gb|AM696395.1|AM696395-12	F: GCTAAATTGCGGCTTCTACC	58.99	50.00	aagag	2	303
	R:GGCTATTCCTCAACCTGTTTGC	62.17	50.00			
gi|186728696|gb|AM696364.1|AM696364-38	F: TGGTTGACCGCAGCATAGT	60.28	52.63	tcctgc	2	343
	R: TGTGCTGCGTGACCTTAGTT	59.51	50.00			
gi|186727742|gb|AM696345.1|AM696345-25	F: CCTACACGCACCAGAACCTT	60.17	55.00	atgagga	2	307
	R: TCTGATCTCTGGCCTGCTCT	60.25	55.00			
gi|186727249|gb|AM696322.1|AM696322-22	F: GTGGGTCAGAAACCCAAGAG	59.55	55.00	cagag	2	310
	R: CAGCCTTTGCCACCAGTATT	60.13	50.00			
gi|186727247|gb|AM696320.1|AM696320-22	F:GGGCCAGTGACAAATGAGAG	60.66	55.00	aga	6	342
	R: CACGACAGTTCACCAAGCAT	59.75	50.00			
gi|186726319|gb|AM696315.1|AM696315-9	F: CTTGCACCCTCCAAGCTATT	59.34	50.00	ca	5	313
	R:GAGGACAACCCAAGCTGAAC	59.70	55.00			
gi|186726315|gb|AM696311.1|AM696311-24	F: CGCTCTTGGTTGCTATGTCA	60.01	50.00	tttcc	2	311
	R: GAGTGGTGTGATGGCAAATG	59.97	50.00			
gi|183206217|gb|AM696051.1|AM696051-29	F: AAGTGGTAGGACCTGGTGGA	59.42	55.00	gtatg	2	357
	R: TTGGAATTCTCTCCCTGCTC	59.36	50.00			
gi|183206214|gb|AM696048.1|AM696048-42	F:GGGCAAAGAAGAGGATCTGA	59.36	50.00	aaagga	2	397
	R:CCAAGGGTAGAATGGGACAA	59.78	50.00			
gi|183206213|gb|AM696047.1|AM696047-35	F: TAGGTGGTTGGGTTGGAGAG	59.96	55.00	gaaaa	2	304
	R: TTCAGAGGTTCCGACTTTGG	60.22	50.00			
gi|183206210|gb|AM696044.1|AM696044-49	F: CAGAAAGGGCTTCGCATAAG	59.97	50.00	gatgtg	2	325
	R: CGAGATGTCCTTCCCACACT	60.11	55.00			
gi|183206208|gb|AM696042.1|AM696042-34	F: AGGATCAGGGTTGAGCATGT	59.54	50.00	actttc	2	394
	R:GCTACATGCAGTGGCAAGAA	60.02	50.00			
gi|183206206|gb|AM696040.1|AM696040-37	F: CTCTGTACTGCATCGGTTGG	59.31	55.00	ttggg	2	352
	R: TTCTCACACCGAGGGTCTCT	59.83	55.00			
gi|183206205|gb|AM696039.1|AM696039-33	F: TCATCAATCTGCGTCTGACC	59.79	50.00	agaatc	2	317
	R:AGAACCAGCAAACCCAGGAT	60.88	50.00			
gi|183206202|gb|AM696036.1|AM696036-28	F: GAGGCAACATCACCCTCCTA	60.07	55.00	ggtgt	2	308
	R: TCATGGACCCACCACTGAAT	61.21	50.00			
gi|183206201|gb|AM696035.1|AM696035-26	F:CTGAAGGGTAGCCAGCAAAG	60.01		gcttt	2	321
	R:CAGCTACTGCAGTTTCCCAGT	59.55				
gi|183206199|gb|AM696033.1|AM696033-32	F: TCCCCAATGGTTCGGTTA	59.70	50.00	cctttt	2	323
	R: TCTGGATTACTGGGCCTTGA	60.59	50.00			
gi|183206198|gb|AM696032.1|AM696032-40	F: CACCCCCTGTCCCTAAGAA	59.90	57.89	gatgaa	2	370
	R: CTTCTTTCCCCTCCACCACT	60.48	55.00			
gi|183206197|gb|AM696031.1|AM696031-45	F: GCTGCACAGGAGTATGCTGA	60.17	55.00	attgt	2	332
	R: CCGAAAGCTATTCAGGTCCA	60.21	50.00			
gi|183206195|gb|AM696029.1|AM696029-32	F: ATCCACGCGTTACTGAGCAT	60.69	50.00	catcaa	2	376
	R: TCACACTTGAAGCATCACAC	60.91	50.00			

Table 3: SSR primers designed from genomic survey sequences

Gen bank no.	Primer sequences(5'-3')	Tm (^{0}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: CGATTCAAACGTCGGTGAG	60.36 60.25	55.00 52.63	aaaat	2	543
gi|149939381|gb|ER896027.1|ER896027-41	F: GTCCTCGCGAATGCATCTA R: GTTCTTTGCGCGAGAGAGTT	59.92 59.76	52.63 50.00	aataaat	2	307
gi|149939380|gb|ER896026.1|ER896026-39	F: AATAAAGGGGGACCACATGC R: TGGGGAGAATAACTCTGACTGG	60.94 60.49	50.00 50.00	aaccc	2	381
gi|149939378|gb|ER896024.1|ER896024-29	F: ATAATGGGGGACCACATGC 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: ATAATGGGGGACCACATGC 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: TGTAACCTTGGCACAACGAG R: CTGTACAGGGGTGTTTAGCTTC	59.76 57.95	50.00 50.00	agttt	2	319
gi|8602604|gb|AZ254289.1|AZ254289-23	F: TGAGGGATCCAAGTCTTTGC R: CACTGGCTTCCCCCAATAA	60.20 60.84	50.00 52.63	agaacc	2	303
gi|8602600|gb|AZ254287.1|AZ254287-37	F: CGCTCATACTAGCTCCCCAAT R: GCTGGCACAAGGGGTTACTA	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	tgtcga	2	325
gi|8602559|gb|AZ254267.1|AZ254267-22	F: CTTGATCAAACTGCCTGCAA R: GCCGGAGTTTGAGTGTCAAT	59.99 60.12	45.00 50.00	aacct	2	331
gi|8602535|gb|AZ254255.1|AZ254255-27	F: GGTGTCATTCAAGGGCATCT R: TCGATTCCTCCTTTGACCAC	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: TCCTCTCCTTCACCTCGTTG R: AACACAGGCTACAGCTCAACC	60.38 59.42	55.00 52.38	tgacaa	2	398
gi|8602510|gb|AZ254243.1|AZ254243-9	F: ATGAGCAAGGGGCAAGTATG R: TTCCCAACAGCTCAGTGTGT	60.10 59.31	50.00 50.00	tcaaag	2	172
gi|8602504|gb|AZ254240.1|AZ254240-18	F: GAGCGTAGGCTTGCTTTGAG R: CACGGGGAGGTAGTGACAAT	60.29 59.84	55.00 55.00	Accca	2	333
gi|8602502|gb|AZ254239.1|AZ254239-25	F: CCAGTGTGGTGGAATTCTGA R: CCTCCAATGGATCCTCGTTA	59.52 59.89	50.00 50.00	ggtgacg	2	328
gi|8602497|gb|AZ254237.1|AZ254237-29	F: TTGCCCCTATCACCTTTCAC R: GTAGACCCGGGTTCCGAAT	59.93 61.09	50.00 57.89	tacag	2	365
gi|8602493|gb|AZ254235.1|AZ254235-30	F: GTGCCCCAACACACTTCTTT R: CTTGCCGTACAACCTCTTGA	60.01 58.92	50.00 50.00	actga	2	304
gi|8602488|gb|AZ254233.1|AZ254233-20	F: GCACCACAATGCATCAACAC R: GAAGCCTGTAGACCCTTGACTC	61.03 59.39	50.00 54.55	tgtcag	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(^{0}C)	GC%
MBSSRG1	HQ148143.1	F:AATTGCAGAATCCCGTGAAC R: AAGAGCGTCTTTGCCTGTTT	$(CGG)_4$	308	58.4	45
MBSSRG2	HQ148143.1	F: GTCGATGACCCAAATCCAAT R: TGCGTTCAAAGACTCGATG	$(TCCTC)_2$	330	58.4	45
MBSSRG3	AY900122.1	F: ATCTGACGAGAGCATGTGGA R: CTCCCCTTTAGCCACAATCA	$(TTGGTG)_2$	325	58.4	50
MBSSRG4	AY900122.1	F: GAAGCGCATTCGTACTGACA R: TACAACCGAAGACACGCAAG	$(GAACA)_2$	326	58.4	50
MBSSRG5	AY683030.1	F: TGATGTGTTCCTCCCGAGTT R: AACAAGTACCCGTTGCCAAG	$(TATTC)_2$	307	58.4	50
MBSSRG6	AY233257.1	F: ACCTTCAGGCTTCAACAACG R: CGACGTAGAAACACACGATCA	$(TGA)_4$	209	58.4	48
MBSSRG7	HQ148143.1	F: GTCGATGACCCAAATCCAAT R: TTGCGTTCAAAGACTCGATG	$(ACGAA)_2$	330	58.4	45
MBSSRG8	HQ148144.1	F: AATTGCAGAATCCCGTGAAC R: AAGAGCGTCTTTGCCTGTTT	$(CGG)_4$	308	58.4	45
MBSSRG9	HQ148144.1	F: CGTAATGCGTCCATACCACA R: CCGATGCTCTTTTTCATGGT	$(CTCCT)_2$	383	59.4	47
MBSSRG10	HQ148144.1	F: CGCCTCCTCTCCTCTTCAG R: CCGATGCTCTTTTTCATGGT	$(ACGAA)_2$	312	61.4	54.1
MBSSRG11	HQ148144.1	F: AATTGCAGAATCCCGTGAAC R: AAGAGCGTCTTTGCCTGTTT	$(CAATC)_2$	308	58.4	45
MBSSRG12	HQ148145.1	F: TTGCAGAATCCTGTGAACCA R: AAGAGCGTCTTTGCCTGTTT	$(CGG)_4$	306	58.4	45
MBSSRG13	HQ148145.1	F: ATCATTGTCGATGCCCAAAC R: AGGATTCTGCAATTCACACCA	$(CTCCT)_2$	301	58.4	45
MBSSRG14	HQ148145.1	F: TTGCAGAATCCTGTGAACCA R: AAGAGCGTCTTTGCCTGTTT	$(CAATC)_2$	306	58.4	45
MBSSRG15	HQ148145.1	F: ATCATTGTCGATGCCCAAAC R: TTGCGTTCAAAGACTCGATG	$(GGAGGGG)_2$	327	58.4	45

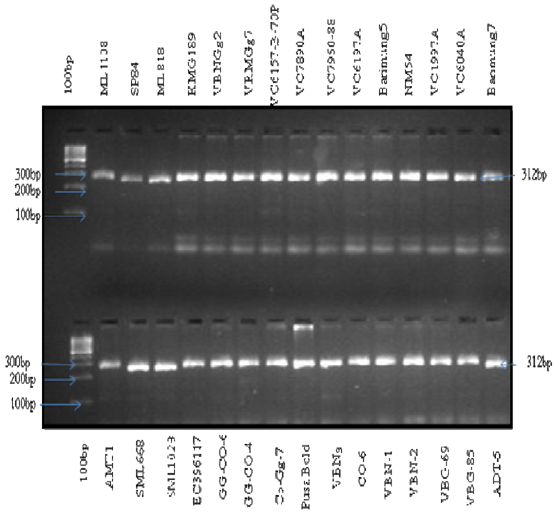

Fig. 2: Amplification by SSR primer MBSSRG10

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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