Indian J. Plant Genet. Resour. 35(1): 80–84 (2022)
DOI 10.5958/0976-1926.2022.00012.2

RESEARCH ARTICLE

Development of Genic Simple Sequence Repeat Markers as Novel Genomic Resources in Dolichos Bean (*Lablab purpureus* L.)

Shweta Kumari[1], Shraddha Ujjainwal[2], Nita Singh[2], Sunil Archak[2] and Dhammaprakash Pandhari Wankhede[2]*

[1]*Centre for Agricultural Bioinformatics, ICAR-Indian Agricultural Statistics Research Institute (IASRI), Library Avenue, Pusa Campus, New Delhi-110012, India*
[2]*ICAR-National Bureau Plant Genetic Resources, Pusa Campus, New Delhi-110012, India*

(Received: 11 March, 2022; Revised: 27 March, 2022; Accepted: 28 March, 2022)

Dolichos bean (*Lablab purpureus* L.) is an old cultivated pulse of Africa, Asia and Australia. In spite of its multiple uses, there are limited genomic resources available, which could facilitate genetic diversity studies and DNA fingerprinting. Here, we report the extraction of SSR markers from the Expressed Sequence Tags (ESTs) of dolichos bean. From a total 1129 unique ESTs of dolichos bean available in public domain, a total of 83 SSRs were identified. Among these EST-SSRs, tri-nucleotide repeats were most abundant (37) followed by tetra-nucleotide repeats (18) and di-nucleotide repeats (15). Functionality of the novel SSRs were validated by successful PCR amplification in nine varieties of dolichos bean using ten SSR markers. The novel EST-SSR markers are expected to be helpful in genetic diversity studies as well as for varietal DNA fingerprinting in this important legume.

Key Words: Dolichos bean, Expressed sequence tags (ESTs), Polymorphic Simple sequence repeats (SSRs)

Introduction

The Dolichos bean (*Lablab purpureus* L.) commonly called as hyacinth bean, field bean, Indian bean, Egyptian kidney bean is an old domesticated pulse and widely cultivated throughout the tropical regions in Africa, Asia and Australia. It is a self-pollinated, bushy semi-erect plant with 2n=22 chromosomes and belongs to Fabaceae family (Ramesh and Byregowda, 2016). While dolichos is used in human diet and animal forage as vegetable, green pods and pulses, it also has utility in intercropping, weed suppressor, and soil erosion retardant. Dolichos, being tolerant to drought and salinity, is grown in a wide variety of climates and soil types. Despite all these advantages, dolichos remained underutilized in terms of cultivation and very limited research was performed for generating genomic resources, diversity analysis and genetic improvement (Ramesh and Byregowda, 2016; Keerthi *et al*., 2018).

Availability of genomics resources in crops helps accelerate studies on genetic diversity, population structure, marker trait association, Quantitative Trait Loci (QTL) identification, gene mapping, comparative genomics and marker assisted breeding which ultimately contribute to genetic improvement and varietal development (Keerthi *et al*., 2018; Kumari *et al*., 2019). At present Simple Sequence Repeat (SSR) markers also known as microsatellite markers are the preferred choice of markers. SSR markers are widely distributed throughout genome, highly variable, co-dominant, multi-allelic and particularly informative (Kumari *et al*., 2019; Wang *et al*., 2011; Desai *et al*., 2021). Expressed Sequence Tag (EST) derived simple sequence repeat markers (EST-SSRs), SSR repeats found in coding sequences, may be generated more rapidly at low cost using the publicly available sequence databases.

There are limited reports on development of SSRs in dolichos bean and most of the markers used are transferred from other species. Zhang *et al*. (2013) has used EST based approach for SSR identification in dolichos bean. However, subsequently, there has been a substantial increase in the submission of dolichos EST sequences in the public sequence databases, which could be used for generation of EST-SSRs. Here we report the development and validation of novel EST-SSRs from dolichos sequences.

**Author for Correspondence: Email- d.wankhede@icar.gov.in*

Materials and Methods

Mining of EST-SSRs

A total of 1,486 ESTs (excluding the previously utilized ESTs) of dolichos bean were downloaded from dbEST database of NCBI. Duplicates were removed using *EGassembler* (Masoudi *et al*., 2006). 1129 unique ESTs were subjected to SSR mining and primer designing using *WebSat* software (Martins *et al*., 2009). The search criteria restricted dinucleotide repeats with at least six repeats and a minimum of five repeats for trinucleotide, tetranucleotide, pentanucleotide, and hexanucleotide repeats. Primers were designed *in silico* using *WebSat* software. Out of 75 primers that were designed, 15 primer pairs were synthesized for laboratory validation.

SSR Marker Analysis

Seeds of nine dolichos varieties (Table 1) were obtained from National Genebank, ICAR-NBPGR, New Delhi. DNA extraction and PCR assay were carried out as per standard practice (Saroha *et al* 2022). 15 primers were checked for amplification in three varieties— Arka Jay, Phule Gauri and ArkaAmogh. Ten primer pairs, producing expected amplicons, were then employed in marker analysis of nine released varieties (Table 1). Each amplicon was scored based on molecular weight by using *Alpha View* software. Data were analysed for polymorphic information content (PIC), Shannon's Information Index, observed and expected heterozygosity using *GenAlEx* v6.5 software (Peakell *et al*., 2012).

Results and Discussion

Identification of EST-SSRs from Dolichos Bean

Out of 1,486 downloaded ESTs from NCBI database, total of 1,129 sequence were filtered after removing the duplicates and used in final analysis. All these unique SSR were searched for presence of different SSRs. Total of 83 SSRs from unique 1129 ESTs were identified. Among these SSRs, tri-nucleotide repeats (37) were predominant followed by tetra-nucleotide repeats (18). This was unlike previous reports in Mung bean, Pigeonpea, Cranberry, black alder Maqui Black and pepper, where dinucleotide SSRs were predominant (Tangphatsornruang *et al*., 2009; Dutta *et al*., 2011; Zhu *et al*., 2012; Anupama *et al*., 2015; Bastías*et al*., 2016; Kumari *et al*., 2019). Dinucleotide SSRs were next abundant (15), followed by pentanucleotide repeats (8) and hexanucleotide repeats (5). Among the tri-nucleotide SSRs, the dominant repeat motif was TGA/TCA (21.6%) where as in di-nucleotide repeats, GA distribution was dominant (21.7%).

Table 1. List of released dolichos varieties used for screening and validation of EST-SSR

Accession No.	Name of the variety
IC0393738	Arka Jay
IC0395441	Phule Gauri
IC0588958	Arka Amongh
IC0584609	IIVR SEM-8, Kashi Haritima
IC0588959	Arka Soumya
IC0588960	Arka Sambhram
IC0589768	JIB (P) 04-14
IC0594178	Tirupati Field Bean-2 (TFB-5)
IC0618490	Chhattisgarh Sem-1 (Indira Sem-1)

For the first time, Zhang *et al*. (2013) had reported 22 EST-SSR in dolichos bean. However, since then, the number of ESTs reported in dolichos bean has increased very significantly. The present study, adds as many as 83 EST-SSRs by utilizing publicly available data to generate genomic resources in dolichos. Out of 83 EST-SSR loci, primers could be designed for 75 SSRs (***Supp Table 1***). BLASTx analysis of 75 SSR loci against *Arabidopsis* genome identified known functions for as many as 39 EST-SSR loci extracted in the present study (Table 2). Important hits comprised diverse gene families such as NAD(P)-binding Rossmann-fold superfamily, homeobox-leucine zipper protein family, ChaC-like family, ribosomal protein S12/S23 family, cupredoxin superfamily protein, etc. associated with important biological process such as senescence, late embryogenesis, somatic embryogenesis, transport protein subunit, Syntaxin of plants, etc.

Validation of EST-SSRs and Diversity Analysis

Functionality of the loci and designed primer-pairs was tested for a subset of 15 EST-SSRs. Ten primer pairs yielded amplification of expected size in three dolichos varieties (Fig. 1A). These 10 SSRs were then employed to carry out marker analysis in nine varieties of *dolichos lablab* (Table 1) to ascertain the utility of these markers for DNA profiling either for genetic diversity studies or for cultivar identification.Three SSR loci—LpSSR1, LpSSR41 and LpSSR43—were found to be polymorphic (Fig. 1B) in nine varieties. Ability of these primers to be used for fingerprinting was estimated by the polymorphic information content (0 to 0.49), Shannon index (0 to 0.687), effective number of alleles (1.0 to 1.976) and expected heterozygosity (0 to 0.494). The

Table 2. Annotation of ESTs harbouring SSRs with reference to *Arabidopsis thaliana*

E-SSR	ESTS ID	Annotation
Lpssr_1	JZ151299.1	Syntaxin of plants
Lpssr_2	JZ151202.1	Single hybrid motif superfamily protein
Lpssr_4	JZ150215.1	NAD (P)-binding Rossmann-fold superfamily protein
Lpssr_5	JZ151139.1	Senescence-associated gene
Lpssr_6	JZ151402.1	Hydroxyproline-rich glycoprotein family protein
Lpssr_10	JZ151044.1	ChaC-like family protein
Lpssr_15	JZ150703.1	Senescence-associated family protein
Lpssr_16	JZ150206.1	Ribosomal protein S25 family protein
Lpssr_17	JZ151108.1	MLP-like protein 43
Lpssr_20	JZ150081.1	Late embryogenesis abundant proteins
Lpssr_24	JZ150859.1	Calcium-binding EF-hand family protein
Lpssr_26	JZ151018.1	Putative thioredoxin
Lpssr_30	JZ151051.1	FKBP-like peptidyl-prolyl cis-trans isomerase family protein
Lpssr_32	JZ151302.1	Somatic embryogenesis receptor-like kinase-like protein
Lpssr_33	JZ150096.1	TIFY domain/Divergent CCT motif family protein
Lpssr_36	JZ151158.1	Chalcone-flavanone isomerase family protein
Lpssr_37	JZ150717.1	Dormancy/auxin associated family protein
Lpssr_38	JZ150951.1	SCAMP1
Lpssr_39	JZ150769.1	SR1
Lpssr_40	JZ151306.1	Alcohol dehydrogenase
Lpssr_41	JZ151154.1	KH domain-containing protein / zinc finger (CCCH type) family
Lpssr_42	JZ151346.1	DHHC-type zinc finger family protein
Lpssr_43	JZ151332.1	Protein N-terminal asparagine amidohydrolase
Lpssr_44	JZ151201.1	Metacaspase 3
Lpssr_45	JZ150363.1	Cupredoxin superfamily protein
Lpssr_47	JZ150148.1	ELIP2
Lpssr_48	JZ151311.1	Single hybrid motif superfamily protein
Lpssr_52	JZ150251.1	Beta-6 tubulin
Lpssr_54	JZ151385.1	Cotton fiber protein
Lpssr_57	JZ150828.1	Ribosomal protein S12/S23 family protein
Lpssr_58	JZ151062.1	cotton fiber protein
Lpssr_63	JZ151251.1	Homeobox-leucine zipper protein family
Lpssr_65	JZ150240.1	PyrD
Lpssr_66	JZ150070.1	Major latex-like protein
Lpssr_69	JZ150331.1	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein
Lpssr_70	JZ150836.1	Hypothetical protein AT1G63310
Lpssr_71	JZ151305.1	Mediator of RNA polymerase II transcription subunit 19a-like protein
Lpssr_73	JZ150142.1	Putative transport protein subunit
Lpssr_74	JZ150963.1	Taximin

low polymorphism observed in the dolichos EST-SSRs could be due to the conserved nature of the genic regions, which are known to be relatively less polymorphic than the intergenic SSRs. Similar observations have been reported in dolichos (Zhang *et al*., 2013) and in other species (Kalia *et al*., 2011; Preeti *et al*., 2020). Additionally, low polymorphism could also be due to the limited number of genotypes used in present study; use of larger panel of varieties and diverse germplasm accessions could possibly unravel the polymorphism at the identified SSR loci.

Conclusion

Dolichos bean has remained as an under-studied species in addition to being an underutilized legume. Availability of genomic resources is lacking in this important legume species and as a result genetic studies have been carried out with inadequate number of markers. EST-SSRs reported in this study, are expected to be valuable additional genomic resource in dolichos bean that can facilitate downstream applications such as diversity analysis and cultivar identification.

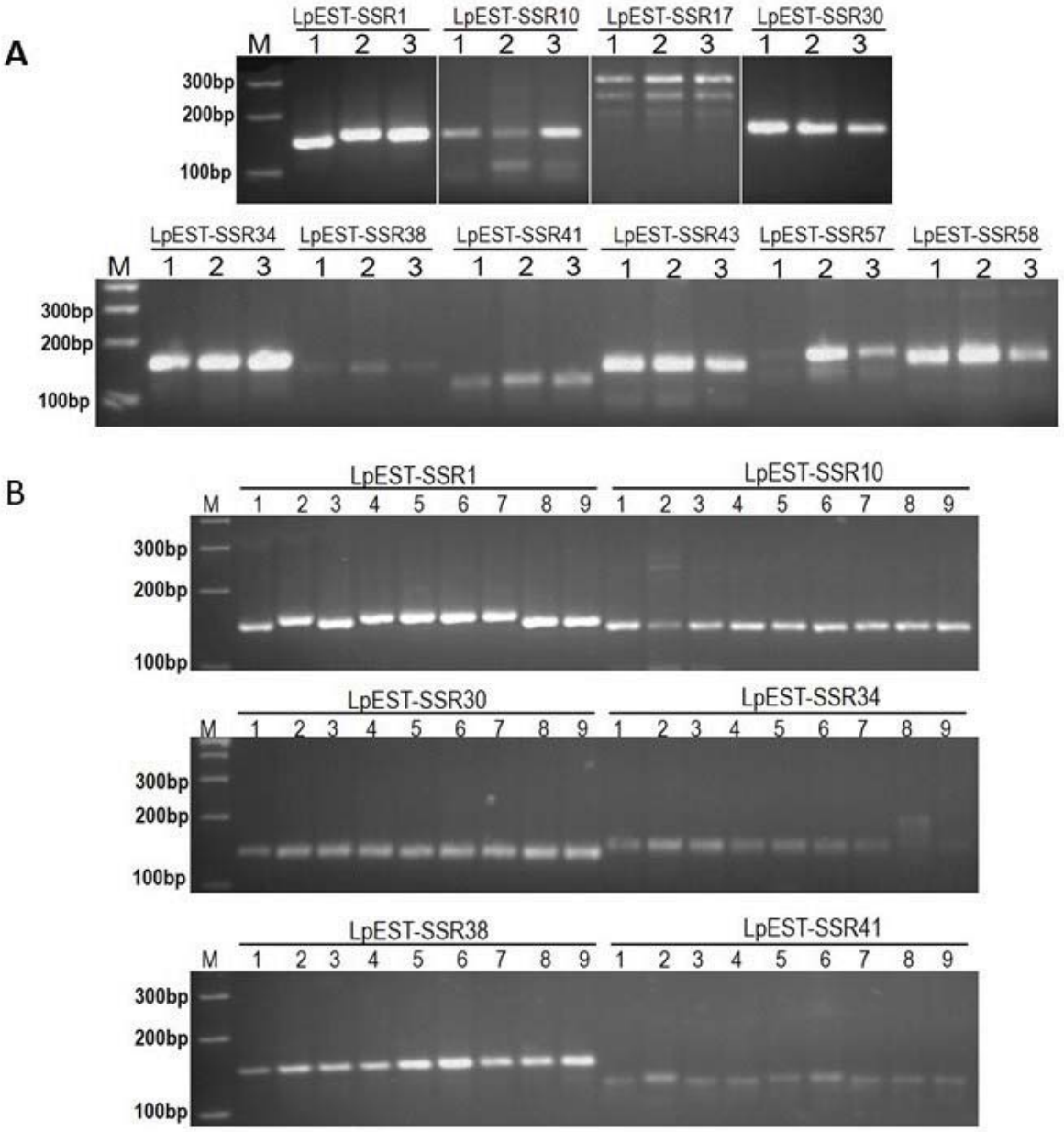

Fig. 1. Validation of EST-SSR markers on dolichos bean varieties. A. Screening of SSR EST-SSR primers for PCR amplification of three varieties. B. Validation of EST-SSR markers on nine released dolichos varieties

Acknowledgement

During the period of study, Shweta Kumari was supported by fellowship from ICAR-IARI, New Delhi. Sunil Archak was supported by ICAR National fellowship. Laboratory facilities at Division of Genomic Resources provided by ICAR-NBPGR gratefully acknowledged.

*Supplementary Table or Figure mentioned in the article are available in the online version.

References

Anupama K, A Cyriac, KV Saji and BK Nirmal (2015) Microsatellite marker based cross species amplification and genetic diversity analysis in the genus *Piper*. *Int. J. adv. res.* **3**: 184-91.

Desai SP, S Ramesh, PV Vaijayanthi, RA Mohan, BR Chandana and B Gonal (2021) SSR markers assay-based establishment of distinctness, uniformity and stability of dolichos bean [*Lablab purpureus* (L.) Sweet var. Lignosus] advanced breeding lines and elite germplasm accessions. *Genet. Resour. Crop Evol.* **68(4)**: 1309-1314.

Dutta S, G Kumawat, BP Singh, DK Gupta, S Singh, V Dogra and NK Singh (2011) Development of genic-SSR markers by deep transcriptome sequencing in pigeonpea [*Cajanuscajan* (L.) Millspaugh]. *BMC Plant Biol.* **11(1)**: 1-13.

Kalia RK, MK Rai, S Kalia, R Singh and AK Dhawan (2011) Microsatellite markers: an overview of the recent progress in plants. *Euphytica.* **177(3)**: 309–334

Keerthi CM, S Ramesh, M Byregowda and PV Vaijayanthi (2018) Simple Sequence Repeat (SSR) Marker assay-based genetic diversity among dolichos bean (*Lablab purpureus* L. Sweet) advanced breeding lines differing for productivity per se traits. *Int J Curr Microbiol Appl. Sci*. **7(5):** 3736-3744.

Kumari R, DP Wankhede, A Bajpai, A Maurya, K Prasad, D Gautam and AB Gaikwad (2019) Genome wide identification and characterization of microsatellite markers in black pepper (*piper nigrum*): a valuable resource for boosting genomics applications. *Plos one*. **14(12)**: e0226002.

Masoudi-Nejad A, K Tonomura, S Kawashima, Y Moriya, M Suzuki, M Itoh and S Goto (2006) EGassembler: online bioinformatics service for large-scale processing, clustering and assembling ESTs and genomic DNA fragments. *Nucleic Acids Res*. **34**: W459-W462.

Martins WS, DCS Lucas, KF Neves and DJ Bertioli (2009) WebSat-A web software for microsatellite marker development. *Bioinformation*. ***3*(6)**: 282.

Peakell R and PE Smouse (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*. **28**: 2537-2539.

Ramesh S and M Byregowda (2016) Dolichos bean (*Lablab purpureus* L. Sweet var. Lignosus) genetics and breeding–present status and future prospects. *Mysore J Agric Sci*. **50(3)**: 481-500.

Saroha A, D Pal, V Kaur, S Kumar, A Bartwal, J Aravind and DP Wankhede (2022) Agro-morphological variability and genetic diversity in linseed (*Linum usitatissimum* L.) germplasm accessions with emphasis on flowering and maturity time. *Genet. Resour. Crop Evol.* **69(1)**: 315-333.

Tangphatsornruang S, P Somta, P Uthaipaisanwong, J Chanprasert, D Sangsrakru, W Seehalak and P Srinives (2009) Characterization of microsatellites and gene contents from genome shotgun sequences of mungbean (*Vigna radiata* (L.) Wilczek). *BMC Plant Biol*. ***9*(1)**: 1-12.

Wang Z, J Li, Z Luo, L Huang, X Chen, B Fang and X Zhang (2011) Characterization and development of EST-derived SSR markers in cultivated sweet potato (*Ipomoea batatas*). *BMC Plant Biol*. **11(1)**: 1-9.

Zhang G, S Xu, W Mao, Y Gong and Q Hu (2013) Development of EST-SSR markers to study genetic diversity in hyacinth bean (*Lablab purpureus* L.). *Plant Omics*. **6(4)**: 295-301.

Zhu H, D Senalik, BH McCown, EL Zeldin, J Speers, J Hyman and JE Zalapa (2012) Mining and validation of pyrosequenced simple sequence repeats (SSRs) from American cranberry (*Vaccinium macrocarpon Ait*.). *Theor. Appl. Genet.* **124(1)**: 87-96.

Supplementary Table 1. Details of EST-SSR markers and respective primers for PCR amplification.

Primer_ID	SSR motif	Forward Primer (5'-3')	Reverse Primer (5'-3')	Annealing Temperature (ºC)	Product size (bp)
Lpssr_1	ATT	CATAGTTCTCCCCATAGTCCT	AACATTATACACACGCATGAA	54	151
Lpssr_2	TC	AGAGAAGAGAGAGATGGCTTG	GAATTCCCATCAACTTTTACC	55	150
Lpssr_3	AGAGA	GGAGAGGAAAAGAGTTGAAGT	AGAAAAGGGTCTGTGAGAGAG	55	129
Lpssr_4	TTC	AGTAGTGGGAGAAAGAGGATG	CGTAGCTTTATTAGCATCACC	54	165
Lpssr_5	TTA	TTGTATTACGGTATCGAGGTT	TGAAGAACTCGGACTTGATAA	54	150
Lpssr_6	ATCA	CGCAAGGTGTAGTAAGAGGTA	GCATCAATTTCCTTAACATGA	55	154
Lpssr_7	TGG	GAACAGTTTTGGGAATTTGA	ACTCTCTCTCCGACGAAAAT	55	184
Lpssr_8	ATGCA	TGAGAACTTGTGCCTTTTTAG	CATACAGATTCTTCTGCTACACA	55	123
Lpssr_9	TATAT	TAGCATACCCTATTGGAAAAA	AACATTTAAACAACCATCACG	55	149
Lpssr_10	AGA	CATCCAAGAAACAAGAAAAAG	GAGAAGGGAGAGAAGAGAAGA	54	147
Lpssr_11	TCA	CAGAACCTATTGCTTTGGAC	GCTTAAAAGTGGGGATACCT	54	151
Lpssr_12	TCTCT	GAGCAGAGAGAAAGAGAGGAG	AACCATGATTTATTGGGATCT	55	166
Lpssr_13	AAG	AATTTCCTTCCACCTTCAAT	GGAGAGAGGGAAATGACG	55	167
Lpssr_14	GCAT	TCATACTTGACAAAGTTGCAG	TTCCCATTAAGAAAAAGAAGG	55	150
Lpssr_15	AAT	GATTCTTGCACTTTAGGGTTT	TCTATTAATTCCCAGATCACG	54	151
Lpssr_16	CTT	GGAGTGATGAGCTTGTATTTG	GCCATTACGGCCTAGTTAC	54	149
Lpssr_17	TTG	CTGCGTGTACTTTATCGTTCT	TTCACATAGAAGAGCACACCT	55	139
Lpssr_18	TCGTTG	CTGCGTGTACTTTATCGTTCT	TTCACATAGAAGAGCACACCT	54	139
Lpssr_19	TTAT	TATTGGACCTTAAATGCACAC	ATTTGTGGCGCTATATAAACA	55	149
Lpssr_20	TCA	GCCCTGAAGTTCATATCTCTT	CAAGAAGAAGAAGGAAGATGG	55	141
Lpssr_21	CTT	TTTCTTCTTCATTCCTCATCA	GGGGAACTGGCTACTCTATTA	54	151
Lpssr_22	GTTT	GAGTGAGAGCCATGTGTTAAG	ATCCAAAGCCATCATTATTCT	55	158
Lpssr_23	CTTT	TAAGAATAATGATGGCTTTGG	ATTTCTCTTGCTCTTGGTCTT	54	144
Lpssr_24	CAA	GGGATTATAACCTCCTCCTTC	TTTGAACACTTGTTGAACCTC	55	145
Lpssr_25	CTG	AAAGCATGTAGTTGAAAATGG	GTGTTGTTAAAGGAGGGTCTT	54	172
Lpssr_26	TTC	CTGATTTAATGGGATTTTCCT	GGAGAATCAGAAAGGGAGATA	54	157
Lpssr_27	GA	AAGCTGCAATAATCAAGTGAG	TTCATGTAGCACACTTCACAC	55	141
Lpssr_28	CAG	ACCATGAGGAGAAAGAAGAAC	CTCACTTGATTATTGCAGCTT	54	156
Lpssr_29	TGA	GTGCTATTGAGGGGACTAGAT	TATGCTCCAATAAAACGCTAT	54	152
Lpssr_30	GGGAAG	AGTTACGGGGAAAAGACTCTA	TTACGTAAGTGCAAGTACCAAG	54	147
Lpssr_31	ATCAA	GGGGGCAAAGAATATATTAAG	AAGAGTGGGTCACAGAAATG	54	143
Lpssr_32	ATG	TAGTTACGGGGGATCTAGC	TGCTCTACCTCCTCATCAATA	54	145
Lpssr_33	AT	TTTCTGAAGCTAACCAATCTG	GTGGCCTTCATACGAATTTAT	55	155
Lpssr_34	AAT	TTTCTGAAGCTAACCAATCTG	GTGGCCTTCATACGAATTTAT	55	155
Lpssr_35	ATAC	TTTCTGAAGCTAACCAATCTG	GTGGCCTTCATACGAATTTAT	55	155
Lpssr_36	TGA	GATGTATACGCATTTGGTGTT	GAAGCCTAACTGTCATGGATA	54	154
Lpssr_37	CCT	ACTACTCCTCCGGTATCTCC	GGAAAACCCGACTATATCTCA	55	156
Lpssr_38	ATTT	ATTATAAGCCACACTCAGCAA	CCATGCTCTCAGCATATACAC	56	146
Lpssr_39	CCG	AAAGGGTAGAGAAAATTCACG	TTGTCAAATAGGTTTGAATGG	55	155
Lpssr_40	AGGT	GTGAAGATTGTGGAGCTCTG	AACTCCTAATTAAAAGGCAACTT	56	157
Lpssr_41	TAT	CCATCTTGTATTAGCCAGGTC	TACGGCAAAACTATAACATGG	56	122
Lpssr_42	AT	AGATGAGGATATCCGAAGATG	TGAGAGTCTCTGCATGTCTTT	56	146
Lpssr_43	TCGT	AGCATCAGCTTGTTCTCAAC	GTTGGTGAGGATGAATGAGTA	56	145
Lpssr_44	ATA	TAGCAAGAACTTGAATTGAGC	AGGTGACTACACTGAAAGCAA	55	151
Lpssr_45	AGATT	GGATTGAGAAAATAGGCAAAT	AACAACCAAAAGAAGCCTAAC	55	138
Lpssr_46	TAAT	GCCTTTGTGTGTGTCTTTAAT	GATCAATGTCAACAGGTTTGT	55	155
Lpssr_47	AGG	GAATTTCGTGCTCACCTTAG	CAGGAGAAGCTTCACTCTCAG	57	148
Lpssr_48	TC	AGTTACGGGAGAGAGAGAAGA	CGAATCAGCATACTTCAAATC	55	139
Lpssr_49	GA	GGAAGAGAGTTCCCTTTTGTA	GGATCATATTGGATCGTTTTT	55	152
Lpssr_50	ACAA	GCCATCCTGTTTCACTTATTT	AAGAAGAAAGGGAAGATGAGA	55	140
Lpssr_51	TTTTA	ATTTGCTCTAGGTCCATTTGT	GTGCTTTCTGTTGAGGTTTTA	55	144
Lpssr_52	ACT	GCCATTACGGCCTAGTTAC	TGTGAAGGATTTCTCTCATTT	54	151

Primer_ID	SSR motif	Forward Primer (5'-3')	Reverse Primer (5'-3')	Annealing Temperature (ºC)	Product size (bp)
Lpssr_53	AAAT	ACATTCAACGTTTTTACGACA	ATGGCATAATATTTGCTTGTG	56	174
Lpssr_54	GA	GTTTTGATGCCATTCTCAAT	CGTGGTATTTATTGCATGATT	55	142
Lpssr_55	AATT	CTCAAATAAAATGGCAAGCTA	GGGAGGAGCATAGTAGGTAGA	55	129
Lpssr_56	TAAA	ACCTAGTGTTTCATTAGTTTTGA	GAAGGATGTTTAATGTTAGGAAA	53	148
Lpssr_57	TGGAAC	GGAGTCCATATTTGGATTTTT	TAGCAAGTCCACACATGATTT	54	159
Lpssr_58	CT	CAAAACACATTCCAATCATTC	AATTTCCATTTCCTCACTCTC	54	148
Lpssr_59	TCTT	GAATCACACCAAGTGTTTGTT	GAATAATGAGCAATAGTGTTGG	54	139
Lpssr_60	TGA	ACACGATTTTCCAACTTAACA	CTAAAGCAGTTTTCTGAACGA	55	180
Lpssr_61	TCA	TTGCAATCTTCTCCTTATTTG	CACCAGATTGATAACGATGAT	55	150
Lpssr_62	TCA	TCTTCTCCTTATTTGCTGTTG	AACATCACCAGATTGATAACG	55	149
Lpssr_63	ATC	CACCTCGTTCTAGGGTTATCT	CAGACAAACCCAATGTTAAAG	55	151
Lpssr_64	TGT	GCATCCCCTTTGATCTATATT	AAACAAACCAAAGAAATCTCC	54	153
Lpssr_65	GA	TCGTATCTGTGTTCGTTTGAT	AGTTACGGGGATGTGTAGTTC	55	149
Lpssr_66	AT	CAGAATTTAGGGAGGTTCTCT	TACGGGGGATACAATATTAGC	55	161
Lpssr_67	CAA	TTCACATAGAAGAGCACACCT	CTGCGTGTACTTTATCGTTCT	55	138
Lpssr_68	ACGACA	TTCACATAGAAGAGCACACCT	CTGCGTGTACTTTATCGTTCT	55	138
Lpssr_69	CA	GACAACACACACAACACACAT	TGAACAAAAACCAATAACACC	55	141
Lpssr_70	ATC	AGAGAAACAAACTTCCAAACC	ACAGATGTGGTTCTATTCTCG	54	149
Lpssr_71	TCT	GTTCCAGAACTGAACCAAAG	AATTAGAATTGACCCGTGAAG	54	145
Lpssr_72	AGAA	AGAAGAGAATTTTGGGTTTTG	CCTCACAAGAACAGGTAAAGA	54	162
Lpssr_73	CCA	CAACAAAGGATATGAAACCAA	GTTAACCTACGTGGAAGGAAG	55	154
Lpssr_74	AG	AAAACACAGTCCAATTTGAAG	AGGAGAGAAAACCAATGAAAG	54	151
Lpssr_75	TAAA	TATATGCTTAAAGGGGAGAGC	CCTGTGAAATTTAAAGCAAAC	54	173

EST-SSR markers shown in bold letters were synthesised and screened against dolichos varieties for PCR amplification. ESTS-SSR markers in bold and underlined have been used for validation in dolichos bean varieties for studying polymorphism and diversity.