


ONLINE RESOURCES



Development and application of genomic resources for comparative and translational genomics in legumes through leveraging genomic sequence of *Medicago truncatula*

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Abstract. The expressed sequence tags (ESTs) of common bean were BLAST aligned with barrel medic genome sequence and developed 1196 conserved intron spanning primers (CISPs) to facilitate genetic studies in legumes. Randomly selected 288 CISPs, representing loci on barrel medic genome, were tested on 10 selected members of legume family. On the source taxa, the highest single copy amplification success rates of 61.8% (barrel medic) and 56.2% (common bean) was obtained. The success rate of markers was 54.5% in cowpea followed by 53.5% in pigeonpea and chickpea, signifying cross taxon amplification and their potential use in comparative genomics. However, relatively low percentages of primer set amplified (40–43%) in soybean, urdbean and peanut. Further, these primers were tested on different varieties of chickpea, pigeonpea and cowpea. The PCR products were sequenced and aligned which resulted in detection of 26 SNPs and eight INDeLs in cowpea, seven SNPs and two INDeLs in chickpea and 27 SNPs and 14 INDeLs in pigeonpea. These SNPs were successfully converted in to size variation for gel-based genotyping. The CISP markers developed in this study are expected to aid in map saturation of legumes and in marker-assisted selection for accelerated crop improvement.

Keywords. basic local alignment search tool; conserved intron spanning primers; single-nucleotide polymorphism; derived cleaved amplified polymorphic sequence; cleaved amplified polymorphic sequence.

Introduction

Limited availability of genomic resources for the economically important legume crops has hampered advances in genetic research and the development of superior crop varieties. Recent advances in sequencing of select botanical models like barrel medic (*Medicago truncatula*) and *Lotus japonicas* and their detailed functional analysis revealed extensive synteny and collinearity among the genome sequences of various legumes belonging to different genera. The availability of expressed sequence tags (ESTs) of few crops like common bean, cowpea and others has enabled the comparative genomic research to develop potential marker systems known as conserved

intron spanning primers (CISPs) which are polymerase chain reaction (PCR)-based and exploit polymorphisms available in the intronic region. The polymorphism information content (PIC) of these markers range from 0.15 to 0.87 in different legumes and large point mutations were observed in the introns (Gupta *et al.* 2012; Kudapa *et al.* 2012, 2014; Zhang *et al.* 2017). These are low-cost and high-throughput DNA markers which support cross species amplification. Abundance and uniform distribution of genetic markers in any plant species is necessary for a number of applications like saturation mapping of the genome, QTL identification and mapping, tagging of vital genes, map-based cloning, marker-assisted selection (MAS), varietal fingerprinting and diversity

analysis at various levels. Single nucleotide polymorphisms (SNPs) and insertions or deletions (INDeLs) which are inexhaustible resources available in a plant genome have become first choice for marker development and are advantageous as they are amenable to high-throughput and cost-effective genotyping (Tsuchihashi and Dracopoli 2002; Kota et al. 2008; Varshney et al. 2009). In the present study, we used ESTs generated from common bean to identify intron flanking primers for genetic analysis in the legume family. These primers also helped in the detection of potential SNPs and/or INDeLs in pigeonpea, chickpea and cowpea, and attempts were made to convert SNPs into PCR-based primers for their use in enriching the existing genetic map.

Materials and methods

Plant materials and DNA extraction

One genotype each from 10 different legumes namely barrel medic (*M. truncatula* Gaertner), pigeonpea (*Cajanus cajan* (L.) Millsp., BRG2), chickpea (*Cicer arietinum* L., Annigeri-1), cowpea (*Vigna unguiculata* (L.) Walp., KBC2), common bean (*Phaseolus vulgaris* L., Arka Suvidha), soybean (*Glycine max* (L.) Merr., MAUS2), blackgram (*V. mungo* (L.) Hepper., TAU1), horsegram (*Macrotyloma uniflorum* (Lam.) Verdc., PHG9), field bean (*Lablab purpureus* L., HA4) and peanut (*Arachis hypogaea* L., GPBD4) was sampled for PCR amplification. Five varieties of pigeonpea (BRG2, TTB7, ICPL 7035, ICPL 8863 and ICPL 87119), nine varieties of chickpea (Annigeri-1 (A-1), WR315, K850, ICCV4958, JG62, ICCV10, KAK2, BG256 and ICCV2) and four varieties of cowpea (KBC-2, IT 38956-1, C-152 and IC-219607) important in breeding programmes were sampled for polymorphism identification. Total genomic DNA was extracted using a modified CTAB method (Saghai-Marooof et al. 1984) and the quality and quantity of extracted DNA was checked on 0.8% agarose gel.

Development of intron length polymorphism primers

A total of 67,429 common bean ESTs downloaded from <https://www.ncbi.nlm.nih.gov> were BLAST ($E < 1 \times 10^{-10}$) aligned to the barrel medic genome (TIGR ver. 3.0; <http://www.tigr.org>) and PCR primers were designed from highly conserved (0–1 mismatch) alignments manually in the initial stages and later an automated approach was used for primer design. Primers were designed such that PCR amplicon spans at least one intron, 17–22-bp long primer sites, 250–1500 bp predicted amplicon size and importantly the designed primer sets should amplify single specific amplicon in barrel medic. All the redundant primers were removed after checking for redundancy on barrel medic genome.

PCR conditions

Reaction conditions were same for all the primers. Reaction mixture included 30 ng genomic DNA, 0.5 mM dNTPs (Sigma-Aldrich, Bengaluru, India), 1 unit of *Taq* DNA polymerase (Sigma-Aldrich), 0.0626 U PR Polymerase (Bangalore Genei, Bengaluru, India), 15 pmol each of forward and reverse primers and 1 × PCR buffer (Sigma-Aldrich) in a total reaction volume of 30 μL. PCR (Eppendorf Mastercycler) cycling parameters were as follows: 94°C for 5 min followed by 94°C for 30 s, 61°C (−0.2°C/cycle) for 45 s, 72°C for 60 s for 16 cycles, followed by 94°C for 30 s, 57°C for 45 s, 72°C for 60 s for 30 cycles and final extension at 72°C for 10 min. PCR products were visualized on 1.5% agarose (Sigma) gel stained with ethidium bromide. Loci were classified (0–2) according to whether they yielded no product (0), a single band (1) and two or more bands (2).

Sequencing of PCR products and designing of derived cleaved amplified polymorphic sequence (dCAPS)

PCR-amplified products were purified using a column-based QIAquick PCR Purification kit (www.qiagen.com).

Table 1. CISPs designed from ESTs of common bean.

Alignment	Number of ESTs	Number of markers	Distribution on barrel medic genome	
			Chromosome	Number of markers
Common bean – barrel medic	67,429	1196	1	139
			2	139
			3	160
			4	183
			5	201
			6	70
			7	165
			8	139
			Total	1196

Table 2. List of 288 CISPs designed from common bean and barrel medic alignments tested across taxa.

Marker name	ESTs	Chr. no.	Forward primer (5'-3')	Reverse primer (3'-5')	Primer start	Primer end	Amplicon size
1	PvMt-001	MtChr1	GTTCGACGATCAATAACAGG	GGAGATTCCTTTTCAACCAGCA	16870815	16870287	528
2	PvMt-002	MtChr1	CACTTCTCAGCAATGTCTGTG	AACCTCGTATTCCAGCTTGTG	7579288	7578535	753
3	PvMt-003	MtChr1	AATCTTCGTCGGCTTC	CATATCCACCAAACGAGTGC	21766334	21767151	817
4	PvMt-004	MtChr1	GGTCTTGATTTCTTTGG	GAGTACAAGGGACCATGGAAG	17888856	17888413	443
5	PvMt-005	MtChr1	TCAACACTCATATAGCACCCAG	AGGGAACACCATCATGAAC	14734544	14733584	960
6	PvMt-006	MtChr1	GGTGGATTTACTGCTTCATGG	GAATGTTGGCATAGCCTCAA	6667889	6668317	428
7	PvMt-007	MtChr2	TTAGCTTCACTCCGACACG	ACCCTGCTGATCAAGTCTCTC	3929009	3929366	629
8	PvMt-008	MtChr2	GGTTTTGAGAAGCCATCAGC	GCCTGTGAGAAGATTCTCTTGC	10158145	10157586	559
9	PvMt-009	MtChr2	CAGTTTGCAATCCCTGCATTA	AGACAGCAGCAGGACCATCAGA	20582477	20582759	282
10	PvMt-010	MtChr2	TGGATGGTCAGTTCTCCAA	CTGGTGAACATGAGCATCAAC	4836910	4837558	648
11	PvMt-011	MtChr2	CATTTGCCTCAGCAAAAGGA	TTCAACTTCAAGGCGATCAG	19749885	19749615	270
12	PvMt-012	MtChr2	CCCTTTCTCAAGTCCAAAGATTC	AGTTGTTGACATGGGGAAGC	7273006	7273754	748
13	PvMt-013	MtChr3	CCTGAATCAAAGATGGAAAGC	GGGGTCAATCTGAGAAATGTT	14849748	14849452	297
14	PvMt-014	MtChr3	GTTCCGCAAGGACCTCATGT	GTTGAAGCAATGTCTCACAGA	25570213	25570911	698
15	PvMt-015	MtChr3	TCCCTTTCTGCTGCTTATC	TTTCAAAGCCTGACCCAGA	21128113	21129311	1198
16	PvMt-016	MtChr3	ATGTGAGTGCTGCAAAACAG	GGTTCTAAACCAGGCTGGA	20011091	20011512	421
17	PvMt-017	MtChr3	TCCCATACAGAGCACCTTCTT	GTCATTAAGCAGATTCTCTCGTC	14835579	14836188	609
18	PvMt-018	MtChr3	GCACCAAAGAGGTTGATGA	TAGTGTCCCATGCCAGAAC	33059074	33058466	608
19	PvMt-019	MtChr4	TGAAGCACCCAATAACAAGC	CCAGATTTGGCTGGCTTG	16669446	16668571	875
20	PvMt-020	MtChr4	CGGATCGTGTGCTCAGAA	TATTGGCAATGTGAGGAAGC	294581	295053	472
21	PvMt-021	MtChr4	CCCCAAAGAGCAAAAGATGCT	GAACCCGAACTTCTTGTGA	27456081	27455239	842
22	PvMt-022	MtChr4	CACCCAGGCTACAACACCT	TCTCCTTTTGCCTTCCCTTG	16670843	16669394	1449
23	PvMt-023	MtChr4	GGATGGGAGCTCAATTGTC	CCCAAGGAATTAGTGTCTTCAG	4365928	4366459	531
24	PvMt-024	MtChr4	GATGTCGGAGCAGTTCAACC	AATCTCATACGGTCGCCCTT	30183209	30182380	829
25	PvMt-025	MtChr5	TAGCGAGGATGAAACTGCTG	ATTTGAAAGCATCCCTGCTC	27906266	27905336	930
26	PvMt-026	MtChr5	GGTTGCCCTGATGGAAG	CAAGTGCCTCTAGCTTTTCA	22847266	22847583	317
27	PvMt-027	MtChr5	AAACCATGGACGGATGATTG	GGGGAAAGTAAATTCAGCA	23593966	23594452	486
28	PvMt-028	MtChr5	TTACTGGAGCTTGCCCTCTC	TACATTCCTCCGAAATTTGG	4157871	4157151	720
29	PvMt-029	MtChr5	AGCTGAAAGATTGTGACCAACC	CTTCAITGTTCTCCCAAAA	3274885	3275353	468
30	PvMt-030	MtChr5	GCATCACCAAGCATGCAAT	GCAGGTAAGTTCCTACACTTG	3311629	3310501	1128
31	PvMt-031	MtChr6	GAAAAGCATTGGCACCTCTT	TTCACCAGCAGCACCAATAG	9939058	9940050	992
32	PvMt-032	MtChr6	CTTTTGTACCGTGTGATAGGTC	AGAATTCCTCATCCCAACA	16073619	16073260	359
33	PvMt-033	MtChr6	TTCAGTCACTCGATGCCAATG	CCCTTGTCTCCATGCATAC	568347	567363	984
34	PvMt-034	MtChr6	GGCCGAATCTCTCGTTACA	CCTTTAGGATCCAGTCAAGC	7946964	7947345	381
35	PvMt-035	MtChr6	AGCGAACCTTCTTTGGTG	CAAGGCTGTGTTATCCATGT	3937515	3938170	655
36	PvMt-036	MtChr6	GGAAGCTGTGATGAACA	TGTTGGACCTTCAATCATGT	4021449	4022198	749
37	PvMt-037	MtChr7	CCAAGCATGGATATGGAAG	GTTGAAGCTGTGGCAGTTCT	27949452	27949708	256
38	PvMt-038	MtChr7	CATCCAAGCCATTGAACAC	GCTCCAATCCCTGTTCAGAG	23095443	23095016	427
39	PvMt-039	MtChr7	GTTTGTCTGTAGTGCCTGA	CGTGGATCAATGCATCACT	12513432	12514921	1489
40	PvMt-040	MtChr7	TCCTTGGGATCTACACCAGTC	TGTTAGGTTCAAACAGACCCAG	21205168	21205603	435
41	PvMt-041	MtChr7	TCTTCTTCTGCTCAGC	CCAAAAGCTTGTCTCAACT	25884293	25884787	494
42	PvMt-042	MtChr7	GATGATGAGAGATGCTGTGC	CAATGTTGTTTGTAGAGCCAGTC	26683710	26683992	282
43	PvMt-043	MtChr8	GCTTCAAACCCGTGGAACCTGT	AGCTATCAAGATGCCAAGTAGC	12921061	12921349	288
44	PvMt-044	MtChr8	GGAACTGCCAAGCCCTGACTA	CCTCCAGCATTTGTCACCTGATT	26839138	26838729	409

Table 2 (cont'd)

Marker name	ESTs	Chr. no.	Forward primer (5'-3')	Reverse primer (3'-5')	Primer start	Primer end	Amplicon size
45	PvMt-045	MtChr8	GCCGTTTCCGCCCTAAGAT	GGCTGTGTGGAATGATAAAGC	22149962	22149555	407
46	PvMt-046	MtChr8	CTTCCAGCTGCAGCATCTT	GATGACAAAACCCCTTGCGAAT	6225243	6226010	767
47	PvMt-047	MtChr8	CGGATTCCTCAAGAGGTCAA	ACTCCATGATGGTTGCCAAT	28382634	28383139	505
48	PvMt-048	MtChr8	CV538180	CGGGGAGCATCTTTGACAT	26578010	26578818	808
49	PvMt-049	MtChr1	ATTTGGTGTGTTGGTGGTC	CCTTTCACAGCTCAACCTT	23978561	23978303	258
50	PvMt-050	MtChr1	GGATTGCAACATTGAGCAG	TTGATGTCCCGCCTAATTGA	12392196	12392598	402
51	PvMt-051	MtChr1	CGACGGATTTACGCTAAACG	CCTCAACTATGGTTTATCC	12366658	12367622	964
52	PvMt-052	MtChr1	GCTTTCGTTTGTGAGAAGAG	GATTTGGGAAATGCCGTAGT	16496200	16495627	573
53	PvMt-053	MtChr1	CAACATAGCGGAGACGAGGT	GTGCGTCTTGGAAACCAAT	6583360	6582784	576
54	PvMt-054	MtChr1	CGCTTCTTTGAGACACCAAC	TGAGTGTCCAGAGTTGGAT	23733213	23734347	1134
55	PvMt-055	MtChr2	GCTGCTCTCTCTCCAAAA	GGAAGCGTGTGAGGTTCTC	10002820	10001297	1523
56	PvMt-056	MtChr2	GCTGCAGAGACTAATGGTTGAA	CCTTGGGAGTCTCAATTTGG	6764806	6765256	450
57	PvMt-057	MtChr2	GAGCCATGGAGGTGAATTA	CAC TGGGGATTTTGAAGTAA	20583079	20583374	295
58	PvMt-058	MtChr2	ATGCTGTGGCTCAACTTGGT	AATGTAGCTGTCTCTGCACCT	6348672	6349951	1279
59	PvMt-059	MtChr2	AGCAGCAATACCTTCATGG	TCAATGGCCTTCTTTAGGG	16372002	16372825	823
60	PvMt-060	MtChr2	CTTTGTGATGCCAAGAAGA	CGTGTTCATCACTACAATCC	25325427	25324385	1042
61	PvMt-061	MtChr3	GTTGGAGTTGGAGATGTTG	TGACACCGTTGAGTAGTAGG	28855095	28885411	316
62	PvMt-062	MtChr3	ATGGCAACATTGCACACAC	GGCATGCTTCTGAGATACAG	15423492	15423075	387
63	PvMt-063	MtChr3	TGCTGCTTCCGATCAAAG	AITTTGGGATGCTTTGGAG	19835012	19835344	332
64	PvMt-064	MtChr3	GTTTGGCATCAGGAAGTTGC	CTTGATTGACCTGGACATGG	19269151	19269550	399
65	PvMt-065	MtChr3	CTCTTGACACCACTCCATCAA	TCCCTCATACATCAGATCTTGG	31487825	31488194	369
66	PvMt-066	MtChr3	TGGACCATGTTCCAGAGTCG	CAATCCCAATCTTCTCATGTGC	20012255	20012225	360
67	PvMt-067	MtChr4	TCCATAGGATCTTCCCTTGA	GACTCCAATGTGGGTGATCC	31564006	31564522	516
68	PvMt-068	MtChr4	GCCATTTGGTGTCTACCTGGA	TGGCTCCAGATTTATGTGT	29443104	29443471	367
69	PvMt-069	MtChr4	ACCACAAATGTTGCCACAGA	GCTCCAATCTTCCGATAGTCAC	31945394	31944869	551
70	PvMt-070	MtChr4	CATCAACGCCACAAGACAC	ATGCTCGGGGTGTTCTTTTC	19707419	19706888	531
71	PvMt-071	MtChr4	ACGAGGTTTTTGCCAGAG	CAAGTGC AAGGGCTATTATCCT	31833836	31834487	651
72	PvMt-072	MtChr4	TTGCAGCATGTGGTGCTAGT	AACATTTCCATGCCACCCTA	32801736	32802057	321
73	PvMt-073	MtChr5	GTTCTTTCAGCAACAACATCAGG	ATGTTTCCAGCACGTGTCA	23290097	23290552	455
74	PvMt-074	MtChr5	AAGTGCAGCCGATCATTG	CTTGTGAAGCCGAAAACAT	2394644	2395624	980
75	PvMt-075	MtChr5	CCTCCCTTGAAAAGTGAACA	CGGTTGACAACTGCTATTCCG	18667603	18666834	769
76	PvMt-076	MtChr5	GACAAAGTGCAGCCTCATCA	TGCGACATATGTGAGAATGC	7604746	7604714	572
77	PvMt-077	MtChr5	GCAGCTTCTTGAAGCACAA	CTTGGGGGAACATCTACAGC	11546006	11546707	701
78	PvMt-078	MtChr5	GCCACATAAAACATGGGTATGC	ATTGTGGCTATTGGGCTTA	8472093	8472641	548
79	PvMt-079	MtChr6	GCACCTTCCACCATCTTC	TCTTCTGTGTGATGCCACA	16087569	16086734	835
80	PvMt-080	MtChr6	GCACCTCAAGGCTGATTGTC	GGCCAAGATACAGGCAAGG	9353884	9353629	255
81	PvMt-081	MtChr6	GAGACAGGAGTTGGAAGAAGA	CTTGTATTCAAGGGCAAGAGG	566231	565974	257
82	PvMt-082	MtChr6	GCAATCCAAACACCAGCTT	CCTTTGTCAGCAACAATGC	9901435	9902311	876
83	PvMt-083	MtChr6	CCCAGAGAACAAGCATGTCA	CCCTTCTTCCAGCTCTTC	17483487	17482637	850
84	PvMt-084	MtChr6	GCTCAGGTAATGTTGGTGGT	TGGCTCTGTCTGTCAATTGG	2596041	2595034	1007
85	PvMt-085	MtChr7	AGCATTTGGGATGCTAGCTG	GTTCCAACATCGGAATCCA	20448770	20447938	832
86	PvMt-086	MtChr7	AAC TGGGTTCAATGCTCCTC	ATTGACCCACAGAAGCTGGA	23415958	23415575	383
87	PvMt-087	MtChr7	TGGTTTTGCAGGAGATGATG	ATCACCTGCCCATCAGGTAG	15425252	15426257	1005

Table 2 (cont'd)

Marker name	ESTs	Chr. no.	Forward primer (5'→3')	Reverse primer (3'→5')	Primer start	Primer end	Amplicon size
88	PvMt-088	MtChr7	AGAGCTCCTGACAAGCTTCTCT	CCGAAGAGTGTCTCTGGTATT	27688424	27688101	323
89	PvMt-089	MtChr7	CAATATCCTTGGGATCTACACC	AGAAGAGTCGCAAGGCAATG	21205163	21205585	422
90	PvMt-090	MtChr7	GGTGTCTCTTCTGGCTCAA	GTCCACAATAGCAGCACAGC	25919480	25920019	539
91	PvMt-091	MtChr8	GCACACAACTTCTCTTTTC	GTTGACATTTCCAGGACGA	1026454	1025535	1099
92	PvMt-092	MtChr8	GTGGAAAATCCACTGATGG	CGAAGGTTCCTTGGTGGATT	20974629	20973537	1092
93	PvMt-093	MtChr8	GCAATGTC AATGATGATCCCTG	ATTTGGTCAACCCAGGA	17713065	17713914	849
94	PvMt-094	MtChr8	ATGTTGATCTCTGTGGAAGC	CCAAGTAGTCTTGCAGCTGAG	31619467	31620136	669
95	PvMt-095	MtChr8	GAACAAGGGGTACTCGAA	TCCTTCTTCAACTCATCCTG	22336004	22337092	1088
96	PvMt-096	MtChr8	GACTGTATCCAGACCAATAGGG	CAATGGTACCGCAAGACTTC	22515790	22516045	255
97	PvMt-097	MtChr1	ACACACAGAGGGCCCTAAA	GCCTCTTCCCACCTCTGA	13861623	13860837	786
98	PvMt-098	MtChr1	GGAAGAAATCATGGAGGTG	ACAATGGATGTCCTTGG	15430353	15429433	920
99	PvMt-099	MtChr1	TCTTGTAAATGGTGGCTTTC	TGTACAGCCAGTTCAGCTTG	23553346	23553953	1393
100	PvMt-100	MtChr1	CACTGTCCATGCCACCAATA	CCCAAAGCTTTACACCAATCA	10264197	10264916	719
101	PvMt-101	MtChr1	TGCTGCACACTGCTCTGTAA	CCACAAGTGTCTGTCTTCA	15075566	15076015	449
102	PvMt-102	MtChr1	CAACACCCCAAGCAAGTTA	AGGACTTGAATGGCAGAGCTA	11867449	11867011	438
103	PvMt-103	MtChr1	CCACATGAGCGAGATTTTG	CACCTTGATACAAAGCCAACC	23022012	23022569	557
104	PvMt-104	MtChr1	GGAGAATTTGAGTGTGCTC	CCTTTGTGACTTCTCCTTTC	5004577	5003601	976
105	PvMt-105	MtChr1	GGAAATCTGAAAAGAGACCAC	TGGTCAAGTGAATAGGGAGTC	22344924	22344128	796
106	PvMt-106	MtChr1	GCCTATGCCCTTGGGTTTAT	CTGCAACTCTGTAACCCAAACC	16264810	16265519	709
107	PvMt-107	MtChr1	CACAAGAAGGAGACTTGTGAGC	TGCAAGTCCACTTCTGTG	23545654	23546765	1111
108	PvMt-108	MtChr1	GCCATCATCTTTCAGTTGGA	GCCCAATTTGGAAGTAAGG	19120625	19120168	457
109	PvMt-109	MtChr2	CAAACTGTGAGGATCTTGTG	CGCTGATCTCACGAACACT	10159561	10158427	1134
110	PvMt-110	MtChr2	TTGTATTCCCAAGCAGGA	ATCGGCAAGTTCCTGTCTCAT	1842890	1844126	1236
111	PvMt-111	MtChr2	CTCCTGAAATGGTTGCTT	CCATAGGCATTTCCAATCTC	1581653	1580742	911
112	PvMt-112	MtChr2	ACGGCAATGGAGGTTT	TTGGTCTGTGGAAACAACACC	23046778	23048212	1434
113	PvMt-113	MtChr2	GACCTCTGTCTGAACTTGG	TTCCCTGTCTCCTTCAATC	2968532	2969876	1344
114	PvMt-114	MtChr2	CCAGGAAACTTCAGTGGAGA	TGCTTGTCTTCTTGACGTTG	17185282	17184736	546
115	PvMt-115	MtChr2	GAATCTATCCCTTCCGCCACA	CAAAGCTTGTAAATGAGCATCAG	4613101	4612212	889
116	PvMt-116	MtChr2	ACCTTTTCTCTGCTGTTCC	AGTCCACAATACCATCCAG	6032165	6031435	730
117	PvMt-117	MtChr2	TGGAAGCTGTGGACCTTCT	GCAAGTATGTTGACAAGAGTG	24596595	24597721	1126
118	PvMt-118	MtChr2	CCGTGTCTAAACCCCAAGAAA	GCAATGAGTCTATGGGCAGT	15231463	15231117	346
119	PvMt-119	MtChr2	CCTTCTGTGATGTTTATAGGG	GCCAGCAGAAGATGGACAT	25580824	25581692	868
120	PvMt-120	MtChr2	AGACATCACCGTTGGAGAC	CGACTGGTTGGCTATCTGG	15443173	15442744	429
121	PvMt-121	MtChr3	CCGTACAAGATGAGGTTTG	CCTTCACTTCTCAGATCGAA	21954116	21954727	611
122	PvMt-122	MtChr3	ATGTAAAGGTTCCGGCAAC	TGGTATACCTCCGTCATCTT	13272497	13273417	920
123	PvMt-123	MtChr3	ATATAGGTTGCCACTTCAGG	TGCTGTGATCTGTACATTCG	30458948	30458527	421
124	PvMt-124	MtChr3	CAAAACCACTCCCTCTGGAA	GACAAATCCAAACCCCTTGC	29058439	29057678	761
125	PvMt-125	MtChr3	CATGGCGAAGATTACGAACA	AAAGAAGCCAACTGAGATGAGG	18322021	18321383	638
126	PvMt-126	MtChr3	TTCTGAGGGCACATCTTGA	GTAGCACTCAAGGATTTGC	32256149	32256643	494
127	PvMt-127	MtChr3	GCTGCAGCAACTTGATCTCTT	AAGACAGCTTCTTGTGGTGA	15354075	15354870	795
128	PvMt-128	MtChr3	TGGTCTTTTTCGGGAAGAAAT	CATGACGTCGAAAACCTTGGGA	965510	966609	1099
129	PvMt-129	MtChr3	TGGACTCATCACTTGTGCTG	ATATGCCCTGGAGGAAAAGA	20293289	20292406	883
130	PvMt-130	MtChr3	CGTCTTGCTCAATGGAGAATC	TGTGCTTCTCCACAGACAAA	12224432	12224936	504

Table 2 (cont'd)

Marker name	ESTs	Chr. no.	Forward primer (5'-3')	Reverse primer (3'-5')	Primer start	Primer end	Amplicon size
131	PvMt-131	MtChr3	CTGCCCTCAATCATGGCTTGT	GCAGAGCTTGCAGAACAAACA	26757486	26757218	268
132	PvMt-132	MtChr3	TCAATTACCTTCTCCACAGCTC	GGAAGCAGAAAGTCGCAAAAGT	25188012	25187124	888
133	PvMt-133	MtChr4	CTGAAGTAGTAAAGGCCACCTG	AGTTCCGGTGAAGTTGGTTGCT	23387016	23386044	972
134	PvMt-134	MtChr4	GGTTGGGTTCAAACTHCCA	TGATGTCGAATGATGGTCA	32107765	32108226	461
135	PvMt-135	MtChr4	AAGCAAGGCAGCTTCTCTCA	GATGCTGAAAGAACTGTTCCAC	22458565	22458222	343
136	PvMt-136	MtChr4	TCAATTGGCAGACTTCATCG	CGTTGCTCTGTCAAAAGTATGC	2704699	2703562	1137
137	PvMt-137	MtChr4	TGATAGGATCTCAGAACCA	CTGGACTACGACCCCAAGAA	24435035	24435546	511
138	PvMt-138	MtChr4	CCCAATTGTGGAACAGAGA	CTCAAAAAGAAATGGCACCAAG	16113870	16112923	947
139	PvMt-139	MtChr4	TCTGGATTTTTCGCAGCAG	GCTACAACACAGCCAAACAAA	26910504	26910949	445
140	PvMt-140	MtChr4	TGTTGGTGAAGTCTGTTGTT	GGCAAGAGCTTCAGCAAAAGA	16041570	16042631	1061
141	PvMt-141	MtChr4	TGTCAAAGAAATCAGCACCA	GGAGAAGCTTTCCTTCGTTG	29175733	29175981	250
142	PvMt-142	MtChr4	TGTTATGGCTGGTGTGTTGG	AAAAGTTTGGCTGCTGTGC	16041403	16042589	1186
143	PvMt-143	MtChr4	GACCGTTGATTCATTTCCAC	GAGGTTGAGTGAAGCCCTGA	12789746	12789454	292
144	PvMt-144	MtChr4	CAGAAAGCAATGTCTACCC	GCCAGAGAAGCAGTGCAA	20962405	20962814	409
145	PvMt-145	MtChr5	CCTAGATGGGTAGATGCTATG	TGGTCACTGACCAATCTTC	19960131	19959416	715
146	PvMt-146	MtChr5	GGTTTGAATCAGCAGATGG	TGACATTCCTCCACCAGACA	27655558	27656922	1364
147	PvMt-147	MtChr5	CTTTGCTGCAATGATAGCC	GAGACACAGCTCTGGTGTTA	11987181	11986623	558
148	PvMt-148	MtChr5	GACTGTTCAAATGGCACACG	TGCTTTAGTCTGCGCCTTC	9108762	9109750	988
149	PvMt-149	MtChr5	AGGTGCAAGCTCACATGTT	TGGTGCACCTCTCTCCTTC	7800040	7799246	794
150	PvMt-150	MtChr5	GGTTCCTACAGAGAAGGACAA	GTGGCATTTGGATCTTTTGG	8007637	8008516	879
151	PvMt-151	MtChr5	GCTTTGTTGGGCAATCTA	CCTCGGATACCTCTGCAAAA	5077193	5075965	1228
152	PvMt-152	MtChr5	TCTTTGAGGGTGTCTGAGT	CATGCTGGATTCATGTCAGG	15673607	15672625	982
153	PvMt-153	MtChr5	CCAGTGGCAGCATACATAA	CGCCCTCAAAGTTCTCTATT	8315457	8314870	587
154	PvMt-154	MtChr5	CCCAATTTGCTGCTCTTC	AGGCGTATGTTATCCAAG	11581917	11582398	481
155	PvMt-155	MtChr5	CAAAGATCACTGGTGAATGTC	TTGCTCTCTCTGAAGAAACC	8007070	8007659	589
156	PvMt-156	MtChr5	ACGGCTATCGTTAATGCTC	AGAAGAGAAACGCCGTCATA	28860273	28859907	366
157	PvMt-157	MtChr6	TAATGCCCTTCTGACCATC	TCAGGTGTGATCGTGTCTTTTC	6756554	6756106	448
158	PvMt-158	MtChr6	CCAGAAAGAAATCCACTTTCA	CAGAAGAAATCCACCCTTCA	9353832	9353526	306
159	PvMt-159	MtChr6	TGAGGAAAGCGATCACTGAG	GGTTGATGGCCCTAATGATG	4442516	4441130	1386
160	PvMt-160	MtChr6	TATAAGAGCAAACGCCAAC	AAGCTTCTTCAATCCAAGCA	11000879	11001481	602
161	PvMt-161	MtChr6	TGGTGTCTGAACCTTCCACCT	AATCCCACTGACCCAGCA	9353414	9354060	646
162	PvMt-162	MtChr6	ATTCATGCCAGATGGGCAAT	TACCCCGTCAAGAAGAAGA	10314452	10315008	553
163	PvMt-163	MtChr6	TGTCGAAGAACAATCTCCTTC	GCCTCCACTCTGCTCAACAA	3966983	3966197	786
164	PvMt-164	MtChr6	TCCAACAATGTTCTCCTGCT	GGATGATAACATAGGCAAGAGC	2810035	2809370	665
165	PvMt-165	MtChr6	GCAGCCCAAAATCTGTCAA	CGTCTCAGGTCGGTTTTGG	5229803	5229534	269
166	PvMt-166	MtChr6	GGCAGGCTCTCAGTGTATAA	CCAACCTCAAGCATGTCAGG	11622101	11621490	611
167	PvMt-167	MtChr6	TCTTTGGGTCAAAACAC	ACCGAAGCTGTGGTTAT	3937252	3938174	922
168	PvMt-168	MtChr6	GCATGTCAAGTCTTGTCTG	CCCTTTCCTTTCAGCTCTTCT	17483499	17482631	868
169	PvMt-169	MtChr7	TGGAGAAGATCTGGCATCAT	GTGAGATCACGACCTGCAA	15425440	15426072	632
170	PvMt-170	MtChr7	GGAGCTATATGCTTCGTTGATG	AAGCACTGGGATGTTATAGC	22887816	22888152	336
171	PvMt-171	MtChr7	AGCACCAATGACAGCAGAGA	GCTCCTGGATGCACTTTTGT	18977669	18977355	314
172	PvMt-172	MtChr7	CGATTTTCGAGTACAACGGGAA	CCCTAACCCAGCAATTACA	21106852	21108244	1392
173	PvMt-173	MtChr7	CCTAGCTCTGAAGGCTTTGAA	AGCTGGTAATTTGGCACCATC	4437689	4436684	1005

Table 2 (cont'd)

Marker name	ESTs	Chr. no.	Forward primer (5'–3')	Reverse primer (3'–5')	Primer start	Primer end	Amplicon size
174	PvMt-174	MtChr7	CAGAAGAAGCGTGACCACAA	TCTACAAAGAGCTTGGAAAGGAG	21029782	21030806	1024
175	PvMt-175	MtChr7	CCGTGGAATACTGATGAATGC	TCAGCCAGAGCTTCTATTGC	22097219	22096328	891
176	PvMt-176	MtChr7	CGTGCTGGAAGAATTGAGG	ACGAAGAGCATCGCATAAG	18387903	18387420	483
177	PvMt-177	MtChr7	GGAGGGGATCAGTTCAAAT	CGTTCCATATCCACCACAA	17510575	17511131	556
178	PvMt-178	MtChr7	TCCACCAGCAAAATGTTACAA	ATGTTCCGGCAGCTTATGAT	16875900	16875270	630
179	PvMt-179	MtChr7	ATTTGGTCTCCCTTTT	TTGTTGTGTCCACCGTTGAG	22284178	22284689	511
180	PvMt-180	MtChr7	GCTGGATTTGACGTAAAGG	TCCCTTAGCAITGGGATGAAC	23224020	23223637	383
181	PvMt-181	MtChr8	GACCAGAAGAAAGATGAGCGTA	TTGGCATCATCTCTCTCC	17300520	17299752	768
182	PvMt-182	MtChr8	GCAITGGCATATTTCTCTC	GGTGTACCCGAATACTCTAAGG	33226261	33224798	1463
183	PvMt-183	MtChr8	TCCTTCTGTTGTTGGGAGGA	CGCCAGCAGACATAACAACA	9392625	9392150	475
184	PvMt-184	MtChr8	TCGTCAAGACCTTTGTGACC	TGCCCTCTTAGCTTCTTCA	10189947	10191007	1060
185	PvMt-185	MtChr8	TGGAGAGTTCGAGACTTGT	AGAGGCAGATCCCTCTGTG	32298411	32297786	625
186	PvMt-186	MtChr8	AAGCAGGTGCAIGACTGG	CCAAGATTTCCATGCTTCCA	23361008	23361346	338
187	PvMt-187	MtChr8	AGAAAGCTGGAAGCACTCCT	TGAATGGCAGCAGCTAGGTT	17710651	17710383	268
188	PvMt-188	MtChr8	GAGGAAGGCATCGAGGAATA	AGCTTGTCTGCCCTGTTGT	24158310	24158921	611
189	PvMt-189	MtChr8	GAGACATCGGTGGCAATTC	TTCCACCGCTTCTTACTCG	3303746	3302546	1200
190	PvMt-190	MtChr8	AGAAAGCTGTGTACCCTTGG	TTGCTCTTGTAGTCAAGATCC	8441028	8441559	531
191	PvMt-191	MtChr8	CAGAGTTAGGAATGCCACCA	TCACCCCTACAGTACCATCA	32377488	32376630	858
192	PvMt-192	MtChr8	ATCCACTTGCCAAATCCACT	CTCGAATTTCCGGTCTTCC	31311117	31311867	750
193	PvMt-1101	MtChr1	AATATGACGCTTCCCTGCT	TTGCGTCAACATACACAT	4433162	4434159	997
194	PvMt-1102	MtChr1	AGCTGCCAAGGATCAATG	GGCTGCTGCTTCAAT	18837957	18838248	291
195	PvMt-1103	MtChr1	CTTGCGTCACTACTTGTGC	CTGTGTGAACCACTCCATCT	22142321	22142698	377
196	PvMt-1104	MtChr1	CATGGTTGATATGGGTTG	TCTTCCCTTGGACCAAGCTG	16488471	16489915	444
197	PvMt-1105	MtChr1	AGTTTTGCCCTCCACT	TACGTGAGGCAAGGAAAGTA	1157087	1157841	754
198	PvMt-1106	MtChr1	GAGAACCCATGACCCACTTG	TTCCATGATCAAGGGCAAC	14748182	14749585	1403
199	PvMt-1107	MtChr1	GGAGTTATGGCTCTGGAAGA	GTTGCCAAGCCTGTTGCTT	5653312	5653681	369
200	PvMt-1108	MtChr1	CCTGTTGGAAGTGAGGCAT	GGTCGCTGAATTCGAATGGT	23706401	23707240	839
201	PvMt-1109	MtChr1	TCCTTGATTTGCTGGTGTTC	GGAAGAAGATGGCGTCTTAG	3441102	3441832	730
202	PvMt-1110	MtChr1	GCAGGCATCAGTCAAGACTTC	GGAAATGTTGCTGGAGATTC	20772649	20773256	607
203	PvMt-1111	MtChr1	CAGGGCAATTTGTTATGCT	GGCAGAAGCCTGAGACTTGTAA	9958846	9959263	417
204	PvMt-1112	MtChr1	CGATGGCATCAGAAAATGAC	CGTGGACGTTCTTCTCTCC	32464190	32464826	636
205	PvMt-1113	MtChr2	CCCTTGAATTTTCAGAGGTTG	TTGCCACTGTCTTGGGAAG	2792158	2793448	1290
206	PvMt-1114	MtChr2	TCCTGCTTTGAACCAACTG	CATTTTCAAGCTCAGACTCCTTC	2187269	2187647	378
207	PvMt-1115	MtChr2	CTGGTGAACATGAGCATCAAC	CAGAGCTACAAGATGAGAACA	6949345	6949972	627
208	PvMt-1116	MtChr2	GCAACATCTGGTGTGTGT	TCCACCCACTCTTGTCTCTC	1732018	1732698	680
209	PvMt-1117	MtChr2	GTTCCGAATGGTGCACAG	CCTTAAAGCTCTGCTTCTATC	22459420	22459704	284
210	PvMt-1118	MtChr2	GGTTATGCTTTGGGATGAT	TGGAAATGTTCTTCACTTGA	20713660	20714120	460
211	PvMt-1119	MtChr2	ACCATGGAGCATCACCTGTT	CAATGACCAAGGAAGAGTTGTG	23820543	23821305	762
212	PvMt-1120	MtChr2	GATTCACGGCAGTTCTGT	TCACTCTTGCAACATCTCCTG	1843684	1844799	1115
213	PvMt-1121	MtChr2	AGCTCGACTCCTTGTACCT	GGAATGGCTGAAAAAGAGATGG	27577819	27578785	966
214	PvMt-1122	MtChr2	CTCCGTGTACAATGCAGTTTG	TGGGAAGAATTTCTTTCAGC	17091596	17092083	487
215	PvMt-1123	MtChr2	ACACTCCCATTTGTTCCATTC	GGTGGAGGAATGATCTTCAAC	17874587	17875002	415
216	PvMt-1124	MtChr2	ACATCAATGGCTGCCTTCC	ATGGCCTACATACCCCTTGG	30048785	30049363	578

Table 2 (cont'd)

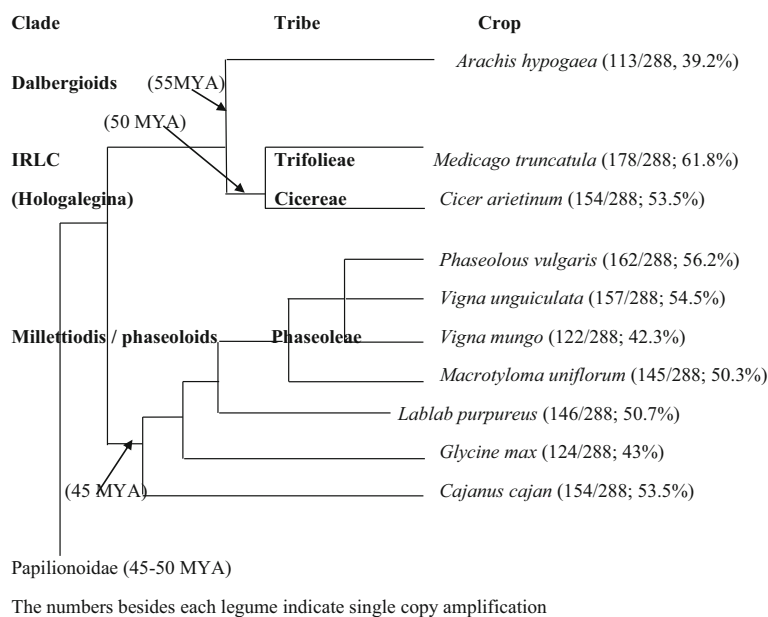
Marker name	ESTs	Chr. no.	Forward primer (5'-3')	Reverse primer (3'-5')	Primer start	Primer end	Amplicon size
217	PvMt-1125	MtChr3	CCATTGCTGTTGATGTTTGG	GACATGTTTCATTGGCAAGC	19684402	19685788	1386
218	PvMt-1126	MtChr3	CAATTTGCAGATGCTTGACG	AGAGCATAGAGACGTGCAAGG	32731883	32732282	399
219	PvMt-1127	MtChr3	CCAAAGCCAAAGTTGAAGA	CAAATTTACCAACGGTCACCT	24297034	24298015	981
220	PvMt-1128	MtChr3	GTGGGAAAGCCACTGAAAT	GCTCCACAGAAACGAAAT	22791581	22792154	573
221	PvMt-1129	MtChr3	ACCACGAAGCCTCAAACAA	ATCACCCCTGAGGTGGATC	30887886	30888326	440
222	PvMt-1130	MtChr3	AGGAAACAACACAGAGTCAC	CCATTTGTGCTCTGATTC	16748996	16749757	761
223	PvMt-1131	MtChr3	TGGTATGGGAGATGATATGG	TAGATGTGCCAAATCCACCA	42058862	42059519	657
224	PvMt-1132	MtChr3	CAGGAAATTTGAGAGCCACA	TGCTCCAACAGGTACACCAC	22054726	22055372	646
225	PvMt-1133	MtChr3	CCACAACACAACTCTCCA	ATGTGGCTTCTCAACTGC	4623265	4623975	710
226	PvMt-1134	MtChr3	CCTTCTCACACCACTCAAT	GTCCAAATGGAITGGCAAG	37586023	37586410	387
227	PvMt-1135	MtChr3	GAGCAAATTTGGATAGGACAAC	TGCTGAGTATTTCCACGTAAGC	30880112	30880986	874
228	PvMt-1136	MtChr3	GGAAAGCTGGAATGCTGA	GGTTTGGAAATCACCTTCC	42379832	42380503	671
229	PvMt-1137	MtChr4	TGGTTGCTGAGATCCTCATC	GGGAGCAAAGTTGTCAITG	30700422	30701805	1383
230	PvMt-1138	MtChr4	ATATGCAAAACCCGGCCATC	TGGAGTGTACCCGTAATTGG	15521266	15522333	1067
231	PvMt-1139	MtChr4	GGAGGTGGAACATTTGATGTG	CGAAGAACCCTCATCAACATC	286551761	28652273	512
232	PvMt-1140	MtChr4	TTGTTGGTGAATGCTGGTGT	GGCAAGAGCTTCAGCAAAGA	20815090	20816150	1060
233	PvMt-1141	MtChr4	CACCAAGCCTTTCAACTCCA	TGTCATTGCGAAGAAAGG	40511288	40512181	893
234	PvMt-1142	MtChr4	TTCCAGAATTCGGGTGCA	TGGGTAACAAGATCGGAAA	39051956	39052835	979
235	PvMt-1143	MtChr4	CCCTCAAGCACCAAAA	TTCTACTGGCACCTACTCG	3682054	3682514	460
236	PvMt-1144	MtChr4	AGGTCTGTGGTTTCAGCTT	CTTAACAGGCCAAGCAAACC	15956953	15957500	547
237	PvMt-1145	MtChr4	GCAGCTTAACCTTTTCCACA	TGGCAAAGTTATCAGGCACAA	24287903	24288958	1055
238	PvMt-1146	MtChr4	GCAATGGAAGAACAATGCTCAG	GGAGACATGGTTAACCGACTTG	7542306	7542591	285
239	PvMt-1147	MtChr4	GGGTGATGCTGTTTCCAAAC	ATAGCAAAGGATGCCCTGAA	19834738	19835195	457
240	PvMt-1148	MtChr4	GAAAGAACAACCCGAGCAT	AAGCCACAGGATGATCTA	24590133	24590751	618
241	PvMt-1149	MtChr5	TGGTCTAAAGGCGATGT	GCAGCTTCTGAACATGTCAC	42006511	42007228	717
242	PvMt-1150	MtChr5	AAAGGATGGCTACCTGGAG	CGTGAACACAGAAAGCATCA	34878468	34879152	684
243	PvMt-1151	MtChr5	TAGCGAGAAGTATGGATGC	TTGAGTGTTTTCCACCTGGTC	29714146	29714641	495
244	PvMt-1152	MtChr5	CTCCTAGTCTGCAATCTGTGTG	TTTGCATATGTGTCCCTCT	9609412	9609944	532
245	PvMt-1153	MtChr5	TGGATTTGACAGGGCACA	TCCTCTTGGAAATCCAGTGC	5926278	5926669	391
246	PvMt-1154	MtChr5	ACCCCAAGCAACCGTAGA	TGTCGCAATGTGGCTTCTA	38936636	38937264	628
247	PvMt-1155	MtChr5	TGGCAACACTTCTATCACTGC	ATTGAAGCAAGGAAAGCCTGA	11339488	11340075	587
248	PvMt-1156	MtChr5	GGACTCAAACCTTGAAGGGATA	TCCTTACCCCTTGTCTGATCC	11033609	11034334	725
249	PvMt-1157	MtChr5	TTGTCTCAAATGGAACACC	CCTGGTTCAGGATTTGGACA	14211211	14212258	1047
250	PvMt-1158	MtChr5	CCTTTGCACCCAAACAAGGA	TGGAGAGGTTGTGTTTGGG	4622682	4623285	603
251	PvMt-1159	MtChr5	CCATCTGAACAACCCCACT	CCAAAGTGGAGCTATTTCTT	26569507	26570619	1110
252	PvMt-1160	MtChr5	CCACACTTGTACTTGCCATT	TGCTCAAAGATGCCAGATG	36394351	36395051	700
253	PvMt-1161	MtChr6	TTACCTGCAAGGCAAAAGT	CCCCAAAGCTCAAAAGATCC	941839	942503	664
254	PvMt-1162	MtChr6	CCAACTATGGTTTGGAAAGACC	TGCAGATGCAGTGATAGAAGTG	11338329	11339517	1188
255	PvMt-1163	MtChr6	ATCTGGTGGCTTATGGGTTT	AAGTTCAATGGCCAACTCCA	3141653	3142421	768
256	PvMt-1164	MtChr6	CAAGGCTGTGGTTATCCATGT	CTTGCACAACAATCTCAGCAG	5183175	5183917	742
257	PvMt-1165	MtChr6	CAATGGATCGAATGGACCAC	CTTGGTGTGCTGGATTCAI	13155767	13156967	1200
258	PvMt-1166	MtChr6	GTTTTCAGGCAGACCATTGC	CAITCCAGCAGCTATCACTCA	13823487	13823756	269
259	PvMt-1167	MtChr6	GAGGAGCTCATCAAGGCAGT	GTTCACTCCATCATCAACTGC	6793020	6793692	672

Table 2 (contd)

Marker name	ESTs	Chr. no.	Forward primer (5'-3')	Reverse primer (3'-5')	Primer start	Primer end	Amplicon size
260	PvMt-1168	MtChr6	TCCACTTGGTCCCTTTTCTCA	AGCCATATGCAACCCCTTCA	3140823	3141580	757
261	PvMt-1169	MtChr6	GCTCCTTATTTGGCTGCAT	GTGCTTAGAGTGAGCGTGTA	16927911	16928604	693
262	PvMt-1170	MtChr6	TTTCCCAACCTCCTTTTGG	AAGGATGCTGCAAGTTGTGG	16359738	16360323	585
263	PvMt-1171	MtChr6	TGACATTTCTTGGTGTC	TGAATGGCTCAGAAAGCTG	16941553	16941973	420
264	PvMt-1172	MtChr6	CCATCTTCAAGCTCTTCC	TTCTGTCAGACTCTCACTGAA	12441120	12441497	377
265	PvMt-1173	MtChr7	GAGGATGCTGTTCAGTTGG	CCCAATCCCTCTGTGCTACCT	25102428	25103251	823
266	PvMt-1174	MtChr7	TCCACTTTTGGCCCTTTGTG	CAGTGTGAGCATCTCCCTGA	18144279	18145223	944
267	PvMt-1175	MtChr7	CGTCATATAGTTGAAGCTGTG	GGCTTTTCTCAATAACCCCAACC	31644493	31645530	1037
268	PvMt-1176	MtChr7	TGTGGTCCACACACACCTC	AACCCCTGTTCGACGAGTCT	34928992	34929523	531
269	PvMt-1177	MtChr7	TTCTTGTGAATCCCCAAC	CCAGAAACCCATGGATGAG	6388020	6388020	814
270	PvMt-1178	MtChr7	GGTGGCTGTGAGAGCATTTG	CATCAGCCTTCAATCTCA	2312775	2313556	781
271	PvMt-1179	MtChr7	GATGCCATGCAGATGC	CTGCTGGTGGATCCTCAAA	5445613	5446476	863
272	PvMt-1180	MtChr7	AGTGGCTTGTATGTAAGTGG	TTCACGTAAAGGCCATCCA	19745354	19745763	409
273	PvMt-1181	MtChr7	GGGTTTCATCAACAGCTCCT	CGTTGTGCTCCAGATCTTT	29707764	29708062	298
274	PvMt-1182	MtChr7	GAAACGTAGCTGCTGGAATGA	GCCCTCAGGGGTATCTTCTCA	24919629	24920149	520
275	PvMt-1183	MtChr7	GGCCAATCCAGCACCA	TGATGAGGCTGCTTTGAGAG	23000082	23000439	357
276	PvMt-1184	MtChr7	GCAGCTGCACAAATCTTC	AAACGTGCAGCTGTAAGG	34779563	34780880	1317
277	PvMt-1185	MtChr8	TCCGTGTGTGGACATCAAT	TCCTTCATTCGCTTTTCTC	36090175	36091480	1305
278	PvMt-1186	MtChr8	GCTTCCAACTCGAAGTACAA	GGCAATTTCAITTTGCTGGTC	79221329	7922015	686
279	PvMt-1187	MtChr8	CTCCACAAGCTCAGTGTA	TTGGGAATGTCTTGTGTA	2232053	2232881	828
280	PvMt-1188	MtChr8	CTGAAACATCTCAGCAAGAGG	TTTTGGTGAACAACCCAGGTG	23819674	23819994	320
281	PvMt-1189	MtChr8	GCCCAACTCAGGATCCTTTT	GACATGCCATAAAATGCAA	24446607	24447080	473
282	PvMt-1190	MtChr8	ATGGCTTGGACCTAGTGCG	GGCCTTGGATCTGCAACTT	33262649	33263558	909
283	PvMt-1191	MtChr8	ATGGAATCCGTGGTAATCG	CCTGGGTAAGCTCAITCAG	23614496	23615692	1196
284	PvMt-1192	MtChr8	TATGCAGATGGGACCCCTGAC	GGTAACCCCTCCCTTGAAGA	33984689	33985142	453
285	PvMt-1193	MtChr8	TGGGGTGAAGAAGCAGTAGG	CAGGTTGAAGCTCCCTCTCT	9637467	9637861	394
286	PvMt-1194	MtChr8	GCTGCATCTGATGCAACTTACC	TTTTGCTGGTGTGGTTCCAG	2598974	2599525	551
287	PvMt-1195	MtChr8	GAGCTGGGGCAGAAATTTATC	GTGAAGGCTAAGGTGGCAA	35500264	35500741	477
288	PvMt-1196	MtChr8	CGAACCTTGCAGAGCTTGA	GTTGACGGCAAAAGTCAGGA	11754467	11755746	1279

Table 3. PCR results of CISPs in selected legumes.

Alignment	Number of markers	Legumes	PCR success			% Success	Single copy amplification (%)
			Blank	Single	Multiple		
Common bean and barrel medic	288	Barrel medic	71	178	39	75.34	61.8
		Cowpea	65	157	66	77.43	54.51
		Chickpea	91	154	43	68.4	53.47
		Pigeonpea	63	154	71	78.12	53.47
		Horsegram	106	145	37	63.19	50.34
		Field bean	96	146	46	66.66	50.69
		Soybean	84	124	80	70.83	43.05
		Groundnut	106	113	69	63.19	39.23
		Blackgram	80	122	86	72.22	42.36
		Common bean	71	162	55	75.34	56.25

**Figure 1.** PCR success rates (single copy amplification) of 288 primers in different legumes (comprehensive legume tree adapted from Gepts et al. 2005).

qiagen.com). After purification, all the products (4 μ L) with the Fermentas GeneRuler 1 kb DNA Ladder were run on 2.0% agarose gel and documented. The PCR products were sequenced at Ocimum Bio-Sciences, Hyderabad, India using an ABI 3730XL sequencer. The sequences were BLAST aligned with the barrel medic genome to check whether orthologous regions were amplified and the amplicons derived from the same primer hit the intended genomic region. Sequences hitting the intended genomic region from different varieties of pigeonpea, chickpea and cowpea were aligned using ClustalW2 (www.ebi.ac.uk/clustalw) to find putative SNPs. Only those regions having base score of more than 20 were used in the identification of SNPs/INDeLs. We used a web-browser-based

program, dCAPS Finder 2.0 (<http://helix.wustl.edu/dcaps/dcaps.html>) to design dCAPS (Neff et al. 1998).

Results and discussion

A total of 1196 nonredundant set of conserved primer pairs were designed from common bean – barrel medic alignments using 67,429 common bean ESTs (table 1). The probable distribution of *in silico* developed common bean-barrel medic (PvMt) CISPs was studied. This study revealed the distribution of the highest number of loci on chromosomes 5 and 4, while chromosome 6 has the lowest number of CISP loci on *M. truncatula*. A

Table 4. SNPs and INDeLs identified in six cowpea varieties from common bean and barrel medic CISPs.

Marker name	Chromosome no.	EST ID	Variety	Variations (SNP or INDeL sequence)	SNP/INDeL	Size (cowpea/Medicago)
PvMt_035	MtChr6	FG230303	C-152	AAAGGGTAGATGATTCATAGCTAAATTTTATC	SNP	541/655
PvMt_103	MtChr1	CV539728	CW-2	AAAGGGTAGATGATTTTCATAGCTAAATTTTATC	SNP	516/557
PvMt_1102	MtChr1	CB541467	CW-2	CITTTGAAAGTTACAGAATGTAATA	INDeL	289/291
PvMt_1103	MtChr1	CB541360	KBC-2	GACGTTGAATAGAGAAAACCTGC	SNP	357/377
PvMt_1103	MtChr1	CB541360	C-152	GACGTTGAATG-AGAAAACCTGC	SNP	357/377
PvMt_1103	MtChr1	CB541360	CW-2	TATGTGTAAGAGGGAAAA	SNP	357/377
PvMt_1103	MtChr1	CB541360	KBC-2	TATGTGTAAGAGGGAAAA	SNP	357/377
PvMt_1103	MtChr1	CB541360	C-152	TATGTGTAAGAGGGAAAA	SNP	357/377
PvMt_1103	MtChr1	CB541360	CW-2	CTGTGTTCAAGTGGTTA	INDeL	357/377
PvMt_1103	MtChr1	CB541360	KBC-2	CTGTGTTCAAGTGGTTA	INDeL	357/377
PvMt_1103	MtChr1	CB541360	C-152	CTGTGTTCAAGTGGTTA	INDeL	357/377
PvMt_1103	MtChr1	CB541360	CW-2	TTATAGATGGATGG-T	SNP	357/377
PvMt_1103	MtChr1	CB541360	KBC-2	TTATAGATGGATGG-T	SNP	357/377
PvMt_1103	MtChr1	CB541360	C-152	TTATAGATGGATGG-T	SNP	357/377
PvMt_1103	MtChr1	CB541360	KBC-2	TAGTTTTGATGAAAAATGTTT	SNP	357/377
PvMt_1103	MtChr1	CB541360	C-152	TAGTTTTGATGAAAAATGTTT	SNP	357/377
PvMt_1103	MtChr1	CB541360	CW-2	TAGTTTTGATGAAAAATGTTT	SNP	357/377
PvMt_1103	MtChr1	CB541360	KBC-2	TTTCCTGATCAAATGIGTATGGT	SNP	357/377
PvMt_1103	MtChr1	CB541360	C-152	TTTCCTGATCAAATGIGTATGGT	SNP	357/377
PvMt_1103	MtChr1	CB541360	CW-2	TTTCCTGATCAAATGIGTATGGT	SNP	357/377
PvMt_1103	MtChr1	CB541360	KBC-2	ATCAATATATAATGAAATAAG	SNP	357/377
PvMt_1103	MtChr1	CB541360	C-152	ATCAATATATAATGAAATAAG	SNP	357/377
PvMt_1103	MtChr1	CB541360	CW-2	ATCAATATATAATGAAATAAG	SNP	357/377
PvMt_1104	MtChr1	CB541110	C-152	TTTGTTTTCAAATGT	SNP	735/444
PvMt_1104	MtChr1	CB541110	CW-2	TTTGTTTTCAAATGT	SNP	735/444
PvMt_1104	MtChr1	CB541110	C-152	TATATTTGTTCTTCGTTTGCCC	INDeL	735/444
PvMt_1104	MtChr1	CB541110	C-152	TATATTTG-TCTTCGTTTGCCC	INDeL	735/444
PvMt_1109	MtChr2	CB541569	IT-38956	AAAAATTCAGATAAAAA	SNP	652/762
PvMt_1109	MtChr2	CB541569	C-152	AAAAATTCAGATAAAAA	SNP	652/762
PvMt_1109	MtChr2	CB541569	KBC-2	AAAAATTCAGATAAAAA	SNP	652/762
PvMt_1109	MtChr2	CB541569	IT-38956	AAAAATTCAGATAAAAA	SNP	652/762
PvMt_1109	MtChr2	CB541569	C-152	AAAAATTCAGATAAAAA	SNP	652/762
PvMt_1119	MtChr6	CB540509	KBC-2	AAAAATTCAGATAAAAA	SNP	652/762
PvMt_1119	MtChr6	CB540509	IT-38956	TAAAAAAGAAAAGCTTTTGCCA	SNP	1253/693
PvMt_1119	MtChr6	CB540509	KBC-2	TAAAAAAGAAAAGCTTTTGCCA	SNP	1253/693
PvMt_1119	MtChr6	CB540509	IT-38956	TGTTTGGGCTGAAAATATATG	SNP	1253/693
PvMt_1119	MtChr6	CB540509	KBC-2	TGTTTGGGTTGAAAATATATG	SNP	1253/693

Table 4 (contd)

Marker name	Chromosome no.	EST ID	Variety	Variations (SNP or INDeL sequence)	SNP/INDeL	Size (cowpea/Medicago)
PvMt_1169	MtChr6	CB540509	IT-38956 KBC-2	GAGGGAAAGAGAAAGATTT GAGGGAAACAGAAAGATTT	SNP	1253/693
PvMt_1169	MtChr6	CB540509	IT-38956 KBC-2	GAAATGCTGTATAAAAAGACC GAAATGTTGTATAAAAAGACC	SNP	1253/693
PvMt_1169	MtChr6	CB540509	IT-38956 KBC-2	TAAAGTTATGGGCTTTCTACT TAAAGTTATGAGICTTTCTACT	SNP	1253/693
PvMt_1169	MtChr6	CB540509	IT-38956 KBC-2	CTACTATATATAGTCATCTTT CTACTATATACTAGTCATCTTT	SNP	1253/693
PvMt_1169	MtChr6	CB540509	IT-38956 KBC-2	ATCTTTCTCAATTTTATCCC ATCTTTCTCATTTTATCCC	SNP	1253/693
PvMt_1169	MtChr6	CB540509	IT-38956 KBC-2	CATTCATTTGAAAACACTACGCTTT CATTCATTTGAAAAGTAGGCTTT	SNP	1253/693
PvMt_1169	MtChr6	CB540509	IT-38956 KBC-2	TTGAAAACACTACGCTTTGAAA TTGAAAAGTAGGCTTTGAAA	SNP	1253/693
PvMt_1169	MtChr6	CB540509	IT-38956 KBC-2	GGTTTTGTTCAAGAAAACAC GGTTTTGTTAAGAAAACAC	SNP	1253/693
PvMt_1169	MtChr6	CB540509	IT-38956 KBC-2	ATTTCAAGGATGATGGA ATTTCAAGAAATGATGGA	SNP	1253/693
PvMt_1169	MtChr6	CB540509	IT-38956 KBC-2	CAGAAAGGACTTAATTTGAAC CAGAAAGGCTTAAATTTGAAC	SNP	1253/693
PvMt_1169	MtChr6	CB540509	IT-38956 KBC-2	AACTTTGTTTACGTTTTCATG AACTTTGTTTACGTTTTCATG	SNP	1253/693
PvMt_1169	MtChr6	CB540509	IT-38956 KBC-2	TCCACTCTCCAAATTTCCAC TCCACTCTCCAA-TTTCA	INDeL	1253/693
PvMt_1169	MtChr6	CB540509	IT-38956 KBC	TTGCCATCT-GAAGTACTAG TTGCCATCTGAAGAAGTACTAG	INDeL	1253/693
PvMt_1174	MtChr7	CB544079	IT-38956 KBC-2	AAATTCGGTGTGCTGTTT AAATTCGGTG-TTGCTGTTT	INDeL	868/944
PvMt_1176	MtChr7	CB543563	KBC-2	CTCTTCTCATGCTACTTGTTTT CTCTTCTGATGCTACTTGTTTT	SNP	448/531
PvMt_1188	MtChr8	CB542012	KBC-2	AAATCCACTG-AAAAAAA AAATCCACTGA AAAAAAAA	INDeL	322/320
PvMt_1193	MtChr8	CB540867	IT-38956 CW-2	AAAGAG-AAATGTA AAAAA-AAATGTA	INDeL	495/394
PvMt_1193	MtChr8	CB540867	IT-38956 CW-2	AAAAAATAAATAATGTA CTTTGAATGGGCTGCT	SNP	495/394
PvMt_1193	MtChr8	CB540867	IT-38956 CW-2	CTTTGAATGGGCTGCT CTTTGAGTGGGCTGCT	SNP	495/394
PvMt_1193	MtChr8	CB540867	IT-38956 KBC-2	TGCTAACTAAAGTGACACCTTT TGCTAACTAAAGTGACACCTTT	SNP	495/394
PvMt_1193	MtChr8	CB540867	IT-38956	TGCTAACTGAGTGACACCTTT	SNP	495/394

Table 5. SNPs and INDeLs among eight pigeonpea genotypes from common bean and barrel medic CISPs.

Marker name	EST ID	Chromosome no.	Variety	Variations (SNP or INDeL sequence)	Position	SNP/INDeL	Size (pigeonpea/Medicago)
PvMt_051	CV542141	MtChr1	ICP7035	GATCTATTCATCAGTTGAAAATTGA	361	SNP	799/964
			TTB-7	GACCTATTCATCAGTTGAAAATTGA	361		
			ICP8863	GACCTATTCATCAGTTGAAAATTGA	360		
PvMt_051	CV542141	MtChr1	ICPL87119	GACCTATTCATCAGTTGAAAATTGA	359	SNP	799/964
			ICP7035	ATGCCACTGATTTATAAGGAATAA	589		
			TTB-7	ATGCCACTGATTTACAAGGAATAA	589		
			ICP8863	ATGCCACTGATTTACAAGGAATAA	588		
			ICPL87119	ATGCCACTGATTTACAAGGAATAA	587		
PvMt_051	CV542141	MtChr1	ICP7035	AGGGTCCATCCTTAAAATGGATTAGAA	459	SNP	799/964
			TTB-7	AGGGTCCGTCCTTAAAATGGATTAGAA	459		
			ICP8863	AGGGTCCGTCCTTAAAATGGATTAGAA	458		
			ICPL87119	AGGGTCCGTCCTTAAAATGGATTAGAA	457		
PvMt_1114	CB542659	MtChr2	ICP7035	GTTTTCTCAAATTCGGTGGGTTTC	388	SNP	1080/378
			GS-1	GTTTTCTCAAATTCGGTGGGTTTC	288		
PvMt_1114	CB542659	MtChr2	ICPL87119	GTTTTCTCAAATTCGGTGGGTTTC	382	SNP	1080/378
			ICP8863	GTTTTCTCAAATTCGGTGGGTTTC	382		
			ICP7035	GAGTCTTTTATAGTTTCCITTTTG	431		
PvMt_1129	CB542481	MtChr3	GS-1	GAGTCTTTTATAGTTTCCITTTTG	431	SNP	826/440
			ICPL87119	GAGTCTTTTATAGTTTCCITTTTG	425		
			ICP8863	GAGTCTTTTATAGTTTCCITTTTG	425		
			ICPL87119	AATCAATAAAAACGTATCGTT	538		
			TTB-7	AATCAATAAAAACGTATCGTT	538		
			ICP7035	AATCAATAAAAACGTATCGTT	543		
			GS-1	AATCAATAAAAACGTATCGTT	538		
			ICP8863	AATCAATAAAAACGTATCGTT	542		
			ICPL87119	TGTCCTGAAATCTTAGTTTTGA	577		
			TTB-7	TGTCCTGAAATCTTAGTTTTGA	577		
PvMt_1176	CB543563	MtChr7	ICP7035	TGTCCTGAAATCTTAGTTTTGA	582	INDeL	474/531
			GS-1	TGTCCTGAAATCTTAGTTTTGA	577		
			ICP8863	TGTCCTGAAATCTTAGTTTTGA	581		
			GS-1	AGGGAAGAGCCCGAA	50		
			ICP7035	AGG-AAGAGCCCGAA	49		
			ICPL87119	AGG-AAGAGCCCGAA	49		
			ICP8863	AGG-AAGAGCCCGAA	43		
			TTB-7	AGG-AAGAGCCCGAA	43		
			GS-1	GCAAAAATGATGCTAAACCA	68		
			ICP7035	GCAAAA-TGATGCTAAACCA	67		
PvMt_1176	CB543563	MtChr7	ICPL87119	GCAAAA-TGATGCTAAACCA	66	INDeL	474/531
			ICP8863	GCAAAA-TGATGCTAAACCA	66		

Table 6. SNPs and INDeLs identified in eight chickpea varieties from common bean and barrel medic CISPs.

Marker name	Chromosome no.	EST ID	Variety	Variations (SNP or INDeL sequence)	Position	SNP/INDeL	Size (chickpea/Medicago)
PvMt_46	MtChr8	FG231803	JG-62	CAAGGACAAAAAACACAGTAAA	508	INDeL	1939/767
PvMt_103	MtChr1	CV539728	WR-315	CAAGGACAAAAAACACAGTAAA	425	SNP	506/557
			KAK-2	CACCAATTATCCATCAAACTC	181		
PvMt_103	MtChr1	CV539728	A-1	CACCAATTATCCATCAAACTC	164	SNP	506/557
			K-850	CACCAATTATGCATCAAACTC	162		
			ICCV-4958	CACCAATTATGCATCAAACTC	162		
			ICCV-2	CACCAATTATGCATCAAACTC	165		
			WR-315	CACCAATTATGCATCAAACTC	166		
			KAK-2	CITTTTAGTTTCTATAGTCT	217		
			A-1	CITTTTAGTTTCTATAGTCT	200		
			K-850	CITTTTAGTTTCTATAGTCT	198		
			ICCV-4958	CITTTTAGTTTCTATAGTCT	198		
			ICCV-2	CITTTTAGTTTCTATAGTCT	201		
PvMt_103	MtChr1	CV539728	WR-315	CITTTTAGTTTCTATAGTCT	202	SNP	506/557
			KAK-2	TCCTTAGACAGATTTTCCATT	234		
			A-1	TCCTTAGACAGATTTTCCATT	217		
			K-850	TCCTTAGACAAATTTTCCATT	215		
			ICCV-4958	TCCTTAGACAAATTTTCCATT	215		
			ICCV-2	TCCTTAGACAAATTTTCCATT	218		
			WR-315	TCCTTAGACAAATTTTCCATT	219		
			KAK-2	CCATTATCTTAGTAATTGTAC	250		
			A-1	CCATTATCTTAGTAATTGTAC	233		
			K-850	CCATTATCTTAGTAATTGTAC	231		
PvMt_103	MtChr1	CV539728	ICCV-2	CCATTATCTTAGTAATTGTAC	234	SNP	506/557
			ICCV-4958	CCATTATCTTAGTAATTGTAC	231		
			WR-315	CCATTGTCTTTGTAAATTGTAC	235		
			KAK-2	AAAAAACATTGTAACCTAAAT	281		
			A-1	AAAAAACATTGTAACCTAAAT	264		
			K-850	AAAAAACATTGTAACCTAAAT	262		
			ICCV-4958	AAAAAACATTGTAACCTAAAT	262		
			ICCV-2	AAAAAACATTGTAACCTAAAT	265		
			WR-315	AAAAAACATTGTAACCTAAAT	266		
			KAK-2	AGGAA-CGAGGTTCAA	356		
PvMt_103	MtChr1	CV539728	A-1	AGGAA-CGAGGTTCAA	339	INDeL	506/557
			K-850	AGGAAACGAGGTTCAA	337		
			ICCV-4958	AGGAAACGAGGTTCAA	337		
			ICCV-2	AGGAAACGAGGTTCAA	340		
			WR-315	AGGAAACGAGGTTCAA	341		
			KAK-2	TGTAATAATGACCAATTATTGTC	400 & 402		
			A-1	TGTAATAATGACCAATTATTGTC	383 & 385		
			K-850	TGTAATAATGACCAATTATTGTC	382 & 384		
			ICCV-4958	TGTAATAATGACTAGTATTGTC	382 & 384		
			ICCV-2	TGTAATAATGACTAGTATTGTC	385 & 387		
WR-315	TGTAATAATGACTAGTATTGTC	386 & 388					

Table 6 (cont'd)

Marker name	Chromosome no.	EST ID	Variety	Variations (SNP or INDeL sequence)	Position	SNP/INDeL	Size (chickpea/Medicago)
PvMt_103	MtChr1	CV539728	KAK-2	TATTGTCATCTTAATTAGTA	410	SNP	506/557
			A-1	TATTGTCATCTTAATTAGTA	393		
			K-850	TATTGTCGCTTAATTAGTA	392		
			ICCV-4958	TATTGTCGCTTAATTAGTA	392		
			ICCV-2	TATTGTCGCTTAATTAGTA	395		
			WR-315	TATTGTCGCTTAATTAGTA	396		
			KAK-2	GTAATAAATTTCTCCCTCCCC	430		
			A-1	GTAATAAATTTCTCCCTCCCC	413		
			K-850	GTAATAAATTTCTCCCTCCCC	412		
			ICCV-4958	GTAATAAATTTCTCCCTCCCC	412		
PvMt_103	MtChr1	CV539728	KAK-2	GTAATAAATTTCTCCCTCCCC	415	SNP	506/557
			ICCV-2	GTAATAAATTTCTCCCTCCCC	416		
			WR-315	GTAATAAATTTCTCCCTCCCC	447		
			KAK-2	CCCCATTTTTTCTCTAAA	447		
			A-1	CCCCATTTTTTCTCTAAA	430		
			K-850	CCCCATTTTTTCTCTAAA	429		
			ICCV-4958	CCCCATTTTTTCTCTAAA	429		
			ICCV-2	CCCCATTTTTTCTCTAAA	432		
			WR-315	CCCCATTTTTTCTCTAAA	433		
			KAK-2	TCTAAAATAATTTCTAGTTA	461		
PvMt_103	MtChr1	CV539728	KAK-2	TCTAAAATAATTTCTAGTTA	444	SNP	506/557
			A-1	TCTAAAATAATTTCTAGTTA	444		
			K-850	TCTAAAATAATTTCTAGTTA	443		
			ICCV-4958	TCTAAAATAATTTCTAGTTA	443		
			ICCV-2	TCTAAAATAATTTCTAGTTA	446		
			WR-315	TCTAAAATAATTTCTAGTTA	447		
			KAK-2	AACTGCAACTTTTTATGGA	488		
			A-1	AACTGCAACTTTTTATGGA	471		
			K-850	AACTGCAACTTTTTATGGA	470		
			ICCV-4958	AACTGCAACTTTTTATGGA	470		
PvMt_103	MtChr1	CV539728	ICCV-2	AACTGCAACTTTTTATGGA	473	SNP	506/557
			KAK-2	CAAAGGCTAACTGCAAAATCC	515		
			A-1	CAAAGGCTAACTGCAAAATCC	498		
			K-850	CAAAGGCTAACTGCAAAATCC	497		
			KAK-2	CAAAGGCTAACTGCAAAATCC	515		
			ICCV-2	CAAAGGCTAACTGCAAAATCC	500		
			KAK-2	CCTGATTTGGAAACCAAAAT	534		
			A-1	CCTGATTTGGAAACCAAAAT	517		
			K-850	CCTGATTTGGAAACCAAAAT	516		
			KAK-2	CCTGATTTGGAAACCAAAAT	534		
PvMt_103	MtChr1	CV539728	ICCV-4958	CCTGATTTGGAAACCAAAAT	516	SNP	506/557
			KAK-2	CCTGATTTGGAAACCAAAAT	534		
			ICCV-2	CCTGATTTGGAAACCAAAAT	534		
			KAK-2	CCTGATTTGGAAACCAAAAT	516		
			ICCV-2	CCTGATTTGGAAACCAAAAT	534		
			ICCV-2	CCTGATTTGGAAACCAAAAT	519		
			ICCV-2	CCAATTTGACCAATCAGAA	646		

Table 6 (contd)

Marker name	Chromosome no.	EST ID	Variety	Variations (SNP or INDeL sequence)	Position	SNP/INDeL	Size (chickpea/Medicago)
PvMt_103	MtChr1	CV539728	ICCV-4958	CCAAATTTGACCATCAGAA	643	SNP	506/557
			K-850	CCAAATTTGACCATCAGAA	643		
			A-1	CCAAATTTGCCCATCAGAA	644		
			KAK-2	CCAAATTTGCCCATCAGAA	661		
			ICCV-2	GTTCCATCTTTGTGGT	839		
			K-850	GTTCCATCTTTGTGGT	835		
			KAK-2	GGTTCACAICTTTGGT	869		
			KAK-2	ACTGCAATTCGGATGGTC	260		
			A-1	ACTGCAATTCGGATGGTC	180		
			K-850	ACTGCAATTCGTATGGTC	257		
PvMt_105	MtChr1	CV540193	ICCV-4958	ACTGCAATTCGTATGGTC	259	SNP	1052/796
			BG-256	ACTGCAATTCGTATGGTC	247		
			WR-315	ACTGCAATTCGTATGGTC	257		
			JG-62	ACTGCAATTCGTATGGTC	148		
			ICCV-2	CGAGGCTAGCTAAACAAAA	51 & 52		
			K-850	CAGAGCTAGCTAAACAAAA	110 & 111		
			JG-62	CAGAGCTAGCTAAACAAAA	105 & 106		
			WR-315	CAGAGCTAGCTAAACAAAA	103 & 104		
			ICCV-4958	CAGAGCTAGCTAAACAAAA	116 & 117		
			KAK-2	CAGAGCTAGCTAAACAAAA	116 & 117		
PvMt_108	MtChr1	CV540411	A-1	CAGAGCTAGCTAAACAAAA	71 & 72	SNP	951/457
			K-850	AAACACTAAA-CAAAAA	550		
			BG-256	AAACACTAAA-CAAAAA	401		
			ICCV-2	AAACACTAAA-CAAAAA	491		
			JG-62	AAACACTAAA-CAAAAA	545		
			WR-315	AAACACTAAA-CAAAAA	543		
			ICCV-4958	AAACACTAAA-CAAAAA	556		
			KAK-2	AAACACTAAA-CAAAAA	556		
			A-1	AAACACTAAA-CAAAAA	511		
			A-1	CTTATAACAA-GCAGGAAAGC	103		
PvMt_110	MtChr1	CV539267	KAK-2	CTTATAACAA-GCAGGAAAGC	54	INDeL	546/1236
			BG-256	CTTATAACAA-GCAGGAAAGC	52		
			JG-62	CTTATAACAA-GCAGGAAAGC	166		
			ICCV-2	CTTATAACAA-GCAGGAAAGC	167		
			K-850	CTTATAACAA-GCAGGAAAGC	172		
			WR-315	CTTATAACAA-GCAGGAAAGC	55		
			ICCV-2	TCTACTTTTAAAAA-CAATTTAA	67		
			JG-62	TCTACTTTTAAAAA-CAATTTAA			
			ICCV-4958	TCTACTTTTAAAAA-CAATTTAA			
			WR-315	TCTACTTTTAAAAA-CAATTTAA			
PvMt_119	MtChr1	CV539711	K-850	TCTACTTTTAAAAA-CAATTTAA		INDeL	1741/868
			KAK-2	TCTACTTTTAAAAA-CAATTTAA			
			BG-256	TCTACTTTTAAAAA-CAATTTAA			
			A-1-23	TCTACTTTTAAAAA-CAATTTAA			

Table 6 (contd)

Marker name	Chromosome no.	EST ID	Variety	Variations (SNP or INDeL sequence)	Position	SNP/INDeL	Size (chickpea/Medicago)		
PvMt_127	MtChr3	CV539523	ICCV-4958	GCACATAA-TCAACTATTGC	556	INDEL	758/795		
			K-850	GCACATAA-TCAACTATTGC	566				
			WR-315	GCACATAA-TCAACTATTGC	563				
			JG11	GCACATAA-TCAACTATTGC	560				
			KAK-2	GCACATAA-TCAACTATTGC	518				
			BG-256	GCACATAA-TCAACTATTGC	513				
			A-1	GCACATAA-TCAACTATTGC	570				
			BG-256	TTTCAGCTTAACAGGGT	81			SNP	Blank/972
			ICCV-4958	TTTCAGCTTAGCAGGGT	91			INDEL	Blank/1137
			ICCV-4958	ACAAA-CAAGA	197				
PvMt_133	MtChr4	CV538123	WR-315	ACAAAACAAGA	72	SNP	Blank/972		
			BG-256	ACAAA-CAAGA	190				
			K-850	ACAAA-CAAGA	189				
			ICCV-2	ACAAA-CAAGA	157				
			A-1	ACAAA-CAAGA	187				
			JG-62	ACAAA-CAAGA	192				
			KAK-2	ACAAA-CAAGA	198				
			ICCV-4958	TCTGAA-CAAATAG	209			INDEL	Blank/1137
			WR-315	TCTGAAACAATAG	84				
			PvMt_136	MtChr4	CV538208			BG-256	TCTGAA-CAAATAG
K-850	TCTGAA-CAAATAG	201							
ICCV-2	TCTGAA-CAAATAG	169							
A-1	TCTGAA-CAAATAG	199							
JG-62	TCTGAA-CAAATAG	204							
KAK-2	TCTGAA-CAAATAG	210							
ICCV-4958	AAAACCAA-ITCAAATTT	226				INDeL	Blank/1137		
WR-315	AAAACCAAATTCAAATTT	101							
BG-256	AAAACCAA-ITCAAATTT	219				SNP	Blank/1137		
K-850	AAAACCAA-ITCAAATTT	218							
ICCV-2	AAAACCAA-ITCAAATTT	186							
A-1	AAAACCAA-ITCAAATTT	216							
JG-62	AAAACCAA-ITCAAATTT	221							
KAK-2	AAAACCAA-ITCAAATTT	227							
ICCV-4958	TTTTCGCTTCTGGTCTGTTG	389							
WR-315	TTTTCGCTTCTGGTCTGTTG	267							
PvMt_136	MtChr4	CV538208	JG-62	TTTTCGCTTCTGGTCTGTTG	390	SNP	Blank/1137		
			K-850	TTTTCGCTTCTGGTCTGTTG	384				
			BG-256	TTTTCGCTTCTGGTCTGTTG	382				
			KAK-2	TTTTCGCTTCTGGTCTGTTG	381				
			ICCV-2	TTTTCGCTTCTGGTCTGTTG	349				
			A-1	TTTTCGCTTCTGGTCTGTTG	379				
			BG-256	TATATAGTCAAGGTCAAATTT	264			SNP	Blank/1228
			WR-315	TATATAGTCAAGGTCAAATTT	238				
			KAK-2	TATATAGTCAAGGTCAAATTT	112				

Table 6 (contd)

Marker name	Chromosome no.	EST ID	Variety	Variations (SNP or INDeL sequence)	Position	SNP/INDeL	Size (chickpea/Medicago)
PvMt_151	MtChr5	CV539567	JG-62	TATATAGTCAGGGTCAAATTT	171	INDeL	Blank/1228
			ICCV-4958	TATATAGTCAGGGTCAAATTT	268		
			A-1	TATATAGTCAGGGTCAAATTT	264		
			K-850	TATATAGTCAGGGTCAAATTT	278		
			K-850	TAAACAAAA-CTAAAAAA	518		
			KAK-2	TAAACAAAAACTAAAAAA	392		
			JG-62	TAAACAAAA-CTAAAAAA	451		
			ICCV-4958	TAAACAAAA-CTAAAAAA	549		
			A-1	TAAACAAAA-CTAAAAAA	544		
			BG-256	TAAACAAAA-CTAAAAAA	544		
PvMt_151	MtChr5	CV539567	WR-315	TAAACAAAA-CTAAAAAA	558	INDeL	Blank/1228
			WR-315	AAAATTATACATGTTT-	651		
			KAK-2	AAAATTATACATGTTT	531		
			K-850	AAAATTATACATGTTT-	584		
			ICCV-4958	AAAATTATACATGTTT-	682		
			A-1	AAAATTATACATGTTT-	677		
			BG-256	AAAATTATACATGTTT-	677		
			JG-62	AAAATTATACATGTTT-	691		
			JG-62	TCCCTAAATCAAAAATCCACGCG	57		
			K-850	TCCCTAATC-AAAATCCACGCG			
PvMt_173	MtChr7	CV539368	A-1	TCCCTAATC-AAAATCCACGCG		INDeL	243/1005
			KAK-2	TCCCTAATC-AAAATCCACGCG			
			WR-315	TCCCTAATC-AAAATCCACGCG			
			BG-256	TCCCTAATC-AAAATCCACGCG			
			ICCV-4958	TCCCTAATC-AAAATCCACGCG			
			K-850	AAGAAAGCAGCTCAGTGAAAACA	217		
			WR-315	AAGAAAGCAGCTTAGTGAAAACA	171		
			K-850	AAAAAATGATGCCAGATTGCAAT	280		
			WR-315	AAAAAATGATGC-AGATTGCAAT	233		
			K-850	AAAAACGGCCTTTGGTGTCAATAAG	258		
PvMt_1147	MtChr4	CB541649	JG-62	AAAAACGGCCTTTAGTGTCAATAAG	296	SNP	601/457
			K-850	ATCTGTGGAGAGAAAGAAAATTT	322		
			WR-315	ATCTGTGGAGAGACAGAAAATTT	360		
			K-850	AGGATACATAAGACAATACAAC	393		
			JG-62	AGGATACATAAGATAATAACAAC	431		
			K-850	AACTATGTTTCAACATATTTAGGA	434		
			ICCV-4958	AACTATGTTTCAACATATCTAGGA	472		
			K-850				
			WR-315				
			K-850				
PvMt_1147	MtChr4	CB541649	JG-62			INDeL	601/457
			K-850				
			WR-315				
			K-850				
			WR-315				
			K-850				
			JG-62				
			K-850				
			WR-315				
			K-850				
PvMt_1196	MtChr8	CB539991	JG-62			SNP	1014/1279
			K-850				
			WR-315				
			K-850				
			JG-62				
			K-850				
			WR-315				
			K-850				
			JG-62				
			K-850				
PvMt_1196	MtChr8	CB539991	JG-62			SNP	1014/1279
			K-850				
			WR-315				
			K-850				
			JG-62				
			K-850				
			WR-315				
			K-850				
			JG-62				
			K-850				
PvMt_1196	MtChr8	CB539991	JG-62			SNP	1014/1279
			K-850				
			WR-315				
			K-850				
			JG-62				
			K-850				
			WR-315				
			K-850				
			JG-62				
			K-850				

Table 7. Polymorphisms detected in three different legumes from common bean and barrel medic CISPs.

	No. of primers	Transitions	Transversions	Total no. of good sequences	Primers with polymorphisms	No. of SNPs	No. of INDeLs	No. of SNPs/kb
Chickpea	41	11	15	312	41	27	14	2.37
Cowpea	34	19	7	256	34	26	8	0.93
Pigeonpea	9	1	4	202	9	7	2	0.52

random set of 288 intron flanking markers (table 2) were tried on 10 diverse legume taxa belonging to both temperate and tropical legumes, namely, barrel medic, pigeonpea (BRG-2), cowpea (KBC-2), chickpea (A1), common bean (Arka Suvidha), soybean (MAUS-2), horsegram (PHG-9), blackgram (TAU-1), field bean (HA-4) and peanut (TMV-2) (table 3). The markers were expected to amplify single specific band as the criteria for primer design were so stringent that the designed primer should amplify single specific amplicon in barrel medic. It was also confirmed by sequencing of PCR products of pigeonpea, chickpea and cowpea varieties. The successful amplification of single-copy loci ranged from 39.2 in peanut (Dalbergoid) to 61.8% in barrel medic (Hologalegina clade). High percentage of amplification success rates was obtained in the source taxa barrel medic (61.8%) and common bean (56.2%). Relatively the same percentage of amplification success rates was obtained in cowpea (54.51%), pigeonpea (53.5%) and chickpea (53.5%) followed by 50% amplification success rates in field bean and horsegram. Comparably low percentages of primer sets amplified in soybean, urdbean and peanut (40–43%) (figure 1). The amplification success rate being high on the source taxa and the average success rate found to be around 45% across different clades, suggested that these primer sets can potentially yield genomic tags for a majority of legume crops. Although the legumes mentioned above belong to different clades, the amplification success using these markers implies high cross species transferability of the primers. In similar studies (Choi *et al.* 2004a, b; Shoemaker *et al.* 2006), successful cross-species genetic markers were developed from the ESTs and these putatively orthologous markers were mapped in barrel medic and a few other legumes. The percentage success rate was relatively low in soybean (43.05%) and groundnut (39.23%). The proportion of primer pairs showing multiple band amplification was higher in some members like field bean and soybean. This is probably because soybean genome underwent polyploidy ~13 Mya (Shoemaker *et al.* 2006). Field bean (*L. purpureus* L.) genome has apparently accumulated a large number of duplications/deletions after it diverged as the maximum number of primers generated multiple bands (Humphry *et al.* 2002). Lower single-copy PCR success rates observed for peanut (39.23%) might be attributed to its evolutionary distant relationship from the source taxa and the barrel medic. High-quality sequences generated from barrel medic, pigeonpea, chickpea and cowpea were examined for AT and GC contents. In all these legumes, AT fraction was found to be more. The sequence analysis presented in the current study supports the earlier conclusions that legume genomes are AT rich. The high primer amplification rate in spite of the AT skews in these legumes points at the significance of these intron spanning primers in comparative genomics in various plant families (Marek and Shoemaker 1997; Garg *et al.* 2011; Varshney *et al.* 2012).

Table 8. dCAPS identified in pigeonpea, cowpea and chickpea.

Marker name	Chromosome no.	SNP	dCAPS primers	Enzyme	Recognition site
Pigeonpea PvMt-051	1	T/C	AACGTAGGATGATTGGCAAGCAATTGTTCCA GATCCTCTTTTCCCGTCCTC	<i>BclI</i>	CCATC
PvMt-051	1	A/G	AACGTAGGATGATTGGCAAGCAATTGTTCCGG CTGAGATGCAGACAATCGTGA	<i>HaeIII</i>	GGCC
PvMt_1174	7	T/A	ATAAACCCCTTGTTCAGAAATCTGCC CTGGGATTCTGTTCTCTTGATG	<i>MaeI</i>	CTAG
Cowpea PvMt_103	8	T/A	CCAGATCAGGCTTCATGGGCTATCGT AAAAACTTGGGCACAGATGC	<i>RsaI</i>	GTAC
PvMt_103	1	C/T	TTTGCTTTCACAGATCAGTAAAGTAG AAAAACTTGGGCACAGATGC	<i>AluI</i>	AGCT
PvMt_1169	1	C/T	AGTACTAGATCATGAAATGTTTGAG AAGGGTCATGAAAACAGCA	<i>AluI</i>	AGCT
PvMt_1169	3	G/C	TGTGTGAGGTTTTTATGAGGGGGAA AAGGGTCATGAAAACAGCA	<i>MboII</i>	GAAGA
PvMt_1169	6	G/A	TTCCAATCCAAAACCTTAAAGTTTCTA AAGGGTCATGAAAACAGCA	<i>MaeI</i>	CTAG
PvMt_1176	7	C/G	TATTATATATGTTGCAACTCTCCT CGAGTCTTAAAGCTTTCCTTGTC	<i>MnlII</i>	CCTC
Chickpea PvMt_151	5	A/G	TATTTTACCACACAAGATATATAGTTA CATGAATCCATCCCTGATCC	<i>MseI</i>	TTAA
PvMt_1196	8	G/A	CTGACTGAGTGGAGACGAAAACGGCCTA TGCTATGCTCTAATGCTGCTG	<i>MseI</i>	TTAA
PvMt_1196	8	A/C	ATTGACACAACATCTGTGGAGAGG ATAGTGTAAAGCGCTGCAA	<i>MboII</i>	GAAGA

Table 9. CAPS identified in pigeonpea, cowpea and chickpea.

Marker name	SNP	SNP sequence	Enzyme	Recognition	RE cut
Pigeonpea					
PvMt_051	T/C	ATGCCACTGATTTATAAGGAATAA ATGCCACTGATTTACAAGGAATAA	PsiI	TTATAA	Wild forward
PvMt_1114	C/A	GAGTCTTTCTAGTTTCCTTTTG GAGTCTTTATAGTTTCCTTTTG	MaeI	CTAG	Wild forward
PvMt_1129	G/A	AATCAATAAAACGTATCGTT AATCAATAAAACATATCGTT	MaeII	ACGT	Wild forward
Cowpea					
PvMt_1103	C/G	TTTCCTGATCAAATGTGTATGGT TTTCCTGATGAAATGTGTATGGT	MboI	GATC	Wild forward
PvMt_1169	G/A	TTAAGTTTATGGGTCTTTCTACT TTAAGTTTATGAGTCTTTCTACT	SimI	GGGTC	Wild forward
PvMt_1169	T/C	CTACTTATATATTAGTCATCTTT CTACTTATATACTAGTCATCTTT	MaeI	CTAG	Mutant forward
PvMt_1169	A/T	ATCTTTCTCAATTTTATCCC ATCTTTCTCATTTTTATCCC	TspEI	AATT	Wild forward
PvMt_1169	C/T	GGTTTTGTCAAGAAAAACAC GGTTTTGTAAAGAAAAACAC	MseI	TTAA	Mutant forward
PvMt_1193	A/G	TGCTAACTAAGTGCACCTTT TGCTAACTGAGTGCACCTTT	BseMII	CTCAG	Mutant reverse
Chickpea					
PvMt_103	C/G	CACCATTTATCCATCAAACCTC CACCATTTATGCATCAAACCTC	CviRI	TGCA	Mutant forward
PvMt_103	G/T	AAAAAACATGTAAACCTAAAT AAAAAACATTTAACCTAAAT	MseI	TTAA	Mutant forward
PvMt_103	T/A	TCTAAAAATATTTCTAGTTTA TCTAAAAATAATTCTAGTTTA	TspEI	AATT	Mutant forward
PvMt_103	G/C	CCTGATTTTGAAACCAAATT CCTGATTTTGCAAACCAAATT	CviRI	TGCA	Mutant forward
PvMt_105	G/T	ACTGCAATTCGGATGGTC ACTGCAATTCGATGGTC	HinfIII	CGAAT	Wild reverse
PvMt_133	A/G	TTCAGCTTAACAGGGT TTCAGCTTAGCAGGGT	MseI	TTAA	Wild forward
PvMt_136	T/G	TTTTCGCTTCTGGTCTGTTTG TTTTCGCTTCGGTCTGTTTG	SimI	GGGTC	Mutant forward
PvMt_151	A/G	TATATAGTCAAGGTCAAATTT TATATAGTCAGGTCAAATTT	SimI	GGGTC	Mutant forward
PvMt_1196	T/C	AACTATGTTTCAACATATTAGGAACAAC AACTATGTTTCAACATATCTAGGAACAAC	MaeI	CTAG	Mutant forward

SNP discovery in pigeonpea, chickpea and cowpea

Introns have less evolutionary constraints when compared with exons. We demonstrated this in our study by studying length polymorphisms and also by detecting relatively good number of SNPs and INDeLs in pigeonpea, chickpea and cowpea. High-quality PCR products obtained by testing them on different varieties of pigeonpea, chickpea and cowpea were sequenced. The PCR products of 222 primer pairs were sequenced and aligned. Manual mining for SNPs/INDeLs was carried out considering those bases whose quality score was more than 20. We found 26 SNPs and eight INDeLs from the alignments of products from 34 primers in cowpea, seven SNPs and two INDeLs from nine primers in chickpea and 27 SNPs and 14 INDeLs from 41 primers in pigeonpea (tables 4–6). Average SNP frequency was calculated considering the number of amplicons for which quality sequences were generated from

different varieties and it was found to be 0.93/kb in cowpea, 2.37/kb in chickpea and 0.52/kb in pigeonpea. Transitions and transversions were found in almost equal proportions in the SNPs identified in chickpea whereas transitions were more in cowpea (table 7).

Conversion of SNPs into PCR-based markers

Detection of restriction sites in the region of SNPs and conversion of SNPs/INDeLs into length polymorphism offers unique opportunities in the genotyping process as it becomes simple gel-based approach and is also cost effective. Among different techniques developed derived cleaved amplified polymorphic sequence (dCAPS) analysis, which uses mismatches in one of the two PCR primers flanking the SNPs to create or remove a restriction endonuclease recognition site in one of the two haplotypes being

assayed is widely used by the plant molecular genetics community as it is gel-based procedure (Michaels and Amasino 1998; Neff et al. 2002). With the help of web-based program dCAPS Finder 2.0 (Neff et al. 2002), we have successfully designed three dCAPS primers each in pigeonpea and chickpea and six in cowpea (table 8). Four dCAPS synthesized for the markers PvMt_051 in pigeonpea, PvMt_103 in cowpea, PvMt_151 and PvMt_1196 in chickpea were successful in producing polymorphism in the respective genotypes of these crops reflecting on the efficacy of dCAPS primers in the genetic studies. We utilized web-based program BIO-EDIT and identified six, three and nine restriction sites in the region of SNPs which can be used directly as CAPS markers in cowpea, chickpea and pigeonpea, respectively (table 9).

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