

Integration of novel SSR and gene-based SNP marker loci in the chickpea genetic map and establishment of new anchor points with *Medicago truncatula* genome

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Abstract This study presents the development and mapping of simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers in chickpea. The mapping population is based on an inter-specific cross between domesticated and non-domesticated genotypes of chickpea (*Cicer arietinum* ICC 4958 × *C. reticulatum* PI 489777). This same population has been the focus of previous studies, permitting integration of new and legacy genetic markers into a single genetic map. We report a set of 311 novel SSR markers (designated ICCM—ICRISAT chickpea

microsatellite), obtained from an SSR-enriched genomic library of ICC 4958. Screening of these SSR markers on a diverse panel of 48 chickpea accessions provided 147 polymorphic markers with 2–21 alleles and polymorphic information content value 0.04–0.92. Fifty-two of these markers were polymorphic between parental genotypes of the inter-specific population. We also analyzed 233 previously published (H-series) SSR markers that provided another set of 52 polymorphic markers. An additional 71 gene-based SNP markers were developed from transcript sequences that are

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highly conserved between chickpea and its near relative *Medicago truncatula*. By using these three approaches, 175 new marker loci along with 407 previously reported marker loci were integrated to yield an improved genetic map of chickpea. The integrated map contains 521 loci organized into eight linkage groups that span 2,602 cM, with an average inter-marker distance of 4.99 cM. Gene-based markers provide anchor points for comparing the genomes of *Medicago* and chickpea, and reveal extended synteny between these two species. The combined set of genetic markers and their integration into an improved genetic map should facilitate chickpea genetics and breeding, as well as translational studies between chickpea and *Medicago*.

Introduction

Chickpea (*Cicer arietinum* L.) is an annual, self-pollinated diploid ($2n = 2x = 16$) species with a relatively small genome of 740 Mbp (Arumuganathan and Earle 1991). Chickpea is also the World's third most widely grown food legume. Over 95% of chickpea production area and consumption occur in developing countries, with India contributing the largest share (65%), followed by Pakistan (9%), Iran (7%), and Turkey (4%) (FAOSTAT database <http://faostat.fao.org/site/567/default.aspx#ancor>, 2007). Cytogenetic and seed protein analyses are consistent with *C. reticulatum* as the wild progenitor of domesticated *C. arietinum*, with southeastern Turkey as the presumed center of origin (Ladizinsky and Adler 1976). Cultivated chickpea is composed of two genetically distinct sub-types that are readily distinguished based on seed size and color: *Desi*, composed of small, brown seeded varieties, and *Kabuli*, composed of large, cream seeded varieties. Due to relatively low rates of polymorphism between cultivated chickpea accessions, inter-specific crosses between *C. arietinum* and *C. reticulatum* have been the primary focus for genetic studies of agronomic traits (see Singh et al. 2008).

A diverse array of technologies is available to identify and monitor DNA polymorphism and as a consequence molecular markers are now routinely used in the breeding programs of several crop species (Varshney et al. 2006, 2007). In the case of chickpea, molecular markers reported in the literature are almost entirely simple sequence repeat (SSR) loci (Choudhary et al. 2006; Hüttel et al. 1999; Lichtenzweig et al. 2005; Sethy et al. 2003, 2006a, b; Winter et al. 1999). Despite considerable effort, low rates of both intra- and inter-specific polymorphism have limited the number of these SSR markers that have been integrated into chickpea genetic maps. A primary goal of the current study was to screen additional molecular markers and thereby enhance the marker density of chickpea genetic maps.

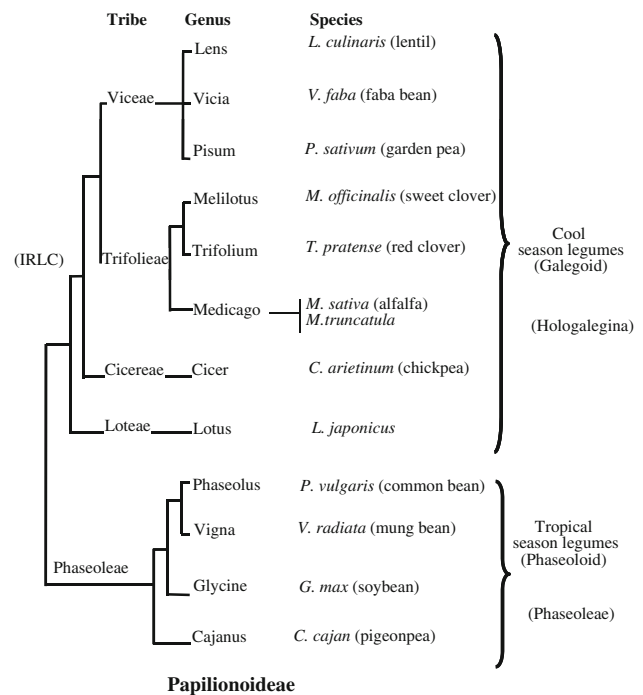


Fig. 1 Phylogenetic relationships: Papilionoideae family. This figure (taken from Choi et al. 2004b) illustrates the phylogenetic relation of chickpea with other legumes. Chickpea and *Medicago* belong to inverted repeat lacking clade (IRLC) of Papilionoideae and constituted the cool season legumes

Chickpea is a close relative of the model legume system *Medicago truncatula* (Fig. 1, reproduced according to Choi et al. 2004b), and thus should benefit from the increasingly detailed description of the structure and function of the *M. truncatula* genome (Cannon et al. 2006). A pressing task for chickpea researchers is to use knowledge gained from the study of reference legumes, such as *M. truncatula*, *Lotus japonicus*, and soybean, to advance genetic improvement of chickpea. Comparative genomics based on orthologous genetic markers offers means to bridge model and crop legumes. Alignment of linkage maps and sequenced orthologous regions between several legume species has revealed an extensive network of macro- and micro-synteny between legume species; importantly, genomic and genetic comparisons of orthologous nodulation genes in several legumes suggests that comparison of genome structure and function may have practical applications to cross-species gene prediction and isolation (see Zhu et al. 2005). The lack of infrastructure (knowledge and physical capacity) in chickpea, however, has limited the potential for cross-genome comparisons and has hampered progress in the area of genomics-assisted breeding (Varshney et al. 2009a).

In view of the above, we sought to enhance marker repertoire and density of genetic maps in chickpea using a combination of several molecular marker sets. We

developed two novel sets of molecular markers based on an SSR-enriched genomic DNA library and gene-based single nucleotide polymorphism (SNP) markers derived from comparison of *Medicago* and chickpea ESTs. These novel genetic markers were analyzed together with published genetic markers to develop a dense genetic map of chickpea. We anticipate that these resources will serve as tools for genomics-assisted breeding in chickpea, and enhance prospects for transfer of knowledge about the structure and function of the *Medicago* genome to chickpea with a final objective of chickpea improvement.

Materials and methods

Plant material and DNA extraction

The cultivated chickpea germplasm line ICC 4958, belonging to *Desi* type and a parent line of inter-specific reference mapping population (*C. arietinum* ICC 4958 × *C. reticulatum* PI 489777) was used to construct microsatellite-enriched library. While two genotypes of chickpea (ICC 4958 and ICC 1882) were used to optimize the polymerase chain reaction (PCR) conditions for newly developed SSR markers, an array of 48 genotypes which includes 33 genotypes from cultivated chickpea (*C. arietinum*) and 15 from wild species of chickpea (7 genotypes from *C. reticulatum*, 2 genotypes from *C. echinospermum* and one each from *C. bijugum*, *C. cuneatum*, *C. judaicum*, *C. microphyllum*, *C. pinnatifidum*, and *C. yamashitae*) was used to assess the polymorphism potential of new set of SSR markers (Table 1).

For integrating the markers into the genetic map, the inter-specific reference mapping population ICC 4958 × PI 489777 comprising of a total of 131 RILs was used. While all 131 RILs were used to score genotyping data for the SSR markers isolated in this study as well as reported by Lichtenzweig et al. (2005), a subset of 94 RILs was used with gene-based markers.

Total genomic DNA was extracted by employing the standardized high throughput mini DNA extraction protocol (as mentioned in Cuc et al. 2008). The quality and quantification of extracted DNA was checked on 1.2% agarose. The DNA was normalized to 5 ng/μl for further use.

Construction of SSR-enriched library

To construct a size-fractionated chickpea genomic DNA library, purified genomic DNA (100 μg) of ICC 4958 was completely digested with *Mbo*I or *Sau*3AI in combination with *Taq*I enzyme. The restricted fragments were separated on low-melting agarose gels, and the gel zone containing

Table 1 List of 48 chickpea genotypes used for calculating polymorphic information content (PIC) of newly developed SSR markers

S No.	Genotype	Botanical variety	Country of origin	Market type
1	ICCV 2	<i>C. arietinum</i>	India	Kabuli
2	ICC 10673	<i>C. arietinum</i>	Turkey	Desi
3	ICC 11944	<i>C. arietinum</i>	Nepal	Desi
4	ICC 12299	<i>C. arietinum</i>	Nepal	Desi
5	ICC 12379	<i>C. arietinum</i>	Iran	Desi
6	ICC 1431	<i>C. arietinum</i>	India	Desi
7	ICC 17116	<i>C. yamashitae</i>	Afghanistan	Wild
8	ICC 17122	<i>C. bijugum</i>	Turkey	Wild
9	ICC 17123	<i>C. reticulatum</i>	Turkey	Wild
10	ICC 17148	<i>C. judaicum</i>	Lebanon	Wild
11	ICC 17152	<i>C. pinnatifidum</i>	Turkey	Wild
12	ICC 17160	<i>C. reticulatum</i>	Turkey	Wild
13	ICC 17162	<i>C. cuneatum</i>	Ethiopia	Wild
14	ICC 17248	<i>C. microphyllum</i>	Pakistan	Wild
15	ICC 1882	<i>C. arietinum</i>	India	Desi
16	ICC 2679	<i>C. arietinum</i>	Iran	Desi
17	ICC 283	<i>C. arietinum</i>	India	Desi
18	ICC 3137	<i>C. arietinum</i>	Iran	Desi
19	ICC 3239	<i>C. arietinum</i>	Iran	Desi
20	ICC 3696	<i>C. arietinum</i>	Iran	Desi
21	ICC 3986	<i>C. arietinum</i>	Iran	Desi
22	ICC 4853	<i>C. arietinum</i>	Unknown	Kabuli
23	ICC 4958	<i>C. arietinum</i>	India	Desi
24	ICC 5002	<i>C. arietinum</i>	India	Desi
25	ICC 506 EB	<i>C. arietinum</i>	India	Desi
26	ICC 5337	<i>C. arietinum</i>	India	Kabuli
27	ICC 6263	<i>C. arietinum</i>	Russia and CISs	Kabuli
28	ICC 7052	<i>C. arietinum</i>	Iran	Desi
29	ICC 7413	<i>C. arietinum</i>	India	Pea
30	ICC 8200	<i>C. arietinum</i>	Iran	Desi
31	ICC 8261	<i>C. arietinum</i>	Turkey	Kabuli
32	ICC 9644	<i>C. arietinum</i>	Afghanistan	Desi
33	ICC V95311	<i>C. arietinum</i>	India	Kabuli
34	JG11	<i>C. arietinum</i>	India	Desi
35	JG62	<i>C. arietinum</i>	India	Desi
36	VIJAY	<i>C. arietinum</i>	India	Desi
37	IG 72933	<i>C. reticulatum</i>	Turkey	Wild
38	IG 72953	<i>C. reticulatum</i>	Turkey	Wild
39	PI 489777	<i>C. reticulatum</i>	Turkey	Wild
40	IG 10419	<i>C. arietinum</i>	Syria	Kabuli
41	IG 6044	<i>C. arietinum</i>	Sudan	Kabuli
42	IG 6899	<i>C. arietinum</i>	Iran	Kabuli
43	IG 7148	<i>C. arietinum</i>	Algeria	Kabuli
44	IG 72971	<i>C. reticulatum</i>	Turkey	Wild
45	IG 73064	<i>C. echinospermum</i>	Turkey	Wild
46	IG 73074	<i>C. echinospermum</i>	Turkey	Wild
47	IG 73082	<i>C. reticulatum</i>	Turkey	Wild
48	IG 7767	<i>C. arietinum</i>	Syria	Kabuli

the fragments of DNA of size 800–1200 bp were excised and ligated into Promega pGEM 3Z(f) vector (Promega, Madison, WI, USA). The vector was transformed into *E. coli* Sure strain—DH10B (Stratagene, Heidelberg, Germany) by electroporation. Approximately 400,000 clones were plated at a density of 20,000 colonies per plate. The masterplates generated were replica-plated on positively charged PVDF macroarrays. Macroarrays were printed using contact printing technology at RZPD GmbH, Berlin, Germany.

For enriching the genomic DNA library, synthetic oligos (GA)₁₀ and (TAA)₁₀ were enzymatically 3' end-labeled with digoxigenated oligonucleotides (DIG Oligonucleotide 3'-End Labeling Kit; Roche, Mannheim, Germany). Subsequently, macroarrays/filters were hybridized with above-mentioned oligo-probes in Roti-Hybri-Quick buffer (Carl Roth GmbH, Karlsruhe, Germany) including 10 µg/ml sheared, denatured *E. coli* DNA to minimize non-specific binding. Filters were hybridized at 55°C overnight and washed three times each for 10 min in 1:2, 1:5, and 1:10 dilutions of the hybridization buffer at 60°C. The digoxigen was detected in a “direct detection assay” performed with the DIG Wash and Block buffer set, and DIG Luminescent detection Kit (Roche, Mannheim, Germany) for chemiluminescent detection with a monospecific antibody coupled to alkaline phosphatase in the presence of CSPD. Filters were exposed to X-ray films (Amersham, Buckinghamshire, UK) with intensifying screens for 4 h or overnight, and the colonies giving strong signals were scraped from the master plates; re-grown; spotted on Hybond N membranes (Amersham, Buckinghamshire, England) to fix the DNA by lysis. Hybridization and chemiluminescent detection was done repeatedly to pick the clones with positive signals. These clones were grown on LB agar plates with ampicillin (100 µg/ml) overnight at 37°C. Aliquots of these colonies were used for colony PCR.

Development of genomic SSR markers

The colonies with high level of signal were used to isolate plasmid DNA using standard alkaline lysis method (Sambrook and Russell 2001). After checking the quality of the plasmid DNA on 0.8% agarose gel, the clones were sequenced using the BigDye Terminator cycle sequencing kit on an ABI3700/ABI3730XL (Applied Biosystems Inc., Foster City, CA, USA). 288 clones were sequenced in both directions using standard T7 promoter and SP6 primers and 19 clones in one direction by using M13-forward sequencing primer at MacroGen (www.macrogen.com) and ICRI-SAT.

The sequences generated were subjected to CAP3, a contig assembly program (<http://pbil.univ-lyon1.fr/cap3.php>) in order to define unigenes. These unigenes were

subjected to MicroSATellite (*MISA*, Varshney et al. 2002) tool to search microsatellites considering minimum ten repeat units of mono- (N), and four repeats of di- (NN), tri- (NNN), tetra- (NNNN), penta- (NNNNN) and hexa- (NNNNNN) nucleotides and compound microsatellites present within a distance of 100 bp. Primer pairs for SSRs were designed using Primer3 program (<http://frodo.wi.mit.edu/>) in batch file, and the SSR markers developed were designated as ICCM (ICRISAT Chickpea Microsatellite) markers (Table 2).

Development of gene-based SNP markers

PCR primers derived from *Medicago* ESTs (expressed sequence tags), *Medicago* BAC (bacterial artificial chromosome)-end sequences, and *M. sativa* cDNA sequences have been described in previous studies (Choi et al. 2004a, b, 2006). To design PCR primers based on chickpea ESTs (Buhariwalla et al. 2005), candidate chickpea transcripts were compared to sequenced *Medicago* BAC clones (<http://www.medicago.org/genome>), and transcripts with high nucleotide identity and low copy representation to the *Medicago* genome were selected for primer designing. Primers were designed from highly conserved coding sequences, to amplify across intron regions (Choi et al. 2004a), using the Lasergene PrimerSelect software package (DNASTar Inc., Madison, WI, USA). Details of these primer pairs are given in Table 3.

The polymorphic gene-based markers between the parents of mapping population were identified essentially as mentioned in Choi et al. (2004a). Each pair of corresponding sequences from genotypes ICC 4958 and PI 489777 was aligned using Sequencher software (Gene Codes, Ann Arbor, MI, USA) to detect SNPs. The sequences with SNPs were transferred to DNA Strider 1.2 (Douglas 2008) to identify restriction site that is coincident with SNPs and cleavable amplified polymorphic sequence (CAPS) assay for genotyping the corresponding SNPs were developed. In cases where a suitable restriction enzyme site was not identified, oligonucleotide primers were designed immediately adjacent to the SNP position, which allows for a single base extension of the SNP site using ABI SNaPshot Multiplexing Kits (Applied Biosystems Inc., Foster City, CA, USA).

Genotyping assay

For both ICCM as well as H-series SSR markers, the forward primers were anchored with M13 tail (CACG ACGTTGTAAACGAC). PCR amplicons generated by SSR markers were analyzed on capillary electrophoresis, while for gene-based SNP markers the CAPS or SNaPshot assays were used for genotyping. For SSR genotyping,

Table 2 Simple sequence repeats (SSR) isolated from microsatellite-enriched library of chickpea

Marker name	GenBank ID	SSR motif ^a	Forward primer (5'–3')	Reverse primer (5'–3')	Product size (bp)	Number of alleles ^b	PIC value
ICCM0001a	F1856656	(AT)5	GAACTTGCCTGGGGACAGG	GAGGTGAGCTGAAAGTGAGGC	246	2	0.05
ICCM0001b	F1856656	(CT)4c(CT)4	TGCACAACGGCTATGTCTTC	GAGGTGAGCTGAAAGTGAGGC	118	9	0.71
ICCM0002	F1856654	(TC)6	TCGTTCTCCGTTATGTGTGC	ATGCCTCCAATAGCATACGG	262	2	0.04
ICCM0003	F1856538	(CT)6	AATGGAA GAACGTCAAGGTG	TTCCACTGGGGCAAAAATAAG	252	7	0.59
ICCM0004	F1856539	(AT)4	CAC TCACACCGCACTTTCA	AAAAGAGAA GCCCACCACAAA	213	4	0.39
ICCM0005a	F1856657	(TC)4N(CT)4N(TC)4	TGCTGACGCACTCGAGGATA	GCAGCAAAAATCAGCACAAA	277	NA	
ICCM0005b	F1856657	(AT)4	TTGGTGCAGATTTTGTGTC	AGATTTGGGGGATAAAAAGGG	241	1	0.00
ICCM0007a	F1856661	(CT)4N(TC)4N(TC)4	TCTACATTTCTCCGTGCC	GAGGAGTGTAGGGGAGAGGG	242	NA	
ICCM0007b	F1856661	(TCC)C)4	CTCTCCCACTCTCCCTTTC	TGAGTAGGATCGTAGTAGGGGG	202	NA	
ICCM0008a	F1856540	(A)10N(A)10	CTCATGGTGGCATTGAGAAA	TCCTTGAATTTTGTAGACACGA	230	2	0.05
ICCM0008b	F1856540	(GA)4N(AG)5t(GA)4	TCGTGTCTCAAAAATTC AAGGA	CAC TC GACCACCACTGCTAA	235	2	0.32
ICCM0008c	F1856540	(CT)4	GGTGGTTGTGGAGGTTGAT	AGGCAACCAATTCATCCTTGT	245	2	0.05
ICCM0009a	F1856541	(GA)4	CAC TTC AAAAGAGTGTGATTTGA	GGTTGAAAAGATGAGTGGTTTTG	189	3	0.10
ICCM0009b	F1856541	(A)11	AAAATATGGAAAGTCGGGCA	TGCATTTTCTTAGCGGTTTTT	257	NA	
ICCM0010a	F1856663	(CT)4	GACGGAAATACGGCTGTTA	GCTGCAATATCTCCGCCTC	113	1	0.00
ICCM0010b	F1856662	(AT)4	ACGCCAATCTTTTGTAGCAC	TCAGCACTGGTGGAAACCAT A	142	12	0.80
ICCM0014a	F1856542	(GA)5	CGTGGTTGTGTGTTTGAGG	TGTGTTTCACTCCCTCTCCC	134	1	0.00
ICCM0014b	F1856542	(TA)5	TTTGGGAACCTTTTCTCATCAA	CCA AATCAATTTTGTGCACTG	254	3	0.16
ICCM0019a	F1856544	(AG)18	TTCCGGTTTGTGATGATAGAAAA	ACTTCCACTTGTTCACATCCG	189	NA	
ICCM0019b	F1856544	(AG)4	CGGATGGAACAAGTGGAAAGT	TCTCGTACCCGGACCAGAGTC	153	6	0.62
ICCM0021a	F1856697	(CA)4N(AT)4N(ATT)5	AAACCGCATTGAGGAATGAC	TTTGCCACGATATGTTCCAGG	232	NA	
ICCM0021b	F1856696	(AT)4	TCTTTCTAAGCAGCTAGGATACGA	AGGAAAGGTGGGATAAATGG	229	NA	
ICCM0022	F1856699	(AT)4	TAAACCGCAATTGACGAATGA	TGAAATTCGCAAGAAATCAAATG	123	18	0.89
ICCM0024	F1856545	(AT)4	CAC TCACACCGCACTTTCA	AAAAGAGAA GCCCACCACAAA	214	3	0.08
ICCM0026	F1856714	(AG)4	CCGGACATTTGTTCTGAAAGT	TCTGTGCAGTGGAGCTATGG	216	2	0.05
ICCM0029	F1856721	(AG)4N(GA)4N(GA)4a(AG)5	CTTCCCTTTTCCAAAATTGA	TCGTTACACAGGTTTTCCCTC	244	1	0.00
ICCM0030a	F1856722	(TC)4N(CT)4N(TC)4	GGCAACGTACGGGATAAATG	GCAGCAAAAATCAGCACAAA	271	1	0.00
ICCM0030b	F1856722	(T)10	TTGTGCTGATTTTGTGTC	TCATCCCATCTCTAAATGTGTCA	257	3	0.09
ICCM0032	F1856546	(GA)4	TCTCTTGACACAAAGTCTGCACAT	CCCAC TCACATGTAGCGAAA	232	1	0.00
ICCM0034	F1856651	(GA)11	TTTGTTTGCGGAGGAATAGG	TCACCTCACACACTTCTTTTC	259	6	0.33
ICCM0035	F1856547	(GT)4	TGAGGGTAAATAAATTTGGTGGC	ATGATTTCCGAGGACAGTGG	101	1	0.00
ICCM0037	F1856548	(AG)4N(GA)4N(AG)4N(GA)5	CAAGCAAATGGGAGACACCTT	CCACCACCTTCAACGTCCTCCT	212	NA	
ICCM0042	F1856740	(CA)4	TTCTGTGATATTCATCAAGGTGG	TTTGACGTACTCTGTGTTTGTTT	259	3	0.08
ICCM0043	F1856552	(GA)6	AAGATTGGATTTCCACAACCG	ACAACCCACCCACACAAAAC	274	7	0.44

Table 2 continued

Marker name	GenBank ID	SSR motif ^a	Forward primer (5'–3')	Reverse primer (5'–3')	Product size (bp)	Number of alleles ^b	PIC value
ICCM0045	F1856553	(TA) ₄ N(TC) ₁₂	TGAATCCCTCAATCCTGTGG	CTTATCTCCCAGTGGCCAG	272	5	0.31
ICCM0052	F1856768	(TAT) ₆	CGTCGTGCCATCTGCTTG	CCAGGTGCAATAGGAAATC	275	2	0.04
ICCM0053	F1856771	(GA) ₃₂ (AG) ₄	TGCGGTCCGACTCTAGAGGAT	CTGAGAAGGAAGCCAACAGG	173	1	0.00
ICCM0059a	F1856779	(GAA) ₄	CGGTCCGATCTGAGGATCACTA	GGGTTCAACTGTTCAGGTTTG	226	NA	
ICCM0059b	F1856779	(AG) ₄ N(GA) ₉ N(GA) ₄	CAAAACCTGAACAGTTGAACCC	AGGTTCTCCCCTCGCTCTCTC	172	3	0.23
ICCM0060	F1856780	(AT) ₄ N(CT) ₄	TGATGAACATGCTAACAACTAACAA	CCAAAGACAACCTGGTGGAGGT	260	2	0.04
ICCM0061	F1856798	(AT) ₆ N(TTA) ₄ (TAA) ₄ N(TTA) ₁₃	ATGCGGTAACTCCTTGACTGG	TGAAATTTGGGTTGGAAAGTTTG	275	5	0.23
ICCM0062	F1856801	(AAT) ₄ N(AAT) ₆	ATGCAACGCCCTAAGTCTCGT	TAGGTACGTTTGGGGTCCCTG	266	3	0.09
ICCM0063	F1856802	(TTA) ₆	TTGATTTATCCTGTGATGCTTTTT	GGCAGTCTGGTCATGTGAAA	194	2	0.04
ICCM0065a	F1856807	(TC) ₆	CATTCCCGCAAAGCTTGTAT	GGTGCCTTGGAGAAAATCAA	137	NA	
ICCM0065b	F1856807	(TC) ₄	CACGGTGTCTTCCACATGAC	TAAGCCAAATACCAAGAGGCG	195	2	0.08
ICCM0066	F1856555	(TC) ₄	ACCATGCTCACCAACTCACA	TCCTTTGACACAAGTCTGCACAT	242	1	0.00
ICCM0067	F1856556	(TC) ₄	AACTGCAACCTCTCTCGCTC	TCCTTTGACACAAGTCTGCACAT	204	1	0.00
ICCM0068	F1856557	(ATT) ₂₂	TCTTCTTTGCTATCTGTCTCGC	TGCATGTCAAACATTTAGACAACTTT	227	14	0.87
ICCM0069	F1856558	(ATT) ₂₂	TCTTCTTTGCTATCTGTCTCGC	TGCATGTCAAACATTTAGACAACTTT	227	13	0.82
ICCM0072	F1856813	(GAA) ₄	CAAGACCTTGAACAGGAGGC	TCCTTCAATTTTCTAACAAATTCATC	200	2	0.14
ICCM0073a	F1856828	(TA) ₄ N(A) ₁₁ N(TA) ₄	TGGATCTGAGGATCTTTGGTGG	TGGATACTATTAAACGAAAAACTAGCG	219	6	0.23
ICCM0073b	F1856828	(TC) ₄	CTTTGTCCACCCACACATCTT	TAGAGAAATGGGGAAAGGGT	217	1	0.00
ICCM0074a	F1856830	(A) ₁₁ N(TC) ₆	AAATCCCAAAATTTAGAGCGG	AACCTTTGAAAAAGGCGGTT	183	9	0.40
ICCM0074b	F1856830	(T) ₁₅	AACCGCCTTTTCAAAGGTT	CCTTCCAGGGGAAAAAGAAA	264	NA	
ICCM0075	F1856559	(AG) ₁₆	CTTTGTACAATAAAATGCAAAAGTAAA	GGAAGCACAGTCTGCACAAA	163	4	0.26
ICCM0076	F1856560	(ATA) ₁₇ N(TAA) ₅	CTCATCGAATAGAACCTACCGA	CCGCTACACCTACAACGGTAA	270	3	0.08
ICCM0077a	F1856561	(AG) ₄ N(T) ₁₀	CCACAAGAAAGACAAAAGGGGA	AAAAAGATGCTAAAACTAAACCAAAGA	217	1	0.00
ICCM0077b	F1856561	(A) ₁₀	GCCGAGAAAAATAAATTTCACCA	GCCGCGACCATTAATTTCTAA	124	2	0.04
ICCM0078a	F1856832	(TAT) ₄ g(ATT) ₆ ag(TAT) ₅ N(AT) ₄	CTGAGGACGTTGGGAATACG	AAAAACTAATCTCGTGTCAAAATCC	280	3	0.22
ICCM0078b	F1856832	(TC) ₄	AATCCCAACGGTGTGAGAGATG	GGCAAGGAGTGGAAAGGGA	279	15	0.62
ICCM0079	F1856562	(TTA) ₆	GAGCTAACGCCCTTCGCTAGA	GAGAGGGATTAACCAAAATAGAGGAA	170	3	0.08
ICCM0080	F1856563	(T) ₁₀	CTGCGGTGACTCTGAGGAT	GAGAATCACGGGTGTTTCAAAG	173	3	0.15
ICCM0081	F1856564	(TAA) ₆	GAGAGGGATTAACCAAAATAGAGGAA	GAGCTAACGCCCTTCGCTAGA	170	3	0.08
ICCM0082	F1856833	(CT) ₁₉ N(TC) ₄	TCACGATCTCACAGAGCCAC	TCCGTGATTCGAGCAACAG	260	3	0.08
ICCM0083a	F1856835	(AT) ₄ N(CT) ₇	CGCTCACACCATCTCACTTC	GAATGGAGGAAATACAGAGTGC	273	NA	
ICCM0083b	F1856837	(A) ₁₃	ATTGAAAAACCAACGCACACA	AGCGACGACAGTGACCTTCT	140	1	0.00

Table 2 continued

Marker name	GenBank ID	SSR motif ^a	Forward primer (5'–3')	Reverse primer (5'–3')	Product size (bp)	Number of alleles ^b	PIC value
ICCM0083c	F1856837	(T)11	TGTTTGTTCCTAGCCACTG	TTGGACAGATTTTGTGTGTTGTT	195	1	0.00
ICCM0084	F1856993	(GA)5	TTTTTGATTGAGCATGCAATGT	GAACCTTTTGGGTCTGTTC	246	2	0.04
ICCM0085	F1856855	(T)10N(TAT)14	GTGGTCCATCTGTCTCGGTT	GGAAAAAGGAGAAAGTGTGGG	210	NA	
ICCM0086a	F1856856	(TA)4N(T)11	TGTGCAATGAGCCTATTGGT	GACAAACAGCGGCATAATCAA	182	NA	
ICCM0086b	F1856856	(AC)4	CTTCCCTTTTACCCCGTTTA	AAAGAAATCGACAATAAAAAGATGA	175	NA	
ICCM0088	F1856861	(T)10N(AT)5	AAAGGAAGGGAAGGAAATGC	GAGTTTGGGCAGGCAATAAA	240	2	0.19
ICCM0089a	F1856863	(A)12	AACACCGACTTTTCCAAAACG	TTTGGGAAATACAACCTTTTGA	204	9	0.45
ICCM0089b	F1856862	(TAT)20	GGGATATCGCCAATATATTTATACC	TTTGGCAACAAAATCCTTTGA	126	NA	
ICCM0090a	F1856864	(TTA)6	ACGGGACTTGGATGACTTTC	AGACCGGTGCTTCTTCTTA	257	NA	
ICCM0090b	F1856864	(TC)5	CTGCCTAGGAAGAAAAGCACG	AAAAATAAATGCGCCGTATGC	160	3	0.39
ICCM0093a	F1856871	(TAT)20	CTTCTGTTATTATCGCCGCC	AGCATCATGGAGCAGAGAGG	223	NA	
ICCM0093b	F1856871	(AT)4	TACCCCTTCTCTCCCCACCT	TCAGTAGTCGGGCAATAGATGA	129	NA	
ICCM0093c	F1856870	(AAAT)4	CCACTTTTAGGGCGCACTTCT	CGACTCATTTTTCACGGACA	197	3	0.24
ICCM0094	F1856872	(AT)4	AGAGGCAAACAAGAACCGAA	AAGGGTAGTGGAGGAATTATGAA	279	3	0.08
ICCM0095a	F1856875	(TC)4	CTCTCCATCCCATCCGACTA	GGAAGCCATATCCAGAGGGT	181	NA	
ICCM0095b	F1856874	(ATTC)4	CGGGACATTTCCGTTAAAAA	CGAGTCGTTTTCTTGGCTTC	171	1	0.00
ICCM0096	F1856877	(AT)4N(CT)4N(TC)4	ACACCCCACTTAATTACACT	GAGAGGTACGAAGCACGAGG	170	9	0.47
ICCM0097a	F1856669	(ATT)12N(TA)4	TGAGGACTGCCAFACTCCAG	TCCCTTTATGAGGGCTTTT	276	3	0.09
ICCM0097b	F1856668	(CTT)4	TCCAAATCCAAAACAACCA	CCTGAGGAGTAAAAGACGGG	130	1	0.00
ICCM0101a	F1856675	(A)13	TAACTGAGTTTCGGGTCCG	CTTAACGGACGTTGTAGGGC	247	NA	
ICCM0101b	F1856674	(AG)4	GAAGACAAAAGGGGCACAA	CCGATTGTTTCAAAGACCAGA	105	2	0.04
ICCM0102	F1856676	(T)12	CACCAAAAAGGGAACTTTCG	AAAAATAGGTGGGGAGGG	167	1	0.00
ICCM0103	F1856678	(A)11	ATGGGGGAATCGGAGACTAA	GGATAGGGAGGAGGGAACAG	110	3	0.18
ICCM0104	F1856566	(TTA)11	CCAAACCTCCAAAATCTGC	TCATTTTGAATTCATTTCTGGG	278	8	0.48
ICCM0105	F1856567	(AT)4	TGCTTCCCTTTTCAATCACCA	TGACAAAAGGACAATAAGTGTTTTA	280	1	0.00
ICCM0106	F1856681	(ATA)7	TGGAAATGCTACCGAATATGG	ACGATCGGAGAGAACGAGAA	267	NA	
ICCM0107a	F1856682	(TCG)4	GCAAAAAGTGTGCTTCCGT	AAGCACATGGCCACTAGCAT	234	1	0.00
ICCM0107b	F1856682	(TA)4	GCAAAAAGTGTGCTTCCGT	AAGCACATGGCCACTAGCAT	234	3	0.16
ICCM0110	F1856568	(AT)4N(TTA)7	AGAGGCAACAAGAACCCGAA	GTAAGAGGGGCAGCTGTTG	183	NA	
ICCM0115	F1856706	(TC)4	ACCCTAAGGGCTCGTTTGTAT	TAGGGATGGAGAGGAGAGCA	245	1	0.00
ICCM0116	F1856709	(T)11	TTTTGGTGCAGAGAAATGGG	GTCTTTCAAGAGGTCCGACG	177	1	0.00
ICCM0117	F1856572	(TAA)4	AGTGACCAGGAAACACGGTC	GCAGAGATTGAATTTTGCCA	254	1	0.00
ICCM0118	F1856573	(T)11	AATTGGGAAGGAAAGCGAGT	TCGCCATTGCAATAATCAAAA	279	NA	

Table 2 continued

Marker name	GenBank ID	SSR motif ^a	Forward primer (5'–3')	Reverse primer (5'–3')	Product size (bp)	Number of alleles ^b	PIC value
ICCM0119	F1856710	(GA) ₄	TTATTGGATGAGTGGATGCG	AATACGTAACCCAAACGTCGC	192	NA	
ICCM0120a	F1856574	(TTA) ₁₂	TGTCGATAAGAGTTTGTATTTTTC	CGTTTTGTTTCATATTCAAACCTCG	220	12	0.75
ICCM0120b	F1856574	(ATT) ₁₃	CGAGTTTGAATAATGAACAACAAACG	GCTTGTAGCTAGGCTCGACTC	166	5	0.29
ICCM0121a	F1856575	(TATT) ₄	CAAAAATTTGGATTCGGGAG	CTATTGCACCTGGGGATACG	238	2	0.09
ICCM0121b	F1856575	(A) ₁₀ N(AT) ₁₇	ACGTAATCCCAAGGTGCAATA	AACAGATGTAGAAAGGTATAATCCATGA	224	1	0.00
ICCM0122	F1856576	(AAT) ₄	GCAACCTGCCATCCATACTT	AGTGAATCAAAATGATGAAAGCA	275	1	0.00
ICCM0123a	F1856577	(TTA) ₁₄	GGATGGTCTGCTGGAAATCAT	AAAGACAACAACAAAAGACAATCAATGT	250	9	0.76
ICCM0123b	F1856577	(TAA) ₂₆	TTTTTACATGATGTCTTTTTTTGTTG	TGAGGACTAAGATAATAGCAATCCAA	201	NA	
ICCM0124	F1856578	(AAT) ₄ aaa(AAT) ₄	CCTCGGGAATTCAACTACCA	TCAAAAATCCACTTTCCACCA	279	5	0.16
ICCM0125a	F1856579	(AAAT) ₄	CGATCTGAGGATCAACTTGTGA	ACTAACCCAGCTCGACCATC	253	NA	
ICCM0125b	F1856579	(TTA) ₅	TATTTATGGTCTGGTCCGGC	CGCTACCAAAATATGGAAGCACT	251	5	0.19
ICCM0127	F1856581	(TAA) ₂₇	TGTTGAAACGAAATTACTCATCG	GGTGGGCTCCTAATGTTTGA	269	6	0.40
ICCM0128a	F1856731	(TA) ₄ N(TAT) ₄	AACCCTAATTTATTTGCACTAATTAATCA	TCAAAAATACGGTAGTAGGATAAAGATGA	161	NA	
ICCM0128b	F1856730	(A) ₁₀	ATTTGGACGATGTGTCGCTT	TTATAGCCCTGCTTTGCTG	266	1	0.00
ICCM0130a	F1856734	(AAT) ₂₂	GGATTTTCGACTTTTATCCCTTTT	CGGACTGGAATCAAAAAGCTC	268	3	0.10
ICCM0130b	F1856734	(ATT) ₅	GAGCTTTTGAATCCAGTCCG	TGTAGGGGTGCAATGTTGTAA	122	1	0.00
ICCM0131	F1856582	(AT) ₄ N(TTA) ₈	AGAGCCAAACAAGAACCAGAA	GTAAAGAGGGGCAGCTGTTG	192	NA	
ICCM0134	F1856748	(A) ₁₁	TTTTTGGAGGACGCTTTGAGT	TGAAGACAGAGACGGTGCAT	109	3	0.08
ICCM0138a	F1856753	(AG) ₄	ATAAATAGCCGGCCACAAGA	CCGATTTGTTTCAAGACCAGA	119	1	0.00
ICCM0138b	F1856752	(ATA) ₁₁	CTGTTGCCGATTTTATATTATTTTT	AAATGTGTTGTTCTGGCCCGT	168	NA	
ICCM0139	F1856755	(ATTC) ₄	TCACGATTTGAATGTCGTG	CGTTTTCCAGCTTCAACAT	151	NA	
ICCM0141	F1856757	(AAT) ₂₀	AAGTATGATGCAGTTCCTCCG	GGCGGAGGGTAATTATTGT	247	NA	
ICCM0142a	F1856759	(AC) ₄	GCAITGGCCAATATCGAAGGT	AGTAGGTGCCAAATGCATCC	206	1	0.00
ICCM0142b	F1856759	(ATT) ₆	GGATGCATTTGGCACCTACT	GAGTTGGTGTAGAAATAGAAATGGA	137	NA	
ICCM0142c	F1856758	(AC) ₇	GGAGGTCCCGAGTCTAAACC	TCTTCAAAGTCTGCTTGACGTGT	105	1	0.00
ICCM0143	F1856761	(AC) ₅	CTGCGGTCGATCTAGAGGAT	AAGGTTGAAGGATGATTGGG	257	NA	
ICCM0150a	F1856583	(ATTC) ₄	TGATTTGAAATGGTCTGTCC	AGTCGTTTTCTCGGCCTCAA	279	1	0.00
ICCM0150b	F1856583	(TAA) ₈ N(AT) ₄	GTAAAGAGGGGCAGCTGTTG	AGAGGCAACAAGAACCAGAA	192	5	0.16
ICCM0152	F1856792	(T) ₁₃ c(CTT) ₄ N(AG) ₅	CGTCTCACGAAGGAGAAGTG	AAAAATTCACCTTGCTAATATTTCCACA	197	1	0.00
ICCM0154	F1856794	(A) ₁₁	AGCTTGTTCGACAGCAGGAT	TCGATGTGAAATATGCCCTCTT	247	1	0.00
ICCM0155	F1856796	(A) ₁₁	GACGGCAGGATTAACCTGCAT	TTCCGATGTGAAATATGCCCTCT	240	2	0.04
ICCM0156a	F1856797	(AT) ₇ N(CA) ₄	TGCAITTCCTCCCTTAAATTTGG	CAGTGGTGGGGAGACAAAAAC	280	2	0.04
ICCM0156b	F1856797	(ATA) ₅ 2N(AAT) ₆	GTTCCTCCGTCCTCCACTTAT	AAACAGTGTAAATGTTGTTGGAAA	276	2	0.08
ICCM0157	F1856584	(GA) ₈	TTTTGTTTTGAGGCAACCACA	AAACAAAACCCAGTGGGAGGT	258	1	0.00

Table 2 continued

Marker name	GenBank ID	SSR motif ^a	Forward primer (5'–3')	Reverse primer (5'–3')	Product size (bp)	Number of alleles ^b	PIC value
ICCM0158	F1856585	(ATA)18N/(TCT)16	CCAAAACACTGACAAGTCCCGT	GGGAAAATATGAGGAAGTTTGG	275	1	0.00
ICCM0159	F1856814	(T)17N/(T)10	TGAAAAATCGAAACCCCTACC	CCTTGTTTTTCAGGGGATTTGT	247	6	0.46
ICCM0160	F1856816	(AAC)4N/(TAA)25	TTGCTTGAACAACACCTTTTCG	CGGGTACAACCCGTAGCAAAT	263	21	0.93
ICCM0161a	F1856818	(AT)4	GATGGTCACTCGGTTTCCGATTC	AACGAGCCCTCTCTGTAAACG	267	4	0.12
ICCM0161b	F1856817	(TAA)4N/(AAT)4	ACTGTCAAGGAAGGAACGGTGT	TCCGTAACAATAAATTTGTGAAGAAA	279	3	0.14
ICCM0162a	F1856586	(ATT)12	TAGCGCAGTCGATCTGAGGA	ACGTAATCCCAAGTGCATAA	272	NA	
ICCM0162b	F1856586	(AAAT)4	GCAAGGCTTTCCCTTGTCA	CGCCGCCAATTTATTTTTA	278	1	0.00
ICCM0162c	F1856586	(T)11N/(TA)4	TAAAAATAAAATTTGGCGGGC	TCGTGTAGGGTGTTTAGGGA	267	3	0.21
ICCM0162d	F1856586	(T)10	GACTCTGCTGGGGACAATTT	CGGGTTTAGAGACCCACTCA	225	1	0.00
ICCM0163	F1856820	(TC)4	CAACGAAATTCATGCTGTGG	TAGGGATGGAGAGGAGAGCA	274	1	0.00
ICCM0165	F1856821	(T)11	CGGACGTACACCTTTTCGTTTC	TGCTTCCGAATAACATAAAGCA	128	NA	
ICCM0166a	F1856824	(T)11	GCCTACTCGCGGATTTTTATC	CCAGGTGCAATAGGGAAATC	276	3	0.09
ICCM0166b	F1856824	(AAAT)7	TGGGGATACGTAGGAGCAAG	TTGGATTCGGGAGTCGATTA	233	NA	
ICCM0166c	F1856824	(AT)5	GCCTACTCGCGGATTTTTATC	CCAGGTGCAATAGGGAAATC	276	2	0.04
ICCM0166d	F1856823	(AT)4	GACTCCCTACCACCTCACA	CGGACCGCACAAAACTAC	275	1	0.00
ICCM0166e	F1856823	(TAT)48	AATAAAAAATCGGAAAGTGGG	TGTGAGGTGGTAGGGGAGTC	276	1	0.00
ICCM0167	F1856588	(ATA)48	GAGTGTACGGGATTTATATGATGA	TCAAAGAAAAGGAACCAAGGC	235	NA	
ICCM0169	F1856589	(TTA)30	AGAGCAAAACAAGAACCCGAA	GTAAGAAGGGGCGAGCTGTTG	251	NA	
ICCM0170a	F1856839	(TA)4	GCTTTGTTGCTTTCGTTCTTTT	AAAGTGTTTGGGTGAGTGG	226	NA	
ICCM0170b	F1856839	(C)10	CTCGCATTCCTTTTCCACTC	GGGGAAAGTATGGGATGAG	154	NA	
ICCM0171	F1856590	(ATT)4	GACCGGATCGTGTCTATAAA	CGTTTTCCCAAGCTTCAACAT	166	1	0.00
ICCM0172	F1856841	(AT)4N/(AT)4	GCAGTCGATCTGAGGATCAAG	TTCACAAGATGTTTTTCAGAACAAAG	278	NA	
ICCM0174	F1856591	(A)11	CAGCGACCTCCTACTGGGTA	CAAAAATGGAGGATTTTTTCTT	175	NA	
ICCM0176	F1856847	(TA)4	ATAGGCTAGACCCGTCGGACA	TCTGAAAATATGATGCAGCCG	273	5	0.16
ICCM0177a	F1856849	(AAT)28c/(ATA)27c/(TAA)4	CTTGAGTTCAAGCCAGAGAGG	GCGTTATTACTGTTACAAATGGCA	279	1	0.00
ICCM0177b	F1856848	(TAT)6N/(TAT)11N (TAT)4N/(TTA)6N/(TAT)8	CCCTTCTTCCATTTCCGAAT	GGGGAGGAGAACGAAAAAGA	273	NA	
ICCM0178	F1856592	(AAT)13	AGTTTGGGTTTACCCGCCT	GAACGGCTCTGTTTCATAAT	280	11	0.83
ICCM0179	F1856850	(TAT)4	AAAGGCCAGTTTACCCGACT	ATTTGATGCAGCAAGCAATG	214	NA	
ICCM0180	F1856852	(TAT)4	AGTCCCTGATCTCCCGAAGT	ATTTGATGCAGCAAGCAATG	179	1	0.00
ICCM0181	F1856593	(ATA)5	CGGGTGTGGATAGCAAGTTT	TCTCTCCTTCTTAATAAAAACAACA	103	1	0.00
ICCM0183	F1856595	(TAT)15	TGAGGACTAAGATAATAGCAATCCAA	TTTTTACATGATGCTTTTTTTGTTG	168	1	0.00
ICCM0185	F1856597	(T)11	AAAAGTTTGGCCCTGGTCTGG	CAITCCATATTCAGTAGCATCCCA	250	6	0.46
ICCM0187	F1856599	(TAT)6	TGACCATCAATCCATTTCTTTTC	TGTTGACGTCTAAATTTGTCCG	280	NA	

Table 2 continued

Marker name	GenBank ID	SSR motif ^a	Forward primer (5'–3')	Reverse primer (5'–3')	Product size (bp)	Number of alleles ^b	PIC value
ICCM0189	FI856601	(TAT) ₆₂	TCCAGTTCCAAATGGCATAA	CCCTTGAGTTCAAGCCAGAG	273	3	0.21
ICCM0190a	FI856602	(ATA) ₆ N(ATA) ₉	GGGGGATTGTCTGAGTTTCA	AAAAAGGCTGGAGACACCTCA	195	7	0.53
ICCM0190b	FI856602	(TAT) ₅	TTTATTGCAGGAAGCGGTTT	CACCACTATCAAAATGCCCT	273	1	0.00
ICCM0191	FI856603	(T) ₁₀	CCTTACGCATAATCGACTCCA	AATTCAAATTGAGTCGCCACC	130	5	0.37
ICCM0192a	FI856879	(TAT) ₁₅ c(ATT) ₁₅	GCTGCCCAAATTTTGACATTA	CCGGGGATCAAATTTCTTCT	279	11	0.88
ICCM0192b	FI856878	(TAA) ₁₅ tg(ATA) ₁₅	CGGACGGGGATAATTTCTTCT	GCTGCCCAAATTTTGACATTA	279	15	0.76
ICCM0193a	FI856881	(TAA) ₅ N(T) ₁₀	TGAACTTCAAACCAAACCAA	TTGTGACAAATTTGAGGGTCT	268	NA	
ICCM0193b	FI856881	(AAT) ₇	TCGATTATAGCTTTATCTTTACCCCTTT	AAAAGTGTGGGAGGGGTTC	242	1	0.00
ICCM0194	FI856883	(A) ₁₀ N(T) ₁₃	CGATTGTCTAGTTTTTAAAAAGAAA	CGACTTCCTGAAAGGAACGAA	200	4	0.22
ICCM0196	FI856605	(ATA) ₅ N(AG) ₆	GTCCGGTGTGGATAGCAAGT	AACACAAATTCCTCAAATAAACAACCT	154	6	0.47
ICCM0197a	FI856885	(T) ₁₃	CGCGTCTAGCAAAAACAAGAA	TTCTCGGCTATAAACATCAA	280	3	0.08
ICCM0197b	FI856884	(TATT) ₇	GATTCGGAGTCCATTACCA	CTATTGCACCTGGGGATACG	241	NA	
ICCM0198	FI856886	(A) ₁₅	CCATCCGAGAAAACCTCGAAA	CAAACGGTATCCATCGGAATC	163	1	0.00
ICCM0199a	FI856889	(A) ₁₀ g(AGAA) ₄	ACCAAAGCAGACCACAACAAT	GTTTTCCCGGCTTCAACAT	265	1	0.00
ICCM0199b	FI856889	(CAAT) ₅	ACCAAAGCAGACCACAACAAT	GTTTTCCCGGCTTCAACAT	265	1	0.00
ICCM0199c	FI856888	(TTA) ₁₅	TTAGAGGCAAAACAGAACCG	ATCTTGAAGTGGCAAAAACG	241	15	0.86
ICCM0200	FI856890	(TAA) ₄	ACGGAGTGACAGGAAAACAC	GCAGACCTACAGAAACAGAGGAA	231	2	0.04
ICCM0201	FI856892	(TAT) ₇ (TTATTG) ₆ ^a (GTTATT) ₄	ATAGAGAGACCCAAAACCGCC	GCCAAAGGCAAAAAGAGATTG	135	NA	
ICCM0202a	FI856894	(T) ₁₀ N(T) ₁₀	CGCCGATCCATTATACTGAC	TTGCCTCTGATTTCTGGTTCA	192	2	0.04
ICCM0202b	FI856894	(TTA) ₁₃	TGAACCAGAAATCAGAGGCAA	CCAAATTTGGTCCGGTTTTTA	207	12	0.77
ICCM0203	FI856606	(CT) ₆	TGGACGTAGGTTGTTGTGGA	TTGGTATCAGTGCCTCGCA	194	1	0.00
ICCM0204	FI856607	(TC) ₇	CACATACACTCCCAATCCC	TGCAGACTGTTTGGTTCGAG	258	1	0.00
ICCM0205	FI856608	(TA) ₉	CGACCATGATTCTCTGATGTG	CACCTCTGCATTTCTTCAAACA	263	8	0.26
ICCM0207	FI856610	(TA) ₄	TCAAACATAAAGCACTCCCC	GGCCATTTGTGTTTGTATGG	182	2	0.04
ICCM0210	FI856898	(TA) ₄ N(TG) ₄ N(CCA) ₄ N(AG) ₁₆	GACCAATGCCCACTTCAACT	AGTTCTGCGAGAGGAATGGA	253	NA	
ICCM0212a	FI856902	(T) ₁₀ N(AT) ₄ N (TA) ₅ N(TA) ₄	TCCTATACCGAAAACCCCAATT	CAAAAATGGATGGATTGTGGG	270	2	0.04
ICCM0212b	FI856901	(GA) ₄	ATTGCCGTTGAGAGAAGTCG	TCGGTCAACCACACTACCAA	183	1	0.00
ICCM0212c	FI856901	(AG) ₈	TTTGGTAGTGTGGTGACCGA	AACCCAAAACCGTGGACTCA	161	6	0.32
ICCM0214a	FI856612	(CT) ₆ N(AC) ₄	CTCTTCAATAGCCCCATCCA	CGTTGGAGAGGCTGAAACAT	249	1	0.00
ICCM0214b	FI856612	(TTTA) ₅	ATGTTTCAGCCTCTCCAACG	TCGCACCTGAACCTCTCTGTG	276	NA	
ICCM0215a	FI856613	(TC) ₅	CCTTCAGTGTGGCTCACA	CTCCAGGAATCCACAGCAAT	253	3	0.15
ICCM0215b	FI856613	(T) ₁₁	AGAATGCTGTGGATTCTTGG	GCAAGCCCCAAAACCTTCAAGA	276	1	0.00

Table 2 continued

Marker name	GenBank ID	SSR motif ^a	Forward primer (5'–3')	Reverse primer (5'–3')	Product size (bp)	Number of alleles ^b	PIC value
ICCM0216a	FI856614	(TG) ₄	CGGGACTTTTCATCTGCTGTT	GTGGGACATCCTCCAAGAAA	200	2	0.04
ICCM0216b	FI856614	(AG) ₅	AAAGCTGGTGGTCGAGCTAA	GACCACCGAACCCAGGATAAA	277	3	0.09
ICCM0219a	FI856906	(ACC) ₄	CCTTTTAAAGGGCTGAAGGCT	TGAAAGAAATGTGGGGGAGAG	203	NA	
ICCM0219b	FI856906	(CT) ₅	TCATCTACCCAAATGCTCC	GTAGTGGGTAGGGGATGGT	240	3	0.10
ICCM0219c	FI856905	(CT) ₄	CTCACCCACACACCTATCC	GGCAAAGGGAGAGAAAGGAAGT	278	NA	
ICCM0219d	FI856905	(TC) ₅	CTCACCCACACACCTATCC	GGCAAAGGGAGAGAAAGGAAGT	278	NA	
ICCM0220	FI856908	(TA) ₄ N(CT) ₄	TCAACCATAAAGCACTCCCC	GGCCATTTGTGTTTTGTATGG	183	1	0.00
ICCM0222	FI856617	(CT) ₅	TCCGATTGGATTTTCAGGAC	GGTATCAGTGACCTCGCCAT	210	1	0.00
ICCM0223a	FI856912	(TCT) ₄	TACAACTTTTGACACCCCGGA	AGTGGCAGTATGCGTTGAGA	224	NA	
ICCM0223b	FI856911	(TC) ₄	GCTCTGTCGGTCTTCTCTGTC	ACAAAAGCGCTCGAATAAGGA	208	NA	
ICCM0224	FI856914	(CT) ₄	ACCACCTTGCTCATCCTCAC	GAGTAGGAGGTGCGAAAACG	274	16	0.74
ICCM0225	FI856915	(CT) ₄	ACGTCCGGATTTGTTCTCAC	ATTATAGGAAGATGGCGGGG	252	6	0.27
ICCM0226a	FI856537	(A) ₁₂	CCAAAGACGCGGATAAATA	AATGCCACCCATAAATTCA	273	1	0.00
ICCM0226b	FI856537	(TA) ₄	GAAAAAGCGCTGTAAAATGGC	CCTCGCATTTTGTCTCAAAG	145	1	0.00
ICCM0228	FI856619	(CT) ₆	TGGACGTAGTTGTTGTGGA	GGACCGGGAGTCCCTTATTA	274	1	0.00
ICCM0229	FI856916	(C) ₁₀	TGTCTTAATCCCTCCTCCCC	AGGGTTTTTTGGTTACCAG	158	9	0.69
ICCM0231a	FI856919	(TTA) ₂ 1N(TC) ₄	CTGGGGATACGTAGGAGCAA	GGAGTGAGATAAGAAAAGAGAGGAGG	278	NA	
ICCM0231b	FI856919	(TC) ₄	ATCCCACTTACCACCCCTTC	GGAAATGAGGAGGATTTGAGA	279	2	0.05
ICCM0232	FI856920	(T) ₁₀	ACGGGAAAGTTTCTGGGTCTT	TAGCGGAGAAAACAGGACTGG	253	3	0.09
ICCM0233a	FI856922	(TA) ₄ N(TTA) ₄ (TTA) ₄	CTCACCACTAGGATGGGAA	AGACTCCCGAGGGATTGACT	242	NA	
ICCM0233b	FI856922	(A) ₁₀	AGTCAATCCCCTCGGGAGTCT	TGTTGAGGGCCTTAGATTGG	256	NA	
ICCM0234a	FI856925	(TTA) ₄	GGGACTACTTTCGCGAATCA	GTGGGTAATCCGTGCGTAAT	229	1	0.00
ICCM0234b	FI856924	(TA) ₄	CAGGCTATGTCATCTCGTGG	CCTGACTGCCACAAGTTTCA	270	1	0.00
ICCM0234c	FI856924	(TCG) ₄	TGCTTCCGTACAGGCTATGTC	CCTGACTGCCACAAGTTTCA	280	1	0.00
ICCM0235a	FI856927	(TC) ₄	TCGCTCATGACAGACTCGAC	GTAGCAGGTGAGATGCACGA	173	5	0.32
ICCM0235b	FI856926	(GT) ₄ c(GT) ₄	GCCGACCCGATTACCTTACT	GAGAGCTAAAGGGGAAGGTGG	176	NA	
ICCM0236a	FI856621	(CGC) ₇	GCTTGTGGCCCTGTATTGTT	AAAACGCAAGCAAAGCAAGT	151	NA	
ICCM0236b	FI856621	(ATT) ₄ N(A) ₁₀	ACTTGTCTTGTGCGTTTT	TTTGTGGGTGGTTGATTTT	219	2	0.04
ICCM0236c	FI856621	(A) ₁₀ N(TA) ₄	AAAATCAAACCAACCCACAAA	ATCACTCTACCGTCAAAACCGA	244	2	0.04
ICCM0237a	FI856622	(AT) ₄ N(ATT) ₆	TCAAACCATCCCTAAAACAATTG	TTCCCTTGGCCATTTATGTTTTG	184	6	0.34
ICCM0237b	FI856622	(A) ₁₃	CAAAAACATAAATGGCAAAAGGAA	TCGTGTTGTAATGTGCCGAT	155	2	0.04
ICCM0237c	FI856622	(AG) ₄ N(GA) ₄	TGTGTTTCTCGATGGCAGAG	CTTTTTCTCCCTTCCACCA	120	1	0.00
ICCM0238	FI856623	(TC) ₄ N(TC) ₄	ACCATAGACGAACCCACCACC	CCAAAGGGGTACAACTGTGT	215	1	0.00
ICCM0240a	FI856650	(TA) ₁₂	ACCCGAAACCCGCAAAATAATA	GCAATGAGACTGGGGTTTTTC	248	NA	

Table 2 continued

Marker name	GenBank ID	SSR motif ^a	Forward primer (5'–3')	Reverse primer (5'–3')	Product size (bp)	Number of alleles ^b	PIC value
ICCM0240b	F1856650	(TA) ₄	ACCCGAACCCGCAAAATA	GCAATGAGACTGGGGTTTC	248	19	0.77
ICCM0242a	F1856929	(AAT) ₁₈	TGCATTCATCTGTTTCGCTC	GAAAAATTTGTGGTTATCCGATTTT	263	8	0.74
ICCM0242b	F1856928	(A) ₁₁	ATCCGCAACACAAACA	CCCTACTCGTAATCGACTCTCG	252	4	0.21
ICCM0242c	F1856928	(TAT) ₅	CAAGTGCAATAGGAAATCCA	ATAGGGCTTTCCACCCGATTT	210	NA	
ICCM0243a	F1856931	(AT) ₅ N(AT) ₄ N(AT) ₈	TCAGGAAACAGACGGAACTTTT	GGGTTCAAATCTATTTGGGC	277	3	0.08
ICCM0243b	F1856931	(AT) ₄	ATTTCGCCCCAATAGGATTT	TTTTTCTATCGGAATATCTCAITTTTCT	280	1	0.00
ICCM0243c	F1856930	(GA) ₄ I ₁ N(AG) ₁₀	ACGACGATTTCTGGATTTTGG	AGTTTTTGGTAGGGGGTTCGAG	237	16	0.88
ICCM0244a	F1856933	(AT) ₈ N(TTA) ₄ (TAA) ₄ N(TTA) ₆ N(ATT) ₄	ATGCGGTAATCCTTGGACTGG	TGCAGGGAATGAAATGTGTGT	252	5	0.25
ICCM0244b	F1856933	(TA) ₅	CACACATTCACCTCCCTGCAA	GATGGAAAGGAGGGGTAAAA	268	NA	
ICCM0245	F1856935	(AG) ₅	GCGGCTGGTTAAGAGTGAG	CCAACACGACCCAAATCAAT	182	6	0.53
ICCM0246a	F1856937	(AT) ₄	TCTGACAGCTCTTGGCTTGA	AACACCCAGACCCCTTTCAT	280	4	0.12
ICCM0246b	F1856937	(TA) ₄	TGAAGAGGAAGAGACGGGAG	AATCCATTTACGGGGGTAGC	268	NA	
ICCM0246c	F1856937	(TATTT) ₄	TGAAGAGGAAGAGACGGGAG	AATCCATTTACGGGGGTAGC	268	1	0.00
ICCM0246d	F1856936	(TC) ₄	GATCACGGTTACGAAATGCAA	TAAAGTTCCCAITGGCTCTG	209	1	0.00
ICCM0247	F1856626	(TTA) ₈	CCTCAATTCATTTTCTTCGG	TTTCCCGATAAAACCATCTGTT	136	2	0.06
ICCM0249	F1856627	(T) ₁₂ N(TAA) ₂₉	TTTCTTCGCATGGGCTTAC	GGAGATTTGTTGGTAGGCTC	193	5	0.16
ICCM0250	F1856940	(TAT) ₄₀	TTTCAAACACAATCTGAACGAGA	CCACCTTCGGGTAGGATACA	231	2	0.04
ICCM0251a	F1856943	(AC) ₄	TCCCTGCTATACACCCATCC	TGGGCATATATGGATCACGA	252	1	0.00
ICCM0251b	F1856943	(CG) ₄	CTACACCCGCCAACCTCTAC	AAGTGATGTGACCGAGCCC	261	1	0.00
ICCM0251c	F1856943	(CA) ₄	AACCCATAATACGGCTCAC	GGGGTGGTAAGGTAGGAGGA	206	2	0.08
ICCM0252a	F1856945	(CCT) ₄	TCTACCTCTCCGCTCTTCCA	TGGTGATAGGTGGTGGTTGA	203	1	0.00
ICCM0252b	F1856944	(T) ₁₁	TTGACGGTGGGGGTATACAT	TCCACACACTCCCACACTCCA	267	NA	
ICCM0253	F1856946	(AT) ₅	TCCCTTACAAGCATTTCCCTG	TGGGGACCGTTTTTTCACCTTA	110	NA	
ICCM0254	F1856628	(TAA) ₄ N(TAA) ₂₉	GCCAAAGCCATTAAACACT	CGTTGTTAAAAACCCGCTTG	273	1	0.00
ICCM0255	F1856629	(ATA) ₈ N(AAT) ₄ N(AT) ₄	GGTACCAGAAATATGGAATGC	TGGCCTGACCTACTTATGGC	272	2	0.04
ICCM0256a	F1856949	(AT) ₄	ACCGTCAITTTCCATACGTC	TGGATCAAGAGGGGAGGATTTG	195	NA	
ICCM0256b	F1856948	(CT) ₄	TTTCTCTTTTGGTGGTTGCC	TTAAGGTTTGGCCACTCCTGG	247	4	0.17
ICCM0256C	F1856948	(TTA) ₁₉	TTAAGCCAGACGTGGGAAAC	AGAAAAGAGGGAAATGGGGA	236	2	0.08
ICCM0257	F1856630	(ATA) ₄₄ N(AAT) ₁₁	TCGTTCCCAACATTCAAAAA	CAATTGCCTTATAGCACAAACA	255	2	0.04
ICCM0258	F1856950	(ATA) ₁₁	TGCATAGGGAAATCAAAACACA	TTATTTCAACCCTCGTGTCCA	271	1	0.00
ICCM0259	F1856631	(TTA) ₁₅	AGAGCAAACAAGAACCGAA	CGAAGCCGAGAAAATGACTC	261	2	0.04
ICCM0261	F1856633	(TAA) ₂₃	GTCCGGGATTCAGTAGGAT	CAAGCCACGGAACTTGTTTT	232	NA	
ICCM0263a	F1856635	(TATT) ₇	CGGGGATAAATCAACACACC	GGGCAAGGTCTTACCCTTGT	265	2	0.08

Table 2 continued

Marker name	GenBank ID	SSR motif ^a	Forward primer (5'–3')	Reverse primer (5'–3')	Product size (bp)	Number of alleles ^b	PI-C value
ICCM0263b	F1856635	(ATA)19	GGTAGAAAAATATTTATGTGTGACCG	CTCGTTCACATACCCGCATA	260	1	0.00
ICCM0265a	F1856636	(AT)5	GGAACTCGGGAATGAAATAGTC	TTGCAAGAAAAACAATTTTAGGA	214	NA	
ICCM0265b	F1856636	(A)13	CGTTTAAATCCTAAAAATGTTTCTTTTG	ACGGCGACAACCAATTAATTC	190	1	0.00
ICCM0265c	F1856636	(TAT)9a(ATT)10N (ATT)11N(AAT)4	TACCGCCACGTTACGGTTTTT	GAAAAATATTTGTGTGTGACCCGA	248	4	0.14
ICCM0266	F1856955	(TAT)31	GAATCGTGAAGGGGAGATTT	GGGGGAATCAAAAAGGCATAG	269	NA	
ICCM0267a	F1856637	(TAT)4	CAACGTCCGTTAAAAACGGTTA	TGGGGATACGTAGGAGCAAG	179	1	0.00
ICCM0267b	F1856637	(TTA)4N(TAA) 8N(ATA)12	CTTGCTCCTACGTATCCCA	AGTCTCGTTACATACCCGCC	272	1	0.00
ICCM0268	F1856957	(CT)7N(TC)4	TTCATCTCTGCCCAAACTCC	TGGGTAGATGGAAGGAGTGG	220	1	0.00
ICCM0269a	F1856959	(AC)4	CCCTCTTTACACCCCACTT	GTAGTGGAGTGGGGCAGGTA	129	2	0.04
ICCM0269b	F1856959	(TC)5	AACATCACTAACCTCCCC	AGGTGTGGTGTGTAAGAGTG	209	NA	
ICCM0270	F1856638	(TAT)16	TCACATACCGCCACAATACG	ACGTATCCCCAGGTGCAATA	276	1	0.00
ICCM0271	F1856639	(GT)4	ACCCGGGTATAAGGTTCCAC	TGCTTGTTTTTCATTTTCATTTTC	240	3	0.11
ICCM0272a	F1856961	(A)12	TTTCCACTTGGAACAGGCTC	AATGGACGATGGTGTGGGTTA	280	3	0.12
ICCM0272b	F1856960	(GA)10N(AG)20	CGCGGTTGAGTTAGAGTGGT	CAAAATCGGGGATTTTGTGTTG	175	12	0.74
ICCM0272c	F1856960	(GA)4	CGCGATTATTACCCACGTTT	GGAAAGGAGGTACCCGGAGTC	249	3	0.09
ICCM0273	F1856963	(TGA)4	TGTAACATCATATCGCCAGC	AGACGTGTAGACAGATGCC	108	2	0.04
ICCM0274	F1856640	(TC)9(TA)15	GACCTACCCCGCAAGTAAT	TTTTTGTCCACACTCACACCT	265	NA	
ICCM0276	F1856965	(C)10	CTCCTACACTGCCTCCCTC	TCATGCTTACTCCGTTGCAG	222	NA	
ICCM0277	F1856642	(TTA)11	GGCAAAACAATAACCGAAACA	GTAAAGAGGGCCAGCTGTTG	196	6	0.30
ICCM0278a	F1856643	(TTA)4	ATAGGGGACCAAAAACCTGCAA	GTGGGTAATCCCGTGCCTAAT	203	1	0.00
ICCM0278b	F1856643	(AT)4	AAAAATACACATCCTGACTGCCA	TTTTGCTTAGACTTGTAGGCATT	159	2	0.17
ICCM0280	F1856969	(T)11	ACTAGATGGTCGCATCCTGG	GGTGAAGGTGTGGATGAGGT	280	1	0.00
ICCM0281a	F1856644	(AC)9	TTCAACCTCCCTACACGTT	GTTCTCTTCTTGTGTTGCC	235	1	0.00
ICCM0281b	F1856644	(AAT)5N(A)11	TGGAAACAACCAAGACCTTCA	GCTGCCACAACAACACTGAGAA	264	2	0.04
ICCM0282a	F1856645	(CGC)7	CCTCGTTGTTGCCCTGTATT	AAAACGCAAGCAAAAGCAAGT	154	3	0.23
ICCM0282b	F1856645	(ATT)4N(A)10	ACTTGTCTTGTGCTTGGGTTTT	TTTTGTGGGTTGGTTGAAATTT	219	NA	
ICCM0282c	F1856645	(A)10N(TA)4	AAAAATCAACCAACCCACAAA	ATCACTACTACCGTCAAAAACGA	250	4	0.25
ICCM0284a	F1856647	(AT)4	CGTATCTACACCCGCCTCA	TGGAATAATCCACTTTGATTTGG	257	3	0.08
ICCM0284b	F1856647	(TA)4	CGTATCTACACCCGCCTCA	TGGAATAATCCACTTTGATTTGG	257	9	0.30
ICCM0285	F1856971	(ATT)5	TGAGGACAAGATTCGGTTCA	AACATGGGGTGTGTTTTCTC	267	1	0.00
ICCM0286a	F1856648	(GA)5	AGCATCACGCATACAGCTTG	ACATTTGGCTCCATTTGTTGG	254	2	0.04
ICCM0286b	F1856648	(AG)4	ACCCCAAAAATGCTGTAGTG	ACGCCCTTTACTGTACGA	276	1	0.00

Table 2 continued

Marker name	GenBank ID	SSR motif ^a	Forward primer (5'-3')	Reverse primer (5'-3')	Product size (bp)	Number of alleles ^b	PIC value
ICCM0288	F1856972	(TAA) _n (TTA) ₄ N(TTA) ₄	TTATTTTTCGGATCCAACGC	GTGATTTTGTTCGGCAAT	278	20	0.88
ICCM0289	F1856976	(T) ₁₃ N(ACA) ₄	CAGCCTCCATGGCATAGATAA	TGCTTGAATGAGTGCAACAA	219	15	0.83
ICCM0290	F1856976	(A) ₁₅	TTGTTGCACTCATTC AAGCA	TTTTTAATTGGGGCAATTGAGC	244	6	0.38
ICCM0291	F1856978	(AT) ₄	AAGTATCAAAATTATACGTCACAAAA	TCATCCTTGTAAAGTCAACCACTT	249	1	0.00
ICCM0292	F1856978	(TAA) ₆	TGGTTGACTTAACAAGGATGAGTG	TCCTCAAGCAGAGGTGGTTC	267	1	0.00
ICCM0293	F1856982	(TAA) ₁₅ g(ATA) ₁₅	AGTGATGCCACGAGAATTGC	CTGGTTCGGAATTGTCATCC	250	5	0.24
ICCM0294	F1856986	(TTA) ₁₅	AGAGGCCAAAACAAGAACCAGAA	CACCCAATTTTGTCCGATTT	185	NA	
ICCM0295	F1856987	(T) ₁₀	GAGGCACCAAATTCGTATCC	CAAAAATTTCTAATTCACCAAGACTTC	256	3	0.09
ICCM0296	F1856987	(TGATT) ₄	CGCCAAGTTTTACTATGTGCTG	TGCTGGATGTTACATAAAACACTCTT	227	1	0.00
ICCM0297	F1856987	(TAA) ₁₈	CATGATTTGATTTGATTTGATTTTC	GGAGTGGGAAAACCTTAAGCC	271	3	0.08
ICCM0298	F1856989	(TC) ₄ N(AAT) ₄	GTGCACCTTGTTCAGCGTTGT	CGCAAAACACACATTCCTCTG	221	5	0.33
ICCM0299	F1856989	(CTT) ₇ N(TCT) ₄	TTATGAAAGCCGAAGCTCGTT	GAGCAGTAAACCTACCCCA	272	NA	
ICCM0300	F1856990	(A) ₁₀	ATGGCCAAAATGAACCTCCAG	AAAAGAGAAGGTTCCATCGG	173	2	0.10
ICCM0301	F1856992	(A) ₁₀	ATGGCCAAAATGAACCTCCAG	AAAAGAGAAGGTTCCATCGG	173	1	0.00

Marker names start with prefix ICCM, which represent *JCRISAT Chickpea Microsatellites*

NA not amplified

^a SSR motifs having "N" nucleotide represent the interruption of few base pairs between two same/different SSR motifs

^b Number of alleles is calculated based on screening 48 chickpea genotypes using touch-down PCR profile of 61–51 °C

Table 3 List of gene-based SNP anchor markers used in comparative mapping of chickpea and *Medicago*

Marker name	Template sequence accession no.	Type	Sequenced Mt/BAC accession no.	Linkage group in <i>M. truncatula</i>	Linkage group in chickpea	Genotyping method	Restriction enzyme	Forward primer (5'–3')	Reverse primer (5'–3')
CAL-TL	AW126242	M/EST	AC149080	1	4	CAPS	Alu I	GTGGAAGGCACCATTGATTGACAAC	TCTTCTCTCAGCCTCTTCAAATGC
CDC2	AW171750	M/EST	AC144481	1	4	SNaPshot	N/A	CAACTTTTGCAAGGTTGCTTTCT	ACTAACACTGGCCACACATCTTCA
CysPr2	AI974635	M/EST	AC148098	1	4	CAPS	Taq I	CCAAAACTTGTCTTACTCTTCAATC	GACAAAACCCACCCAGACAATCAACTAG
HRIP	AW126332	M/EST	NA	1	4	SNaPshot	N/A	GGAAAAATTATCTCTCCAAATGGGGTA	AAAAATAGCACTGCACCAAAAAGTGCTG
ppPF	AI974685	M/EST	NA	1	4	SNaPshot	N/A	TCTGCCACCACAACAACACTAC	AAAAATTTGTTCTGAACACTCACTTGAAGCCA
REP	AA660953	M/EST	AC133709	1	4	CAPS	Taq I	CTCCATTTCCGGTTCGTTCC	CACCGGTTGCCCTCCAGAC
RL3	AI974458	M/EST	AC125473	1	4	CAPS	Hind III	TTCAATCGATAAGAATATCGCTTTG	TGTGTGCATACCAAGCCTTG
TC76700	TC76700	M/EST	AC122171	1	4	CAPS	Hinc II	CCAAAGACCCAGTTCGTGT	GTTGCATGGTTGACATCAGG
TC86606	TC86606	M/EST	AC139748	1	4	SNaPshot	N/A	TGAAAAATGGCAATGTGGAGA	TGGGATTCCTCATCTCTCAG
TC87270	TC87270	M/EST	AC124216	1	4	CAPS	Dde I	TGGCTTCTTTCCGGTCTCTGT	TCAAAACCAACGCAATTTGGTA
TC88727	TC88727	M/EST	AC137895	1	6	SNaPshot	N/A	ATCAGGCAGAACTTGTCTCGT	AGAGTGCCCGGTATATTCCT
DSI	AA660976	M/EST	AC122168	1	4	SNaPshot	N/A	GAAAGCCAAAAGTATGAAGGGCCACGCAC	CATGGTGCAATAAATCAACCAAGACATC
ChitinaseII	NA	NA	~AC137554	2	1	Pfaff and Kahl (2003)		AGCACATGAACCAATCCACA	CATGGTTGCAATTTCAACGTC
ACCO	AI974230	M/EST	AC121236	2	1	CAPS	Stu I	GAAAGATGGCGCAAAAAGAAAGT	CGATGTGCTGTGCATCTCGTTAAGTTCCCT
AJ005043	AJ005043	Ca/EST	~AC127170	2	1	SNaPshot	N/A	TTTTCCGGGAACCTGCTTATGG	CATGGAAGTGGACCCGTTTTT
CPCB2	AW191283	M/EST	NA	2	1	CAPS	Dra I	AGA AAGAGTGAAGTCTGTGGATCTACATC	GGAATGAACAGCCACACACCTAATGTAAATC
DMI-1	AY497771	Gene	AC140550	2	1	CAPS	Hinc II	ACCTTCTTTTCCCTTGGCAAT	ACCGCATACAACGGCTAAACCC
TC77488	TC77488	M/EST	AC126013	2	1	CAPS	Afl III	ATGCTTGCAGATCTCTGCTTT	GATCTGCCTCAACTCCAAAGC
I433P	AI974411	M/EST	AC144342	3	5	SNaPshot	N/A	AAGGTTTTCTACCTTAAAGATGAAGGGAG	GTTTAGCAAAATGTCAGGGCAGCA
AF457590	AF457590	Ca/EST	~AC122728	3	5	SNaPshot	N/A	CTCCCTCTCTATCGCCCTCT	TGTGAAAGGGTTGGTGTGAA
AIGP	AW125928	M/EST	NA	3	5	CAPS	BSPW I	CTGATAGGGCCAGGAGGCGGGAAGA	GTTTTTAGCATTTGGACGAATGGTTGGT
CysPr1	AI974595	M/EST	AC131026	3	5	CAPS	Ear I	GAGAAATCAAAAGAAATTAAGACAAAGA	GAAAGAAITTCATGGGGAGCAAAAGT
Ms/U515	AJ410128	Ms/EST	NA	3	5	SNaPshot	N/A	GTTAAGGGAAACCATGACAACCCACA	CCAGGCTCGGATTGAGCAGGGGTTTGT
Ms/U83	AJ410159	Ms/EST	NA	3	5	SNaPshot	N/A	TGGAAAACTTGGATCGATCCTGCTCA	TGATAGTCTGCAACAAAGTCCAGCA
P40	AJ006759	Ca/cDNA	~AC143340	3	5	Pfaff and Kahl (2003)		TTTTTAAACGCCCGCAATGA	CAGAAAAGCAATGGTGGGAAT
TC80362	TC80362	M/EST	AC137828	3	5	CAPS	Ase I	GCTTGAGCCTCCAGAGATTG	TGATAAGCCTTGCAGTGTGC
TRPT	AA660362	M/EST	AC122170	3	5	SNaPshot	N/A	CAACAACCTAGTATAGCGATCAGTG	CATTCAAAGCCCAACCAAGTT
DNABP	AI737524	M/EST	AC121244	4	6	CAPS	Afl III	CCCTATGAGCTTGGGTTTGTCT	CTCATGGCATACGTTTTCAGC
EST948	AA661051	M/EST	AC145021	4	6	CAPS	Dde I	GCAGGGTTTCCGCTCCAGTG	AACCTAATGAATGATTGGAAAGGTTAGCG
Ms/U40	AJ410120	Ms/EST	NA	4	6	SNaPshot	N/A	AAGAAATGAGGAAGAGGAATCAACA	GGTTCCTAAAGGAACAATGATGACA
MTU07	AI737610	M/EST	NA	4	6	CAPS	Bsm I	CAGACACCCAAAGAAATACAGAA	GATGACCAGAGCCTAATACTATTATGACT
TC78756	TC78756	M/EST	AC144502	4	6	CAPS	Alu I	GGAGGAGGAGGAATCAGG	ATGTGGAGAGATGGTGAGC
TC79726	TC79726	M/EST	AC130805	4	6	CAPS	Clal	TGCTGAAAGGAGGATTCAGG	ACAGCATTTGTGAGCAACCCAC
TC88598	TC88598	M/EST	AC135316	4	6	CAPS	Bcl I	AGATGGGAGATTGATGCTG	AAGCAACATGCAGTGAGCTG
TGDH	AA660742	M/EST	NA	4	6	SNaPshot	N/A	CGGTGGCTTCAFCGGTTCT	GACGTGATTTGTAATCAGCAGGAGTA
iRALS	AW126282	M/EST	AC125476	4	6	CAPS	Msp I	GGTCTCGAGCTGTTTTTGGAGAAG	GCAATTCCTCTCAGCTAAAAGTG

Table 3 continued

Marker name	Template sequence accession no.	Type	Sequenced Mt BAC accession no.	Linkage group in <i>M. truncatula</i>	Linkage group in chickpea	Genotyping method	Restriction enzyme	Forward primer (5'–3')	Reverse primer (5'–3')
U71	AJ410150	Ms/EST	NA	5	8	SNaPshot	N/A	GCTGAAGCTGAAGGTTTTCG	CGCATTTTTATGGATGAAGAGA
X60755	X60755	Ca/EST	~AC137669	5	8	SNaPshot	N/A	AGGTGCCATAGGAAGACACG	CCCAATCTTTTTCTCCACA
AB025002	AB025002	Ca/EST	~AC122727	5	2	CAPS	EcoR V	TTCTTCGATCATGTGCAACTT	CGTGACCAAGCTTCGTAGTA
AJ005041	AJ005041	Ca/EST	~AC122727	5	2	CAPS	Dra I	AGCGTCTAGCCAGCATCAAT	CCATGGCTTCTTACCCTTCA
AJ404640	AJ404640	Ca/EST	~AC131455	5	2	CAPS	Pst I	TGGAGAAGATGAGGGGAATG	TCAAAGGATGCCAAATCACA
CYSK	AW207985	Mt/EST	AC135320	5	8	SNaPshot	N/A	GGAAATGCTAAAGATGTTACAGAATTGA	AATGAGCACACTCTGTCCAGGTGTGA
CYSS	AW127154	Mt/EST	AC135320	5	8	CAPS	Bgl II	CTGATGCAGAGAGAAAGGGGCTTATCA	CCAAATCAGCTCCAAAAGCTAATAGAATGA
DK242R	AQ917211	Mt/BES	NA	5	2	CAPS	BsmA I	CGTATGTTTAAATCCGTTAGTCCGTCCT	GCTTGCCTTAGATATTTGGCACCTCA
FENR	AW127593	Mt/EST	AC138010	5	8	CAPS	Hph I	ATGCTTATGCCAAAAGATCCAAATGC	CTCACAGCAAAGTCGAGCCTGAAGT
Ms/U393	AJ410119	Ms/EST	NA	5	2	SNaPshot	N/A	ATTGGAGAAGGGCAATCCACCACA	TCCCTTCAATCTATCCATCCCAAGA
Ms/U89	AJ410164	Ms/EST	NA	5	8	CAPS	EcoR V	CATATGAACAGTTGAAAAGTGGATGA	CAATATGGCCAAACATTAATAAATGGCA
TCMO	AW127521	Mt/EST	AC141923	5	5	CAPS	Hph I	GTCTACGGCGAAACATTTGGCGTAAAATGC	CAATTTGCAGCAACATTTGATGTTCTCAACA
TC87369	TC87369	Mt/EST	AC124591	6	2	CAPS	Pvu I	ATAGTGGACTTCGGCGAGAA	TGACGGGATCTTTTCTTTG
AGT	AW126002	Mt/EST	NA	7	3	CAPS	Xba I	GATTTGGGCTCAATCTTCTTGTGTGCA	CCTGAAAGGGGAAAATTGCCACATTGA
AJ004917	AJ004917	Ca/EST	~AC136505	7	3	CAPS	Dde I	CCAAAGAAACCCAGTGGATGT	GCAGCATCAAGATCACGAAA
AJ012739	AJ012739	Ca/EST	~AC130801	7	3	CAPS	Dra I	CTTCAGTCAGGAGGGAGACG	TGCAAAATTCCTGCTACAAGGA
AJ291816	AJ291816	Ca/EST	~AC140025	7	3	SNaPshot	N/A	TTGGAGGTGCTGTTGATGTA	TGC AAAATGCTTGCCAAAATAG
DK225L	AQ917191	Mt/EST	AC137994	7	3	CAPS	Hinc II	TGTCCTTGCTTCTTATCTCTTCTTCA	AGCAGCAACAACACTTACAACAACACTC
ENOL	AA660534	Mt/EST	NA	7	3	CAPS	Dde I	TTCCATCAAGGCCGTCAGA	TTTGACCAACCCCAATTCATT
MS/U380	AJ410118	Ms/EST	NA	7	3	CAPS	Taq I	CACCTATTGCAATTTCCATGCTTCA	CAGTTGTTGTAGCAAGGGCACA
RNAH	AJ974503	Mt/EST	AC123899	7	3	CAPS	Mbo II	GCTTCCACCAGCTGATACAGG	TTAGCCCTAGCAAGAATGTCACCTG
TC76881	TC76881	Mt/EST	AC136505	7	3	CAPS	Hph I	TTCTTGGGGAACAGAACAGG	GCAGCATCAAGATCACGAAA
TC78638	TC78638	Mt/EST	AC135795	7	3	CAPS	Acc I	TGATTTGCAAAGCAGGTTGAG	CGGCATTCAGTAGGAAGCTC
TC84431	TC84431	Mt/EST	AC139355	7	3	CAPS	Nsi I	AGAAAGGACTTGGGCAACCT	CCAATACTGCTCTTTTGGT
TC86212	TC86212	Mt/EST	AC124963	7	3	CAPS	Hinc II	GTGGCTCTTGTGTGCTGTA	AGCCAGGATGAGGCACCTCA
TC88512	TC88512	Mt/EST	AC136974	7	3	SNaPshot	N/A	CTACTGTTGGACGGGTTGCT	AACCTGGGGCAATTTGTGTAA
TC88726	TC88726	Mt/EST	AC121242	7	3	SNaPshot	N/A	GAGGAGAATACGCCATCCAA	GGATAGTGTGGGCTGCATCT
AJ276270	AJ276270	Ca/EST	~AC122726	8	7	SNaPshot	N/A	GCTGCGAATTTTGACCTAGC	TGCCCTTGCAGATTACAGTTG
AJ276275	AJ276275	Ca/EST	~AC140032	8	7	SNaPshot	N/A	TTTTTGCCTGGACC AAAATC	AAGGCTTGTACTGCCCTTGA
AJ489614	AJ489614	Ca/EST	~AC138087	8	7	SNaPshot	N/A	CGAGGAAGAAATGGCAAAGAG	AACAAGCCAAATGAGGGAGTG
CoA-O	AJ974546	Mt/EST	AC119408	8	7	CAPS	Nla III	TTTTGGGGAAATAATGGAAAGTCT	CTCGGGCAAATGTTGAAAATTC
CPOX2	AW127442	Mt/EST	NA	8	7	SNaPshot	N/A	AAAGAAATCATGCTCCTCAAGCTG	CTTCTCTTGTGGAAAGTCAGCA
EST671	AA660779	Mt/EST	NA	8	7	CAPS	Hpa II	GGTGTATCTATGAAAGAGGCCTCATTGG	TGTCCTGGGTATGTAGAAAAGCCTCAC
FIS-1	AJ974522	Mt/EST	AC122726	8	7	SNaPshot	N/A	TCAGTGAATGAGGGTTTTTCTACG	CTGTTTCACTCAACTTCAGCAACTTT
Ms/U82	AJ410158	Ms/EST	NA	8	7	SNaPshot	N/A	TTCTGTAACGAGATGTTTGTGTGGC	CAAAATGAAGAAAAGAGGAATGAAGGT

Table 3 continued

Marker name	Template sequence accession no.	Type	Sequenced Mt BAC accession no.	Linkage group in <i>M. truncatula</i>	Linkage group in chickpea	Genotyping method	Restriction enzyme	Forward primer (5'–3')	Reverse primer (5'–3')
AJ004960	AJ004960	Cu/EST	~AC136142	Unknown	2	CAPS	BstNI	GCCTGGTGTGATCGTTACCT	ATGA AACCCGGCAAGACTTG
Ms/L591	AJ410091	Ms/EST	~AC148970	Unknown	3	CAPS	Hind III	GGCAGCTATAAAATCAAGTATCATGC	TGCCACTTGGCCAAAGGACTCATT

"~" denotes that the putative orthologs of the chickpea genes are located in the corresponding *M. truncatula* sequences (Genbank accession numbers). TC#s are from TIGR gene index database (<http://www.tigr.org>)
Mt, *Medicago truncatula*; Ms, *Medicago sativa*; Ca, *Cicer arietinum*; N/A, not available; N/A, not applicable

PCR was carried out in 5 µl reaction volume in GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). The reaction mixture contained final concentration of 5 ng/µl of template DNA, 0.5 mM dNTPs, 0.5 µM of M13 tailed forward, 1 µM of reverse primer, 1 µM of M13 labeled primer, 0.75 mM of MgCl₂, 0.1 U of Taq DNA polymerase (AmpliTaq Gold), and 1× PCR buffer (AmpliTaq Gold). An initial denaturation was given for 15 min at 94°C. Subsequently, ten touch-down PCR cycles comprising of 94°C for 20 s, 61/60/55°C (depending on the marker as given in Table 2, ESM Table 1) for 20 s, and 72°C for 30 s were performed. These cycles were followed by 35 cycles of 94°C for 10 s with constant annealing temperature of 54/56/48°C (depending on marker and touch-down profiles as given in Table 2, ESM Table 1) for 20 s, and 72°C for 30 s, and a final extension was carried out at 72°C for 20 min. The amplified products were separated by capillary electrophoresis using ABI PRISM® 3700 DNA analyzer, and allele calling was carried out as given in Varshney et al. (2009b).

For SNP genotyping, in CAPS assay, 1.5 µl PCR product of 94 RILs was digested with the corresponding restriction enzymes. Each digestion reaction contained 2–5 U of the corresponding restriction enzyme and 1× compatible buffer in a total volume of 10 µl. Enzyme digestions were incubated at the appropriate temperature for at least 4 h. Digestion products were separated and scored as mentioned in Choi et al. (2004a). In case of SNaPshot assay, the ABI SNaPshot Multiplexing Kits was used following the same protocol as suggested by the manufacturer, except that 0.5 µl SNaPshot mix for a single marker was used (see Choi et al. 2004a).

Polymorphism assessment of SSR markers

While ICCM-series markers were screened on the panel of 48 diverse genotypes including the parents of the inter-specific mapping population (Table 1), the H-series markers were screened on only two parental genotypes (ICC 4958 and PI 489777). Allelic data obtained for the SSR markers were subjected to AlleloBin program (http://www.icrisat.org/gt-bt/download_allelebin.htm) for allele calling based on the repeat units of SSR motif for corresponding markers. In case of ICCM markers, the binned allelic data were used to calculate polymorphic information content (PIC) value of the markers by using the PowerMarker V3.25 program (<http://statgen.ncsu.edu/powermarker/>).

Linkage analysis and map construction

Genotyping data for both ICCM- and H-series polymorphic markers were generated on 131 recombinant inbred lines (RILs) of the mapping population and for 94 RILs in case

of gene-based SNP markers. In addition, marker genotyping data for 407 marker loci were compiled (Huettel et al. 2002; Pfaff and Kahl 2003; Tekeoglu et al. 2000; Winter et al. 1999, 2000).

Marker genotyping data were analyzed using the χ^2 test to assess the goodness-of-fit to the expected 1:1 segregation ratio for each marker. Subsequently, genotyping data for all the markers, including those with distorted segregation, were used for linkage analysis using MAPMAKER/EXP 3.0 (Lander et al. 1987). Marker loci were first divided into linkage groups at a LOD score of 16 and a recombination fraction of 0.37 by two-point analysis using the ‘group’ command. Marker order in the linkage groups was determined using the multi-point analysis ‘try’ command of the program. Most likely order of the loci within the group was determined using multipoint ‘compare’ command. The ungrouped marker loci were also attempted to integrate into genetic map at a smaller LOD value (up to 6). The map distances were calculated by applying the ‘Kosambi’ mapping function (Kosambi 1944) as per MAPMAKER/EXP 3.0 program. Residual heterozygosity was not considered in linkage mapping.

Results

Isolation and characterization of simple sequence repeats

A genomic DNA library composed of ca. 400,000 clones was constructed from the ICC 4958 genotype. Hybridization of this library with GA and TAA oligo probes yielded 359 clones that were sequenced and assembled into a set of 115 contig and 342 singleton DNA sequences, which we refer to as genome survey sequences (GSS). These sequences were submitted to National Centre for Biotechnology Information (NCBI) and respective GenBank accessions are mentioned in Table 2.

Two hundred and ninety-nine of the 457 GSSs were determined to contain a total of 643 SSRs, with 165 GSSs containing more than one SSR. As depicted in Fig. 2, di- and tri-nucleotide repeats were the most abundant (39 and 40%, respectively), with mono-nucleotide and tetra-nucleotide repeats representing 16 and 3% of cases, respectively. Other types of SSRs had <1% representation. In terms of repeat motifs, the tri-nucleotide repeat motif TAA/ATT was most common, accounting 36.8% of all repeat, followed by the di-nucleotide repeat GA/CT at 19.2%.

These SSR loci were categorized into two groups based on the length of their SSR tracts: Class I SSRs (>20 nucleotides in length) and Class II containing SSRs (>12 but <20 nucleotides in length) (Fig. 3). Considering only perfect SSRs, which is the set of SSRs that contain a single motif (e.g., TAA), we observed uneven distribution between

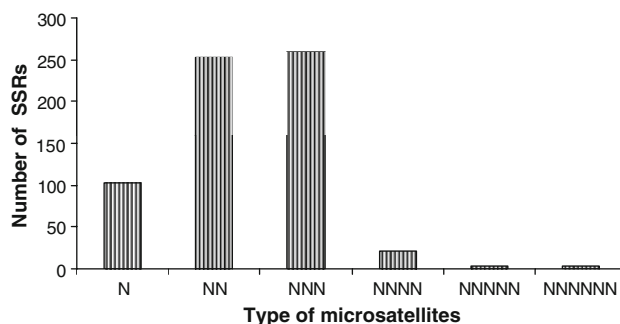


Fig. 2 Frequency of microsatellites based on type of repeat motifs in microsatellite-enriched library of chickpea. Frequency of tri-nucleotide repeats were higher among the chickpea microsatellite markers followed by di-nucleotide repeats. N, mono-nucleotide repeats; NN, di-nucleotide repeats; NNN, tri-nucleotide repeats; NNNN, tetra-nucleotide repeats; NNNNN, penta-nucleotide repeats, NNNNNN, hexa-nucleotide repeats

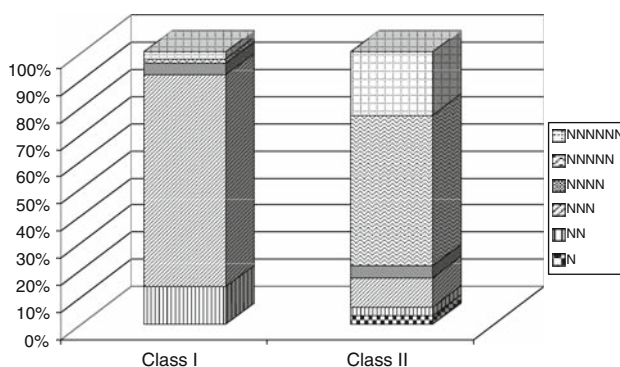


Fig. 3 Distribution of Class I and Class II repeats in newly isolated chickpea microsatellites. Class I microsatellites are with >20 nucleotides in length and Class II repeats contain perfect SSRs with >12 but <20 nucleotides in length. Among Class I repeats, tri-nucleotide repeats were abundant followed by di-nucleotide repeats, while in Class II repeats, penta-nucleotide repeats contributed highest, followed by hexa-repeats. N, mono-nucleotide repeats; NN, di-nucleotide repeats; NNN, tri-nucleotide repeats; NNNN, tetra-nucleotide repeats; NNNNN, penta-nucleotide repeats, NNNNNN, hexa-nucleotide repeats

Classes I and II. In particular, the longer Class I SSRs were substantially enriched for tri-nucleotide repeats, which represented 77% of all Class I repeats. A similar uneven distribution was noted for other repeats, but most notably the penta-nucleotide repeats, which comprised 55% of all Class II repeats and less than 2% of all Class I repeats.

Similarity analysis was performed for all 457 GSSs using BLASTN and BLASTX algorithms, and significant similarity was determined at an Expect value threshold of $\leq 1E-05$ (Table 4). Relatively few of the GSS sequences had E values that surpassed this score, irrespective of the species data set under analysis. This is consistent with the expectation that randomly selected short genomic sequences only occasionally correspond to gene coding

Table 4 Functional annotation of ICCM sequences with EST databases

BLAST algorithm	Database	Number of entries in database searched	Number of sequences showing similarity	Number of sequences with significant similarity (<1E-05)	Percentage of sequences with expected values <1E-05	Median expected values
BLASTN	Ca_EST	7,097	450	26	5.69	2E-60
	Mt_EST	249,625	449	76	16.63	5E-22
	Lj_EST	158,135	449	48	10.50	1E-16
	Pv_EST	83,448	449	35	7.66	4E-26
	Vu_EST	183,757	440	49	10.72	1E-14
	Gm_EST	880,561	440	73	15.97	3E-14
	Ah_EST	41,489	227	14	3.06	6E-15
	At_EST	1,527,298	444	44	9.63	1E-11
	Os_EST	1,220,877	285	20	4.38	2E-19
	Pa_EST	418,223	452	48	10.50	1E-12
BLASTX	Uniprot	385,721	409	137	29.98	3E-12

The database were downloaded from NCBI in May–June, 2008

BLASTN, nucleotide BLAST; BLASTX, protein BLAST; EST, expressed sequence tags; Ca, *Cicer arietinum*; Mt, *Medicago truncatula*; Lj, *Lotus japonicus*; Pv, *Phaseolus vulgaris*; Vu, *Vigna unguiculata*; Gm, *Glycine max*; Ah, *Arachis hypogaea*; At, *Arabidopsis thaliana*; Os, *Oryza sativa*; Pa, *Populus alba*

regions that will match EST data sets. Nevertheless, in cases where BLAST hits with e-value lower than 1E-05 threshold were recorded, the degree of similarity, expressed as either nucleotide identity of deduced protein similarity, was highest for phylogenetically related species, decreasing in rank order of phylogenetic distance (i.e., *Medicago* > lotus > soybean = cowpea = common bean > poplar > *Arabidopsis* > rice). Among these sequences, 40 were identified as related sequences in all three analyzed cool season legumes, i.e., chickpea, *Medicago*, and Lotus (Hologalegina clade; see Fig. 1), while 29 sequences had similarity with all three analyzed warm season legumes, i.e., soybean, common bean, and cowpea (Phaseoleae clade). Only 21 sequences were identified as similar sequences in both Hologalegina and Phaseoleae species. Two of these GSSs (FI856609 and FI856659) showed significant similarity with sequences of all the plant species analyzed in the present study (see ESM Table 2).

With the objective of annotating these newly isolated GSSs, all 457 GSSs were analyzed for BLASTX analysis using UniProt database. 137 of these GSSs (29.9%) showed homology to the UniProt database at a relatively relaxed cutoff value of $\leq 1E-05$. Among these, 84 unique protein sequences were used for deriving respective gene ontology (GO) (see ESM Table 3). The GO studies permitted assignment of 64 sequences to biological process, 64 to cellular component, and 67 to molecular function ontologies. According to the GO schema, single proteins typically have more than one Ontology assignment.

Development of novel SSR genetic markers

All SSR containing GSSs (299) were analyzed by means of Primer3, yielding a list of potential oligonucleotide primers from which 311 primer pairs were selected and synthesized. Where feasible primer pairs were designed for more than one SSR in a single GSS with the goal of increasing the conversion of GSSs into useable genetic markers.

Primer pairs were screened for amplification of DNA from two chickpea genotypes, i.e., ICC 4958 and ICC 1882 (Table 2). This analysis provided a set of 234 markers (75%) with scorable amplicons. Screening of these 234 markers on 48 genotypes of chickpea further defined a subset of 147 polymorphic markers (62.82%), with allele content ranging from 2 to 21 and an average of five alleles per marker. Among these 147 polymorphic sites, 56 were polymorphic exclusively in wild species, 8 were polymorphic exclusively in cultivated and 83 of them were polymorphic across wild and cultivated species of chickpea.

We refer to these new polymorphic SSR markers as ICCM (ICRISAT Chickpea Microsatellite) markers. Allelic data obtained from 48 genotypes were used to calculate the PIC value of each ICCM marker, and thus infer the discriminatory power of these ICCM markers. PIC values ranged from 0.04 to 0.92 with an average of 0.26. Twenty-six markers displayed the minimum PIC value of 0.04 each, while marker ICCM0160 had both the highest PIC value (0.92) and the highest number of alleles (21), followed by marker ICCM0022 with 18 alleles and a PIC value of 0.89

(Table 2). As has been observed in previous studies of SSRs from plant species (Temnykh et al. 2001), Class I SSRs (41 of 57) were on average more polymorphic than Class II SSRs (106 of 177), with mean PIC values of 0.38 and 0.22, respectively. Nevertheless, a higher fraction of the polymorphic SSRs identified in this study were from Class II (106) compared to Class I (41), owing to the increased abundance of Class II SSRs in our data set. Consistent with their overall abundance in Class I SSRs (Fig. 3), tri-nucleotide repeats (20) constituted major part of the Class I polymorphic sites, with compound repeats (18) comprising the next largest fraction of Class I ICCM markers. In contrast, di-nucleotide repeats were relatively rare in the total Class II data set, but comprised the largest fraction of polymorphic Class II ICCM markers (47); similar to Class I markers, compound repeats (30) constituted of the second most common fraction of Class II polymorphic sites.

In addition to the ICCM markers developed in this study, we also analyzed a set of 233 markers developed primarily by Lichtenzveig et al. (2005); these are the so-called “H-series” SSR markers. One-hundred fifty-three H-series markers yielded scorable amplicons in two PCR profiles (ESM Table 1). Both the ICCM and H-series SSR markers were tested for polymorphism between chickpea ICC 4958 and PI 489777, the parents of the inter-specific mapping population. From this analysis we identified 104 SSRs (52 ICCM and 52 H-series) that were suitable as genetic markers in the inter-specific cross, with polymorphism rates of 33.9 and 22.2% for the H-series and ICCM SSR markers, respectively.

Development of gene-based SNP markers

A set of 246 gene-specific primers, developed earlier by Choi et al. (2004a) based on gene sequences of *M. truncatula* and *M. sativa*, were used to amplify DNA of the parental genotypes of the inter-specific mapping population of chickpea. One-hundred four (~42%) of these primer pairs showed strong single fragments on 1% agarose gels; these amplicons were re-sequenced in both mapping parents of the inter-specific cross (ICC 4958 and PI 489777), quality-scored, and trimmed to yield 96 pairs of high quality sequences. Additional 25 primer pairs were designed based on chickpea EST sequences that possessed high similarity to previously mapped *Medicago* genes, yielding 18 additional high-quality sequence pairs. Alignment of the 114 ICC 4958 and PI 489777 sequence pairs revealed SNPs in 80 (~70%) genes. Seventy-one of these genes contained SNPs that could be converted to reliable genotyping assays using either CAPS or SNaPshot protocols (Table 3). Two additional gene-based markers, P40 and chitinase II, were also used for genetic analysis; these genes were previously

mapped in chickpea by Pfaff and Kahl (2003), while their putative orthologs have been mapped in *M. truncatula* by Choi et al. (2004a).

Construction and features of the genetic map

The inter-specific cross between ICC 4958 × PI 489777 is maintained as an advanced recombinant inbred population that has been used in numerous genetic studies (Huettel et al. 2002; Pfaff and Kahl 2003; Winter et al. 2000). Although the number of markers previously analyzed in this population is relatively large (407 loci), a high percentage of the markers are anonymous sequences (e.g., RFLP) and/or exhibit dominant patterns of inheritance (e.g., AFLP). Thus, in many cases, these legacy genetic maps are based on molecular markers that are either difficult to apply or to reproduce. With the intent of extending this genetic map, and enhancing the number of easily scorable markers, we genotyped the 123 new molecular markers (52 ICCM SSR loci and 71 gene-based SNP loci) and 52 previously published H-series SSR loci described above, and combined the genotype data with that of the 407 previously published loci. Linkage relationships were evaluated using MAPMAKER/EXP 3.0.

As shown in Fig. 4, 47 (90.3%) of 52 ICCM marker loci, 46 (88.4%) of 52 H-series SSR loci, all (100%) of 71 gene-based marker loci, and 357 (87.7%) of 407 legacy marker loci coalesced to yield eight linkage groups, in agreement with eight chickpea chromosomes. The linkage groups were numbered according to Winter et al. (2000), using marker loci that were common to both studies. This revised genetic map contains 521 marker loci, with an average inter-marker distance of 4.99 cM and spanning 2,602.1 cM. Considering the 740-Mbp physical size of the chickpea genome (Arumuganathan and Earle 1991), and ignoring the fact that rates can vary widely within the genome, 1 cM distance in the present map equates to roughly 285 kbp. With the exception of linkage group (LG) 8, which has relatively few genetic markers (25 markers), the average number of markers per linkage group was 71 ± 8.9 . LG 8 was also the shortest linkage group based on genetic distance, spanning 124.7 cM; however, in general LG size was not well correlated with the number of markers. As described below, comparative mapping with *Medicago truncatula* revealed that the entirety of chickpea LG8 corresponds to one arm of *Medicago* Chr5, adding further credibility to its assignment as a physically short linkage group.

Comparative linkage analysis between *Medicago* and chickpea genomes

As shown in Fig. 5, the 71 gene-based SNP markers are distributed among eight major linkage groups of chickpea,

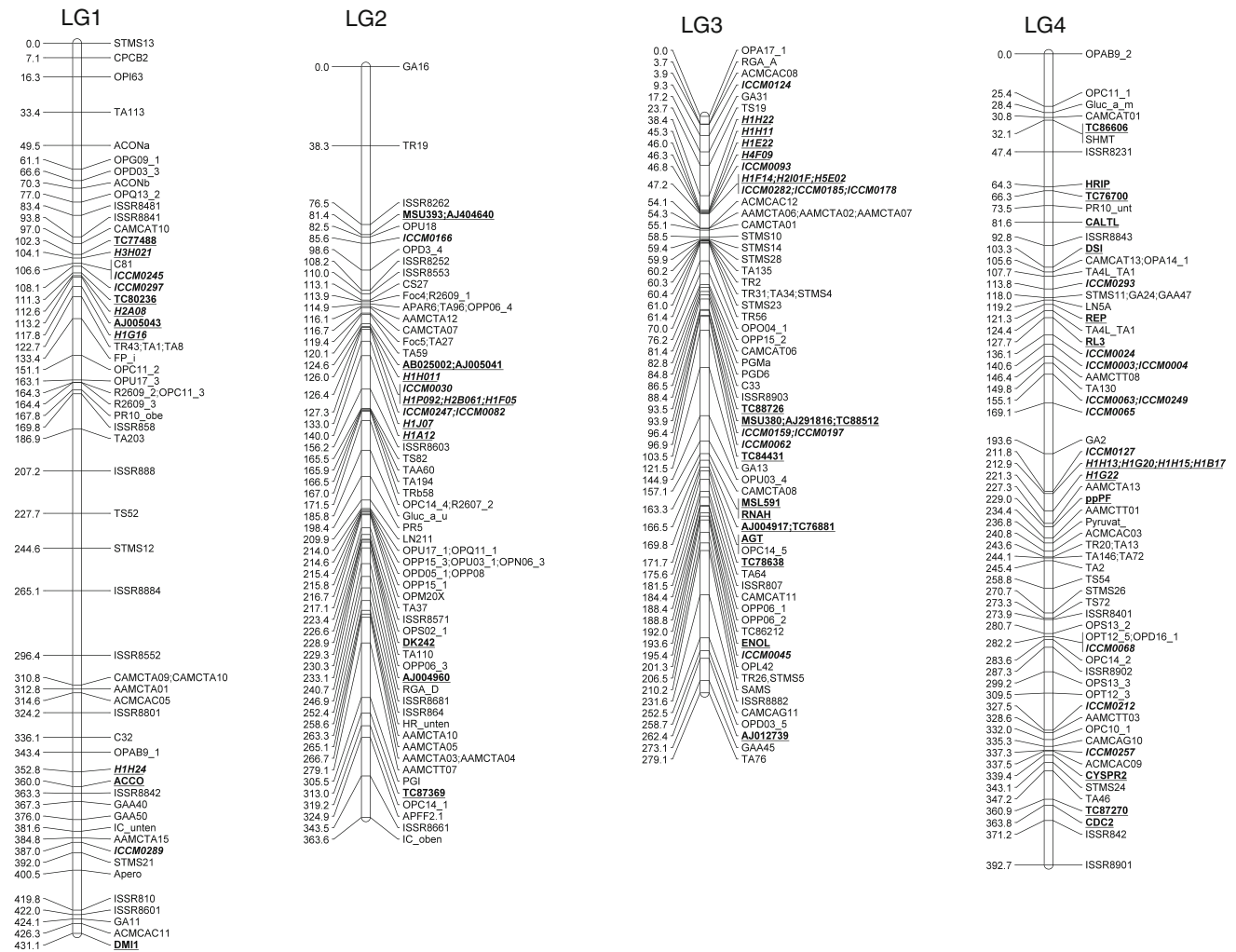


Fig. 4 An integrated genetic map of chickpea based on recombinant inbred lines of *C. arietinum* (ICC 4958) × *C. reticulatum* (PI 489777). Map was constructed using MAPMAKER/EXP 3.0 with Kosambi mapping function. Distances between the loci (in cM) are shown to the left of the linkage group and all the loci are at the right side of the map. Newly developed SSR markers developed from microsatellite-

enriched library (ICCM-series) are **bold and italicized**; SSR markers taken from Lichtenzveig et al. (2005) are **bold, italicized, and underlined**; SNP markers which were used as the anchor markers in comparative mapping of chickpea and *Medicago* were depicted as **bold and underlined**. Linkage groups (LGs) are designated according to the map of Winter et al. (2000)

facilitating comparison of genome structure between *M. truncatula* and chickpea. The respective *M. truncatula* and chickpea LGs are numbered according to Choi et al. (2004a) and Winter et al. (2000). Alignment of conserved genes between the two genetic maps reveals a high level of synteny between the two genomes. In particular, the *M. truncatula* linkage groups 1, 2, 3, 4, 7, and 8 correspond to chickpea linkage groups 4, 1, 5, 6, 3, and 7, respectively. Despite the overall high level of synteny between these six pairs of linkage groups, intra-chromosomal segment rearrangements reduce co-linearity (but not synteny) between *M. truncatula* LG1 (MtLG1) and chickpea LG4 (CaLG4). In contrast to the conserved synteny noted for Mt–Ca linkage group pairs 1–4, 2–1, 3–5, 4–6, 7–3, and 8–7,

one-to-one relationships do not hold true for *M. truncatula* linkage groups 5 and 6 and chickpea linkage groups 2 and 8. In particular, *M. truncatula* LG5 can be aligned with both chickpea LG2 and LG8. We note that CaLG8 appears to be derived entirely from one arm of MtLG5, consistent with its short genetic distance and small number of genetic markers, described above. In several cases, conserved markers mapped to non-syntenic positions between the two genomes (e.g., CDC2 and TCR8727 on MtLG1, DNABP on MtLG4, and TCMO on MtLG5), which may reflect translocation or duplication events involving single genes or small chromosomal segments, or the mapped loci may correspond to paralogous genes. Mt-LG6 could not be effectively aligned to any of the chickpea linkage groups (Fig. 5),

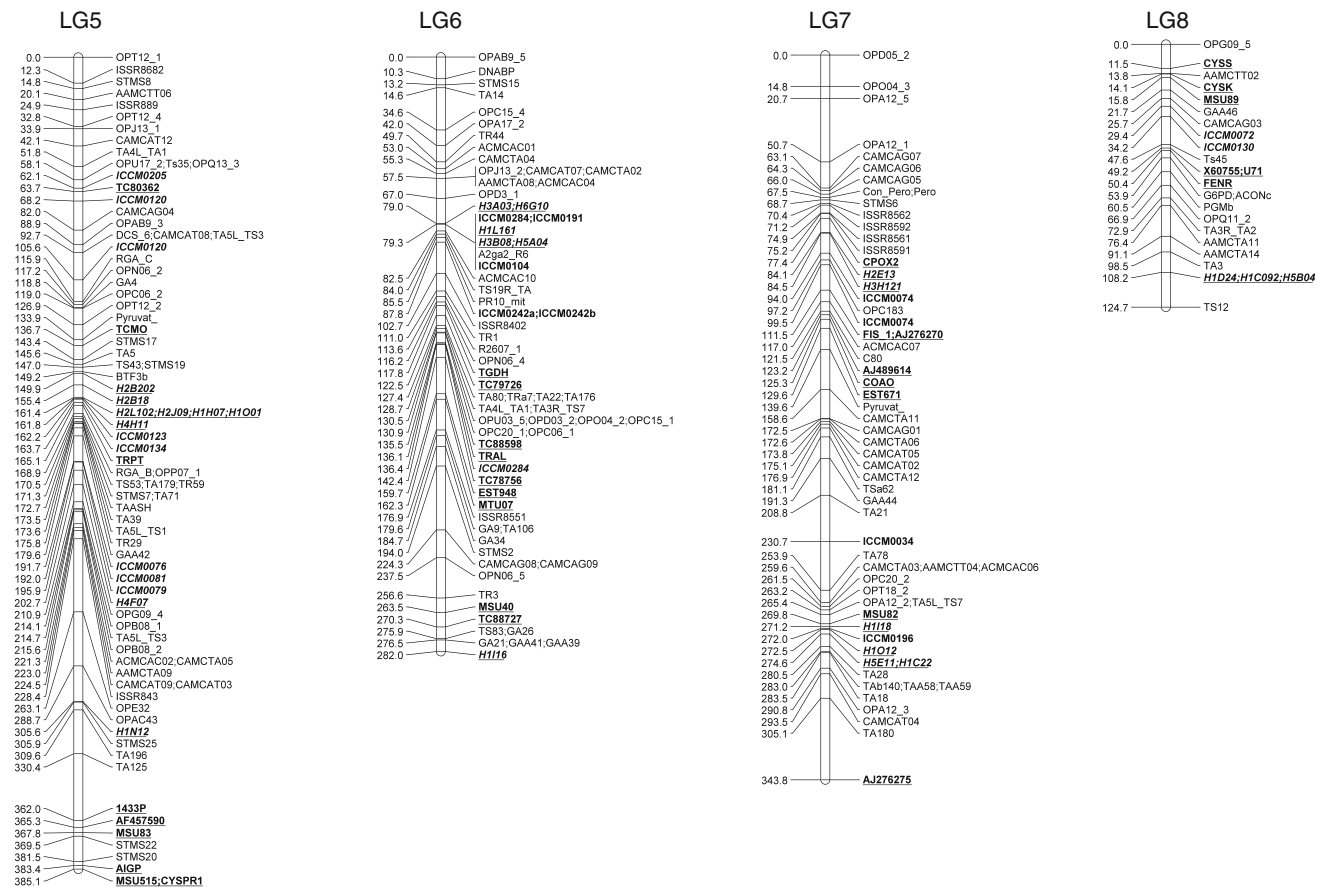


Fig. 4 continued

consistent previous reports describing Mt LG6 as rich in heterochromatin (Kulikova et al. 2001) and having a relatively low content of transcribed genes (Choi et al. 2004a).

Comparison of resistance gene homologs (RGH) between *Medicago* and chickpea

The majority of functionally characterized disease resistance (*R*) genes encode a nucleotide-binding site (NBS) and a leucine-rich repeat (LRR) region (Hulbert et al. 2001). NBS-LRR genes have been deeply surveyed and characterized in *M. truncatula* (Zhu et al. 2002; Ameline-Torregrosa et al. 2008), with >330 NBS-LRR genes having known genetic positions. In contrast, chickpea RGHs are not thoroughly surveyed, and only a limited number of sequences from degenerate PCR are available in the public databases (Meyers et al. 1999; Huettel et al. 2002). Nevertheless, several phylogenetically distinct RGH classes have been placed on the genetic map of chickpea (Huettel et al. 2002), thus facilitating the comparative genome analysis presented here.

Comparative phylogenetic analysis of RGH sequences from *M. truncatula* with those from chickpea is illustrated

in Fig. 6. To highlight the comparison, only those *M. truncatula* sequences that are relevant to the mapped chickpea sequences are shown. In the TIR-NBS-LRR subfamily, chickpea RGH-G (CAC86496 on CaLG6; Huettel et al. 2002) is highly similar to several *M. truncatula* TIR-NBS-LRR genes located on MtLG4, in a region syntenic to *Cicer* LG6 that also contains chickpea RGH-G (Huettel et al. 2002). Similarly, chickpea RGH-B (CAC86491; Huettel et al. 2002) is a CC-NBS-LRR gene that is closely related to several CC-NBS-LRR genes located in a cluster at the top of MtLG3, in a region of the *Medicago* genome syntenic with the terminus of CaLG5 that contains RGH-B (Huettel et al. 2002). A lack of synteny was observed for chickpea RGH-D (TIR-NBS-LRRs represented by sequences CAC86454, CAC86455, CAC86493, AF186626, and AF186629; Huettel et al. 2002), which is located at the top of CaLG2; the closest homologs of RGH-D in *M. truncatula* (i.e., BAC AC144658) are localized to the distal region of MtLG4. We note that the bottom of CaLG2 harbors numerous active resistance genes against two of the most important diseases of chickpea (*Fusarium* wilt and *Ascochyta* blight). At present, no RGHs have been reported mapped close to these resistance phenotypes (Winter et al.

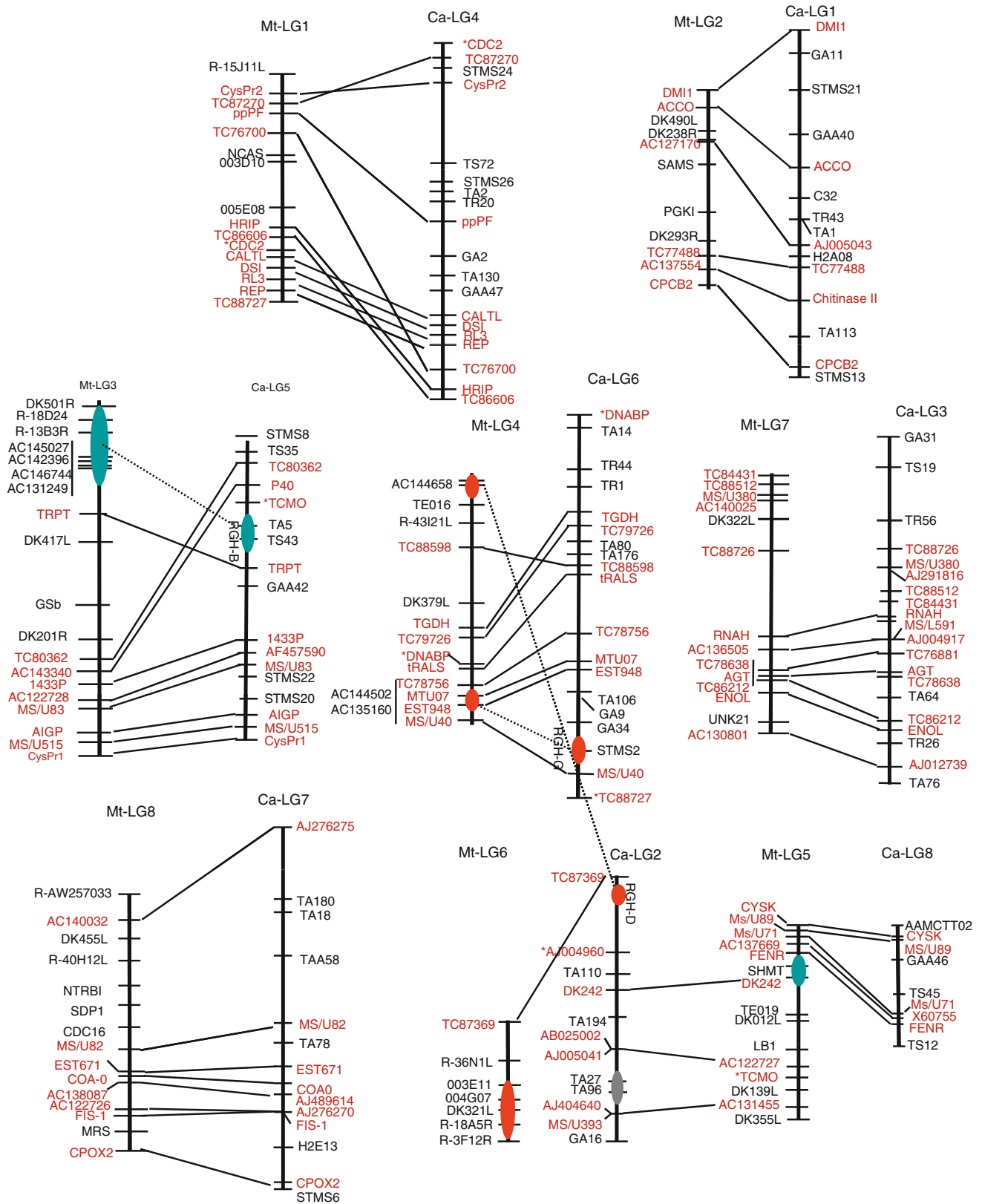


Fig. 5 Comparative map of *Medicago* and chickpea. Gene-based SNP markers (marked in red color) were used as the anchor markers in comparative analysis of chickpea and *Medicago* genome. The resistance gene homologs (RGH) are depicted as oval structures and their homo-

logs in *Medicago* are shown with connecting dotted lines. Solid lines show the macro-synteny observed across chickpea and *Medicago* with respect to 71 gene-based markers

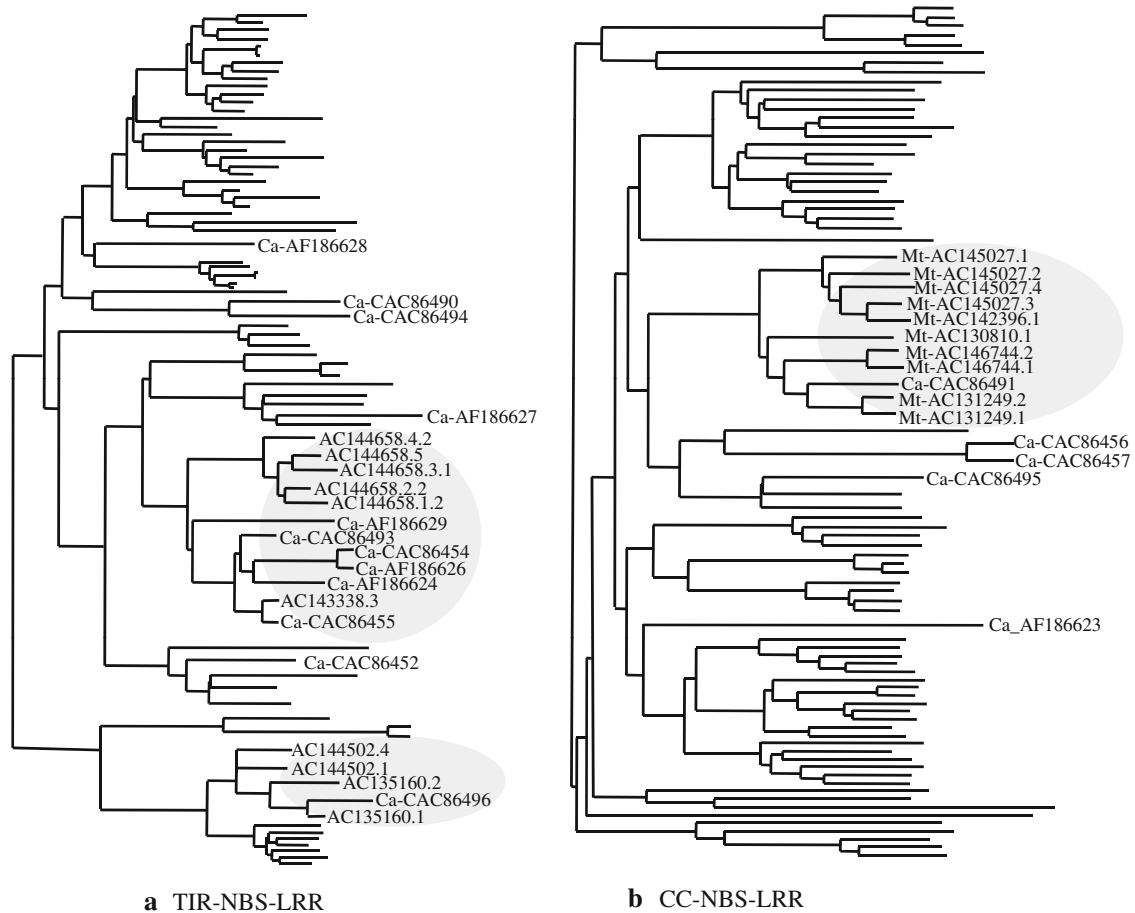


Fig. 6 Comparison of RGH sequences in *Medicago* and chickpea. To highlight the comparison between the chickpea and *Medicago* RGHs, only those *Medicago* sequences that are relevant to the mapped chickpea sequences have been shown in this figure. In the TIR-NBS-LRR subfamily, chickpea RGH-G (CAC86496 on Ca-LG6) was found highly similar to several *Medicago* TIR-NBS-LRR genes (**a**) located on BAC

clones AC144502 and AC135160. AC144502 and AC135160 were closely linked on Mt-LG4, in a region syntenic to Ca-LG6 that also contained chickpea RGH-G. In the CC-NBS-LRR (**b**) subfamily (Ca-LG5), chickpea RGH-B (CAC86491) was closely related to several CC-NBS-LRR genes located on *Medicago* BAC clones AC145027, AC142396, AC130810, AC146744, and AC131249

2000; Pfaff and Kahl 2003; Sharma et al. 2004). Moreover, the low frequency of comparative molecular markers around these *R* gene regions in both *M. truncatula* and chickpea complicate precise statements regarding the relationship of these genome regions.

Discussion

SSR markers have become common place for plant genetics and breeding applications. Despite the fact that hundreds of SSR markers have been identified and tested in chickpea (Hüttel et al. 1999; Sethy et al. 2006a, 2006b; Winter et al. 1999; Lichtenzveig et al. 2005), the narrow genetic background of cultivated chickpea germplasm has limited their application, and thus there exists a need to develop a larger set of novel genetic markers. With the objective of enriching the marker repertoire of chickpea, we

have contributed novel SSR markers derived from a genomic library enriched for GA and TAA repeat motifs and a set of gene-based SNP markers. The basis of our marker discovery work was *C. arietinum* genotype ICC 4958, which is being used as a reference genotype for genomic and genetic resource by the chickpea community.

In the present study, 65.4% of hybridizing genomic clones in our SSR-enriched library yielded 643 SSRs. This rate of SSR recovery is comparable with previous studies, for example in peanut where 68% of hybridizing clones yielded SSRs (Cuc et al. 2008). Moreover, the relatively high abundance of tri- and di-nucleotide repeats that we observed is consistent with previous studies in chickpea (Hüttel et al. 1999; Lichtenzveig et al. 2005; Winter et al. 1999). Among the SSRs identified here, the most common SSR motifs were TAA/ATT repeats and GA/CT repeats. This result reflects the fact that our enrichment targeted TAA and GA motifs, and it is consistent with previous

studies in chickpea (Hüttel et al. 1999; Lichtenzveig et al. 2005; Winter et al. 1999), other legume species (Akkaya et al. 1992; Cregan et al. 1994; Mun et al. 2006), and even in cereal species (Varshney et al. 2002; Jayashree et al. 2006).

Temnykh et al. (2001) developed a scheme to classify SSRs according to length, in which Class I and Class II SSRs are greater than or less than 20 bp, respectively. This division based on sequence length has practical utility, because Class I SSRs are generally more polymorphic and thus more desirable as genetic markers. The majority of SSRs isolated from our SSR-enriched library belong to Class II, though as expected the Class I SSRs had higher rates of polymorphism. A useful measure of polymorphic potential for any genetic marker is its polymorphism information content value, or PIC value. PIC values provide information on the probability that a given marker will be polymorphic between any two individuals in a population, and thus are a function both of allele frequencies and allele number. Screening of the ICCM-series markers on 48 genotypes revealed that average PIC value of SSR markers having Class I repeats (0.38) was higher than that of Class II repeats (0.22). The majority of the Class I repeats were tri-nucleotide repeats, consistent with the known utility of tri-nucleotide repeats as genetic markers in plants (Varshney et al. 2005).

Polymorphic information content value was also analyzed in relation to repeat unit type and length. Among di-, tri-, and tetra-nucleotide repeats, tri-nucleotide repeats showed higher polymorphism (average PIC = 0.33) with average allele number of 5.7 per marker. Markers with mono-nucleotide repeats showed the least polymorphism (average PIC = 0.197). Relatively longer repeats appear to have contributed to the higher level of polymorphism as compared to di-nucleotide repeats (Gupta and Varshney 2000). It was also observed that among tri-nucleotide SSRs, the SSR markers based on (TAA/TTA) repeat motifs displayed higher polymorphism (average PIC = 0.35) with an average allele number of 6.12 per marker. Similarly, among di-nucleotide repeats SSR markers based on TA/AT repeat motifs had a higher average PIC value (0.27) compared to others with an average of 6.1 alleles. In fact, the earlier studies in chickpea also revealed the abundance of TAA/TTA (tri-nucleotide) and TA/GA (di-nucleotide) SSR motifs and the extensive polymorphism found with markers containing these repeat motifs (Hüttel et al. 1999; Lichtenzveig et al. 2005). PIC values of compound SSRs (average PIC = 0.29) were comparable with tri-nucleotide repeats with 5.68 alleles per marker. This can be attributed to the fact that the markers with compound SSRs have more than one SSR motif, which increases their chances to be polymorphic markers.

We assessed the potential identity of SSR-related sequences by performing BLAST analyses versus plant EST data sets, and based on GeneOntology analysis

through UniProt. Less than one-third of the SSR-associated GSS sequences had significant hits in these databases, though where hits were recorded the derived annotations add a potentially useful data type to the marker metadata. Not surprisingly, chickpea GSS sequences (from which the SSRs were derived) had higher similarity to ESTs from other legume species, and overall higher similarity to dicot outgroups (i.e., poplar and *Arabidopsis*) than to monocot (i.e., rice) data sets.

Comprehensive genetic map of chickpea

An inter-specific mapping population derived from ICC 4958 (*C. arietinum*) and PI 489777 (*C. reticulatum*) was used to incorporate novel microsatellite and gene based markers. This mapping population has been widely used in past by chickpea community in order to incorporate several hundred microsatellite markers (Winter et al. 2000) and gene-based markers (Pfaff and Kahl 2003). The diverse genetic background of the parents provides for higher rates of polymorphism not only at the genetic level but also at phenotypic levels such as resistance to *Fusarium* wilt (Winter et al. 2000) and *Ascochyta* blight (Rakshit et al. 2003), facilitating trait mapping. Therefore, this population is generally considered as the international reference mapping population.

The present genetic map of chickpea represents 521 marker loci, spanning 2,602 cM with an average inter-marker distance of 4.99 cM. The order of common marker loci defined in present map agrees with earlier reports from Winter et al. (2000). However, the current map differs considerably from that of Winter et al. (2000) in having eight linkage groups, in agreement with eight chromosomes, whereas the Winter et al. (2000) map was composed of 16 linkage groups. There are probably at least two factors that contribute to this condensation of linkage groups: first, the new markers identified in the present study act as bridge points between the Winter et al. linkage groups, and second, essentially all of the markers mapped in the current study behave a co-dominant genetic features, which adds considerable power to the genetic evaluation compared to a high fraction of dominant markers in earlier studies. Importantly, the comparative analyses to *Medicago* support a simple assignment of eight chickpea linkage groups to eight chromosomes.

Comparative mapping of chickpea and *Medicago*

Mappig of the gene-based markers from *Medicago* in the genetic map of chickpea showed not only a high level of macrosynteny but also revealed features of structural divergence between the two genomes. Six of the eight linkage groups display a one-to-one correspondence between the

Medicago and chickpea, suggesting that these linkage groups reflect the genome of the common Galegoid clade legume ancestor. *Medicago* LG5 and LG6, and chickpea LG2 and LG4, appear to have a more complicated ancestry, consisting of a minimum of several chromosomal translocation events. Thus, Mt-LG 5 is essentially a composite of portions of LG2 and LG8 of chickpea. Several research groups have compared genome structure between *Medicago* and various crop legumes (see Zhu et al. 2005). Our current results extend the comparative network to include chickpea, by demonstrating broad conservation of genome macrostructure between chickpea and *Medicago*.

One goal of comparative genetic analyses is to transfer information from well-characterized reference species to less well-characterized crops with an eye toward crop improvement. Among the agronomic targets in chickpea is resistance to several economically important pathogens; candidate genes for disease resistance are the conserved family of NBS-LRR resistance gene homologs (RGH). Several phylogenetically distinct RGH classes have been placed on the genetic map of chickpea (Huettel et al. 2002), thus facilitating the comparative genome analysis between chickpea and *Medicago*. In particular, we have documented two cases of syntenic NBS-LRR clusters that contain co-phyletic genes in each species. Interestingly, Ca-LG2 is known to harbor active resistance genes against *Fusarium* wilt and *Ascochyta* blight. At present, no RGHs have been reported mapped close to these resistance phenotypes. Nevertheless, the facts that a single conserved gene (TC87369) maps to the top terminal region of both Mt-LG6 and Ca-LG2, and that both linkage groups are rich in NBS-LRR genes and/or active disease resistance genes (Sharma et al. 2004; Zhu et al. 2002), may suggest shared ancestry of Mt-LG6 and Ca-LG2, though such speculation needs to be verified by more detailed study of the respective genome regions.

Similar observations of NBS-LRR synteny have been made for resistance gene homologs within the Solanaceae (Grube et al. 2000) and between *Medicago* and pea (*Pisum sativum*) (Zhu et al. 2002). However, the limited numbers of comparative molecular markers (gene-based SNPs) around these *R* gene regions in both *Medicago* and chickpea precludes precise statements regarding the relationship of these genome regions. Although the current analysis is based on a relatively small number of comparative markers, the potential of more detailed analyses to predict gene content and chromosomal structure in chickpea by reference to *Medicago* seems clear.

Conclusion

A set of 311 novel microsatellite markers were developed from microsatellite-enriched library in order to increase the

genomic resources in chickpea. In total 147 potential SSR marker loci were found based on diversity pattern of SSR loci on a panel of 48 diverse chickpea genotypes. These markers should have utility for genetic analysis of a range of chickpea mapping populations and as anchor markers in comparative mapping to other legumes.

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