Theor Appl Genet (2010) 120:1415–1441
DOI 10.1007/s00122-010-1265-1

ORIGINAL PAPER

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

Spurthi N. Nayak · Hongyan Zhu · Nicy Varghese · Subhojit Datta · Hong-Kyu Choi · Ralf Horres · Ruth Jüngling · Jagbir Singh · P. B. Kavi Kishor · S. Sivaramakrishnan · Dave A. Hoisington · Günter Kahl · Peter Winter · Douglas R. Cook · Rajeev K. Varshney

Received: 3 September 2009 / Accepted: 27 December 2009 / Published online: 23 January 2010

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

Communicated by H. T. Nguyen.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-010-1265-1) contains supplementary material, which is available to authorized users.

S. N. Nayak · N. Varghese · J. Singh · D. A. Hoisington · R. K. Varshney (✉)
Centre of Excellence in Genomics (CEG), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502324, Andhra Pradesh, India
e-mail: r.k.varshney@cgiar.org

S. N. Nayak · P. B. Kavi Kishor
Department of Genetics, Osmania University, Hyderabad 500007, Andhra Pradesh, India

H. Zhu · S. Datta · H.-K. Choi · D. R. Cook
Department of Plant Pathology, University of California, Davis, CA 95616, USA

H. Zhu
Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546, USA

S. Datta
Indian Institute of Pulses Research, Kanpur 208024, Uttar Pradesh, India

H.-K. Choi
Department of Genetic Engineering, Dong-A University, Busan 604-714, South Korea

R. Horres · R. Jüngling · G. Kahl
University of Frankfurt, Max von Laue Str. 9, 60439 Frankfurt am Main, Germany

J. Singh · S. Sivaramakrishnan
Department of Agricultural Biotechnology, Acharya N.G. Ranga Agricultural University (ANGRAU), Hyderabad 500030, Andhra Pradesh, India

G. Kahl · P. Winter
GenXPro GmbH, Frankfurter Innovationszentrum Biotechnologie (FIZ), Altenhöferallee 3, 60438 Frankfurt am Main, Germany

R. K. Varshney
Genomics Towards Gene Discovery Subprogramme, Generation Challenge Programme (GCP), CIMMYT, Int APDO Postal 6-641, 06600 Mexico DF, Mexico

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; Lichtenzveig 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.

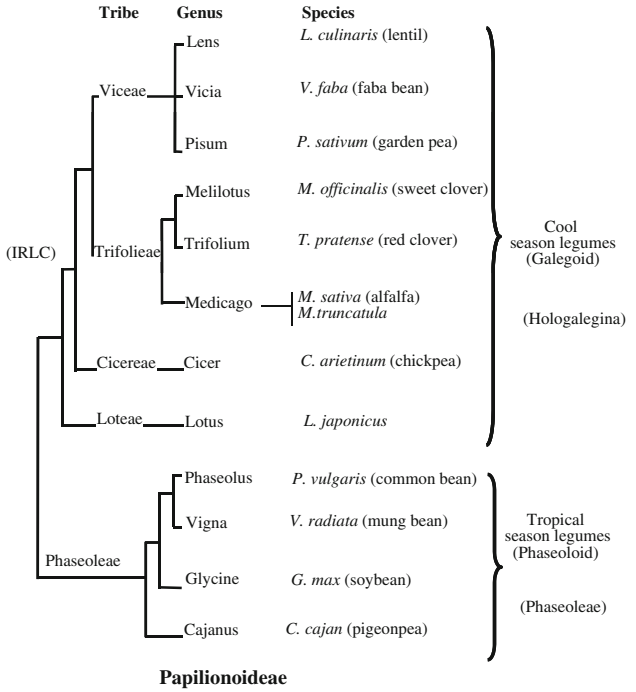


Fig. 1 Phylogenetic relationships: Papilionoideae family. This figure (taken from Choi et al. 2004b) illustrates the phylogenetic relation of chickpea with other legumes. Chickpea and *Medicago* belong to inverted repeat lacking clade (IRLC) of Papilionoideae and constituted the cool season legumes

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 Lichtenzveig 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-fractioned 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	*C. arietinum*	India	Kabuli
2	ICC 10673	*C. arietinum*	Turkey	Desi
3	ICC 11944	*C. arietinum*	Nepal	Desi
4	ICC 12299	*C. arietinum*	Nepal	Desi
5	ICC 12379	*C. arietinum*	Iran	Desi
6	ICC 1431	*C. arietinum*	India	Desi
7	ICC 17116	*C. yamashitae*	Afghanistan	Wild
8	ICC 17122	*C. bijugum*	Turkey	Wild
9	ICC 17123	*C. reticulatum*	Turkey	Wild
10	ICC 17148	*C. judaicum*	Lebanon	Wild
11	ICC 17152	*C. pinnatifidum*	Turkey	Wild
12	ICC 17160	*C. reticulatum*	Turkey	Wild
13	ICC 17162	*C. cuneatum*	Ethiopia	Wild
14	ICC 17248	*C. microphyllum*	Pakistan	Wild
15	ICC 1882	*C. arietinum*	India	Desi
16	ICC 2679	*C. arietinum*	Iran	Desi
17	ICC 283	*C. arietinum*	India	Desi
18	ICC 3137	*C. arietinum*	Iran	Desi
19	ICC 3239	*C. arietinum*	Iran	Desi
20	ICC 3696	*C. arietinum*	Iran	Desi
21	ICC 3986	*C. arietinum*	Iran	Desi
22	ICC 4853	*C. arietinum*	Unknown	Kabuli
23	ICC 4958	*C. arietinum*	India	Desi
24	ICC 5002	*C. arietinum*	India	Desi
25	ICC 506 EB	*C. arietinum*	India	Desi
26	ICC 5337	*C. arietinum*	India	Kabuli
27	ICC 6263	*C. arietinum*	Russia and CISs	Kabuli
28	ICC 7052	*C. arietinum*	Iran	Desi
29	ICC 7413	*C. arietinum*	India	Pea
30	ICC 8200	*C. arietinum*	Iran	Desi
31	ICC 8261	*C. arietinum*	Turkey	Kabuli
32	ICC 9644	*C. arietinum*	Afghanistan	Desi
33	ICC V95311	*C. arietinum*	India	Kabuli
34	JG11	*C. arietinum*	India	Desi
35	JG62	*C. arietinum*	India	Desi
36	VIJAY	*C. arietinum*	India	Desi
37	IG 72933	*C. reticulatum*	Turkey	Wild
38	IG 72953	*C. reticulatum*	Turkey	Wild
39	PI 489777	*C. reticulatum*	Turkey	Wild
40	IG 10419	*C. arietinum*	Syria	Kabuli
41	IG 6044	*C. arietinum*	Sudan	Kabuli
42	IG 6899	*C. arietinum*	Iran	Kabuli
43	IG 7148	*C. arietinum*	Algeria	Kabuli
44	IG 72971	*C. reticulatum*	Turkey	Wild
45	IG 73064	*C. echinospermum*	Turkey	Wild
46	IG 73074	*C. echinospermum*	Turkey	Wild
47	IG 73082	*C. reticulatum*	Turkey	Wild
48	IG 7767	*C. arietinum*	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 ICRISAT.

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 ACGTTGTAAAACGAC). 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	FI856656	(AT)5	GAACTTGCTTGGGGACAGG	GAGGTGAGCTGAAGTGAGGC	246	2	0.05
ICCM0001b	FI856656	(CT)4tc(CT)4	TGCACAACGGCTATGTCTTC	GAGGTGAGCTGAAGTGAGGC	118	9	0.71
ICCM0002	FI856654	(TC)6	TCGTTCTCCGTTATGTGTGC	ATGCCTCCAATAGCATACGG	262	2	0.04
ICCM0003	FI856538	(CT)6	AATGGAAGAACGTCAGGGTG	TTCCACTGGGGCAAAATAAG	252	7	0.59
ICCM0004	FI856539	(AT)4	CACTCACACACGCACTTTCA	AAAAGAGAAGCCCACCAAAA	213	4	0.39
ICCM0005a	FI856657	(TC)4N(CT)4N(TC)4	TGCTGAGCGATCTGAGGATA	GCAGCAAAAATCAGCACAAA	277	NA	
ICCM0005b	FI856657	(AT)4	TTGGTGCAGATTTTTGTTGC	AGATTTGGGGGATAAAAGGG	241	1	0.00
ICCM0007a	FI856661	(CTC)4N(TC)4N(TC)4	TCTACATTCTCTCCGTGCCC	GAGGAGTGTAGGGGAGAGGG	242	NA	
ICCM0007b	FI856661	(TCCTC)4	CTCTCCCCACTCTCCCTTTC	TGAGTAGGATCGTAGTAGGGGG	202	NA	
ICCM0008a	FI856540	(A)10N(A)10	CTCATGGTGGCATTGAGAAA	TCCTTGAATTTTTGAGACACGA	230	2	0.05
ICCM0008b	FI856540	(GA)4N(AG)5t(GA)4	TCGTGTCTCAAAAATTCAAGGA	CACTCGACCACCACTGCTAA	235	2	0.32
ICCM0008c	FI856540	(CT)4	GGTGGTTTGTGGAGGTTGAT	AGGCAACCATTCATCCTTGT	245	2	0.05
ICCM0009a	FI856541	(GA)4	CACTTCAAAAGAGTGTTTGATTTGA	GGTTTGAAAGATGAGTGGTTTTG	189	3	0.10
ICCM0009b	FI856541	(A)11	AAAATATGGAAAGTCGGGCA	TGCATTTTCTTAGCGGTTTTT	257	NA	
ICCM0010a	FI856663	(CT)4	GACGGAAATACGGCTGGTTA	GCTGCAATATACTCCGCCTC	113	1	0.00
ICCM0010b	FI856662	(AT)4	ACGCCAATTCTTTTGAGCAC	TCAGCACTGGTGGAACCATA	142	12	0.80
ICCM0014a	FI856542	(GA)5	CGTGGTTGTGTTGTTTGAGG	TGTGTTCATCTCCCTCTCCC	134	1	0.00
ICCM0014b	FI856542	(TA)5	TTTGGGAACCTTTCTCATCAA	CCAAATCATTTTTGTGCACTG	254	3	0.16
ICCM0019a	FI856544	(AG)18	TTCCGGTTGTTGAGTAGAGAAA	ACTTCCACTTGTTCCATCCG	189	NA	
ICCM0019b	FI856544	(AG)4	CGGATGGAACAAGTGGAAGT	TCTCGTACCGGACCAGAGTC	153	6	0.62
ICCM0021a	FI856697	(CA)4N(AT)4N(ATT)5	AAACCGCATTGAGGAATGAC	TTTGCCACGATATGTTCAGG	232	NA	
ICCM0021b	FI856696	(AT)4	TCTTTCTAAGCAGTCTAGGATACGA	AGGAAGGGTGGGATAATTGG	229	NA	
ICCM0022	FI856699	(AT)4	TAAACCGCATTGACGAATGA	TGAATTCGCAAGAATCAAATG	123	18	0.89
ICCM0024	FI856545	(AT)4	CACTCACACACGCACTTTCA	AAAAGAGAAGCCCACCAAAAA	214	3	0.08
ICCM0026	FI856714	(AG)4	CCGGACATTGTTCTGAAGGT	TCTGTGCAGTGGAGCTATGG	216	2	0.05
ICCM0029	FI856721	(AG)4N(GA)4N(GA)4a(AG)5	CTTCCCCTTTCCAAAATTGA	TCGTTCACAGGTTTTCCCTC	244	1	0.00
ICCM0030a	FI856722	(TC)4N(CT)4N(TC)4	GGCAACGTACGGGATAAATG	GCAGCAAAAATCAGCACAAA	271	1	0.00
ICCM0030b	FI856722	(T)10	TTGCTGCTGATTTTTGTTGC	TCATCCATCATTCTAAATGTGTCA	257	3	0.09
ICCM0032	FI856546	(GA)4	TCTCTTGACACAAGTCTGCACAT	CCCACTCACATGTAGCGAAA	232	1	0.00
ICCM0034	FI856651	(GA)11	TTTGTTTGCGGAGGAATAGG	TCACCTCACCACACTTCTTTTC	259	6	0.33
ICCM0035	FI856547	(GT)4	TGAGGGTAAATAAATTGGTGGC	ATGATTTCCGAGGACAGTGG	101	1	0.00
ICCM0037	FI856548	(AG)4N(GA)4N(AG)4N(GA)5	CAAGCAATGGGAGACACCTT	CCACCACTTCAACGTCTCCT	212	NA	
ICCM0042	FI856740	(CA)4	TTCGTGATATTCATCAAGGTGG	TTTGACGTACTCTGTGTTTTGTTT	259	3	0.08
ICCM0043	FI856552	(GA)6	AAGATTGGATTCCACAACGC	ACAACCCACCCACACAAAC	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	FI856553	(TA)4N(TC)12	TGAATCCCTCAATCCTGTGG	CTTATCTCCCCAGTTGCCAG	272	5	0.31
ICCM0052	FI856768	(TAT)6	CGTCGTGTCCATCTGTCTTG	CCAGGTGCAATAGGGAAATC	275	2	0.04
ICCM0053	FI856771	(GA)32t(AG)4	TGCGGTCGACTCTAGAGGAT	CTGAGAAGGAAGCCAACAGG	173	1	0.00
ICCM0059a	FI856779	(GAA)4	CGGTCGATCTGAGGATCACTA	GGGTTCAACTGTTCAGGTTTG	226	NA	
ICCM0059b	FI856779	(AG)4N(GA)9N(GA)4	CAAACCTGAACAGTTGAACCC	AGGTTCTCCCTCGCTCTCTC	172	3	0.23
ICCM0060	FI856780	(AT)4N(CT)4	TGATGAACATGCTAACAAACTAACAA	CCAAGACAACTGGTGGAGGT	260	2	0.04
ICCM0061	FI856798	(AT)6N(TTA)4(TAA)4N(TTA)13	ATGCGGTAATCCTTGACTGG	TGAAATTGGGTTGGAAGTTTG	275	5	0.23
ICCM0062	FI856801	(AAT)4N(AAT)6	ATGCAACGCCTAAGTCTCGT	TAGGTACGTTTGGGGTCCTG	266	3	0.09
ICCM0063	FI856802	(TTA)6	TTGATTTATCCTGTGATGCTTTTT	GGCAGTCTGGTCATGTGAAA	194	2	0.04
ICCM0065a	FI856807	(TC)6	CATTCCCGCAAAGCTTGTAT	GGTGCCTTGGAGAAAATCAA	137	NA	
ICCM0065b	FI856807	(TC)4	CACGGTGTCTTCCACATGAC	TAAGCCAATACCAAGAGGCG	195	2	0.08
ICCM0066	FI856555	(TC)4	ACCATGCTCACCAACTCACA	TCTCTTGACACAAGTCTGCACAT	242	1	0.00
ICCM0067	FI856556	(TC)4	AACTGCAACCTCTCTCGCTC	TCTCTTGACACAAGTCTGCACAT	204	1	0.00
ICCM0068	FI856557	(ATT)22	TCTTCTTTGCTATCTGTCTCGC	TGCATGTCAAACATTAGACAACTTT	227	14	0.87
ICCM0069	FI856558	(ATT)22	TCTTCTTTGCTATCTGTCTCGC	TGCATGTCAAACATTAGACAACTTT	227	13	0.82
ICCM0072	FI856813	(GAA)4	CAAGACCTTGAACAGGAGGC	TCTTTCAATTTTCTAACAATTTCATC	200	2	0.14
ICCM0073a	FI856828	(TA)4N(A)11N(TA)4	TCGATCTGAGGATCTTTGGTG	TGGATACTATTAACGAAAAACTAGCG	219	6	0.23
ICCM0073b	FI856828	(TC)4	CTTGTCCACCCCACATTCTT	TAGAGAAATGGGGGAAGGGT	217	1	0.00
ICCM0074a	FI856830	(A)11N(TC)6	AAATCCCAAATTTAGAGCGG	AACCTTTGAAAAAGGCGGTT	183	9	0.40
ICCM0074b	FI856830	(T)15	AACCGCCTTTTTCAAAGGTT	CCTTCCAGGGGAAAAAGAAA	264	NA	
ICCM0075	FI856559	(AG)16	CTTTGTAACAATAAAATGCAAAGTAAA	GGAAGCACAGTCTGCACAAA	163	4	0.26
ICCM0076	FI856560	(ATA)17N(TAA)5	CTCATCGAATAGAACCTACCGA	CCGCTACACCTACAACGGTAA	270	3	0.08
ICCM0077a	FI856561	(AG)4N(T)10	CCACAAGAAGACAAAGGGGA	AAAAAGATGCTAAAACTAAACCAAAGA	217	1	0.00
ICCM0077b	FI856561	(A)10	GCCGAGAAAATAAATTTCACCA	GCCGCGACCATTAATTCTAA	124	2	0.04
ICCM0078a	FI856832	(TAT)4 g(ATT)6ag(TAT)5N(AT)4	CTGAGGACGTTGGGAATACG	AAAAACTAATCTCGTGTTCAAATCC	280	3	0.22
ICCM0078b	FI856832	(TC)4	AATCCCAACGGTGAGAGATG	GGACAAGGAGTGGAAGGGA	279	15	0.62
ICCM0079	FI856562	(TTA)6	GAGCTAACGCCTTCGCTAGA	GAGAGGGATTAAAACAAATAGAGGAA	170	3	0.08
ICCM0080	FI856563	(T)10	CTGCGGTGACTCTGAGGAT	GAGAATCACGGGTGTTTCAAG	173	3	0.15
ICCM0081	FI856564	(TAA)6	GAGAGGGATTAAAACAAATAGAGGAA	GAGCTAACGCCTTCGCTAGA	170	3	0.08
ICCM0082	FI856833	(CT)19N(TC)4	TCACGATCTCACAGAGCCAC	TCCGTGATTCTGAGCAACAG	260	3	0.08
ICCM0083a	FI856835	(AT)4N(CT)7	CGCTCACACCATCTCACTTC	GAATGGAGGAAATACAGAGTGC	273	NA	
ICCM0083b	FI856837	(A)13	ATTGAAAAACCACGCACACA	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	FI856837	(T)11	TGTTTGTTGCCTAGCCACTG	TTGGACAGATTTTTGTTGTTTGTT	195	1	0.00
ICCM0084	FI856993	(GA)5	TTTTGATTGAGCATGCAATGT	GAACCTTTTGAGGTCTGTTGC	246	2	0.04
ICCM0085	FI856855	(T)10N(TAT)14	GTGGTCCATCTGTCTCGGTT	GGAAAAGGAGAAAGTTGTGGG	210	NA	
ICCM0086a	FI856856	(TA)4N(T)11	TGTGCATTGAGCCTATTGGT	GACAACAGCGGCATAATCAA	182	NA	
ICCM0086b	FI856856	(AC)4	CTTCCCCTTTACCCCGTTTA	AAAGAAATCGACAATAAAAGAGTGA	175	NA	
ICCM0088	FI856861	(T)10N(AT)5	AAAGGAAGGGAAGGAAATGC	GAGTTTGGGCAGGCAATAAA	240	2	0.19
ICCM0089a	FI856863	(A)12	AACACCGACTTTCCAAAACG	TTTGGGAAATACAACCTTTGAA	204	9	0.45
ICCM0089b	FI856862	(TAT)20	GGGATATCGCCAATATATTTTATACC	TTTGGCAACAAATCCTTTGA	126	NA	
ICCM0090a	FI856864	(TTA)6	ACGGGACTTGGATGACTTTC	AGACGCGTGCTTTCTTCCTA	257	NA	
ICCM0090b	FI856864	(TC)5	CTGCCTAGGAAGAAAGCACG	AAAATAAATGCGCCGTATGC	160	3	0.39
ICCM0093a	FI856871	(TAT)20	CTTCTGTTATTATCGCCGCC	AGCATCATGGAGCAGAGAGG	223	NA	
ICCM0093b	FI856871	(AT)4	TACCCTTTCTCTCCCCACCT	TCAGTAGTCGGGCAATAGATGA	129	NA	
ICCM0093c	FI856870	(AAAT)4	CCACTTTTAGGCGCACTTCT	CGACTCATTTTTCACGGACA	197	3	0.24
ICCM0094	FI856872	(AT)4	AGAGGCAAACAAGAACCGAA	AAGGGTTAGTGGAGGAATTATGAA	279	3	0.08
ICCM0095a	FI856875	(TC)4	CTCTCCATCCCATCCGACTA	GGAAGCCATATCCAGAGGGT	181	NA	
ICCM0095b	FI856874	(ATTC)4	CGGGACATTTCCGTTAAAAA	CGAGTCGTTTTCTTGGCTTC	171	1	0.00
ICCM0096	FI856877	(AT)4N(CT)4N(TC)4	ACACCCCACCCTAATTACACT	GAGAGGTACGAAGCACGAGG	170	9	0.47
ICCM0097a	FI856669	(ATT)12N(TA)4	TGAGGACTGCCATACTCCAG	TCCCCTTTATGAGGGCTTTT	276	3	0.09
ICCM0097b	FI856668	(CTT)4	TCCAATTCCAAAACACACCA	CCTGAGGAGTAAAAGACGGG	130	1	0.00
ICCM0101a	FI856675	(A)13	TAACTGAGTTTCGGGTTCCG	CTTAACGGACGTTGTAGGGC	247	NA	
ICCM0101b	FI856674	(AG)4	GAAGACAAAAAGGGGCACAA	CCGATTGTTTCAAGACCAGA	105	2	0.04
ICCM0102	FI856676	(T)12	CACCAAAAAGGGAACTTTCG	AAAAATAGGGTGGGGAGGG	167	1	0.00
ICCM0103	FI856678	(A)11	ATGGGGGAATCGGAGACTAA	GGATAGGGAGGAGGGAACAG	110	3	0.18
ICCM0104	FI856566	(TTA)11	CCAAACCTCCAAAAATCTGC	TCATTTTTGATTCATTCTTGGG	278	8	0.48
ICCM0105	FI856567	(AT)4	TGCTTCCTTTTCAATCACCA	TGACAAAGGACAAATAAGTGTTTTA	280	1	0.00
ICCM0106	FI856681	(ATA)7	TGGAATTGCTACCGAATATGG	ACGATCGGAGAGAACGAGAA	267	NA	
ICCM0107a	FI856682	(TCG)4	GCAAAAAGTGTTGCTTCCGT	AAGCACATTGCCACTAGCAT	234	1	0.00
ICCM0107b	FI856682	(TA)4	GCAAAAAGTGTTGCTTCCGT	AAGCACATTGCCACTAGCAT	234	3	0.16
ICCM0110	FI856568	(AT)4N(TTA)7	AGAGGCAAACAAGAACCGAA	GTAAAGAGGGGCAGCTGTTG	183	NA	
ICCM0115	FI856706	(TC)4	ACCCTAAGGGCTCGTTTGAT	TAGGGATGGAGAGGAGAGCA	245	1	0.00
ICCM0116	FI856709	(T)11	TTTTGGTGCAAGAGAATGGG	GTCTTTCAAGAGGTCGCAGC	177	1	0.00
ICCM0117	FI856572	(TAA)4	AGTGACCAGGAAACACGGTC	GCAGAGATTGAATTTTGCCA	254	1	0.00
ICCM0118	FI856573	(T)11	AATTGGGAAGGAAAGCGAGT	TCGCCATTGCAATAATCAAA	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	FI856710	(GA)4	TTATTGGATGAGTGGATGCG	ATTACGTAACCCAACGTCGC	192	NA	
ICCM0120a	FI856574	(TTA)12	TGTCTCGATAAGAGTTTGTTATTTTTC	CGTTTTGTTTCATATTCAAACTCG	220	12	0.75
ICCM0120b	FI856574	(ATT)13	CGAGTTTGAATATGAAACAAAACG	GCTTGTAGCTAGGCTCGACTC	166	5	0.29
ICCM0121a	FI856575	(TATT)4	CAAAAATTTGGATTCGGGAG	CTATTGCACCTGGGGATACG	238	2	0.09
ICCM0121b	FI856575	(A)10N(ATA)17	ACGTATCCCCAGGTGCAATA	AACAGATGTAGAAGGTATAATCCATGA	224	1	0.00
ICCM0122	FI856576	(AAT)4	GCAACCTGCCATCCATACTT	AGTGAATCAAATGATGAAAGCA	275	1	0.00
ICCM0123a	FI856577	(TTA)14	GGATGGTCTGCTGGAATCAT	AAAGACAACAAAAAGACAATCATGT	250	9	0.76
ICCM0123b	FI856577	(TAA)26	TTTTTACATGATTGTCTTTTTGTTG	TGAGGACTAAGATAATAGCAATCCAA	201	NA	
ICCM0124	FI856578	(AAT)4aaa(AAT)4	CCTCGGGAATTCAACTACCA	TCAAAAATCCACTTTCCACCA	279	5	0.16
ICCM0125a	FI856579	(AAAT)4	CGATCTGAGGATCAACTTGTGA	ACTAACCACCGTCGACCATC	253	NA	
ICCM0125b	FI856579	(TTA)5	TATTTATGGTCTGGTCCGGC	CGCTACCAAATATGGAACGACT	251	5	0.19
ICCM0127	FI856581	(TAA)27	TGTTGAACGAATTTACTCATCG	GGTGGGCTCCTATTGTTTGA	269	6	0.40
ICCM0128a	FI856731	(TA)4N(TAT)4	AACCCTAATTTATTTGCACTATTATCA	TCAAAATACGGTAGTAGGATAAGATGA	161	NA	
ICCM0128b	FI856730	(A)10	ATTTGGACGATGTGTCGCTT	TTATAGCCCCTGCTTTGCTG	266	1	0.00
ICCM0130a	FI856734	(AAT)22	GGATTTCGACTTTTATCCCTTTTT	CGGACTGGAATCAAAAGCTC	268	3	0.10
ICCM0130b	FI856734	(ATT)5	GAGCTTTTGATTCCAGTCCG	TGTAGGGGTGCATGGTGTAA	122	1	0.00
ICCM0131	FI856582	(AT)4N(TTA)8	AGAGCCAAACAAGAACCGAA	GTAAAGAGGGGCAGCTGTTG	192	NA	
ICCM0134	FI856748	(A)11	TTTTGGAGGCAGCTTTGAGT	TGAAGACAGAGACGGTGCAT	109	3	0.08
ICCM0138a	FI856753	(AG)4	ATAAATAGCCGGCCACAAGA	CCGATTGTTTCAAGACCAGA	119	1	0.00
ICCM0138b	FI856752	(ATA)11	CTGTTGCCGATTTTATATTATTTTT	AAATGTGTTGTTCTGGCCGT	168	NA	
ICCM0139	FI856755	(ATTC)4	TCACGATTTGAATGGTCGTG	CGTTTTCCCAGCTTCAACAT	151	NA	
ICCM0141	FI856757	(AAT)20	AAGGTATGATGCAGTTCCCG	GGGCGGAGGGTAATTATTGT	247	NA	
ICCM0142a	FI856759	(AC)4	GCATTGCCAATATCGAAGGT	AGTAGGTGCCAAATGCATCC	206	1	0.00
ICCM0142b	FI856759	(ATT)6	GGATGCATTTGGCACCTACT	GAGGTTGGTGTAGAATAGAATGGA	137	NA	
ICCM0142c	FI856758	(AC)7	GGAGGTCCCGAGTCTAAACC	TCTTCAAGTCTGCTTGACGTGT	105	1	0.00
ICCM0143	FI856761	(AC)5	CTGCGGTCGATCTAGAGGAT	AAGGTTGAAGGATGATTGCG	257	NA	
ICCM0150a	FI856583	(ATTC)4	TGATTGAAATGGTCGTGTCC	AGTCGTTTTCTCGGCTTCAA	279	1	0.00
ICCM0150b	FI856583	(TAA)8N(AT)4	GTAAAGAGGGGCAGCTGTTG	AGAGGCAAACAAGAACCGAA	192	5	0.16
ICCM0152	FI856792	(T)13c(CTT)4N(AG)5	CGTCTCACGAAGGAGAAGTG	AAAAATTCACTTGCTAATATTTCACA	197	1	0.00
ICCM0154	FI856794	(A)11	AGCTTGTTCGACAGCAGGAT	TCGATGTGAATATGCCCTCTT	247	1	0.00
ICCM0155	FI856796	(A)11	GACGGCAGGATTAACTGCAT	TTCGATGTGAATATGCCCTCT	240	2	0.04
ICCM0156a	FI856797	(AT)7N(CA)4	TGCATTCCCCTTTAATTTGG	CAGTGGTGGGGAGACAAAAC	280	2	0.04
ICCM0156b	FI856797	(ATA)52N(AAT)6	GTTTCTCCGTCCGCACTTAT	AAACAGTGTAAATTGTTGTTGGAAA	276	2	0.08
ICCM0157	FI856584	(GA)8	TTTGTTTTGAGGCAACCACA	AAACAAACCCAGTGGGAGGT	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	FI856585	(ATA)18N(TCT)16	CCAAAACTGACAAGTCCCGT	GGGAAAATATGAGGAAGTTTGC	275	1	0.00
ICCM0159	FI856814	(T)17N(T)10	TGAAAAATCGGAAACCCTACC	CCTTGTTTTCAGGCGATTGT	247	6	0.46
ICCM0160	FI856816	(AAC)4N(TAA)25	TTGCTTGAAACAACCTTTCG	CGGGTACAACCGTAGCAAAT	263	21	0.93
ICCM0161a	FI856818	(AT)4	GATGGTCATCGGTTCGATTC	AACGAGGCCCTCTGTAACG	267	4	0.12
ICCM0161b	FI856817	(TAA)4N(AAT)4	ACTGTCAGGAAGGAACGGTG	TCCGTAACAAAAATTGTGAAGAAA	279	3	0.14
ICCM0162a	FI856586	(ATT)12	TAGCGCAGTCGATCTGAGGA	ACGTATCCCCAGGTGCAATA	272	NA	
ICCM0162b	FI856586	(AAAT)4	GCAAGGTCTTTCCCTTGTCA	CGCCGCCAATTTTATTTTTA	278	1	0.00
ICCM0162c	FI856586	(T)11N(TA)4	TAAAAATAAAATTGGCGGCG	TCGTGTTAGGGTGTTTAGGGA	267	3	0.21
ICCM0162d	FI856586	(T)10	GACTCTGCTGGGGACAATTT	CGGGTTTAGAGACCCACTCA	225	1	0.00
ICCM0163	FI856820	(TC)4	CAACGAATTTCATGCTGTGG	TAGGGATGGAGAGGAGAGCA	274	1	0.00
ICCM0165	FI856821	(T)11	CGGACGTACACCTTTCGTTC	TGCTTCCGAATAACATAAAGCA	128	NA	
ICCM0166a	FI856824	(T)11	GCCTACTCGCGGATTTTTATC	CCAGGTGCAATAGGGAAATC	276	3	0.09
ICCM0166b	FI856824	(AAAT)7	TGGGGATACGTAGGAGCAAG	TTGGATTCGGGAGTCGATTA	233	NA	
ICCM0166c	FI856824	(AT)5	GCCTACTCGCGGATTTTTATC	CCAGGTGCAATAGGGAAATC	276	2	0.04
ICCM0166d	FI856823	(AT)4	GACTCCCCTACCACCTCACA	CGGACGCGACAAAAACTAC	275	1	0.00
ICCM0166e	FI856823	(TAT)48	AATAAAAATGCGGAAGTGGG	TGTGAGGTGGTAGGGGAGTC	276	1	0.00
ICCM0167	FI856588	(ATA)48	GAGTGTACGGGGATTATATGATGA	TCAAGAAAAGGAACCAAGGC	235	NA	
ICCM0169	FI856589	(TTA)30	AGAGGCAAACAAGAACCGAA	GTAAAGAGGGGCAGCTGTTG	251	NA	
ICCM0170a	FI856839	(TA)4	GCTTTGTTGCTTTCGTTCTTTT	AAAGTGTTTGGGGTGAGTGG	226	NA	
ICCM0170b	FI856839	(C)10	CTCGCATTCCTTTTCCACTC	GGGGGAAAGTATGGGATGAG	154	NA	
ICCM0171	FI856590	(ATTC)4	GACCGGGATCGTGTCATAAA	CGTTTTCCCAGCTTCAACAT	166	1	0.00
ICCM0172	FI856841	(AT)4N(AT)4	GCAGTCGATCTGAGGATCAAG	TTCACAAGATGTTTTCAGAACAAAG	278	NA	
ICCM0174	FI856591	(A)11	CAGCGACCTCCTACTGGGTA	CAAAAATGGAGGATTTTTCCTT	175	NA	
ICCM0176	FI856847	(TA)4	ATAGGCTAGACCGTCCGACA	TCTGAAATATGATGCAGCCG	273	5	0.16
ICCM0177a	FI856849	(AAT)28c(ATA)27c(TAA)4	CTTGAGTTCAAGCCAGAGAGG	GCGTTATTACTGTTACAAATGGCA	279	1	0.00
ICCM0177b	FI856848	(TAT)6N(TAT)11N (TAT)4N(TTA)6N(TAT)8	CCCTTCTTCCATTTCCGAAT	GGGGAGGAGAACGAAAAAGA	273	NA	
ICCM0178	FI856592	(AAT)13	AGTTTGGGTTTCACCGCCT	GAACGCGCTCTGTTCATAAT	280	11	0.83
ICCM0179	FI856850	(TAT)4	AAAGGCCAGTTTACCCGACT	ATTTGATGCAGCAAGCAGTG	214	NA	
ICCM0180	FI856852	(TAT)4	AGTCCCTGATCTCCCGAAGT	ATTTGATGCAGCAAGCAGTG	179	1	0.00
ICCM0181	FI856593	(ATA)5	CGGGTGTGGATAGCAAGTTT	TCTCTCCTTCCTAATAAAACAAACA	103	1	0.00
ICCM0183	FI856595	(TAT)15	TGAGGACTAAGATAATAGCAATCCAA	TTTTTACATGATTGTCTTTTTGTTG	168	1	0.00
ICCM0185	FI856597	(T)11	AAAGTTTGGCCTGGTCTGG	CATTCCATATTCAGTAGCATTCCA	250	6	0.46
ICCM0187	FI856599	(TAT)6	TGACCATCAATCCATTTCTTTTC	TGTTGACGTCTAATTTTGTCCG	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)6N(ATA)9	GGGGGATTGTCTGAGTTTCA	AAAAGGCTGGAGACACCTCA	195	7	0.53
ICCM0190b	FI856602	(TAT)5	TTTATTGCAGGAAGCGGTTT	CACCACTATCAAATGCCCCT	273	1	0.00
ICCM0191	FI856603	(T)10	CCTTACGCATAATCGACTCCA	AATTCAATTGAGTCGCCACC	130	5	0.37
ICCM0192a	FI856879	(TAT)15c(ATT)15	GCTGCCCAAATTTTGACATTA	CCGGGGATCAAATTCTTCTT	279	11	0.88
ICCM0192b	FI856878	(TAA)15tg(ATA)15	CGGACGGGGATAATTCTTCT	GCTGCCCAAATTTTGACATTA	279	15	0.76
ICCM0193a	FI856881	(TAA)5N(T)10	TGAACTTTCAAAACCAAACCAA	TTGTGACAATTTGAGGGTTCT	268	NA	
ICCM0193b	FI856881	(AAT)7	TCGATTATAGCTTTATCTTTACCCTTT	AAAAGTGTTGGGAGGGGTTC	242	1	0.00
ICCM0194	FI856883	(A)10N(T)13	CGATTGTCCTAGTTTTAAAAAGAAA	CGACTTCCTGAAGGAACGAA	200	4	0.22
ICCM0196	FI856605	(ATA)5N(AG)6	GTCGGGTGTGGATAGCAAGT	AACACAATTCCTCAAATAACAAACT	154	6	0.47
ICCM0197a	FI856885	(T)13	CGCGTCTAGCAAAACAAGAA	TTCTCGGCCTATAAACATCAA	280	3	0.08
ICCM0197b	FI856884	(TATT)7	GATTCCGGAGTCCATTACCA	CTATTGCACCTGGGGATACG	241	NA	
ICCM0198	FI856886	(A)15	CCATCCGAGAAAACTCGAAA	CAACGGTATCCATCGGAATC	163	1	0.00
ICCM0199a	FI856889	(A)10 g(AGAA)4	ACCAAGCAGACCACAACAAT	GTTTTCCCGGCTTCAACAT	265	1	0.00
ICCM0199b	FI856889	(CATT)5	ACCAAGCAGACCACAACAAT	GTTTTCCCGGCTTCAACAT	265	1	0.00
ICCM0199c	FI856888	(TTA)15	TTAGAGGCAAACCAGAACCG	ATCTTGAAGTGGGCAAAACG	241	15	0.86
ICCM0200	FI856890	(TAA)4	ACGGAGTGACCAGGAAACAC	GCAGACCTACAGAAACAGAGGAA	231	2	0.04
ICCM0201	FI856892	(TAT)7(TTATTG)6[a] (GTTATT)4	ATAGAGAGACCCAAACCGCC	GCCAAAGGCAAAAGAGATTG	135	NA	
ICCM0202a	FI856894	(T)10N(T)10	CGCCGATCCATTATACTGAC	TTGCCTCTGATTCTGGTTCA	192	2	0.04
ICCM0202b	FI856894	(TTA)13	TGAACCAGAATCAGAGGCAA	CCAATTTGGTCCGGTTTTTA	207	12	0.77
ICCM0203	FI856606	(CT)6	TGGACGTAGGTTGTTGTGGA	TTGGTATCAGTGACCTCGCA	194	1	0.00
ICCM0204	FI856607	(TC)7	CACATACACTCCCCAATCCC	TGCAGACTGTTTGGTTCGAG	258	1	0.00
ICCM0205	FI856608	(TA)9	CGACCATGATTCTCTGATGTG	CACCTCTGCATTTCTTCAAACA	263	8	0.26
ICCM0207	FI856610	(TA)4	TCAACCATAAAGCACTCCCC	GGCCATTTGTGTTTTGTATGG	182	2	0.04
ICCM0210	FI856898	(TA)4N(TG)4N(CCA)4N(AG)16	GACCATTGCCCACTTCAACT	AGTTCTGCGAGAGGAATGGA	253	NA	
ICCM0212a	FI856902	(T)10N(AT)4N(TA)5N(TA)4	TCCTATACCGAAAACCCCATT	CAAAATGGATGGATTGTGGG	270	2	0.04
ICCM0212b	FI856901	(GA)4	ATTGCCGTTGAGAGAAGTCG	TCGGTCACCACACTACCAAA	183	1	0.00
ICCM0212c	FI856901	(AG)8	TTTGGTAGTGTGGTGACCGA	AACCCAAAAACGTGGACTCA	161	6	0.32
ICCM0214a	FI856612	(CT)6N(AC)4	CTCTTCAATAGCCCCATCCA	CGTTGGAGAGGCTGAAACAT	249	1	0.00
ICCM0214b	FI856612	(TTTA)5	ATGTTTCAGCCTCTCCAACG	TCGCACCTGAACTCTCTGTG	276	NA	
ICCM0215a	FI856613	(TC)5	CCTTCAGTGTTTGGCTCACA	CTCCAGGAATCCACAGCATT	253	3	0.15
ICCM0215b	FI856613	(T)11	AGAATGCTGTGGATTCCTGG	GCAAGCCCAAAACTTCAAGA	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	CGGGACTTTCATCTGCTGTT	GTGGGACATCCTCCAAGAAA	200	2	0.04
ICCM0216b	FI856614	(AG)5	AAAGCTGGTGGTCGAGCTAA	GACCACCGAACCAGGATAAA	277	3	0.09
ICCM0219a	FI856906	(ACC)4	CCTTTTAAGGGCTGAAGGCT	TGAAAGAATGTGGGGGAGAG	203	NA	
ICCM0219b	FI856906	(CT)5	TCATCCTACCCAATTGCTCC	GTAGTGGGGTAGGGGATGGT	240	3	0.10
ICCM0219c	FI856905	(CT)4	CTCACCCACCACACCTATCC	GCGAAGGGAGAGAAGGAAGT	278	NA	
ICCM0219d	FI856905	(TC)5	CTCACCCACCACACCTATCC	GCGAAGGGAGAGAAGGAAGT	278	NA	
ICCM0220	FI856908	(TA)4N(CT)4	TCAACCATAAAGCACTCCCC	GGCCATTTGTGTTTTGTATGG	183	1	0.00
ICCM0222	FI856617	(CT)5	TCCGATTGGATTTTCAGGAC	GGTATCAGTGACCTCGCCAT	210	1	0.00
ICCM0223a	FI856912	(TCT)4	TACAACTTTTGACACCGCGA	AGTGGCAGTATGCGTTGAGA	224	NA	
ICCM0223b	FI856911	(TC)4	GCTCTGTCGGTCTTCCTGTC	ACAAAGCGCTCGAATAAGGA	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	CCAACGACGCGGATAAATA	AATGCCCACCCATAATTTCA	273	1	0.00
ICCM0226b	FI856537	(TA)4	GAAAAAGCGCTGTAAATGGC	CCTCGCATTTTGTTCTCAAAG	145	1	0.00
ICCM0228	FI856619	(CT)6	TGGACGTAGGTTGTTGTGGA	GGACCGGGAGTCCCTTATTA	274	1	0.00
ICCM0229	FI856916	(C)10	TGTCTTATTCCTCCTCCCCC	AGGGGTTTTTGGGTTACCAG	158	9	0.69
ICCM0231a	FI856919	(TTA)21N(TC)4	CTGGGGATACGTAGGAGCAA	GGAGTGAGATAAGAAAGAGAGGAGG	278	NA	
ICCM0231b	FI856919	(TC)4	ATCCCACCTTACCACCCTTC	GGAATGAGGAGGGATTGAGA	279	2	0.05
ICCM0232	FI856920	(T)10	ACGGGAAGTTTCTGGGTCTT	TAGCGGAGAAACAGGACTGG	253	3	0.09
ICCM0233a	FI856922	(TA)4N(TTA)4t(TTA)4	CTCACCACTAGGGATGGGAA	AGACTCCCGAGGGATTGACT	242	NA	
ICCM0233b	FI856922	(A)10	AGTCAATCCCTCGGGAGTCT	TGTTGAGGGCCTTAGATTGG	256	NA	
ICCM0234a	FI856925	(TTA)4	GGGACTACTTTCGCGATTCA	GTGGGTAATCCGTGCGTAAT	229	1	0.00
ICCM0234b	FI856924	(TA)4	CAGGCTATGTCATCTCGTCG	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)4c(GT)4	GCCGACCCGATTACCTTACT	GAGAGCTAAGGGGAAGGTGG	176	NA	
ICCM0236a	FI856621	(CGC)7	GCTTGTTGCCCTGTATTGGT	AAAACGCAAGCAAAGCAAGT	151	NA	
ICCM0236b	FI856621	(ATT)4N(A)10	ACTTGCTTTGCTTGCGTTTT	TTTGTGGGTTGGTTGATTTT	219	2	0.04
ICCM0236c	FI856621	(A)10N(TA)4	AAAATCAACCAACCCACAAA	ATCACTCTACCGTCAAAACGA	244	2	0.04
ICCM0237a	FI856622	(AT)4N(ATT)6	TCAACCATCCCTAAAAACATTTG	TTCCTTTGCCATTTATGTTTTG	184	6	0.34
ICCM0237b	FI856622	(A)13	CAAAACATAAATGGCAAAGGAA	TCGTGTTGTATTGTGCCGAT	155	2	0.04
ICCM0237c	FI856622	(AG)4N(GA)4	TGTGTTTCTCGATGGCAGAG	CTTTTTCTCCCTTTCCACCA	120	1	0.00
ICCM0238	FI856623	(TC)4N(TC)4	ACCATAGACGAACCACCACC	CCAAGGGGGTACAACTTGTGT	215	1	0.00
ICCM0240a	FI856650	(TA)12	ACCCGAACCCGCAAATAATA	GCAATGAGACTGGGGTTTTC	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	FI856650	(TA)4	ACCCGAACCCGCAAATAATA	GCAATGAGACTGGGGTTTTC	248	19	0.77
ICCM0242a	FI856929	(AAT)18	TGCATTCATCTGTTTCGCTC	GAAAATATTTGTGGTTATCCGATTTT	263	8	0.74
ICCM0242b	FI856928	(A)11	ATCCGCAACACAACAAAACA	CCCTACTCGTAATCGACTCTCG	252	4	0.21
ICCM0242c	FI856928	(TAT)5	CAAGTGCAATAGGGAAATCCA	ATAGGGCTTTCCACCGATTT	210	NA	
ICCM0243a	FI856931	(AT)5N(AT)4N(AT)8	TCAGGAACAGACGGAACTTTTT	GGGTTCAAATCCTATTGGGC	277	3	0.08
ICCM0243b	FI856931	(AT)4	ATTTGCGCCCAATAGGATTT	TTTTTCTATCGGAATATCTCATTTTCT	280	1	0.00
ICCM0243c	FI856930	(GA)41N(AG)10	ACGACGATTCTGGATTTTGG	AGTTTTGGTAGGGGGTCGAG	237	16	0.88
ICCM0244a	FI856933	(AT)8N(TTA)4(TAA)4N(TTA)6N(ATT)4	ATGCGGTAATCCTTGACTGG	TGCAGGGAGTGAATGTGTGT	252	5	0.25
ICCM0244b	FI856933	(TA)5	CACACATTCACTCCCTGCAA	GATGGAAGGGAGGGGTAAAA	268	NA	
ICCM0245	FI856935	(AG)5	GCGGCTGGTTTAAGAGTGAG	CCAACACGACCCAAATCAAT	182	6	0.53
ICCM0246a	FI856937	(AT)4	TCTGACAGCTCTTGCCTTGA	AACACCCAGACCCCTTTCAT	280	4	0.12
ICCM0246b	FI856937	(TA)4	TGAAGAGGAAGAGACGGGAG	AATCCATTTACGGGGGTAGC	268	NA	
ICCM0246c	FI856937	(TATTT)4	TGAAGAGGAAGAGACGGGAG	AATCCATTTACGGGGGTAGC	268	1	0.00
ICCM0246d	FI856936	(TC)4	GATCACGGTTACGAATGCAA	TAAGGTTCCCATTGGCTCTG	209	1	0.00
ICCM0247	FI856626	(TTA)8	CCTCAATTCATTTTTCTTCGG	TTTCCCGATAAACCATCTGTT	136	2	0.06
ICCM0249	FI856627	(T)12N(TAA)29	TTTCTTCGCATGGGCTTAAC	GGAGATTTGTTGGGTAGGCTC	193	5	0.16
ICCM0250	FI856940	(TAT)40	TTTCAAACACAATCTGAACGAGA	CCACCTTCGGGTAGGATACA	231	2	0.04
ICCM0251a	FI856943	(AC)4	TCCCTGCTATACACCCATCC	TGGGCATATATGGATCACGA	252	1	0.00
ICCM0251b	FI856943	(CG)4	CTACACCCGCCAACCTCTAC	AAGTGTATGTGACCGAGCCC	261	1	0.00
ICCM0251c	FI856943	(CA)4	AACCCATAATACGCGCTCAC	GGGGTGGTAAGGTAGGAGGA	206	2	0.08
ICCM0252a	FI856945	(CCT)4	TCTACCTCTCCGCTCTTCCA	TGGTGATAGGTGGTGGTTGA	203	1	0.00
ICCM0252b	FI856944	(T)11	TTGACGGTGGGGGTATACAT	TCCACACACTCCCACTACCA	267	NA	
ICCM0253	FI856946	(AT)5	TCCCTTACAAGCATTCCCTG	TGGGGACCGTTTTTCACTTA	110	NA	
ICCM0254	FI856628	(TAA)4N(TAA)29	GCCAAGCCCATTAAAACACT	CGTTGTTAAAAACCGCGTTG	273	1	0.00
ICCM0255	FI856629	(ATA)8N(AAT)4N(AT)4	GGCTACCGAATATGGAATGC	TGGCCTGACCTACTTATGGC	272	2	0.04
ICCM0256a	FI856949	(AT)4	ACCGCTCATTTCCATACGTC	TGGATCAAGAGGGAGGATTG	195	NA	
ICCM0256b	FI856948	(CT)4	TTTCTCTTTTGGTGGTTGCC	TTAAGGTTTGCCACTCCTGG	247	4	0.17
ICCM0256C	FI856948	(TTA)19	TTAAGCCAGACGTGGGAAAC	AGAAAGAAGGGAAATGGGGA	236	2	0.08
ICCM0257	FI856630	(ATA)44N(AAT)11	TCGCTTCCAACATTCAAAAA	CAATTGCACTTATAGCACAAACA	255	2	0.04
ICCM0258	FI856950	(ATA)11	TGCATAGGGAAATCAAAACACA	TTATTTCACCGTCGTGTCCA	271	1	0.00
ICCM0259	FI856631	(TTA)15	AGAGGCAAACAAGAACCGAA	CGAAGCCGAGAAAATGACTC	261	2	0.04
ICCM0261	FI856633	(TAA)23	GTCCGGGGATTCAGTAGGAT	CAAGCCACGGAACTTGTTTT	232	NA	
ICCM0263a	FI856635	(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]	PIC value
ICCM0263b	FI856635	(ATA)19	GGTAGAAAATATTTATGTGTTGACCG	CTCGTTCACATACCGCCATA	260	1	0.00
ICCM0265a	FI856636	(AT)5	GGAACTCGGGATTGAAATAGTC	TTGCAAAGAAAACAATTTTAGGA	214	NA	
ICCM0265b	FI856636	(A)13	CGTTTAATCCTAAAATTGTTTTCTTTG	ACGGCGACAACCATTAATTC	190	1	0.00
ICCM0265c	FI856636	(TAT)9a(ATT)10N (ATT)11N(AAT)4	TACCGCCACGTTACGTTTTT	GAAAATATTTGTGTGTTGACCGA	248	4	0.14
ICCM0266	FI856955	(TAT)31	GAATCGTGAGGGGGAGATTT	GGGGGAATCAAAAGGCATAG	269	NA	
ICCM0267a	FI856637	(TATT)4	CAACGTCCGTTAAAACGGTTA	TGGGGATACGTAGGAGCAAG	179	1	0.00
ICCM0267b	FI856637	(TTA)4N(TAA) 8N(ATA)12	CTTGCTCCTACGTATCCCCA	AGTCTCGTTCACATACCGCC	272	1	0.00
ICCM0268	FI856957	(CT)7N(TC)4	TTCATCTCTGCCCAAACTCC	TGGGTAGATGGAAGGAGTGG	220	1	0.00
ICCM0269a	FI856959	(AC)4	CCCTCTTTACACCCCACCTT	GTAGTGGAGTGGGGCAGGTA	129	2	0.04
ICCM0269b	FI856959	(TC)5	AACATCACTAACCCTCCCCC	AGGTGTGGGTGGTAGGAGTG	209	NA	
ICCM0270	FI856638	(TAT)16	TCACATACCGCCACAATACG	ACGTATCCCCAGGTGCAATA	276	1	0.00
ICCM0271	FI856639	(GT)4	ACCCGGGTATAAGGTTCCAC	TGCTTGTTTTTCATTTTCATTTTC	240	3	0.11
ICCM0272a	FI856961	(A)12	TTTCCACTTGGAACAGGCTC	AATGGACGATGGTTGGGTTA	280	3	0.12
ICCM0272b	FI856960	(GA)10N(AG)20	CGCGGTTGAGTTAGAGTGGT	CAAATCGGGGATTTTGTTTG	175	12	0.74
ICCM0272c	FI856960	(GA)4	CGCGATTATTACCCACGTTT	GGAAAGGAGGTACCGGAGTC	249	3	0.09
ICCM0273	FI856963	(TGA)4	TGTAACTCATCATCGCCAGC	AGACGTGTAGACAGATGCCC	108	2	0.04
ICCM0274	FI856640	(TC)9(TA)15	GACCCTACCCCGCAAGTAAT	TTTTTGTCCACACTCACACCT	265	NA	
ICCM0276	FI856965	(C)10	CTCCTACACTGCCTCCCCTC	TCATGCTTACTCCGTTGCAG	222	NA	
ICCM0277	FI856642	(TTA)11	GGCAAACAATAACCGAAAACA	GTAAAGAGGGCCAGCTGTTG	196	6	0.30
ICCM0278a	FI856643	(TTA)4	ATAGGGGACCAAAACTGCAA	GTGGGTAATCCGTGCGTAAT	203	1	0.00
ICCM0278b	FI856643	(AT)4	AAAATACACATCCTGACTGCCA	TTTTGCTTAGACTTGTAGGCATT	159	2	0.17
ICCM0280	FI856969	(T)11	ACTAGATGGTCGCATCCTGG	GGTGAAGGTGTGGATGAGGT	280	1	0.00
ICCM0281a	FI856644	(AC)9	TTCAACCCTCCCTACACGTT	GTTCTCTTCTTGCTGGTGCC	235	1	0.00
ICCM0281b	FI856644	(AAT)5N(A)11	TGGAACAACCAAGACCTTCA	GCTGCCACAAACACTGAGAA	264	2	0.04
ICCM0282a	FI856645	(CGC)7	CCTCGTTGTTGCCCTGTATT	AAAACGCAAGCAAAGCAAGT	154	3	0.23
ICCM0282b	FI856645	(ATT)4N(A)10	ACTTGCTTTGCTTGCGTTTT	TTTGTGGGTTGGTTGAATTTT	219	NA	
ICCM0282c	FI856645	(A)10N(TA)4	AAAATTCAACCAACCCACAAA	ATCACTCTACCGTCAAAACGA	250	4	0.25
ICCM0284a	FI856647	(AT)4	CGTATCTACACCCGCACTCA	TGGAAAATCCACTTTGATTGG	257	3	0.08
ICCM0284b	FI856647	(TA)4	CGTATCTACACCCGCACTCA	TGGAAAATCCACTTTGATTGG	257	9	0.30
ICCM0285	FI856971	(ATTC)5	TGAGGACAAGATTCCGTTCA	AACATGCGGGTTGTTTTCTC	267	1	0.00
ICCM0286a	FI856648	(GA)5	AGCATCACGCATACAGCTTG	ACATTGGCTCCATTGTTTGG	254	2	0.04
ICCM0286b	FI856648	(AG)4	ACCCCCAAAATGCTGTAGTG	ACGCCCCTTTACTGTTACGA	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	FI856972	(TAA)4N(TTA) 4N(TTA)4	TTATTTTTCGGATCCAACGC	GTGATTTTTGTTCGGCCATT	278	20	0.88
ICCM0289	FI856976	(T)13N(ACA)4	CAGCCTCCATGGCATAGATAA	TGCTTGAATGAGTGCAACAA	219	15	0.83
ICCM0290	FI856976	(A)15	TTGTTGCACTCATTCAAGCA	TTTTTATTGGGGCATTGAGC	244	6	0.38
ICCM0291	FI856978	(AT)4	AAGTATTCAATTATACGTGCACAAAA	TCATCCTTGTTAAGTCAACCACTT	249	1	0.00
ICCM0292	FI856978	(TAA)6	TGGTTGACTTAACAAGGATGAGTG	TCCTCAAGCAGAGGTGGTTC	267	1	0.00
ICCM0293	FI856982	(TAA)15tg(ATA)15	AGTGATGCCACGAGAATTGC	CTGGTTCGGAATTGTCATCC	250	5	0.24
ICCM0294	FI856986	(TTA)15	AGAGGCAAACAAGAACCGAA	CACCCAATTTTGTCCGATTT	185	NA	
ICCM0295	FI856987	(T)10	GAGGCACCAAATTCGTATCC	CAAAATTTTCTAATTCACCAAGACTTC	256	3	0.09
ICCM0296	FI856987	(TGATT)4	CGCCAAGTTTTACTATGTGCTG	TGTCTGGATGTTACATAAACACTCTT	227	1	0.00
ICCM0297	FI856987	(TAA)18	CATGATTTGATTTGATTTGATTTC	GGAGTGGGAAACCTTAAGCC	271	3	0.08
ICCM0298	FI856989	(TC)4N(AAT)4	GTGCACTTGTTCAGCGTTGT	CGCAAACACACATTCCTCTG	221	5	0.33
ICCM0299	FI856989	(CTT)7N(TCT)4	TTATGAAGCCGAAGCTCGTT	GAGCAGTAAACGTACCCCCA	272	NA	
ICCM0300	FI856990	(A)10	ATGGCCAAAATGAACTCCAG	AAAAGAGAAGGTTCCATCGG	173	2	0.10
ICCM0301	FI856992	(A)10	ATGGCCAAAATGAACTCCAG	AAAAGAGAAGGTTCCATCGG	173	1	0.00

Marker names start with prefix ICCM, which represent *IC*RISAT *C*hickpea *M*icrosatellites

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 *M. truncatula*	Linkage group in chickpea	Genotyping method	Restriction enzyme	Forward primer (5′–3′)	Reverse primer (5′–3′)
CALTL	AW126242	Mt/EST	AC149080	1	4	CAPS	Alu I	GTGGAAGGCACCATTGATTGACAAC	TCTTCTTCTCAGCCTCTTCAAATGC
CDC2	AW171750	Mt/EST	AC144481	1	4	SNaPshot	N/A	CAACTTTGCAAGGGTGTTGCTTTCT	ACTAACACCTGGCCACACATCTTCA
CysPr2	AI974635	Mt/EST	AC148098	1	4	CAPS	Taq I	CCAAAAACTTGCTTCTATACTCTTCATTC	GACAAAACCCACCCAGACAAATCAACTAG
HRIP	AW126332	Mt/EST	NA	1	4	SNaPshot	N/A	GGAAAAATTTATCCTCCAAATTGGGGGTA	AAAAATAGCAGTGCACCAAAAAGTGCTG
ppPF	AI974685	Mt/EST	NA	1	4	SNaPshot	N/A	TCTCGCCACCAACAACAACTAC	AAAAATTGTTCATGAACACTCACTTGAAGCCA
REP	AA660953	Mt/EST	AC133709	1	4	CAPS	Taq I	CTCCATTTCCCGTTCGTTCG	CACCGGTTGCCCTCCAGAC
RL3	AI974458	Mt/EST	AC125473	1	4	CAPS	Hind III	TTCAATCGATAAGAATACTGCTTTG	TGTGTGTCATACCAGCCTTG
TC76700	TC76700	Mt/EST	AC122171	1	4	CAPS	Hinc II	CCAAAGACCCAGTTCGTGTT	GTTGCATGGTTGACATCAGG
TC86606	TC86606	Mt/EST	AC139748	1	4	SNaPshot	N/A	TGAAAATGGCAATGTGGAGA	TTGGGATTCCTCATCCTCAG
TC87270	TC87270	Mt/EST	AC124216	1	4	CAPS	Dde I	TGGCTTCTTTCGGTCTCTGT	TCAAAACCACGCATTTGGTA
TC88727	TC88727	Mt/EST	AC137895	1	6	SNaPshot	N/A	ATCAGGCAGAACTTGCTCGT	AGAGTGCCCGGTATATTCCT
DSI	AA660976	Mt/EST	AC122168	1	4	SNaPshot	N/A	GAAGCCAAAAAGTATGAAGGGCCACGCAC	CATGGTGCATAAAACTCAACCAAGACATC
ChitinaseII	NA	NA	~AC137554	2	1	Pfaff and Kahl (2003)		AGCACATGAACCAATCCACA	CATGGTTGCAATTTCACGTC
ACCO	AI974230	Mt/EST	AC121236	2	1	CAPS	Stu I	GAAGATGGCGCAAAAAGAAAGT	CGATGTGTCGTGTCATCTCGTTAAGTTCCCT
AJ005043	AJ005043	Ca/EST	~AC127170	2	1	SNaPshot	N/A	TTTTCGGGAACTGCTTATGG	CATGGAAGTGGACCGTTTTT
CPCB2	AW191283	Mt/EST	NA	2	1	CAPS	Dra I	AGAAAGAGTGAAGTCTGTGGATCTACATC	GGATGAACAGCCACACACCTAATGTAATC
DMI-1	AY497771	Gene	AC140550	2	1	CAPS	Hinc II	ACCCTTCTTTCCTTGGCATT	ACCGCATACAACGCTAAACC
TC77488	TC77488	Mt/EST	AC126013	2	1	CAPS	Afl III	ATGCTTGCAGATCCTGCTTT	GATCTGCCTCAACTCCAAGC
1433P	AI974411	Mt/EST	AC144342	3	5	SNaPshot	N/A	AAGGTTTTCTACCTTAAGATGAAGGGAG	GTTTAGCAAGATTGCAGGCACGA
AF457590	AF457590	Ca/EST	~AC122728	3	5	SNaPshot	N/A	CTCCCTCTCTATCGCCCTCT	TGTGAAAGGGTTGGTGTGAA
AIGP	AW125928	Mt/EST	NA	3	5	CAPS	BSPW I	CTGATAGGGCCAGGAGGCAGGGAAGA	GTTTTTTAGCATTTGGACGAATGGTTGGT
CysPr1	AI974595	Mt/EST	AC131026	3	5	CAPS	Ear I	GAGAATTCAAAGAAGAAATTAAGACAAAGA	GAAGAATTCATGGGGAGCAAAGT
Ms/U515	AJ410128	Ms/EST	NA	3	5	SNaPshot	N/A	GTTAAGGGAACCATGACAACCACA	CCAGGCTCGGATTGAGCAGGGTTTGT
Ms/U83	AJ410159	Ms/EST	NA	3	5	SNaPshot	N/A	TGGAAAACTTGATGGATCCTGCTCA	TGATAGTCCTGCAACAAGTCCAGCA
P40	AJ006759	Ca/cDNA	~AC143340	3	5	Pfaff and Kahl (2003)		TTTTTAAACGCCGCAATGA	CAGAAAGCAATGGTGGGAAT
TC80362	TC80362	Mt/EST	AC137828	3	5	CAPS	Ase I	GCTTGAGCCTCCAGAGATTG	TGATAAGCCTTGCAGTGTGC
TRPT	AA660362	Mt/EST	AC122170	3	5	SNapshot	N/A	CAACAACCTAGTATAGCGATCAGTG	CATTCAAAGCCCACCAAGTT
DNABP	AI737524	Mt/EST	AC121244	4	6	CAPS	Afl III	CCCTATGAGCTTGGGTTTGTCT	CTCATGGCATACGTGTTCAGC
EST948	AA661051	Mt/EST	AC145021	4	6	CAPS	Dde I	GCAGGGGTTTCGCTCCAGTG	AACTTAATGAATGATTGGAAGGTTTAGCG
Ms/U40	AJ410120	Ms/EST	NA	4	6	SNaPshot	N/A	AAGAATGAGGAAGAGGAATTCAACA	GGTTCCTAAGGAACAATGATGACA
MTU07	AI737610	Mt/EST	NA	4	6	CAPS	Bsm I	CAGACACCCAAAGAATTACCAGAA	GATGACCAGAGCCTAATACTATTTATGACT
TC78756	TC78756	Mt/EST	AC144502	4	6	CAPS	Alu I	GGAGGAGGAGGAAGATCAGG	ATGCTGGAGAGATGGTGAGC
TC79726	TC79726	Mt/EST	AC130805	4	6	CAPS	Cla I	TGCTGAAGGGAGGATTCAAG	ACAGCATTGTGAGCAACCAC
TC88598	TC88598	Mt/EST	AC135316	4	6	CAPS	Bcl I	AGATGGGGAGATTGATGCTG	AAGCAACATGCAGTGAGCTG
TGDH	AA660742	Mt/EST	NA	4	6	SNaPshot	N/A	CGGTGGCTTCATCGGTTCT	GACGTGTATTGTAATCAGCAGGAGTA
tRALS	AW126282	Mt/EST	AC125476	4	6	CAPS	Msp I	GGTCTGCGAGCTGTTTTTGGAGAAG	GCAATTCCCTCCTCAGCTAAAAGTG

Table 3 continued

Marker name	Template sequence accession no.	Type	Sequenced Mt BAC accession no.	Linkage group in *M. truncatula*	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	CCCAATCTTTTTCTCCCACA
AB025002	AB025002	Ca/EST	~AC122727	5	2	CAPS	EcoR V	TTCCTCGATCATGTCGAACTT	CGTGCACCAGCTTCGTAGTA
AJ005041	AJ005041	Ca/EST	~AC122727	5	2	CAPS	Dra I	AGCGTCTAGCCAGCATCAAT	CCATGGCTTCTTACCCTTCA
AJ404640	AJ404640	Ca/EST	~AC131455	5	2	CAPS	Pst I	TGGAGAGAATGAGGGGAATG	TCAAAGGATGCCAAATCACA
CYSK	AW207985	Mt/EST	AC135320	5	8	SNaPshot	N/A	GGAATTGCTAAAGATGTTACAGAATTGA	AATGAGGACACTCTGTCCAGGTGTGA
CYSS	AW127154	Mt/EST	AC135320	5	8	CAPS	Bgl II	CTGATGCAGAAGAGAAGGGGCTTATCA	CCAATTCAGCTCCAAAAGCTAATAGAATGA
DK242R	AQ917211	Mt/BES	NA	5	2	CAPS	BsmA I	CGTATGTTTAATCCGTTAGTCCGTCTT	GCTTGCTTAGATATTTGGCACTTCA
FENR	AW127593	Mt/EST	AC138010	5	8	CAPS	Hph I	ATGCTTATGCCAAAAGATCCAAATGC	CTCACAGCAAAGTCGAGCCTGAAGT
Ms/U393	AJ410119	Ms/EST	NA	5	2	SNaPshot	N/A	ATTGGAGAAGGGCAATCCTCCACCA	TCCCTTCATTCTATCCATCCCAAGA
Ms/U89	AJ410164	Ms/EST	NA	5	8	CAPS	EcoR V	CATATGAACAGTTGAAAGTGGATGA	CAATATGGCCAACATTAAAAAATGGCA
TCMO	AW127521	Mt/EST	AC141923	5	5	CAPS	Hph I	GTCTACGGCGAACATTGGCGTAAAATGC	CAATTGCAGCAACATTGATGTTCTCAACA
TC87369	TC87369	Mt/EST	AC124591	6	2	CAPS	Pvu I	ATAGTGGACTTCGGCGAGAA	TGACGGGGATCTTTTCTTTG
AGT	AW126002	Mt/EST	NA	7	3	CAPS	Xba I	GATTTGGGCCTCATTCCTTCTTGTGTGCA	CCTGAAGGGGGAAAATTGCCCACATTGA
AJ004917	AJ004917	Ca/EST	~AC136505	7	3	CAPS	Dde I	CCAAGGAAACCAGTGGATGT	GCAGCATCAAGATCACGAAA
AJ012739	AJ012739	Ca/EST	~AC130801	7	3	CAPS	Dra I	CTTCAGTCAGGAGGGAGACG	TGCAAATTTCGCTACAAGGA
AJ291816	AJ291816	Ca/EST	~AC140025	7	3	SNaPshot	N/A	TTGGAGGTGCTGGTGATGTA	TGCAAATGCTTGCCAAATAG
DK225L	AQ917191	Mt/EST	AC137994	7	3	CAPS	Hinc II	TGTCCTTGCTTCTTATCCTTCCTTCA	AGCAGCACAACAACTTACAACAACTC
ENOL	AA660534	Mt/EST	NA	7	3	CAPS	Dde I	TTCCATCAAGGCCCGTCAGA	TTGCACCAACCCCATTCATT
MS/U380	AJ410118	Ms/EST	NA	7	3	CAPS	Taq I	CACTCATTGCAATTTCCATGCTTCA	CAGTTGTTGTAGCAAGGGCACA
RNAH	AI974503	Mt/EST	AC123899	7	3	CAPS	Mbo II	GCTTCCACCAGCTGATACACG	TTAGCCCTAGCAAGAATGTCACTG
TC76881	TC76881	Mt/EST	AC136505	7	3	CAPS	Hph I	TTCTTGGGGAACAGAACAGG	GCAGCATCAAGATCACGAAA
TC78638	TC78638	Mt/EST	AC135795	7	3	CAPS	Acc I	TGATTGCAAAGCAGGTTGAG	CGGCATTCAGTAGGAAGCTC
TC84431	TC84431	Mt/EST	AC139355	7	3	CAPS	Nsi I	AGAAAGGACTTGGGCAACCT	CCAATACGTGCCTCTTTGGT
TC86212	TC86212	Mt/EST	AC124963	7	3	CAPS	Hinc II	GTGGCTCCTTGTGTGCTGTA	AGCCAGGATGAGGCACTCTA
TC88512	TC88512	Mt/EST	AC136974	7	3	SNaPshot	N/A	CTACTGTTGGACGGGTTGCT	AACCTGGGGCATTTGTGTAA
TC88726	TC88726	Mt/EST	AC121242	7	3	SNaPshot	N/A	GAGGAGAATACGCCATCCAA	GGATAGTGTGGGCTGCATCT
AJ276270	AJ276270	Ca/EST	~AC122726	8	7	SNaPshot	N/A	GCTGCGAATTTTGACCTAGC	TGCCTTTGCAGATTCAGTTG
AJ276275	AJ276275	Ca/EST	~AC140032	8	7	SNaPshot	N/A	TTTTTGCCTGGACCAAATTC	AAGGCTTGTACTGCCCTTGA
AJ489614	AJ489614	Ca/EST	~AC138087	8	7	SNaPshot	N/A	CGAGGAAGAATGGCAAAGAG	AACAAGCCAATGAGGGAGTG
CoA-O	AI974546	Mt/EST	AC119408	8	7	CAPS	Nla III	TTTGGGGGAAATAATGGAAGTCT	CTCGGGCAATGTTGAAAAATC
CPOX2	AW127442	Mt/EST	NA	8	7	SNaPshot	N/A	AAAGAATCATGTCCTCAAGCTG	CTTCTTCTTGTGGAAGTCAGCA
EST671	AA660779	Mt/EST	NA	8	7	CAPS	Hpa II	GGTGTTATCTATGAAAGAGGCCTCATTGG	TGTCCTTGGGTATGTAGAAAAGCCTTCAC
FIS-1	AI974522	Mt/EST	AC122726	8	7	SNaPshot	N/A	TCAGTGATTGAGGGTTTTTCTACG	CTGTTTCATCAACTTCAGCAACTTT
Ms/U82	AJ410158	Ms/EST	NA	8	7	SNaPshot	N/A	TTCGTCAACGAGATGTTTGTGTTGGC	CAAATTGAAGAAAAGAGGAATGAAGGT

Table 3 continued

Marker name	Template sequence accession no.	Type	Sequenced Mt BAC accession no.	Linkage group in *M. truncatula*	Linkage group in chickpea	Genotyping method	Restriction enzyme	Forward primer (5′–3′)	Reverse primer (5′–3′)
AJ004960	AJ004960	Ca/EST	~AC136142	Unknown	2	CAPS	BsiN I	GCCTGGTGTGATCGTTACCT	ATGAAACCGGCAAAGACTTG
Ms/L591	AJ410091	Ms/EST	~AC148970	Unknown	3	CAPS	Hind III	GGCAGCTATAAAATCAAGTATCATGC	TGCCACTTCGCCAAAGGACTCATTA

"~" 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*; NA, 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 $MgCl_2$, 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_allelobin.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

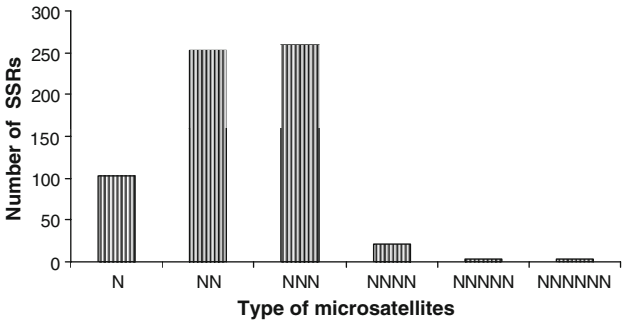


Fig. 2 Frequency of microsatellites based on type of repeat motifs in microsatellite-enriched library of chickpea. Frequency of tri-nucleotide repeats were higher among the chickpea microsatellite markers followed by di-nucleotide repeats. N, mono-nucleotide repeats; NN, di-nucleotide repeats; NNN, tri-nucleotide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

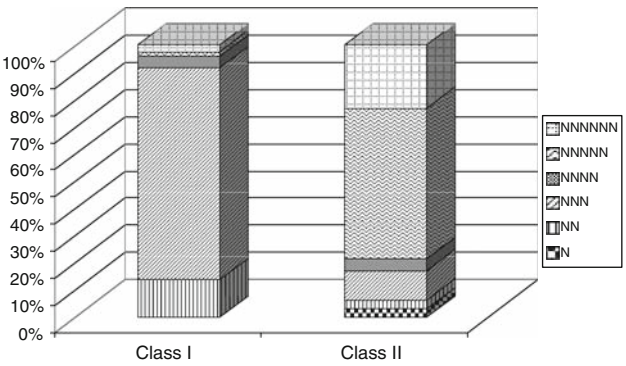


Fig. 3 Distribution of Class I and Class II repeats in newly isolated chickpea microsatellites. Class I microsatellites are with >20 nucleotides in length and Class II repeats contain perfect SSRs with >12 but <20 nucleotides in length. Among Class I repeats, tri-nucleotide repeats were abundant followed 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 ≤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 ≤ 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 that 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,

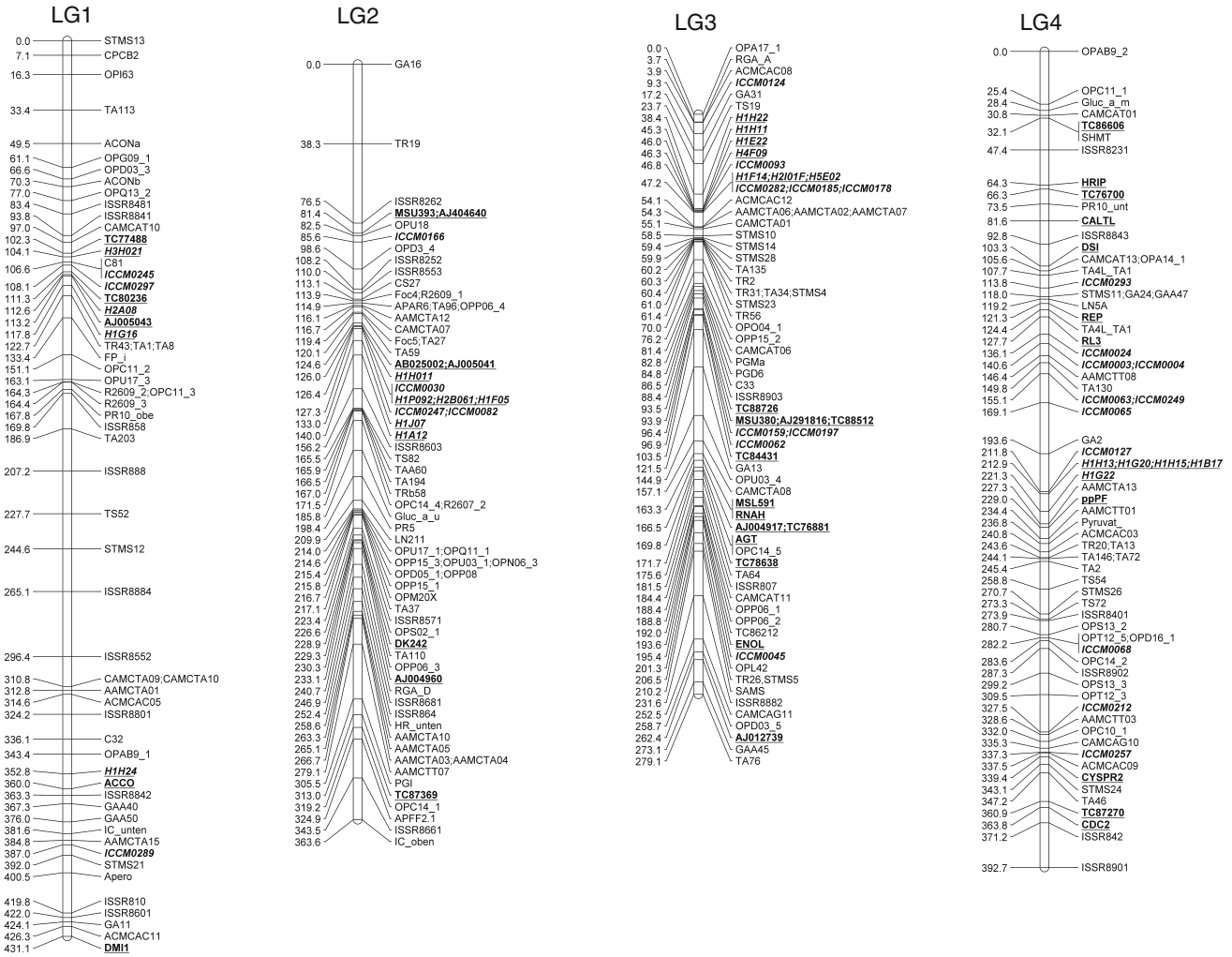


Fig. 4 An integrated genetic map of chickpea based on recombinant inbred lines of *C. arietinum* (ICC 4958) × *C. reticulatum* (PI 489777). Map was constructed using MAPMAKER/EXP 3.0 with Kosambi mapping function. Distances between the loci (in cM) are shown to the *left* of the linkage group and all 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 TC88727 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),

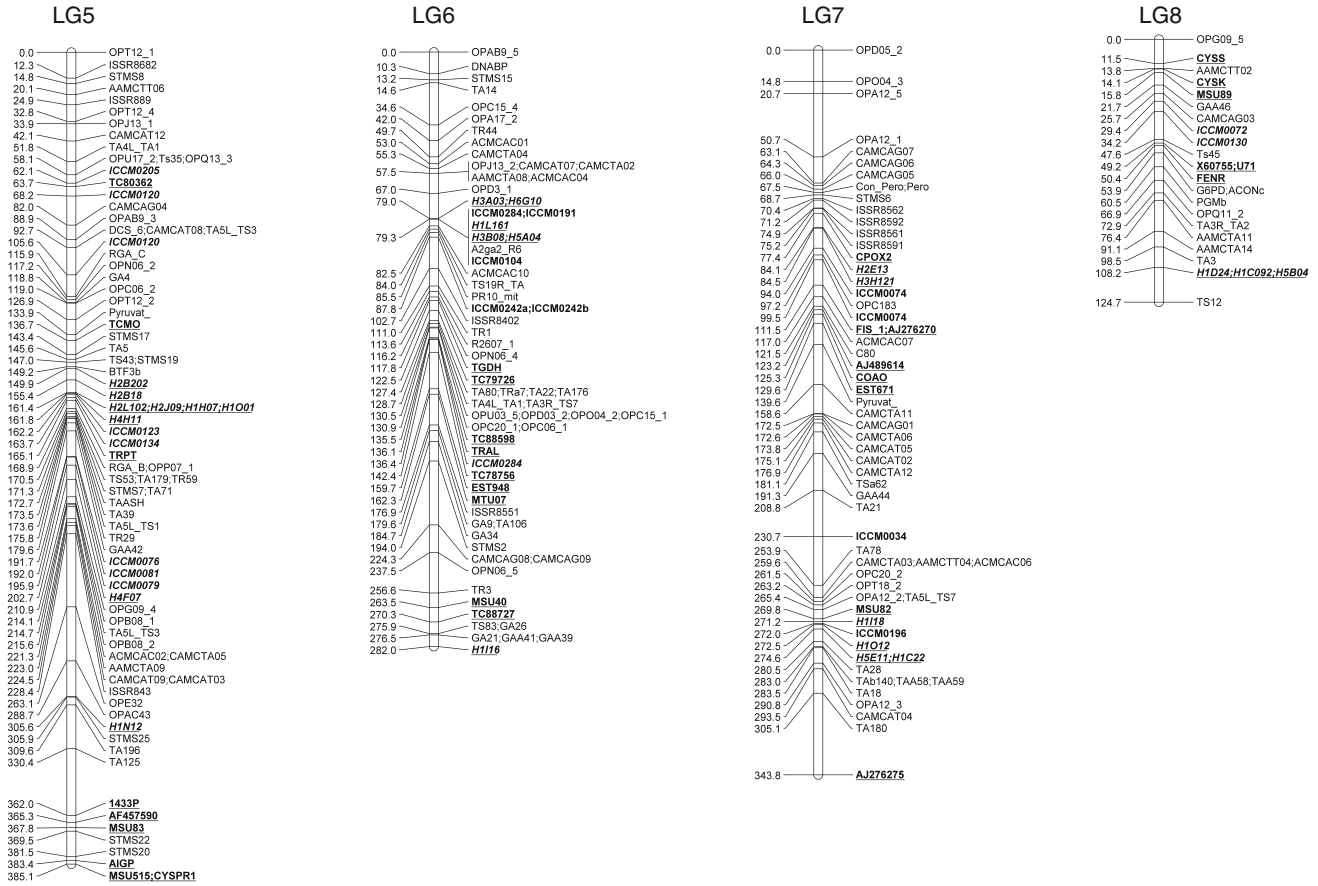


Fig. 4 continued

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.

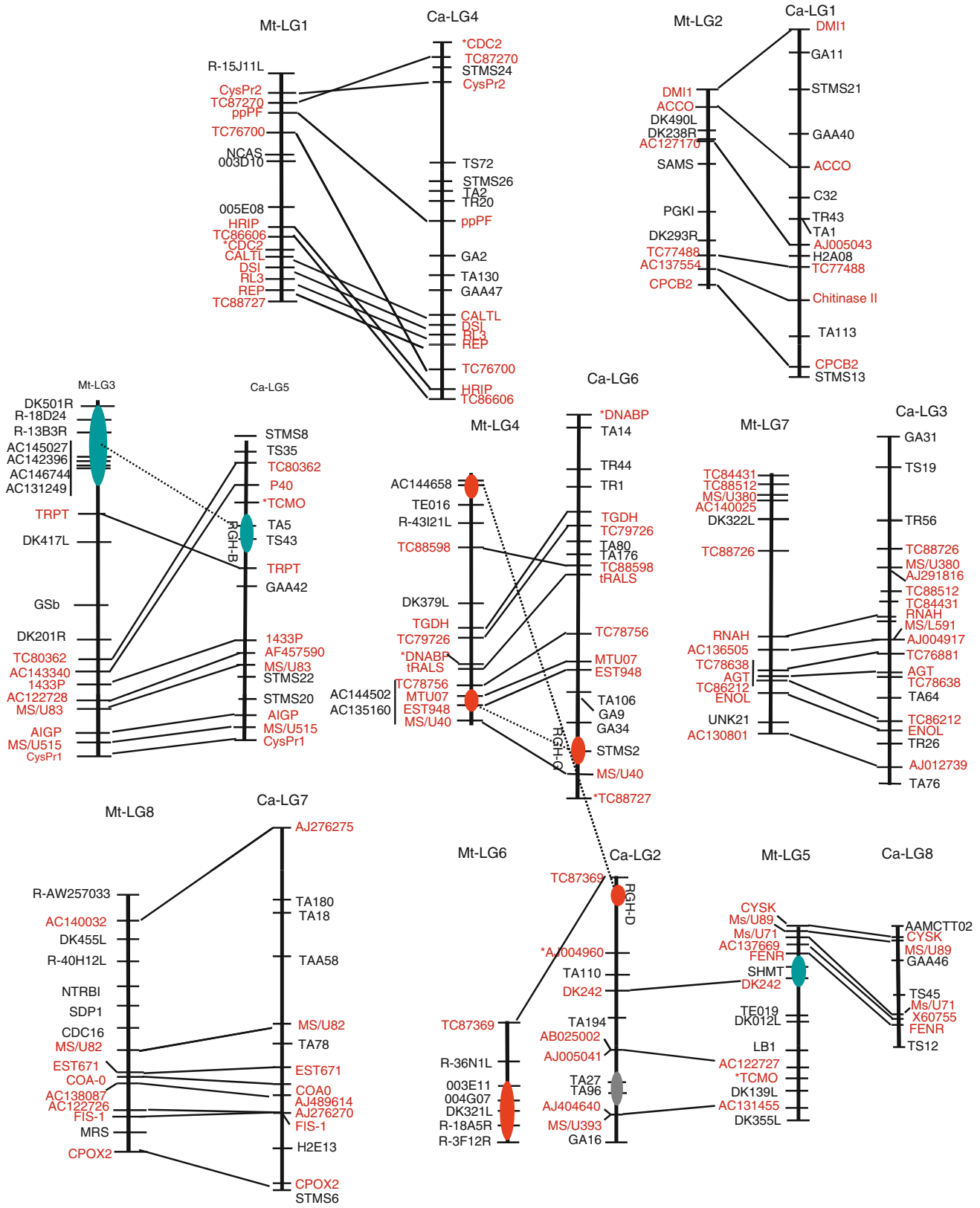
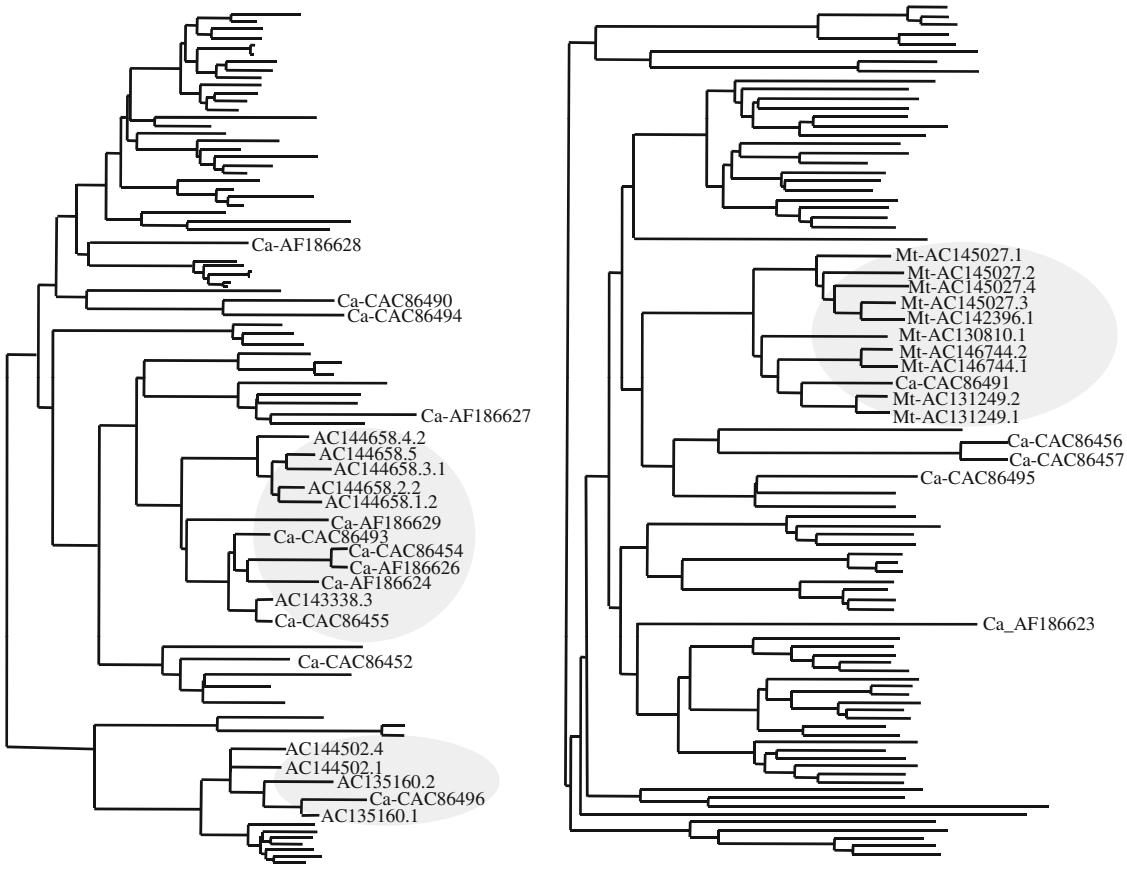


Fig. 5 Comparative map of *Medicago* and chickpea. Gene-based SNP markers (marked in *red* color) were used as the anchor markers in comparative analysis of chickpea and *Medicago* genome. The resistance gene homologs (RGH) are depicted as *oval* structures and their homologs in *Medicago* are shown with connecting *dotted lines*. *Solid lines* show the macrosynteny observed across chickpea and *Medicago* with respect to 71 gene-based markers



a TIR-NBS-LRR **b** CC-NBS-LRR

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 were 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.

Acknowledgments Thanks are due to Prathima Juvvadi, Gudipati Srivani and Abdul Gafoor for their technical assistance. Financial support from Tropical Legume I of Generation Challenge Program (GCP, http://www.generationcp.org) of CGIAR and National Fund of Indian Council of Agricultural Research of Government of India are gratefully acknowledged. The financial support by National Science Foundation Grant 0110206 to DRC is acknowledged. Council of Scientific and Industrial Research (CSIR), Government of India is acknowledged for providing fellowship to SNN and the Indian Council of Research NATP-HRD program for providing fellowship to SD. We thank Dr. Fred Muehlbauer of the USDA-ARS for providing the chickpea recombinant inbred population and genetic marker data.

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