Large-scale development of cost-effective SNP marker assays for diversity assessment and genetic mapping in chickpea and comparative mapping in legumes

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Summary

A set of 2486 single nucleotide polymorphisms (SNPs) were compiled in chickpea using four approaches, namely (i) Solexa/Illumina sequencing (1409), (ii) amplicon sequencing of tentative orthologous genes (TOGs) (604), (iii) mining of expressed sequence tags (ESTs) (286) and (iv) sequencing of candidate genes (187). Conversion of these SNPs to the cost-effective and flexible throughput Competitive Allele Specific PCR (KASPar) assays generated successful assays for 2005 SNPs. These marker assays have been designated as Chickpea KASPar Assay Markers (CKAMs). Screening of 70 genotypes including 58 diverse chickpea accessions and 12 BC₃F₂ lines showed 1341 CKAMs as being polymorphic. Genetic analysis of these data clustered chickpea accessions based on geographical origin. Genotyping data generated for 671 CKAMs on the reference mapping population (Cicer arietinum ICC 4958 × Cicer reticulatum PI 489777) were compiled with 317 unpublished TOG-SNPs and 396 published markers for developing the genetic map. As a result, a second-generation genetic map comprising 1328 marker loci including novel 625 CKAMs, 314 TOG-SNPs and 389 published marker loci with an average inter-marker distance of 0.59 cM was constructed. Detailed analyses of 1064 mapped loci of this second-generation chickpea genetic map showed a higher degree of synteny with genome of Medicago truncatula, followed by Glycine max, Lotus japonicus and least with Vigna unguiculata. Development of these cost-effective CKAMs for SNP genotyping will be useful not only for genetics research and breeding applications in chickpea, but also for utilizing genome information from other sequenced or model legumes.

Introduction

Chickpea (Cicer arietinum) is the third most important legume crop, a source of dietary protein and a beneficial agricultural crop in the semi-arid regions of the world. The development of sustainable high yielding varieties against persisting abiotic stresses and biotic stresses is a prerequisite to meet the world hunger. Molecular breeding strategies have been adopted to improvise crop improvement programmes in several crops including legumes such as soybean and common bean (see Chamarthi et al., 2011). In case of chickpea, progress in the area of implementation of markers in breeding programmes, however, has been relatively slow. Availability of limited molecular markers coupled with narrow genetic diversity has been the major constraints to hamper development of genetic maps and undertaking trait mapping studies. Marker genotyping cost is another critical factor that determines adoption of markers in breeding programmes as it involves genotyping of large number of segregating lines.

Among different marker systems, simple sequence repeats (SSRs) and SNPs are the markers of choice for genetics and plant breeding applications (Close et al., 2009; Gupta and Varshney, 2000). Although the genotyping assays are expensive and/or time consuming, the SSR markers have been an inevitable choice till date in many crop species including chickpea for large-scale characterization of germplasm collections (Upadhyaya et al., 2008), construction of genetic maps (Choudhary et al., 2009; Nayak et al., 2010; Thudi et al., 2011; Winter et al., 1999) and QTL identification (Aryamanesh et al., 2010; Santra et al., 2000). On the other hand, SNPs are biallelic and the most abundant genetic variations, which are evenly distributed in higher frequencies throughout the genome of most plant species (Allen et al., 2011; Yan et al., 2009). As these markers are amenable for automation and high-throughput approach, the genotyping costs for SNPs can be lowered down. As a result, SNP genotyping of large-scale segregating populations as well as germplasm collections becomes cost-effective for developing high-density genetic maps, genome-wide

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association mapping, marker-assisted selection (MAS) and genomic selection (GS) studies (see Varshney, 2010).

Depending on the sample size and number of markers to be analysed, medium- to high-throughput assay platforms such as BeadXpress and GoldenGate assays from Illumina Inc. (San Diego, CA) with varying set of multiplexes (96, 384, 768 or 1536 SNPs per assay) are available. Such platforms have been developed and used in several crop species such as barley (Close et al., 2009), wheat (Akhunov et al., 2009), maize (Yan et al., 2009), oil seed rape (Durstewitz et al., 2010), soybean (Hyten et al., 2008), cowpea (Muchero et al., 2009), pea (Deulvot et al., 2010) and chickpea (Choudhary et al., 2012; R.V. Penmetsa, N. Carraquilla-Garcia, A.D. Farmer, R.K. Varshney, D.R. Cook, unpublished data). These platforms, however, are cost-effective only when a minimum of 96, 384, 762 or 1536 SNPs are used for genotyping a large number of genotypes (R.R. Mir, P.J. Hiremath, O. Riera-Lizarazu, R.K. Varshney, unpublished results). In cases of molecular breeding applications such as MAS where only few markers are required for genotyping a large number of segregating lines, Illumina-based genotyping assays do not seem to be cost-effective. In such cases, Competitive Allele Specific PCR (KASPar) assay from KBiosciences (Hertfordshire, UK) (http://www.kbioscience.co.uk) seems to be an attractive marker genotyping assay (Allen et al., 2011; Cortes et al., 2011). KASPar assay is a PCR-based novel homogeneous fluorescent SNP genotyping system. It is a very flexible

assay and can be carried out on undefined set of markers (http:// www.kbioscience.co.uk/reagents/KASP_manual.pdf, http://www. kbioscience.co.uk/download/KASP.swf).

This study has been undertaken in chickpea with the following objectives: (i) to compile a large set of informative SNPs, (ii) to develop KASPar assays for cost-effective SNP genotyping, (iii) to analyse genetic diversity in the selected *Cicer* spp. accessions, (iv) to develop a second-generation genetic map based on SNPs, and (v) to determine the extent of genetic synteny of chickpea with some closely related legume species.

Results

Large-scale identification of SNPs

With an objective of developing the cost-effective KASPar assays for chickpea genetics and breeding applications, 2486 informative SNPs were compiled following four approaches (Figure 1).

Solexa/Illumina sequencing

Solexa/Illumina 1G sequencing was carried out on total RNA samples of four genotypes, namely ICC 4958, ICC 1882, ICC 506-EB and ICCC 37 of the cultivated species (*C. arietinum*), and one genotype (PI 489777) of wild species (*Cicer reticula-tum*) (Hiremath *et al.*, 2011). In total, approximately 96 million Solexa/Illumina sequence reads were generated (Table 1). After



Figure 1 A schematic representation to select the informative SNPs for conversion into KASPar assay and their utilization for genetic mapping and germplasm analysis. A total of four approaches—(i) Solexa/Illumina sequencing, (ii) tentative orthologous genes (TOGs), (iii) mining of expressed sequence tags from public domain (iv) and allele-specific resequencing—were used to identify a set of 2486 nonredundant SNPs. Although efforts were made to develop KASPar assays for all SNPs, successful assays were developed for 2005 SNPs. Screening of these assays on 58 *Cicer* spp. accessions showed polymorphism with 1341 CKAMs, including 119 CKAMs showed polymorphism with JG 11 and ICC 4958, the parental lines of 12 BC₃F₂ lines analysed. Furthermore, genotyping data were generated for 651 CKAMs on 131 RILs of the interspecific mapping populations, of which 625 CKAMs were integrated into the chickpea genetic map.

Table 1 A summary of identification of single nucleotide polymorphisms (SNPs) based on Solexa/Illumina sequencing

Genotype	Treatment	Average read length (bp)	No. of reads (million)	Total number of SNPs (≥3 read depth, frequency difference of ≥0.75 and ≤0.25)
PI 489777	_	36	26.3	10 368
ICC 4958	Drought stress	36	15.6	586
ICC 1882	Drought stress	36	22.1	
ICC 506 EB	Helicoverpa stress	36	5.2 1	4677
ICCC 37	Helicoverpa stress	36	26.8	

aligning these sequence reads with the chickpea transcriptome assembly (CaTA) comprising 103 215 tentative unique sequences (TUSs) (Hiremath *et al.*, 2011) using Alpheus pipeline (Miller *et al.*, 2008) and pair-wise comparison of parental genotypes considering selection criteria such as read depth of \geq 3 and frequency difference of \geq 0.75 and \leq 0.25 (Azam *et al.*, 2012), a total of 15 361 SNPs in 9517 TUSs were selected (Table 1). By comparing the identified SNPs across the three parental combinations, 14 454 unique SNPs were identified from 9517 nonredundant TUSs. To select nonredundant SNPs, all the 14 454 SNPs in 9517 TUSs were compared with already available SNPs developed in other studies (Gujaria *et al.*, 2011; Nayak *et al.*, 2010; R.V. Penmetsa, N. Carraquilla-Garcia, A.D. Farmer, R.K. Varshney, D.R. Cook, unpublished results). As a result, a final set of 1409 SNPs from 1409 TUSs was selected.

Mining of sanger ESTs

On the basis of cluster analysis of 27 259 Sanger expressed sequence tags (ESTs), 9569 unigenes including 2431 contigs and 7138 singletons were identified in an earlier study (Varshney *et al.*, 2009). A set of 729 contigs having ESTs from at least two genotypes and read depth of \geq 5 was explored for SNP selection. An SNP with high polymorphism information content (PIC) value (\geq 0.5) and having at least 50 bp window on either sides was considered from each contig. Finally, a nonredundant set of 286 SNPs from 286 TUSs were selected (Figure 1).

Allele-specific sequencing of candidate genes

Allele resequencing of 220 genes on a set of 2–20 genotypes representing nine *Cicer* species provided 1893 SNPs in our earlier study (Gujaria *et al.*, 2011). By considering the criteria of selecting one SNP with higher PIC value from each gene and 50-bp region on both flanking side of the SNP, a total of 183 SNPs present in 183 genes were selected. In addition, four SNPs coming from two drought-responsive genes (Nayak *et al.*, 2009) were also selected (Figure 1).

Allele-specific sequencing of TOGs

With a goal of identification of cross-species genetic markers, allele-sequencing was conducted on ICC 4958 and PI 489777 for a total of 1440 tentative orthologous genes (TOGs) (R.V. Penmetsa, N. Carraquilla-Garcia, A.D. Farmer, R.K. Varshney, D.R. Cook, unpublished data). On the basis of SNP analysis on this data set, a GoldenGate assay was developed for 768 SNPs including 733 SNPs from TOGs and 155 SNPs from other sources. Genotyping of the reference mapping population with this GoldenGate assay integrated a total of 450 SNPs including 429 TOG-SNPs onto the genetic map. On the basis of design-

able criteria for KASPar assays, a total of 604 TOG-SNPs including 410 mapped and 194 unmapped SNPs were selected (Figure 1).

In brief, a set of 2486 SNPs including 1409 SNPs from Solexa/Illumina sequencing, 286 SNPs from mining Sanger ESTs, 187 SNPs from allele-specific sequencing of candidate genes and 604 TOG-SNPs was assembled (Table S1). It is important to mention here that except for the 187 SNPs from allele resequencing of candidate genes and 604 SNPs from TOGs, the assembled SNPs were not validated earlier. Therefore, the compiled SNPs can be considered as putative SNPs.

Development and validation of KASPar assay

The selected set of 2486 SNPs was used for developing KASPar assays (Table S1). The developed KASPar assays have been designated as Chickpea KASPar Assay Markers (CKAMs). All 2486 CKAMs were used for validation on a panel of 70 genotypes (Table S2). These genotypes include 55 lines/varieties of the cultivated species (*C. arietinum*) from 11 countries, three accessions from the wild species (*C. reticulatum*) and 12 BC₃F₂ lines generated after introgressing a genomic region containing QTLs for several drought tolerance traits from ICC 4958 into JG 11 by using marker-assisted backcrossing approach (unpublished results).

A total of 2005 (80.6%) CKAMs were validated of the 2486; of these, 1341 (66.8%) CKAMs were polymorphic among 58 genotypes, 664 (33.1%) were monomorphic in the genotypes tested, and 481 (19.4%) failed to generate a useful amplification signal (Table S1, Figure 2). No attempt was made to redesign the primer for failed CKAMs. A comparison of SNP predicted *in silico* (assembled) and alleles called in the KASPar assays for the 2005 validated CKAMs showed 100% consistency. The PIC values for the polymorphic CKAMs varied between 0.02 and 0.50 with an average of 0.12 (Table S1).

Analysis of CKAMs on the parental genotypes of the mapping populations showed higher polymorphisms in interspecific (*C. arietinum* × *C. reticulatum*) crosses than in intraspecific (*C. arietinum* × *C. arietinum*) crosses. Among interspecific crosses, maximum number of polymorphisms (930 CKAMs) was observed in the reference mapping population (ICC 4958 × PI 489777) followed by crosses segregating for *Helicoverpa* resistance, that is, ICC 3137 × IG 72953 (620 CKAMs) and ICC 3137 × IG 72933 (276 CKAMs). In the case of the intraspecific crosses, maximum polymorphism was identified between Arerti and ICC 4958 (159 CKAMs), which represent parents of MABC population for improvement of chickpea for drought tolerance. The polymorphism status of CKAMs between different parental combinations is given in Table 2.



Figure 2 Snapshots showing SNP genotyping with KASPar assays. Different possible scenarios of SNP genotyping in germplasm collection (a–c) and interspecific RIL mapping population (d–f) have been shown. Marker genotyping data generated for each genotype were used for allele calling using the automatic allele calling option. Allelic discrimination (two alleles) for a particular marker in the genotypes examined has been shown on a scatter plot with axes 'X' and 'Y'. The snapshot (a) shows monomorphic pattern, that is, occurence of only one allele (blue spots) for CKAM0790 marker. In the snapshot (b), polymorphism pattern, that is, occurence of two alleles (blue and red spots) for CKAM1175 marker in almost equal proportion in the germplasm collection, has been shown. All germplasm accessions show homozygosity for the corresponding alleles, and one accession shows missing data (pink spot). The snapshot (c) shows heterozygosity, that is, occurence of both alleles (green spots) for CKAM1802 marker in nine germplasm accessions in addition to occurence of two alleles in homozygous condition in several accessions (blue and red spots) and three accessions with missing data. The snapshot (d) shows occurence of one allele (red spots) in majority of RILs, except two RILs with the other allele (blue spots) and two RILs with missing data (brown spots). Two clusters of about 50% of RILs each with one allele (blue and red spots) along with two RILs with missing data in majority of the lines.

Genetic diversity analysis

Genotyping data obtained for all 1341 polymorphic CKAMs on 58 chickpea genotypes (Table S3) were used for assessing the genetic diversity and understanding their genetic relationships. Genetic dissimilarity between different pairs of genotypes varied from 0.02 (ICC 7554 and ICC 3137) to a maximum of 0.74 (PI 48977 and IG 72933) with a mean of 0.37. On the basis of the dissimilarity data and UPGMA method, a hierarchical cluster analysis was performed on all the 58 genotypes using DARwin V5.0.128 software (Perrier *et al.*, 2003) (Figure 3). In the dendrogram, the genotypes were grouped into two discrete major clusters: the Cluster-I comprised only two wild species (*C. reticulatum*) genotypes (IG 72953 and PI 489777), and the Cluster-II

comprised 56 genotypes of *C. arietinum* species, with an exception of one genotype IG 72933, belonging to *C. reticulatum* species, that branches off sequentially at the base of the dendrogram closer to the Cluster-I. In the Cluster-II, few landraces and cultivars from India (Annigeri, ICC 4593, ICCC 37, ICCV 05530), Ethiopia (Arerti), Mexico (ICC 12037) and Israel (ICC 7571) formed a clear outlying group, with the remaining 48 genotypes clustering into two main groups—the Cluster-IIa and the Cluster-IIb. The Cluster-IIa has 13 genotypes that mainly belong to Afghanistan (2), Chile (1), Ethiopia (1), Iran (4), Portugal (1), Turkey (1), Mexico (1) and former USSR (2). The Cluster-IIb is comprised of 35 genotypes, of which 33 belong exclusively to India, one to Iran and one to Cyprus. Within the Cluster-IIb,

Parental genotypes of		Marker data available for	Polymorphic
segregating population	Features of segregating populations	both parental lines	markers (%
Interspecific mapping populations (Cicer	arietinum × Cicer reticulatum)		
ICC 4958 × PI 489777	International reference mapping population	1900	930 (48.9)
ICC 3137 × IG 72953	Helicoverpa resistance	1744	620 (35.6)
ICC 3137 × IG 72933	Helicoverpa resistance	1839	276 (15.0)
Intraspecific mapping populations (C. ari	etinum \times C. arietinum)		
ICC 4958 × ICC 1882	Drought tolerance and root traits	1966	148 (7.5)
ICC 283 × ICC 8261	Drought tolerance and root traits	1960	58 (3.0)
ICC 6263 × ICC 1431	Salinity tolerance	1966	54 (2.7)
JG 62 × ICCV 05530	Fusarium wilt (FW), Ascochyta blight (AB), Botrytis grey mould (BGM)	1947	32 (1.6)
Annigeri × ICC 4958	Root traits	1939	125 (6.4)
ICC 506-EB × Vijay	Helicoverpa resistance	1969	27 (1.4)
Marker-assisted backcrossing (MABC) po	pulations		
Arerti × ICC 4958	Introgressing root trait QTL	1964	159 (8.1)
Ejere \times ICC 4958	Introgressing root trait QTL	1967	140 (7.1)
ICC 97105 × ICC 4958	Introgressing root trait QTL	1981	147 (7.4)
ICCV 10 × ICC 4958	Introgressing root trait QTL	1982	136 (6.8)
ICCV 95423 × ICC 4958	Introgressing root trait QTL	1984	124 (6.2)
JG 11 × ICC 4958	Introgressing root trait QTL	1986	119 (6.1)
DCP 92-3 × ICC 4958	Introgressing root trait QTL	1982	137 (6.9)
KAK 2 × ICC 8261	Introgressing root trait QTL	1967	40 (2.0)
ICCV 92318 (Chefe) × ICC 8261	Introgressing root trait QTL	1971	37 (1.9)
C 214 × ILC 3279	Introgressing AB resistance	1963	53 (2.7)
C 214 × WR 315	Introgressing FW resistance	1934	15 (0.8)
Phule G5 \times Vishal	Introgressing FW resistance	1954	27 (1.4)
Phule G12 × WR 315	Introgressing FW resistance	1980	26 (1.3)
JG 74 × JG 14	Introgressing FW resistance	1959	51 (2.6)
JG 74 × WR 315	Introgressing FW resistance	1970	35 (1.8)
Annigeri × WR 315	Introgressing FW resistance	1934	34 (1.8)
Annigeri × ICCV 10	Introgressing FW resistance	1935	29 (1.5)
Marker-assisted recurrent selection (MAR	RS) mapping populations		
JG 130 × ICCV 05107	Enriching drought tolerance alleles	1977	31 (1.6)
ICCV 2 × JG 11	Enriching salinity tolerance alleles and early flowering	1973	30 (1.5)
JG 11 × ICCV 04112	Enriching drought tolerance alleles	1975	27 (1.3)

Table 2 CKAMs-based polymorphisms in some segregating populations of chickpea

SNP, single nucleotide polymorphisms.

ICC 1882 is separated from the rest of the genotypes. Overall, the clustering pattern showed a distinctive grouping of genotypes into separate clusters based on their geographical origin and also based on species background (Figure 3a).

Relationship of BC₃F₂ lines with the recurrent parent

A set of 12 BC₃F₂ generated after introgressing a genomic region containing QTLs for several drought tolerance–related traits in JG 11 variety after maker-assisted backcrossing (MABC) with ICC 4958 genotype were tested with all 2005 CKAMs to assess the genome recovery of JG 11 parent in the MABC lines. As a result, 108–117 markers showed similarity between the given BC₃F₂ line and JG 11 (Table S4). In brief, the tested BC₃F₂ lines showed genome recovery of JG 11 from 91% (BC₃F₂_170, BC₃F₂_187, BC₃F₂_195) to 98% (BC₃F₂_120, BC₃F₂_248) (Figure 3b). Furthermore, comparison of the BC₃F₂ lines with ICC 4958 showed the presence of allele of ICC 4958 in the BC₃F₂ lines for 10 CKAMs (CKAM0017, CKAM1802, CKAM1444, CKAM0042, CKAM0043, CKAM1641, CKAM1963, CKAM1933, CKAM1709 and CKAM1604). These markers seem

to be the potential mappable markers in the genomic region transferred from ICC 4958 to JG 11.

Second-generation genetic map of chickpea

The reference mapping population (ICC 4958 \times PI 489777) was targeted for integrating CKAMs in the genetic map of chickpea. In this context, a total of 930 CKAMs showed polymorphism between the parental genotypes. The polymorphic CKAMs include 503 Solexa/Illumina SNPs, 377 TOG-SNPs and 50 candidate gene sequencing-based SNPs. As genotyping data were already available on the reference mapping population for all 371 TOG-SNPs via GoldenGate assay, only 118 markers representing all the linkage groups were selected for genotyping via KASPar assays mainly for quality control. Therefore, genotyping data were generated on the reference mapping population for a total of 671 CKAMs (503 Solexa/Illumina SNPs, 50 candidate genes SNPs and 118 TOG-SNPs). High-guality genotyping data, however were generated for 651 CKAMs (492 Solexa/Illumina SNPs, 46 candidate genes SNPs and 112 TOG-SNPs). Analysis of genotyping data showed Mendelian segregation ratio for a total



Figure 3 Genetic relationships in germplasm and BC_3F_2 lines. Hierarchical clustering of chickpea accessions was carried out based on UPGMA using DARwin. The part (a) of the figure shows phylogenetic relationships among 58 germplasm lines based on allelic data for 1341 CKAMs. All the genotypes analysed could be grouped into two main clusters (I and II). The Cluster-I comprised two wild species genotypes (*Cicer reticulatum*) and Cluster-II comprises accessions mainly of *Cicer arietinum* species coming from 11 different countries. The part (b) of the figure shows genetic dissimilarity of 12 BC_3F_2 lines with JG 11, the recurrent parent.

of 525 markers, and the remaining 126 (19.3%) markers exhibited segregation distortion (Table S5) owing to skewed occurrence/distribution of one of the two parental alleles or high percentage (60%) absence of allele data (Figure 2d,e,f).

As genotyping data were available for a total of 429 TOG-SNPs via GoldenGate assay (R.V. Penmetsa, N. Carraquilla-Garcia, A.D. Farmer, R.K. Varshney, D.R. Cook, unpublished data) and high-guality genotyping data were generated for 112 TOG-SNPs from this set via KASPar assay in the study, the genotyping data for the remaining 317 TOG-SNPs generated via GoldenGate assay were added to the data set of 651 CKAMs. In addition, genotyping data were also assembled for (i) 61 genic molecular markers (GMMs) including 31 CGMMs, 15 CIS-Rs and 15 ICCeMs (Gujaria et al., 2011), and (ii) 335 legacy markers including SSRs from different sources (H-series, ICCMs, CAMs, SSRs-Frankfurt University, ISSRs), SNaPshot assays-based SNPs, CAPS, DArTs (Thudi et al., 2011), and RAPDs. In summary, genotyping data were compiled for 1364 markers and used for constructing the genetic map. The most likely order of the markers was determined based on the verified position of

GMMs (Gujaria *et al.*, 2011), TOG-SNPs (R.V. Penmetsa, N. Carraquilla-Garcia, A.D. Farmer, R.K. Varshney, D.R. Cook, unpublished data) and legacy markers (Nayak *et al.*, 2010; Thudi *et al.*, 2011). By using JOINMAP v 4.0 program (Van Ooijen *et al.*, 2006), a total of 1328 markers were mapped onto eight linkage groups (CaLG01–CaLG08) as per the nomenclature given in Thudi *et al.* (2011). The developed genetic map spans a total of 788.6 cM distance with an average intermarker distance of 0.59 cM (http://cmap.icrisat.ac.in/cmap/sm/cp/hiremath/) (Figure 4). Details about different type of markers integrated in this map are given in Table 3. The number of markers per linkage group varied from 107 (CaLG08) to 255 (CaLG04). The total distance of individual linkage groups ranged from 70.5 (CaLG08) to 116.6 cM (CaLG01).

Uneven distribution and clustering of markers was observed along the length of all the chickpea linkage groups in this map. Occurrence of both minor (3–5 cM) and major (>5 cM) gaps between adjacent loci was observed (Table 4). A detailed observation revealed extensive clustering of CKAMs and TOG-SNPs near the telomeric regions of CaLG03, CaLG06, CaLG07 and

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CaLG01 (A)	CaLG01 (B)	CaLG01 (C)	CaLG01 (D)	CaLG02 (A)	CaLG02 (B)
0.0 - GA11	30.5 - TOG898575	ISSR858	90.5 - CKAM0414	0.0 - H4H12	ISSR8561
1.7 TOG905558	30.9 OPAB9-1	TOG894619	90.8 TOG903590	5.2 / DK225	50.3 AJ005041
2.9 DMI1	31.8 CKAM1643	61.4 TOG897323	91.2 TOG902578	10.3 1 TOG898594	AB025002
4.3 4.5	32.1 CKAM0934 33.5 ICCM0289	TOG899509 TOG902765	92.1 CKAM1562 93.9 TOG895899	15.2	50.6 ICCM0082
6.0 TOG901896	33.6 TOG930652	TOG903021	94.1 TOG898532	CKAM1287	50 0 M ICCM0247
6.2 CISR008	33.9 CKAM1971	62.0 CCM0297	94.5 [/]	17.0 CKAM1544	51.0 H1P092
8.1 / TOG896385	34.7 TOG894864	62.7 TA8	= CKAM1097	CKAM1819	51 1 JULT ICCM0030b
8.8 TOG901542	35.8 AAMCTA15 37.7 CKAM1968	62.9 TA1 63.9 TR43	96.0 ^J CKAM1309 CKAM1863	17.1 CKAM1695	51.4 - CGMM098
11.0 CKAM0888	CKAM0752	TOG898978	TOG895054	18.0 CKAM0835	51.5 CGMM010
12.0 CKAM0541	CKAM0016	64.4 ICCM0245	96.2 TOG898024	18 2 CKAM0723	52.4 - CKAM1101
14.8 TOG896074	39.1 CKAM0360	64.8 TOG910457	CKAM1701	// ICKAMITI90	52.6 CGMM053
15.7 CKAM0947	CKAM1064 CKAM1194	65.6 H = Mt6879803 CGMM051	96.9 97.0 CKAM1786	18.3 CKAM1254 19.6 TOG895817	CKAM1975
16.6 STMS21		66.6 TOG918864	TOG898468	21.0 TOG906560	52.7 CKAM0874
17.2 CKAM1140	40.7 TA203	66.7 - H3H021	97.4 TOG947627	21.7 JICCeM035	CKAM0891
ICKAM1645	42.2 CKAM1074	67.9 H1G16	99.4 CKAM1893	22.8 TOG917884 23.8 Gm2076345	52.9 CKAM1378
18.1 CKAM1424 23.1 TOG894483	42.2 CKAM2003	68.8 TC77488 70.2 TOG897376	99.8 CKAM0651 CKAM1217	23.8 Gm2076345 25.1 CKAM1564	53.1 CKAM0759
23.8 CKAM1904	42.3 CKAM1638	70.8 ISSR8841	100.5 CGMM031	25.4 CKAM0561	53.7 CKAM1575
CKAM0928	43.3 CKAM0307	74.1 ISSR8552	101.2 CKAM0745	25.7 TOG897285	53.8 - CKAM1855
CKAM0784	44.5 cpPb678248	75.0 TOG897239	101.6 CKAM1338	27.8 TOG895245	I GRAMITZIZ
23.9 CKAM0207	45.0 ACCO	76.1 ISSR8481 76.2 TOG897728	102.8 - CKAM1767 102.9 - CKAM0170	29.3 GA16 29.8 TOG895030	54.0 CKAM0793 54.1 CKAM1719
CKAM1914	45.5 TOG895237	77.9 CISR064	103.1 CKAM1166	30.0 TR19	54.6 CKAM1333
CKAM1474	45.7 TOG894516	80.6 ISSR888	103.2 · CKAM0742	30.7 - ICCeM033	54.8 - TOG895412
CKAM1082	46.0 R2609-3	81.0 TA113	105.6 CPCB2	32.4 ISSR8262	54.9 CKAM0892
26.1 CKAM1404 CKAM1515	46.4 - TOG902030	82.5 ACONa	106.2 CGMM018 109.7 ICCeM055	32.7 TOG915502 TOG935983	CKAM1647
26.6 CKAM0463	H1H24	83.0 TOG938507	112.7 STMS13	34.0 - CKAM1273	55.9 CKAM1242
26.7 CKAM1054	48.3 TOG902569	83.5 TOG905474	116.6 ICCeM063	34.1 CKAM0320	CKAM1494
29.0 CKAM1593	48.9 GAA40			35.8 CKAM1428	56.0 TOG896190
29.5 CKAM1774 CKAM1302	49.1 • TOG897333 49.7 • R36082y	CaLG03 (B)	CaLG03 (C)	38.1 CKAM1282 38.3 CKAM1436	56.1 CKAM1844 56.2 TOG915207
	50.7 TOG961504	042000 (2)	. ,	39.4 CGMM015	56.4 CKAM0019
CaLG03 (A)	50.8 ISSR8842	28.8 1 CKAM0232	46.5 ICCeM018 46.6 CaM0658	39.5 CKAM0901	56.5 TOG919333
	51.8 TOG905290	28.9 CKAM0560		39.6 CKAM0173	56.6 CKAM0391
0.0 - CKAM0314	52.2 C32 54.0 TOG898940	30.6 CKAM0554	46.7 H1F14	40.0 ISSR8553 40.8 CKAM1277	CKAM1121
3.6 \ / CKAM0592	54.1 ISSR8801	30.6 30.9 CKAM0520	ICCM0093c	41.3 CKAM0755	CKAM0948
6.4 CKAM0441	TOG912303	ICKAM0257	47.2 Gm2077934	TOG894061	56.8 CKAM0796
6.8 CKAM0692	54.8 TOG894192	31.3 CKAM0174	47.8 H2I01F	CKAM1824	CKAM1080
8.6 CKAM0265	55.6 H2A08	33.1 33.4 CKAM0586	48.4 TOG960856	41.4 CKAM0325 CKAM1763	57.0 TOG895601 TOG912685
9.3 CKAM0612	56.9 TOG900394	33.4 33.6 CKAM0586 CKAM0213	48.6 4 F ICCM0185	CKAM1537	TOG901841
9.4 CKAM0588	57.2 TC80236	34.6 ICCM0282c	48.8 Gm2123242	42.1 CGMM012	TOG897346
9.6 CKAM0639 9.6 CKAM0493	58.2 TOG901047	35.4 CKAM0191	50.1 Gm2113547 51.0 MSL591	42.5 AJ404640	57.1 TOG903853
11.1 CKAM0422	58.7 AJ005043	35.6 CKAM0660	H1H11	42.6 MSU393 42.9 TOG895374	TOG915900 TOG918275
11.7 CKAM0636	TOG905379	CKAM0557	51.6 TOG910318	43.5 PGI	TOG923111
12.2 CKAM0291	^{59.0} TOG913108	35.7 CKAM0628	51.7 TOG937303	ISSR8252	57.2 ISSR8592
12.3 CKAM0438 13.9 CKAM0375	59.1 TOG897499	CKAM0474	52.3 5 5 CGMM039 52.7 TOG898975	CGIVIIVI037	58.3
CKAM0358	59.2 CaM0403 59.4 AGL112	CKAM0573	52.9 TOG897513	44.6 TA194 44.8 TOG898285	58.4 CKAM0791
14.0 CKAM0622	59.6 TOG897579	35.8 CKAM0455	53.6 - 53.6 - 55 H5E02	44.9 TAA60	58.6 CKAM0820
- CKAM0596	59.9 CGMM042	36.0 TS19	54.2 TR2	45.3 ICCM0166a	58.7 CKAM1340
15.5 EKAM0185		36.6 CKAM0295	54.7 TOG894002 55.3 Tp6849720	45.9 TS82	59.1 CKAM1312
17.4 CKAM0356		36.7 CKAM0272	RNAH	46.4 47.4 CGMM007	59.7 CGMM030 60.4 CGMM043
10.2 CKAM0681		36.8 STMS28	56.1 AJ004917	47.7 TA96	61.4 H1J07
18.3 CKAM0525		37.3 TA135	56.6 TC76881	47.8 TOG895747	62.8 DK242
20.8 CKAM0499 21.8 CKAM0571		CKAM0512	56.9 CGMM014 58.0 TOG894920	48.0 TA59	63.3 ISSR8603
21.8 CKAM0571 22.1 CKAM0673		37.7 - TR31	58.4 TOG894263	48.1 TOG924480 48.2 TA27	64.9 H1A12 66.2 CKAM0634
22.5 CKAM0553		38.0 - STMS14	58.8 TOG894351	48.9 CKAM1738	66.6 CKAM1382
		R3608-3	59.2 CISR111	50.0 H2B061	68.2 CKAM0246
		38.4 CKAM0294	60.0 · AGL23 61.0 · AGT		CKAM1257 69.8 - CKAM1425
		38.5 - CKAM0638 38.6 - ICCM0124	62.2 GA31	CaLG03 (D)	69.8 - CKAM1425 CKAM1484
		38.6 ICCM0124 39.5 STMS10	63.9 TA64	. ,	70.3 CKAM1820
		39.8 CKAM0488	65.0 Ca21249	AGL94	71.3 CKAM1669
		39.9 CKAM0163	65.4 TOG903883 65.7 TC78638	71.4 ICCM0045 TOG894927	CKAM1747 CKAM1783
		40.4 CKAM0261 40.6 H4F09	68.7 TOG896159	72.1 CISR108	CKAM1264
		40.8 CKAM0387		74.6 cpPb491550	71.4 CKAM1348
		41.5 CKAM0468		75.2 CKAM0645 75.8 TC86212	CKAM1136
		41.7 MSU380		75.8 77.9 ENOL	CKAM0811
		42.4 ICCM0159 42.9 TC88726		79.6 TR26	CKAM1717
		43.2 TC88512	CaLG03 (E)	81.1 cpPb677322	72.1 CKAM1607
		44.2 ICCM0178	Π		CKAM1678
		44.6 ICCM0062 45.0 ICCM0197a			72.2 CKAM0212 CKAM1463
		45.0 ICCM0197a 45.6 H1E22		85.7 TOG908483	72.5 CKAM0921
		45.7 ICCeM028	97.1 TA76	86.9 TOG902798	CKAM0029
		AJ291816		87.9 AJ012739 cpPb488939	74.7 TA110 76.0 AJ004960
		46.0 TOG905013 TOG917730		10pr 0400339	76.0 AJ004960 76.7 CGMM013
		TC84431	101.9 - GAA45		77.7 TOG903882
				—	82.9 ISSR864
					85.6 TOG903783 88.8 ISSR8661
					88.8 92.9

Figure 4 A second-generation genetic map of chickpea. The genetic map based on reference mapping population (ICC 4958 × PI 489777) is comprised of a total of 1328 marker loci including newly developed 625 CKAMs, 314 tentative orthologous genes (TOGs)-SNPs (R.V. Penmetsa, N. Carraquilla-Garcia, A.D. Farmer, R.K. Varshney, D.R. Cook, unpublished data) and 389 published marker loci in earlier studies. Eight different linkage groups are shown and designated as CaLG01 to CaLG08. For the visualization of marker names and orders, each LG has been split into 2–5 parts. For instance, four LGs, namely CaLG02, CaLG07 and CaLG08, are split into A and B parts; three LGs, namely CaLG04, CaLG05 and CaLG06, are split into A, B and C parts; the CaLG01 is divided into A, B, C and D parts; and CaLG03 is divided into A, B, C, D and E parts. Map distances (cM) are presented on the left side of the bars, and corresponding markers are listed on the right side of the bars. Each marker class is colour coded as follows: *green*, CKAMs; *red*, TOGs-SNPs; *black*, CGMMs; *dark blue*, CISRs; *golden yellow*, ICCeMs; *light blue*, DArTs; and *brown*, legacy markers. High resolution genetic map is available at http::cmap.icrisat.ac.in/cmap/sm/cp/hiremath/.

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CaL 0.0	.G04 (A)
7.3 —	ISSR842
10.9 12.6 14.1 14.4 17.2 20.1 22.1 23.0 24.1 25.7 26.4 25.7 26.4 25.7 28.5 28.7 30.4 30.5 30.6 30.8 30.9	CDC2 TOG899684 TC87270 TOG897469 cpPb675919 TOG894099 cpPb491143 TOG896166 TA46 TOG902037 CKAM1037 CKAM1933 CGMM089 TOG895163 ICCM0257 CKAM1076 TOG945905 ICKAM179 ICKAM179 ICKAM179 ICKAM179 ICKAM179 ICKAM179 ICKAM1691 CKAM1742 ICKAM1598 ICKAM1618 CKAM149 CKAM1405 CKAM1

CaLG	04 (B)	Cal
36.1 \	CGMM079	64.1
36.3	TOG897521	64.2
36.4	ICCM0212c	64.6
36.9 37.3	r STMS26 r TS72	65.1 65.2
38.2	CKAM0476	65.3
38.5	CKAM1517 CKAM1280	65.7
38.6	CKAM1248	66.1
39.1	TOG895229 TOG913370	66.7 67.1
39.8	TA13	67.3
39.9 [/] /=	4TR20 TOG917721	67.4
40.6	CKAM0798	67.7 - 67.8 -
40.9	H1G22	68.1
41.6	TA146	68.5
41.9	CKAM1364	68.8
42.9	TA72 TOG922889	69.7 - 70.0 -
43.4	GA2	70.1
43.6	• TOG906662	70.5
43.9	• TOG913321	71.0
44.4	• TA130 • TOG900323	71.1 ⁻ 71.6 ⁻
45.6	ppPF	71.7
45.7	TOG897326	73.0
46.3	CKAM0244	73.4 ⁻ 73.7 ⁻
48.4	CaM0507	73.9
49.1	1H1H13 H1G20	74.0
49.8	ICCM0127	74.1
49.9	+ tk_515 HTOG927609 ICCM0249	74.4 ⁻ 74.6 ⁻
50.4	TOG895060 TOG903155	74.7
51.0	TOG908504	74.8
51.1	TOG902016 TOG899516 TOG901045	74.9
51.2	TOG896733	75.1
51.6	ICCM0068	75.2
52.3 - 52.5 -	CKAM0770 CKAM1576	75.3 ⁻ 75.4 ⁻
52.7	CKAM0847 CKAM1469	76.5
52.8	CKAM1088	77.5
52.9	CKAM1636 CKAM1198	78.0
	CKAM1384 CKAM1790	78.2 - 78.3 -
53.0	CKAM0869 CKAM0875	78.6
53.1	CKAM1210	78.8
53.3 53.4	CKAM1359	78.9 ⁻ 79.3 ⁻
	CKAM1589 CKAM1999	79.4 ⁻
53.5	CKAM1581 CKAM1458	
	CKAM1185 CKAM1754 CKAM1690	79.7 ⁻ 79.9 ⁻
53.7	CKAM1447	80.0
53.8 ⁻	CKAM1587	80.2
53.9	CKAM1099 CKAM1826	80.3
54.1	CKAM0812 CKAM0978	81.0
	CKAM1056 CKAM1328	82.3
54.2	CKAM1356 CKAM0750 CKAM1832 CKAM1788	83.1 ⁻
	CKAM1832 CKAW1788	83.2
54.4	ICKAM1796 CKAM1481	84.2 ⁻
54.5	· CKAM1417 · H1H15	84.3
55.2 ⁻ 55.4 ⁻	Gm2084815	84.6 ⁻ 84.7 ⁻
56.2	CKAM0885	85.0
56.8 ⁻	ICCeM015	85.1
57.0 - 57.7 -	+ CKAM1330 + CKAM1455 CKAM0966	85.2
58.2	ICCM0063	86.2
58.9 ⁻	CKAM0769 CKAM1191	86.5
59.0 -	CKAM0923 CKAM1129	88.0 ⁻ 89.0 -
59.1 ⁻	TOG935579	89.0 89.1
59.5 ⁻	CKAM0814 CKAM0701	89.2
59.8	CKAM1432 CKAM1721	89.9 ⁻ 90.2 ⁻
59.8 - 59.9 -	CKAM1623	90.2 - 90.5 -
60.3	CKAM1984	91.7
	CKAM1726 CKAM1220	91.9
60.7	CKAM1488 CKAM0443 CKAM0920 CKAM1624	92.0 ⁻ 92.2 ⁻
61.8	CKAM0787	92.2 92.5
61.9	CKAM1535	
62.5 ⁻ 62.7 ⁻	CKAM1177 CKAM1354 CKAM0737	
63.0	TOG910718	

LGC	04 (C)
	- CKAM1062 CKAM1681
E	CKAM0747 CKAM1045
	TOG897715 ICCM0004
	TOG895578 RL3
E	LG99 TOG903593
	TOG896007 CKAM0649
	CKAM1808
FI	CKAM1394 CGMM036
	CISP5_dcaps CGMM074
	· ICCM0293 · TOG895535
E	CKAM1270
	CKAM1760 CKAM0944
	CKAM1619 CKAM1508
E	CKAM1353
F	CKAM0646
	CKAM1770 CKAM1146
	• TOG900006 • CKAM0826
	• TOG896540 • CKAM1716
E	· CKAM1012 · TOG901549
	CKAM1450 CKAM1010 CKAM0285
	CKAM1776 CKAM0990
	CKAM0897 CGMM044
	+ DSI + TOG908289 TOG903969
	CALTL
	• TOG895877 • TOG904041
	1TOG894357 TOG898075 1TOG896981 TOG922990
	CKAM1205
	TOG910388 TOG916001 ICCeM036 TOG900053
	• TOG906507 • GAA47
	• GA24 • STMS11
	TOG901184 TOG897003 TOG898887
	TOG910862 CKAM1502
	CKAM0979 CKAM1501
	CKAM0434 TOG896495
	· CGMM002 · TOG910203
	CKAM1471 CKAM1096
	TOG894321 TOG896976 TOG901729 TOG930121
	1TOG910567 CKAM1349 1TOG896911
	• TOG896066 • TC86606
	CKAM1703 CKAM0338
	CKAM0000 CKAM1043 CKAM0925
	CKAM1063
	CKAM1232 TOG899054
	+ TOG908396 + HRIP
	TOG912749 TC76700

CaLG	605 (A)						
0.0 - TOG896852 CGMM023							
0.0							
5.1	- ISSR8682						
0.1							
7.6	— cpPb326684						
9.1	- Ca14402150174189						
10.4	TOG913042						
10.5	STMS20						
11.9	— STMS8						
12.9	— CGMM038						
14.5	— ISSR889						
16.7 \	CKAM0196						
17.5	AIGP						
19.5	/ CKAM1193						
19.6	CKAM1640						
21.6	CKAM1289						
22.2	CKAM1812						
23.7	LG103 cpPb488668						
24.8	CKAM0338						
25.4	CKAM1946						
25.6	7 TOG901215						
26.4	TOG895760						
27.8	CKAM0732						
28.5	CKAM1422						
29.5	/ MSU83						
31.3	TOG903079						
32.2	7 TOG905363						
33.1	CKAM1000						
33.2	TOG916130						
33.5	CKAM1042						
34.5	TOG918373						
36.4	CKAM1632						
36.9	- CKAM1158						
37.5	- CKAM0310						
38.6	CKAM0941						
38.7	TOG900269 TOG899751						
39.7	CpPb489565						

Figure 4b (Continued)

Large-scale	development	of	chickpea	KASPar	assays	9	
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0.0 2.6 -2.9 -3.3 -

3.4

3.5

4.4 5.7 6.7 7.0 8.0 8.4 8.7 9.9 10.1 10.2 10.4 10.5

10.8

10.9 11.1 11.2

11.3 13.1 13.4 14.5 15.2 16.2 16.4

18.6 18.7

18.9 19.2

19.3

19.4

19.5

19.6 19.7 19.8 19.9 20.0 20.1

20.2 20.7

21.1 21.2

21.7 22.7 23.7 25.8 27.3 27.9 28.0

28.1 28.9

29.8

30.9

31.7 32.0

32.2 32.3 33.6 34.9

CaLG	05 (B)	CaLG05 (C)
41.0 \	/ M1433P	80.1 TA71
42.5	Gm2091985 TOG894358 TOG895900 CISR022	80.3 TA179 80.6 TRPT
42.5	TOG896702 TOG904656	81.0 STMS19
43.7	- Ts35	81.8 / ICCM0076 82.8 / Mt133126
43.9	TOG894965 ICCeM041	82.8 J Mt133126 83.0 TA5
46.5	- CKAM0905	85.0 TA39
47.3	cpPb675429 TOG899600	87.1 CGMM050 87.2 TR29
48.1	TOG896050	88.5 GAA42
48.4	LCCM0205	89.3 ICCM0079
48.8	TC80362 CKAM1768 CKAM1860	89.4 CaM0038 89.9 TOG927739
50.5	CKAM0788	90.2 // TOG915805
50.6 J	CKAM1024 CKAM1448	90.6 J - 4 TOG896285 CaM0639
52.0	ICCM0120b	91.9 J TOG897350 TOG915278
52.9	CKAM1810	TOG915293 TOG936005
53.0 53.2	CKAM1586 CKAM1120 TOG897884	92.0 TOG895876
53.6	CKAM1526 CKAM1464	92.1 TOG902902 TOG907096
	CKAM1621 CKAM1068	92.2 TOG896967 92.3 TOG894267 TOG900450
53.7	CKAM0351	TOG907169 TOG894043
55.0	CKAM1006	93.0 TOG904027
56.9 57.1	CISR027 CISR016	95.1 TOG895467 TOG903716 TOG912629
58.0	TOG898007 CKAM1186	95.6 TOG903808
58.4	CKAM1053	
58.8	CKAM0960 CKAM0539	
	CKAM1233	
59.4 59.6	CKAM1020 TOG894669 TOG902476	
59.9	CKAM1720	
60.7 61.2	• CKAM1155 • CKAM1649	
61.7	CKAM11045	
61.8	CKAM1017 CKAM0971	
61.9 62.1	TOG903813	
62.4	· TOG895630	
63.0	· cpPb350493 CKAM1224 TOG903928	
63.5	CKAM0278	
63.6	· CKAM1677 · CKAM1002	
63.7 - 65.2 -	CKAM1710	
65.3	CKAM0827 CKAM0830	
65.8	CKAM1430 CKAM1805	
65.9	TOG900987	
66.8 69.0	GA4 CKAM1718	
69.5	CKAM1538	
co. c	CKAM1722 TOG903939 CKAM0400 CKAM1292	
69.6	CKAM1442	
69.7	CKAM1052 CKAM1039	
69.9 - 70.0 -	CKAM1769 CKAM1252 CKAM1237	
70.1	CKAM1171 CKAM1444	
	CKAM1434 CKAM1414 CKAM1413 CKAM1126	
70.4 ⁻	CKAM1733 CKAM0668	
70.7	CKAM1030 CKAM0927 CKAM1316	
70.7 ⁻ 70.8 ⁻	CKAM1316	
70.9	CKAM1075 CKAM0722	
71.3 ⁻ 71.4 ⁻	CKAM0937 CKAM1830	
71.5	TOG913047 CKAM1261	
72.0 ⁻	TOG917719 CKAM1665 CKAM1663	
73.6	CKAM2004	
74.5	CISR025	
75.0 - 75.4 -	• TCMO • TOG899130	
75.8	H2B202	
75.9 ⁻ 76.1 ⁻	• H4H11 • H1O01	
76.1	H2J09	
76.4	ICCM0123a	
76.5 ⁻ 76.9 ⁻	+ H2L102 + H1H07	
77.5	H4F07	
78.0 ⁻ 78.3 -	ICCM0134 TOG895545 TOG918150	
78.6	ICCM0081	
78.8	TOG902462 TOG910676 TOG895816	
79.2 79.9	TS43	

CaL	.G(06 (A)	CaLG06 (B)
0.0 -/	∩	MTU07	37.0 \ TC78756
		TOG898284	37.4 H1116
2.6 \ 2.9	╞	- CKAM0967	39.0 40.4 CGMM020
3.3 -		CKAM1758	41.1 CKAM0010
		CKAM1825 CKAM1041	41.9 Pc1722835
3.4 ^J		CKAM1629	42.2 42.4 TC88727
		CKAM0775	42.7 CaM0421
3.5		CKAM1403 CKAM1921	43.3 ICCM0284a
4.4		CKAM1347	43.6 43.7
5.7		EST948	43.9 H3A03
6.7		CKAM0190 CKAM1522	44.1 ICCM0104 44.2 H6G10
7.0		CKAM1351	CaM1402
8.4	F	CKAM0953	44.4 H1L161
8.7 ⁻ 8.9 -	H	CKAM1612 CKAM0914	44.5 H3B08
9.9		CKAM1553	44.7 CaM0733
0.1	E	• CKAM0996	44.9 ICCM0242b
0.2		CKAM0662	45.2 - CISR030
0.5		CKAM0819	45.9 TOG905619
	Н	CKAM0906 CKAM0876	46.1 TOG906575
	Н	CKAM1386	46.2 - TC88598 47.4 - cpPb679928
		CKAM1689	48.2 STMS2
0.8		CKAM1872 CKAM0792	48.3 ICCeM039
	P	CKAM0792 CKAM0845	50.2 - TC79726 50.7 - CISR036
	Н	CKAM1707	52.3 TA80
		CKAM1247 CKAM1125	53.2 TOG896584
0.9		CKAM0822	53.7 - TA176 55.9 - TR1
1.1 -	П	CKAM1653	58.5 📲 🔤 TR44
1.2 ⁻ 1.3 ⁻	П	CKAM1162	59.7 - 63.0 - TOG906936
3.1		CKAM0911	63.4 TOG894270
3.4		CKAM0411 CKAM1049	63.6 ISSR8402
4.5 ⁻ 5.2 ⁻		CKAM1583	64.0 65.3 TOG913329
6.2		CKAM0895	69.1 DNABP
6.4 8.6		CKAM0034	CaLG06 (C)
8.7		CKAM1541	73.4 \ \ \ 70G910601
8.9		CKAM1431	73.6 TOG896498
9.2		CKAM0943	74.0 TOG901133 74.3 cpPb323556
9.3		CKAM0871	75.0 TOG900075
0.0		CKAM1563 CKAM1704	75.3 // TOG907934
9.4		CKAM0735	75.7 J TOG902560
9.4		CKAM1766	80.0 TOG897362
		CKAM0021 CKAM0801	80.4 TOG914210 81.8 TOG908356
		CKAM1676	TOG916065
9.5		CKAM1100 CKAM1013	TOG897351
9.5		CKAM1399	82.1 TOG897956
		CKAM0870	TOG903023
		CKAM1420 CKAM1592	TOG903841 TOG914910
9.6 ⁻		CKAM1928	TOG914910
9.7		CKAM1005	82.2 TOG898370
9.8 ⁻ 9.9 ⁻		CKAM1144	TOG902834
0.0		CKAM1765	82.9 - TOG899640
0.1 ⁻ 0.2 ⁻		CKAM1437	83.2 TOG899062 83.9 TOG898533
0.2 20.7		CKAM0982	
1.1 -		CKAM1250 CKAM1014	84.3 TOG902919
1.2		CKAM1014	84.4 TOG899538
1.7		CKAM1878	85.6 TOG894408
2.7		• TOG897528 • CGMM034	86.0 TOG919211
3.7 ⁻ 5.8 ⁻		CISR029	86.2 TOG894314 TOG903717
7.3		GA9	86.4 TOG909974
7.9 ⁻ 8.0 ⁻		CKAM1290	86.6 TOG894415 87.1 TOG901904
8.1		TA106	88.9 TOG922092
8.9		CKAM1477	89.0 - TOG895029
9.8		CKAM0332	89.2 - TOG896103 TOG906599
0.9		CKAM1285	90.1 TOG899728
1.7		CKAM1715	90.5 TOG899657 96.6 TOG900222
2.0		CKAM0695	96.6 TOG900222 97.4 TOG946804
2.2		CKAM1195	99.8 TOG894248
2.3		CKAM0848 CKAM1408	99.9 TOG917794 103.2 TOG907144
3.6		CISR041	106.4 TOG916452
4.9		GA34	106.6 ^J ^I TOG897973

Figure 4c (Continued)

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CaLG07 (A)	CaLG)7 (B)	CaLG	08 (A)	CaLG	08 (B)
0.0 - AJ276275	60.4 🖂 🚘	- CKAM1590	0.0	- CISR066		CKAM1301
2.9 \ cpPb677096		CKAM1089	0.7	CISR067	37.6	CKAM1600
7.9] / cpPb490874	60.5 -	CKAM1147	1.9	AAMCTT02		CKAM1732
13.3 1 / ICCM0034		CKAM1605	2.4	CYSK		CKAM1706
19.0 1 CaM0598	61.2	CKAM1608	3.4	CYSS	38.0	CKAM1286
22.4 H5E11	61.3	CKAM1339	4.7	ICCM0072		ICKAM1836
24.1 H1C22	61.7	TOG903027	5.1	cpPb172945	38.1	CKAM1547
24.9 MSU82	61.9	CKAM0706	5.8	MSU89		CKAM1519
25.6 TH1118	62.5	STMS25	6.7	CKAM1493	38.2	CKAM1667
26.8 - AGL178	62.6	CKAM0846	6.8	CKAM1018		CKAM1344
27.9 H1012		CKAM1485	6.9	CKAM0711	38.3	CKAM0779
29.4 ICCM0196	64.0 ⁻	CKAM1499		CKAM1460	41.1	TOG904000
31.8 TA18		CKAM0894		CKAM1124	41.4	TA3R-TA23L
34.0 TAA58		CKAM1035	8.0	CKAM1611	41.8	• TOG919655
35.0 - TOG903911	.	CKAM1178	// N	CKAM1483	42.4	• TOG901744
35.2 - ISSR8231	64.1	CKAM1734	8.1	CKAM1523	44.6	CGMM017
36.9 - GAA44		CKAM1843		CKAM1245	45.7	• TOG919584
38.5 - EST671		CKAM1468	8.9	CKAM0802	45.8	TOG902063
40.5 - cpPb677011	64.2	CKAM1497		CKAM1773	46.2	PGMb
43.6 - COAO	64.6	CKAM1845	10.2	CKAM0761	47.9	• TOG895142
44.8 - TA180	64.0	CKAM0749	11.2	CKAM1574		CKAM1903
TOG897306	64.9	CKAM1775		CKAM1782	48.7	CKAM1651
15 0 TOG908917	65.3	TOG918556	11.5	CKAM1288	50.0	CKAM0343
45.0 TOG916106	65.6	CKAM0168		TOG895871	51.0	• TOG924405
TOG927781	66.9	• TOG896040	11.9	CKAM0340	52.2	H1D24
45.4 ISSR8591	67.0	CKAM0984	42.0	TOG896172	52.4	H1C092
46.3 - AJ489614	67.6	CKAM1123	12.9	CKAM1345	52.7	H5B04
48.3 - CPOX2	67.6	CKAM1546	14.2	ICCM0130a	56.4	CKAM1410
49.3 - H3H121	67.7	CKAM1200	15.8	CISR054	57.5	CKAM1740
49.5 - H2E13		CKAM1265		CKAM0544	57.8	CKAM1685
49.6 - TOG897618	67.0	CKAM1670	17.1	CKAM0999	57.9	CKAM1393
50.5 W	67.9	CKAM1977	18.2	CKAM1407	58.2	CKAM0430
51.0 - STMS6		CKAM1202	18.8	CKAM0014	E0.2	CKAM1397
51.7 - ICCM0074a	69.0	ICCeM029	19.8	CKAM0709	58.3	CKAM1614
52.3 V OPC11-1	69.5	TA4L-TA191R_291-284	20.8	CKAM1840	60.7	CGMM028
53.2 - CGMM041	60.6	CKAM1630	21.4	H3C11	64.3	TA3
53.4 TOG894885	69.6	CKAM1539	21.7	CKAM1634	67.9	TS12
53.6 CPPb677907	69.7	CKAM1555	22.3	CKAM0932	70.5	LICCeM054
	70.8	CKAM2005	22.3	CKAM1631		
54.0 AJ276270	71.6	CKAM1646	24.1	CKAM1750		
54.6 WE FIS-1	71.8	CKAM0935	24.5	· U71		
55.2 / ICCM0074b	71.9	ICCM0065b	24.9	• X60755		
55.5 CGMM004	73.2	· OPA14-1		CKAM1772		
56.6 CKAM1990	79.0	• TOG896873	25.7	CKAM0342		
	79.3	TOG901547		CKAM0856		
57.1 CKAM0795		TOG905278	26.3	CKAM0900		
	81.6	• TA4L-TA199R-3_300	26.6	CKAM1512		
CKAM0366	87.5	CKAM1550	27.1	CKAM1871		
57.2 CKAM1960	92.0	OPS13-3	27.6 -	CKAM1402		
CKAM1543	93.7	CKAM0166	27.7	CKAM1743		
57.6 CKAM0460	106.9	ISSR8902		CKAM1461		
I CKAW0654	112.1	VISSR8401	28.4	CKAM1978		
CKAM1529 CKAM1498			29.0	CKAM1456 CKAM1908		
57.7 CKAM1498			29.2	TOG902768		
CKAM1748			29.2	FENR		
58.3 TOG908268			30.1	Ts45		
TOG905371			31.2	CKAM1889		
CKAM1181			32.1	TOG919502		
CKAM1693			32.2	OPQ11-2		
58.9 CKAM0837			32.9	CKAM0229		
CKAM1662			33.3	CKAM1854		
CKAM0910			34.5	CKAM1890		
59.0 - CKAM1303				TOG903898		
59.2 TOG946834				CKAM1727		
TOG900261			35.4	CKAM0918		
59.3 cpPb675317				CKAM0730		
59.4 TOG905443			35.6	CKAM1159		
CKAM1304			36.1	TOG897619		
59.6 CKAM0610						
TOG899683						
59.7 CKAM1073						

Figure 4d (Continued)

CaLG08 (Figure 4). In the case of CaLG01, CaLG02, CaLG04 and CaLG05, more CKAMs were clustered near the subtelomeric regions.

Comparison of the developed genetic map with other chickpea maps

The developed genetic map with 1328 marker loci was compared with the 1291 loci genetic map (Thudi *et al.*, 2011) and 300 loci transcript map of Gujaria *et al.* (2011). The details of comparison of these maps are available at http://cmap.icrisat.ac.in/cmap/sm/cp/hiremath/. These comparisons reflect a greater congruency in terms of grouping of markers into specific linkage groups. A few exceptions were also observed. For instance, TA4L-TA199R-3_300 and TA4L-TA191R_291-284 loci were mapped on LG04 by Thudi *et al.* (2011) and on LG06 by Gujaria *et al.* (2011); these loci have been assigned to CaLG07 in the present map. Similarly, the marker loci TA5L-TS38R-1_470 and TA5L-TS129R_208 that were present on LG05 and LG08 of genetic maps developed by Thudi *et al.* (2011) and Gujaria *et al.* (2011), respectively, could not be assigned to any

Table 3 Distribution of markers on the	second-generation lin	kage map of chickpea
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Marker type		Chickpea linkage group								Tatal manlana
	Total markers used	CaLG01	CaLG02	CaLG03	CaLG04	CaLG05	CaLG06	CaLG07	CaLG08	Total markers mapped
CKAMs	651	52	81	57	132	90	86	59	68	625
TOG-SNPs	317	56	29	16	67	58	56	19	13	314
Published marker loci										
GMMs										
CGMMs	32	4	10	2	6	3	2	2	2	31
CISRs	15	2	-	2	-	4	4	-	3	15
ICCeMs	15	2	2	2	2	1	1	1	1	12
Legacy markers										
H-series	44	4	7	6	5	7	5	6	4	44
ICCMs	46	3	4	9	10	7	6	5	2	46
CAMs	10	1	-	1	1	2	4	1	-	10
SSRs	93	14	11	16	14	14	10	9	5	93
ISSRs	26	8	8	-	2	2	1	5	-	26
SNaPshot assay-based SNPs	79	8	8	18	12	8	8	10	7	79
CAPS	13	-	1	4	2	2	1	-	-	10
DArTs	19	1	-	3	2	5	2	5	1	19
RAPD	4	1	-	-	-	-	-	2	1	4
Total no. of markers	1364	156	161	136	255	203	186	124	107	1328
Total distance (cM)		116.6	92.94	101.8	92.5	95.6	106.6	112.1	70.5	788.6
Average intermarker distance (cM)		0.75	0.58	0.75	0.36	0.47	0.57	0.90	0.66	0.59

SNP, single nucleotide polymorphisms; SSR, simple sequence repeats; TOG, tentative orthologous genes.

Linkage group (LG)	5		Intermarker distance	No. of clusters	Genetic mapping position and number of markers (in parenthesis) in clusters observed				
CaLG01	156	116.6	0.75	3	23 (8), 39 (5), 61 (6)				
CaLG02	161	92.94	0.58	7	17 (6), 41 (6), 53 (5), 56 (6), 57 (8), 71 (7), 72 (8)				
CaLG03	136	101.8	0.75	1	35 (8)				
CaLG04	255	92.5	0.36	5	52 (8), 53 (19), 54 (14), 60 (7), 30 (11)				
CaLG05	203	95.6	0.47	5	53 (7), 58 (7), 69 (10), 70 (15), 91 (6)				
CaLG06	186	106.6	0.57	4	3 (7), 10 (15), 19 (20), 82 (12)				
CaLG07	124	112.1	0.90	2	57 (11), 64 (12)				
CaLG08	107	70.5	0.66	2	8 (8), 38 (7)				
Total	1328	788.6	5.04	29					
Average	166	98.58	0.63	3.6					

linkage group in this genetic map. Apart from these shifts in marker locations, no other discrepancy was observed.

Genome relationships of chickpea with closely related legume species

We combined both the genetic map position information for chickpea loci and genome sequence information of closely related species of different clades to evaluate the degree of synteny between genomes of chickpea and other related legume species. A set of 1064 of 1328 mapped loci for which both genetic map positions and sequence information were available were compared with genome assemblies of *Medicago truncatula* (Mt 3.5), *Lotus japonicus* (Lj 2.5 pseudo-molecules), soybean (*Glycine max*) (Glyma1) and the genetic map of cowpea (*Vigna unguiculata*, Muchero *et al.*, 2009) (Figure 5).

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In the case of chickpea and *Medicago*, 555 unique chickpea loci showed significant matches with 1558 genomic regions on *Medicago* chromosome (Table 5). Most of the chickpea loci have ≥ 2 matches in *Medicago*. About 111 chickpea loci from CaLG01 showed similarity with Mtchr02 genomic regions. Similarly, loci from CaLG02 showed maximum matches to Mtchr05, followed by CaLG03 with Mtchr07, CaLG04 with Mtchr01, CaLG05 with MtChr03, CaLG06 with Mtchr04, CaLG07 with MtChr04, and CaLG08 with MtChr05. In brief, each linkage group of chickpea showed considerable synteny with one or more chromosomes of *Medicago*, although internal duplication of DNA sequences/blocks was not observed (Figure 5a).

In the comparison of chickpea with soybean, 494 chickpea unique loci matched 1798 short stretches distributed on different chromosomes of soybean (Glyma1 assembly) (Figure 5b, Table S6). Each chickpea marker locus showed similarity to



Figure 5 Genome relationships of chickpea with closely related legume species. Homologous relationship of chickpea genome with four legume species, that is, *Medicago truncatula* (a), soybean (b), *Lotus japonicus* (c) and cowpea (d), has been shown by comparing sequence data of 1064 mapped markers of chickpea with genome sequence of *Medicago* (Mt 3.5), *L. japonicus* (Lj 2.5 pseudomolecules), soybean (Glyma1 genome assembly) and cowpea genetic map (Muchero *et al.*, 2009). Maximum similarity was observed with *Medicago* (1558), followed with soybean genome (1798), *Lotus* (438) and least with cowpea (55). The percentage of matches in each species is in congruence with their phylogenetic distances.

Chickpea linkage groups	Number of chickpea unique loci	Medicago truncatula chromosomes									
		MtChr01	MtChr02	MtChr03	MtChr04	MtChr05	MtChr06	MtChr07	MtChr08	MtChr0	Total
CaLG01	69	9	111	18	16	22	9	19	8	15	227
CaLG02	61	7	9	10	14	90	23	12	8	7	180
CaLG03	62	12	25	30	20	41	15	99	26	12	280
CaLG04	95	104	1	7	24	12	11	9	10	19	197
CaLG05	93	11	3	129	13	14	5	9	13	19	216
CaLG06	76	6	5	8	87	39	11	11	51	9	227
CaLG07	46	1	7	5	54	8	3	2	11	1	92
CaLG08	53	4	2	3	4	99	6	14	1	6	139
Total	555	154	163	210	232	325	83	175	128	88	1558

Table 5 Mapping of chickpea marker loci on Medicago chromosomes

*The numbers shown in bold represent the highest matches between chickpea and Medicago.

approximately 3–4 regions on Glyma1. This reflects the number of matches one would expect to see based on the one round of whole genome duplication in soybean. Only 267 unique chickpea loci matched with 438 regions on *Lotus* (Table S7, Figure 5c). In the case of cowpea in which genetic map was used for the comparison, least matches were observed between chickpea and cowpea genomes. Only 50 unique chickpea loci showed synteny with 55 loci of cowpea map (Table S8, Figure 5d).

Discussion

Cost-effective KASPar assays for SNP genotyping

Until recently, SSR markers were the commonly used markers for chickpea genetics research and breeding applications (Upadhyaya et al., 2011). Nevertheless, in some cases, genetic maps have also been developed using DArTs (Thudi et al., 2011), CISRs (Gujaria et al., 2011) and SNPs/CAPs (Choudhary et al., 2012; Gujaria et al., 2011; Nayak et al., 2010). With the availability of whole genome or EST sequences in many crop species, the use of SNP markers has been proven attractive for high-throughput use in molecular breeding (Rafalski, 2002; Varshney, 2010). High-throughput SNP genotyping platforms such as Illumina's GoldenGate or Infinium assays are being used for large-scale SNP genotyping. While the high-throughput SNP genotyping platforms are very useful for rapid genotyping of mapping population or germplasm collections, they are not generally economical for projects such as in silico SNP validation, gene-specific SNP assays, marker saturation in the regions of interest and marker application projects that utilizes defined set/panel of smaller number of SNP markers on varying number of genotypes. In such cases, SNP genotyping technologies such as arrayed primer extension reaction (APEX) (Podder et al., 2008), dynamic allele-specific hybridization (DASH) (Podder et al., 2008), molecular beacons (Mhlanga and Malmberg, 2001), primer extension followed by MALDI-TOF (alternative to Sequenom's assays) (Sauer et al., 2000) and KASPar assay (http://www.kbioscience.co.uk/reagents/KASP.html) have been developed. While choosing a particular SNP genotyping platform, several features such as the reproducibility, accuracy, capability of multiplexing, the level of throughput, time consumption and cost (considering both the equipments required and the cost per genotype) need to be considered. As molecular breeding applications, generally, require screening of large populations with a few markers, this study developed costeffective KASPar marker assays for SNP genotyping in chickpea.

A total of 2486 SNPs were assembled from different sources for developing KASPar assays. KASPar assays developed for chickpea have been referred as CKAMs. Genotyping of these 2486 CKAMs on a panel of 70 genotypes provided a validated set of 2005 CKAMs. This includes KASPar assays for 539 TOG-SNPs that were initially assayed on GoldenGate assays. Conversion of these TOG-SNPs into KASPar assay will facilitate use of TOG-SNPs in chickpea genetics and breeding application.

To compare the success rate of converting putative SNPs into successful and informative KASPar assays, amplification and polymorphism statistics were checked across the four sets of SNPs. The set of markers that gave higher rate of failures were those SNPs identified from alignments of Sanger ESTs (172 SNP markers, i.e. 60% of a total of 286). The possible reasons could be attributed primarily to (i) SNPs were mined from the ESTs with sequencing artefacts, (ii) frequency of one of two alleles for a given SNP is very low in the EST data set, and (iii) all the genotypes for which EST-based mining approach provided SNPs were not included in the genotype panel used in the current study (Varshney *et al.*, 2009). The remaining number of markers that could not be validated include 222 (15.7% of total of 1409) from Alpheus pipeline predicted SNPs, 65 SNPs (10.7% out of 604) from TOG-SNPs and 22 SNPs (11.7% out of 187) from allele resequencing data. Overall, the KASPar assay has shown 81% validation success rate in our study. Comparison of costs and time involved in genotyping the SNPs via KASPar assays and GoldenGate assays for the same set of SNPs in this study, showed superiority of KASPar assays over GoldenGate assays, especially when limited number of SNPs (<500) are genotyped with <100 lines.

The PIC values of validated CKAMs varied from 0.02 to 0.50 with an average of 0.12. Low range of PIC value of CKAMs is not unexpected as genetic variation in the chickpea gene pool is limited (Nayak *et al.*, 2010; Thudi *et al.*, 2011). Also, this study identifies polymorphic markers (15–930) for different mapping populations segregating for drought, salinity, *Fusarium wilt*, Ascochyta blight, etc. It, therefore, provides opportunities for mapping resistance to biotic and tolerance to abiotic stresses in chickpea.

Diversity analysis and molecular breeding applications

This study demonstrates the suitability of KASPar assays for SNP genotyping for understanding the relationships in the germplasm collection as well as for molecular breeding applications. Despite using a wide diverse collection of genotypes with all 2005 CKAMs, an overall success rate of 81% was achieved. The genetic dissimilarity analysis of the germplasm accessions determines relationships of accessions with each other. The dendrogram developed based on genetic dissimilarity coefficient depicted clear clustering of chickpea accessions into two main clusters as per their geographical origin and species type of all 58 accessions (55 accessions of C. arietinum species and three accessions of C. reticulatum species) analysed. Two accessions of C. reticulatum are resolved as a separate group; however, IG 72933, a C. reticulatum, was found closer to C. arietinum. Similar results were observed in an earlier genetic diversity study using 513 SSR markers in which the IG 72933 genotype showed 40% similarity with the C. arietinum genotypes (Gudipati, 2007). The Cluster-II contained more geographically divergent material of the C. arietinum species. As expected, accessions of all Indian origin formed a separate clade, and the remaining accessions from other countries were grouped into another clade (IIa). Overall, these results are in general congruence with earlier studies and indicate that the cluster topology is reliable.

The study also demonstrates the utility of CKAMs for assessing the genome recovery of BC_3F_2 lines. This study identified five lines ($BC_3F_2_120$, $BC_3F_2_170$, $BC_3F_2_187$, $BC_3F_2_195$ and $BC_3F_2_268$) with > 95% genome recovery of JG 11 in MABC experiments. These lines may be used for multi-location field trials for evaluating agronomic performance as well as for developing the near isogenic lines (NILs) for fine mapping the QTLs.

Second-generation genetic map of chickpea with more anchoring points with other legume genomes

As expected, the number of polymorphic markers observed between interspecific mapping populations is higher than intra-

specific mapping populations. For instance, maximum number of polymorphic markers is 930 (ICC 4958 × PI 489777) in interspecific crosses as compared with 159 (Arerti \times ICC 4958) in intraspecific crosses. As ICC 4958 × PI 489777 population is a reference mapping population, genotyping data were generated for the polymorphic CKAMs. Although genotyping data were earlier generated for TOG-SNPs on the mapping population via GoldenGate assays (R.V. Penmetsa, N. Carraquilla-Garcia, A.D. Farmer, R.K. Varshney, D.R. Cook, unpublished data), a set of 118 TOG-SNPs distributed on all eight LGs was also targeted for generating genotyping data via KASPar assays for quality control purpose. Comparison of high-quality data for 112 markers generated via KASPar assay with that of Golden-Gate assay showed no discrepancy. After assembling genotyping data for 539 remaining CKAMs, 317 TOGs and 396 marker loci from other sources (Gujaria et al., 2011; Navak et al., 2010; R.V. Penmetsa, N. Carraguilla-Garcia, A.D. Farmer, R.K. Varshney, D.R. Cook, unpublished data; Thudi et al., 2011), genotyping data for a total of 1364 marker loci were considered for mapping. As a result, a comprehensive genetic map comprising 1328 marker loci including 939 new marker loci (625 CKAMs, 314 TOGs-SNPs) and 389 already published mapped marker loci was developed. The second-generation genetic map has a coverage of 788.6 cM genetic distance. On an average, each of the linkage group has 166 markers with an average distance of 98.6 cM. This map has probably the highest number of gene-based SNP markers (1088) mapped in chickpea so far. Earlier to this map, Gujaria et al. (2011) developed a transcript map with 126 gene-based markers and Choudhary et al. (2012) developed a genetic map with 406 marker loci including 177 gene-based markers. This map has approximately eightfold gene-based markers as compared with the abovementioned studies. Another important feature with this genetic map is the availability of cost-effective KASPar assays for the mapped gene-based markers that can be used in any number as well as on a variable number of lines. The guality and accuracy of the second-generation genetic map was evaluated by comparing it with several genetic maps developed in earlier studies (Gujaria et al., 2011; Nayak et al., 2010; Thudi et al., 2011; Winter et al., 1999).

Clustering of two or more markers is a commonly occurring phenomenon observed in several earlier genetic maps of chickpea (Nayak *et al.*, 2010; Thudi *et al.*, 2011; Winter *et al.*, 1999). Only CKAMs and TOG-based SNPs were clustered, which constitute a large proportion of mapped markers [i.e. 625 CKAMs and 314 TOG-SNPs (939, 71%) of 1328] compared with other marker types. This clustering may be attributed mainly to random selection of markers from the closely spaced regions of the genome that have undergone comparatively less number of recombination events.

As a complement to the gene-based linkage map developed in this study, we compared the sequences of these mapped loci with genome assemblies/genetic maps of four legume species (*Medicago*, *Lotus*, cowpea and soybean). Through the comparative analysis, high conservation of synteny was observed between chickpea and *Medicago*, whereas lowest level of synteny conservation was observed between chickpea and cowpea. Apparently, during the time of analysis genome sequence information was not available for cowpea; hence, the analysis was carried out by comparing with high-density linkage map developed by Muchero *et al.* (2009) available then. As a result, least similarity was identified between chickpea and cowpea,

although chickpea is phylogenetically closer to cowpea than it is to soybean, which shares the same common ancestor relative to the ancestor of chickpea, Medicago and Lotus (Wojsciechowski et al., 2004). In all the other cases, high level of similarity was observed (>70%, 1E-05) between sequences of chickpea, and those of compared legumes, however, are often punctuated or interrupted by chromosomal rearrangements, thereby resulting in disruption of the linear order of the genes. Subsequently, these variations (insertion, deletion, duplication or rearrangements) form the basis for evolution of diverse genomes. One or more chickpea loci match to a single locus on Medicago chromosome, and similar pattern was observed for the remaining three legume genomes with chickpea. This may reflect segmental duplication events of chromosomal stretches, or the mapped loci may correspond to paralogous genes or same gene family members. Recent analysis of Medicago genome has revealed that higher rates of mutations and chromosomal rearrangements are known to have occurred after the whole genome duplication event as compared with other model legumes such as Glycine max and L. japonicus (Young et al., 2011).

A number of chickpea unique loci matching to different chromosomal regions on Mt 3.5, Glyma1, Lj 2.5 and cowpea genetic map were identified. Of the 69 chickpea unique loci that mapped on 227 regions distributed over eight chromosomes of Medicago, approximately 49% (i.e. 111 of 227) matched to the MtChr02 and the remaining 116 were similar to those on other chromosomes. Only 53 loci are in linear order with Mtchr02 chromosomal regions, and the remaining are in nonlinear positions. These findings support the earlier reports by Choi et al. (2004), Nayak et al. (2010) and Zhu et al. (2005) that one to one synteny does not hold true between chickpea and the compared legume species, and the synteny is restricted only to small genetic or genomic intervals (Young et al., 2011). Our comparative results showed that regions of CaLG02 and CaLG08 are strongly similar to Mt05, which in turn shows high similarity to regions on Gm01, Gm02 and Gm11, which are consistent with the findings of Young et al. (2011).

Conclusions

The study reports compilation of a large number of SNPs and their conversion into cost-effective KASPar assays. A set of 2005 KASPar assays have been developed for accelerating chickpea genetics research and breeding applications. Together with these markers and recently developed SSR markers from genomic libraries (Nayak et al., 2010) and BAC-end sequences (Thudi et al., 2011), DArT markers (Thudi et al., 2011), CISRand CAPS-based CGMMs, >10 000 markers have become available in chickpea. The available marker resource should be able to tackle the issue of narrow genetic diversity in the gene pool as it is now possible to identify reasonable number of polymorphic markers in any given combination of cross. Genetic structure information gained on 58 chickpea accessions may be useful in finding suitable parental combinations for developing the new mapping populations segregating for different traits of interest to chickpea breeders. Furthermore, a number of polymorphic markers were identified in many existing mapping populations that can be used for developing genetic maps and mapping of different agronomic traits. Many polymorphic markers were found to be common in many mapping populations,

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revealing their usefulness in providing bridging markers and for comparing different chickpea maps. Developed genetic map is the most enriched genetic map for gene-based markers. This map should be useful not only in comparing different chickpea genetic maps, but also in anchoring the physical map, currently underway, as well as establishing more anchor points among genomes of chickpea and other legume species.

Experimental procedure

Plant material and DNA extraction

A set of 70 different chickpea genotypes was used for validation of SNPs using KASPar assays. Details of these genotypes are given in Table 2 and Table S2. Furthermore, a set of 131 recombinant inbred lines (RILs) derived from the cross between ICC 4958 (*C. arietinum*) and PI 489777 (*C. reticulatum*) was used for genetic mapping.

Total genomic DNA of all the accessions was extracted from leaves of two-week-old seedlings using high-throughput mini DNA extraction protocol as mentioned in Cuc *et al.* (2008). The quality and quantity of extracted DNAs were assessed on 0.8% agarose gel. The DNA was normalized to 5 ng/ μ L for genotyping.

RNA Sequencing by Solexa/Illumina

Five different chickpea genotypes, viz. ICC 4958, ICC 1882, PI 489777, ICC 506 and ICCC 37, which are parents of different mapping populations, were selected for RNA sequencing. Roots of 22-day-old seedlings of ICC 4958 and ICC 1882 were subjected to drought stresses, and subsequently total RNA was extracted from both genotypes (Hiremath et al., 2011). About 22-day-old leaves of ICC 506 and ICCC 37 were infested with larvae of Helicoverpa armigera for a period of 5 days under green house conditions (temperature of 28 ± 5 °C and relative humidity of >65%). After a brief infestation period, leaf samples from both genotypes were harvested for total RNA extraction. Total RNA was also extracted from 22-day-old root tissues of PI 489777, a wild species genotype. Subsequently, the total RNA samples of all the genotypes were sent for Solexa/Illumina sequencing at National Center for Genome Research (NCGR), USA.

Development and analysis of KASPar assays

For developing the KASPar assays, 50 bp upstream and 50 bp downstream flanking sequences around the variant position (SNP) were selected (Table S1). Subsequently, KASPar assays for the targeted SNPs were carried out at KBioscience, UK. Complete details on principle and procedure of the assay are available at http://www.kbioscience.co.uk/reagents/KASP_manual.pdf and http://www.kbioscience.co.uk/download/KASP.swf. On the basis of the fluorescence obtained, allele call data are viewed graphically as a scatter plot for each marker assayed using the SNPViewer. The consistency between the predicted SNP and assayed ones was checked for each SNP marker.

Evaluation of polymorphism in chickpea accessions

The PIC refers to the value of a marker for detecting polymorphism within a given germplasm, depending on the number of detectable alleles and the distribution of their frequency. In this study, the PIC value of markers was calculated using the following formula (Anderson *et al.*, 1993):

$$PIC = 1 - \sum_{i=1}^{n} p_i^2$$

Where 'n' denotes the total number of alleles and 'p' refers to the frequency of the 'i'th allele at a genetic locus in different genotypes.

Genetic diversity analysis

To evaluate the relationship between chickpea germplasm accessions, SNP allele call data obtained for polymorphic markers were used for calculating both pair-wise genetic distance and per cent dissimilarity matrix to construct a dendrogram using DARwin V5.0.128 software (darwin.cirad.fr/darwin/ Home.php, Perrier *et al.*, 2003). Cluster analysis was carried out using the UPGMA method.

Genetic mapping

Genotyping data obtained using KASPar assays (CKAMs) were compiled with the marker data for TOGs-SNPs (R.V. Penmetsa, N. Carraquilla-Garcia, A.D. Farmer, R.K. Varshney, D.R. Cook, unpublished data) and selected markers from all 8 linkage groups mapped in earlier studies (Gujaria et al., 2011; Nayak et al., 2010; Thudi et al., 2011). Segregation data for CKAMs were tested for goodness of fit to the expected Mendelian ratio of 1:1 using chi-square (χ^2) analysis (P < 0.05). All markers were primarily divided into linkage groups using the 'group' command of MAPMAKER/EXP 3.0 program (Lander et al., 1987). However, to construct high-guality genetic map, those markers grouped by MAPMAKER were mapped using JOINMAP 4 program (Stam, 1993; Van Ooijen, 2006; http://www.kyazma. nl/index.php/mc.JoinMap/). 'Kosambi' mapping function was used to calculate centimorgan (cM) distances. LOD values ranging from 3 to 7 were considered for grouping and mapping. MAPCHART (2.1v) was used for drawing maps (Voorips, 2002, http://www.biometris.wur.nl/uk/Software/MapChart/).

Comparative mapping between chickpea and closer legumes

Sequences data for mapped chickpea marker loci were gueried using BLAST against genomes of *M. truncatula* (Mt 3.5), L. japonicus (Lj 2.5 pseudomolecules), soybean (Glyma1 genome assembly) and cowpea genetic map (Muchero et al., 2009). All the databases mentioned are available at http://comparativelegumes.org/. Hits matching a minimum of 70% sequence identity were retained for comparative study. Identification of homologous blocks was performed using I-ADHoRE v2.1 (Vandepoele et al., 2002). For the purpose of developing Circos images, cM distances on the chickpea linkage groups were scaled up by a factor of 250 000 to match similar base pair lengths of the chromosomes of other legumes' genomes. Visualization of blocks was performed with Circos26. Scales along the outer edge of the chickpea linkage groups show actual cM distances, while the scale along the outer edge of the Medicago chromosomes are in Mb.

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Supporting information

Additional Supporting information may be found in the online version of this article:

Table S1 Details on 2486 selected SNPs along with their SNP variants, 50 bp upstream and downstream flanking sequence, marker ID and PIC values.

 Table S2
 Details on 70 chickpea genotypes used for validation of 2486 CKAMs.

Table S3 Details of genotyping data for 1341 CKAMs on 58 chickpea genotypes.

Table S4 Details of genotyping data for 119 CKAMs on 12 BC_3F_2 lines along with parental genotypes ICC 4958 and JG 11. **Table S5** Details of genotyping data on 131 RILs for 651

CKAMs and their respective Chi-square (χ^2) test values.

Table S6 Detailed results on comparison of mapped marker loci of chickpea with soybean (*Glycine max*) genome.

Table S7 Detailed results on comparison of mapped marker loci of chickpea with *Lotus japonicus* genome.

Table S8 Detailed results on comparison of mapped marker loci of chickpea with cowpea genetic map.

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