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Genome-wide development and deployment of informative intron-spanning and intron-length polymorphism markers for genomics-assisted breeding applications in chickpea

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ABSTRACT

The discovery and large-scale genotyping of informative gene-based markers is essential for rapid delineation of genes/QTLs governing stress tolerance and yield component traits in order to drive genetic enhancement in chickpea. A genome-wide 119169 and 110491 ISM (intron-spanning markers) from 23129 *desi* and 20386 *kabuli* protein-coding genes and 7454 *in silico* InDel (insertion-deletion) (1–45-bp)-based ILP (intron-length polymorphism) markers from 3283 genes were developed that were structurally and functionally annotated on eight chromosomes and unanchored scaffolds of chickpea. A much higher amplification efficiency (83%) and intra-specific polymorphic potential (86%) detected by these markers than that of other sequence-based genetic markers among *desi* and *kabuli* chickpea accessions was apparent even by a cost-effective agarose gel-based assay. The genome-wide physically mapped 1718 ILP markers assayed a wider level of functional genetic diversity (19–81%) and well-defined phylogenetics among domesticated chickpea accessions. The gene-derived 1424 ILP markers were anchored on a high-density (inter-marker distance: 0.65 cM) *desi* intra-specific genetic linkage map/functional transcript map (ICC 4958 × ICC 2263) of chickpea. This reference genetic map identified six major genomic regions harbouring six robust QTLs mapped on five chromosomes, which explained 11–23% seed weight trait variation (7.6–10.5 LOD) in chickpea. The integration of high-resolution QTL mapping with differential expression profiling detected six including one potential serine carboxypeptidase gene with ILP markers (linked tightly to the major seed weight QTLs) exhibiting seed-specific expression as well as pronounced up-regulation especially in seeds of high (ICC 4958) as compared to low (ICC 2263) seed weight mapping parental accessions. The marker information generated in the present study was made publicly accessible through a user-friendly web-resource, "Chickpea ISM-ILP Marker Database". The designing of multiple ISM and ILP markers (2–5 markers/gene) from an individual gene (transcription factor) with numerous aforementioned desirable genetic attributes can widen the user-preference to select suitable primer combination for simultaneous large-scale assaying of functional allelic variation, natural allelic diversity, molecular mapping and expression profiling of genes among chickpea accessions. This will essentially accelerate the identification of functionally relevant molecular tags regulating vital agronomic traits for genomics-assisted crop improvement by optimal resource expenses in chickpea.

1. Introduction

Development and large-scale genotyping of informative sequence-based genetic markers in bi-parental mapping populations and natural core/minicore germplasm lines is essential

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to identify and map the functionally relevant genes/QTLs (quantitative trait loci) governing important agronomic traits for genomics-assisted crop improvement of chickpea. To accomplish these objectives, effective deployment of sequence-based robust SSR (simple sequence repeat), SNP (single nucleotide polymorphism) and insertion-deletion (InDel) markers developed at a genome-wide and/gene level has been made to expedite diverse high-throughput genetic analysis including QTL and trait association mapping in chickpea [1–27]. Despite these efforts, very limited number of trait-associated QTLs have been fine-mapped and validated in multiple genetic backgrounds (diverse mapping populations) of chickpea. This essentially restrains the identification and use of informative markers tightly linked to QTLs/genes regulating traits of agricultural importance that could be exploited efficiently for marker-assisted genetic improvement in chickpea. In this context, especially the development of numerous gene-based markers revealing wider genomic distribution as well as higher intra-specific polymorphic potential among chickpea accessions using a simpler affordable marker genotyping assay is prerequisite. This will essentially accelerate construction of high-density genetic linkage maps and fine-mapping/map-based cloning as well as high-resolution association mapping in order to identify potential genes/QTLs and natural allelic variants controlling diverse stress tolerance and yield component traits for marker-aided genetic enhancement of chickpea with a narrow genetic base.

In recent years, intron-spanning markers (ISM) and/or intron-length polymorphism (ILP) markers have been developed successfully at a genome-wide scale targeting the introns of annotated genes, which are effectively employed in various large-scale genotyping applications of multiple major food crop plants [4,5,28–42]. The ISM and ILP markers are now considered as a preferred ideal marker system in plant genetic analysis due to their diverse array of desirable inherent genetic attributes comparable with other commonly adopted sequence-based markers and wide genomic distribution specifically in the gene regions of crop plant genomes. The added-advantage of these markers has been realized currently in view of their efficiency to reveal direct reflection of allelic variation/diversity within the genes. These informative genic markers thus have significance to rapidly establish marker-trait association and targeted mapping of genes/QTLs governing vital agronomic traits in crop plants.

More recently, ISM and ILP markers with dense genome-wide coverage have been developed successfully involving and comparing all the intronic sequence components of genes annotated from the completely sequenced genomes of two rice accessions [43]. The practical utility of these gene-based markers in various genomics-assisted breeding applications including genetic diversity analysis, construction of high-density genetic linkage maps, evolutionary studies, mapping of genes/QTLs regulating important agronomic traits and marker-assisted breeding has been well-demonstrated in rice. With the availability of draft genome sequences of two cultivated *desi* (ICC 4958) and *kabuli* (CDC Frontier) accessions, it is now possible to develop ISM primarily at a genome-wide scale in chickpea by targeting all individual introns present in the genes annotated from their genomes. In order to convert ISM into ILP markers, each intron of these genes can be scanned for InDels by comparing the whole genome sequences of two *desi* and *kabuli* chickpea accessions. The ILP markers are especially developed and amplified targeting multiple InDels within an individual intron at a time, thereby have pronounced likelihood potential of detecting intra-/inter-specific polymorphism as compared to InDel markers among chickpea accessions. Subsequently, the correctly annotated polymorphic introns can be validated/genotyped in diverse accessions by designing and amplifying ISM and ILP primers from the exonic sequences flanking the target introns. The afore-mentioned strategy of developing ISM and ILP markers will provide user a wider flexibility to select diverse combinations of informative primers from an individual gene revealing reproducible PCR amplification by their convenient genotyping in a simpler affordable gel-based assay in chickpea. Consequently, these markers could be efficient in detecting higher polymorphic potential for differentiation of closely-related cultivated accessions along with precise assaying of differential expression profiles across tissues/stages of accessions more efficiently in chickpea. Essentially, marker genotyping and differential gene expression profiling can be assayed simultaneously with an identical set of ISM and ILP markers, which will be useful in targeted mapping of differentially expressed genes directly on the genome and identification of diverse arrays of potential genes for successful rapid quantitative dissection of complex traits and genetic enhancement studies in chickpea.

In view of above prospects, the present study developed genome-wide ISM and ILP markers by targeting/comparing individual introns of genes annotated from the sequenced draft whole genomes of *desi* (ICC 4958) and *kabuli* (CDC Frontier) chickpea accessions. The utility of these developed markers in detecting potential for amplification, polymorphism and molecular diversity among cultivated *desi* and *kabuli* accessions as well as realistic assaying and accurate estimation of differential gene expression profiles in diverse seed developmental stages of low and high seed weight chickpea accessions was accessed. The informative well-validated ISM and ILP markers were utilized to construct a high-resolution intra-specific genetic linkage map for identification and molecular mapping of major genes harbouring seed weight QTLs in chickpea. In order to make all the designed chickpea ISM and ILP markers publicly accessible at a genome-wide scale, a user-friendly web resource, “Chickpea ISM-ILP Marker Database” was developed.

2. Materials and methods

2.1. Development of ISM and ILP markers

For developing genome-wide ISM and ILP markers, the individual intronic sequence components of each protein coding gene annotated from the sequenced *desi* (ICC 4958, CGAP v2.0 [44]), and *kabuli* (CDC Frontier v1.0 [45]), draft genomes were retrieved. To develop ISMs, forward and reverse primers from the 100-bp exonic sequences flanking each intron of genes were designed individually employing custom-made Primer3 perl scripts as per Badoni et al. [43]. To convert ISMs into InDels-led ILP markers at a genome-wide scale, intronic-InDels between *kabuli* and *desi* genomes were detected by comparing the individual intronic sequences of each gene annotated from *kabuli* genome with corresponding orthologous genomic sequences of sequenced *desi* genome. The uniqueness of primers designed both for ISM and ILP markers in two chickpea genomes was ascertained following Wang et al. [29] and Badoni et al. [43]. We determined the genomic distribution of ISM and ILP markers in diverse coding and non-coding sequence component of chickpea genes structurally and functionally annotated on eight chromosomes and unanchored scaffolds of both *desi* and *kabuli* genomes.

2.2. Experimental validation, amplification and polymorphic potential of markers

To determine the amplification and polymorphic potential of markers, ISM/ILP markers designed from the diverse protein-coding genes including transcription factors (at least one marker/gene) exhibiting ≥4 bp InDel-based *in silico* fragment length polymorphism between CDC Frontier (*kabuli*) and ICC 4958 (*desi*) accessions were screened. These selected markers were phys-

ically mapped on eight chickpea chromosomes and unanchored scaffolds of both *desi* and *kabuli* genomes. The ISM/ILP markers were PCR amplified with the genomic DNA isolated from 32 including 20 *desi* and 12 *kabuli* chickpea accessions (Supplemental file 1) following Jhanwar et al. [46] and Kujur et al. [7]. The PCR products of amplified ISM/ILP markers exhibiting ≥10-bp *in silico* fragment length polymorphism were resolved in 2.5% agarose gel and fragment size (bp) of amplicons was determined against 50-bp DNA ladder size standard. The PCR product of amplified ISM/ILP markers revealing <10-bp *in silico* fragment length polymorphism was purified and sequenced using automated 96 capillary ABI 3730 × l DNA Analyzer (Applied Biosystems, USA) as per Kujur et al. [7] and Saxena et al. [13]. The genotyping data of experimentally validated ISM and ILP markers was analysed using PowerMarker v3.51 [47] to measure the average polymorphic alleles per marker, per cent polymorphism and polymorphism information content (PIC) among chickpea accessions.

2.3. Molecular diversity analysis

To determine the molecular diversity and phylogenetic relationships among 32 *desi* and *kabuli* accessions (Supplemental file 1), the genotyping data of informative polymorphic ISM and ILP markers physically mapped across eight chickpea chromosomes and unanchored scaffolds were analysed in PowerMarker v3.51. For this, the marker genotyping data was analysed through Nei and Li similarity coefficient-based neighbor joining (NJ) method of PowerMarker for clustering analysis and construction of an unrooted phylogenetic tree among accessions as per Kujur et al. [7,9].

2.4. Genetic map construction and QTL mapping

We selected the ISM and ILP markers exhibiting polymorphism between high (*C. arietinum desi* accession ICC 4958 with 100-seed weight: 35.4 g) and low (*C. arietinum desi* accession ICC 2263 with 100-seed weight: 13.6 g) seed weight parental accessions of a 190 F_7 RIL (recombinant inbred line) mapping population (ICC 4958 × ICC 2263) from our marker polymorphism study. The genomic DNA isolated from mapping parents and RIL individuals were PCR amplified and genotyped using the informative markers as per aforementioned agarose gel- and amplicon sequencing-led genotyping strategies. The genotyping data of markers exhibiting goodness-of-fit to the expected Mendelian 1:1 segregation ratio based on χ^2-test ($p < 0.05$) was analysed by JoinMap v4.1 (http://www.kyazma.nl/index.php/mc.JoinMap) at a higher LOD threshold (4.0) with Kosambi mapping function to measure the linkage analysis among the markers used. A high-density genetic map was constructed by integrating the markers into defined linkage groups (LGs) based on their centi Morgan (cM) genetic distances and corresponding marker physical positions (bp) on the chromosomes, and visualized using CIRCOS as per Kujur et al. [9].

The 190 individuals and parental accessions of a F_7 RIL mapping population (ICC 4958 × ICC 2263) were grown in the field during the crop growing season for two consecutive years (2013 and 2014) with at least two replications (as per randomized complete block design) and phenotyped for 100-seed weight (g). The coefficient of variation (CV), broad-sense heritability (H^2) and frequency distribution of seed weight in the RIL mapping population were estimated as per Bajaj et al. [16]. For molecular mapping of major seed weight QTLs, the genotyping data of ISM and ILP markers genetically mapped on eight chickpea chromosomes were integrated with multi-location/years replicated 100-seed weight field phenotypic data of 190 RIL mapping individuals and parental accessions employing the composite interval mapping (CIM) function (LOD threshold score >4.0 with 1000 permutations and $p < 0.05$ significance) of QTL Cartographer v2.5 [48] and MapQTL 6 [49]. The phenotypic variation explained (PVE) and additive effect specified by each major grain weight QTL at a significant LOD were estimated following Bajaj et al. [17].

2.5. Expression profiling

To evaluate the potential of ISM and ILP markers for precise estimation of differential expression profiles of genes (from which these markers were developed), we designed primer-pairs from 100-bp flanking exonic sequences of introns in such a way that the markers should amplify 56–196 bp (mean 117 bp) amplicon product size in the cDNA of chickpea accessions used. The total RNA was isolated from diverse vegetative and reproductive tissues (shoot, root, leaf, flower bud and pod) and two seed developmental stages (early cell division phase during 10–20 days after podding/DAP and late maturation phase during 21–30 days after DAP) of low and high seed weight RIL mapping parental accessions (ICC 4958 and ICC 2263). The isolated RNA was digested with DNase (QIAGEN, USA) and purified using RNeasy MinElute Cleanup Kit (QIAGEN, USA), and the purified RNA was tested for high-quality using NanoDrop 2000c Spectrophotometer (NanoDrop products, USA). The cDNA made from this high-quality RNA was amplified with ISM/ILP primers designed from selected chickpea genes along with internal control elongation factor 1-alpha (*EF1α*) using the semi-quantitative and quantitative RT-PCR assays as per Kujur et al. [7] and Bajaj et al. [16]. Three independent biological replicates of each sample and two technical replicates of each biological replicate with no template and primer as control were included in the quantitative RT-PCR assay. The expression level of genes estimated in diverse tissues and seed developmental stages of low and high seed weight chickpea mapping parental accessions were compared among each other and along with control (vegetative leaf tissue of respective accessions) to scan the differentially regulated genes. A heat map illustrating the differential expression profiles of ISM/ILP marker-containing genes was constructed using the TIGR MultiExperiment Viewer (MeV, http://www.tm4.org/mev).

2.6. Construction of ISM and ILP marker database

We constructed an online user-friendly three-layered architecture-led web resource "Chickpea ISM-ILP Marker Database" using MySQL ver. 5.6.12 (www.mysql.com) at back-end and PHP ver. 5.4.16 (www.php.net) at front-end, following the detail methods of Badoni et al. [43]. All the ISM and ILP markers designed from the genes annotated from both *desi* and *kabuli* draft whole genomes were stored in this database for unrestricted public use. The online database was currently hosted on a Linux operating system-based HP Server (Intel Xeon quad core processors with 256 GB of random access memory) and compatible with various commonly used browsers like Firefox and Chrome.

3. Results and discussion

3.1. Genome-wide development and genomic distribution of chickpea ISM and ILP markers

We developed a total of 119169 and 110491 genome-wide ISMs from the introns of 23129 and 20386 protein-coding *desi* and *kabuli* chickpea genes, respectively (Fig. 1A, C, D, Supplemental file 2). Of these, 105756 and 98807 ISMs designed from the introns of 18810 *desi* and 17674 *kabuli* genes, respectively were physically mapped on eight chromosomes. The remaining 13413 and 11684 ISMs developed from 4319 and 2712 *desi* and *kabuli* genes, respectively were mapped on the unanchored scaffolds of chickpea genome (Fig. 1A, C, D, Supplemental file 2). Highest number of 17909 and 15955 ISMs were designed from the intronic sequences

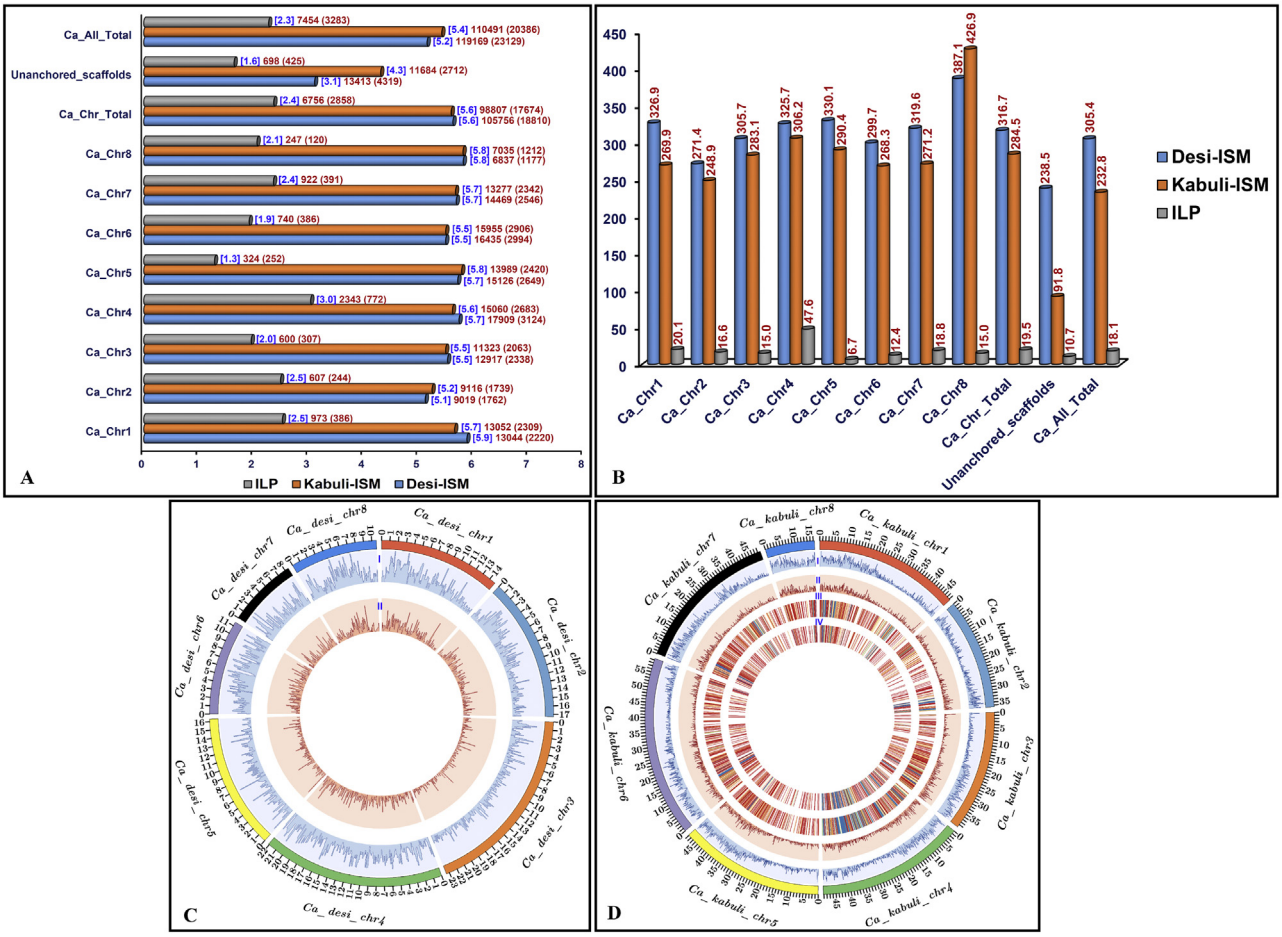

Fig. 1. Genome-wide distribution pattern [frequency (A) and relative density (B)] of 119169 (23129 genes) *desi* and 110491 (20386) *kabuli* ISM and 7454 (3283) ILP markers developed from the intronic sequences of genes annotated on eight chromosomes and unanchored scaffolds of chickpea. Numbers in the square brackets and parentheses indicate the frequency (number of ISMs/ILPs per gene) of markers and ISM/ILP markers-containing *desi* and *kabuli* chickpea genes, respectively. (C) Genomic distribution of 119169 *desi* ISMs physically mapped on eight chromosomes and unanchored scaffolds of chickpea are depicted by a Circos circular ideogram. The circles I and II represent 119169 and 48835 ISMs designed from the total 23129 protein-coding *desi* genes and 8615 TF-encoding *desi* genes, respectively. (D) Genomic distribution of 110491 *kabuli* ISMs and 7454 ILP markers physically mapped on eight chromosomes and unanchored scaffolds of chickpea are depicted by a Circos circular ideogram. The circles I and II indicate 110491 and 48146 ISMs developed from the total 20386 protein-coding *kabuli* genes and 8302 TF-encoding genes, respectively. The circles III and IV illustrate 7454 and 3427 ILP markers developed from the total 3283 protein-coding *kabuli* genes and 1443 TF-encoding genes, respectively. The outermost circles signify the physical size (Mb) of eight chromosome-pseudomolecules coded with multiple colours. The detail information regarding ISM and ILP markers are mentioned in the Supplemental files 2 and 3.

of 3124 and 2906 genes annotated on the chromosomes 4 and 6 of *desi* and *kabuli* chickpea genomes, respectively. Lowest number of 6837 and 7035 ISMs were developed from the intronic sequences of 1177 and 1212 genes annotated on the *desi* and *kabuli* chromosome 8, respectively. We observed almost a similar trend of frequency distribution of ISMs both within the *desi* and *kabuli* genes annotated on nearly all eight chickpea chromosomes (Fig. 1A, C, D, Supplemental file 2). In *desi* genome, this ranged from 5.1 ISMs per gene on chromosome 2–5.9 ISMs per gene on chromosome 1, with a mean of 5.2 ISMs per gene. In *kabuli* genome, this varied from 5.2 ISMs per gene on chromosome 2–5.8 ISMs per gene on chromosomes 5 and 8, with an average of 5.4 ISMs per gene (Fig. 1A). In the unanchored scaffolds of *desi* and *kabuli* genomes, the frequency of ISMs was 3.1 and 4.3 ISMs per gene, respectively. At whole *desi* and *kabuli* genome level, maximum density of physically mapped ISMs was observed on chromosome 8 (*desi*: 387.1 ISMs/Mb and *kabuli*: 426.9 ISMs/Mb), while minimum on chromosome 2 (*desi*: 271.4 ISMs/Mb and *kabuli*: 248.9 ISMs/Mb), with an average of 305.4 and 232.8 ISMs/Mb, respectively (Fig. 1B). In the unanchored scaffolds of *desi* and *kabuli* genomes, the density of ISMs was 238.5 and 91.8 ISMs/Mb, respectively.

A significant difference observed in the frequency of ISM-carrying genes annotated between *desi* (23129 genes) and *kabuli* (20386) genomes is possibly due to availability of uneven high-quality draft sequence assembly of these two chickpea cultivars with varied genome coverage employing totally different computational genomics tools and annotation pipelines accompanied with non-uniform criteria. This resulted in sequencing of different genomic regions of *desi* and *kabuli* genomes and annotation of smaller number of genes in *kabuli* (28269) as compared to *desi* (30257). In this perspective, development of ISMs from the genes at a genome-wide scale can be enriched in chickpea by deploying improved version of both *desi* (CGAP v2.0 [44]) and *kabuli* (CDC Frontier v1.0 [45]) genomes with high-quality large-size chromosome pseudomolecules and unanchored scaffolds as reference and comparison of their individual ISM outputs. This strategy holds much relevance for discovering more robust and non-erroneous common as well as unique ISMs representing redundant and non-redundant fractions of *desi* and *kabuli* genomes, aside their annotated genes (90% genes especially supported with global transcriptome profiling-led gene transcription data), thereby driving large-scale genome-wide ISMs development in chickpea. Essentially, only 25.2% (5835 of total 23129 ISMs-containing *desi* genes)

and 28.7% (5846 of total 20386 *kabuli* genes) genes with 29865 (25.1% of total 119169 ISMs) and 29845 (27% of total 110491 ISMs) ISMs were found to be common between *desi* and *kabuli* genomes, respectively (Supplemental file 2). The ISMs developed from the remaining 71.3–74.8% genes were unique covering only the different non-redundant regions of *desi* and *kabuli* genomes. Therefore, use of both *desi* and *kabuli* genomes as reference for developing ISMs appears more advantageous for widening their practical applicability in genomics-assisted breeding as well as crop improvement concerning varied phenotypic and genetic diversity characteristics of *desi* and *kabuli* chickpea. The two major chickpea cultivars- *desi* and *kabuli* with different plant characteristics representing diverse gene pools-based geographical distribution are distinguished primarily by small/large seed size, angular/owl seed shape, thicker/thinner seed coat, dark yellow/beige seed coat colour and pink/white flower colour [50,51]. Significant efforts have been made to understand the molecular diversity and domestication pattern among *desi* and *kabuli* accessions using traditional- and NGS-led genome-wide and gene-derived SSR and SNP markers in chickpea. Based on these, a clear differentiation and clustering of accessions into distinct *desi* and *kabuli* cultivar groups as per their parentage, geographical origin and adaptive environment is apparent [7,9,13,18,24,25,45,52]. However, the aforesaid studies also highlighted the contribution of complex breeding history involving inter-crossing/introgression coupled with strong adaptive selection pressure towards existence of admixed domestication pattern-based population genetic structure among *desi* and *kabuli* chickpea accessions. Considering the uneven pseudomolecule/scaffold assembly and non-uniform annotation-led genome sequencing efforts as well as distinct phenotypic and genetic diversity characteristics of *desi* and *kabuli*, development of ISMs from the draft whole genomes including cultivar-specific genes of both these sequenced accessions will be useful to expedite various genomics-assisted breeding applications in chickpea.

With an effort to convert ISMs to ILP markers at a genome-wide scale, 32618 InDels were identified within the introns of genes annotated between *desi* (ICC 4958) and *kabuli* (CDC Frontier) genomes. This comparison of intronic-InDel polymorphism between *desi* and *kabuli* genomes, 7454 ISMs were converted into ILP markers targeting introns of 3283 genes, which were well-distributed and physically mapped on eight chromosomes and unanchored scaffolds of chickpea genome (Fig. 1A, C, D, Supplemental file 3). Highest number of 2343 ILP markers developed from the introns of 772 genes were physically mapped on chromosome 4, whereas it was lowest on chromosome 8 (247 ILP markers in 120 genes). The *in silico* fragment length polymorphism detected by ILP markers between *desi* and *kabuli* genomes based on sum of InDels-size (bp) variation within introns of genes ranged from 1 to 45 bp, with a mean of 4.4 bp. The ILP markers exhibiting 1–4 bp (74.3%, 5541 of 7454 markers) InDels-based fragment length polymorphism within the introns of genes between *desi* and *kabuli* genomes were found abundant (Supplemental file 3). About 14.4% (1073) and 11.3% (840) ILP markers revealed 5–10 bp and 11–45 bp intronic InDels-based fragment length polymorphism, respectively. A varied trend of frequency distribution of ILP markers was observed in all eight chromosomes and unanchored scaffolds of chickpea genome (Fig. 1A). However, this ranged from 1.3 to 3.0 ILP markers per gene, with a mean of 2.3 ILP markers per gene. The genome coverage based on density of physically mapped ILP markers exhibited their maximum density on chromosome 4 (47.6 ILP markers/Mb) and minimum on chromosome 5 (6.7 ILPs/Mb), with a mean of 18.1 ILP markers/Mb (Fig. 1B). In the unanchored scaffolds of chickpea genome, the density of ILP markers was 10.7 ILP markers/Mb. Summarily, a random uneven genomic distribution of physically mapped ISM and ILP markers pertaining to their abundance and relative density across eight chickpea chromosomes and unanchored scaffolds was observed. Notably, 6869 (5.8% of total 119169 ISMs) and 2397 (2.2% of total 110491 ISMs) ISMs were found to be present in the 1532 (6.6% of total 23129 *desi* genes) and 441 (2.2% of total 20386 *kabuli* genes) duplicated genes annotated individually from *desi* and *kabuli* genomes, respectively. This included two ILP markers in the two duplicated *kabuli* genes (Supplemental files 2, 3). Interestingly, a higher density and wider genome coverage of gene-derived *desi* (305.4 ISMs/Mb) and *kabuli* (232.8 ISMs/Mb) ISMs, but a lower density of ILP markers (18.1 ILPs/Mb) as compared to that of multi-allelic SSR (144–157 SSRs/Mb) and InDel (61.4 InDels/Mb) markers (physically mapped on eight chickpea chromosomes) was evident [21,24]. Therefore, ISMs with dense genome-wide coverage will be useful for diverse high-throughput genetic analysis including high-resolution QTL and association analysis in mapping population and natural germplasm lines to identify potential genes/QTLs regulating traits of agronomic importance in chickpea.

The functional annotation of ISMs-containing chickpea genes revealed the occurrence of 817 (0.7% of total 119169 ISMs) and 29 (0.3% of total 110491 ISMs) ISMs in the 250 (1.1% of total 23129 ISMs-carrying *desi* genes) and 7 (0.03% of total 20386 *kabuli* genes) transposable elements (TE)-related genes annotated from *desi* and *kabuli* genomes, respectively (Supplemental file 2). Notably, 81053 and 71956 ISMs developed from the introns of 12504 and 10915 *desi* and *kabuli* genes, respectively as well as 4737 ILP markers (Intronic InDel-based *in silico* fragment length polymorphism ranged from 1 to 45-bp) designed from the 1977 genes were primarily involved in multiple cellular, biological and molecular processes of crop plants (Supplemental files 2 and 3). The KOG-based functional annotation of these ISM and ILP markers-carrying *desi* and *kabuli* genes revealed their enrichment for post-translational modification, protein turnover and chaperones (O, 7.9–9.1%), signal transduction mechanisms (T, 7.5–7.6%) and unknown expressed proteins (S, 5.9–6.2%), beside the general function prediction (R, 15.6–20.1%) (Fig. 2A, Supplemental files 2 and 3). Moreover, 48835 *desi* and 48146 *kabuli* ISM and 3427 ILP markers-containing 8615, 8302 and 1443 genes (representing 58 TF gene family), respectively belonging to *bHLH* (9.5–10.8%), Trihelix (8.5%), *NAC* (6.7–7.0%), *Myb* (6.2%) and *B3* (5.9–6.1%) TF families were found predominant in chickpea (Fig. 2B–D, Supplemental files 2 and 3). In order to access the functional significance of identified genome-wide ISM and ILP markers for subsequent fine-mapping/map-based cloning of major QTLs/genes, these markers were annotated in the genes underlying the known QTLs documented previously for double podding, vernalisation, nodulation, drought tolerance, and *Ascochyta* blight and *Fusarium* wilt resistance traits in chickpea [15,53–60]. Based on these analyses, 52879 ISMs and 9322 ILP markers in the 9625 and 3021 *kabuli* genes regulating aforementioned diverse a/biotic stress tolerance and yield component traits were identified (Supplemental file 4).

The designing of multiple *desi* and *kabuli* ISM (5.2–5.4 ISM/gene) and ILP (2.3 ILP/gene) markers in individual genes can impart greater flexibility to molecular geneticists/breeders for screening appropriate primer combination exhibiting robust marker amplification (with a mean amplicon product size of 528 bp) and detecting higher potential of functional allelic polymorphism especially among cultivated (*desi* and *kabuli*) chickpea accessions. This will essentially expedite identification of functionally relevant genes by high-resolution QTL mapping and association analysis to efficiently dissect the complex quantitative traits for genomics-assisted crop improvement in chickpea. The genome-wide ISM and ILP markers developed from diverse trait-associated functionally annotated known and candidate genes/QTLs can be utilized as an instant genomic resource for rapidly establishing marker-trait linkages and identification/fine-mapping of molecular tags governing vital agronomic traits to drive marker-assisted foreground and background selection-led genetic enhancement in chickpea.

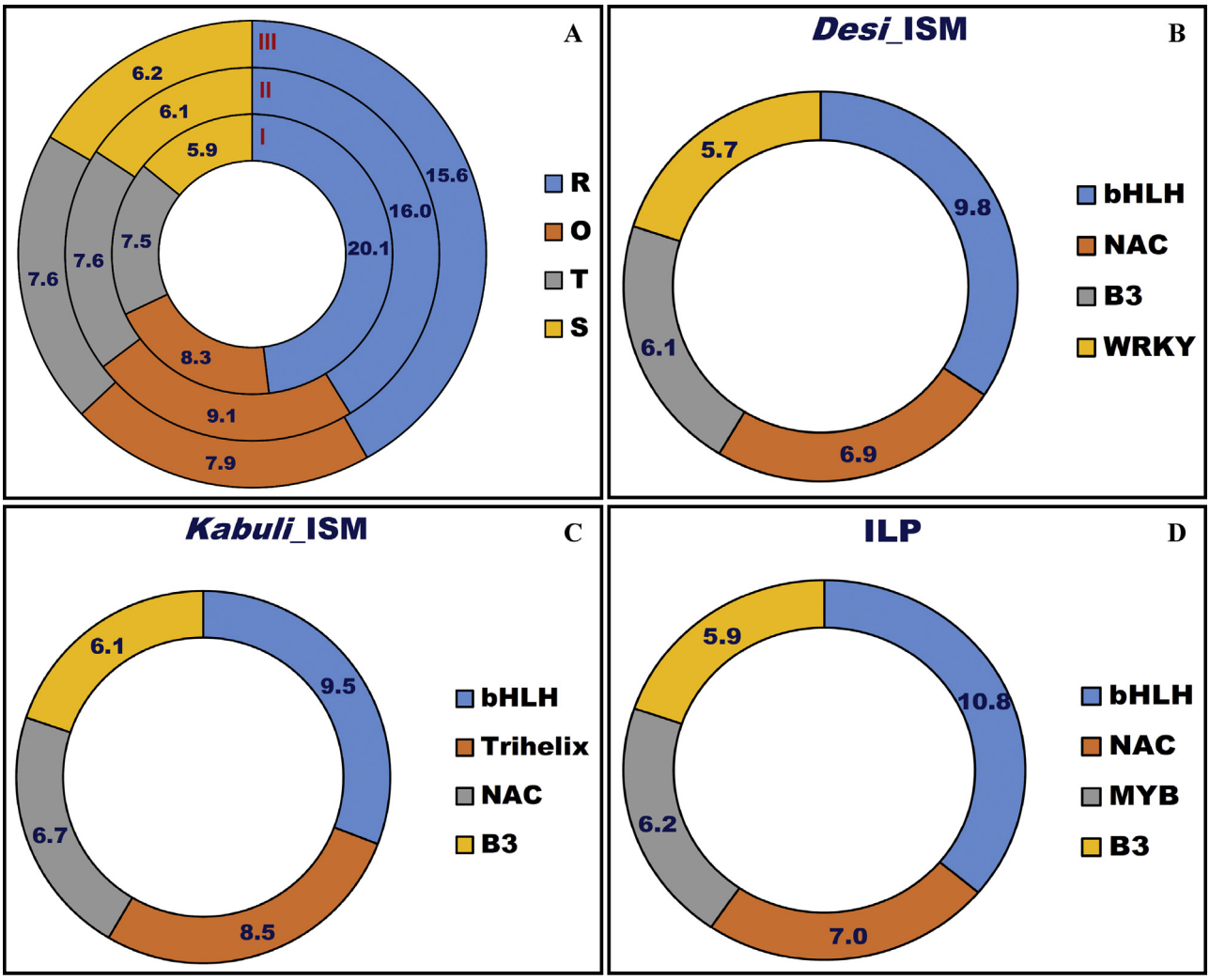

Fig. 2. (A) Proportionate distribution of 81053 (12504 genes) *desi* and 71956 (10915) *kabuli* ISMs as well as 4737 (1977) ILP markers designed from the chickpea genes representing diverse KOG-based functional classes. Frequency (%) distribution of 48835 (8615 genes) *desi* (B) and 48146 (8302) *kabuli* ISMs (C) including 3427 (1443) ILP (D) markers developed from the different TF-encoding genes of chickpea.

3.2. High marker polymorphic and molecular diversity potential

To evaluate the amplification, polymorphism and genetic diversity potential of ISM and ILP markers, a set of 2405 ILP markers (representing 2019 ISMs with at least one marker in 1604 genes) exhibiting ≥4 bp InDel-based *in silico* fragment length polymorphism between *kabuli* (CDC Frontier) and *desi* (ICC 4958) accessions were selected. These markers were physically mapped on genes annotated across eight chromosomes and unanchored scaffolds of *kabuli* genome. All the 2405 ISM/ILP markers were validated experimentally using the genomic DNA of one chickpea accession (ICC 4958), the genome sequence from which ISM and ILP markers were designed originally. Of these, 1993 ISM/ILP markers amplified single reproducible PCR amplicons in 2.5% agarose gel with a mean amplification success rate of 82.9% (Fig. 3). The validated ISM/ILP markers were further genotyped in 32 cultivated *desi* and *kabuli* chickpea accessions using the agarose gel- and amplicon sequencing-based assays to assess their polymorphic potential among these accessions. The amplified markers along with 1718 (86.2%) polymorphic markers genotyped in 24 chickpea accessions overall detected 4981 alleles with an average PIC of 0.70. The number of alleles detected by the ILP markers among chickpea accessions varied from 1 to 3 alleles with a mean 2.5 alleles per marker (Fig. 3). A higher polymorphic potential of markers was observed among 20 accessions belonging to *desi* (1538 markers, 77.2% polymorphism and mean PIC: 0.67) than that of 12 accessions representing *kabuli* (1365, 68.5% and 0.61) chickpea. The potential of markers to detect polymorphism between *desi* and *kabuli* cultivars (1617, 81.1% and 0.68) was much higher as compared to that within cultivated *desi* and *kabuli* chickpea (Fig. 3). The amplified 1679 (84.2%) ILP markers revealing *in silico* InDel-based fragment length polymorphism between *desi* and *kabuli* accessions were validated experimentally using agarose gel- and PCR amplicon sequencing-based assays in chickpea.

Higher average amplification success rate (82.9%) as well as intra–specific polymorphic potential (86.2%) detected by the ISM and ILP markers among *desi* and *kabuli* cultivated chickpea accessions are much higher than that documented especially with random genome-wide SSR markers (~35%) and *in silico* polymorphic SSR markers (50–60%) [2,4,7,13,18,24,61,62], but comparable to genome-wide InDel markers (~83%) [21]. These informative gene-based ISM and ILP markers with a higher potential of detecting intra-specific polymorphic potential among domesticated accessions at a genome-wide scale even by a simpler cost-effective agarose gel-based assay could serve as an ideal resource for their immense use in various genomics-assisted breeding applications of chickpea. Essentially, the validation and genotyping of ILP markers overall ascertained the correspondence of expected *in silico* frag-

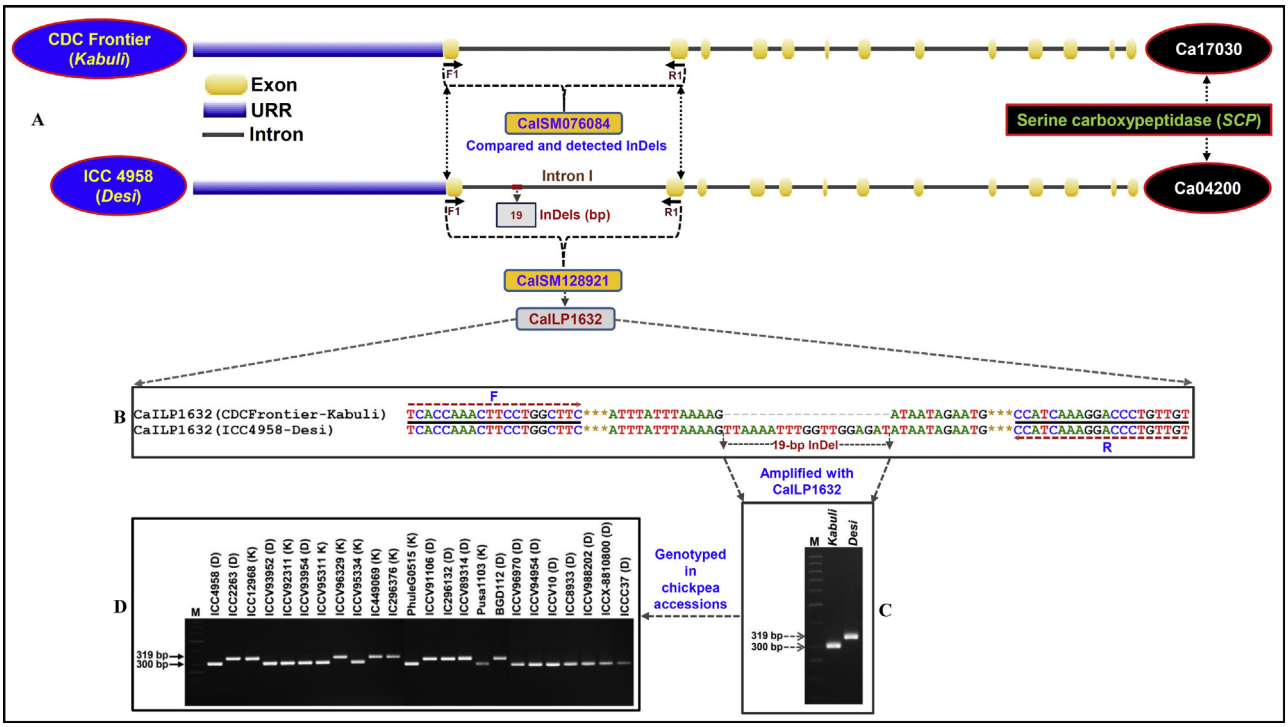

Fig. 3. Graphic representation illustrating the major steps followed for efficient designing (A, B), large-scale experimental validation (C) and genotyping (D) of *desi* (CaISM128921) and *kabuli* (CaISM076084) ISM and ILP (CaILP1632) markers derived from the intronic sequence component of a serine carboxypeptidase (*SCP*) gene to access its potential for amplification (C) and polymorphism (D) among a representative 24 cultivated *desi* (D) and *kabuli* (K) chickpea accessions. The forward (F) and reverse (R) primers designed from the exonic sequence of a *SCP* gene flanking the intron without any InDel and with a 19-bp intronic-InDel were developed as ISM and ILP markers, respectively (A, B). URR: upstream regulatory region. The identities of ISM and ILP markers along with their detailed information are provided in the Supplemental files 2 and 3.

ment length polymorphism based on sum of InDels-size occurred in each intron of genes with their actual amplicon fragment size variation detected experimentally using the gel- and amplicon sequencing-based assays between *kabuli* and *desi* chickpea accessions. This infers practical applicability of these markers for large-scale genotyping applications, which can be complemented further with user-preference marker selection (screening markers based on their predetermined intronic-InDel size) by optimal use of resources in chickpea. Therefore, the ISM and ILP markers with diverse desirable genetic attributes like simplicity of their *in silico* discovery, robustness in high-throughput genetic analysis and efficiency in detecting functional allelic variation within the gene sequence components of multiple cultivated accessions can serve as a preferred marker resource for genomics-assisted breeding applications of chickpea in laboratories with minimal infrastructural facilities. The ISM and ILP markers developed from the coding, but non-coding gene sequence components of *desi* and *kabuli* genomes essentially have greater efficacy to recover the recurrent parent genome than that of random markers (representing mostly the non-coding regions of genome) in marker-assisted background selection, which is of much preferred as far as phenotype is concerned. Henceforth, these genic ISM and ILP markers will expedite the process of marker-assisted genetic improvement at a genome-wide scale by targeting multiple stress tolerance and yield component traits in chickpea.

The estimation of genetic diversity level among 32 *desi* and *kabuli* chickpea accessions using 1718 polymorphic ISM/ILP markers (mapped on eight chromosomes and unanchored scaffolds) exhibited a broader range of distance coefficient from 0.19 to 0.81 with an average of 0.60. The level of functional molecular diversity detected by genic ISM and ILP markers among domesticated chickpea accessions is higher/comparable to that documented earlier using genome-wide SSR and InDel markers [21,24]. In this context, a wider level of functional genetic diversity uncovered by gene-derived ISM and ILP markers assumes great significance for expediting various genomics-assisted breeding applications in chickpea at a genome-wide scale. The phylogenetic relationship among 32 *desi* and *kabuli* cultivated chickpea accessions was determined and illustrated by an unrooted phylogenetic tree (Fig. 4). The ISM and ILP markers clearly differentiated all these accessions from each other and clustered into two major groups, namely *desi* and *kabuli*. This collectively infers the correspondence of clustering patterns observed among these cultivated chickpea accessions with the known cultivar-specific origination, pedigree relationships and parentage [2,4,7,9,24,52].

3.3. Construction of a high-resolution ISM/ILP marker-based genetic linkage map

To construct a high-density genetic linkage map, 1424 ILP markers (physically mapped on eight chromosomes) revealing polymorphism between two parental accessions (ICC 4958 and ICC 2263) were genotyped among 190 RIL individuals of a F_7 chickpea mapping population (ICC 4958 × ICC 2263). The marker linkage analysis mapped 1424 markers across eight chromosomes of a constructed chickpea genetic map (Table 1, Fig. 5). The genetic map covered a total map length of 807.48 cM with a mean map-density (inter-marker distance) of 0.65 cM. Highest and lowest number of markers were mapped on chromosomes 4 (458 markers) and 8 (55), respectively (Table 1). Longest and shortest map-length spanning 194.85 and 57.03 cM were obtained in chromosomes 4 and 8, respectively. The most and least saturated genetic maps were observed in chromosomes 4 (mean inter-marker distance 0.43 cM) and 8 (1.04 cM), respectively (Table 1).

The genic ISM and ILP markers have significance in differentiating the homozygous and heterozygous RIL mapping individuals,

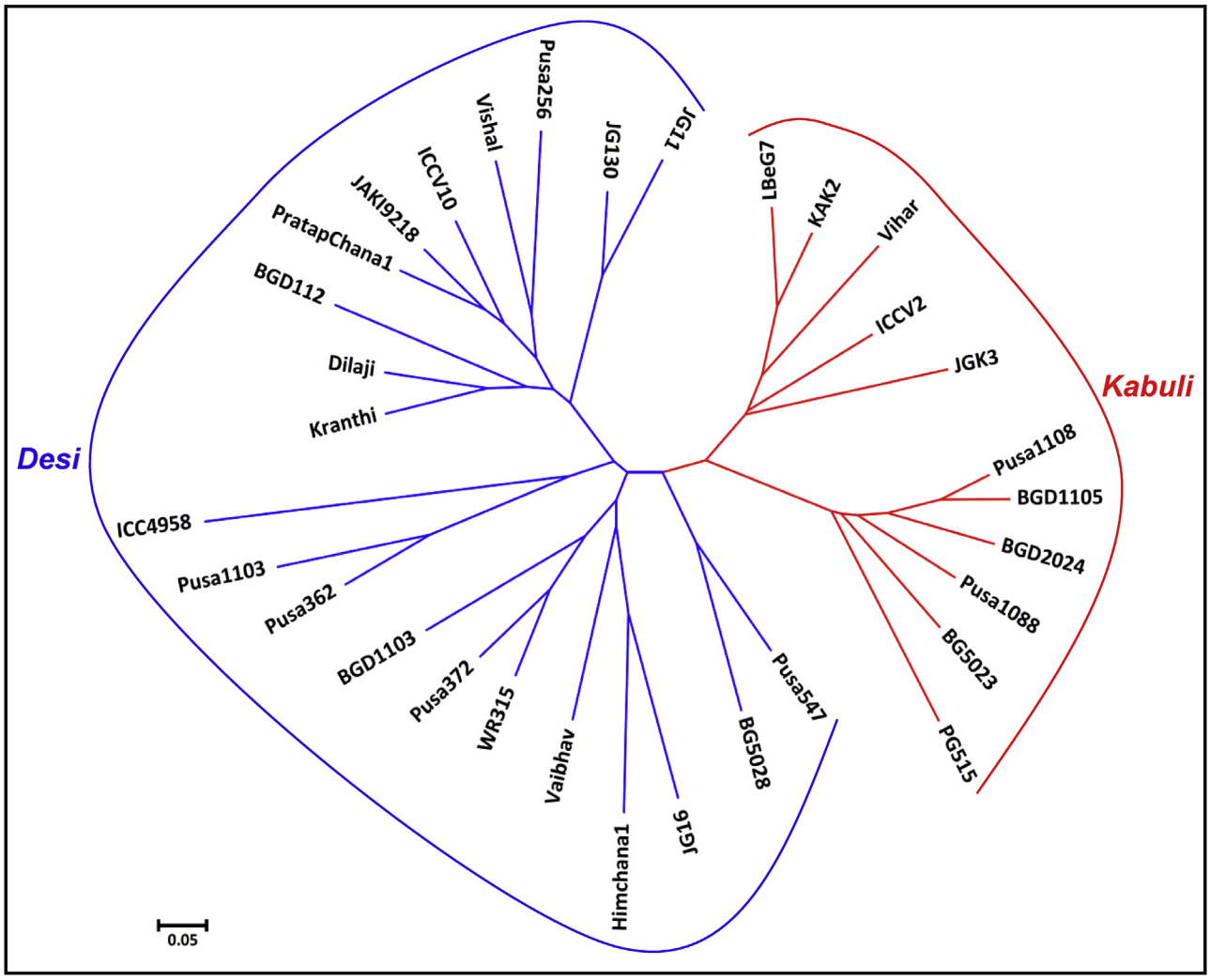

Fig. 4. An unrooted phylogenetic tree illustrating the genetic relationship among cultivated 32 *desi* and *kabuli* chickpea accessions based on Nei and Li's similarity coefficient using 1718 polymorphic ISM/ILP markers.

Table 1
ISM/ILP markers mapped on eight chromosomes of an intra-specific chickpea genetic linkage map (ICC 4958 × ICC 2263).

Linkage groups (LGs)/chromosomes (Chr)	ISM/ILP markers mapped	Map-length covered (cM)	Mean inter-marker distance (cM)
*Ca*LG(Chr)1	197	112.09	0.57
*Ca*LG(Chr)2	120	87.10	0.73
*Ca*LG(Chr)3	156	90.50	0.58
*Ca*LG(Chr)4	458	194.85	0.43
*Ca*LG(Chr)5	95	73.51	0.77
*Ca*LG(Chr)6	152	84.39	0.56
*Ca*LG(Chr)7	191	108.01	0.57
*Ca*LG(Chr)8	55	57.03	1.04
Total	1424	807.48	0.65

thereby exhibited co-dominant genetic inheritance pattern similar to other commonly utilized SSR and InDel markers for construction of genetic linkage map and QTL mapping in chickpea. This infers the broader practical applicability of these developed genome-wide genic markers for construction of high-resolution genetic linkage maps (functional transcript maps) and efficient molecular mapping of QTLs/genes governing stress tolerance and yield component traits in chickpea. A 1424 ISM/ILP markers-led high-density intra-specific genetic linkage map (inter-marker distance of 0.65 cM) (ICC 4958 × ICC 2263) constructed in the present study is comparable/highly saturated than that documented yet in multiple intra- and inter-specific mapping population-derived genetic maps (0.21–8.01 cM) of chickpea [2–7,12,14,16,17,21,25–27]. Therefore, a high-density intra-specific genetic linkage map constructed by us has significance to be utilized as a reference for quick molecular mapping of high-resolution QTLs/genes regulating diverse agronomic traits in chickpea.

3.4. Molecular mapping of major seed weight QTLs

A significant phenotypic variation for seed weight (100-seed weight: 10.6–38.9 g with an 18.3 g mean ± 5.4 S.D., CV: 29.5%

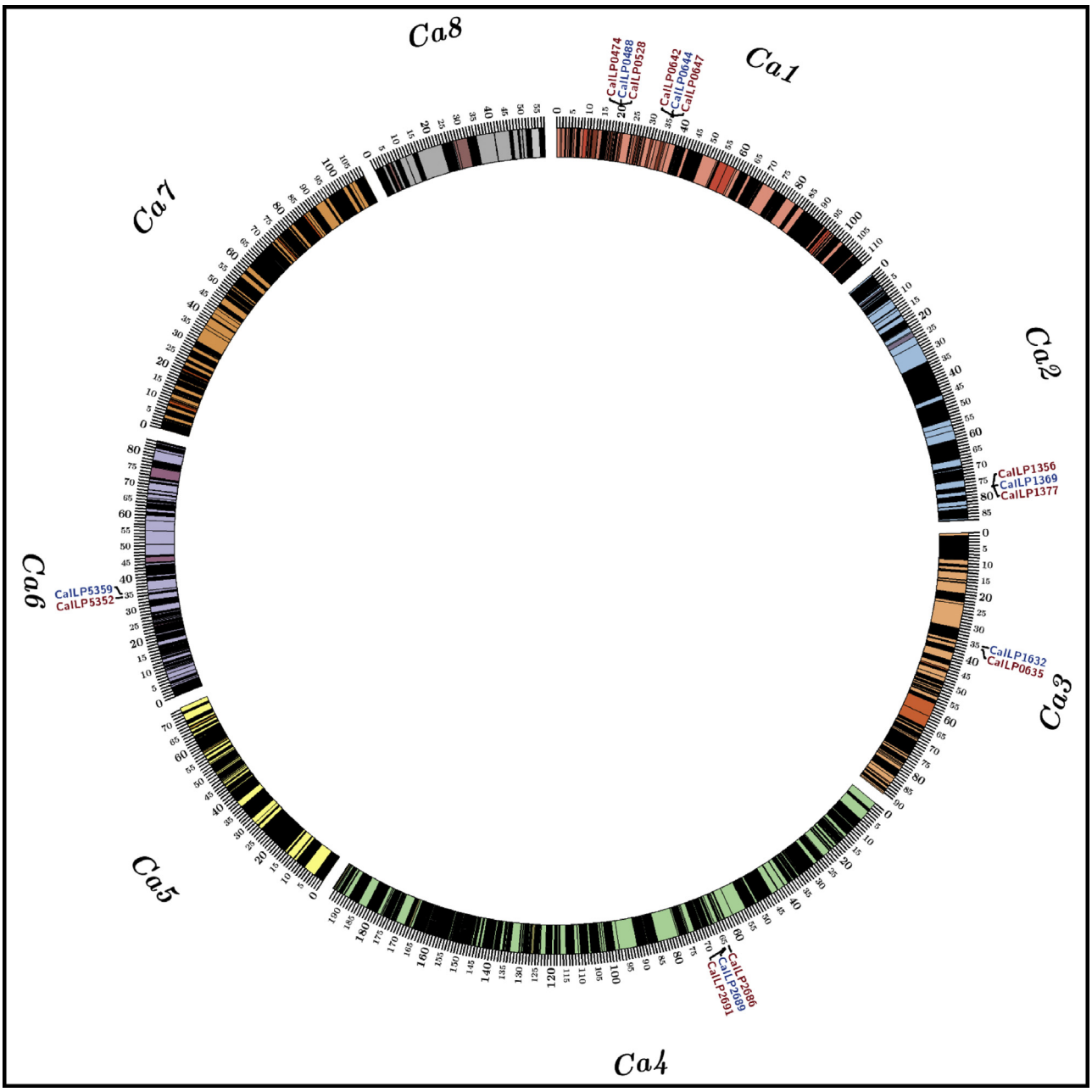

Fig. 5. (A) Molecular mapping of six major seed weight QTLs on a high-density intra-specific genetic linkage map/transcript map (ICC 4958 x ICC 2263) constructed by integrating 1424 gene-derived ILP markers on eight chickpea chromosomes, which is depicted by a Circos circular ideogram. The outer circles represent the diverse genetic map length (cM) (spanning 5 cM uniform genetic distance intervals between bins) of eight chromosomes coded with multiple colours. The constructed high-resolution genetic map identified six ILP markers-containing candidate genes at six major genomic regions harbouring seed weight QTLs mapped on five chickpea chromosomes 1, 2, 3, 4 and 6. The ILP markers flanking and tightly linked to the major seed weight QTLs highlighted with red and blue colour, respectively. The detail information on ISM and ILP markers and seed weight QTLs are mentioned in Table 2 and Supplemental files 2 and 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and 87% H^2) trait in 190 individuals and two parental accessions of a RIL mapping population (ICC 4958 × ICC 2263) was obtained. We observed a normal frequency distribution, including a bi-directional transgressive segregation of seed weight in an aforementioned RIL mapping population (Supplemental file 5). The two years multi-location replicated field phenotyping data of seed weight and genotyping information of 1424 ILP markers genetically mapped on eight chickpea chromosomes were integrated for molecular mapping of major seed weight QTLs. This analysis detected six major genomic regions harbouring six robust QTLs associated with seed weight that were mapped on five chickpea chromosomes (1, 2, 3, 4 and 6) (Table 2, Fig. 5). Highest number of two major seed weight QTLs were mapped on chromosome 1. The individual major QTL explained 11–23% phenotypic variation (R^2) for seed weight trait at 7.6–10.5 LOD (Table 2). The PVE (phenotypic variation explained) measured for all six major QTLs in combination was 32.8%. Six major genomic regions underlying these seed weight QTLs spanned (0.25 cM on chromosome 1–1.86 cM on chromosome 4) with 23 ILP markers, were mapped on six different genomic regions of five chromosomes. All six major seed weight QTLs revealed positive additive gene effect (2.0–3.8) for seed weight with major allelic contribution from a high seed weight parental chickpea accession ICC 4958. The integration of genetic and physical maps of *kabuli* genome detected six genes with ILP markers

Table 2
Molecular mapping of major seed weight QTLs in chickpea.

[a]QTLs	LGs/ Chromosomes	Marker intervals with genetic positions (cM)	QTL physical intervals (bp)	Markers tightly linked with seed weight QTLs [genetic (cM) and physical (bp) positions]	Structural annotation	Protein-encoding genes	LOD	PVE (R^2%)	A
Caq^lSW1.1	1	CaILP0474 (19.32)-CaILP0528 (19.57)	7884794–7991529	CaILP0488 [(19.48) and (7950391)]	Ca08045	Cytochrome P450	9.8	16	2.5
Caq^lSW1.2	1	CaILP0642 (35.33)-CaILP0647 (35.82)	14421165–14620090	CaILP0644 [(35.73) and (14581851)]	Ca07077	No apical meristem (NAM) transcription factor	10.0	19	2.8
Caq^lSW2.1	2	CaILP1356 (76.97)-CaILP1377 (77.15)	32203753–32282290	CaILP1369 [(77.09) and (32257214)]	Ca10289	Homeobox transcription factor	8.7	12	3.2
Caq^lSW3.1	3	CaILP1632 (35.01)-CaILP1635 (35.98)	15424043–15838572	CaILP1632 [(35.01) and (15424043)]	Ca17030	Serine carboxypeptidase	10.5	23	3.8
Caq^lSW4.1	4	CaILP2686 (64.30)-CaILP2691 (66.16)	16197589–16665407	CaILP2689 [(65.63) and (16532811)]	Ca05471	Basic leucine zipper (*bZIP*) transcription factor	8.5	15	2.4
Caq^lSW6.1	6	CaILP5352 (34.48)-CaILP5359 (35.55)	23776052–24522143	CaILP5359 [(35.56) and (24522143)]	Ca27823	Cytochrome P450	7.6	11	2.0

(revealing 4–32 bp InDels-based fragment length polymorphism among *desi* and *kabuli* chickpea accessions) tightly linked to six major QTLs governing seed weight (single marker analysis-based high-resolution QTL mapping) in chickpea (Table 2, Fig. 5).

To assure the robustness and novelty of our identified QTLs, the genomic regions underlying six major seed weight QTLs were compared with that documented in previous QTL mapping studies utilizing multiple inter- and intra-specific chickpea mapping populations [7,8,10,11,14–17,22,23]. We could not find any correspondence of these identified seed weight QTLs with known QTLs reported earlier based on their congruent genetic or physical positions on chickpea chromosomes. This infers that major seed weight QTLs identified in our study are novel and possibly exhibit population-specific genomic distribution. It is possibly due to varied effect of population-specific genetic inheritance pattern, which especially rely upon the genetic constitution of parental accessions selected to develop mapping populations with contrasting agronomic traits between the past and present studies.

3.5. Differential expression profiling of genes with ISM/ILP markers

To assess the potential of developed genic ISM and ILP markers for accurate assaying of differential expression pattern of genes (from which these markers are derived), semi-quantitative and quantitative RT-PCR assays were performed using the RNA isolated from vegetative and reproductive tissues (shoot, root, leaf, flower bud and pod) and two seed developmental stages (early cell division and late maturation phase occurring at 10–20 and 21–30 DAP, respectively) of low and high seed weight mapping parental accessions (ICC 4958 and ICC 2263). In the perspective of identifying genes associated with seed weight in chickpea, the ISM and ILP markers designed from each of six genes underlying six major seed weight QTLs (delineated by high-resolution QTL mapping) were selected for their differential expression profiling in above-said five vegetative and reproductive tissues and two seed developmental stages of ICC 4958 and ICC 2263 using semi-quantitative and quantitative RT-PCR assays. The use of ISM/ILP markers derived from six chickpea genes in semi-quantitative RT-PCR assay amplified single reproducible PCR amplicons of expected product size (bp) across all samples resolved in agarose gel.

For quantitative RT-PCR assay, the selected six genes-derived ISM/ILP markers were amplified using the cDNA of shoot, root, leaf, flower bud, pod and two seed developmental stages of RIL mapping parental accessions-ICC 4958 and ICC 2263 (at least three biological replicates) with no template and primer as control. This amplified single gene-specific PCR product of desired fragment size, which was further ascertained through single peak-led melting-curve analysis of individual gene. Accordingly, we measured and compared the amplification curves and cycle threshold (C_T) of all gene-based markers across all three biological replicates of aforesaid vegetative and reproductive tissues and two seed developmental stages of ICC 4958 and ICC 2263. All six genes with ISM/ILP markers exhibited high seed-specific expression in both ICC 4958 and ICC 2263 (Fig. 6). These genes with markers exhibited pronounced up-regulated expression (>8-fold) pattern in two seed developmental stages as compared to control vegetative and reproductive tissues (shoot, root, leaf, flower bud and pod) of ICC 4958 and ICC 2263 (Fig. 6). Interestingly, six ILP markers-derived from genes underlying seed weight major QTLs revealing InDel-based *in silico* fragment length polymorphism (4–32 bp) between *desi* and *kabuli* chickpea accessions exhibited higher differential expression (>two-fold up-regulation) especially in seed developmental stages of high seed weight mapping parental accession (ICC 4958) as compared to low seed weight parent (ICC 2263) (Fig. 6). Interestingly, serine carboxypeptidase protein-coding gene of these revealing

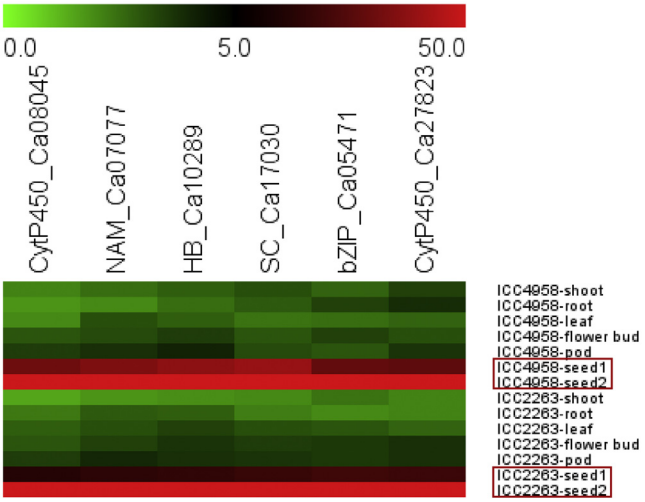

Fig. 6. The differential expression profiles of six genes with ILP markers underlying major seed weight QTLs (delineated by high-resolution QTL mapping) in the vegetative and reproductive tissues (shoot, root, leaf, flower bud and pod) and two seed developmental stages (seed1 and seed2: early cell division and late maturation phase occurring at 10–20 and 21–30 DAP, respectively) of low and high seed weight mapping parental accessions (ICC 4958 and ICC 2263). The green, black and red colour scale (mentioned at the top) represent the low, medium and high level of average log signal expression values of genes in various tissues/stages, respectively. The marker-carrying genes and tissues/stages utilized for expression profiling are specified on the top and right side of an expression map, respectively. The detail structural and functional annotation of ILP markers-containing genes are provided in Table 2 and Supplemental files 2 and 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

pronounced up-regulation (>4-fold) in high than that of low seed weight mapping parental accessions during seed development, was found to be most promising for seed weight regulation in chickpea.

Collectively, the ISM and ILP markers developed from the genes have added-advantages for assaying precise and robust differential expression profiles of corresponding genes in diverse developmental stages/tissues of *desi* and *kabuli* chickpea accessions at a genome-wide scale. Moreover, the designing of multiple ISM and ILP markers from individual genes also provides flexibility to users to select desirable primer combination for reproducible amplification and realistic measurement of differential expression pattern of target genes especially in RT-PCR assay. Therefore, the efficacy of these gene-based markers for simultaneous assaying of DNA-based high-throughput genotyping, functional allelic diversity and expression profiling accurately in a diverse array of domesticated *desi* and *kabuli* chickpea accessions was evident. These useful genetic characteristics of markers will further accelerate the molecular mapping of major QTLs/eQTLs (expression QTLs) and differentially expressed genes regulating diverse vital agronomic traits in chickpea. It could essentially assist us in rapid delineation of trait-associated functionally relevant molecular tags at a genome-wide scale for marker-assisted genetic enhancement of chickpea.

3.6. Significance of ISM and ILP markers in delineation of candidate genes regulating seed weight in chickpea

The mapping of high-resolution major seed weight QTLs on a ISM and ILP markers-anchored high-density intra-specific genetic linkage map (ICC 4958 × ICC 2263) and their effective integration with differential expression profiling delineated six potential candidate genes [cytochrome P450, no apical meristem (*NAM*), homeobox, serine carboxypeptidase (*SCP*), basic leucine zipper (*bZIP*) and cytochrome P450] underlying six major QTLs governing seed weight in chickpea (Fig. 5, Table 2). Of these, one strong seed weight-associated serine carboxypeptidase (*SCP*) gene with ILP markers exhibiting 19-bp InDel-based fragment length polymorphism among *desi* and *kabuli* accessions was significantly expressed and highly up-regulated especially in later seed developmental stages of high seed weight mapping parental accessions. This is coherent with previous map-based cloning and functional genomic studies, which demonstrates the efficacy of this gene in regulation of seed size/weight by modulating brassinosteroid (BR) signalling pathways in rice and *Arabidopsis* [63–66]. The seed weight-associated three TF genes (Homeobox, *bZIP* and *NAM*) with ILP markers identified in chickpea are reportedly involved in controlling seed growth and development as well as determination of

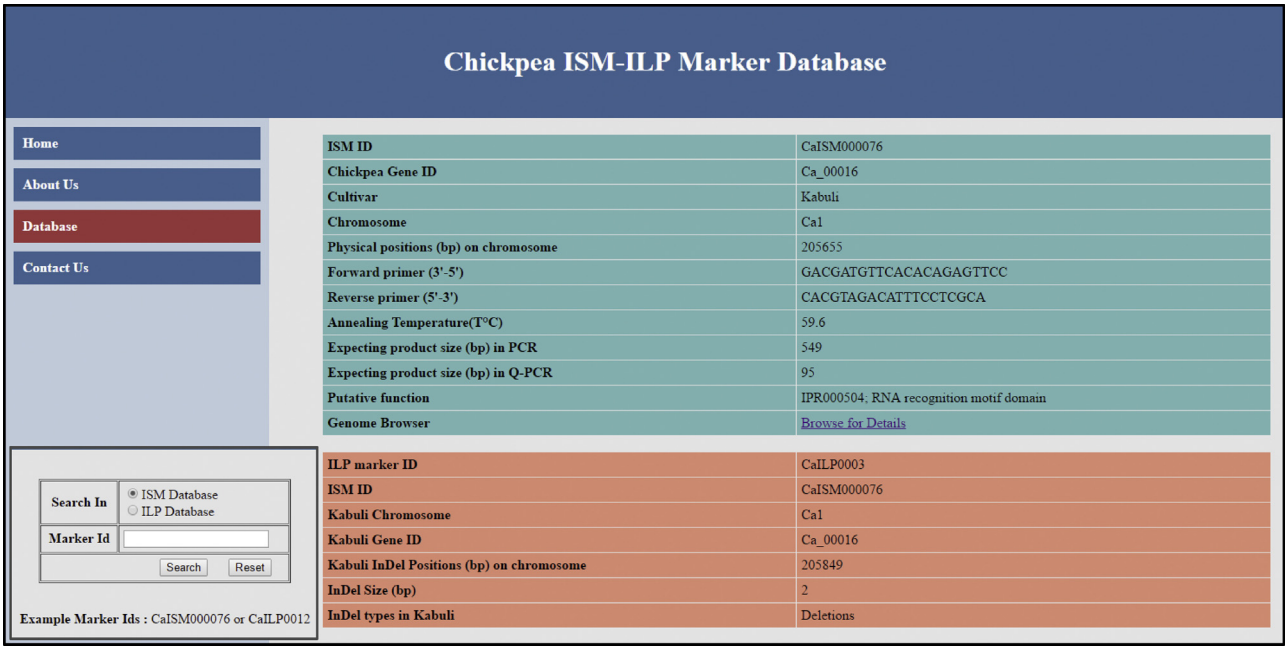

Fig. 7. Snapshots depicting the characteristic features and advantages of diverse interfaces included in a public web-resource "Chickpea ISM-ILP Marker Database". The snapshots were selected from the database webpages developed.

seed size/weight in multiple crop plants [67–77]. The integration of trait association analysis with QTL mapping, differential expression profiling and LD (linkage disequilibrium)-based marker haplotyping have ascertained the trait association potential of *bZIP* and *NAC* TF gene-derived SNPs mapped on the chromosomes 1 and 2 governing seed weight in chickpea [7,8]. The potential involvement of cytochrome P450 protein-coding gene in regulation of seed development including seed size has been well-documented in many monocot and dicot plant species [78–81]. In these context, the gene-based ISM/ILP markers identified by us thus have significance to be utilized in marker-aided genetic improvement for developing varieties with higher seed size/weight and yield in chickpea.

3.7. Characteristics and efficacy of chickpea ISM and ILP marker database

We developed an online marker database portal named as "Chickpea ISM-ILP Marker Database" for querying and visualization of ISM and ILP markers developed from the draft *desi* and *kabuli* genomes in order to expedite their unrestricted public access for various genomics-assisted breeding applications in chickpea. The database majorly contained diverse web-based interfaces with prime search options including Search by cultivars, Search by marker IDs, Search by gene locus IDs and Search by gene function. Currently, the database contains the information on genome-wide 119169 *desi* and 110491 *kabuli* ISMs as well as 7454 ILP markers along with their cultivar-specific origination, gene structural and functional annotation, forward/reverse primer sequences, expected PCR/Q-PCR product size (bp), annealing temperature (T°C) and InDel characteristics in the ISM/ILP marker regions. In addition, the database contained several user-friendly key options like displaying the search results in a tabular format to visualize and batch-download the ISM and ILP markers designed from gene sequence components of *desi* and *kabuli* genomes. In addition, the search outputs of ISM and ILP markers can be displayed by their hyperlinks to the genome browsers with genes annotated on the *desi* (CGAP v2.0, [44]) and *kabuli* (CDC Frontier v1.0, [45]) genomes. The database is publicly accessible via the Internet using web-links: http://webapp.cabgrid.res.in/chickpea/ and http://bioinformatics.iasri.res.in/chickpea/. The snapshots of the "Chickpea ISM-ILP Marker Database" are depicted in Fig. 7.

Our study primarily demonstrated the efficacy of designing multiple ISM and ILP markers from the intronic sequence components of genes with dense genome-wide coverage and their broader applicability in expediting the multiple genomics-assisted breeding applications of chickpea. Therefore, this provides a wider flexibility to molecular geneticists/breeders for screening diverse desirable combination of informative ISM and ILP markers from an individual gene producing reproducible amplification (83% efficiency) including a higher polymorphic potential (86%) for differentiation of domesticated *desi* and *kabuli* accessions more efficiently to drive large-scale genetic analysis in chickpea. The added-advantage of gene-derived ISM and ILP markers as compared to other sequence-based markers with regard to their simplicity in discovery and greater potential of detecting functional allelic polymorphism among cultivated *desi* and *kabuli* accessions in the gene regions of genome even by a cost-effective agarose gel-based assay was evident. Consequently, these markers also have utility for assaying a wider functional genetic diversity and realistic measurement of phylogenetic relationships among cultivated accessions during chickpea domestication. The proficiency of these gene-based markers in construction of high-density genetic linkage map/functional transcript map and molecular mapping of high-resolution major seed weight QTLs was apparent in chickpea. In these perspectives, unrestricted public access of these informative gene-based markers developed at a genome-wide scale (mapped on eight chromosomes and unanchored scaffolds) has been made available to the chickpea scientific community by a user-friendly web-resource, "Chickpea ISM-ILP Marker Database", with a primary objective to expedite genomics-assisted breeding applications including marker-aided foreground and background analysis and genetic enhancement in chickpea.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.plantsci.2016.08.013.

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