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Identification of candidate genes for dissecting complex branch number trait in chickpea

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ABSTRACT

The present study exploited integrated genomics-assisted breeding strategy for genetic dissection of complex branch number quantitative trait in chickpea. Candidate gene-based association analysis in a branch number association panel was performed by utilizing the genotyping data of 401 SNP allelic variants mined from 27 known cloned branch number gene orthologs of chickpea. The genome-wide association study (GWAS) integrating both genome-wide GBS- (4556 SNPs) and candidate gene-based genotyping information of 4957 SNPs in a structured population of 60 sequenced *desi* and *kabuli* accessions (with 350–400 kb LD decay), detected 11 significant genomic loci (genes) associated (41% combined PVE) with branch number in chickpea. Of these, seven branch number-associated genes were further validated successfully in two inter (ICC 4958 × ICC 17160)- and intra (ICC 12299 × ICC 8261)-specific mapping populations. The axillary meristem and shoot apical meristem-specific expression, including differential up- and down-regulation (4–5 fold) of the validated seven branch number-associated genes especially in high branch number as compared to the low branch number-containing parental accessions and homozygous individuals of two aforesaid mapping populations was apparent. Collectively, this combinatorial genomic approach delineated diverse naturally occurring novel functional SNP allelic variants in seven potential known/candidate genes [*PIN1* (PIN-FORMED protein 1), *TB1* (teosinte branched 1), *BA1*/*LAX1* (*BARREN STALK1*/*LIKE AUXIN1*), *GRAS8* (gibberellic acid insensitive/GAI, Repressor of ga13/RGA and Scarecrow8/SCR8), *ERF* (ethylene-responsive element-binding factor), *MAX2* (more axillary growth 2) and lipase] governing chickpea branch number. The useful information generated from this study have potential to expedite marker-assisted genetic enhancement by developing high-yielding cultivars with more number of productive (pods and seeds) branches in chickpea.

1. Introduction

Plant architecture is a complex dynamic characteristic that determines the shape, size, geometry and external structure of a plant [1]. Shoot branching, a key determinant of plant architecture undermines the ability of a plant to produce side shoots from its axillary buds [2]. Branching density and pattern is a key yield-component trait, as increasing shoot branching can be translated into increase biomass and seed/pod production. Shoot branching plays a key role in adaptation of plant to their local environment by changing the shape of plant. The number and size of the branches formed determine the total area of the plant and the spatial distribution of leaf area in the canopy. The amount of light absorbed by leaves of the plants make them compete with the neighboring plants in terms of light interception and capturing of other resources, thereby reducing the chances of weed growth. Branching exerts its impact on dry matter accumulation and assimilates partitioning into the vegetative compartment and the reproductive growth [3]. Branching also affects developmental phenotypes, including flowering time and reproductive success in plant [4]. The process of axillary shoot branch formation is controlled by a complex interaction between genetically regulated developmental process and the environment [5,6]. Branching pattern and its density determined by number of branches per plant, is a complex trait and governed by many genes/QTLs (quantitative trait loci). More-

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over, this trait was most probably targeted for domestication and selective breeding [7]. Considering the importance of branch number in yield improvement and adaption to the environment, it is imperative to identify the underlying heritable forces and potential genes/QTLs regulating this complex trait. Molecular dissection of branch number trait will also pave the way to uncover the regulatory and domestication pattern of genes/QTLs governing this trait. Significant efforts have been made toward deciphering the complex genetic architecture of branching pattern and density in several crop species, including *Arabidopsis*, pea, soybean, rice, maize and barley [8–15]. In legumes, a number of loci, including *RAMOSUS* (*ram, rms1, rms2, rms3, rms4* and *rms5*) as well as *Branching* (*Br1* and *Br2*) have been identified as regulatory factors controlling branching patterns and density in pea and soybean [3,12].

Chickpea [*Cicer arietinum* (L.)] is an economically important food legume with a genome size of ~740 Mbp. It is a self-pollinated annual diploid crop species having indeterminate and branched growth habit. The cultivated chickpea plant is generally erect or semi-erect with primary, secondary and tertiary branching, resembling a small bush. Unlike cultivated species, the wild species of chickpea has prostrate growth habit [16]. The effect of branching on seed and pod yield as well as water-use efficiency is extensively studied and well documented in chickpea [17]. Only limited number of QTLs/genes regulating branch number have been identified utilizing QTL mapping and trait association analysis [18–23]. However, these identified QTLs/genes are yet to be deployed in marker-assisted selection for developing cultivars with high branch number in chickpea.

Considering the significance of integrated genomics-assisted breeding approach for quantitative dissection of complex yield component traits in crop plants, it would be interesting to employ this strategy in natural and mapping populations to identify functionally relevant molecular tags (markers, genes/QTLs and alleles) regulating branch number in chickpea [20,24–29]. This will provide much needed inputs for genetic dissection of complex branch number trait, which can be subsequently utilized in marker-assisted breeding program leading to development of superior cultivar with a desirable high attribute of branch number/density in chickpea. The increase in branch density as determined by number of productive (pod and seed-bearing branches) branches per plant can also enhance the pod and seed yield in chickpea, which is indispensable to feed the fast growing population for sustaining food security.

Keeping above in view, an integrated genomics-assisted breeding approach involving SNP (single nucleotide polymorphism) marker-based genome-wide association study (GWAS) and candidate gene-based association analysis, QTL mapping and differential gene expression profiling was utilized in the present study to delineate novel allelic variants in the genes associated with branch number in chickpea.

2. Materials and methods

2.1. Mining and genotyping of genome-wide SNPs

We selected 60 phenotypically and genotypically diverse *desi* (17 accessions) and *kabuli* (43) *Cicer* accessions (representing various eco-geographical regions of 21 countries of the world) (Supplemental file 1) from the chickpea germplasm collections (16991, including 211 minicore germplasm lines) available at ICRISAT Genebank following the methods of Kujur et al. [25,26]. For large-scale mining and high-throughput genotyping of genome-wide SNPs, the genomic DNA was isolated from *Cicer* accessions were used to constitute a 96-plex GBS library. The library was sequenced (100-bp single end) employing Illumina HiSeq2000 NGS platform as per the modified procedures of Elshire et al. [30] and Kujur et al. [31]. The Bowtie v2.1.0 [32] and reference-based GBS pipeline/genotyping approach of STACKS v1.0 (http://creskolab.uoregon.edu/stacks) were employed for de-multiplexing and mapping of high-quality FASTQ sequence reads onto the reference *kabuli* draft chickpea genome [32] and detection of high-quality SNPs from 60 accessions as per Kujur et al. [31]. The structural and functional annotation of GBS-derived SNPs detected in different coding and non-coding sequence components of genes and genomes (chromosomes/pseudomolecules and scaffolds) were performed according to *kabuli* genome annotation [33].

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2.2. Discovery and genotyping of candidate gene-derived SNPs

A selected 27 cloned genes/QTLs known to be involved in regulation of branch/tiller/panicle numbers in diverse crop plants, including *Arabidopsis thaliana*, tomato, *Medicago*, soybean, maize, rice and barley [13] were acquired for large-scale discovery and high-throughput genotyping of gene-based SNPs. The coding sequences (CDS) of these known genes were sequence homology (BLAST) searched against the CDS of *kabuli* genes to identify the best true chickpea gene orthologs. All the CDS and 2000-bp upstream and 1000-bp downstream regulatory regions of identified true *kabuli* chickpea gene orthologs (*E*-value: 0 and bit score ≥500) were targeted to design (Batch Primer3, http://probes.pw.usda.gov/batchprimer3) the multiple overlapping forward and reverse primers with expected amplification product size of 500–700 bp. The gene-derived primers were PCR amplified using the genomic DNA of 60 *Cicer* accessions (selected for genome-wide GBS-based SNP genotyping). The amplified PCR fragments were purified, cloned and sequenced by an automated DNA sequencer (Applied Biosystems, ABI 3730xl DNA Analyzer, Vernon Hills, Illinois, USA). The high-quality sequences were aligned and compared among accessions, and SNPs were detected in diverse sequence components of orthologous chickpea genes following Kujur et al. [24] and Saxena et al. [34].

2.3. Phylogenetic tree construction, population structure and LD patterns

The high-quality SNP genotyping data among 60 *Cicer* accessions were analysed with PowerMarker v3.51 [35] and MEGA v6.0 [36] to construct an unrooted neighbour-joining (NJ)-based phylogenetic tree (with 1000 bootstrap replicates) among accessions. The population genetic structure among accessions was determined by a model-based program STRUCTURE v2.3.4 following Kujur et al. [24,25] and Saxena et al. [34]. The genome-wide and candidate gene-based genotyping data of SNPs physically mapped on eight *kabuli* chromosomes were analysed by PLINK and the full-matrix approach of TASSELv5.0 [34,37]. Based on these, the genome-wide LD patterns (r^2, frequency correlation among pair of alleles across a pair of SNP loci) and LD decay (by plotting average r^2 against 50 and 20 kb uniform physical intervals across chromosomes) in population was determined.

2.4. Phenotyping for branch number

Sixty *Cicer* accessions were planted in a single row with a plant density of 35 × 10 cm and raised in the experimental field following RCBD (randomised complete block design) with at least two replications. Following the above criteria, these accessions were grown and phenotyped for two consecutive years (2011 and 2012) during crop growing season at two diverse geographical locations (New Delhi; latitude/longitude: 28.4°N/77.1°E and