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ORIGINAL ARTICLE

Genetic dissection of plant growth habit in chickpea

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Abstract A combinatorial genomics-assisted breeding strategy encompassing association analysis, genetic mapping and expression profiling is found most promising for quantitative dissection of complex traits in crop plants. The present study employed GWAS (genome-wide association study) using 24,405 SNPs (single nucleotide polymorphisms) obtained with genotyping-by-sequencing (GBS) of 92 sequenced *desi* and *kabuli* accessions of chickpea. This identified eight significant genomic loci associated with erect (E)/semi-erect (SE) vs. spreading (S)/semi-spreading (SS)/prostrate (P) plant growth habit (PGH) trait differentiation regardless of diverse *desi* and *kabuli* genetic backgrounds of chickpea. These associated SNPs in combination explained 23.8% phenotypic variation for PGH in chickpea. Five PGH-associated genes were validated successfully in E/SE and SS/S/P PGH-bearing parental accessions and homozygous individuals of three intra- and interspecific RIL (recombinant inbred line) mapping populations as well as 12 contrasting *desi* and *kabuli* chickpea germplasm accessions by selective genotyping through Sequenom MassARRAY. The shoot apical, inflorescence and floral meristems-specific expression, including upregulation (seven-fold) of five PGH-associated genes especially in germplasm accessions and homozygous RIL mapping individuals contrasting with E/SE PGH traits was apparent. Collectively, this integrated genomic strategy delineated diverse non-synonymous SNPs from five candidate genes with strong allelic effects on PGH trait variation in chickpea. Of these, two vernalization-responsive non-synonymous SNP alleles carrying SNF2 protein-coding gene and B3 transcription factor associated with PGH traits were found to be the most promising in chickpea. The SNP allelic variants associated with E/SE/SS/S PGH trait differentiation were exclusively present in all cultivated *desi* and *kabuli* chickpea accessions while wild species/accessions belonging to primary, secondary and tertiary gene pools mostly contained prostrate PGH-associated SNP alleles. This indicates strong adaptive natural/artificial selection pressure (Tajima's D 3.15 to 4.57) on PGH-associated target genomic loci during chickpea domestication. These vital leads thus have potential to decipher complex transcriptional regulatory gene function of PGH trait differentiation and for understanding the selective sweep-based PGH trait evolution and domestication pattern in cultivated and wild chickpea accessions adapted to diverse agroclimatic conditions. Collectively, the essential inputs generated will be of profound use in marker-assisted genetic enhancement to develop cultivars with desirable plant architecture of erect growth habit types in chickpea.

Keywords Chickpea · GWAS · Plant growth habit · QTL · SNP

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Introduction

Chickpea (*Cicer arietinum* L.) is a diploid annually grown self-pollinated food legume crop with a high economic value having a genome size of ~740 Mbp and known to be nutritionally rich (Kumar et al. 2011; Gaur et al. 2012). The cultivated chickpea (*desi* and *kabuli*) representing diverse genepools with contrasting agronomic traits believed to have originated from its wild *C. reticulatum* progenitor for all of which the draft genomes have been sequenced recently (Jain et al. 2013; Varshney et al. 2013, 2014; Parween et al. 2015; Gupta et al. 2016). The cultivated *desi* and *kabuli* chickpea usually exhibit erect to semi-erect plant growth habit while accessions belonging to wild species commonly have prostrate growth habit. This indicates that plant growth habit could be a common target trait for both domestication and artificial breeding in chickpea (Wang and Li 2008; Meyer et al. 2012).

The plant growth habit is a vital agronomic trait in chickpea that varies widely among *desi*, *kabuli* and wild accessions (Upadhyaya et al. 2006). The chickpea germplasm accessions are primarily classified and characterized into five plant growth habit types—erect (0–15° angle of primary branches from the vertical axis at mid-pod filling stage), semi-erect (16–25°), semi-spreading (26–60°), spreading (61–80°) and prostrate (branches creeping above the ground) (Upadhyaya et al. 2008). The growth habit is a crucial determinant of plant architecture and plays a key role in influencing various seed and pod yield component traits in chickpea as well as adaptation of its plant-type to diverse agroecological environments. The plant growth habit is thus a complex yield contributing quantitative trait and usually governed by convoluted interplay of multiple genes/QTLs (quantitative trait loci) especially regulating diverse developmental processes in chickpea (Benlloch et al. 2015).

Considering the agronomic importance of growth habit in defining ideal plant architecture and seed/pod yield enhancement as well as affecting plant adaption to its environment, it is imperative to dissect the genetic and molecular basis underlying this broad trait variation existing among natural germplasm accessions of chickpea (Wang and Li 2008; Meyer et al. 2012). Attempts have been made to develop improved cultivars with desirable plant type by exploiting diverse potential wild *Cicer* species accessions in introgression breeding program of chickpea genetic enhancement (Cobos et al. 2009; Aryamanesh et al. 2010; Benlloch et al. 2015). However, the genetic drag of undesirable traits like spreading/prostrate growth habit and late flowering is the major hindrance to such process of conventional introgression breeding for chickpea genetic improvement. To substantiate these efforts, genomics-assisted breeding strategy by molecular mapping of major QTLs predominantly associated with erect/semi-erect and prostrate growth habits on multiple interspecific genetic linkage maps (*C. arietinum* × *C. reticulatum*) have been deployed to understand the complex genetic architecture and inheritance pattern of plant growth habit trait variation in chickpea (Cobos et al. 2009; Aryamanesh et al. 2010). For instance, a candidate genetic locus (*Hg*/*hg*) underlying a major erect/prostrate QTL has been mapped on the linkage group 3 of an interspecific genetic map (semi-erect *C. arietinum desi* cv. ICCL 81001 × prostrate *C. reticulatum* wild cv. Cr5-9) (Cobos et al. 2009). Alongside, another candidate genetic locus (*Prostrate*) harbouring a major prostrate QTL has been mapped on the linkage group 3 of an interspecific genetic map (semi-erect *C. arietinum desi* cv. ICC 3996 × *C. reticulatum* wild cv. ILWC 184) (Aryamanesh et al. 2010). These studies infer that the genetic analysis of plant growth habit trait is mostly limited to estimation of heritability and genetic mapping of few long marker interval-spanning major growth habit QTLs due to their quantitative and polygenic nature of inheritance in chickpea (Cobos et al. 2009; Aryamanesh et al. 2010). However, these major QTLs governing growth habit are yet to be validated across diverse genetic backgrounds and environments prior to their deployment in fine-mapping/map-based gene isolation and marker-assisted breeding program for developing improved cultivars of desirable plant type in chickpea. In this context, comprehensive understanding on complex genetic inheritance pattern by quantitative dissection of plant growth habit is essential to decipher the regulatory function and domestication pattern of genes/QTLs controlling these traits in chickpea.

Considering the efficacy of integrated genomics-assisted breeding strategy (association study, QTL mapping and expression profiling) for genetic dissection of various complex quantitative traits in crop plants, this approach can be deployed in large-scale natural and mapping populations to delineate functionally relevant potential molecular tags (markers, genes/QTLs, alleles and haplotypes) regulating plant growth habit in chickpea (Bajaj et al. 2015a, b, 2016; Das et al. 2015, 2016; Kujur et al. 2015a, b; Upadhyaya et al. 2015, 2016). The aforesaid integrated strategy will also assist us in unravelling the molecular genetic basis of natural plant growth habit trait variation existing in various cultivated and wild accessions of chickpea adapted to diverse agroclimatic regions. The resultant findings on complex plant growth habit trait dissection can essentially be utilized in genomics-assisted breeding program to develop superior cultivars with preferred genetic attributes of erect plant type in chickpea. The development of cultivars with restructured erect plant type will not only facilitate higher pod/seed yield and mechanization of harvesting in chickpea but also mitigate the effects of acute climate change for sustaining global food security.

In light of the above, the present study employed an integrated genomics-assisted breeding strategy involving genome-wide association study (GWAS), selective genotyping and differential expression profiling to delineate functionally relevant natural allelic variants in potential candidate

gene(s) regulating plant growth habit traits, with an eventual objective of developing ideal erect new plant type for accelerating chickpea genetic enhancement.

Materials and methods

Phenotyping for plant growth habits

A total of 92 including 39 *desi* and 53 *kabuli* chickpea accessions were grown in the field following randomised complete block design (RCBD) with at least two replications during normal crop season for two consecutive years (2012 and 2013) at two diverse geographical locations (Patancheru, Hyderabad: latitude 17° 3′ N/longitude 77° 2′ E and New Delhi: 28° 4′ N/77° 2′ E) of India (Table S1). In addition, 190 individuals representing each of two intraspecific F_7 RIL (recombinant inbred lines) mapping populations: ICC 13523 (erect *kabuli* landrace) × ICC 12299 (semi-spreading *desi* landrace) and ICC 4958 (semi-erect *desi* landrace) × ICC 12299 as well as one interspecific F_8 RIL mapping population: ICC 4958 (semi-erect *desi* landrace) × ICC 17160 (prostrate wild accession) were grown as per aforementioned strategies. The angle of primary branches from the vertical axis of each accession and RIL mapping individual (2–4 representative plants from each accession/individual) were measured at their mid-pod filling stage. The germplasm accessions and mapping individuals of chickpea were classified predominantly into diverse plant growth habit types, including erect (E), semi-erect (SE), semi-spreading (SS) and spreading (S) in accordance with the estimation of 0–15, 16–25, 26–60, 61–80° angles of their primary branches, respectively, from the vertical while prostrate (P) growth habit was measured based on flatness of branches over the ground.

The diverse statistical parameters, including coefficient of variation (CV), analysis of variance (ANOVA) and frequency distribution of diverse plant growth habit (PGH) traits phenotyped among accessions and RIL mapping individuals were estimated following Bajaj et al. (2015a). The inheritance pattern of PGH traits was determined by measuring the effect of genotypes (accessions) (G), environments (experimental years and/or geographical locations) (E) and G × E interactions using ANOVA. The broad-sense heritability [$H^2 = \sigma^2g/(\sigma^2g + \sigma^2ge/n + \sigma^2e/nr)$] of PGH traits was measured employing optimum criteria including σ^2g (genetic), σ^2ge (G × E) and σ^2e (error) variance with *n* (number of experimental years/environments) = 2 and *r* (number of replicates) = 2.

Trait association mapping

The genotyping data including structural/functional annotation information of *kabuli* reference genome- and de novo-based SNPs mined from the sequenced 92 (39 *desi* and 53 *kabuli*) chickpea accessions using a GBS (genotyping-by-sequencing)-assay were acquired (Kujur et al. 2015a, c). For GWAS of PGH traits, the genetic diversity information including phylogenetic tree, PCA (principal component analysis) and population structure among 92 chickpea accessions were obtained from our previous study (Kujur et al. 2015a, c). The population structure ancestry coefficient (Q), kinship matrix (K) and PCA (P) data along with genome-wide SNP genotyping and PGH phenotyping information of 92 accessions were analysed through mixed model (P + K, K and Q + K)-based CMLM (compressed mixed linear model) and P3D (population parameters previously determined, Zhang et al. 2010; Kang et al. 2011)/EMMAX (efficient mixed model association eXpedited) approaches of GAPIT (Lipka et al. 2012) as described previously (Kujur et al. 2015a; Bajaj et al. 2016; Upadhyaya et al. 2015, 2016). To ascertain the accuracy and validity of SNP marker-trait association, the observed and expected $-\log_{10}(P)$ value relative distribution estimated for each PGH-associated genomic locus was compared based on quantile-quantile plot. Subsequently, the correction of their adjusted *P* value threshold of significance for multiple comparison was performed by false discovery rate (FDR cut-off ≤0.05, Benjamini and Hochberg 1995) following Kujur et al. (2015a), Upadhyaya et al. (2015, 2016) and Bajaj et al. (2016). The degree of association of SNP loci with diverse PGH traits was measured by the R^2 using a model with the SNPs and adjusted *P* value adopting a FDR-controlling strategy. The SNP loci in the genomic/gene regions exhibiting significant association with PGH traits at the lowest FDR adjusted *P* values (threshold $P < 10^{-8}$) and highest R^2 (degree of SNP marker-trait association) were identified in chickpea.

Validation of PGH-associated SNPs in natural and mapping populations

To ensure the potential of detected genomic SNP loci for PGH trait association, these SNPs were targeted to validate among natural germplasm accessions as well as in two intraspecific F_7 RIL mapping populations: (ICC 13523 × ICC 12299) and (ICC 4958 × ICC 12299) and one inter-specific F_8 RIL mapping population: (ICC 4958 × ICC 17160) contrasting with diverse PGH traits in chickpea. For this, three parental accessions and 10 of each homozygous RIL individuals of three aforesaid mapping populations along with 12 *desi* and *kabuli* germplasm accessions (selected from 92 accessions used for GWAS) exhibiting variations for diverse contrasting PGH traits (E, SE, SS, S and P) were selected for genomic DNA isolation. The PGH-associated SNPs showing polymorphism between the parents of three mapping populations were further genotyped in the aforementioned homozygous mapping individuals and germplasm accessions using MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) SNP genotyping assay as per Saxena et al. (2014a, b). The PGH-associated SNP allelic

variants (scanned by GWAS) identified from homozygous accessions/individuals were correlated with diverse PGH traits to validate the trait association potential of target genomic loci. Furthermore, the physical positions (bp) of PGH-associated SNPs localized on chromosomes were correlated with that of multiple known major PGH QTLs mapped earlier on the inter-specific genetic linkage maps to determine the validity and novelty of these GWAS-derived major genomic loci identified in chickpea.

Differential expression profiling

To determine the expression pattern of candidate genes ensured by GWAS and selective genotyping, the differential expression profiling of PGH-associated genes was performed using the semi-quantitative and quantitative RT-PCR assays. The RNA was isolated from vegetative and reproductive tissues [leaf, root, shoot apical meristem (SAM), inflorescence and floral meristems] of parental accessions and two of each homozygous individuals derived from aforementioned three mapping populations as well as four *desi* and *kabuli* germplasm accessions contrasting with diverse (E/SE and P/SS/S) PGH traits (Table S2). The isolated RNA was amplified with the gene-specific primers and differential expression analysis was performed following Bajaj et al. (2015c) and Upadhyaya et al. (2015). RNA isolated from three independent biological replicates of each sample and two technical replicates of each biological replicate with no template and primer as control were used in the quantitative RT-PCR assay. One microgram of high quality total RNA was utilized for cDNA synthesis using first strand cDNA synthesis kit (Applied Biosystems, USA). The cDNA (1:100 dilution) along with 1X Fast SYBR Green Master Mix (Applied Biosystems) and 200 nM of forward and reverse gene-specific primers in a total reaction volume of 10 μl were amplified in 7500 Fast Real-Time PCR system (Applied Biosystems). The differential expression profiles of genes among tissues/stages of germplasm accessions and RIL mapping individuals were determined as per Bajaj et al. (2015c) and Upadhyaya et al. (2015). An internal control gene elongation factor 1-alpha (*EF1α*) exhibiting consistent expression across diverse tissues and developmental stages of chickpea accessions/individuals was utilized for normalization of expression value. The significant difference in gene expression was determined by a LSD-ANOVA significance test using SPSS 17.0 (http://www.spss.com/statistics) and visualized with a heat map employing MultiExperiment Viewer (MeV, http://www.tm4.org/mev).

Natural allelic diversity in PGH-associated candidate genes

To determine natural allelic diversity, PGH-associated candidate genes validated by GWAS, selective genotyping and differential expression profiling were selected. The 100-bp sequence regions flanking the associated SNP loci were PCR amplified using the genomic DNA of 79 wild chickpea accessions (belonging to primary, secondary and tertiary gene pools) and 92 cultivated (*desi* and *kabuli*) chickpea accessions (selected for GWAS). A representative set of 79 wild accessions were selected from five annual wild species, namely, *C. reticulatum* (14 accessions), *C. echinospermum* (8), *C. judaicum* (22), *C. bijugum* (19) and *C. pinnatifidum* (15) belonging to primary and secondary gene pools, and one perennial species *C. microphyllum* of tertiary gene pool. The PCR amplicons were cloned and sequenced, and SNPs were mined among accessions following Kujur et al. (2013), Saxena et al. (2014a) and Upadhyaya et al. (2015). To estimate various nucleotide diversity measures such as average pairwise nucleotide diversity ($\theta\pi$) and Watterson's estimator of segregating sites ($\theta\omega$) and Tajima's D, a 100-kb non-overlapping sliding window approach of TASSEL v5.0 (http://www.maizegenetics.net) was utilized as per Xu et al. (2011) and Kujur et al. (2015c).

Results and discussion

GWAS of PGH traits in chickpea

An optimized NGS-based GBS strategy was utilized to discover and genotype genome-wide SNPs and subsequently in GWAS for identification of potential genes and natural allelic variants regulating diverse PGH traits in chickpea. In the present study, 24,405 high-quality SNPs discovered from 92 sequenced *desi* and *kabuli* accessions employing reference genome (16376)- and de novo (8029)-based GBS assays were further integrated with replicated multilocation/year PGH field phenotyping information to perform GWAS of PGH traits in chickpea (Table S3). The high-quality 14,115 SNPs physically mapped on eight *kabuli* chickpea chromosomes were submitted to NCBI dbSNP (http://www.ncbi.nlm.nih.gov/SNP/snp_viewTable.cgi?handle=NIPGR) with SNP submission (SS) accession numbers (2,728,910,337 to 2,728,924,439) for unrestricted public access (Table S3). The aforesaid SNP genotyping information-led neighbour-joining phylogenetic tree, high-resolution population genetic structure and PCA differentiated all 92 accessions from each other and clustered into two distinct populations; POP I and POP II representing primarily the *kabuli* and *desi* chickpea cultivars with E, SE, S and SS plant growth habits. This agreed well with our previous studies that utilized genome-wide GBS-derived SNPs for determining the evolutionary relationship and population genetic structure among a similar set of 92 *desi* and *kabuli* chickpea accessions (Kujur et al. 2015a, c). The normal frequency distribution along with a broader PGH phenotypic variation and 80% broad-sense heritability (H^2) among 92 chickpea accessions was evident. We utilized

the field phenotyping data of accessions exclusively revealing consistent phenotypic expression (sustained by high H^2) of PGH traits across two geographical locations/year for subsequent SNP marker-trait association analysis. These observations indicated that the 92 natural chickpea germplasm accessions representing 16 diverse ecogeographical regions of the world screened by us for GWAS are rich in allelic diversity for diverse PGH traits. Therefore, the selected accessions can serve as a useful genetic resource for mining novel functional allelic variants to expedite trait association mapping of PGH at a whole genome and/or gene-level in chickpea. This will essentially accelerate identification of potential genes and natural allelic variants governing diverse PGH traits at a genome-wide scale in domesticated chickpea.

The CMLM and P3D/EMMAX model-based association mapping approach (FDR cut-off ≤0.05) identified eight genomic loci (gene-based SNPs) exhibiting significant association ($P \leq 10^{-8}$) with diverse PGH traits in chickpea (Fig. 1, Table 1). This included seven SNP loci which were physically mapped on six chromosomes. The remaining one SNP locus was represented from scaffold region of chickpea genome. Maximum number of three PGH-associated SNPs were mapped on chromosome 4 (Fig. 1, Table 1). One of eight PGH-associated genomic SNP loci was derived from the intergenic region; whereas, rest seven SNP loci were represented from diverse coding (six non-synonymous SNPs) and non-coding intronic (one SNP) sequence components of seven *kabuli* genes (Table 1). The detected eight significant SNPs explained 10–17% phenotypic variation for diverse PGH traits observed in 92 chickpea accessions. The total phenotypic variation for PGH traits explained by eight SNPs in combination was 23.8%. Six SNP loci from the six candidate genes were significantly associated with E/SE vs. P/SS/S PGH trait differentiation in chickpea (Table 1). The remaining one of each intergenic and gene-derived SNP were identified to be associated with E/SE vs. SS/S PGH trait differentiation. The significant association of eight SNP loci mapped in more than one genomic regions (genes) with diverse PGH traits infers complex genetic inheritance pattern and regulation of these target quantitative traits in chickpea. A significant difference concerning the association potential of SNP loci with PGH traits in two different *desi* and *kabuli* populations could not be observed despite diverse genetic architecture of PGH traits in 92 accessions belonging to two populations (POP I and II) and along entire population. Henceforth, PGH-associated genomic loci (genes) identified by us employing GWAS have efficacy to be deployed in marker-aided genetic enhancement of chickpea. Remarkably, strong association of non-synonymous coding SNPs-containing two genes, namely, major intrinsic protein (MIP) [glutamic acid (GAA) to glycine (GGA)] and sucrose non-fermenting (SNF2) [leucine (CTT) to phenylalanine (TTT)] with E/SE vs. P/SS/S PGH trait differentiation (P: 1.2–1.5 × 10^{-10} with 15–17% PVE) as compared to that of other six associated SNPs was apparent (Table 1). The non-synonymous SNPs from the candidate genes associated with diverse PGH traits delineated in our study using GWAS have functional significance towards rapid dissection of these complex quantitative traits in chickpea. The non-synonymous SNPs in the genes are known to alter their transcriptional regulatory mechanism for controlling diverse yield and quality component traits in chickpea (Kujur et al. 2013, 2014; Bajaj et al. 2015a, b, c; Upadhyaya et al. 2015). Aside, the added advantages of large-scale SNPs discovered especially from the genes annotated on the chromosomes for high-resolution trait association mapping to delineate potential genes regulating vital agronomic traits have been well documented in crop plants with small diploid and large complex polyploid genomes (Akpinar et al. 2017). Therefore, clues obtained from our study further can essentially be employed for establishing rapid marker-trait linkages and efficient PGH trait-regulatory gene identification and characterisation in chickpea. The GWAS is a much proficient and attractive strategy for quantitative dissection of diverse complex agronomic traits in crop plants including chickpea

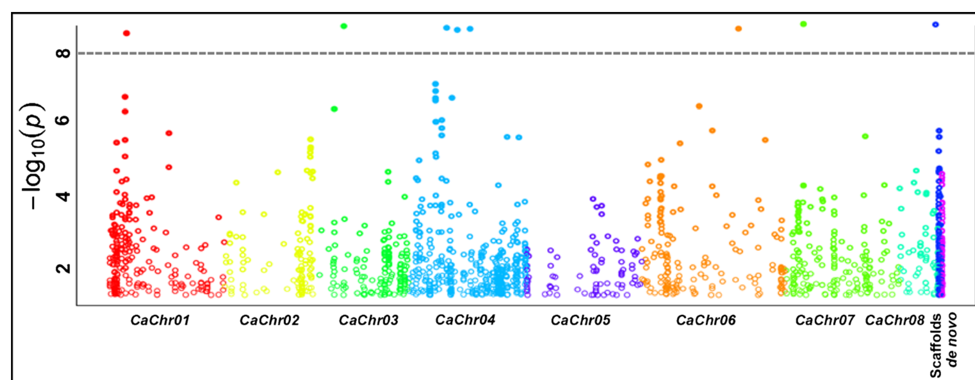

Fig. 1 GWAS-derived Manhattan plot illustrating significant (FDR cut-off <0.05) -$\log_{10}(P)$ values (*y*-axis) associated with S/SE vs. P/SS/S PGH trait differentiation using 24,405 reference genome and de novo-based GBS-SNPs. The *x*-axis represents the genomic distribution of SNPs physically mapped on eight chromosomes and scaffolds of chickpea genome. The SNPs revealing significant association with diverse PGH traits at a cut-off *P* value ≤1 × 10^{-8} are marked with a *dotted line*

Table 1 Eight genomic/gene-derived SNP loci regulating diverse plant growth habit traits delineated by integrating GWAS with selective genotyping and expression profiling

SNP IDs	*Kabuli* chromosomes	SNP physical positions (bp)	SNPs	Gene accession IDs	Structural annotation	Encoded protein	Known/putative functions	Association analysis		Traits associated
								P	PVE (%)	
CaKSNP735	*Ca_Kabuli_Chr1*	6262577	[T/C]	Ca07886	CDS (non-synonymous)	Isoleucine (ATT) to Threonine (ACT)	Unknown expressed protein	1.6×10^{-8}	12	SE/E vs. SS/S/P
CaKSNP5885[a,b]	*Ca_Kabuli_Chr4*	11231137	[A/G]	Ca04355	CDS (non-synonymous)	Glutamic acid (GAA) to Glycine (GGA)	Major intrinsic protein	1.2×10^{-10}	17	SE/E vs. SS/S/P
CaKSNP5889	*Ca_Kabuli_Chr4*	11246093	[G/A]	–	Intergenic	–	–	2.4×10^{-8}	10	SE/E vs. SS/S
CaKSNP6404[a,b]	*Ca_Kabuli_Chr4*	18071114	[C/A]	Ca18648	CDS (non-synonymous)	Alanine (GCG) to Glutamic acid (GAG)	Ankyrin repeat-containing protein	1.0×10^{-9}	12	SE/E vs. SS/S/P
CaKSNP3572[a,b]	*Ca_Kabuli_Chr3*	8933409	[A/C]	Ca25280	Intron	–	ABC transporter	1.0×10^{-8}	10	SE/E vs. SS/S/P
CaKSNP10843	*Ca_Kabuli_Chr6*	29205940	[T/C]	Ca17457	CDS (non-synonymous)	Leucine (CTT) to Proline (CCT)	DNA ligase	1.2×10^{-8}	11	SE/E vs. SS/S
CaKSNP12117[a,b]	*Ca_Kabuli_Chr7*	7756455	[C/T]	Ca13243	CDS (non-synonymous)	Leucine (CTT) to Phenylalanine (TTT)	SNF2-related	1.5×10^{-10}	15	SE/E vs. SS/S/P
CaKSNP15974[a,b]	*Ca_Kabuli_Scaffold682*	157238	[G/C]	Ca23185	CDS (non-synonymous)	Serine (AGC) to Threonine (ACC)	Transcriptional factor B3	1.4×10^{-8}	11	SE/E vs. SS/S/P

[a] Selective genotyping

[b] Differential expression profiling

(Feuillet et al. 2012; Thudi et al. 2014; Kujur et al. 2015a; Upadhyaya et al. 2015, 2016; Bajaj et al. 2016; Liu et al. 2016). In this perspective, a high-resolution GWAS approach deployed by us to scale down the potential genomic loci/genes governing quantitative traits of PGH at a genome-wide scale appears much expedient for understanding the complex genetic architecture of target traits in order to drive genomics-assisted crop improvement of chickpea (Kujur et al. 2015a; Bajaj et al. 2016; Upadhyaya et al. 2015, 2016).

Validation of PGH-associated genomic loci by selective genotyping in mapping and natural populations

To validate the PGH-associated SNP loci, SNPs revealing parental polymorphism were genotyped in 10 of each homozygous individuals derived from two intraspecific [(ICC 13523 × ICC 12299) and (ICC 4958 × ICC 12299)] and one interspecific (ICC 4958 × ICC 17160) RIL mapping populations as well as among 12 *desi* and *kabuli* germplasm accessions contrasting with E/SE and S/SS plant growth habit traits. Four non-synonymous SNPs and one intronic SNP-containing candidate genes (major intrinsic protein, ankyrin repeat domain containing protein, ABC transporter, sucrose non-fermenting protein and B3 transcription factor) exhibiting significant association ($P = 1.37 \times 10^{-8}$) with PGH traits (based on our GWAS) were validated in both intra-and interspecific mapping populations contrasting for diverse PGH traits by selective genotyping (Figs. S1, 2, 3, Table 1). Interestingly, the occurrence of identical E/SE and S/SS-associated alleles derived from these five gene-based SNP loci in parental accessions (ICC 13523, ICC 12299, ICC 4958 and ICC 17160) and homozygous mapping individuals contrasting with E/SE and S/SS PGH traits was observed (Figs. S1, 2, 3). Likewise, five gene-derived SNP alleles were validated in E/SE (ICC 16814, ICC 6306, ICC 7323, ICC 11284, ICC 1161, ICC 15061 and ICC 8261) and S/SS (ICC 5590, ICC 11584, ICC 1164, ICC 13044 and ICC 15888) PGH-bearing natural germplasm accessions (Figs. S1, 2, 3). In order to ascertain the accuracy/validity and novelty of five SNP loci-carrying genes for PGH trait association (validated by GWAS and selective genotyping) in chickpea, the outcome of the current study was compared and correlated with that of previous studies on molecular mapping of known major PGH QTLs documented in this important food legume crop. To perform this, markers linked/flanking the known major PGH QTLs (reported by previous QTL mapping studies) were selected for their further validation in the PGH-specific 92 *desi* and *kabuli* natural chickpea germplasm accessions and three inter-/intra-specific mapping populations constituted in our study. This revealed correspondence of one intronic SNP-containing gene (ABC transporter) harbouring the known major PGH (E/P) QTLs mapped on linkage group 3 between past and present studies based on congruent flanking/linked marker physical positions (bp) on the chickpea chromosomes (Cobos et al. 2009; Aryamanesh et al. 2010). In this context, seven novel and one previously documented SNPs-containing known/candidate genes governing diverse PGH traits delineated in our study could be considered as potential candidates for their utilization in marker-assisted genetic improvement with an ultimate objective of developing restructured ideal erect plant type in chickpea.

Validation of PGH-associated genes through expression profiling

The PGH-associated eight genes, including five validated by GWAS and selective genotyping were chosen for differential expression analysis to infer the functional regulatory patterns of these genes governing PGH traits in chickpea. The differential expression analysis of PGH trait-associated genes in multiple vegetative and reproductive tissues of diverse (E/SE and P/SS/S) PGH-bearing chickpea germplasm accessions and RIL mapping individuals were performed (Table S3, Fig. 4). Five candidate genes validated by GWAS and selective genotyping revealed SAM, inflorescence and floral meristems-specific expression (>5-fold) compared with vegetative leaf and root tissues of E/SE and S/SS/P PGH-bearing parents, homozygous mapping individuals and germplasm accessions of chickpea. All these five genes exhibited pronounced differential upregulation (>7-fold, $P \leq 0.001$) in SAM, inflorescence and floral meristems of E/SE as compared to that of S/SS/P PGH-bearing individuals/accessions (Fig. 4). Interestingly, these five meristem-specific differentially expressed genes exhibiting significant association with diverse PGH traits (validated by GWAS and selective genotyping) mostly contained non-synonymous coding SNPs. Remarkably, two strong PGH-associated non-synonymous SNPs-containing genes (encoding major intrinsic protein and SNF2 protein) revealed pronounced upregulation (>10-fold, $P \leq 0.0001$) in SAM, inflorescence and floral meristems of E/SE than that of S/SS/P PGH-bearing individuals/accessions of chickpea (Fig. 4). Therefore, it would be interesting to correlate these non-synonymous novel SNP allelic variants mined from different sequence components of the genes with diverse PGH trait association potential and differential expression profiles underlying plant growth habit trait regulation in chickpea. Altogether, five potential differentially expressed genes with non-synonymous functional SNP allelic variants governing PGH traits were delineated in our study by integrating GWAS with selective genotyping and differential expression profiling in natural and mapping populations of chickpea.

The meristem-specific expression, including enhanced upregulation and transcript accumulation of five PGH-associated candidate genes (encoding major intrinsic protein, ankyrin repeat domain containing protein,

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CaKSNP12117-SNF2-ICC13523   TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCACTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProLeuLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-ICC4958    TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCACTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProLeuLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-ICC16814   TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCACTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProLeuLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-ICC6306    TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCACTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProLeuLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-ICC7323    TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCACTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProLeuLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-ICC11284   TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCACTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProLeuLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-ICC1161    TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCACTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProLeuLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-ICC15061   TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCACTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProLeuLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-ICC8261    TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCACTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProLeuLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-HEM        TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCACTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProLeuLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-HSEM       TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCACTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProLeuLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
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E/SE accessions, mapping parents and homozygous RIL individuals

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CaKSNP12117-SNF2-ICC12299   TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCATTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProPheLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-ICC5590    TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCATTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProPheLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-ICC17160   TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCATTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProPheLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-ICC11584   TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCATTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProPheLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-ICC1164    TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCATTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProPheLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-ICC13044   TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCATTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProPheLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-ICC15888   TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCATTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProPheLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-HSM        TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCATTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProPheLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-HSSM       TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCATTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProPheLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-HPM        TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCATTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProPheLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-Cr         TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCATTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProPheLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-Ce         TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCATTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProPheLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-Cj         TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCATTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProPheLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-Cb         TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCATTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProPheLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-Cp         TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCATTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProPheLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
CaKSNP12117-SNF2-Cm         TATATGGGTCAGATTTTGAAGAAAATATGTGATCATCCATTTCTGTTGACAAAGAGGGCAGCTGAGGATGTGTTGGAAGGG
                            TyrMetGlyGlnIleLeuLysLysIleCysAspHisProPheLeuLeuThrLysArgAlaAlaGluAspValLeuGluGly
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SS/S/P accessions, mapping parents and homozygous RIL individuals

Fig. 2 The PGH-associated SNP allelic variants (C/T) exhibiting missense non-synonymous amino acid substitutions [(leucine (CTT) to phenylalanine (TTT)] in a vernalization-responsive candidate gene (encoding SNF2 protein) differentiated the E/SE from S/SS/P PGH-bearing mapping parents, homozygous RIL individuals and cultivated/wild accessions of chickpea by selective genotyping. The sequence regions carrying the non-synonymous SNPs are highlighted with *dotted boxes*. The details regarding the validated SNP loci are mentioned in Tables 1 and S3. HEM/HSEM/HSM/HSSM/HPM: homozygous erect/semi-erect/spreading/semi-spreading/prostrate mapping individuals, *Cr Cicer reticulatum*, *Ce C. echinospermum*, *Cj C. judaicum*, *Cb C. bijugum*, *Cp C. pinnatifidum* and *Cm C. microphyllum*

ABC transporter, sucrose non-fermenting protein and B3 transcription factor) scaled down in the present study have ascertained their significant role in controlling growth and development of multiple crop plants. The transcriptional regulation, including differential accumulation of transcripts of these genes in the SAM, inflorescence and floral meristems and their interactions with multiple genes particularly governing the biosynthesis, accumulation, transport and signalling of hormones during vegetative and reproductive growth have been well understood in diverse crop plants, including legumes. The gene encoding plasma membrane major intrinsic proteins (MIPs) is known to be an essential component in controlling major aspects of photosynthesis and plant growth/development (Kelly et al. 2014). The gene encoding ankyrin repeat domain-containing protein plays a key role in plant morphogenesis and architecture by regulating gene expression and modulating meristematic activity of SAM in crop plants (Sharma and Pandey 2016). The transcriptional regulation and function of ankyrin repeat domain-containing protein in influencing plant growth and development has also been elucidated by several previous studies (Nodzon et al. 2004). One of the vernalization-responsive sucrose non-fermenting (SNF2) protein-coding gene plays dynamic role in reduced growth habit without altering other morphological features by its transcript accumulation and chromatin remodelling/modification including maintenance of SAM in multiple plant species (Wang and Li 2008; Ryan and Owen-Hughes 2011; Sacharowski et al. 2015; Folta et al. 2016). A *SNF2* chromatin-remodelling protein-coding gene also controls

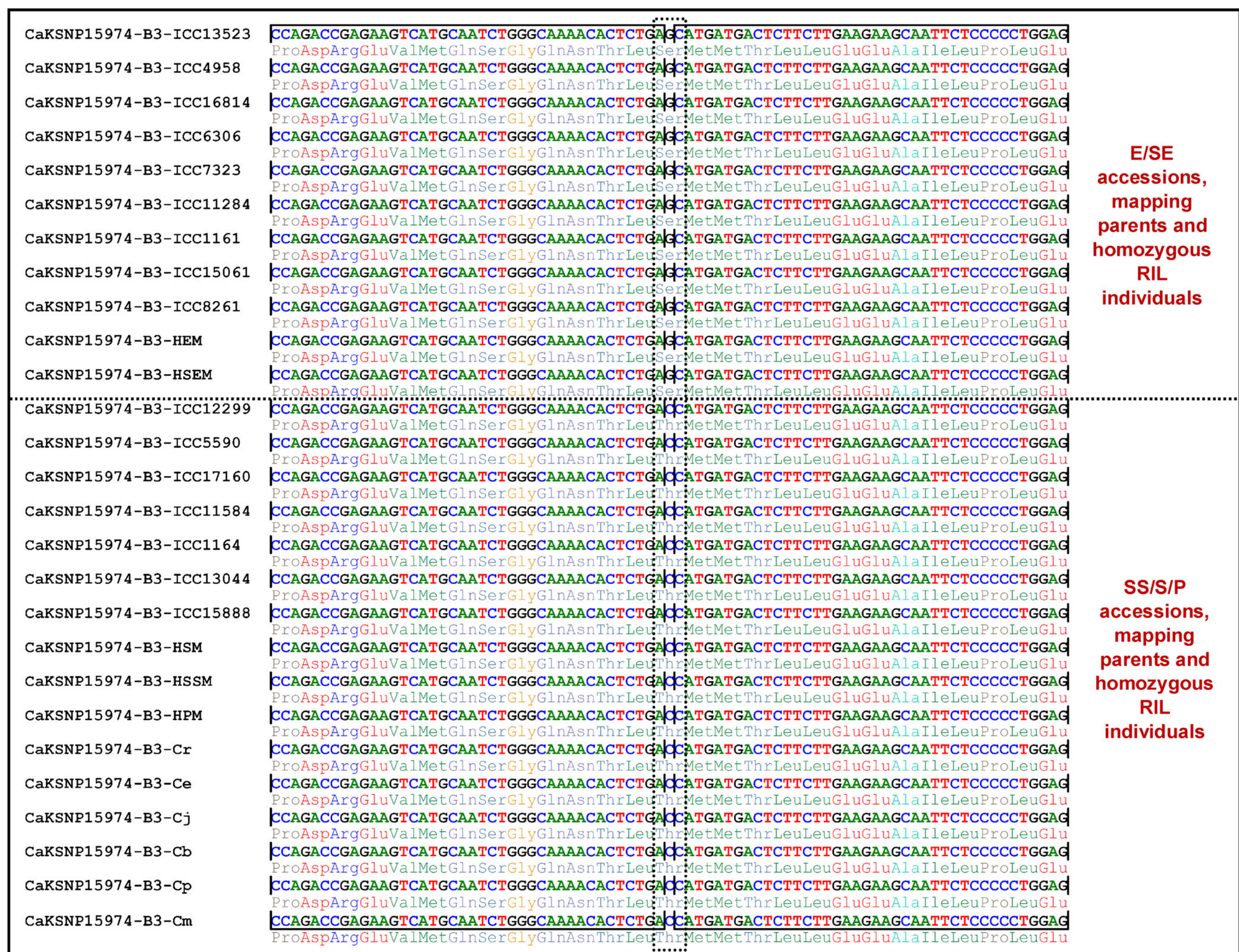

Fig. 3 The PGH-associated SNP allelic variants (G/C) exhibiting missense non-synonymous amino acid substitutions [serine (AGC) to threonine (ACC)] in a vernalization-responsive candidate gene (encoding B3 transcription factor) differentiated the E/SE from S/SS/P PGH-bearing mapping parents, homozygous RIL individuals and cultivated/wild accessions of chickpea by selective genotyping. The sequence regions carrying the non-synonymous SNPs are highlighted with *dotted boxes*. The details regarding the validated SNP loci are mentioned in the Tables 1 and S3. HEM/HSEM/HSM/HSSM/HPM: homozygous erect/semi-erect/spreading/semi-spreading/prostrate mapping individuals, *Cr Cicer reticulatum*, *Ce C. echinospermum*, *Cj C. judaicum*, *Cb C. bijugum*, *Cp C. pinnatifidum* and *Cm C. microphyllum*

expression and activation of key flowering genes by directly binding to proximal promoter regions of these genes. A *SNF2* gene is thus reportedly involved in regulation of both vernalization/flowering responses as well as inflorescence architecture-based plant growth habit trait variation in crop plants. The gene encoding ATP-binding cassette (ABC) transporter underlying a previously reported known PGH major QTLs (mapped on chromosome 3) plays a major role in steering phytohormones to integrate endogenous and exogenous signals and thereby assists in synchronizing plant growth with environmental and developmental changes (Cobos et al. 2009; Aryamanesh et al. 2010; Kang et al. 2011; Borghi et al. 2015). The B3 transcription factor family encompassing several distinct gene families, including the LAV [leafy cotyledon2 (LEC2)-abscisic acid insensitive3 (ABI3)-VAL], ARF (auxin response factor), RAV [related to ABI3 and VP1 (viviparous1)] and REM (reproductive meristem) families like *VRN1* (vernalization1) gene, are known to be involved in growth and development as well as flowering and vernalization responses in crop plants (Agarwal et al. 2011). The accumulation of transcripts of DNA ligase-encoding gene is known to enhance plant growth and development (Waterworth et al. 2010). The aforementioned previous reports altogether suggest that the novel functionally relevant natural allelic variants regulating PGH traits scaled down by an integrated genomic approach (GWAS, selective genotyping and expression profiling) can be deployed for rapid quantitative

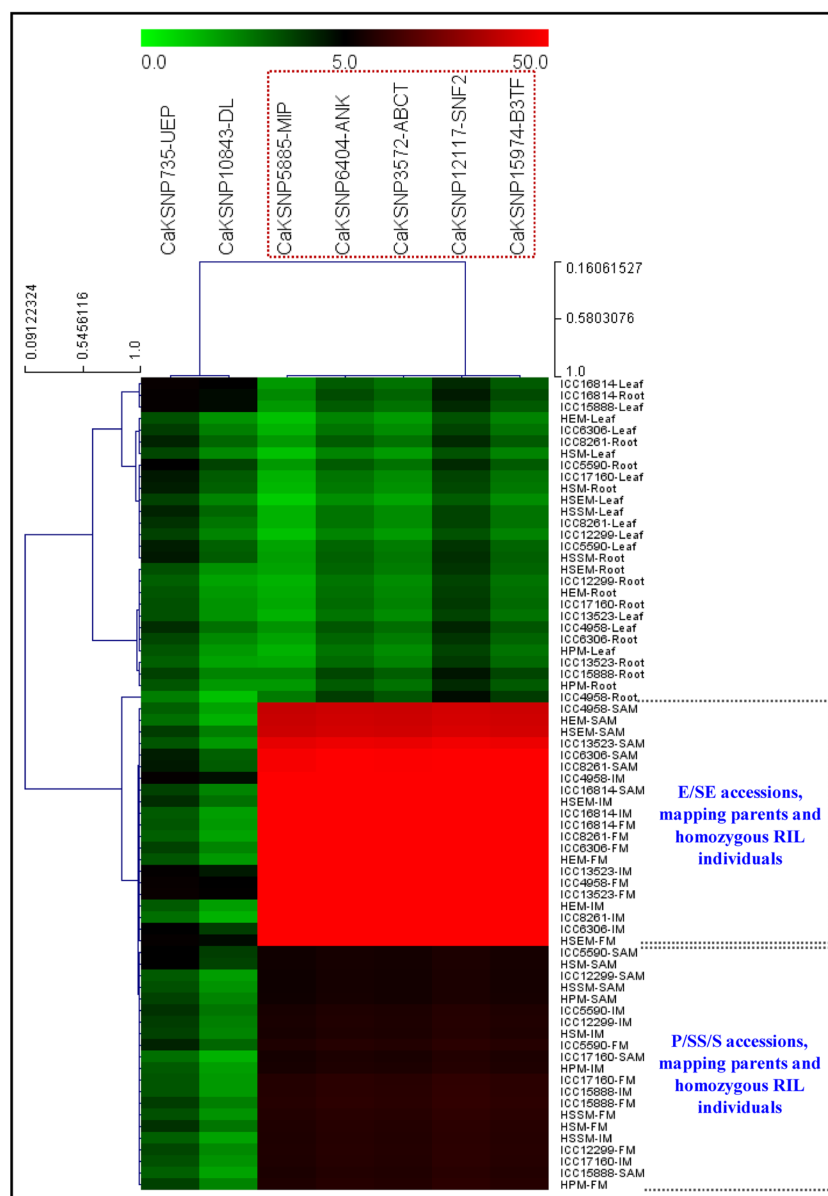

Fig. 4 Hierarchical cluster display illustrating the differential expression profiles of seven PGH-associated genes (validated by association analysis and selective genotyping) in vegetative and reproductive tissues of multiple germplasm accessions and homozygous RIL mapping individuals contrasting with diverse PGH traits (Table S2) using quantitative RT-PCR assay. The colour scale at the top represents the average log signal expression values of genes in different tissues; in which *green*, *black* and *red colour* denote low, medium and high level of expression, respectively. The details regarding SNPs and genes are mentioned in the Tables 1 and S3. The tissues and genes used for expression profiling are mentioned on the *right* and *top side* of expression map, respectively. The genes exhibiting shoot apical meristem (SAM), inflorescence meristem (IM) and floral meristem (FM)-specific expression and differentially upregulated especially in E/SE PGH-bearing germplasm accessions, mapping parents and homozygous RIL individuals are indicated with a red colour box. The HEM/HSEM/HSM/HSSM/HPM: homozygous erect/semi-erect/spreading/semi-spreading/prostrate mapping individuals

dissection of complex plant growth habit traits in chickpea. These informative molecular tags thus have potential to accelerate marker-assisted genetic enhancement for developing genetically tailored chickpea cultivars with ideal plant architecture of erect growth habit.

Understanding the natural/functional allelic diversity-based selection and domestication pattern of PGH-associated candidate genes

To infer the natural/functional allelic diversity-led selection and domestication pattern of PGH traits, five PGH-associated genes validated by GWAS, selective genotyping and expression profiling were selected. The PCR amplicons of 100 bp flanking gene regions of these associated SNPs were sequenced among 79 wild chickpea accessions (representing primary, secondary and tertiary gene pools) and compared that with 92 cultivated *desi* and *kabuli* chickpea accessions (used for GWAS) for mining natural SNP allelic variants. This exhibited the presence of identical PGH-associated SNP alleles in the five genes which were polymorphic among the E/SE and S/SS PGH-bearing accessions belonging to *desi* and *kabuli* cultivar groups as well as 79 prostrate PGH-bearing wild chickpea accessions.

The PGH-associated five SNPs-containing genes were selected to compare and deduce the nucleotide diversity measures as well as the direction and magnitude of natural selection acting on these gene loci across cultivated and wild

accessions during chickpea domestication. These genes exhibited almost a similar trend of nucleotide diversity ($\theta\pi$ and $\theta\omega$) and Tajima's D-based selection occurring on SE/E/S/SS PGH-bearing 92 *desi* and *kabuli* chickpea accessions. The extreme reduction of natural nucleotide diversity level ($\theta\pi$: 0.41–0.67 and $\theta\omega$: 0.47–0.63) specifically in SE/E/S/SS PGH-bearing cultivated *desi* and *kabuli* chickpea accessions as compared to P PGH-bearing annual/perennial wild accessions ($\theta\pi$: 2.57–3.14, $\theta\omega$: 2.87–3.29) was evident. The reduced nucleotide diversity in cultivated accessions was further supported well with enhanced Tajima's D (3.15 to 4.57) and strong positive selection than that of wild species/accessions encompassing primary, secondary and tertiary gene pools. These findings clearly provide definite evidence regarding signature of strong positive selection on five PGH-associated gene-derived SNP loci in SE/E/S/SS PGH-bearing *desi* and *kabuli* chickpea accessions in contrast to prostrate PGH-bearing wild accessions during chickpea domestication. This is agreed well with enduring positive selection for diverse plant architecture contributing traits like plant growth habit suggesting its significance as a target trait for both domestication and artificial breeding in crop plants, including chickpea (Wang and Li 2008; Meyer et al. 2012). The detailed analysis involving the entire natural allelic variants/potential locus targeted by positive selection in PGH-associated genes by constituting molecular haplotypes and selective sweep mapping is required to gain a deeper insight into the complex plant growth habit trait evolution and domestication in chickpea. This will be further useful to decipher the molecular genetic mechanism underlying fixation of such complex growth habit plant architectural quantitative traits especially in domesticated E/SE/S/SS *desi* and *kabuli* chickpea accessions that are adapted to diverse agroclimatic conditions as compared to their prostrate PGH-bearing wild progenitors.

Vernalization is a crucial key response of plant growth habit and flowering time which has been considered the most vital evolutionary bottleneck during chickpea domestication. This has led to evolution of presently cultivated vernalization insensitive E/SE PGH-bearing *desi* and *kabuli* accessions especially from the vernalization sensitive P PGH-bearing wild progenitor *C. reticulatum* (Abbo et al. 2014). This implicates that PGH is a major adaptive domestication trait targeted for selective breeding of chickpea accessions growing in diverse agroclimatic conditions. Interestingly, the presence of both SNP allelic variants mined from two vernalization-responsive candidate genes [encoding SNF2 protein (C/T) and B3 transcription factor (G/C)] associated significantly with E/SE vs. S/SS/P PGH trait differentiation was observed in 92 E/SE and S/SS PGH-bearing cultivated *desi* and *kabuli* accessions. In contrast, the occurrence of only the vernalization-responsive S/SS/P PGH trait-associated gene [encoding SNF2 protein (T) and B3 transcription factor (C)]-derived SNP alleles was observed in 79 wild accessions representing primary, secondary and tertiary gene pools. The nucleotide diversity ($\theta\pi$ and $\theta\omega$) and Tajima's D estimation further inferred the influence of very strong positive selection (with enhanced D) in favour of retention of E/SE-associated 'C' SNP in the vernalization responsive SNF2 protein-coding gene specifically in *desi* and *kabuli* accessions towards assortment of more preferential E/SE PGH traits in chickpea. Similarly, the preferential selection in favour of retention of E/SE-associated 'G' SNP in the vernalization responsive B3 transcription factor gene especially in E/SE PGH-bearing *desi* and *kabuli* accessions was evident. These findings may be a result of extensive contribution of four sequential bottlenecks (including vernalization) coupled with artificial selection/modern breeding efforts that are constantly practiced during the genetic improvement program of chickpea for developing cultivars with desirable erect plant growth habit of high preference (Jain et al. 2013; Kujur et al. 2013; Varshney et al. 2013; Abbo et al. 2014; Saxena et al. 2014a, b). These findings infer that the natural SNP allelic variants discovered in the two vernalization-responsive genes are possibly associated with plant growth habit trait evolution, and therefore, the growth habit is expected to represent an important component of domestication trait in chickpea. Therefore, it would be interesting to target these potential genes for mining novel functional allelic variants and haplotypes towards understanding the natural adaptation-based plant growth habit trait evolution and domestication pattern in cultivated and wild chickpea.

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