Genetic dissection of photosynthetic efficiency traits for enhancing seed yield in chickpea

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Funding Information
Department of Biotechnology (DBT), Ministry of Science and Technology

Abstract
Understanding the genetic basis of photosynthetic efficiency (PE) contributing to enhanced seed yield per plant (SYP) is vital for genomics-assisted crop improvement of chickpea. The current study employed an integrated genomic strategy involving photosynthesis pathway gene-based association mapping, genome-wide association study, quantitative trait loci (QTL) mapping, and expression profiling. This identified 16 potential single nucleotide polymorphism loci linked to major QTLs underlying 16 candidate genes significantly associated with PE and SYP traits in chickpea. The allelic variants were tightly linked to positively interacting QTLs regulating both enhanced PE and SYP traits as exemplified by a chlorophyll A-B binding protein-coding gene. The leaf tissue-specific pronounced up-regulated expression of 16 associated genes in germplasm accessions and homozygous individuals of mapping population was evident. Such combinatorial genomic strategy coupled with gene haplotype-specific association and in silico protein–protein interaction study delineated natural alleles and superior haplotypes from a chlorophyll A-B binding (CAB) protein-coding gene and its interacting gene, Timing of CAB Expression 1 (TOC1), which appear to be most promising candidates in modulating chickpea PE and SYP traits. These functionally pertinent molecular signatures identified have efficacy to drive marker-assisted selection for developing PE-enriched cultivars with high seed yield in chickpea.

KEYWORDS
chickpea, GWAS, photosynthesis, QTL, SNP, yield

1 | INTRODUCTION

In order to sustain global food security, it is imperative to enhance the yield and productivity of crop plants, especially of the staple food crops such as major cereals and legumes. The food we consume is the resultant of millions of years of evolution which provided the green plants the ability to fix the atmospheric carbon dioxide into carbohydrate using the solar energy. The photosynthetic carbon metabolism (PCM) is thus considered as one of the major contributors to crop grain yield. Since long time, researchers all over the world have made substantial efforts to manipulate the metabolic enzymes controlling photosynthesis in order to enhance the crop yield and productivity. The ribulose-1,5-bisphosphate carboxylase (RuBisCO) is considered as one of the major target of this manipulation, and by engineering of this metabolic enzyme, photosynthetic carbon fixation gain could increase by 30% in the environment (Zhu, Ort, Whitmarsh, & Long, 2004). Therefore, a better understanding on regulation of photosynthesis metabolism at a global scale using advanced structural, functional, and comparative genomics as well as molecular genetics strategies is essential to accomplish the prime objective of crop yield enhancement. In this perspective, a PCM-associated HIGHER YIELD RICE transcription factor enhancing photosynthesis and subsequent
grain yield during normal and stress (drought and high-temperature) conditions is found most promising in rice (Ambavaram et al., 2014). Moreover, the manipulation of cytochrome b6/f complex in the electron transport chain appears to be a potential target for enhancing photosynthetic rates and yield in crop plants (De Souza et al., 2017; Yamori et al., 2016). The aforesaid studies collectively highlighted that photosynthesis is the most vital and basic metabolic pathway essential for energy generation and survival and overall growth and development of crop plants. Henceforth, the PCM and major traits contributing to high photosynthetic efficiency (PE) need to be optimized at a global scale employing diverse genomics-assisted breeding and functional genomic approaches for the increase in crop yield and productivity. Recently, a genome-wide association study (GWAS) of diverse chlorophyll traits including total chlorophyll content is found efficient to identify genomic loci (genes) governing PCM for genetic improvement of legumes including soybean (Dhanapal et al., 2016). Unfortunately, no such information pertaining to PCM regulation is available in chickpea that necessitates comprehensive understanding on complex genetic architecture of vital traits contributing to high PCM and PE as well as increased seed yield in this legume crop.

Chickpea (Cicer arietinum), a vital food legume crop, is primarily represented by two of its domesticated desi and kabuli cultivars exhibiting distinct agro-morphological characteristics. Numerous genetic resources in form of germplasm accessions, landraces, cultivated varieties, genetic stocks, and wild species accessions as well as advanced generation mapping population contrasting for diverse agronomic traits are accessible in chickpea at various international and national Genbank. The draft genome sequences of desi and kabuli and its progenitor wild Cicer reticulatum accession are decoded recently in chickpea (Gupta et al., 2016; Jain et al., 2013; Parween et al., 2015; Varshney, Song, et al., 2013). Moreover, the transcriptome and genome resequencing of multiple cultivars (desi and kabuli) and wild chickpea accessions generated a diverse array of genes, transcription factors, and regulatory networks as well as simple sequence repeat and single nucleotide polymorphism (SNP) markers at a genome-wide scale (Pandey et al., 2016; Varshney, Mohan, et al., 2013). The available sequence information is also useful in deciphering the structural and functional perspectives of protein coding genes controlling diverse known regulatory pathways/networks especially underlying the basic metabolic processes such as photosynthesis at a whole genome level in crop plants (http://www.genome.jp/kegg/pathway.html).

Development of high-yielding climate resilient cultivars in chickpea is the prime focus worldwide. Increasing photosynthetic carbon fixation rate comes up as an attractive strategy for further yield enhancement in this vital legume food crop. This requires a comprehensive understanding of the photosynthetic gene regulatory pathways/networks and manipulation of gene(s) involved to achieve high PE and increased seed yield. The rapid genetic dissection of complex PE traits controlling increased seed yield is now certainly feasible through deployment of an integrated genomics-assisted breeding strategy involving association mapping, quantitative trait loci (QTL) mapping, expression profiling, and molecular haplotyping in chickpea (Kale et al., 2015; Kujur et al., 2015, 2015b, 2016). The combinatorial genomic strategy will be useful to scan functionally pertinent novel molecular tags including potential genes and superior alleles influencing PE for enhancing yield in chickpea.

In these perspectives, the present study employed high-resolution gene-based association analysis and GWAS integrated with QTL mapping and transcript profiling in phenotypically well-characterized natural germplasm accessions and mapping population to scan the most promising molecular signatures (SNP marker, genes/QTLs and natural alleles) regulating high PE vis-à-vis increased seed yield per plant (SYP) in chickpea.

2 | MATERIALS AND METHODS

2.1 | Genetic resource and phenotyping

An association panel of 92 accessions including 38 desi and 54 kabuli core, minicore, and reference core germplasm accessions representing diverse ecogeographical locations of 20 countries of the world was constituted in accordance with Kujur et al. (2015a; 2015b; Table S1). These were grown in the experimental field during normal crop season (October to February) as per randomized complete block design and/or alpha-lattice design with two replications following the standard agronomic practices. To develop an intraspecific mapping population contrasting with PE traits, two accessions, ICC 4958 (desi landrace originated from central India) and ICC 12299 (desi landrace from Nepal) exhibiting high and low PE, respectively, were selected from our aforesaid constituted association panel of chickpea. Subsequently, an intraspecific F2 recombinant inbred lines (RIL) mapping population (ICC 4958 × ICC 12299) composing of 236 individuals was grown in field according to aforesaid strategies.

These natural germplasm accessions and mapping population were phenotyped for diverse PE and SYP traits for successive years (2013 and 2014) at International Crops Research Institute for the Semi-Arid Tropics, Hyderabad (latitude 17°3′N/longitude 77°2′E). For determining PE, diverse major traits including total chlorophyll content, chlorophyll fluorescence, and CO2 assimilation rate were measured. To estimate chlorophyll content (CC), the total chlorophyll was extracted from the liquid nitrogen-grounded homogenate of 10–15 days old seedlings (fresh weight of 0.5 g) with 2 ml chilled 80% acetone, and their concentration were measured (mg/g fresh weight) by an ultraviolet-visible spectrophotometer at 663, 645, and 470 nm following the methods of S. C. Saxena et al. (2013). For precise estimation of chlorophyll content, third and fourth leaves (representing the plant canopy) of 30–40 days after sowing (DAS) grown plants were considered to be the most suitable leaf positions for chlorophyll measurement in chickpea. These leaf tissues were used for SPAD chlorophyll meter reading (SCMR) by SPAD-502 Plus meter (KONICA MINOLTA CO. LTD., JAPAN) as per Kashiwagi, Krishna, Singh, and Upadhyaya (2006). The chlorophyll fluorescence (CF) and CO2 exchange rate were measured from the third and fourth attached leaves of 60–90 DAS old plants by estimating the maximal quantum yield of PSII (Fv/Fm) employing the modulated chlorophyll fluorometer and LI-6400XT (LI-COR Inc., USA) as per Ambavaram et al. (2014). Especially, the mean CO2 exchange rate was estimated by selecting three to five representative plants, after 6hr of illumination.
with a daily continuous photoperiodic cycle of 10-hr light and 14-hr
dark at the 22–24 °C leaf temperature, 400–500 μmol/s CO₂ and 65–70% relative humidity. To determine the CO₂ exchange rate
precisely, CO₂ assimilation rate at increasing CO₂ concentration
(CAR↑CO₂ [μmol·CO₂·m⁻²·s⁻¹]) and CO₂ assimilation rate at increasing
light intensity (CAR↑LI [μmol·CO₂·m⁻²·s⁻¹]) were measured fol-
lowing Ambavaram et al. (2014). Three biological replicates, each
with three technical replicates, were used for measuring the individual
target traits in accessions and RIL mapping individuals of chickpea.
SYP was measured by weighing the mean weight (g) of fully matured
dried seeds (10% moisture content) harvested from 5 to 10 representa-
tive plants of each germplasm accession and RIL mapping individual.
The genetic inheritance pattern of all these studied traits using diverse
statistical parameters including coefficient of variation (CV), broad-
sense heritability (H²), Pearson’s correlation coefficient (r), and fre-
cquency distribution was determined in an association panel and a
RIL mapping population as per the methods described earlier (Bajaj,
Upadhyaya et al., 2015). CV was determined by estimating the ratio
of standard deviation to the mean for each individual environment.
We measured the significant effect between gene/genotype and envi-
ronment by estimating the genotypes (G; accessions/mapping
individuals) and phenotyping experimental years/environments (E)
interaction (G × E) using analysis of variance as per Upadhyaya et al.
(2016). The analysis of variance outcomes were further used to esti-
mate the broad-sense heritability σ²g (σ²g + σ²ge n + σ²e nr) follow-
ing σ²g (genetic), σ²ge (G × E) and σ²e (error) variance with n (number of
experimental years/environments) = 2 and r (number of replicates) = 2.

2.2 Targeted gene amplicon resequencing-based
SNP mining and genotyping

A selected set of candidate genes annotated from kabuli genome and
reported to be involved in photosynthesis-related metabolic pathways
cam00195, cam00196, and cam00710) in chickpea was retrieved
from Kyoto Encyclopedia of Genes and Genomes (KEGG) database
(http://www.genome.jp/kegg/pathway.html). The coding DNA
sequences (CDS) of these genes (NCBI C. arietinum Annotation
Cicer_arietinum/101/%23BuildInfo) were BLASTN searched against
the previously released CDS of kabuli genes (Varshney, Mohan,
et al., 2013; http://gigadb.org/dataset/100076) to obtain the best
possible true gene homologues (E: 0 with ≥500 bit score) of chickpea.
This exertion was performed to synchronize the version of reference
kabuli genome-led gene annotation information utilized in the afore-
said candidate gene-based SNP analysis with that of whole genome
genotyping-by-sequencing (GBS)-derived SNP genotyping data avail-
able with us for GWAS of PE and SYP traits in chickpea.

The identified chickpea genes were further resequenced using the
genomic DNA of 92 diverse desi and kabuli germplasm accessions
(association panel) of chickpea employing the multiplexed amplicon
reseque uneasy strategy (TrueSeq Custom Amplicon v1.5) of Illumina
MiSeq next-generation sequencer (Illumina, USA). The 2 kb upstream
regulatory regions (URRs), exons/CDS, introns, and 2 kb downstream
regulatory regions (DRRs) of these genes were selected for designing
and synthesizing the custom oligo-probes (producing amplicons with
mean size of 500 bp per reaction) using Illumina Design Studio. The
probe-pooling, template libraries-constitution, sample-specific indices
addition to individual libraries and normalization of uniquely tagged
pooled amplicon libraries were performed as per M. S. Saxena, Bajaj,
Das, et al. (2014) and Malik et al. (2016). Accordingly, the sequencing
of generated clusters by Illumina MiSeq platform, mapping of
sequenced gene amplicons of each chickpea accession using the
pseudomolecules of kabuli chickpea genome (http://gigadb.org/
dataset/100076), and discovery of high-quality SNPs among acces-
sions were carried out as per M. S. Saxena, Bajaj, Das, et al. (2014)

2.3 Trait association mapping

For association mapping, the genotyping and comprehensive annota-
tion information of candidate gene-derived SNPs and genome-wide
SNPs discovered from the resequenced 92 desi and kabuli chickpea
accessions (association panel) employing a GBS assay were obtained
(Kujur et al., 2015, 2015a, 2015b). Subsequently, the population struc-
ture, principal component analysis (PCA), and linkage disequilibrium
(LD) decay measured among accessions were acquired from the previ-
ous study of Kujur et al. (2015a, 2015b). The accessions revealing high
H² for the studied PE and SYP traits across two diverse environments
were acquired for association study. Consequently, environment-wise
phenotyping information of these said traits measured from the indi-
vidual selected accession were utilized to calculate its average trait
value for association study. The SNP genotyping data were integrated
with multi-environment field phenotyping information of PE and SYP
traits as well as kinship (K), population structure (Q), and PCA (P) infor-
mation of accessions (association panel). This was performed by mixed
model (P + K, K and Q + K)-led compressed mixed linear model
(CMLM) and P3D (Kang et al., 2010; Z. Zhang et al., 2010)/efficient
mixed model association expedition (EMMAX) strategies of genome
association and prediction integrated tool (GAPIT) (Lipka et al.,
2012). The reliability of SNP marker-trait association was ascertained
by using the quantile–quantile (Q–Q) plot-based false discover rate
(FDR) (cut-off ≤0.05, Benjamini & Hochberg, 1995) correction for mul-
tiple comparison between observed/expected -log₁₀(p) value and
adjusted p value threshold of significance, measured in each trait-asso-
ciated genomics loci. The genomic SNP loci associated with PE and
SYP traits at a lowest FDR adjusted p value (cut-off p < 1 × 10⁻⁵)
and highest R² were considered significant.

2.4 QTL mapping

The GBS-derived high-quality SNPs (differentiating 92 accessions)
showing polymorphism between two parental accessions (ICC 4958
and ICC 12299) were genotyped using the genomic DNA of 236 mapping
individuals from a RIL population (ICC 4958 × ICC 12299) through Sequenom MALDI-TOF MassARRAY assay (http://www.
sequenom.com) as per M. S. Saxena, Bajaj, Das, et al. (2014) and M.
S. Saxena, Bajaj, Kujur et al. (2014). The significant SNP genotyping
data were analysed by JoinMap 4.1 (http://www.kyazma.nl/index.php/mc.
JoinMap) with Kosambi mapping function and using high logarithm
of odds (LOD) threshold (≥5.0) to estimate the linkages among the SNPs. A high-resolution intraspecific genetic linkage map was constructed by assigning the SNPs into defined linkage groups (LGs; designated as LG1 to LG8)/chromosomes in accordance with their centiMorgan (cM) genetic distances and respective marker physical positions (bp) on chromosomes and further visualized by MapChart v2.2 (Voorrips, 2002).

For molecular mapping of major QTLs governing PE and SYP traits, the genotyping information of SNPs genetically mapped on a high-density linkage map (with eight chromosomes/LGs) was correlated with multienvironment PE and SYP trait field phenotypic data of RIL mapping individuals and parents using a composite interval mapping (CIM) function of MapQTL 6 (Van Ooijen, 2009). For QTL mapping, the LOD cutoff score >5.0 with 1,000 permutation at a p < .05 was considered most significant in CIM. Accordingly, the phenotypic variation explained (PVE) and additive effect specified by each major QTL on PE and SYP traits at a significant LOD were determined. The main-effect QTLs (M-QTLs) were identified using a CIM function of MapQTL 6 and QTLNetwork v2.0 (http://ibi.zju.edu.cn/software/qtlnetwork). The M-QTLs and the QTLs involved in epistatic (Q × Q) and QTLs by environment (Q × E) interactions as defined as epistatic QTLs (E-QTLs) were identified using the three-loci QTL interface of Genotype Matrix Mapping program v.2.1 (www.kajusa.or.jp/GMM). The detail M-QTL and E-QTL mapping strategy followed was adopted from Gautami et al. (2012) and Varshney et al. (2014).

2.5 Differential expression profiling

RNA was isolated from vegetative and reproductive tissues (shoot, root, young/mature leaf [third/fourth leaves of 30–40 DAS grown...
<table>
<thead>
<tr>
<th>Traits studied</th>
<th>Genetic resource utilized</th>
<th>Environments</th>
<th>Geographical location with experimental years</th>
<th>Mean ± SD (standard deviation)</th>
<th>Range</th>
<th>Coefficient of variation (CV %)</th>
<th>Heritability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll content (CC; mg/g-FW)</td>
<td>92 germplasm accessions</td>
<td>Environment I</td>
<td>Hyderabad (2013)</td>
<td>1.21 ± 0.30</td>
<td>0.55–1.84</td>
<td>24.79</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Environment II</td>
<td>Hyderabad (2014)</td>
<td>1.44 ± 0.27</td>
<td>0.68–2.08</td>
<td>25.69</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Overall environments</td>
<td></td>
<td>1.33 ± 0.33</td>
<td>0.63–1.99</td>
<td>24.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>236 RILs (ICC 4958 × ICC 12299)</td>
<td>Environment I</td>
<td>Hyderabad (2013)</td>
<td>1.12 ± 0.30</td>
<td>0.49–2.13</td>
<td>26.79</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Environment II</td>
<td>Hyderabad (2014)</td>
<td>1.05 ± 0.27</td>
<td>0.43–2.05</td>
<td>25.71</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Overall environments</td>
<td></td>
<td>1.03 ± 0.28</td>
<td>0.45–2.02</td>
<td>27.18</td>
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<tr>
<td>SPAD chlorophyll meter readings (SCMR)</td>
<td>92 germplasm accessions</td>
<td>Environment I</td>
<td>Hyderabad (2013)</td>
<td>50.10 ± 4.19</td>
<td>40.80–60.75</td>
<td>8.36</td>
<td>80</td>
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<td></td>
<td></td>
<td>Environment II</td>
<td>Hyderabad (2014)</td>
<td>55.13 ± 5.07</td>
<td>45.70–63.19</td>
<td>9.20</td>
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<tr>
<td></td>
<td></td>
<td>Overall environments</td>
<td></td>
<td>52.13 ± 4.07</td>
<td>43.80–61.98</td>
<td>7.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Environment II</td>
<td>Hyderabad (2014)</td>
<td>51.17 ± 3.89</td>
<td>43.80–63.15</td>
<td>7.60</td>
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<td></td>
<td></td>
<td>Overall environments</td>
<td></td>
<td>48.34 ± 3.59</td>
<td>40.80–61.02</td>
<td>7.43</td>
<td></td>
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<tr>
<td>Chlorophyll fluorescence (CF; Fv/Fm)</td>
<td>92 germplasm accessions</td>
<td>Environment I</td>
<td>Hyderabad (2013)</td>
<td>0.83 ± 0.033</td>
<td>0.79–0.90</td>
<td>3.98</td>
<td>81</td>
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<tr>
<td></td>
<td></td>
<td>Environment II</td>
<td>Hyderabad (2014)</td>
<td>0.91 ± 0.047</td>
<td>0.88–0.95</td>
<td>5.96</td>
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<td>Overall environments</td>
<td></td>
<td>0.86 ± 0.025</td>
<td>0.81–0.92</td>
<td>2.91</td>
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<td></td>
<td>236 RILs (ICC 4958 × ICC 12299)</td>
<td>Environment I</td>
<td>Hyderabad (2013)</td>
<td>0.89 ± 0.033</td>
<td>0.85–0.99</td>
<td>3.71</td>
<td>80</td>
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<tr>
<td></td>
<td></td>
<td>Environment II</td>
<td>Hyderabad (2014)</td>
<td>0.81 ± 0.021</td>
<td>0.78–0.90</td>
<td>2.59</td>
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<tr>
<td></td>
<td></td>
<td>Overall environments</td>
<td></td>
<td>0.85 ± 0.026</td>
<td>0.80–0.94</td>
<td>3.06</td>
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<td>CO2 assimilation rate at increasing CO2 concentration (CAR-CO2↑; μmol CO2 m⁻² s⁻¹)</td>
<td>92 germplasm accessions</td>
<td>Environment I</td>
<td>Hyderabad (2013)</td>
<td>25.19 ± 1.25</td>
<td>20.79–27.89</td>
<td>4.96</td>
<td>85</td>
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<td></td>
<td></td>
<td>Environment II</td>
<td>Hyderabad (2014)</td>
<td>20.11 ± 1.11</td>
<td>17.76–23.16</td>
<td>5.52</td>
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<td></td>
<td>Overall environments</td>
<td></td>
<td>22.12 ± 1.13</td>
<td>19.80–24.86</td>
<td>5.11</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Overall environments</td>
<td></td>
<td>20.09 ± 1.63</td>
<td>16.70–25.85</td>
<td>8.11</td>
<td></td>
</tr>
<tr>
<td>CO2 assimilation rate at increasing light intensity (CAR-LI↑; μmol CO2 m⁻² s⁻¹)</td>
<td>92 germplasm accessions</td>
<td>Environment I</td>
<td>Hyderabad (2013)</td>
<td>15.17 ± 1.08</td>
<td>12.55–16.92</td>
<td>7.12</td>
<td>82</td>
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<tr>
<td></td>
<td></td>
<td>Environment II</td>
<td>Hyderabad (2014)</td>
<td>20.18 ± 1.27</td>
<td>15.65–22.31</td>
<td>6.29</td>
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<td></td>
<td>Overall environments</td>
<td></td>
<td>17.09 ± 1.17</td>
<td>14.70–19.92</td>
<td>6.85</td>
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<td>236 RILs (ICC 4958 × ICC 12299)</td>
<td>Environment I</td>
<td>Hyderabad (2013)</td>
<td>12.54 ± 1.41</td>
<td>10.74–18.79</td>
<td>11.24</td>
<td>81</td>
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<td>Environment II</td>
<td>Hyderabad (2014)</td>
<td>18.89 ± 1.68</td>
<td>14.74–23.88</td>
<td>8.89</td>
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<tr>
<td></td>
<td></td>
<td>Overall environments</td>
<td></td>
<td>15.78 ± 1.52</td>
<td>12.60–21.12</td>
<td>9.63</td>
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<tr>
<td>Seed yield per plant (SYP; g)</td>
<td>92 germplasm accessions</td>
<td>Environment I</td>
<td>Hyderabad (2013)</td>
<td>15.19 ± 1.91</td>
<td>10.57–20.47</td>
<td>12.57</td>
<td>83</td>
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<tr>
<td></td>
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<td>Environment II</td>
<td>Hyderabad (2014)</td>
<td>10.87 ± 1.64</td>
<td>7.45–14.58</td>
<td>15.09</td>
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<td>Overall environments</td>
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<td>12.78 ± 1.87</td>
<td>9.37–17.69</td>
<td>14.63</td>
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<td>Environment II</td>
<td>Hyderabad (2014)</td>
<td>9.69 ± 2.66</td>
<td>3.45–18.87</td>
<td>27.45</td>
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<tr>
<td></td>
<td></td>
<td>Overall environments</td>
<td></td>
<td>10.43 ± 2.98</td>
<td>4.17–20.95</td>
<td>28.57</td>
<td></td>
</tr>
</tbody>
</table>

Note. RIL = recombinant inbred line.
TABLE 2  Genomic loci associated with major photosynthetic efficiency (PE) and seed yield per plant (SYP) traits in chickpea

<table>
<thead>
<tr>
<th>Photosynthesis-related metabolic pathway genes</th>
<th>KEGG photosynthesis pathway module</th>
<th>Gene accessions IDs</th>
<th>SNP IDs</th>
<th>Kabuli chromosomes</th>
<th>SNP physical positions (bp)</th>
<th>Structural annotation</th>
<th>Amino acid substitutions</th>
<th>Differential gene expression (tissue-specific up-regulation) in leaves of high PE/SYP accessions*</th>
<th>Association analysis</th>
<th>PE and SYP traits associated</th>
<th>p</th>
<th>PVE (%)</th>
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<tr>
<td>Oxidoreductase FAD/NAD(P)</td>
<td>F-type ATPase</td>
<td>Ca_07996</td>
<td>Ca17446222</td>
<td>Ca_Kabuli_Chr1</td>
<td>7,446,222 (T/C)</td>
<td>CDS (nonsynonymous)</td>
<td>TTG (leucine) – TCG (serine)</td>
<td>2.6</td>
<td>CC, SCMR, CF, CARTCO2, and CARTLI</td>
<td>1.0 × 10^-8</td>
<td>5.8</td>
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<tr>
<td>Phosphoenolpyruvate carboxylase</td>
<td>CAM_dark</td>
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<td>1.5 × 10^-8</td>
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<td>Iron–sulphur protein</td>
<td>Cytochrome b/f complex</td>
<td>Ca_14123</td>
<td>Ca113563280</td>
<td>Ca_Kabuli_Chr1</td>
<td>13,563,280 (G/C)</td>
<td>DRR</td>
<td>-</td>
<td>4.3</td>
<td>CC, SCMR, CF, CARTCO2, and CARTLI</td>
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<td>TAC (tyrosine) – TAG (ammon)</td>
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<td>Phosphate dehydrogenase</td>
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<td>GCC (alanine) – GAC (aspartic acid)</td>
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<td>Ca658843981</td>
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<td>ACC (threonine) – TCC (serine)</td>
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<td>TAC (tyrosine) – TGC (cysteine)</td>
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<td>Ca_Kabuli_Chr7</td>
<td>19,530,542 (T/G)</td>
<td>CDS (nonsynonymous)</td>
<td>AAT (asparagine) – AAG (lysine)</td>
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<td>CC, SCMR, CF, CARTCO2, and CARTLI</td>
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<td>Calvin cycle</td>
<td>Ca_11440</td>
<td>Ca88024702</td>
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<td>8,024,702 (T/C)</td>
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<td>Scaffold354218070的精神翻译</td>
<td>Ca_27778</td>
<td>Scaffold3542</td>
<td>Ca_Kabuli_Chr8</td>
<td>18,070</td>
<td>-</td>
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TABLE 2 (Continued)

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<th>KEGG photosynthesis metabolic pathway</th>
<th>KEGG photosynthesis pathway module</th>
<th>Gene accession IDs</th>
<th>SNP IDs</th>
<th>Kabuli chromosomes</th>
<th>SNP positions</th>
<th>Structural annotation</th>
<th>Amino acid substitutions</th>
<th>Differential gene expression (tissue-specific up-regulation) in leaves of high PE/SYP accessions*</th>
<th>Association analysis</th>
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<td>Photosynthesis-related</td>
<td>Photosystem II</td>
<td>Ca_24986</td>
<td>Scaffold89207917</td>
<td>Scaffold9</td>
<td>207,917</td>
<td>(C/A)</td>
<td>URR</td>
<td>11.3</td>
<td>CC, SCMR, CF, CAR, CO2, and SYP*</td>
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</tbody>
</table>

Note. KEGG = Kyoto Encyclopedia of Genes and Genomes; CAM = crassulacean acid metabolism; URR = upstream regulatory region; DRR = downstream regulatory region; SNP = single nucleotide polymorphism; PVE = phenotypic variation explained; CC = chlorophyll content; SCMR = SPAD chlorophyll meter reading; CF = chlorophyll fluorescence; CAR = CO2 assimilation rate at increasing CO2 concentration; CAR = CO2 assimilation rate at increasing light intensity.

* Fold change calculated with respect to leaf tissue in high as compared to low accessions/RIL mapping individuals. Details regarding SNPs are mentioned in the Table S2.

2.7 | Trait association potential of interacting genes

2.6 | In silico protein-protein interaction study

To scan the known/candidate protein-protein interactions based on experimental data, computational prediction and public literature survey, the amino acid sequence encoded by an Arabidopsis homologue of a strong PE, and SYP trait-associated gene were analysed in silico. Significant difference in gene expression was, estimated and differential expression profiles were visualized with a heat map by MultiExperiment Viewer (http://www.tm4.org/mev).
differential expression potential of gene haplotypes with the studied PE and SYP traits were performed in accordance with Kujur et al. (2015, 2015b) and M. S. Saxena, Bajaj, Das, et al. (2014).

3 | RESULTS

3.1 | Large-scale SNP genotyping discovers genome-wide and photosynthesis pathway gene-derived novel alleles for genomics-assisted breeding applications of chickpea

The large-scale genotyping of SNPs discovered from 136 photosynthesis metabolic pathways-related genes among 92 desi and kabuli accessions representing an association panel detected 3,338 high-quality SNPs from different coding and noncoding sequence components of these genes with a mean frequency of 24.5 SNPs/gene (Table S2). The sequencing of 96-plex ApeKI GBS libraries constructed from 92 accessions (with an average of 2.1 million reads per chickpea accession) produced 207.9 million high-quality sequence reads. Of these, 149.8 million sequence reads produced from chickpea accessions were mapped on kabuli reference genome according to their unique physical position (bp). The sequencing information obtained in this study was submitted to a NCBI-short read archive database (www.ncbi.nlm.nih.gov/sra) with accession number SRX845396 for unrestricted public access. In total, 11,079 high-quality SNPs (with ≥10 read-depth and ≥20 SNP base quality in individual accession) were detected from 92 accessions using kabuli reference (4,314 SNPs)- and de novo (6,765)-based GBS strategy (Figure 1a,b; Table S2). Notably, 3,651 and 663 reference-based SNPs were mapped on eight chromosomes and unanchored scaffolds of kabuli chickpea genome, respectively. This underlines greater utility of GBS assay in fast high-throughput discovery and genotyping of high-quality SNPs altogether at a whole genome level in chickpea. In this context, GBS-derived genome-wide SNPs discriminating domesticated desi and kabuli accessions discovered in the current study have much implications for their immense use and broader practical applications in genomics-assisted breeding and further genetic enhancement studies of chickpea.

Notably, 7,652 including 3,338 gene-derived and 4,314 genome-wide GBS-SNPs were mapped across chromosomes (6,826 SNPs) and unanchored scaffolds (826) of kabuli genome (Figure 1a, b; Table S2). The highest number of 907 SNPs (21%) discovered from 291 genes were mapped on Chromosome 4, whereas maximum of 613 (18.4%) genome-wide SNPs were localized on Chromosome 1. The detailed structural annotation of 3,338 gene-based and 4,314 genome-wide SNPs demonstrated the occurrence of 5,933 (77.5%) and 1,719 (22.5%) SNPs in 1,410 genes and intergenic regions, respectively (Figure 1b,c; Table S2). The gene-derived SNPs included the highest and lowest proportion of 42.7% (2,531) and 0.3% (20) SNPs in the introns and URRs of 631 and 4 genes, respectively. The 1,901 coding SNPs consisted of 59% (1,130 SNPs) and 41% (771) synonymous and nonsynonymous (missense/non-sense) SNPs, respectively (Figure 1b,c; Table S2). The functional
TABLE 3 Molecular mapping of major photosynthetic efficiency and seed yield QTLs in chickpea

<table>
<thead>
<tr>
<th>QTLs*</th>
<th>LGs/ Chromosomes</th>
<th>Marker intervals with genetic positions (cM)</th>
<th>QTL physical intervals (bp)</th>
<th>Markers tightly linked to QTLs with genetic (cM) and physical positions (bp)</th>
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<tr>
<td>CaqPE1.1</td>
<td>CaLG(Chr)1</td>
<td>CaqSNP807(C/T): 20.54 to CaqSNP1313 (G/C): 26.40</td>
<td>CaqSNP807(C/T): 7,050,958 to CaqSNP1313 (G/C): 13,680,008</td>
<td>Ca17446222(T/C): 21.86 and 7,446,222 Ca112641213(C/T): 23.87 and 12,641,213 Ca113563280(G/C): 25.53 and 13,563,280</td>
</tr>
<tr>
<td>CaqPE1.2</td>
<td>CaLG(Chr)1</td>
<td>CaqSNP1875(T/G): 78.45 to CaqSNP2061 (C/T): 84.70</td>
<td>CaqSNP1875(T/G): 40,358,747 to CaqSNP2061 (C/T): 46,920,524</td>
<td>Ca142189380(A/G): 81.49 and 42,189,380</td>
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<tr>
<td>CaqPE2.2</td>
<td>CaqSYP2.1</td>
<td>CaqSNP3281(T/C): 57.80 to CaqSNP3283 (C/A): 61.53</td>
<td>CaqSNP3281(T/C): 35,601,657 to CaqSNP3283 (C/A): 35,612,587</td>
<td>Ca23343577(A/G): 58.27 and 3,343,577</td>
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<tr>
<td>CaqPE4.1</td>
<td>CaLG(Chr)4</td>
<td>CaqSNP5697(C/T): 22.45 to CaqSNP5710 (A/C): 26.48</td>
<td>CaqSNP5697(C/T): 8,669,515 to CaqSNP5710 (A/C): 8,805,578</td>
<td>Ca48779743(C/G): 24.17 and 8,779,743</td>
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<tr>
<td>CaqPE5.1</td>
<td>CaLG(Chr)5</td>
<td>CaqSNP8486(G/A): 3.14 to CaqSNP8622 (T/C): 5.68</td>
<td>CaqSNP8486(G/A): 31,176,844 to CaqSNP8622(T/C): 33,673,894</td>
<td>Ca533245382(C/A): 3.97 and 33,245,382</td>
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<td>CaqPE6.1</td>
<td>CaLG(Chr)6</td>
<td>CaqSNP13971(G/A): 32.54 to CaqSNP10345(T/C): 36.81</td>
<td>CaqSNP13971(G/A): 11,665,233 to CaqSNP10345(T/C): 15,861,278</td>
<td>Ca614019110(G/C): 34.89 and 14,019,110</td>
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<td>CaqPE6.2</td>
<td>CaLG(Chr)6</td>
<td>CaqSNP11526(A/G): 62.12 to CaqSNP11598(G/A): 66.78</td>
<td>CaqSNP11526(A/G): 58,237,503 to CaqSNP11598(G/A): 58,908,024</td>
<td>Ca658843981(A/T): 64.87 and 58,843,981</td>
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<tr>
<td>CaqPE7.1</td>
<td>CaqSYP7.1</td>
<td>CaqSNP12106(T/C): 53.45 to CaqSNP12681(C/T): 59.12</td>
<td>CaqSNP12106(T/C): 7,580,158 to CaqSNP12681(C/T): 20,496,303</td>
<td>Ca78419380(A/G): 55.87 and 8,419,380 Ca710909055(A/G): 57.34 and 10,909,055 Ca711324664(C/G): 58.17 and 11,324,664 Ca719530542(T/G): 58.95 and 19,530,542</td>
</tr>
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</table>

Note. LGs = linkage groups; cM = centiMorgan; PE = photosynthetic efficiency; SYP = seed yield per plant; LOD = logarithm of odds; PVE = phenotypic variation explained; DRR = downstream regulatory region; CC = chlorophyll content; SCMR = SPAD chlorophyll meter reading; CF = chlorophyll fluorescence; CAR†CO2 = assimilation rate at increasing CO2 concentration; CAR†LI = CO2 assimilation rate at increasing light intensity; A = additive effect.

*aCaqPE1.1 (Cicer arietinum QTL for photosynthetic efficiency on Chromosome 1 Number 1) and CaqSYP2.1 (C. arietinum QTL for seed yield per plant on Chromosome 2 Number 1). Proportion of PVE by QTLs; PE positive additive effect infers alleles from high PE and SYP mapping parental chickpea accession ICC 4958. Details regarding SNPs are mentioned in the Tables S2 and S5.

3.2 | Association mapping identifies potential genomic loci governing PE and SYP traits in chickpea

To perform gene-based association mapping and GWAS, we employed genome-wide GBS (11,079 SNPs) and gene-based (3,338 SNPs) SNP genotyping data of 14,417 SNPs assayed in 92 chickpea accessions (association panel; Table S2). The use of these 14,417 SNPs, in determining high-resolution population structure and PCA, discriminated all 92 desi and kabuli accessions from each other and overall grouped into two distinct populations, POP I and POP II. The LD decay estimation in an association panel using 7,652 SNPs (4,314 genome-wide and 3,338 gene-based SNPs) that were mapped across eight chromosomes, illustrated LD decay (r^2 reduced to half of its highest value) nearly at 200–300 kb physical distance of chromosomes. A wider level of phenotypic variation (2.91–24.81% CV) and normal frequency distribution of PE LI) and SYP traits in an association panel representing two population groups based on multienvironment field phenotyping data was apparent (Table 1; Figure S1). All accessions representing an association panel revealed high H2 (varied from 80% to 85%) for the six studied traits across environments. Maximum significant (p < .0001) positive correlation among five PE traits (mean r: 0.97) and minimum positive correlation between PE and SYP traits (0.61) were observed in an association panel. A higher significant difference (p < .0001) among 92
accessions (association panel) both for PE (CC, SCMR, CF, CAR↑CO₂, and CAR↑LI) and SYP traits was observed despite substantial environmental effect on these traits (Table S3). A significant interaction between genotypes (G)/accessions and environment (E) for the said traits based on G × E variance was apparent. For gene-based association mapping and GWAS, genotyping data of 14,417 SNPs assayed among 92 accessions (association panel) were integrated with their multi-environments field phenotyping data of PE (CC, SCMR, CF, CAR↑CO₂, and CAR↑LI) and SYP traits. The CMLM-led association study at a significant FDR cut-off ≤0.05 detected 16 genomic loci revealing association with six PE (CC, SCMR, CF, CAR↑CO₂, and CAR↑LI) and SYP traits at a p ≤ 10⁻⁸ which were got validated across two environments (Table 2; Figure 2). The association potential of genomic SNP loci with PE (CC, SCMR, CF, CAR↑CO₂, and CAR↑LI) was apparent. The highest four trait-associated SNPs were mapped on Chromosome 1 followed by three SNPs on Chromosome 7. Ten and 6 of 16 trait-associated loci were derived from diverse coding (10 nonsynonymous SNPs) and noncoding (one URR-SNP and five DRR-SNPs) regions of 16 genes, respectively (Table 2; Figure 2). Thirteen SNPs derived from different coding and noncoding sequence components of 13 genes were associated with five PE (CC, SCMR, CF, CAR↑CO₂, and CAR↑LI) traits, whereas three nonsynonymous coding SNPs from three genes were associated with both PE and SYP traits. The PVE determined by 16 PE and SYP trait-associated individual loci of 16 genes among 92 chickpea accessions varied from 5.0% to 12.0% R². All these 16 trait-associated loci altogether in combination gave 18.4% PVE. Three individual nonsynonymous SNPs-containing three genes showing significant association with both PE and SYP traits revealed 10.2–12.0% PVE (combined PVE: 20.6%), whereas 13 PE-associated individual gene-based SNPs revealed 5.0–6.3% PVE (10.2%) in chickpea. We observed a strong association of one coding SNP (A/G) revealing nonsynonymous amino acid substitution (Isoleucine [ATC] to Valine [GTC]) in a chlorophyll A-B binding protein-coding gene (1.2 × 10⁻⁷ P with 12% R²) as compared with other 15 identified genomic loci with PE (CC, SCMR, CF, CAR↑CO₂, and CAR↑LI) and SYP traits. Henceforth, this gene was considered as a promising candidate for dissection of PE and SYP traits in chickpea.
FIGURE 3 Nine genomic regions harbouring 11 major QTLs associated with five photosynthetic efficiency (chlorophyll content, SPAD
chlorophyll meter reading, chlorophyll fluorescence, CO2 assimilation rate at increasing CO2 concentration, and CO2 assimilation rate at
increasing light intensity) and seed yield traits mapped on six chromosomes of a high-density genetic linkage map (ICC 4958 x ICC
12299) of chickpea. The genetic distance (cM) and identity of the single nucleotide polymorphism (SNP) loci mapped on
chromosomes are denoted on left and right sides of chromosomes, respectively. The details of SNPs flanking and tightly linked to major QTLs
are provided in Tables 3 and S5. Orange and yellow square boxes represent the QTLs associated with photosynthetic efficiency and seed yield
traits, respectively, mapped on chromosomes of a genetic map. The SNPs flanking and tightly linked to major QTLs are depicted with blue and red
colour lines, respectively.
3.3 | Molecular mapping of QTLs ascertains association potential of genomic loci for PE and SYP traits in chickpea

We constructed an intraspecific genetic map (ICC 4958 × ICC 12299) of chickpea by integrating 589 SNPs across eight LGs (LG1–LG8; Tables S4 and S5). The genetic linkage map covered a total map length of 728.65 cM with a map density that defined as mean intermarker distance of 1.24 cM (Table 3; Figure 3). The highest and lowest saturated genetic maps were LG4 and LG3 with the map densities of 0.90 and 1.82, respectively. This high-density map characteristic of a constructed genetic linkage map reflected its utilization in high-resolution QTL mapping of agronomic traits in chickpea. The field phenotyping of a RIL mapping population (ICC 4958 × ICC 12299) exhibited a wider level of PE (CC, SCMR, CF, CAR↑CO2, and CAR↑LI) and SYP trait variation (3.06–28.57% CV) and high $H^2$ (80–83%) among 236 mapping individuals and parental accessions across two environments (Table 1; Figure S1). The normal frequency distribution including bidirectional transgressive segregation of these studied traits in RILs emphasizes the higher proficiency of a generated RIL population (ICC 4958 × ICC 12299) for subsequent QTL mapping study in chickpea (Figure S1). We observed a significant difference ($p < .0001$) among RIL mapping individuals for PE (CC, SCMR, CF, CAR↑CO2, and CAR↑LI) and SYP traits even though these traits were influenced by significant environmental effect as evident from $G \times E$ interaction variance (Table S3).

The QTL mapping was performed by combining genotyping information of 589 genetically mapped SNPs and two environments PE and SYP trait field phenotyping data of RIL mapping individuals along with parental accessions. This detected 11 M-QTLs (5.0–11.7 LOD) harbouring nine genomic regions governing PE and SYP traits were mapped on six chickpea LGs/chromosomes (except 3 and 8; Table 3; Figure 3). The PVE determined by individual M-QTL for PE and SYP traits varied from 8.8–20.8% $R^2$. The PVE measured for all 11 M-QTLs in combination was 24.8%. All these 11 M-QTLs were got validated across two environments evaluated and thereby considered as robust QTLs governing PE and SYP traits in chickpea. Seven M-QTLs associated with PE traits (8.6–10.5 PVE with 5.0–7.6 LOD) were mapped on seven different unique genomic regions of five chromosomes (Table 3; Figure 3). The remaining four M-QTLs governing both PE (13.0 to 20.8 PVE with 10.1 to 11.7 LOD) and SYP (19.7 to 20.1 PVE with 10.5 to 11.2 LOD) traits were mapped on the identical genomic regions of Chromosomes 2 and 7. The detected 11 M-QTLs exhibited positive additive gene effect for PE (3.8–6.7) and SYP (4.8–6.3) traits inferring the effective contribution of alleles derived from a high PE and SYP mapping parental accession ICC 4958 on these loci for enhancing the target traits (Table 3). Three-loci interaction Genotype Matrix Mapping-based QTL analysis detected 28 E-QTLs for PE and SYP traits of which nine E-QTLs (26.3–43.5% PVE) corresponded to M-QTLs mapped on chromosomes (Table 3). The effective integration of our association and QTL mapping outcomes revealed that SNPs annotated from 14 candidate genes linked to 11 major M-QTLs had potential for significant association with PE and SYP traits in chickpea (Tables 2 and 3). Notably, one coding SNP (A/G) revealing nonsynonymous amino acid substitution (Isoleucine [ATC] to Valine [GTC]) in a chlorophyll A-B binding protein-coding gene mapped on a 3,343,577 bp (58.27 cM) genomic interval of CaqPE2.2 and CaqSYP2.1 M-QTLs exhibited strong association potential for both PE and SYP traits based on association analysis (12.0% PVE with 1.2 × 10$^{-9}$ P) and QTL mapping (20.8% PVE with 11.7 LOD; Tables 2 and 3). Moreover, at these two CaqPE2.2 and CaqSYP2.1 M-QTL regions, maximum positive additive effects and thus highest positive interactions of QTLs/alleles governing both enhanced PE and SYP traits derived from a high PE and SYP mapping parental accession ICC 4958 was evident. In these perspectives, nonsynonymous SNP allelic variants of a chlorophyll A-B binding protein-coding gene governing both PE (CC, SCMR, CF, CAR↑CO2, and CAR↑LI) and SYP traits tightly linked to CaqPE2.2 and CaqSYP2.1 major QTLs (validated by GWAS and QTL mapping), respectively, was selected as potential candidate for further validation by transcript profiling in chickpea.

3.4 | Differential expression profiling validates regulatory function of associated genes for PE and SYP traits in chickpea

The differential expression profiling of 16 PE and SYP trait-associated genes (validated through association and QTL mapping) was performed in multiple vegetative and reproductive tissues (shoot, root, young/mature leaf, flower bud, young pod, and seed) of parental accessions (ICC 4958 and ICC 12299) and two of each homozygous individuals from a RIL mapping population (ICC 4958 × ICC 12299) using quantitative RT-PCR assay (Table 2). Eight desi and kabuli germplasm accessions with contrasting PE and SYP traits were also included for expression profiling (Table S6). All 16 PE and SYP trait-associated genes were significantly up-regulated (≥fourfold) in young/mature leaves (third/fourth leaf of 30–40 DAS grown plants) as compared with respective vegetative and reproductive tissues of germplasm accessions, RIL individuals, and mapping parents (Table 2; Figure 4). The up-regulation (≥threefold) of all 16 trait-associated genes in high than that of low PE and SYP RILs and accessions was observed. Four genes with nonsynonymous and regulatory SNPs were extremely up-regulated (≥10-fold) in leaves of high than that of low PE and SYP RILs and accessions (Table 2; Figure 4). Notably, a chlorophyll A-B binding protein-coding gene with a nonsynonymous SNP revealing strong association with PE and SYP traits exhibited pronounced up-regulation (14.9-fold) in young/mature leaves of high as compared with low PE and SYP RIL mapping individuals, parents, and germplasm accessions (Table 2; Figure 4).

3.5 | Molecular haplotyping identifies natural allelic variants and superior haplotypes of interacting genes governing PE and SYP traits in chickpea

The in silico protein–protein interaction study enabled to identify eight Arabidopsis homologues of chickpea genes possibly interacting with a strong PE and SYP trait-associated chlorophyll A-B binding protein-coding gene. The high-resolution candidate gene-based
The molecular haplotyping of a strong PE and SYP trait-associated chlorophyll A-B binding protein-coding gene (Ca_10519) and its interacting trait-associated gene TOC1 (Ca_13165) using 33 and 22 SNPs including two functionally relevant nonsynonymous trait-linked SNPs respectively constituted two major haplotypes for each gene with the high LD resolution (Figure 5a--d). The gene haplotype-specific association analysis identified two major haplotypes, HAP A (30% to 37% PVE with $10^{-7}$ to $10^{-11}$ P) and HAP B (41% to 49% PVE with $10^{-12}$ to $10^{-14}$ P), from the CDS regions of each two genes represented by significant proportion of desi and kabuli germplasm accessions exhibiting strong association with low and high PE efficiency as well as SYP trait differentiation, respectively, in a constituted association panel (Figure 5e). The haplotype-specific differential expression profiling targeting these two potential trait-associated genes (chlorophyll A-B binding protein-coding gene and TOC1) depicted pronounced up-regulation (>sevenfold) of HAP B in the young/mature leaves of germplasm accessions contrasting with high PE and SYP traits as compared with that of HAP A in the accessions contrasting with low PE and SYP traits (Figure 5f). This implicates the functional significance of natural allelic variants and haplotypes identified from two genes in regulating PE traits contributing for seed yield enhancement of chickpea.

**FIGURE 4** Differential expression profiles of 16 PE and SYP trait-associated genes (validated by high-resolution association analysis and QTL mapping) in vegetative/reproductive tissues (root, shoot, young leaf, mature leaf, flower bud, young pod, and seed) of germplasm accessions and homozygous mapping individuals and parental accessions of a recombinant inbred line (RIL) mapping population (ICC 4958 × ICC 12299) contrasting with high and low PE and SYP traits using quantitative real time polymerase chain reaction assay. The green, black, and red color in colour scale at the top represent low, medium, and high level of average log signal expression value of genes in different tissues, respectively. A strong PE and SYP trait-associated gene exhibiting enhanced up-regulation especially in young and mature leaf of accessions and RIL mapping individuals contrasting with high photosynthetic efficiency and seed yield is highlighted with red box. The detail information regarding genes used for expression study are mentioned in the Table 2. The tissues and genes selected for expression profiling are indicated on right and upper portion of expression map, respectively, H-PE/SYP and L-PE/SYP = high and low photosynthetic efficiency and seed yield per plant [Colour figure can be viewed at wileyonlinelibrary.com]
namely, F-type ATPase, crassulacean acid metabolism-light/dark, Cytochrome b6/f-complex, Calvin cycle, Photosystem II, and photosynthetic transporter of photosynthesis pathways in crop plants including chickpea. Among these, the basic-leucine zipper (bZIP) transcription factor, protein kinase, and chlorophyll A-B binding protein exhibiting strong association (>10% PVE) with PE and SYP traits across two environments appear to be highly promising. The bZIP is known to govern transcriptional gene regulatory networks underlying growth, development, and abiotic stress responses in crop plants. The involvement of two bZIP transcription factors in transcriptional regulation of Rubisco activase gene (GmRCAα) required for the light activation of most vital photosynthetic pathway enzyme, Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), is well documented in soybean (J. Zhang et al., 2016). The use of combinatorial genomic approach delineated a highly up-regulated chlorophyll A-B binding protein-coding gene influencing both PE and SYP traits in chickpea.

The functional significance of this gene with nonsynonymous SNP alleles in PE and SYP trait regulation was evident from its strong trait association potential and tight linkage with robust M-QTLs (CaqPE2.2 and CaqSYP2.1) governing the both enhanced PE and SYP traits positively. This is further supported well with young/mature leaf-specific expression of this gene especially in germplasm accessions and homozygous RIL mapping individuals with contrasting high PE and SYP traits. The chlorophyll A-B binding protein, belonging to the light harvesting complex of thylakoid membrane, functions in transfer of light energy to the reaction centre. The light harvesting chlorophyll binding (LHCB) proteins are reported to be involved in various growth, development, and drought stress responses in crop plants (Xu et al., 2012). An allelic variant of barley LHCB protein coding gene, Lhcb1, is known to be associated with a number of agro-economic traits (Xia et al., 2012). Five SNPs in Lhcb1 gene are found to be significantly associated with diverse agronomic traits including,
spike length, grain number per spike, and thousand grain weight in barley (Xia et al., 2012). A diverse array of proteins belonging mostly to photosystem complex has been reported to be interacting with the *Arabidopsis* homologue of our strong PE and SYP trait-associated chlorophyll A-B binding (CAB1) protein-coding gene. Among the chickpea homologues of these proteins, TOC1 was found to be strongly associated with photosynthesis efficiency and seed yield traits based on association analysis, QTL mapping, and expression profiling in chickpea. This gene is well characterized as a key regulator of circadian rhythm in *Arabidopsis*. Though direct interaction between TOC1 and CAB1 has not been reported but it regulates expression of CAB1 through transcriptionally regulating *LATE ELONGATED HYPOCHOTYL* and *CIRCADIAN CLOCK ASSOCIATED 1*. Both these genes are reported to be the positive regulator of CAB1 and at the same time represses TOC1 expression during the day hours (Alabadi et al., 2001). These results hint a salient role of circadian rhythm for increasing PE in crop plants. The high-resolution association analysis, QTL mapping, and gene expression profiling combined with molecular haplotyping/LD mapping and haplotype-specific gene expression study delineated two superior haplotypes from each chlorophyll A-B binding protein-coding gene and its interacting TOC1 gene regulating both PE and SYP traits in chickpea. Higher heritability of these trait-associated molecular tags underlying robust QTLs across environments suggests their broader practical applicability in genetic enhancement studies of chickpea. This infers the functional relevance of molecular signatures scanned from these two potential genes for rapid dissection of complex PE and SYP traits in chickpea. A much comprehensive analysis of transcriptional regulation of aforesaid high PE and SYP trait-associated genes including two highly-promising genes and deciphering their subsequent role in control of photosynthesis metabolism pathway toward enhancing seed yield is essential for further deployment of the molecular tags in genomics-assisted crop improvement of chickpea. Though the current study utilized a small size association panel (92 accessions) for association mapping study, however, association potential of genes/alleles for PE and SYP traits was ascertained through high-resolution QTL mapping, expression profiling, and molecular haplotyping of interacting genes. Henceforth, the promising molecular signatures regulating PE and SYP traits delineated in the current study deploying an integrated genomics-assisted breeding strategy will be useful to develop high seed-yielding cultivars enriched with PE traits in chickpea.

**ACKNOWLEDGEMENTS**

The financial support provided by the Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India, is acknowledged.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.