Planta (2024) 259:17
https://doi.org/10.1007/s00425-023-04294-x

ORIGINAL ARTICLE

Dissecting chickpea genomic loci associated with the root penetration responsive traits in compacted soil

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Received: 30 August 2023 / Accepted: 14 November 2023 / Published online: 11 December 2023
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Abstract

Main conclusion **Soil compaction reduces root exploration in chickpea. We found genes related to root architectural traits in chickpea that can help understand and improve root growth in compacted soils.**

Abstract Soil compaction is a major concern for modern agriculture, as it constrains plant root growth, leading to reduced resource acquisition. Phenotypic variation for root system architecture (RSA) traits in compacted soils is present for various crops; however, studies on genetic associations with these traits are lacking. Therefore, we investigated RSA traits in different soil compaction levels and identified significant genomic associations in chickpea. We conducted a Genome-Wide Association Study (GWAS) of 210 chickpea accessions for 13 RSA traits under three bulk densities (BD) (1.1BD, 1.6BD, and 1.8BD). Soil compaction decreases root exploration by reducing 12 RSA traits, except average diameter (AD). Further, AD is negatively correlated with lateral root traits, and this correlation increases in 1.8BD, suggesting the negative effect of AD on lateral root traits. Interestingly, we identified probable candidate genes such as *GLP3* and *LRX* for lateral root traits and *CRF1-like* for total length (TL) in 1.6BD soil. In heavy soil compaction, *DGK2* is associated with lateral root traits. Reduction in laterals during soil compaction is mainly due to delayed seedling establishment, thus making lateral root number a critical trait. Interestingly, we also found a higher contribution of the GxE component of the number of root tips (Tips) to the total variation than the other lateral traits. We also identified a pectin esterase, *PPE8B*, associated with Tips in high soil compaction and a significantly associated SNP with the relative change in Tips depicting a trade-off between Tips and AD. Identified genes and loci would help develop soil-compaction-resistant chickpea varieties.

Keywords Ethylene · GWAS · Legumes · Root length · Root penetration · Soil compaction · SNPs

Abbreviations

AD	Average diameter (mm)
BD	Bulk density
GWAS	Genome-Wide Association Study
LR	Lateral root
PA	Projected area
PR	Primary root
PVE	Phenotypic variation explained
RSA	Root system architecture
SA	Surface area (cm^2)
Tips	Root tips
Tips_RE	Relative change in total number of tips
TL	Total length (cm)
RE	Relative change in
RV	Total volume (cm^3)

Communicated by Dorothea Bartels.

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Introduction

Chickpea (*Cicer arietinum* L.) ($2n = 16$) is the second most widely grown pulse crop with high nutritional benefits contributing to global food security (Varshney et al. 2013, 2019; Madurapperumage et al. 2021). An alarming increase in the human population in concert with changing environmental conditions threatens crop growth and development (Satterthwaite et al. 2010; Rehman and Khan 2019). To meet the growing demand for food production, agriculture is being

modernized. Heavy farm machinery used more frequently negatively impacts soil quality and root growth. It causes soil mechanical impedance or compaction in response to physical consolidation. Soil compaction reduces soil porosity, restricts water and nutrient infiltration, reduces aeration, creates anaerobic conditions, destroys structure, and limits root growth (Schneider et al. 2021). These effects restrict seed germination and root exploration of soil, thus affecting global grain yield production (Ishaq et al. 2001; Lipiec et al. 2012; Cambi et al. 2018).

Roots undergo adaptive growth responses to deal with compacted soils and penetrate cracks. Compact soil significantly impacts root phenotype (Lynch et al. 2014; Vanhees et al. 2020). In such soil, roots tend to become thicker, stunted, and more resistant to buckling and deflection when encountering rugged terrain (Whiteley et al. 1982; Jin et al. 2013). Thicker roots are related to higher penetration pressure (Popova et al. 2016); however, in recent reports, an increase in root diameter is associated with lower penetration (Huang et al. 2022). Nevertheless, this increase in diameter can provide an advantage in terms of higher water uptake, compensating for reduced root length and surface area (Lynch et al. 2014).

Apart from root morphological changes, the plant undergoes various anatomical adaptations. Predicting root penetration and strength in maize is better accomplished by examining cortical cell wall thickness, area, cortical cell count, and stele diameter rather than root diameter (Chimungu et al. 2015). Also, smaller outer cortical cells improve hard soil penetration (Chimungu et al. 2015). Maize plants with deeper roots tend to have thinner layers of cortical cells from the third node regions and increased aerenchyma in the fourth node regions (Vanhees et al. 2022). Similar variations have been observed in other crops such as rice, soybean, wheat, and maize (Chimungu et al. 2015; Vanhees et al. 2020; Schneider et al. 2021; Mondal et al. 2022). Further, the root cortex develops anatomical modifications such as aerenchyma and multiseriate sclerenchyma (MCS) to increase penetration and withstand external pressure (Schneider et al. 2021). These root morphological and anatomical responses in compacted soil are under the strict control of molecular and hormonal regulations (Pandey et al. 2021).

Root growth response is conventionally thought to be the inability of the root to overcome the high external pressure of mechanical impedance (Passioura 2002; Correa et al. 2019), which is recently found to be the complex phytohormonal response (Pandey et al. 2021). Compacted soil restricts ethylene diffusion to the nearby soil, thus accumulating ethylene near plant roots (Pandey et al. 2021). The plant senses this accumulated ethylene as the signal for compacted soil, leading to root extension inhibition and favoring radial swelling (Huang et al. 2022). Ethylene-response-mutants of rice and Arabidopsis were narrow and could penetrate better in compacted soil. Ethylene further deploys auxin and abscisic acid (ABA) as downstream signals to modify rice root cell elongation and radial expansion, causing root tips to swell and reducing their ability to penetrate compacted soil (Huang et al. 2022). ABA causes cortical cell radial expansion. Here, rice mutants of ABA biosynthetic genes have attenuated cortical cell radial expansion in compacted soil. Auxin further aids by altering the cell elongation as *osaux1* mutants penetrate compacted soil better than the wild-type roots and do not exhibit cortical cell radial expansion (Huang et al. 2022). Despite the molecular players known in model crops, there is still limited understanding of the root responses in compacted soil for legumes, especially in one of the essential commercial legumes, chickpea.

Harnessing natural variation in root system architecture (RSA) under compaction is an effective strategy for dissecting the root response of chickpeas in compacted soil (Thudi et al. 2014; Varshney et al. 2019; Lynch 2022). Gene banks worldwide have conserved over 90,000 chickpea accessions, yet the full extent of the crop's diversity remains unexplored due to the limited availability of phenotypic data for crucial adaptive traits (Upadhyaya et al. 2011). To identify genetic variation associated with chickpeas' ability to penetrate compact soil, we investigated 210 chickpea accessions in normal and two levels of compacted soil. The study aims to find the genetic variants responsible for higher root growth irrespective of compaction. We performed a Genome-Wide Association Study (GWAS) with RSA traits for variant identification. In the present study, we have identified significant associations and underlying genes responsible for root architecture modification in response to soil compaction. This information could benefit future research and breeding programs, as it provides a foundation for understanding the responsiveness and genetic basis of chickpea root architectural traits in compacted soil.

Materials and methods

Plant material and growth conditions

The present study comprises 210 diverse chickpea (*Cicer arietinum* L.) accessions from the chickpea mini-core collection conserved at gene banks (Suppl. Table S1). These chickpea mini-core accessions are also a part of the 3 K pangenome project containing 3600 genotypes (Varshney et al. 2021). The mini-core panel comprises diverse accessions from more than 25 countries and regions, including commercial varieties and landraces (Suppl. Table S1). The experiment was set up at the National Institute of Plant Genome Research, New Delhi, from November 2022 to January 2023 in a polyhouse with natural conditions. Before sowing, chickpea seeds were overnight

soaked in water. The soaked seeds were planted in cylindrical PVC pipes with 5.7, 7.5, and 8.3 kg of sandy loam soil filled up to 26 cm in height. The chickpea association panel was phenotyped for three soil compaction treatments, i.e., zero compacted soil treatment with 5.7 kg soil and 1.1 bulk density g/cm^3 (BD), moderately compacted soil stress treatment with 7.5 kg soil and 1.6 BD g/cm^3, and heavily compacted soil stress treatment with 8.3 kg soil and 1.8 BD g/cm^3 compaction of soil stress in five replications for 9 days (Suppl. Fig. S1 a-j). The field soil was initially screened and filtered using a 2 mm hole-size iron construction sand sieve mesh (1.3 × 2 m). Initially, 15 % soil moisture was adjusted, and throughout the experiment, equal watering was done in all the treatments. The soil compaction was carried out using a hand-held soil compaction proctor.

Phenotyping of chickpea accessions for root penetration and root system architecture-related traits under compacted soil

Nine days after germination (DAG), chickpea seedlings were carefully removed from the compacted soil without causing any damage to the roots (Suppl. Fig. S1 f–j). Washed chickpea seedlings were fixed in FAA fixative solution comprising formaldehyde 10 % + ethanol 50 % + acetic acid 5 % + 35 % water (by vol.) (Ruzin 1999). Chickpea seedlings were scanned using EPSON Perfection V850 Pro scanner, and traits were measured using winRHIZHO software (Le Marié et al. 2014). Here, a total of 13 root architecture traits were considered, including total root length (TL_cm), total root surface area (SA_cm^2), the average diameter (AD_mm), total root volume (RV_cm3), number of tips, primary root length (PRL_cm), lateral root length (LRL_cm), primary root surface area (PRSA_cm), lateral root surface area (LRSA_cm), primary root projected area (PRPA), lateral root projected area (LRPA), primary root volume (PRVol), and lateral root volume (LRVol). The raw root architecture data were subjected to pre-processing for outlier removal. The clean data was used for ANOVA and correlation analysis using R. Exploratory data analysis of phenotypic traits were performed using R and MS Excel, and boxplots and bar graphs were generated using ggplot2 (Wickham 2011). Correlation plots were generated using the corrplot package (Wei et al. 2017). G x E analysis using a mixed model was performed using statgenGxE and statgenSTA packages (van Rossum et al. 2017). Additionally, plasticity indexes were calculated for all 13 traits by calculating the relative change between compacted (1.6 BD and 1.8 BD) and non-compacted (1.1 BD) soil.

The chickpea whole-genome sequencing (WGS) data retrieval, quality checks, and genome-wide variant detection

The whole-genome sequencing (WGS) data of 210 chickpea accessions were retrieved from the NCBI sequence reads archive (SRA) database (PRJNA657888) (Varshney et al. 2021). Further, SRA data for 210 mini-core chickpea accessions was processed with SRA Toolkit 3.0.5 (Leinonen et al. 2010) and faster-dump to get fastq paired-end reads. Paired-end reads were cleaned with AfterQC 0.9.7, (Chen et al. 2017), and quality was checked using FastQC (version 0.11.9) (Brown et al. 2017). The reads were mapped to the chickpea CDC frontier reference genome (Varshney et al. 2013) using BWA (version 0.7.12) (Li 2013). The SAM files were converted to BAM files using samtools (version 1.18) (Danecek et al. 2021). The variant calling was performed using the Genome Analysis Toolkit (GATK version 4.4.0.0) program's HaplotypeCaller function (McKenna et al. 2010). The variant calling files (VCFs) were merged using the CombinedGVCF function in GATK. The combined VCF was filtered for missing percentage (keeping sites present in 90 % of genotypes), Minimum mapping quality (mQ = 30), read depth, and minor allele frequency (maf > 0.05) using vcftools (version 4.2) (Danecek et al. 2011; Li 2011). The heterozygous calls were converted to missing using TASSEL software, and sites were filtered for maf > 0.05 and site minimum count of 95 % to identify high-quality SNPs for association analysis (Bradbury et al. 2007).

Phylogenetic diversity and population structure

The population structure of 210 chickpea accession was performed using the maximum likelihood method in Admixture 1.30 (Alexander and Lange 2011). The Admixture *K* values were set to *K* = 1 to 10, and each step iterated ten times with 2000 bootstrapping. A phylogenetic tree was constructed for 210 chickpea accessions using the neighbor-joining method (NJ) using TASSEL and R package tree view. Further, genetic principal components analysis was calculated, and a plot was generated using SNPRelate and GAPIT R packages (Lipka et al. 2012; Wang and Zhang 2021).

GWAS to identify significant associations for root architecture traits in compacted soil

The GWAS was performed using the BLINK statistical model in the GAPIT R package (Lipka et al. 2012). Significant loci obtained from the GAPIT (based on Bonferroni correction) were considered for further analysis. Out of all the significant loci, only those showing significant

allelic effects were considered for further identification of candidate genes. For the identification of candidate genes, we identified linkage disequilibrium (LD) blocks within the 300 kb region from the significant loci, i.e., 150 kb upstream and downstream of the significant loci, based on the average LD decay of the chickpea genome (Upadhyaya et al. 2016). Further, LD was calculated using the full matrix option in TASSEL (Bradbury et al. 2007) of the LD block containing the significant loci. Within the LD block, only those SNPs in LD ($R^2 \geq 0.8$) with significant loci were considered further along with significant SNP. Genes adjoining (within 2 kb) of the SNPs were considered as potential candidate genes for further analysis. Only those genes were considered that are in the same LD block as the significant loci. The LD blocks were estimated, and plots were generated using LDBlockShow (version 1.40) (Dong et al. 2021).

Gene expression analysis using qRT-PCR

Chickpea accession ICC4958 was used to study the expression of different putative genes playing a role under soil compaction. Root samples were taken at 9 DAG from three soil compaction treatments (1.1 BD, 1.6 BD, and 1.8 BD). Tissues were frozen in liquid nitrogen and stored at − 80 °C. High-quality RNA was extracted from the chickpea root tissues using the TRIZOl method (Ambion by Life Technologies, Carlsbad, CA, USA). Isolated RNA was subjected to DNase treatment (DNase I, RNase-free (1 U/µL), Thermo Scientific), and cDNA was prepared using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Primers used in the study were designed from the CDS region of genes using NCBI Primer-BLAST (Ye et al. 2012), preferably from the intron–exon junction, and best-suited ones with minimum self-complementarity index were selected (cf Suppl. Table S7). Gene expression profiling was performed using Applied Biosystems 7900 Fast Real-Time PCR machine. The relative expression of genes (Log2 fold change) in roots under compaction treatments (BD 1.6 and BD1.8) with respect to non-compacted soil (BD1.1) was calculated using the ΔΔCt method (Livak and Schmittgen 2001). The expression of *Initiation factor 4 alpha (IF4α)* (a housekeeping gene) was taken as the endogenous control as per previous studies for comparing stress samples in chickpea (Garg et al. 2010). The experiment was performed on three biological replicates.

Results

The phenotypic variation of chickpea root system architecture traits response under compacted soil

Chickpea accessions were phenotyped for 13 RSA traits under three levels of soil compactions with five replications. Comparing RSA traits between treatments, it was observed that, except for average diameter (AD), all remaining RSA traits showed a decrease in their values with increasing soil compaction (Suppl. Table S2; Fig. 1a–i). In 1.1 BD soil, the population mean of AD was 1.06 mm, which was increased to 1.25 mm in 1.6 BD soil and 1.29 mm in 1.8 BD soil. Contrastingly, the population mean of total length (TL) in 1.1 BD was 27.27 cm, decreasing to 11.33 cm in 1.6 BD soil and 8.61 cm in 1.8 BD soil. Lateral root length was higher in uncompacted soil, but this trend is reversed in compacted soil. Thus, compacted soil delays RSA establishment, resulting in shorter overall root length and increased diameter.

All the traits showed significant genotype, environment, and genotype X environment (G × E) variation (Suppl. Table S3). Maximum G × E contribution was shown by root volume (RV) and primary root volume (PRVol), followed by AD. Length traits displayed the least G x E contribution, suggesting low G × E variation in these traits, depicting lower variation in plasticity. By comparing variance with increasing soil compaction, we observed a reduction in variance in all the traits except AD. Higher G × E contribution and increased variance with soil compaction in AD depicted higher genetic variation in response to increasing diameter. Similarly, plasticity indexes of total RV and PRVol showed high variance, followed by PRPA, PRSA, and AD. Interestingly, a high variance was only present in plasticity indexes for 1.8 BD soil, not 1.6 BD soil, depicting higher plasticity variation in 1.8 BD soil compared to 1.6 BD soil (Suppl. Table S4).

In 1.1 BD soil, total length (TL) showed a weak positive correlation for primary root traits and a strong positive correlation for lateral root traits. However, surface area (SA) displayed the opposite trend, depicting laterals contributing more towards total root length and primary root contributing more towards total root surface area. Primary and lateral root traits showed no correlation with each other in 1.1 BD soil. The AD showed a negative correlation between lateral root and branching traits but displayed a positive correlation for primary root traits. In 1.6 BD and 1.8 BD soils, TL and SA showed similar trends in correlation with primary and lateral root traits. However, a weak positive correlation is observed among lateral and primary root traits in 1.6 BD soil, which vanishes in 1.8 BD soil (Suppl. Figs. S2a–c and S3a–f).

Interestingly, the positive correlation between AD and primary root length (PRL) in 1.1 BD soil vanishes in 1.6

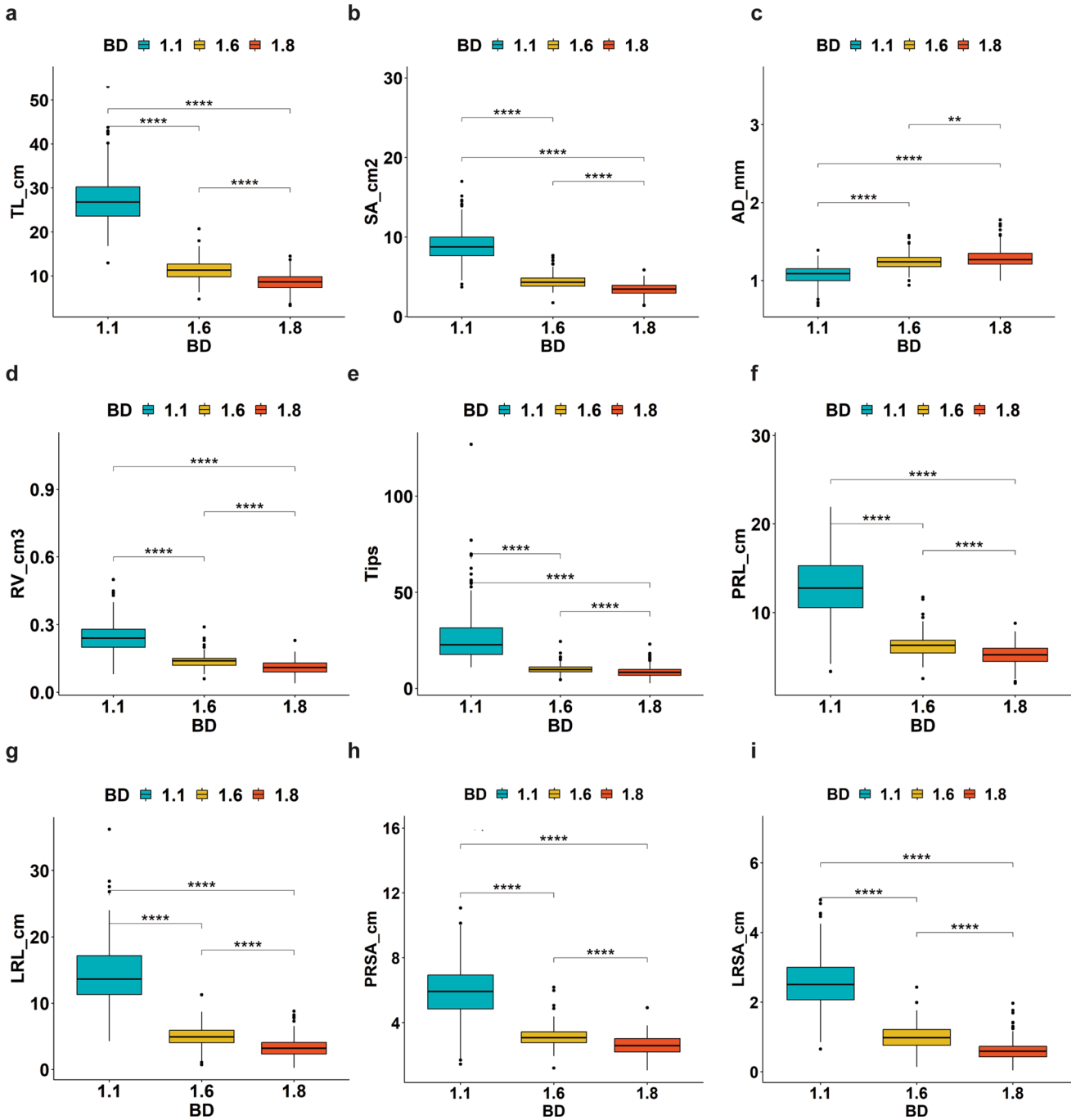
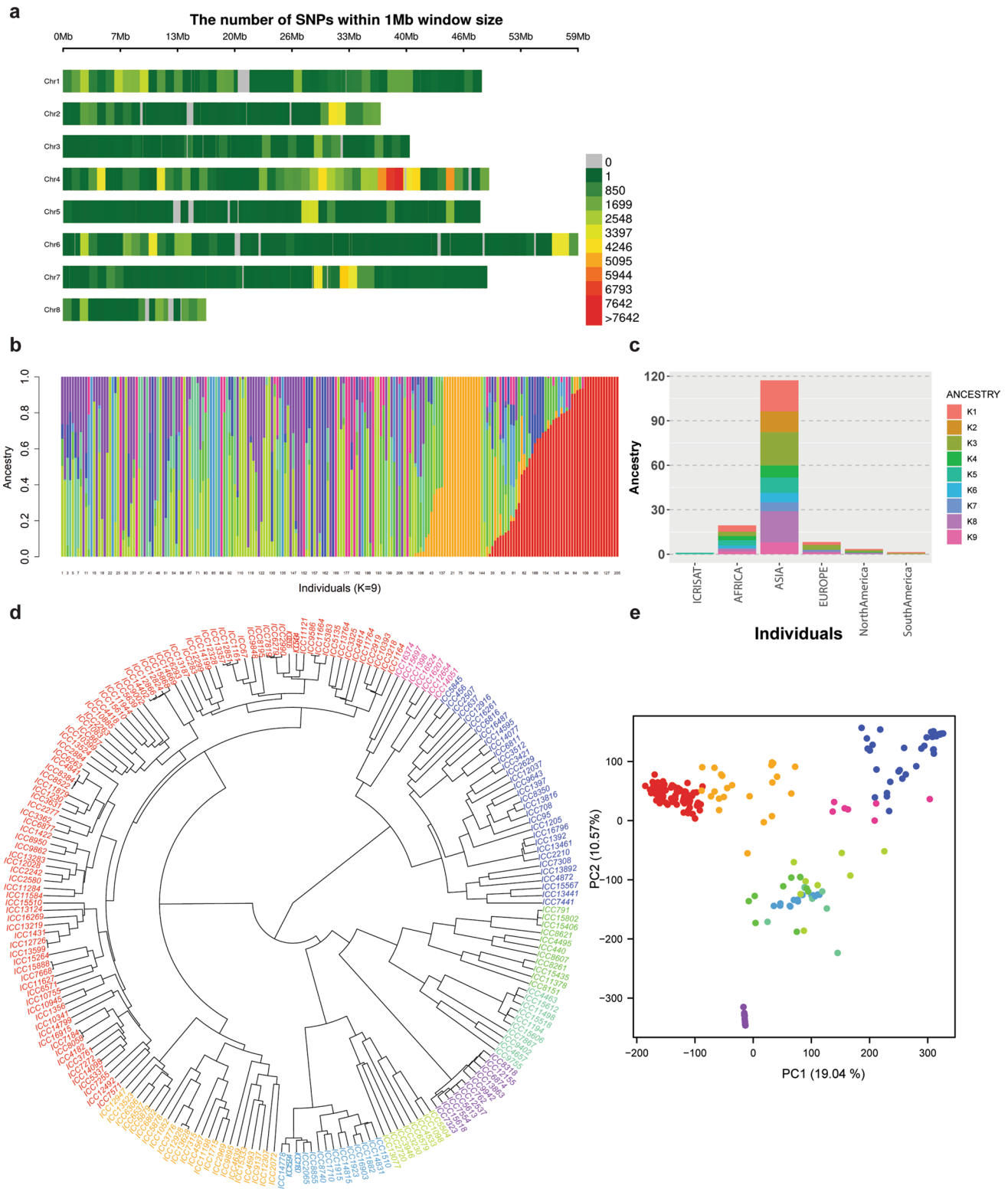


Fig. 1 Phenotyping of chickpea mini-core GWAS panel for root system architecture (RSA) traits under three treatments (1.1BD, 1.6 BD, and 1.8 BD) of compacted soil. Box plots depict phenotypic variation and the difference between the three treatments for total root length (**a**, TL) root surface area (**b**, SA), average root diameter (**c**, AD), root volume (**d**, RV), number of root tips (**e**, Tips), (**f**, PRL), lateral root length (**g**, LRL), primary root surface area (**h**, PRSA), lateral root surface area (**i**. LRSA). The central line in the box plots depicts the population median for the trait in a particular treatment. Here, except AD, all the remaining traits decrease with increasing soil compaction. Comparison between treatments were analyzed using ANOVA followed by a post-hoc test ($P<0.05$) ($n=210$). Significant comparisons were depicted using *. The * indicate level of significance as, $*P<0.05$, $**P<0.01$, $***P<0.001$

BD and 1.8 BD soils. It suggested that roots with larger diameter are not related to longer primary roots in moderate and heavy soil compaction, which were in uncompacted soil. As 1.1 BD soil, AD negatively correlates with lateral and branching traits in 1.6 BD and 1.8 BD soils. However, the correlation is weaker in 1.8 BD soil. This suggests



a trade-off between lateral root development and average diameter in compacted soil. The resources required for increasing diameter in compacted soil could possibly deter resource availability for the lateral root development (Suppl. Figs. S2a–c and S3a–f).

Phylogenetic diversity and population structure

After variant calling and filtering, 238,240 single-nucleotide polymorphisms (SNPs) were extracted to perform GWAS with RSA traits in response to compact soil (Fig. 2a). The

◂**Fig. 2** The phylogenetic variation, population structure, and genetic diversity of chickpea mini-core GWAS panel. **a** A density plot was created using high-quality SNPs to show the distribution of 200 k SNPs across the chickpea genome. Here, green indicates less SNP density and red indicates higher SNP density. **b** The population variation was identified using admixture software; the *K* value varies from 1 to 10 in a 10 iteration, and each iteration was run at 2000 bootstrapping. The bar plot was plotted to identify the distribution of phylogenetic diversity and population STRUCTURE. The bar plot represents the K-9 population among the 210 min-core chickpea accessions. **c** The bar plot shows the distribution of chickpea accession in nine populations in six groups. **d** Dendrogram depicts the clustering of chickpea accessions into nine sub-populations. **e** Principal component analysis (PCA) of 210 chickpea accessions. Here, the PCA biplot (PC1 vs. PC2) depicts diversity in the population, where PC1 explains 19.04 % variation, and PC2 explains 10.57 % variation

extracted SNPs were used to study population structure and genomic variation in the GWAS panel. Population admixture showed an optimum K-value of 9 and was selected based on the minimum cross-validation error, which was fixed to retain pure accessions by eliminating admixtures (Fig. 2b, c). The bar plot designates the distribution of nine chickpea population in six geographical groups. Asia region comprises the highest number of accessions belonging to nine population clusters. Further, phylogenetic diversity analysis and genetic principal component analysis (PCA) obtained a similar clustering pattern, where PC1 and PC2 explained 19.04 % and 10.57 % variation, respectively (Fig. 2d–e).

Genome-wide association mapping of SNPs linked with root penetration and RSA-responsive traits

The GWAS was performed using the BLINK model to identify significantly associated SNPs for RSA traits under three different bulk densities soil. In control 1.1 BD soil, we have identified two significant SNPs at chromosome 4, i.e., S4_38707673 (*P*-2.09E–08) and S4_665844 (*P*-2.81E–06), linked with AD and TL traits (Suppl. Fig. S4a–d; Table 1). Their phenotypic variance explained (PVE %) percentage varies from 38.12 % to 29.86 %. In moderate 1.6 BD soil compaction treatment, we identified four significant SNPs: S7_32834852 (*P*-6.47E–07), S7_29444770 (*P*-1.92E–06), S8_3754663 (*P*-4.03E–06), and S7_29511774 (*P*-4.04E–06). The most significant SNP, S7_32834852, was associated with lateral root surface area (LRSA) (*P*-4.01E–06), lateral root volume (LRVol) (*P*-2.91E–06), LRPA (*P*-2.58E–06), TL (*P*-6.47E–07), and their PVE were 28.28 %, 63.04 %, 27.52 %, and 27.17 %, respectively (Fig. 3a–f; Suppl. Figs. S5 a-h; S6 a-f; S7a–f; Table 1). This was followed by S7_29444770, which was associated with lateral root length (LRL) (*P*-1.92E–06), lateral root projected area (LRPA) (*P*-2.58E–06), and LRSA (*P*-4.01E–06), with 61.26 %, 9.79 %, and 8.80 % PVE, respectively. The SNP S8_3754663 linked with primary root volume (PRVol) (*P*-4.03E–06), and SA (1.10E–06), and the PVE were 17.71 % and 15.14 %. The SNP, S7_29511774, was associated with TL (*P*-4.04E–06) with a PVE of 7.81 % (Figs. 4a–f; Suppl. Fig. 5a–h; S6a–f; S7a–f; Table 1).

We identified seven significant SNPs in 1.8 BD soil (Suppl. Fig. S8 a-f; Suppl. Fig. S9 a-f; Suppl. Fig. S10 a-f; Suppl. Fig. S11a–d). The most significant SNP, S6_34682862, was associated with AD (*P*-4.21E–11), PRVol (*P*-6.53E–09), and total root volume RV (*P*-1.34E–09); their PVE varies between 59.81 %, 42.49 %, and 48.29 %, respectively. Followed by S6_6702892 SNP associated with LRPA (*P*-5.91E–08), LRSA (*P*-5.82E–08), and LRVol (*P*-5.89E–08), their PVE varies between 41.62 %, 42.24 %, and 39.84 %, respectively. The SNP S4_22818995 linked with PRL (*P*-9.64E–08) showed 39.07 % PVE. S4_22984656 was associated with PRPA (*P*-1.83E–08) and PRSA (*P*-1.47E–08) and showed a PVE of 46.5 % and 46.74 %, respectively. The SNP S4_30653480 (*P*-5.76E–09) was associated with SA (49.60 % PVE), and S1_25029792 (*P*-1.89E–10) and S4_3178953 (*P*-1.14E–09) were associated with Tips; their PVE were 13.10 % and 20.89 %, respectively (Suppl. Figs. S8a–f; S9a–f; S10a–f; S11a–d; Table 1).

Similarly, plasticity indexes were used to find significant associations for root plasticity in compacted soil. For plasticity indexes of 1.6 BD, we identified nine significant SNPs. Three SNPs were identified to be associated with volume-related traits. S1_17932204 was associated with RV_RE (*P*-4.06E–09) and PRVol_RE (P-1.36E–08), showing 8.55 % and 7.86 % PVE. In addition, S4_25935749 was associated with RV_RE (*P*-5.68E–11) and PRVol_RE (*P*-5.59E–12), showing 49 % and 54.4 % PVE. For LRVol_RE, S1_46735079 (*P*- 1.26E-07) showed a significant association with PVE 35.58 %. For area-related traits, S3_27560701 showed the highest significance with PRPA_RE (*P*-1.50E–12) and PRSA_RE (*P*-9.70E–13), explaining 19.7 % and 20.2 % PVE. Followed by S7_5017663, which showed significant associations with PRPA_RE (*P*-1.31E–10) and PRSA_RE (*P*-1.06E–10), explaining 24.1 % and 24.3 % PVE. For PRL_RE, S3_27560701 (*P*-1.84E–15) showed the most significant association with PVE, 37.8 %. Further, S3_27904102 explained 34.86 % PVE for PRL_RE (*P*-1.38E–08) (Suppl. Table S5 and Suppl. Fig. S12a–j).

We identified four significant SNPs for plasticity indexes of 1.8BD, three at chromosome 4 and one at chromosome 6, which were linked with PRL_RE, PRPA_RE, PRSA_RE, PRVol_RE, and Tips_RE, having their PVE varying between 58 % and 11 %. The most significant SNP, S4_25390989, was associated with PRL_RE (*P*-1.71E–07), showing 31.82 % PVE and followed by S4_30228728, associated with primary root related traits, PRL_RE (*P*-2.54E–14), PRPA_RE (*P*-2.95E–18), PRSA_RE (*P*-3.11E–18) and PRVol_RE (*P*-1.03E–17), having their PVE 49.39 %, 58.51 %, 58.33 %

Table 1 List of significantly associated SNPs with root system architectural traits in three levels of soil compaction

Treatment	SNP	Region	Chromosome	Position	*P* value	Maf	Effect	PVE (%)	Trait
1.1BD	S4_665844	Intergenic	4	665,844	2.81E–06	0.152381	– 3.57658	29.86244	TL
	S4_38707673	Promotor	4	38,707,673	2.09E–08	0.27619	– 0.10277	38.11789	AD
1.6BD	S7_29444770	Intergenic	7	29,444,770	1.92E–06	0.07619	1.51647	61.26125	LRL
	S7_29444770	Intergenic	7	29,444,770	2.58E–06	0.07619	0.098244	9.790604	LRPA
	S7_32834852	Intergenic	7	32,834,852	3.82E–06	0.054762	– 0.0945	27.5185	LRPA
	S7_29444770	Intergenic	7	29,444,770	4.01E–06	0.07619	0.303585	8.804953	LRSA
	S7_32834852	Intergenic	7	32,834,852	4.01E–06	0.054762	– 0.29711	28.28022	LRSA
	S7_32834852	Intergenic	7	32,834,852	2.91E–06	0.054762	– 0.00669	63.03652	LRVol
	S8_3754663	Intergenic	8	3,754,663	4.03E–06	0.32381	– 0.0138	17.71467	PRVol
	S8_3754663	Intergenic	8	3,754,663	1.10E–06	0.32381	– 0.32747	15.14327	SA
	S7_29511774	Promotor	7	29,511,774	4.04E–06	0.069048	1.993197	7.81357	TL
	S7_32834852	Intergenic	7	32,834,852	6.47E–07	0.054762	– 2.15485	27.16725	TL
1.8BD	S6_34682862	Intergenic	6	34,682,862	4.21E–11	0.069048	– 0.12073	59.81478	AD
	S6_6702892	Genic	6	6,702,892	5.91E–08	0.057143	– 0.07016	41.61769	LRPA
	S6_6702892	Genic	6	6,702,892	5.82E–08	0.057143	– 0.22128	42.23659	LRSA
	S6_6702892	Genic	6	6,702,892	5.89E–08	0.057143	– 0.00434	39.83886	LRVol
	S4_22818995	Intergenic	4	22,818,995	9.64E–08	0.195238	0.894435	39.07356	PRL
	S4_22984656	Intergenic	4	22,984,656	1.83E–08	0.190476	– 0.15776	46.49552	PRPA
	S4_22984656	Intergenic	4	22,984,656	1.47E–08	0.190476	– 0.49685	46.74342	PRSA
	S6_34682862	Intergenic	6	34,682,862	6.53E–09	0.069048	– 0.02188	42.4871	PRVol
	S6_34682862	Intergenic	6	34,682,862	1.34E–09	0.069048	– 0.02049	48.28974	RV
	S4_30653480	Promotor	4	30,653,480	5.76E–09	0.364286	0.647109	49.5962	SA
	S1_25029792	Genic	1	25,029,792	1.89E–10	0.240476	– 1.45328	13.09835	Tips
	S4_3178953	Intergenic	4	3,178,953	1.14E–09	0.157143	– 1.93528	20.88846	Tips

Maf, minor allele frequency; *PVE*, phenotype variance explained; *SNP*, single-nucleotide polymorphism; *BD*, bulk density; *TL_cm*, total root length, *SA_cm2*, surface area; *AD_mm*, average diameter; *RV_cm3*, root volume; Tips; *PRL_cm*, primary root length; *LRL_cm*, lateral root length; *PRSA_cm*, primary root surface area; *LRSA_cm*, lateral root surface area; *PRPA*, primary root projected area; *LRPA*, lateral root projected area; *PRVol*, primary root volume; *LRVol*, lateral root volume

and 57.39 %, respectively. Further, SNP S4_33340818 was identified, linked with PRPA_RE (*P*-2.34E–09), PRSA_RE (*P*-2.17E–09) and PRVol_RE (*P*-9.207E–10) having PVE of 11.83 %, 12.09 %, and 13.86 % respectively. Finally, the Tips_RE (*P*-2.06E–10) associated SNP, S6_34682862, was also identified, which had its PVE of 40.91 % (Suppl. Table S5 and Suppl. Fig. S13a, b).

Identification of potential candidate genes

Only those SNPs were retained from the GWAS result for further analyses, which showed a significant allelic effect. In 1.1 BD soil, SNPs associated with AD and TL showed significant allelic effects. For AD-associated SNP, S4_38707673 (G/A), the A allele showed broader AD than the G allele. For S4_38707673, we identified six genes in LD and lie in the same LD block. The significant SNP is localized within a bidirectional sugar transporter *SWEET1* (LOC101498274) gene (Suppl. Fig. S14 a). For TL-associated SNP, S4_665844 (G/C), the C allele showed longer roots than the G allele. The significant SNP is localized in the promoter of the *low psii accumulation 1* (LOC101508210) gene, and apart from LPA1, no other gene was found to be in LD with the SNP within the 300 kb window (Table 2; Suppl. Fig. S14b).

In moderate soil compaction of 1.6 BD soil, two SNPs, S7_29511774 and S7_32834852, showed significant allelic effect. S7_29511774 (T/C) is associated with TL, and the T allele showed longer roots compared to the C allele. A total of 11 genes were found to be in LD and in the same LD block with significant SNP (Suppl. Fig. S15 a-e). Among the 11 genes, *cytokinin response factor-1-like (CRF1-like*) (LOC101501547) is an ethylene-responsive transcription factor family protein and could play an important role in regulating root length in compacted soil. SNP S7_32834852 (C/T) was significantly associated with TL, LRSA, LRL, and LRPA. In this, the C allele showed longer roots and a larger surface area compared to the T allele. Within the 300 kb window, 15 genes were found to be in LD and the same LD block with the significant SNP. In upstream of the significant SNP, the nearest gene was *pollen-specific leucine-rich repeat extensin-like protein 3 (LRX)* (LOC101506805), and in

downstream the closest gene was *AP2-like ethylene-responsive transcription factor AIL1* (LOC101506248). Both the SNPs were in significant LD with ethylene-responsive genes, suggesting a vital role of ethylene during soil compaction in chickpea (Table 2; Suppl. Fig. S15a–e).

Under heavy soil, compaction of 1.8 BD soil, three SNPs, S1_25029792, S6_6702892, and S6_34682862, displayed a significant allelic effect. S1_25029792 (T/A) was associated with the number of tips, and the A allele resulted in more tips than the T allele. Two genes were found in LD with the significant SNP (LOC101495273, LOC101514396). Both of these genes were *pectinesterase/pectinesterase inhibitor PPE8B-like*. The second SNP, S6_6702892 (G/A), was associated with LRPA and LRSA, and the A allele resulted in a larger surface area than the G allele. The significant SNP lies within the *diacylglycerol kinase 2* (*DGK2*) (LOC101500556) gene, and none of the neighboring genes within the 300 kb window was found to be in LD with the significant SNP (Table 2; Suppl. Fig. S16a–f). The third SNP, S6_34682862 (T/A), was associated with AD, PRVol, and total root volume RV, and the A allele showed more volume and broader diameter compared to the T allele. The SNP lies in an intergenic region, and none of the genes are in LD with the significant SNP. This could be due to the low density of markers in this region. The candidate gene can be identified by increasing marker density in the region; however, the nearest gene from the significant SNP was *peroxisomal membrane protein 11B* (LOC101501092), around 6.6 kb from the SNP.

Plasticity indexes for moderately compacted soil have four SNPs showing significant allelic effects: S1_17932204, S1_46735079, S3_24492256, and S3_27904102. S1_17932204 (C/A) was associated with PRVol_RE and RV_RE, and the C allele showed a lower volume reduction than the T allele. Four genes were in the same LD block and LD with the significant SNP (Suppl. Table S6 and Suppl. Fig. S17). For LRVol_RE, we found S1_46735079 (C/A) significantly associated and showed a significant allelic effect. Three genes are in the same LD block and LD with the significant SNP. Among these genes, two were uncharacterized, and one was helicase *SEN1* (LOC101504865). For PRPA_RE and PRSA_RE, SNP S3_24492256 (G/C) showed a significant association and allelic effect (Suppl. Table S6 and Suppl. Fig. S17). Here, the C allele showed a lower reduction in PRPA_RE and PRSA_RE than the G allele. The SNP lies within the 2 kb of *cyclin U4-1-like* (LOC101500825), and another gene *putative glucan endo-1,3-beta-glucosidase* (LOC101493810) was in the same LD block and LD with the significant SNP (Suppl. Tables S6 and S17). Only one SNP (S6_34682862 (T/A)) associated with Tips_RE showed a significant allelic effect for highly compacted soil. Here, the A allele showed a lower reduction in the number of Tips than the T allele (Suppl. Tables S6 and S17).

Differential expression analysis of putative candidate genes for RSA traits in compacted soil

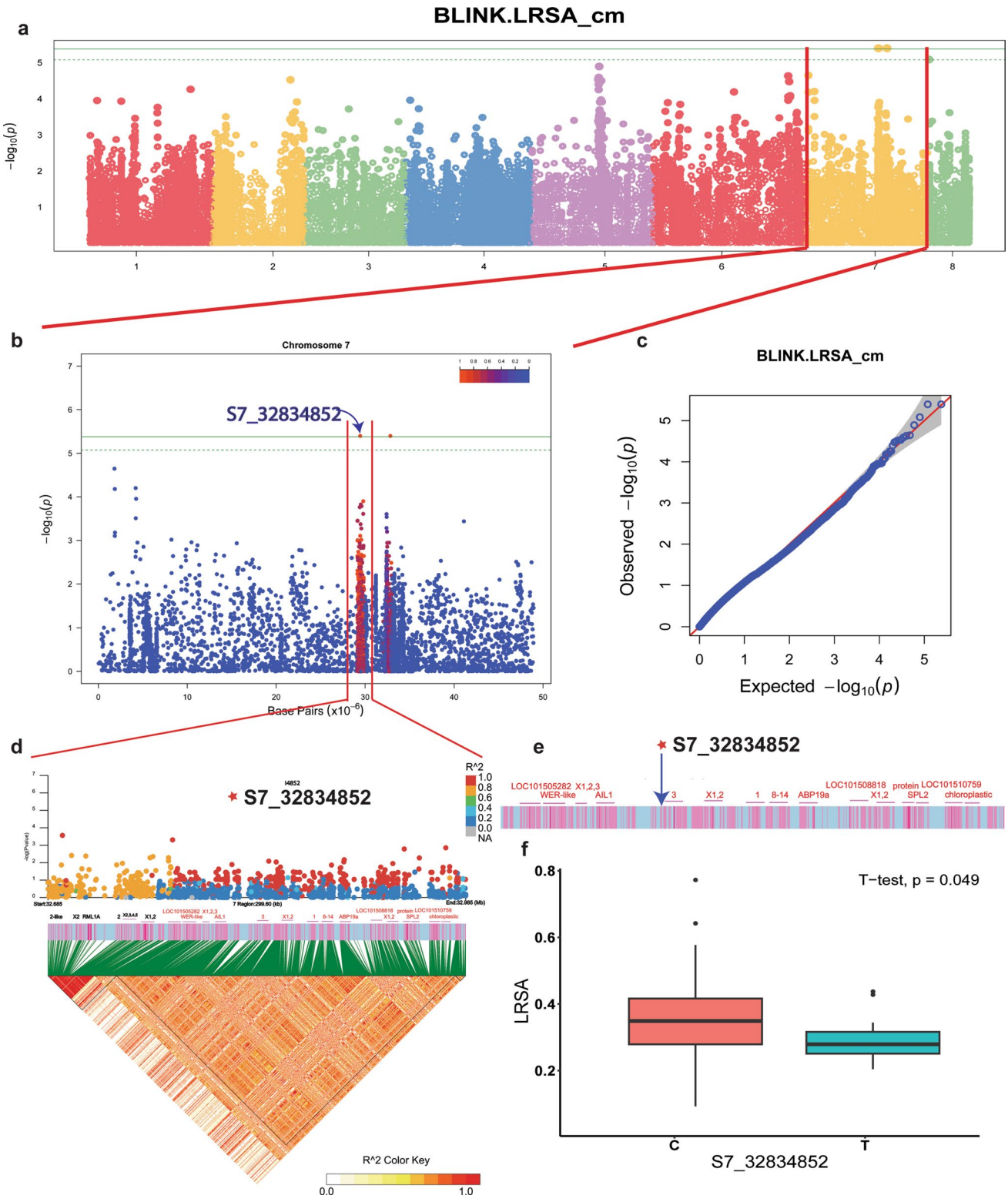
We studied the expression behavior of the 21 putative candidate genes for three different SNPs. In 1.6 BD soil, we studied one SNP, S7_32834852, associated lateral root traits and showed a significant allelic effect. S7_32834852 had 15 genes in the same LD block with it, and among those 15 genes, *germin-like protein 3 (GLP3)* (LOC101507652) showed the highest fold change in response to compaction of 1.6 BD. In addition, another gene associated with this SNP i.e., *LRX* (LOC101506805), also showed higher fold change upon soil compaction of 1.6 BD (Fig. 5a). For SNP, S7_29511774, we checked 12 genes, and among them, *CRF1-like* (LOC101501547) was highly upregulated in 1.6 BD soil (Fig. 5b).

In heavy compaction of soil 1.8 BD, two SNPs, S1_25029792 and S6_6702892, associated with lateral root traits had significant allelic effects. Among the two pectin esterase genes found in LD with significant SNP (S1_25029792), one of them (LOC101495273) is highly induced (Fig. 5c). It showed remarkably high fold change in both 1.6 BD and 1.8 BD soils in comparison to 1.1 BD soil. The second SNP, S6_6702892, linked to lateral root characteristics, is located in the intronic region of *DGK2* (LOC101500556). Interestingly, this gene had high expression in roots grown in 1.8 BD soil, not in 1.6 BD soil (Fig. 5d).

Discussion

Soil compaction has become a significant challenge for agriculture productivity due to the use of heavy machinery in modern farming practices. Addressing this issue is crucial to ensure food security. Roots growth gets hindered in compacted soil as they struggle to penetrate hard soil. This decrease in root growth reduces soil exploration for water and minerals, ultimately causing overall growth retardation and making plants more sensitive to soil compaction (Tracy et al. 2012). However, genetic variations are present in plants, which resuls in varying sensitivity to soil compaction-induced root shortening (Colombi and Walter 2017). Therefore, we evaluated 210 chickpea accessions to understand the important root traits under different degrees of soil compaction. We aimed to identify accessions not deterred by compacted soil and the associated genetic variations.

High-strength soil leads to changes in root system architecture (RSA), including reduced length, surface area, and volume of both primary and lateral roots; contrastingly, during soil compaction, the average diameter increased with an increase in soil compaction. We observed a positive correlation between average root diameter (AD) and primary



root length (PRL) in uncompacted soil, which vanishes in compacted soil in chickpea. This suggests neither positive nor negative correlation of AD with penetration ability in compacted soil. However, a broader diameter in maize is positively correlated with higher penetration (Chimungu et al. 2015). Contrastingly, in recent reports, wider diameter is shown to be negatively associated with penetration in rice (Huang et al. 2022). These contrasting results suggest inter-species variation in the ability of thicker roots to penetrate hard soil. In our case, no correlation is observed between

◂**Fig. 3** Genome-wide association mapping of chickpea lateral root traits under moderately compacted soil (1.6 BD) using BLINK GWAS model. Four significant SNPs: S7_32834852, S7_29444770, S8_3754663, and S7_29511774 were identified associated with root traits in moderately compacted 1.6 BD soil. The SNP S7_32834852 is associated with lateral root surface area (LRSA), lateral root projected area (LRPA), lateral root volume (LRVol), and total root length (TL) in chr7 under 1.6 BD soil. **a** The Manhattan plot represents chromosome-wise SNP distribution and significant SNPs associated with LRSA. **b** Regional association of SNPs in chromosome 7 with LRSA. **c** The QQ plot shows the significant SNPs linked with LRSA. **d** The LD block was plotted around SNP S7_32834852 for the 300 kb region (upstream 150 kb and downstream 150 kb). The LD block plot shows the distribution of candidate genes in the 300 kb region and **f** genes linked with chickpea root-responsive traits. **e** The SNP S7_32834852 shows a significant allelic effect (C/T) for LRSA. In the Manhattan plot, a dotted line represents the threshold value calculated using FDR at $P < 0.05$, and a green line represents the Bonferroni threshold at $P < 0.05$. Comparison between two alleles is made using Student's *T* test, and the *P* value of the test is depicted in the plot

AD and PRL, possibly due to the engagement of additional traits influencing root penetration in hard soils.

Further, AD and lateral root traits negatively correlated in uncompacted and compacted soils, suggesting a trade-off between both traits. With increasing root diameter and decreasing lateral length in compacted soil, this trade-off between AD and lateral root traits could have more aggravated consequences for already reduced root exploration in compacted soil. Therefore, the increase in root diameter could reduce root-soil exploration in compacted soil, leading to reduced penetration, as observed in rice (Huang et al. 2022).

Apart from AD, all other root traits showed strikingly reduced standard deviation and variance with an increase in the level of soil compaction. Similar results were observed in tomato, where roots showed higher variation in root traits in uncompacted compared to compacted soil (Tracy et al. 2012). This could be due to restricted growth in compacted soil, and maybe the variants responsible for variation in root growth in uncompacted soil are downstream of the pathway, resulting in reduced growth during soil compaction.

In the present GWA study, we have identified several significant SNPs and candidate genes linked with root penetration and RSA-related traits under various levels of soil compaction. Here, we discussed candidate genes for only those SNPs with significant allelic effects.

Two SNPs displayed significant allelic effects in moderate soil compaction of 1.6 BD. Among the two SNPs, S7_32834852 (C/T) is significantly associated with lateral root traits such as LRPA, LRSA, and LRVol. Fifteen genes are in LD and lie in the same LD block with significant SNP; among those 15 genes, *GLP3* and *LRX* showed the highest induction in response to soil compaction of 1.6 BD. GLPs are highly expressed and conserved across different plant species, and several have root-specific expression under various stresses (Zaynab et al. 2022). In Arabidopsis, overexpression of *PD-GLP1* and *PD-GLP2* leads to a decrease in primary root length and an increase in lateral root length (Ham et al. 2012). Similarly, AtLRX2 and AtLRX6 are involved in lateral root formation, which suggests that LRX could also be involved in lateral root development (Baumberger et al. 2003; Lewis et al. 2013). At this point, we could delimit two genes among the 17, and further characterization of both genes is required to confirm their role in soil compaction.

The other SNP, S7_29511774 (T/C), is associated with TL, and 11 genes are in LD and lie in the same LD block with significant SNP. Among these 11 genes, *CRF1-like* (LOC101501547) is a transcription factor belonging to the ERF family containing the AP2 domain. Interestingly, *CRF1-like* also showed increased expression in soil compaction of 1.6 BD and 1.8 BD relative to 1.1 BD. Thus, suggesting a probable role of CRF1-like during soil compaction. AtCRFs are involved in controlling root growth as their quadruple mutant (*atcrf1/3/5/6*) displays reduced primary root length and lateral number (Raines et al. 2016). In Arabidopsis, CRFs are cytokinin response factors containing the CRF and AP2 domains (Rashotte and Goertzen 2010). A similar protein was reported in *Spirogyra*, annotated as *CRF1*, but lacked the CRF domain; however, it displayed steady upregulation upon ethylene treatment (Van de Poel et al. 2016). Ethylene is also reported to induce the expression of *SlCRF4* (Shi et al. 2012). Interestingly, changes in ethylene levels within the plant and in the immediate vicinity affect root growth in compacted soil. During soil compaction, ethylene accumulates near the root surface due to less diffusion to the surrounding compacted soil (Pandey et al. 2021). Ethylene employs other downstream signals like ABA and auxin to mediate the soil compaction response, such as cortical radial cell expansion, and reduce primary root length, respectively (Huang et al. 2022). As AtCRFs are known cytokinin response factors, it would be interesting to look into the involvement of cytokinin in regulating root growth along with auxin and ABA in compacted soil. Until now, no study has been related to cytokinin involvement in compacted soil; however, cross-talk of ethylene and cytokinin is well known for regulating primary root growth (Artner and Benkova 2019). Cytokinin induces ethylene biosynthesis, and ethylene is involved in activating cytokinin signal transduction through multi-step phosphorelay resulting in root growth inhibition (Hansen et al. 2009; Zdarska et al. 2019; Yamoune et al. 2023). Thus, it could be possible that CRFs play an essential role in this cross-regulation to cause root growth inhibition during soil compaction.

In heavy soil compaction of 1.8 BD, three SNPs showed significant allelic effects for lateral root traits. S1_25029792 (T/A) is significantly associated with the total number of tips and represents the lateral root number. Two pectin esterase

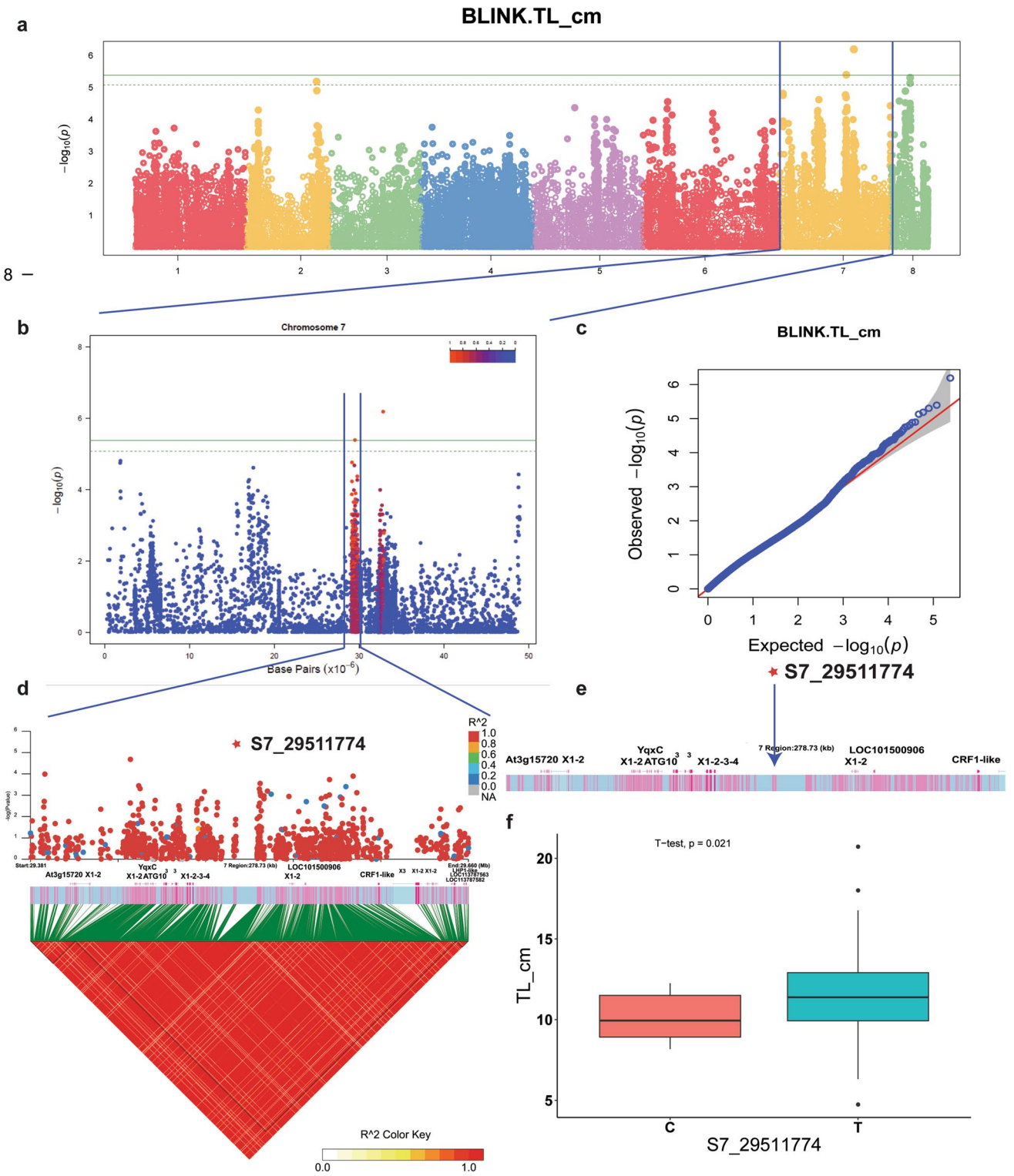


Fig. 4 Genome-wide association mapping of chickpea total root length (TL) under moderately compacted (1.6 BD) soil. The GWAS identified significant SNP, S7_29511774 associated with TL. **a** The Manhattan plot represents chromosome-wise SNP distribution and significant SNPs associated with TL. **b** Regional association of SNPs in chromosome 7 with TL. **c** The QQ plot shows significant SNPs linked with chickpea TL. **d** The LD block plotted around the SNP, S_29511774 within the 300 kb region. **e** Significant candidate genes lie in the 300 kb region and the same LD block with the significant SNP. **f** Box plot depict significant allelic effect (C/T) for TL. In the Manhattan plot, a dotted line represents the threshold value calculated using FDR at $P < 0.05$, and a green line represents the Bonferroni threshold at $P < 0.05$. Comparison between two alleles is made using Student's T test, and the P value of the test is depicted in the plot

Table 2 List of candidate genes for associated SNPs having significant allelic effects in three levels of soil compaction

Treatment	SNP	Chromosome	Start	End	Strand	Name	GeneID	Trait
1.1BD	S4_38707673	4	38,709,058	38,712,057	−	Bidirectional sugar transporter SWEET1	LOC101498274	AD
		4	38,719,693	38,726,950	−	Probable beta-1,4-xylosyltransferase IRX10	LOC101498610	AD
		4	38,735,097	38,737,160	−	F-box/LRR-repeat protein At3g48880-like	LOC101490603	AD
		4	38,657,327	38,660,738	+	Scarecrow-like protein 1	LOC101496871	AD
		4	38,661,832	38,670,111	−	Small RNA degrading nuclease 5, transcript variant X3	LOC101497200	AD
		4	38,672,007	38,681,861	−	Transcriptional corepressor SEUSS, transcript variant X2	LOC101497744	AD
	S4_665844	4	662,477	664,062	−	Protein LOW PSII ACCUMULATION 1, chloroplastic	LOC101508210	TL
1.6BD	S7_29511774	7	29,545,578	29,549,349	+	Uncharacterized LOC101500595, transcript variant X1	LOC101500595	TL
		7	29,555,795	29,556,602	+	Uncharacterized LOC101500906	LOC101500906	TL
		7	29,602,150	29,603,464	−	Ethylene-responsive transcription factor CRF1-like	LOC101501547	TL
		7	29,611,931	29,629,024	+	Septin and tuftelin-interacting protein 1 homolog 1-like, transcript variant X3	LOC101501015	TL
		7	29,632,232	29,640,557	+	Uncharacterized LOC101501329, transcript variant X1	LOC101501329	TL
		7	29,447,127	29,451,441	−	Uncharacterized hydrolase YNR064C, transcript variant X2	LOC101497804	TL
		7	29,452,039	29,456,543	+	Putative rRNA methyltransferase YqxC	LOC101498329	TL
		7	29,457,251	29,461,090	−	Ubiquitin-like-conjugating enzyme ATG10	LOC101498673	TL
		7	29,465,703	29,469,815	−	FRIGIDA-like protein 3	LOC101499213	TL
		7	29,470,860	29,475,419	−	FRIGIDA-like protein 3	LOC101499533	TL
		7	29,479,991	29,485,255	+	Probably inactive leucine-rich repeat receptor-like protein kinase At5g48380, transcript variant X2	LOC101499833	TL
	S7_32834852	7	32,838,446	32,839,896	+	Pollen-specific leucine-rich repeat extensin-like protein 3	LOC101506805	LRPA,LRSA,LRVol,TL
		7	32,854,376	32,856,865	−	Growth-regulating factor 5-like, transcript variant X2	LOC101507142	LRPA,LRSA,LRVol,TL

Table 2 (continued)

Treatment	SNP	Chromosome	Start	End	Strand	Name	GeneID	Trait
		7	32,875,595	32,876,495	+	Germin-like protein subfamily 3 member 1	LOC101507652	LRPA,LRSA,LRVol,TL
		7	32,885,582	32,886,400	+	Germin-like protein 8–14	LOC101507977	LRPA,LRSA,LRVol,TL
		7	32,900,262	32,901,223	+	Auxin-binding protein ABP19a	LOC101508289	LRPA,LRSA,LRVol,TL
		7	32,916,357	32,922,743	+	Uncharacterized LOC101508818	LOC101508818	LRPA,LRSA,LRVol,TL
		7	32,927,074	32,933,901	+	Mitochondrial intermembrane space import and assembly protein 40 homolog, transcript variant X2	LOC101509141	LRPA,LRSA,LRVol,TL
		7	32,941,271	32,943,735	+	Nodulation-signaling pathway 1 protein	LOC101510109	LRPA,LRSA,LRVol,TL
		7	32,944,247	32,949,145	−	E3 ubiquitin-protein ligase SPL2	LOC101510434	LRPA,LRSA,LRVol,TL
		7	32,959,641	32,960,803	+	Uncharacterized LOC101510759	LOC101510759	LRPA,LRSA,LRVol,TL
		7	32,961,153	32,964,970	−	Probable GTP diphosphokinase RSH2, chloroplastic	LOC101511084	LRPA,LRSA,LRVol,TL
		7	32,773,988	32,787,409	+	Uncharacterized LOC101505282	LOC101505282	LRPA,LRSA,LRVol,TL
		7	32,788,725	32,790,420	+	Transcription factor WER-like	LOC101505610	LRPA,LRSA,LRVol,TL
		7	32,796,149	32,800,530	+	Ubiquitin-like protein ATG12, transcript variant X2	LOC101505935	LRPA,LRSA,LRVol,TL
		7	32,804,776	32,809,499	+	AP2-like ethylene-responsive transcription factor AIL1	LOC101506248	LRPA,LRSA,LRVol,TL
BD1.8	S1_25029792	1	25,076,107	25,081,016	−	Pectinesterase/pectinesterase inhibitor PPE8B-like, transcript variant X2	LOC101495273	Tips
		1	25,117,567	25,123,901	−	Pectinesterase/pectinesterase inhibitor PPE8B-like	LOC101514396	Tips
	S6_6702892	6	6,701,817	6,709,783	−	Diacylglycerol kinase 2, transcript variant X1	LOC101500556	LRSA, LRPA, LRVol
	S6_34682862	6	NA	NA	NA	NA	NA	AD, RV, PRVol

BD, bulk density; *SNP*, single-nucleotide polymorphism; *TL_cm*, total root length; *AD_mm*, average diameter; *RV_cm3*, root volume; *Tips*, number of root tips; *LRL_cm*, lateral root length; *LRSA_cm*, lateral root surface area; *LRPA*, lateral root projected area; *PRVol*, primary root volume; *LRVol*, lateral root volume

genes were found to be in LD with significant SNP, and among the two, LOC101495273 is highly induced upon heavy soil compaction. It suggests the probable role of pectin esterase in lateral root formation during soil compaction. Pectin esterase acts on cell wall pectin and de-methyl-esterify galacturonic acid residues of homogalacturonan pectin. Desertification by pectin esterase can either lead to cell wall stiffening or loosening, depending on the esterification pattern (Micheli 2001; Willats et al. 2001). Cell wall remodeling by pectin esterases is required for lateral root formation as the ratio between pectin esterification and deesterification is vital for the processes before lateral root primordia formation (Wachsman et al. 2020). In Arabidopsis, single and double mutants of *pme2* and *pme3* display reduced lateral root numbers, and

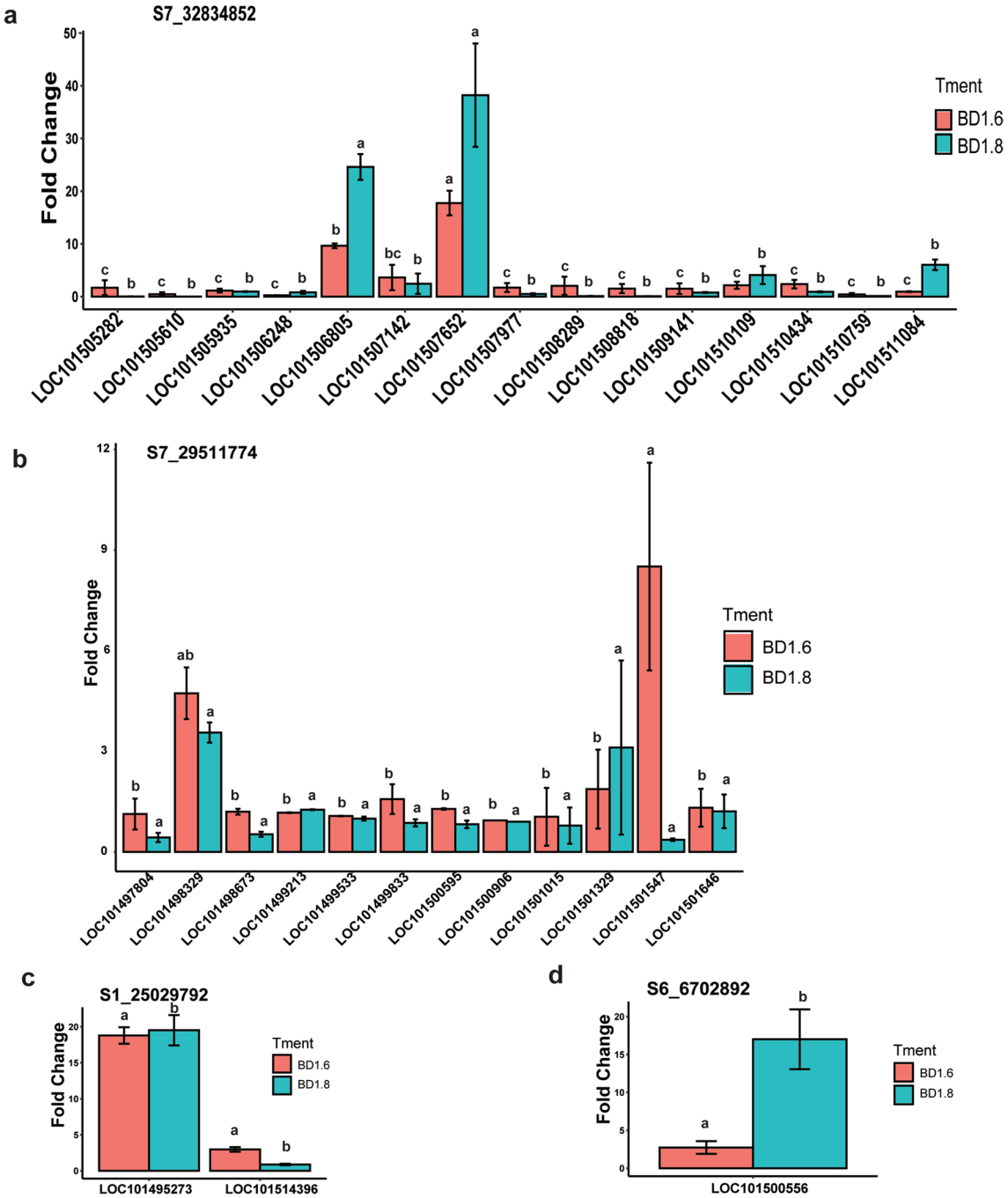


Fig. 5 Validation of candidate genes from associated loci with significant allelic effect using real-time qPCR **a** Expression of 15 putative candidate genes in moderate (1.6 BD) and heavy (1.8 BD) soil compaction for SNP, S7_32834852 associated with lateral root traits in moderate compaction. Out of these 15 genes, *GLP3* (LOC101507652) and *LRX* (LOC101506805) showed significantly elevated expression. **b** Expression of 12 candidate genes in 1.6 BD and 1.8 BD soil for SNP, S7_29511774. Out of these 12 genes, we found highly elevated expression of *CRF-1-like* (LOC10501547) in moderately compacted soil. **c** Expression pattern of candidate genes for two SNPs, S1_25029792 and S6_6702892, associated with root tips and lateral root traits for heavy soil compaction. Of two pectin esterase genes in LD with the first SNP, pectinesterase/pectinesterase inhibitor *PPE8B-like* (LOC101495273) showed induced expression under both compaction treatments. **d** For the second SNP, the expression pattern of *DGK2* (LOC101500556) shows specifically elevated expression under high compaction, BD1.8. Relative expression plotted to show log twofold change using ΔΔCt method. Comparisons between genes within a treatment were analyzed using ANOVA followed by a Tukey's Honest Significant Difference (HSD) post-hoc test ($P < 0.05$)

overexpression of *PME5* leads to a reduction in pre-branching site numbers (Wachsman et al. 2020). As discussed, soil compaction delays seedling root architecture establishment (Bassett et al. 2005), resulting in smaller laterals. Thus, the induction of pectin esterase during soil compaction could be an important adaptive strategy by plants to regulate the process required before lateral root primordia formation. Further characterization of the gene and allelic variants will lead to a better understanding of the involvement of pectin esterase in lateral root formation during soil compaction.

The second SNP, S6_6702892 (G/A), is significantly associated with LRPA, LRSA, and LRVol and lies within the intron of *DGK2* (LOC101500556). Interestingly, *CaDGK2* is induced only during heavy soil compaction and not in moderate soil compaction, suggesting that this gene specifically works in heavy soil compaction. DGKs phosphorylate diacylglycerol to form phosphatidic acid (PA). Diacylglycerol is known to promote lateral root formation, and PA reduces lateral root development. *Osdgk1* mutants have more diacylglycerol and lead to higher lateral root density. Upon PA treatment, lateral root density was restored to WT (Yuan et al. 2019). This suggests the critical role of *DGK2* in regulating lateral root development in compacted soils.

In terms of plasticity, the contribution of the genotype x environment (G x E) component to the total variation is more for root volume (RV), primary root volume (PRVol), and AD, followed by surface area and least in length traits. Interestingly, we found a significant association (S1_17932204) with plasticity indexes of RV, PRVol, and RV in 1.6 BD soil, showing a significant allelic effect with all three traits. On comparing primary and lateral root traits, primary root traits displayed more G x E contribution than lateral root traits. However, the total number of tips shows comparable G x E contribution as primary root traits. Here, we found a significantly associated SNP with the plasticity index of Tips (S6_34682862) in 1.8 BD soil. Interestingly, the same SNP is associated with AD, RV, and PRVol in 1.8 BD soil; however, it has opposite effects on traits. The T allele leads to a larger diameter and volume in 1.8 BD soil but results in a higher reduction of the number of tips, leading to genotypes with the A allele thinner and less reduction in the number of root tips. Hence, it could be concluded that a larger diameter/volume is traded off with a decrease in the number of laterals and length of laterals during soil compaction, leading to reduced soil exploration. Thus, genotypes with thinner roots and profound laterals could be more beneficial during soil compaction due to higher soil exploration.

Conclusion

Soil compaction in chickpea leads to severe reduction in root system architecture (RSA) traits, except average diameter (AD), which increased with increasing soil compaction. We did not find any direct negative correlation of AD with primary root length (PRL) in compacted soil; however, we identified that the aggravated trade-off between AD and laterals in compacted soil could make increased AD responsible for deteriorated soil exploration in terms of reduced lateral roots. Interestingly, we identified significant associations for various RSA traits and their plasticity indexes for moderate (1.6 BD) and high (1.8 BD) soil compaction, leading to the identification of important probable candidate genes regulating root architecture in compacted soil. These findings will be crucial in developing chickpea varieties resistant to soil compaction.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00425-023-04294-x.

Acknowledgements RD and US acknowledge the research fellowship from DBT funded project (BT/PR31665/AGIII/103/1172/2019). PSK and MP acknowledge fellowship from DBT, India. BS acknowledge a fellowship from Indo-Swiss joint research project (BT/IN/Swiss/46/JG/2018 by DBT, India. JG was supported by NIPGR core grant. We thank Swarup K Parida for sharing chickpea seeds.

Author contributions RD, PSK, MP conducted experiments, analyzed data and wrote the manuscript. US and BS helped with the experiments. JG designed the project, supervised experiments, analyzed data and wrote the manuscript.

Data Availability Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Conflict of interest The authors declare no conflict of interest.

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