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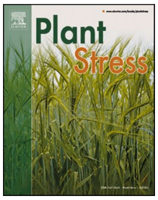

Elucidating the genomic regions through genome-wide association study (GWAS) for root traits in cowpea (*Vigna unguiculata* (L) Walp) mini-core collection☆

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ABSTRACT

Enhancing the root traits through selection and breeding efforts can significantly improve the acquisition of soil resources, consequently boosting crop production in marginal environments. Cowpea is one of the important legume crops in arid and semi-arid regions, grown mostly in marginal environments under drought conditions. The roots play a crucial role in plant's ability to survive and adapt under such water stressed conditions. The understanding of cowpea genetic architecture related to root system architecture (RSA) remains limited due to difficulties in accurately measuring root traits. In the present study, a set 110 diverse mini-core collection of cowpea from IITA, Nigeria was evaluated for thirteen (13) different root-shoot traits under controlled conditions in the green house. Significant variation was recorded for all the root traits in the mini-core set. Most promising genotype for most of the root traits, *viz.*, root volume, dry root weight, root biomass density and tap root diameter were identified. The correlation analysis revealed significant positive association ($p < 0.001$) of root weight with shoot weight (0.85), root volume ($r = 0.61$), and tap root diameter (0.56). In the principal component analysis (PCA), first three principal components explained the variance of 64.31%, with PC1 contributing 44.43%, PC2 10% and PC3 9.88%. All the root-shoot traits contribute positive variation to PC1especially dry root weight and dry shoot weight that had high component loading and in PC2 root shoot ratio had high loading. The root-shoot trait data was used with 6,574 good quality SNP data for discovery of genes/QTLs/marker-trait associations for root traits. The analysis of results of genome wide association study (GWAS) led to the identification of 52 unique SNPs associated significantly ($p < 10^{-4}$) with thirteen root-shoot traits. Highest number of SNPs was found associated with root weight and chromosome 5 was identified as SNP hotspot for harboring majority of significant SNPs. We further delve candidate genes within 50 kb around the identified SNP that led to the identification of several important candidate genes underlying genomic regions responsible for root-shoot traits in cowpea. The promising cowpea genotypes for root traits and promising MTAs/candidate genes will prove useful in cowpea improvement programs including improvement of drought tolerance in cowpea.

1. Introduction

The current projections estimate the global population to stand around 9 billion by 2050 (Alexandratos and Bruinsma, 2012) with the situation is complicated by the looming threats of climate change. Thus, implications present a daunting challenge before the humanity to achieve food and livelihood security especially in developing countries, farmers living over there have limited adaptive capacities (Mahendra,

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2012). Legumes, especially in marginal farming systems across the globe deliver important food, nutritional and ecosystem services to farm communities. They provide important sources of oil, fiber, and protein-rich food and feed while supplying nitrogen (N) to agro-ecosystems via their unique ability to fix atmospheric N_2 through symbiosis involving soil bacteria “rhizobia”. Among the food legumes, cowpea (*Vigna unguiculata* (L) Walp) plays a considerable role in ensuring food security and generating income in many tropical and sub-tropical regions (Carlos, 2000; Tharanathan and Mahadevamma, 2003). It is a warm weather crop and necessitates lower amounts of rainfall compared to the majority of other crops so it is suitable for production in semi-arid and arid regions of lowland tropics and sub-tropics of the world (Emongor, 2007). Cowpea is cultivated already in marginal environments commonly experiencing drought, low fertility and pest attack and these constraints are likely to become more severe with the effect of climate change (Yadav et al., 2015). Besides being suitable for limited resource conditions, it is important for the crop to be capable of efficient resource acquisition from the soil. This could be achieved through efficient root system architecture of crop. Breeding efforts have so far mainly targeted above-ground traits of crop plants. However, an underexploited breeding strategy is trait-based selection focused on linking specific root traits to efficient resource acquisition (Cattivelli et al., 2008; Lynch 2015).

The root systems of plants are fundamental for obtaining essential soil resources. The root architecture changes are associated with the alterations in how roots explore and exploit soil volumes as well as nutrient-rich patches (Lynch, 2007). Root phenotyping is as important as shoot phenotyping, since roots are the main responsible organ for the plant’s performance in relation to water and nutrient absorption (Wasaya et al., 2018). Thus, for improvement of the cowpea, understanding the role of the root system in the diversity and performance of genotypes is crucial. Although there has been limited exploration into the impacts of genetic and phenotypic variation in cowpea root system architecture (RSA) traits on the crop performance (Burridge et al., 2016, 2017; De Barros et al., 2007). However considerable genetic diversity has been reported in cowpea for root system architecture (RSA) traits associated with growth in nutrient-poor and arid environments (Krasilnikoff et al., 2001, 2003; Singh et al., 2002; Matsui and Singh, 2003; Gull et al., 2020). In earlier investigations, it has been pinpointed, specific cowpea root traits that play crucial role in soil phosphorus (P) acquisition and enhance usage efficiency (Kugblenu et al., 2014).

Likewise, deep root systems have been reported as advantageous, particularly in conditions of drought (Agbicodo et al., 2009; De Barros et al., 2007; Matsui and Singh, 2003). However, it must be noted that there could be some additional construction and maintenance costs for plants investing in deeper roots, especially under conditions of limited available water (Hall, 2012). Examining the presence of genetic diversities and the corresponding variations in plasticity responses within cowpea can aid in formulating effective breeding strategies for enhanced efficiency in resource acquisition and utilization in relation to RSA traits.

The visualization and measurement of root systems for the use of RSA traits in conventional breeding pose considerable challenges. Root systems exhibit dynamism and numerous phenotypic RSA features are transiently expressed, resulting in marked variability in RSA for the same genotype (Orman-Ligeza et al., 2014). Soils are inherently heterogeneous and opaque, making it currently impractical to conduct screening in fields for many aspects of RSA. Additionally, achieving appropriate phenology is linked with agro-climatic studies (Gregory et al., 2009). Therefore, the measurement and utilization of RSA traits in breeding should be precisely targeted and tailored to specific environments, to enhance effectiveness and minimize costs (de Dorlodot et al., 2007). Root system of crop plants is very important for imparting drought stress tolerance. For instance, it is desirable to have a very deep, extensive, highly branched, and wide-spreading root system for imparting drought tolerance (Kramer, 1969). In order to optimize access to increased soil moisture under drought, plants undergo adaptations by developing greater rooting depth and increasing root biomass (Blum, 2010; Fenta et al., 2014). In legume crops, drought tolerance is also associated with the diameter and distribution of metaxylem vessels, which play a significant role in governing root conductivity (Purushothaman et al., 2013). Besides the development of deep and proliferative roots, drought stress induces plasticity responses in root systems. This includes an enhancement in the number of fibrous roots, a reduction in lateral root diameter, and changes in root biomass (Meister et al., 2014; Salazar-Henao et al., 2016). Plants possess the capacity to tailor their root architecture to adapt to different environments, including stressful conditions, by integrating genetic programs that regulate root growth (Jovanovic et al., 2007). Plants possessing diverse genetic and nutritional conditions shows significant variations in root structure, encompassing differences in the number and distribution of root systems. This adaptability allows plants to efficiently recover when transitioning from stressful to normal conditions (López-Bucio et al., 2003).

The root structural phenes of most annual plants including cowpea are fairly consistent, but the number, placement, and growth direction of each root in a system are highly plastic, even among genetically identical plants (Malamy, 2005). Root system development is an important agronomic trait of cowpea plant. The architectural and anatomical root traits in a given environment allow plants to survive periods of water or nutrient deficit and effectively forage the resources (Klepper, 1992). The selection and breeding for improved root phenotypes can improve the acquisition of soil resources, thus leading to improved crop production in marginal environments. Breeding programs could utilize the strategy of selection for root phenotypes to enhance production in various challenging environments. Plants with a strong, deep root system are better at scavenging for available moisture and nutrients that makes them to grow under adverse conditions.

With the advancement of genome sequencing and bioinformatics technologies, approaches such as association and linkage mapping have come a long way to unravel the genetic diversity of targeted traits across crops. Genome-wide association studies (GWAS) are frequently used to identify new genes and QTLs by locating significant allelic differences in candidate genes underpinning quantitative and complicated traits, such as those linked to growth, development, stress tolerance, and nutritional quality (Kumar et al., 2015). Taking advantage of this approach, the study aimed to utilize this GWAS approach to identify QTL through their non-random association with genetic markers. Although GWAS has become a widely used tool in quantitative genetic analysis, even in cases where marker density is high and a heterogeneous diversity panel is utilized, small effect QTL often go undetected in the case of highly polygenic traits (Brachi et al., 2011). GWAS have the capability to offer more specific insights into the genetic control of traits and identify potential candidate genes by indicating their locations.

Genes/QTLs have been used in cowpea to analyze genetic control of several agronomical, yield traits, biotic and abiotic stresses. For instance, quantitative trait loci (QTL) have been discovered in cowpea for key traits including tolerance to drought (Muchero et al., 2010, 2013), seed quality (Lucas et al., 2013b), resistance to root-knot nematodes (Huynh et al., 2016), root pathogens including *Macrophomina phaseolina* (Muchero et al., 2011) and Fusarium wilt (Pottorff et al., 2014, 2012), insects (Huynh et al., 2015; Lucas et al., 2013a; Kpoviessi et al., 2022), and the parasitic weed Striga (Ouedraogoet al. 2012), pod length (Xu et al., 2017), flowering time (Paudel et al., 2021; Seo et al., 2020), and seed size (Lo et al., 2019). However, there are limited reports available in cowpea, where genes/QTLs have been mapped for root traits in this important grain legume crop. Therefore, efforts have been undertaken during the present study to evaluate a set of diverse one hundred ten genotypes from the mini-core collection of IITA, Nigeria in the green house of Genetics and Plant Breeding, Faculty of Agriculture, Wadura campus, SKUAST-Kashmir, Kashmir, India. Data was recorded on thirteen different root-shoot traits. The trait data thus recorded has been used in combination with DArTseq SNPgenotypic data to identify marker-trait associations (MTAs)/genes/QTLs for cowpea root-shoot traits. The important MTAs reported will prove useful for cowpea breeding community in the world.

2. Materials and methods

2.1. Plant material and environment conditions

A set of 110 cowpea (*Vigna unguiculata* (L) Walp) diverse mini-core collection genotypes derived from International Institute of Tropical Agriculture (IITA), Nigeria core collection was used during the present study (**S** Table 1). The cowpea mini-core collection was evaluated under greenhouse conditions in Western Himalayan region of Kashmir, India. The evaluation of cowpea lines was carried out during the year 2020–2021 under greenhouse conditions of Division of Genetics and Plant Breeding, Faculty of Agriculture, Wadura, Sopore (34° 17′ N and 74° 33′ E at an altitude of 1594 masl). The set of 110 cowpea lines was selected after evaluating the cowpea mini-core collection of 350 accessions under the field condition at Division of Genetics and Plant Breeding, Faculty of Agriculture, Wadura, Sopore. The set of accessions was reduced for root trait evaluation based on the performance of the cowpea lines in field for different traits especially response to photoperiod of temperate conditions of Western Himalayas of Kashmir region. Under field conditions it was observed that number of accessions could not reach to flowering stage, the growth was limited only up to vegetative phase. This was due to the response of plants to varying photoperiod as it effect greatly on reproductive development of plant although some genotypes are insensitive (Ellis et al., 1994). However, the vegetative phase of plant is least or not affected by photoperiod (Wienk, 1963; Craufurd et al., 1997). Therefore, based on trait performance including their adaptability in Western Himalayas, we shortlisted the set of 110 genotypes for the study of RSA during the present study.

2.2. Trait phenotyping

For phenotyping different root traits, the cowpea genotypes were planted in green house under completely randomized experimental design with three replications. The plants were grown in 1.3 m high poly vinyl chloride (PVC) columns with 20 cm internal diameter. The growth medium was a mixture of soil and sand. The medium was chosen in order to ensure correspondence between greenhouse and field screening systems. Using sand alone results in long roots (offering less friction) while as using soil alone greatly impedes root growth on account of formation of hard pan. The columns were regularly irrigated in order to avoid water stress. At the end of the experiment, the roots were harvested carefully from columns at the stage of booting, without any breakage and the soil from the columns was sieved to derive all possible root fractions for unbiased estimate of root biomass. The roots thus harvested were washed with a mild detergent solution to remove sand/soil and other impurities, rinsed with tap water to remove excess soap and dried in shade. The cleaned roots were used in recording different root traits. This method is easy and cost effective for studying root traits and enables to retain almost whole of the root with least destruction.

The roots were harvested 60 days after sowing at booting stage. The thirteen different root-shoot traits were recorded in cowpea population. The detail of observed traits and its description is given in (Table 1; Fig. 1**)**.

Table 1
Details of thirteen different root-shoot traits in cowpea recorded during the present study.

S. No.	Trait	Description
1	Root depth (cm)	Length of the longest root from base of stem.
2	Fresh root weight (g)	Weight of total root biomass.
3	Dry root weight (g)	Weight of dry root biomass.
4	Fresh shoot weight (g)	Weight of total above ground biomass.
5	Dry shoot weight (g)	Weight of dried above ground biomass.
6	Root shoot ratio	Ratio of dry root weight to dry shoot weight
7	Root volume (cm^3)	Measured as the deviation of volume in a measuring cylinder and converted into root volume by formula ($\pi r^2 h$).
8	Adventitious root number	The number of roots in the hypocotyl region.
9	Tap root diameter	The diameter of tap root measured by using vernier caliper
10	Stem diameter	The diameter of stem measured by using vernier caliper.
11	Branching density	The number of first order branches on the primary root counted at upper 5–10 cm of the root.
12	Basal root number	The number of roots emerging from the base of the stem.
13	Root biomass density ($gmcm^{-3}$)	Measured as ratio of dry root weight to volume of PVC column. This trait measures the exploration capacity of root in a given volume of soil and is an indirect measure of metabolic cost of roots. It helps to identify roots which can explore more area in a given root biomass.

2.3. Trait data analysis

The phenotypic data was recorded on thirteen different root-shoot traits, mentioned in Table 1. The traits recorded were analyzed for significant variations and basic statistic description using R software Version 4.3. The analysis of variances for root-shoot traits was done using package *Agricolae*. Correlation and Principal Component Analysis was also done using R software Version 4.3. The correlation between different root-shoot traits was obtained using package *Metan.* For principal component analysis Factoextra and Factominer packages of R software were used in order to know the contribution of different traits in the variances.

2.4. Marker genotyping and marker data

Leaf samples were harvested from three-week-old seedlings of each

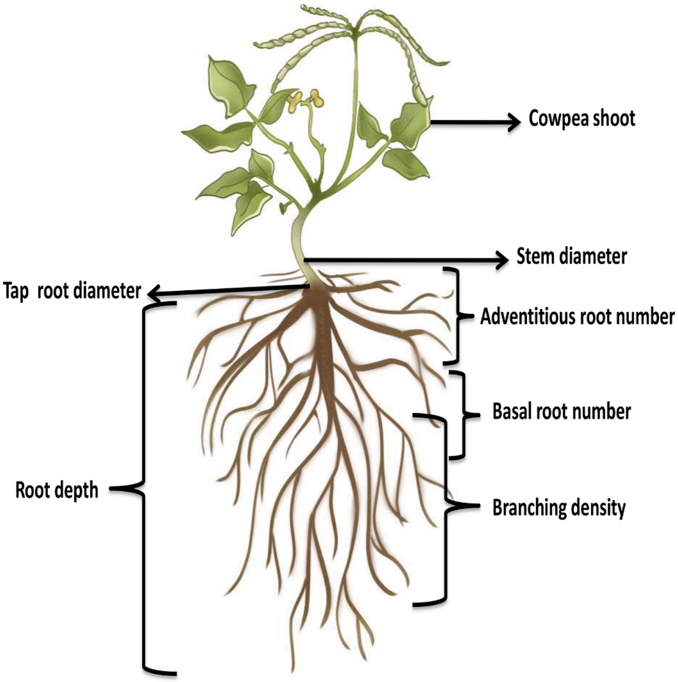


Fig. 1. A hypothetical cowpea plant showing above ground and below ground parts. The figure shows the different root trait in cowpea plant that were measured during the present study.

of the 376 cowpea accessions and preserved at -80 °C to lyophilize all the samples. The extraction of genomic DNA (gDNA) was carried out following the Diversity Array Technology (DArT) DNA extraction protocol available at www.diversityarrays.com/files/DArT_DNA_isolation.pdf. The extracted gDNA underwent for quality control through 1% agarose gel electrophoresis and quantification using a Nanodrop 2000 spectrophotometer (Thermo Scientific) in accordance with the manufacturer's instructions. High-quality DNA samples (100 ng/μL) were sent to DArT Pty Ltd in Canberra, Australia, for genotyping using high-depth DArTseqTM technology (Jaccoud et al., 2001). The procedures for complexity reduction, cloning, library construction, and cleaning followed the methods outlined by Egea et al. (2017).

A total of 19,359 SNPs genome-wide were discovered in 351 accessions of 376 total mini-core collections, using high-depth DArTseq SNP genotyping. Further, these SNPs were filtered fora subset of 109 cowpea accessions, monomorphic markers, minor allele frequency (MAF) $\geq$ 0.01, and $<$ 0.20 missing data were applied to eliminate poor-quality SNPs. A total of 6574 good quality SNPs from 109 accessions were retained for the subsequent genome-wide association study (GWAS). The skewed distribution of p-values indicated the significant result occurrence (**S** Fig. 1).

2.5. *Genome wide association analysis*

To identify the marker trait associations (MTAs), software program TASSEL3.0 (Trait Analysis by aSSociation, Evolution and Linkage) was used. GWAS was performed with 109 diverse cowpea genotypes (**S** Table 1) using two different models including the general linear model (GLM) based on the Q-matrix and the mixed linear model (MLM) based on both the Q-matrix and the kinship matrix (K-matrix) derived from the marker data using the TASSEL software program. The significant MTAs were described based on p-value and minimum allele frequencies. The negative log (1/n) was used to establish the significant threshold (Wang *et al*., 2012; Yang *et al*., 2013). Loci with minimum allele frequency <0.05 were filtered out. Only dimorphic variation sites were retained. The SNPs were screened based on the secondary allele frequency being >0.05 and p value <0.05 for significant MTAs. The Manhattan and QQ plots were generated using the software program TASSEL (Figs. 6 **and** 7; **S Fig** 2 **and** 3).

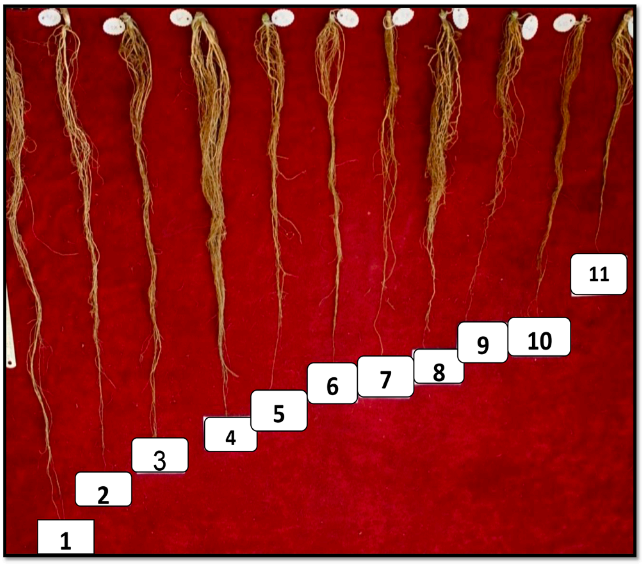


Fig. 2. Variation among cowpea accessions for different root traits observed during present study (1. TVu-16,449, 2. TVu-14,759,3. TVu-14,248, 4. TVu-374, 5. TVu-15,143, 6. TVu-523, 7. TVu-109, 8. TVu-2398, 9. TVu-1036, 10. TVu-969,11. TVu-946).

2.6. *Candidate genes identification*

The significant SNPs/MTAs that were discovered using both GLM and MLM models of GWAS were further analyzed for the candidate genes by using corresponding physical position of SNP-QTL in cowpea reference genome *Vigna unguiculata* v1.1, corresponding annotation information (Vunguiculata_469_v1.1.annotation_info.txt) and gff3 file for annotation/gene (Vunguiculata_469_v1.1.gene.gff3.gz). A genomic region of 50 kb upstream and downstream of the significant SNPs was searched, and gene models were extracted to identify candidate genes. Identified gene models and their encoding protein products were also subsequently further searched in literature for their functions in crops.

3. Result

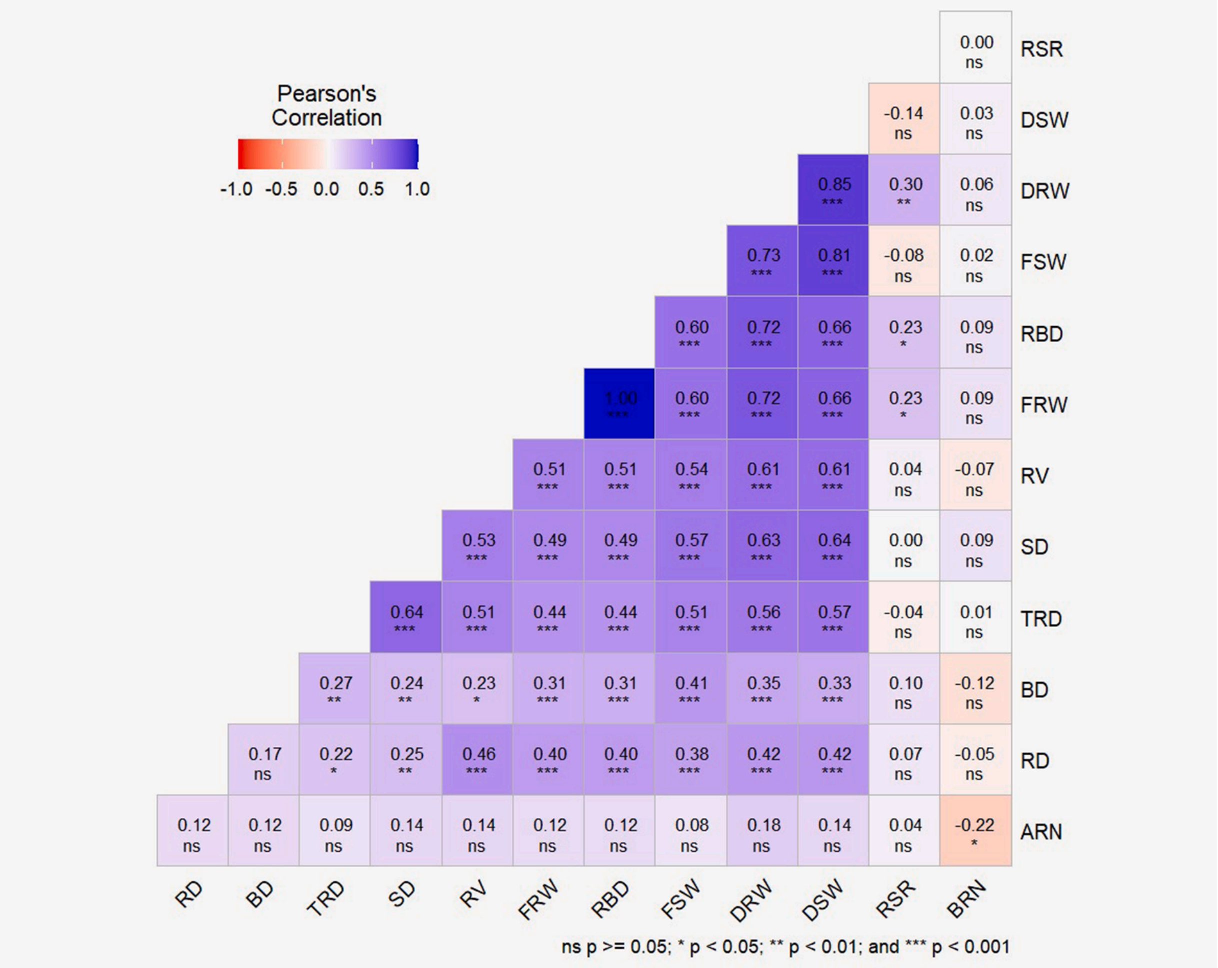
3.1. *Trait variation for different root-shoot traits*

A subset of 111 accessions of the cowpea core collection, including one check variety (Shalimar Cowpea-01), were phenotyped for thirteen different root-shoot traits under greenhouse conditions. Thirteen root-shoot traits among all the cowpea diverse lines were observed at the optimum stage, after carefully harvesting roots from the PVC columns. Significant variation ($p < 0.05$) was observed for all thirteen root-shoot traits recorded among cowpea genotypes (Table 2; Fig. 2). Among cowpea genotypes under study genotype "TVu-14,172″ was found promising for most of the traits, *viz.*, root volume (84.9 cm^3), dry root weight (25.5 gm), root biomass density (0.00307 $gmcm^{-3}$), tap root diameter (15 mm), fresh root weight (99.5 gm), fresh shoot weight (206 gm) and dry shoot weight (109.5 gm). The maximum root depth (114 cm) was recorded in genotype "Tvu-14,890″. The maximum root shoot ratio (0.59) was recorded in genotype "Tvu-13,778″. Branching density was found to be highest (45) in genotype "TVu- 9620″. The genotype "TVu-1477″ was found to possess highest number (20) of adventitious roots. The highest stem diameter (21.5 mm) was found in genotype "TVu-14,172″. The highest number of basal roots (25) was found in genotype "TVu-113″. The descriptive statistics for traits revealed that the adventitious root number is most variable trait as depicted by it's broad range (number varies from 0 to 20) and showing highest coefficient of variation (CV = 18.98%). The root traits namely dry root weight, root volume, biomass density, basal root number, and root shoot ratio, also showed highly significant variations (Table 3).

3.2. *Correlation and principal component analysis (PCA)*

The correlations worked out between various traits during the present study revealed that the estimates of phenotypic correlation were found to be significantly positive for majority of traits (Fig. 3). Root weight showed a significant positive ($p < 0.001$) association with shoot weight (0.85), root volume(r = 0.61), and tap root diameter (0.56). Fresh root weight was positively correlated with root biomass density (r = 1). On the other hand, a significant negative ($p < 0.05$) correlation was also found between the adventitious root number and basal root number (r = -0.22).

The Principal Component Analysis (PCA) was done for thirteen root-shoot traits and variability was found to be concentrated in first three components (Table 4, Fig. 4). In the rest of principal components variance explained was irrelevant as eigen values were below unity. The first three principal components explained the cumulative variance of 64.31%, with PC1 contributing 44.43%, followed by PC2 and PC3 accounting for 10% and 9.88% variation respectively. The PCA biplot (Fig. 5) depicting the variation contributed by different traits was constructed using first two principal components, contributing the total variation of 54.43%. The biplot depicted that first principal component explained 44.43% variation mainly contributed by dry root weight and dry shoot weight as indicated by component loading. Similarly, PC2 explained 10% of variation mainly contributed by root shoot ratio.



	RD	BD	TRD	SD	RV	FRW	RBD	FSW	DRW	DSW	RSR	BRN
RSR												0.00 ns
DSW											-0.14 ns	0.03 ns
DRW										0.85 ***	0.30 **	0.06 ns
FSW									0.73 ***	0.81 ***	-0.08 ns	0.02 ns
RBD								0.60 ***	0.72 ***	0.66 ***	0.23 *	0.09 ns
FRW							1.00 ***	0.60 ***	0.72 ***	0.66 ***	0.23 *	0.09 ns
RV						0.51 ***	0.51 ***	0.54 ***	0.61 ***	0.61 ***	0.04 ns	-0.07 ns
SD					0.53 ***	0.49 ***	0.49 ***	0.57 ***	0.63 ***	0.64 ***	0.00 ns	0.09 ns
TRD				0.64 ***	0.51 ***	0.44 ***	0.44 ***	0.51 ***	0.56 ***	0.57 ***	-0.04 ns	0.01 ns
BD			0.27 **	0.24 **	0.23 *	0.31 ***	0.31 ***	0.41 ***	0.35 ***	0.33 ***	0.10 ns	-0.12 ns
RD		0.17 ns	0.22 *	0.25 **	0.46 ***	0.40 ***	0.40 ***	0.38 ***	0.42 ***	0.42 ***	0.07 ns	-0.05 ns
ARN	0.12 ns	0.12 ns	0.09 ns	0.14 ns	0.14 ns	0.12 ns	0.12 ns	0.08 ns	0.18 ns	0.14 ns	0.04 ns	-0.22 *

ns p >= 0.05; * p < 0.05; ** p < 0.01; and *** p < 0.001

Fig. 3. Correlation heatmap for thirteen root-shoot traits. Figure shows the phenotypic association between different root traits under study in cowpea diverse germplasm, showing positive and negative relation among different traits studied.

Table 2
Analysis of variance (ANOVA) for thirteen root and shoot traits recorded for 111 cowpea genotypes under greenhouse conditions.

SOV	DF	RD	RV	FRW	FSW	DRW	DSW	RSR	ADRN	TRD	SD	BD	BRN	RBD
Genotype	110	906.30**	473.01**	718.86**	3701.19**	36.30**	747.67**	0.02**	25.79**	9.01**	18.96**	143.71**	32.29**	0.00**
Error	222	3.65	8.15	2.25	1.00	0.25	2.25	0.001	0.93	0.28	0.26	4.81	1.10	0.00

RD-root depth; RV-root volume; FRW-fresh root weight; FSW-fresh shoot weight; DRW-dry root weight; DSW-dry shoot weight; RSR-root shoot ratio; ADRN-adventitious root number; TRD-tap root diameter; SD-stem diameter; BD-branching density; BRN-basal root number; RBD-root biomass density; SOV-source of variation.

3.3. *Marker-trait associations (MTAs)*

The genome-wide association analysis was conducted using a set of SNPs uniformly distributed across all the chromosomes of cowpea and the trait data of all the thirteen traits (root depth, root volume, adventitious root number, branching density, root biomass density, basal root number, tap root diameter, stem diameter, fresh root weight, dry root weight, fresh shoot weight, dry shoot weight, root shoot ratio). The marker-trait associations (MTAs) were declared significant only when its p-values were $< 10^{-4}$. A total of 71 SNPs were found to be associated significantly with the thirteen root-shoot traits under study (**S** Table 2). The identified SNPs were distributed across the 11 cowpea chromosomes. Besides the SNP distributed over the 11 chromosomes, several SNPs were displaying their location on chromosomes 12 and 13, which are actually unlinked, thus do not map on any chromosome. However, these markers were left in place because they continue to demonstrate a strong correlation with trait attributes. The number of significant marker-trait associations detected, varied from one MTA for root depth to seventeen MTAs for dry root weight. Nevertheless, a number of SNPs found in the MTAs were associated with more than one trait; as a result, 52 distinct marker trait associations were found for each of the thirteen traits (**S** Table 3). Among all the MTAs, a set of only 10 MTAs were detected by using both GLM and MLM models and these MTAs have been declared as very promising and important for cowpea breeding programs (Table 5). These ten distinct SNPs were discovered to be extremely important ones, that showed association with nine different root-shoot traits (Adventitious root number, root biomass density, dry root weight, dry shoot weight, fresh root weight, fresh shoot weight, stem diameter, root volume, root shoot ratio). Among these ten highly significant SNPs, 14081978|F|0–13:T>C-13:T>C was found to be associated with three root-shoot traits (dry root weight, dry shoot weight and fresh root weight), located on chromosome 5, position 2881638 and 25354610|F|0–28:*G*>*A*-28:*G*>*A* was found to be associated with 5 root-shoot traits (dry root weight, dry shoot weight, fresh root weight, fresh

Table 3
Basic phenotypic description of thirteen root-shoot traits in diverse cowpea germplasm.

Root trait	Mean±SE	Minimum	Maximum	CV(%)
RD(cm)	74.00 ± 1.10	27.77	114	2.58
RV(cm^3)	25.28 ± 1.60	14.15	84.90	11.29
FRW(gm)	26.50 ± 0.86	3.50	99.50	5.66
FSW(gm)	90.96 ± 0.50	19.80	206.00	1.09
DRW(gm)	4.44 ± 0.20	0.17	25.50	11.27
DSW(gm)	26.24 ± 0.8	2.75	119.50	5.71
RSR	0.18 ± 0.50	0.03	0.59	16.67
ARN	5.09 ± 0.30	0.00	20.00	18.98
TRD(mm)	7.44 ± 0.20	4.10	15.00	7.05
SD(mm)	8.72 ± 1.20	4.20	21.50	5.86
BD	16.37 ± 0.60	7	45	13.36
BRN	8.82 ± 0.00	2	25	11.89
RBD(gmcm^{-3})	0.000843 ±	0.00011	0.00307	5.64

RD-root depth; RV-root volume; FRW-fresh root weight; FSW-fresh shoot weight; DRW-dry root weight; DSW-dry shoot weight; RSR-root shoot ratio; ADRN-adventitious root number; TRD-tap root diameter; SD-stem diameter; BD-branching density; BRN-basal root number; RBD-root biomass density.

Table 4
Eigen values and variations accounted by first three principal components. The table depicts that the majority of the variation is accumulated in first three principal components.

Principal component	PC1	PC2	PC3
Eigen value	5.77	1.30	1.28
Percent variation accounted	44.43	10.00	9.88
Cumulative variation accounted	44.43	54.43	64.31

shoot weight and stem diameter), locate on chromosome 4, position 27763272.

In addition, a set of 11 SNPs was also identified to show significant association with more than one trait (Table 6). Out of 71 significantly associated SNPs with thirteen root-shoot traits studied, eight SNPs were found to be significantly associated with root volume located on chromosome number 3, 4, 5, 9, 10 and 13 (**S** Table 4); eight were showing association with dry shoot weight located on chromosome number 3, 4, 5, 11 and 13; seven were significantly associated with adventitious root number located on chromosome number 3, 5, 7, 8, 11, and 13; seven SNPs were associated significantly with fresh root weight on chromosome number 3, 4, 5, 6, 7, and 9; five were significantly associated with root biomass density located on chromosome number 4, 5, 6, 7,and 13;

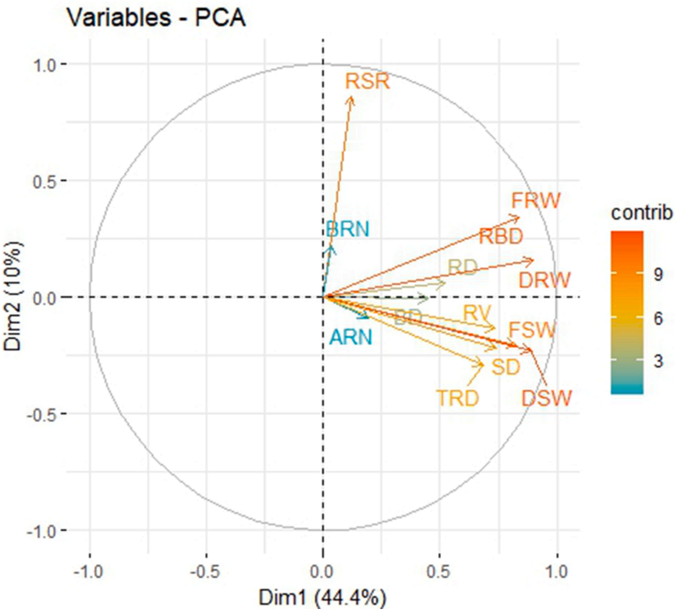


Fig. 5. PCA biplot of thirteen root-shoot traits of diverse cowpea germplasm. The proportion of total variance explained by first and second principal component is given in brackets. The arrows indicating direction of loading for each trait and red arrows are indicating high contributions to the PCs.

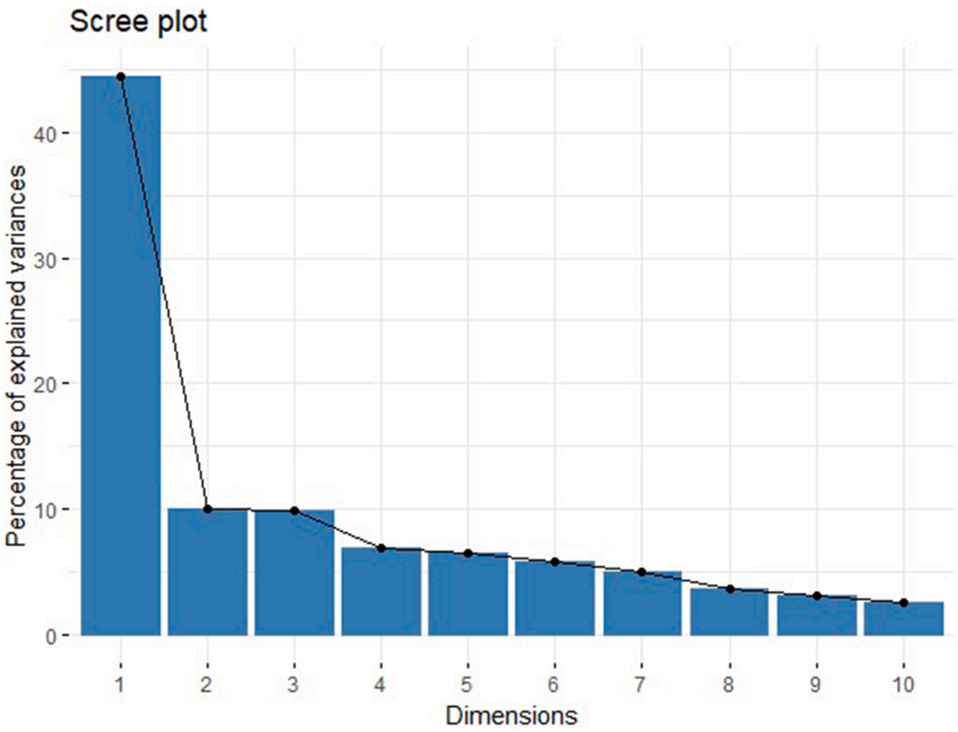


Fig. 4. Histogram depicting the percentage of varianes explained by different principal components, showing that first three components are accumulating majority variation.

Table 5
MTAs identified in the present study including those markers that were showing significant associations in both MLM and GLM models for different traits analyzed.

S.NO	Marker	Trait	Chromosome number	Position	Marker Rsquare(%) (GLM/MLM)	Model
1	14,060,175|F|0–24:*A*>*G*-24:*A*>*G*	Adventitious root number	VU_13	1472	18.62/ 19.73	GLM/MLM
2	14,085,648|F|0–21:C>T-21:C>T	Rootbiomassdensity	VU_13	1517	22.16/ 22.16	GLM/MLM
3	35,154,814|F|0–32:*A*>*G*-32:*A*>*G*	Adventitious rootnumber	VU11_OLD9	218,407	25.85/ 22.27	GLM/MLM
4	14,075,447|F|0–48:*G*>*A*-48:*G*>*A*	Rootbiomassdensity	VU04_OLD11	1,200,123	28.42/ 28.42	GLM/MLM
5	14,081,978|F|0–13:T>C-13:T>C	Dry root weight	VU05_OLD1	2,881,638	61.15/ 55.20	GLM/MLM
		Dry shootweight			35.71/ 32.57	GLM/MLM
		Fresh rootweight			55.52/ 51.03	GLM/MLM
6	42,215,508|F|0–47:*T*>*G*-47:*T*>*G*	Stemdiameter	VU03_OLD3	7,509,057	20.39/ 23.49	GLM/MLM
7	14,086,086|F|0–44:C>T-44:C>T	Root shoot ratio	VU03_OLD3	11,805,787	20.75/ 20.87	GLM/MLM
8	25,354,610|F|0–28:*G*>*A*-28:*G*>*A*	Dry rootweight	VU04_OLD11	27,763,272	51.62/ 41.97	GLM/MLM
		Dryshootweight			29.29/ 25.20	GLM/MLM
		Fresh rootweight			42.84/ 37.28	GLM/MLM
		Fresh shootweight			22.60/ 22.20	GLM/MLM
		Stemdiameter			27.64/ 23.70	GLM/MLM
9	25,366,829|F|0–64:*A*>*T*-64:*A*>*T*	Root volume	VU09_OLD8	28,989,833	23.35/ 23.80	GLM/MLM
10	14,078,337|F|0–15:*A*>*G*-15:*A*>*G*	Dry rootweight	VU07_OLD2	29,718,708	31.19/ 23.29	GLM/MLM

four SNPs were significantly associated with each of basal root number, tap root diameter and stem diameter located on chromosome number 3, 4, 5, 7, 8, 10, 11 and 13; three SNPs were significantly associated with fresh shoot weight located on chromosome number 5, 4, and 3; for root shoot ratio two SNPs were found to be significantly associated both located on chromosome number 3. In case of root depth and branching density only one SNP was identified to be significantly associated located on chromosome number 3 and 13 respectively. The highest number of significant SNP associations was identified for dry root weight, in which seventeen SNPs were found to be significantly associated located on chromosome number 2, 3, 4, 5, 6, 7, 8, 9, 11. All the SNPs discovered were distributed among all the chromosomes of cowpea but chromosome number 5 was identified as SNP hotspot for harboring highest number of significant SNPs particularly at position 2881638 and also highly significant SNPs after applying Bonferroni correction. The chromosome 5 possess significant SNPs for nine different root-shoot traits (Root volume, root biomass density, dry root weight, dry shoot weight, fresh root weight, fresh shoot weight, stem diameter, tap root diameter and adventitious root number) and also highly significant SNPs for dry root weight and dry shoot weight were found on this chromosome. The phenotypic variation explained by the 10 MTAs (Table 5) varied from 18.62% by "14060175|F|0–24:*A*>*G*-24:*A*>*G*" for Adventitious root number to 61.15% by "14081978|F|0–13:T>C-13:T>C" for Dry root weight. Most of these MTAs are very major effect MTAs.

3.4. *Candidate gene analysis*

Candidate gene analysis was performed by searching gene models within 50 kb region above and below from the SNP position in cowpea reference genome database (https://phytozome-next.jgi.doe.gov/info/Vunguiculata_v1_1). The identification of candidate genes in long genomic ranges would be tedious task and would result in an exhaustive list. Thus, we enlisted the genes within range of 50 kb of every MTA. The genomic region of 10 significant SNPs in both GLM and MLM approaches (SNP position ± 50 kb) harbored a total of 77 candidate genes (**S** Table 5). Out of these 77 genes, there were twenty-one such candidate genes whose role was found to be directly or indirectly associated with the root traits (Table 7). These genes were found to be associated with seven different SNPs. The remaining genes identified were found to have various cellular components, biological and molecular functions.

4. Discussion

Cowpea is a vital annual legume crop grown throughout world, particularly in Africa followed by Brazil. In India, cowpea is grown in semi-arid regions usually as intercrop with cereals but also in rotation as sole crop (Sharawyand El-Fiky, 2003). It is a source of relatively low-cost plant protein and other essential nutrients globally. It is the main staple food in Sub-Saharan Africa supporting millions of people there (Enyiukwu et al., 2018). Cowpea also thrives very well under low soil fertility and dry land conditions that makes it one of the most resilient legume crops that is suitable for growing in low input and water limited conditions (Nkhoma et al., 2020). A mini-core collection of IITA was phenotyped and data was recorded on thirteen root-shoot traits. The data recorded was used in combination with DArTSNP genotypic data for discovery of genes/QTLs/markers for root-shoot traits.

4.1. *Root-shoot trait variation in cowpea mini-core collection*

In the present study phenotyping of thirteen root-shoot traits viz., root depth, root volume, fresh root weight, dry root weight, root shoot ratio, adventitious root number, tap root diameter, basal root number, branching density, root biomass density, fresh shoot weight, dry shoot weight and stem diameter have been done. All the traits showed significant ($P < 0.01$) differences among all genotypes. Root depth and primary root elongation have been reported to be better parameters for selection than root length density and dry root matter in legumes (Hamblin and Tennat, 1987) and these parameters have been found varying significantly in the set of cowpea genotypes under study. The phenotyping of root traits in cowpea have been done earlier in different studies, Adu et al. (2019), IttahandArua (2017), Comas et al. (2012), Gwathmey et al. (1992), Uga et al. (2013) and all these studies have reported significant variation for different root traits among cowpea genotypes. The exceptional non-significant results were reported for root depth, dry root matter, root shoot ratio by Matsui and Singh (2003) but in our study all these three traits were found to vary significantly which is desirable for selection of better root phenotype. Krasilnikoff et al. (2003) studied cowpea root traits and found significant variations for root length and root hairs and also found their association with phosphorus uptake. The cowpea root architecture has also been studied by Burridge et al. (2016) in which significant variation was observed for basal root whorl number (BRWN) and for basal root number (BRN) under filed conditions. In this study significant differences have also been found for hypocotyl root number and branching, basal root growth angle, BRWN, BRN, and primary root length among the cowpea genotypes within environment and within year. Plant roots play an important role in plant growth by exploiting resources in soil through uptake of water and nutrients. Root systems of the plant are critical for improving the soil acquisition. Thus, breeding for root traits is a potential source for progress of intensified crop production system on limited arable land and in changing climate (Orman-Ligeza et al., 2014). Root traits are useful in enhancing plant productivity under changing climatic conditions especially drought conditions. Thus, it is important to understand

Table 6

The MTAs identified in the present study; table depicts those association where each marker is showing association with more than one trait under study.

<table>
<tr><th>S. NO</th><th>Marker</th><th>Chromosome number</th><th>Position</th><th>Trait</th></tr>
<tr><td>1</td><td>14,085,648|F|0–21:C>T-21:C>T</td><td>VU_13</td><td>1517</td><td>Branchingdensity
Rootbiomassdensity</td></tr>
<tr><td>2</td><td>25,355,835|F|0–14:C>G-14:C>G</td><td>VU03_OLD3</td><td>689,150</td><td>Root volume
Tap rootdiameter</td></tr>
<tr><td>3</td><td>14,081,978|F|0–13:T>C-13:T>C</td><td>VU05_OLD1</td><td>2,881,638</td><td>Dry rootweight
Dry shootweight
Fresh rootweight
Fresh shootweight
Root volume
Stemdiameter
Tap rootdiameter</td></tr>
<tr><td>4</td><td>25,365,226|F|0–7:C>T-7:C>T</td><td>VU08_OLD5</td><td>17,406,046</td><td>Adventitiousrootnumber
Dry rootweight</td></tr>
<tr><td>5</td><td>14,075,872|F|0–36:*A*>*G*-36:*A*>*G*</td><td>VU06_OLD6</td><td>26,599,103</td><td>Dry rootweight
Fresh rootweight</td></tr>
<tr><td>6</td><td>25,354,610|F|0–28:*G*>*A*-28:*G*>*A*</td><td>VU04_OLD11</td><td>27,763,272</td><td>Dry rootweight
Dry shootweight
Fresh rootweight
Fresh shootweight
Stemdiameter
Tap rootdiameter</td></tr>
<tr><td>7</td><td>14,078,337|F|0–15:*A*>*G*-15:*A*>*G*</td><td>VU07_OLD2</td><td>29,718,708</td><td>Dry rootweight
Fresh rootweight
Tap rootdiameter</td></tr>
<tr><td>8</td><td>25,363,957|F|0–33:*T*>*G*-33:*T*>*G*</td><td>VU09_OLD8</td><td>32,254,007</td><td>Dry rootweight
Fresh rootweight</td></tr>
<tr><td>9</td><td>14,082,997|F|0–24:*T*>*G*-24:*T*>*G*</td><td>VU03_OLD3</td><td>59,983,984</td><td>Dry shootweight
Fresh shootweight</td></tr>
<tr><td>10</td><td>35,153,546|F|0–19:*G*>*A*-19:*G*>*A*</td><td>VU03_OLD3</td><td>60,072,273</td><td>Dry rootweight
Fresh rootweight</td></tr>
<tr><td>11</td><td>28,497,654|F|0–42:C>T-42:C>T</td><td>VU03_OLD3</td><td>62,906,101</td><td>Dry rootweight
Fresh rootweight</td></tr>
</table>

the interaction between root and surrounding soil environment which could be improved through root phenotyping.

4.2. Correlationand PCA

In the present study, positive correlation has been identified between root weight with root volume, tap root diameter, root biomass density and shoot weight which indicates that these traits could be improved simultaneously and these traits may be controlled by same set of genes/QTLs/MTAs. Ittah and Arua (2017) previously reported on the correlation of cowpea root traits with yield traits, albeit they were unable to obtain significant results for all traits. Another study by Gull et al. (2020) examined the correlation between cowpea root and shoot traits and reported that leaf area was inversely correlated with tissue biomass density but favorably correlated with rooting depth and root volume. The quantity of leaves had a negative correlation with tissue biomass density but a positive correlation with rooting depth, root volume, and shoot biomass. Various other studies have also revealed correlations between various agro-morphological and yield features in cowpea (Singh et al., 2003; Sahai et al., 2013; Meena et al., 2015; Nguyen et al., 2019; Manggoel et al., 2012; Sofi et al., 2022).

In addition to correlation analyses, we also conducted principal component analysis to identify the root feature that contributed the most to variation recorded in the set of diverse cowpea germplasm. Dry root weight, fresh root weight, root shoot ratio, and dry shoot weight were the characteristics that were most significant contributors. Thus, while choosing cowpea genotypes for stressed environments with limited water supply, these characteristics may be taken into account. Similar PCA results have been reported by Santos et al. (2023), where the PCA of cowpea root attributes was conducted, including yield traits. The primary contributors to variation were the dry root weight and the root shoot ratio. PCA in cowpea reported by Adu et al. (2019), revealed that traits related to soil and root tissue angles, shoot and root diameter, root biomass, hypocotyl root length, root count, and lateral root density were identified among the top 50% of the most crucial traits contributing to variation. The PCA for cowpea root and seed traits have also been done by Mohammed et al. (2022). In this study, seed traits were found associated with PC1 and most root traits related to PC2 indicating that seed dimension phenotypes were correlated but showed no association with seedling root traits. Therefore, these traits are important considerations in endeavors to breed for improved genotypes in cowpea

4.3. Discovery of QTLs for root-shoot traits in cowpea

Cowpea is one of the most important climate resilient, drought tolerant legume crops grown in arid and semi-arid regions of the world (Munoz-Amatriain*et al.*, 2017). The crop can thrive well under such stress condition with well-established root system. This enables the shoot to facilitate water uptake throughout the plant. Consequently, the size, properties, and distribution of the root system ultimately dictate the plant’s access to water, setting limits on shoot functioning. This relationship can be linked to an analogy where a horse drives a cart, and the characteristics of the cart set limits on the capacity of the horse (Nardini et al., 2002; Sperry et al., 2002). However, in cowpea, there is a significant need for extensive research to comprehend the genetic makeup of root traits, considering the limited availability of studies in this context. Therefore, a notable area of research interest lies in improving root traits that facilitate the efficient deployment of tissues for the exploration of soil water, specifically contributing to the sustained productivity of crops under water deficit conditions. Thus, the study aimed to reveal trait specific regions/QTLs in cowpea genome that are associated with the different root traits that will fill the essential gap in cowpea improvement to develop genotypes with better root architecture.

GWAS offers much higher mapping resolutions for identification of genes/QTLs associated with different traits in large populations (Mamo et al., 2014). Thus, exploring the advantages of GWAS using SNPs and with the aim to fill the research gap in genomic studies of root traits in cowpea, present study reports the association of SNPs with thirteen different root-shoot traits in diverse cowpea germplasm. In the present study, a set of 52 unique SNPs were reported to be significantly associated for thirteen different root traits. The association was significantly validated based on p values and r^2 values. Out of these discovered SNPs, 10 SNPs/MTAs were such that were found to be highly significant as showed association in both MLM and GLM model analysis (Table 5). These MTAs were largely major effect MTAs explain large proportion of phenotypic variation. These markers showed association with nine root-shoot traits namely, adventitious root number, root biomass density, dry root weight, fresh root weight, dry shoot weight, fresh shoot weight, stem diameter, root shoot ratio and root volume. Among the

Table 7

Candidate genes identified associated with different SNPs. The table includes only those gene having their probable functions associated directly or indirectly with the root trait of cowpea.

S. No.	GeneID	Start-position	EndPosition	Function	SNP-QTL	Chromosome number	QTL-position	Role	Refs.
1	Vigun04g110600	27,713,355	27,715,529	PTHR31744:SF10 - NAC DOMAIN CONTAINING PROTEIN 87	25,354,610|F|0–28:*G*>*A*-28:*G*>*A*	VU04_OLD11	27,763,272	Associated with plant abiotic stress (drought and salinity) responses, make them potential candidates for imparting stress tolerance.	Nakashima et al. (2009), Jeong et al. (2010)
2	Vigun04g110800	27,776,968	27,780,555	MYB FAMILY TRANSCRIPTION FACTOR-RELATED				involved in regulating responses to environmental stresses such as drought, salt, and cold; also well-known role of plant MYB TFs in roots is controlling the cell cycle.	Yanhui et al. (2006), Agarwal et al. (2006), Chen et al. (2022)
3	Vigun04g111000	27,794,078	27,796,003	RING ZINC FINGER PROTEIN // RING/U-BOX DOMAIN—CONTAINING PROTEIN				mainly function as E3 ubiquitin ligases, and play important roles in plant growth, development, and the responses to abiotic stresses such as drought, salt, temperature, reactive oxygen species, and harmful metals.	Han et al. (2022)
4	Vigun04g081200	12,220,602	12,225,175	CHLORIDE CHANNEL // CHLORIDE CHANNEL PROTEIN CLC-A-RELATED	14,075,447|F|0–48:*G*>*A*-48:*G*>*A*	VU04_OLD11	1,200,123	their functions range from ion homeostasis to cell volume regulation, transepithelial transport, and regulation of electrical excitability. Thus have role in salinity tolerance	Jentsch et al. (2002), Nedelyaeva et al. (2022)
5	Vigun04g081500	12,280,847	12,283,013	Ulp1 protease				affects several important biological processes in plants including response to abiotic stress. Also play a role in drought tolerance in Arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa)*	Murtas et al. (2003), Catala et al. (2007); Choudhary et al. (2009), Park et al. (2011)
6	Vigun04g081900	12,375,943	12,376,769	EF-HAND CALCIUM-BINDING DOMAIN CONTAINING PROTEIN				plays a role in plant responses to bicarbonate, salt and osmotic stresses	Chen et al. (2015)
7	Vigun04g082200	12,439,744	12,445,149	Leucine Rich Repeat (LRR_1) // Protein tyrosine kinase (Pkinase_Tyr) // Leucine rich repeat (LRR_8)				required for root-hair development also encode proteins associated with sterol-rich lipid rafts	Jones et al. (2006)
8	Vigun05g034800	2,849,920	2,850,580	histone H3 (H3)	14,081,978|F|0–13:T>C-13:T>C	VU05_OLD1	2,881,638	dynamics of histones H3.1 and H3.3 characterize cells with different division potential in the growing root and a longer G2 phase in cells undergoing the last cell cycle before differentiation	Otero et al. (2016)

(*continued on next page*)

Table 7 (*continued*)

S. No.	GeneID	Start-position	EndPosition	Function	SNP-QTL	Chromosome number	QTL-position	Role	Refs.
9	Vigun05g035300	2,882,992	2,885,234	UDP-glucuronosyl/UDP-glucosyltransferase				confers drought tolerance in spring wheat and plays an important role in root and stem development of abiotic and biotic stresses	Zahra et al. (2022), Ouyang et al. (2012)
10	Vigun05g035800	2,921,191	2,923,445	CYTOCHROME P450 FAMILY PROTEIN-RELATED				control cell division and cell expansion, vascular differentiation, fruit growth, root development, and flower formation. protect plants from drought	Minerdi et al. (2013), Mao et al. (2013)
11	Vigun05g035900	2,927,061	2,930,565	F-box-like				can inhibit the growth of primary roots and promote the growth of root hairs by increasing the content of ethylene through the karrikinssignaling pathway.	Carbonnel et al. (2020)
12	Vigun07g179700	29,687,331	29,699,778	MATH/TRAF domain	14,078,337|F|0–15: A>G-15: A>G	VU07_OLD2	29,718,708	these are pivotal in modulating plant development and environmental stress responses including drought, salinity.	Dai et al. (2023)
13	Vigun07g180200	29,722,306	29,726,315	Auxin efflux carrier				regulates root and lateral root development, root gravitropism, root hair development.	Swarup and Bhosale (2019)
14	Vigun07g180400	29,733,893	29,737,636	UDP-glucose-cellulose glucosyltransferase				roles in growth regulation and development, in protection against pathogens and abiotic stresses and in adaptation to changing environments.	Gharabli et al. (2023)
15	Vigun09g131900	29,035,562	29,041,584	OSMOTIC STRESS POTASSIUM TRANSPORTER // POTASSIUM TRANSPORTER 2	25,366,829|F|0–64: A>T-64: A>T	VU09_OLD8	28,989,833	maintains root meristem activity and root growth by regulating K+ and auxin homeostasis in response to low-K+ stress	Zhang et al., al. (2020)
16	Vigun11g001400	184,234	188,386	E3 UBIQUITIN-PROTEIN LIGASE ATL59-RELATED	35,154,814|F|0–32: A>G-32: A>G	VU11_OLD9	218,407	play important roles in hypocotyl elongation, root development; major role in maintaining cell viability in the root apical meristem	Christians et al. (2002), Koiwai et al. (2007), Xie et al. (2002)
17	Vigun11g001700	200,119	202,747	CATION/H(+) ANTIPORTER 1-RELATED				involved in the homeostasis of K+, Na+, and pH of the cell under salinity stress conditions	Ali et al. (2020)
18	Vigun11g001900	211,936	215,754	ALCOHOL DEHYDROGENASE RELATED				plays an important role in plant survival under anaerobic conditions	Shen et al. (2021)
19	Vigun03g089200	7,481,704	7,485,869	mitogen-activated protein kinase kinasekinase 1, plant (MEKK1P)	42,215,508|F|0–47: T>G-47: T>G	VU03_OLD3	7,509,057	involved in efficient transmission of specific stimuli and also involved in the regulation of the antioxidant defense	Sinha et al. (2011)

(*continued on next page*)

Table 7 (*continued*)

S. No.	GeneID	Start-position	EndPosition	Function	SNP-QTL	Chromosome number	QTL-position	Role	Refs.
								system in response to stress signaling	
20	Vigun03g089400	7,506,810	7,513,052	HOMEOBOX-LEUCINE ZIPPER PROTEIN ATHB-14-RELATED				regulation of root hair development in a subset of epidermal cells	Di Cristina et al. (1996)
21	Vigun03g089600	7,536,715	7,541,825	nuclear transcription factor Y, alpha (NFYA)				play pivotal roles in symbiotic root nodule development, abscisic acid (ABA)-regulated seed germination, primary root development	

different root-shoot traits studied highest number and highly significant SNPs were found associated with dry root weight. In the correlation and PCA analysis, dry root weight has also been observed to have significant positive correlation with other traits and also having high loading in variance contribution. Thus, dry root weight could be considered as a promising trait for improvement of cowpea genotypes in terms of root architecture. GWAS for root traits in cowpea have also been reported earlier by Burridge et al. (2017), and in their study 21 significantly associated markers have been identified. The SNPs associated with the root traits namely dry root weight, fresh root weight, dry shoot weight, fresh shoot weight, tap root diameter, stem diameter, root volume were found to be co-localized in our study located on chromosome 5 position 2881638. These traits have important role in establishment of better root architecture. The QTLs co-localized and their positive impact on median root width and seed weight per plant has been reported by Muchero et al. (2013), suggest that a moderately large, broad cone-shaped root system is likely to be beneficial in most environments. SNPs associated with tap root diameter have been found co-localized

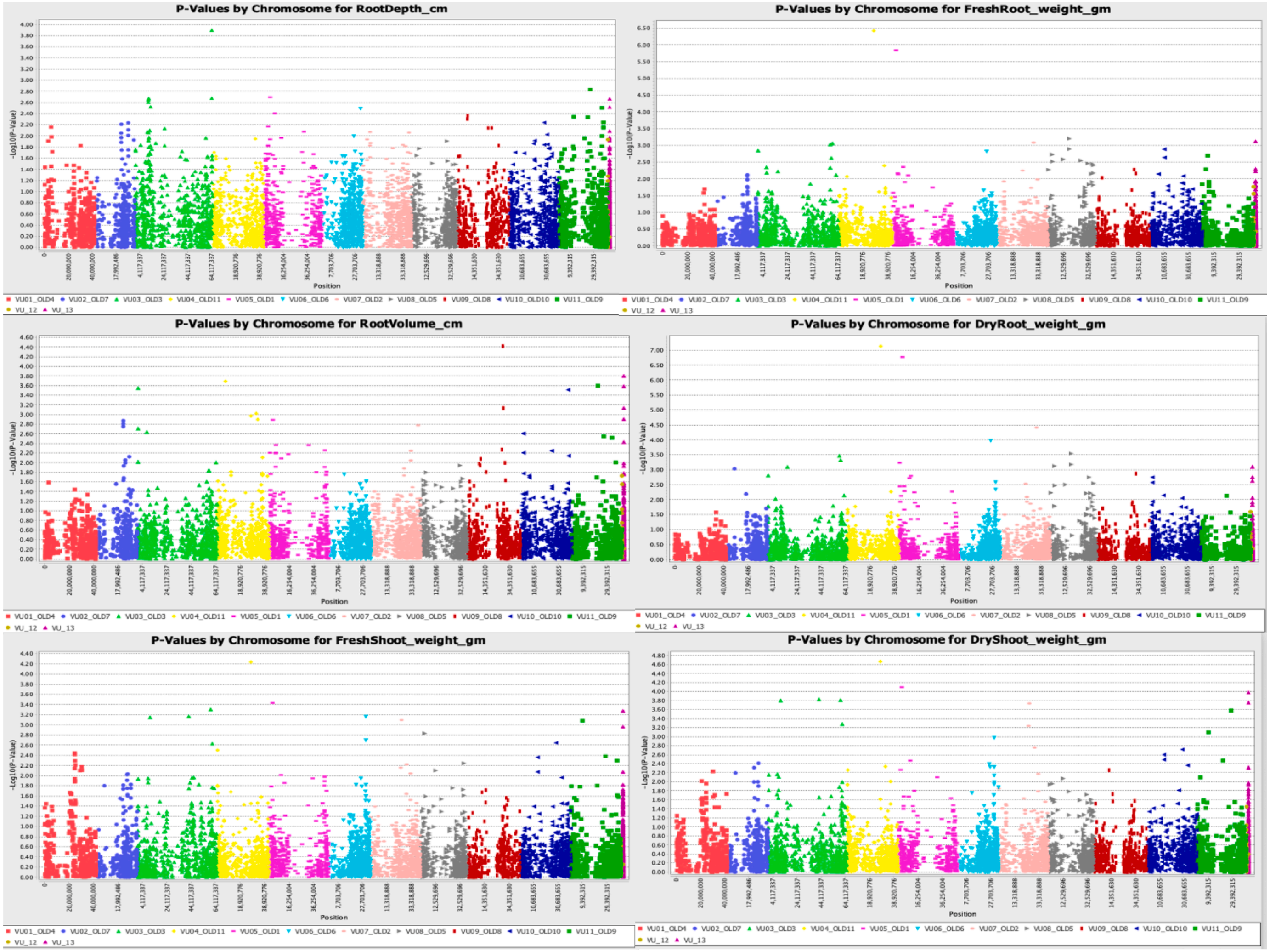
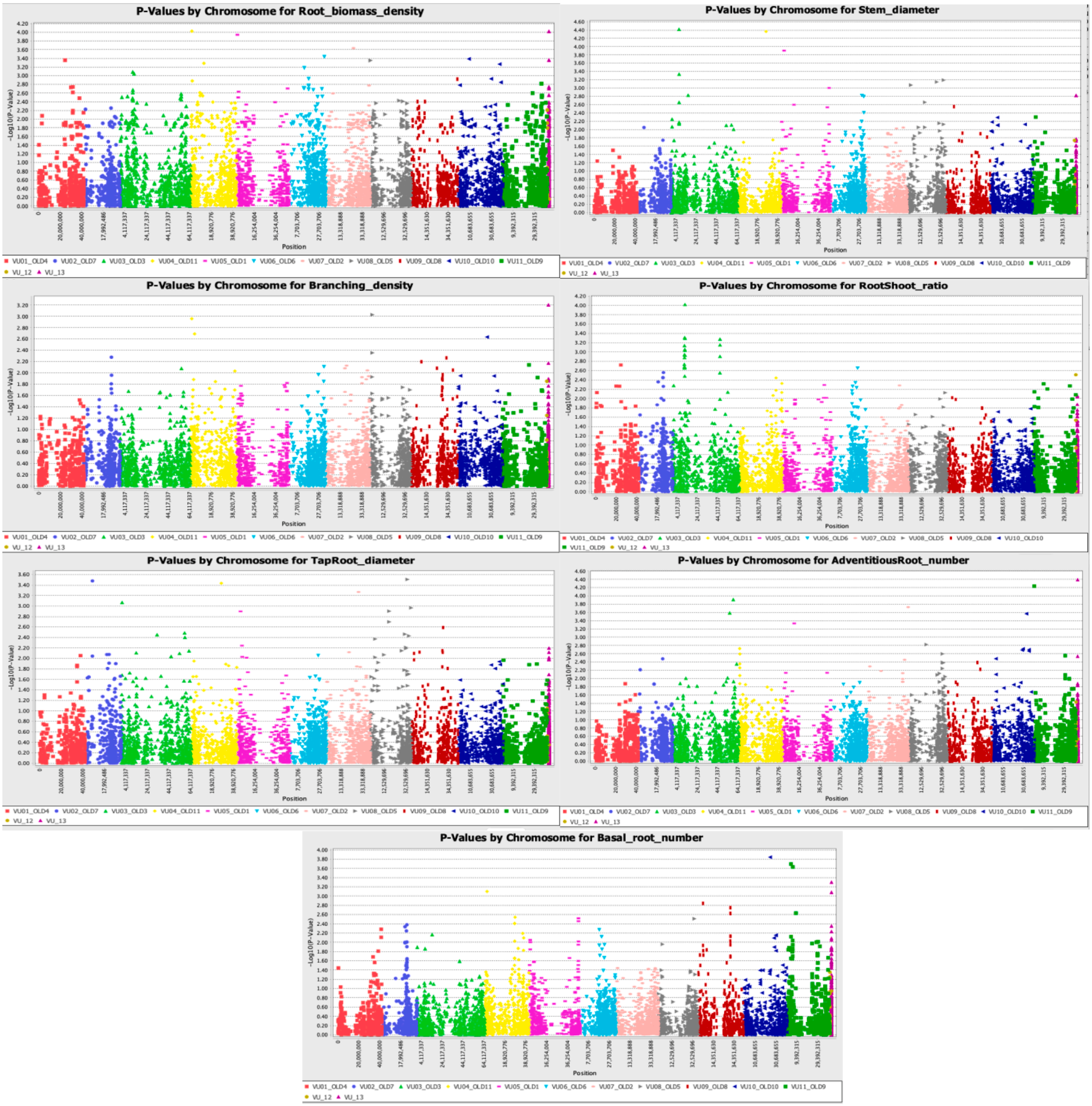


Fig. 6. Manhattan plots showing significant MTAs identified using MLM of software program TASSEL for thirteen root-shoot traits (13 plots for 13 traits). The significant QTLs are dots falling towards the top of plot depicting their location on X-axis.



P-Values by Chromosome for Root_biomass_density

P-Values by Chromosome for Stem_diameter

P-Values by Chromosome for Branching_density

P-Values by Chromosome for RootShoot_ratio

P-Values by Chromosome for TapRoot_diameter

P-Values by Chromosome for AdventitiousRoot_number

P-Values by Chromosome for Basal_root_number

Fig. 6. (*continued*).

with root weight, root volume in our study. The tap root or hypocotyl diameter serves useful as a reserve of carbohydrates for future reallocation in a perennial, fodder or multiple harvests cropping system (Gwathmey et al., 1992). Thus, co-localization of markers for these traits is validating the correlation among these traits.

The significant associations among different root traits with number of SNPs identified are of vital importance and can be used in marker-assisted selection to improve cowpea root architecture. The present GWAS analysis led to the identification of promising QTLs for all thirteen root-shoot traits of cowpea under study (Fig. 6). Among these the QTLs identified through both GLM and MLM models (Table 5) are highly significant and thus of great importance. These QTLs can be effectively used in future studies for mapping of genes associated with particular root trait under study.

4.4. *Candidate gene discovery*

The candidate genes for root traits have been identified for several legume crops (Azeem et al., 2019; Bhaskarla et al., 2020; Wu et al., 2021). However, to the best of our knowledge, this is the first study, where candidate genes have been identified for root-shoot traits in cowpea.The candidate genes identified in this study have been found to have a role in various biological functions associated with roots, such as root growth, development, and response to abiotic stress (Table 7). The genes identified, including “Vigun04g082200” associated with SNP (14075447|F|0–48:*G*>*A*-48:*G*>*A*), related to root biomass density have

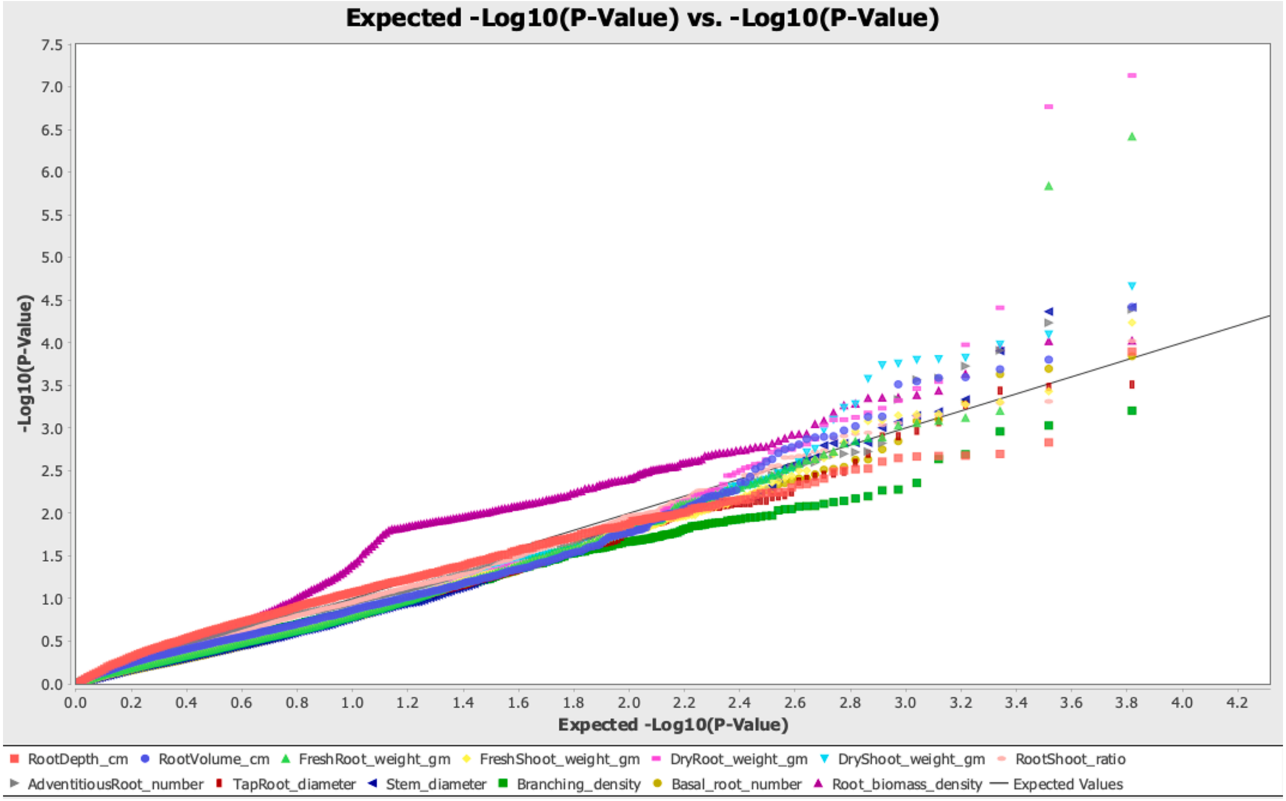


Fig. 7. QQ-plot of MLM model for all thirteen root-shoot traits studied. Those traits falling above the threshold linear line are showing significant marker trait associations.

been reported earlier to be responsible for the development of root hairs (Jones et al., 2006) and therefore, validates our results. Similarly, another gene (Vigun05g035900) identified, associated with the SNP (14081978|F|0–13:T>C-13:T>C) related to root weight have been reported to promote the growth of root hairs by increasing content of ethylene through karrikins signaling pathway in an earlier study (Carbonnel et al., 2020). The gene "E3 UBIQUITIN-PROTEIN LIGASE ATL59-RELATED" (Vigun11g001400) associated with adventitious root number identified in our study have been reported earlier to play important roles in hypocotyl elongation, root development and also in maintaining cell viability in the root apical meristem in rice (Christians et al., 2009; Koiwai et al., 2007;Xie et al., 2002). Most of the genes uncovered in this research have been found to be associated with conferring tolerance to abiotic stresses such as drought and salinity in cowpea. The root system is crucial for absorbing water and nutrients, offering structural support, and enhancing tolerance to abiotic stresses, thus is of paramount importance (Hussain et al., 2018; 2020). Notably, a robust root system architecture contributes to enhanced growth and heightened stress resilience (Uematsu et al., 2012; Hussain et al., 2020). Previous report of candidate gene identification for drought tolerance in cowpea have discovered seven markers that are homologous to sequences isolated from cowpea or other plant systems under drought or abiotic stress conditions. Additionally, two of these candidate genes have showed homology with coding sequences for a multidrug resistance protein 3 and a photosystem I assembly protein ycf3 (Muchero et al., 2010). Candidate gene analysis have been done earlier in cowpea for various other traits besides root traits including leaf morphology, seed coat color, disease resistance (Pottorff et al., 2014b, 2012b; Muchero et al., 2011), nematode resistance (Santos et al., 2018), insect resistance (Miesho et al., 2019), flowering time (Paudel et al., 2019).

5. Conclusion

The efficiency of water and nutrient uptake in crops is considerably influenced by the root system architecture (RSA). Root traits play an essential role in enhancing plant productivity, especially under challenging climatic conditions such as drought. Therefore, it is crucial to understand the dynamic interaction between roots and the surrounding soil environment. This understanding can be advanced through effective root phenotyping, contributing to improved resilience and performance of plants in adverse conditions. In the present study, the variation for root traits was explored using association mapping for 110 diverse cowpea mini-core collection. In total 52 unique significant SNPs were identified to be associated with thirteen root-shoot traits in cowpea. Out of which majority number of SNP associations were identified in root weight. The root weight was also found to show significant positive correlation with other traits as well. So, efforts for improvement of root weight could lead to simultaneous improvement of other root traits as well. This study expands our understanding of dissecting the genetic architecture of root system architecture (RSA) traits, laying the foundation for the genetic enhancement of root traits in cowpea breeding. Additionally, the extensive research on cowpea root features has yielded important insights into the underground architecture of the plant and its implications for overall plant performance. The study of root characteristics like depth, branching patterns, and root hair density has revealed significant elements of the cowpea's capacity to adapt to a variety of environmental circumstances. Knowing these characteristics can greatly aid in the creation of cowpea varieties that are more resilient and productive, especially in light of climate change and shifting agricultural environments. Genetic locus information, particularly SNPs associated with these traits, provided potential for discovering candidate genes, that is the first report of candidate gene analysis for root traits in cowpea to the best of our knowledge. This will help in understanding molecular mechanisms, and creating molecular markers useful in future breeding programs. The results of this study lay the groundwork for further investigations that will try to decipher the complex relationship between root characteristics and cowpea's stress-response, which will eventually lead to the development of sustainable farming methods.

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CRediT authorship contribution statement

Aaqif Zaffar: Writing – original draft, Software, Investigation, Data curation, Formal analysis. **Rajneesh Paliwal:** Writing – original draft, Supervision, Formal analysis. **Michael Abberton:** Writing – original draft, Supervision, Investigation, Funding acquisition, Conceptualization. **Sabina Akhtar:** Formal analysis, Software, Writing – review & editing. **Rafiq Ahmad Mengnoo:** Writing – original draft, Investigation, Data curation. **Aamir Nazir Sheikh:** Writing – original draft, Software, Resources, Formal analysis, Data curation. **Parvaze Ahmad Sofi:** Writing – original draft, Software, Methodology. **Mohd Ashraf Bhat:** Writing – original draft, Visualization, Resources, Methodology, Conceptualization. **Reyazul Rouf Mir:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data is available in the manuscript.

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Supplementary materials

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