Received: 4 August 2023 | Accepted: 24 January 2024

DOI: 10.1002/tpg2.20436

The Plant Genome

ORIGINAL ARTICLE

Multi-locus genome-wide association study reveal genomic regions underlying root system architecture traits in Ethiopian sorghum germplasm

Masarat Elias[1] | **Diriba Chere**[2] | **Dagnachew Lule**[3] | **Desalegn Serba**[4] | **Alemu Tirfessa**[5] | **Dandena Gelmesa**[1] | **Tesfaye Tesso**[2] | **Kassahun Bantte**[6] | **Temesgen M. Menamo**[6]

[1]School of Plant Science, Haramaya University, Dire Dawa, Ethiopia

[2]Department of Agronomy, Kansas State University, Manhattan, Kansas, USA

[3]Ethiopia Agricultural Transformation Institute, Addis Ababa, Ethiopia

[4]United States Department of Agriculture, Agricultural Research Service, U.S. Arid Land Agricultural Research Center, Maricopa, Arizona, USA

[5]Ethiopian Institute of Agricultural Research (EIAR), Melkassa Agricultural Research Center, Adama, Ethiopia

[6]Department of Plant Science and Horticulture, Jimma University, Jimma, Ethiopia

Correspondence
Temesgen M. Menamo, Department of Plant Science and Horticulture, Jimma University, P. O. Box 307, Jimma, Ethiopia.
Email: temesgen2008@hotmail.com

Assigned to Associate Editor Xianran Li.

Abstract

The identification of genomic regions underlying the root system architecture (RSA) is vital for improving crop abiotic stress tolerance. To improve sorghum (*Sorghum bicolor* L. Moench) for environmental stress tolerance, information on genetic variability and genomic regions linked to RSA traits is paramount. The aim of this study was, therefore, to investigate common quantitative trait nucleotides (QTNs) via multiple methodologies and identify genomic regions linked to RSA traits in a panel of 274 Ethiopian sorghum accessions. Multi-locus genome-wide association study was conducted using 265,944 high-quality single nucleotide polymorphism markers. Considering the QTN detected by at least three different methods, a total of 17 reliable QTNs were found to be significantly associated with root angle, number, length, and dry weight. Four QTNs were detected on chromosome SBI-05, followed by SBI-01 and SBI-02 with three QTNs each. Among the 17 QTNs, 11 are colocated with previously identified root traits quantitative trait loci and the remaining six are genome regions with novel genes. A total of 118 genes are colocated with these up- and downstreams of the QTNs. Moreover, five QTNs were found intragenic. These QTNs are S5_8994835 (number of nodal roots), S10_55702393 (number of nodal roots), S1_56872999 (nodal root angle), S9_1212069 (nodal root angle), and S5_5667192 (root dry weight) intragenic regions of *Sobic.005G073101, Sobic.010G198000*, *Sobic.001G273000*, *Sobic.009G013600*, and *Sobic.005G054700*, respectively.

Abbreviations: FASTmrEMMA, factored spectrally transformed multi-locus random-SNP-effect EMMA; FASTmrMLM, factored spectrally transformed multi-locus random-SNP-effect MLM; ISIS EM-BLASSO, iterative sure independence screening EM-Bayesian LASSO; MAF, minor allele frequency; ML-GWAS, multi-locus genome-wide association study; MLM, mixed linear model; mrMLM, multi-locus random-SNP-effect MLM; mrMLM.GUI, multi-locus random-SNP-effect mixed linear model with graphical user interface; pKWmEB, polygenic-background-control-based Kruskal–Wallis test plus empirical Bayes; pLARmEB, polygenic-background-control-based least angle regression plus empirical Bayes; QTN, quantitative trait nucleotide; RSA, root system architecture.

Particularly, *Sobic.005G073101*, *Sobic.010G198000*, and *Sobic.009G013600* were found responsible for the plant growth hormone-induced RSA. These genes may regulate root development in the seedling stage. Further analysis on these genes might be important to explore the genetic structure of RSA of sorghum.

Plain Language Summary

Sorghum is an important crop for many people, but it can be affected by drought and other environmental factors. Root system architecture has been identified as a critical factor for drought adaptation in the crop. This study looked at the genetic makeup of sorghum plants to find specific parts of their DNA that are linked to traits related to the roots. The researchers found 17 regions of the DNA that are associated with traits like the angle, number, length, and weight of the roots. Some of these regions contain genes that we already knew were important for root traits, while others are new discoveries. Five of these regions are within genes that control plant growth hormones and may affect how the roots develop. Understanding these genes could help us improve the ability of sorghum plants to tolerate environmental stress. Further study of these genes could help us better understand and improve the root system of sorghum plants.

1 | INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is a diploid C4 cereal with a chromosome number of $2n = 2x = 20$ and genomic size of 750 Mbp (Paterson et al., 2009). The species *S. bicolor* consists of the cultivated sorghum and a group of semi-wild and wild plants. The cultivated sorghum exhibits significant genetic and morphological divergence and is categorized into five races—bicolor, guinea, caudatum, kafir, and durra—based on inflorescence, grain, and glume characteristics (Harlan & de Wet, 1972). It is an ancient grain with wide adaptability worldwide (Boyles et al., 2019) and is extensively cultivated in tropical and temperate regions (Borrell et al., 2021). It ranks as the fifth most important cereal globally, following maize, rice, wheat, and barley (FAO et al., 2021).

Sorghum plays a vital role as a staple food for millions of people, particularly in sub-Saharan Africa (Cuevas et al., 2018). It serves as a primary source of energy, protein, vitamins, and minerals in many households (Winn et al., 2009). Additionally, the by-products of sorghum are used as fodder, building materials, fencing, and in the production of brooms (Dahlberg et al., 2011). Ethiopia is the second-largest producer of sorghum in Africa after Nigeria, with an estimated national average productivity of 2 tonne per hectare (FAO et al., 2021).

Although sorghum is an important crop for millions of people, its productivity is affected by various biotic and abiotic constraints. These constraints include diseases, insect pests, weeds, nutrient deficiency, aluminum toxicity, drought, salinity, waterlogging, and high temperature stress (Hao et al., 2021). Drought is the most important yield-limiting factor, particularly in moisture-stressed agroecologies (Girma, Nida et al., 2020). Furthermore, drought is the main cause of sorghum genetic erosion (Teshome & Zhang, 2019), with significant landraces disappearing as a result of crop failure caused by severe drought. Numerous efforts have been made to understand the genetic and physiological mechanisms that contribute to drought resistance in crop plants (Demelash et al., 2023; Tebeje et al., 2020; Wondimu et al., 2020). Accordingly, root system architecture (RSA) has been identified as a critical factor for drought adaptation (Demelash et al., 2021; Menamo et al., 2023; Singh et al., 2010).

The importance of roots in accessing water and nutrients from the soil is well recognized and has been linked to crop efficiency in water and nutrient use (Comas et al., 2013). Because roots are underground, obtaining comprehensive data on root phenotypes in plants grown in their natural environment has been difficult (Singh et al., 2011). Nevertheless, controlled environment studies have shown that root architecture plays a significant role in both water and nutrient uptake, affecting aboveground plant performance (Bucciarelli et al., 2021; Xu et al., 2022). The nodal root angle is an important aspect of sorghum's RSA, influencing the elongation and spatial distribution of roots in the soil profile and potentially impacting drought adaptation (Joshi et al., 2017; Singh et al.,

2011). Root angle has recently received increased attention as an important trait for breeding drought tolerance in sorghum (Demelash et al., 2021; Demelash et al., 2023; Girma, Mekbib et al., 2020).

Information on a local genetic material is invaluable to plant breeders when deciding which crosses to make and for germplasm conservation. It also aids in the characterization and classification of accessions into heterotic groups, as well as in realizing potential genetic gain through selection (Mofokeng, 2015). Furthermore, the marker-trait association using high-throughput genotype data is rapidly expanding due to advancements in the scale, accuracy, and cost of high-throughput sequencing and genotyping. Genome-wide association study (GWAS) is an effective and efficient tool for identifying genome–phenotype associations and the causative loci/candidate genes (Alqudah et al., 2020). GWAS is critical for understanding the genetic architecture of complex traits (Mathew et al., 2019). With recent advances in next-generation DNA sequencing technologies and large-scale precision phenotyping, GWAS is used to identify candidate genes associated with traits of interest (Brachi et al., 2011; Girma et al., 2019). While GWAS has been used in several studies to identify genomic regions with important traits in sorghum (Adeyanju et al., 2015; Cuevas et al., 2018; Wondimu et al., 2023), there are still significant gaps in our understanding of the genetic basis of many important traits in this crop, particularly RSA (Girma et al., 2019). Single-locus genome-wide association study using ordinary mixed models may not fully account for small effect loci. As a result, multi-locus genome-wide association study (ML-GWAS) models have been suggested as a potential solution to this issue (Rakitsch et al., 2013). ML-GWAS models are considered more efficient and reliable than SL-GWAS models for mapping genomic regions because they simultaneously estimate all-marker effects (Zhong et al., 2021).

Ethiopia has a high genetic diversity in sorghum and its wild relatives, making it an important resource for global sorghum improvement (Girma et al., 2019; Shegro et al., 2013). The Ethiopian National Sorghum Improvement program aims to boost productivity by utilizing these germplasm resources in collaboration with national, regional, and international research programs (Gebrie & Genet, 2019). Genetic variability is essential for improving traits through breeding and has a direct impact on the genetic gain achieved through selection. To effectively utilize these resources in breeding programs, however, they must be characterized for key agronomic, physiological, and nutritional traits. Understanding genetic diversity is therefore vital for both the utilization and conservation of genetic resources (Menamo et al., 2021).

Despite the importance of genetic variation in RSA, information on sorghum is limited. However, the Ethiopian sorghum germplasm exhibits high diversity in shoot and root traits. Understanding the genetic variability in RSA is crucial for sorghum improvement programs because it enables the development of desired root system characteristics for specific environments (Singh et al., 2010; Singh et al., 2011). Some of these traits may be especially useful for adaptation, and when combined with other known traits, can contribute to improving drought tolerance through breeding. Therefore, the objectives of this study were to investigate common quantitative trait nucleotides (QTNs) via multiple methodologies and identify genomic regions associated with RSA traits using GWAS in Ethiopian sorghum germplasm in order to formulate genome-assisted drought tolerance breeding strategies.

Core Ideas

- The study used multi-locus genome-wide association to identify 17 quantitative trait nucleotides (QTNs) significantly associated with root traits.
- Eleven QTNs colocated with previously identified root trait quantitative trait loci, while six were novel regions.
- Several genes were found within the 17-QTNs, located within up- and down-streams of linkage-disequilibrium decay regions.
- Five QTNs were found in the intragenic region of the five genes.
- This high-resolution germplasm characterization provides a critical dataset for exploring sorghum root genetics.

2 | MATERIALS AND METHODS

2.1 | Genetic materials

A total of 274 genotypes were used for this experiment. These genotypes were obtained from the Sorghum National Research Program, Melkasa Agricultural Research Center (MARC). The materials were collected from various regions of the country, representing a spectrum of sorghum growing agroecologies and various sorghum races and maintained in MARC (Table S1).

2.2 | Root growth condition

The experiment was conducted in the greenhouse at the Horticulture and Plant Science Department, College of Agriculture and Veterinary Medicine, Jimma University, Ethiopia. The mean daily air temperature during the growing season in the greenhouse ranged from 27.2˚C to 35˚C. The greenhouse

allowed for the transmission of 60% of incident photosynthetically active radiation for 12 h day light. Plants were watered once or twice a week, depending on the environmental conditions. A total of 1.5 kg of diammonium phosphate (DAP) and 1 kg of urea were applied as plant nutrients across the 500 chambers. DAP was applied in granular form during sowing, while urea was applied in liquid form when the plants reached the third leaf stage. The nutrients were evenly distributed in each chamber.

2.3 | Root system architecture phenotyping procedure and design

The experiment was laid out in a row-column design with three and four (checks) replications in 1 year. The experiment was conducted using a high-throughput root phenotyping platform (Demelash et al., 2021; Singh et al., 2011) consisting of 500 pairs of Perspex plates (40-cm deep, 35-cm wide, and 3-mm thick). The screening was conducted using 10 tubs with 50 root chambers. The nodal root angle was measured from the first flush of nodal roots at 2 cm from the base of the plant (Duresso et al., 2023; Menamo et al., 2023; Singh et al., 2011) using *Opengelphoto.tcl* software developed by the University of Queensland (www.activestate.com/activetcl). The root angle for each plant was averaged across four observations (left and right of each plant for both sides of the chamber). After root imaging, each chamber was laid flat, the top perspex plate was removed, and root length measurement was taken on the longest first flush nodal roots, and then numbers of nodal roots were counted from the base of the plants. Root samples were air dried on blotting paper and then oven-dried for 3 days at 60°C to determine root dry weight (g/plant).

2.3.1 | Phenotypic data analysis

Data were analyzed using a mixed linear model (MLM) allowed using "asreml-R" R package (Gilmour et al., 2006):

REML mixed model:

$$y = X\tau + Zu + e,$$

where y is the measured data for each trait; τ is the fixed effects (genotypes) in the trial; X is the design matrix for fixed effects; u is the random effects (columns and rows); Z is the design matrix for random effects; and e is the residual error effects. The genetic parameters including genotypic variance, phenotypic variance, genotypic coefficient of variance, phenotypic coefficient of variance, and heritability were estimated using *variability* R package in R software (Popat et al., 2020). Genotypic variance ($\sigma^2{}_g$) was estimated using $\sigma^2 g = (\mathrm{MS}g - \mathrm{MS}e)/r$, where MS$g$ is the mean square of genotypes, MSe is the mean square of error, and r is the number of replications. Phenotypic variance (σ_p^2) was estimated using $\sigma^2 p = \sigma_g^2 + \sigma_e^2$, where ($\sigma_e^2$) is the error mean square. Heritability in the broad sense (H^2) was computed using the formula suggested by Pariyar et al. (2021).

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + (\sigma_e^2/n)},$$

where $\sigma^2 g$ is the genotypic variance; $\sigma^2 e$ is the phenotypic variance; and n is the number of replicates.

The best linear unbiased prediction (BLUP) value was estimated using META-R package (Alvarado et al., 2020) and used for GWAS analysis.

2.4 | Genome wide association study analysis

Genotyping by sequencing (GBS) procedures (Elshire et al., 2011) were conducted using the ApeKI restriction enzyme (recognition site, G| CWCG). The GBS library was sequenced on Illumina HiSeq 2500 lanes following the manufacturer's protocol. The single nucleotide polymorphism (SNP) markers were extracted from the SNP data developed from resequencing of 1628 sorghum accessions at University of Wisconsin, Biotechnology Center (Girma, Nida et al., 2020). The dataset was developed through Purdue University Research Repository (Girma et al., 2019). The SNPs dataset was filtered to exclude SNPs with minor allele frequency (MAF) < 0.05 or missing values greater than 25%, and the remaining missing values were imputed using the Beagle 5.0 software package (Browning et al., 2018), yielding a total of 265,944 SNPs. Six ML-GWAS models were used for marker-trait association analysis, including multi-locus random-SNP-effect MLM (mrMLM) (Wang et al., 2016), factored spectrally transformed multi-locus random-SNP-effect MLM (FASTmrMLM) (Tamba & Zhang, 2018), factored spectrally transformed multi-locus random-SNP-effect effecient mixed-model association (FASTmrEMMA) (Wen et al., 2018), pLARmEB (Zhang et al., 2017), pkWmEB (Ren et al., 2018), and iterative sure independence screening EM-Bayesian LASSO (ISIS EM-BLASSO) (Tamba et al., 2017), all of which were implemented in the "mrMLM.GUI)" R package (where mrMLM.GUI is the mlmmulti-locus random-SNP-effect mixed linear model with graphical user interface) (Wen et al., 2018).

The QTNs with an logarithm of the odds (LOD) score of at least 4.00 were thought to be significantly associated with RSA traits of the test statistics $p = Pr(x_1^2 > 4x4.61) = 0.00002$, whereas $4.0 \times \ln 100 = 4.0 \times 4.61$ is converted from LOD 4 to its corresponding likelihood ratio test, which, under the null hypothesis, follows a chi-square distribution with 1 degree of freedom (Wang et al., 2016). In

TABLE 1 Fixed effect variance, random effects variance, mean, and range for key root traits of 274 Ethiopian sorghum accessions at sixth-leaf stage.

Traits	Unit	Genotype ($n = 274$)	Replication ($n = 3$)	Mean	Range	Heritability (%)
Root angle	Degree	14.12**	8.31	11.03	8.9–21.3	44.38
Root length	cm	78.05**	31.53	67.46	43.5–69.7	17.33
Root number	Number	5.16**	3.7	12.34	7.0–15.7	56.97
Root dry weight	g	0.03**	0.07	0.55	0.17–0.77	49.32

**Significantly different at <.01.

addition, only SNP markers identified in at least three models were designated as reliable RSA-associated QTNs. Similarly, QTNs that were detected in three or more models and showed phenotypic variation ($R^2 > 10\%$) were designated as major QTNs. Population structure and Kinship matrix (K) for our accessions have been previously estimated (Girma, Nida et al., 2020). Similarly, we allowed mrMLM.GUI software package to calculate population structure and kinship matrix (K) internally. The resulting −log10 (P) values from the ML-GWAS approach were used to draw the Manhattan and Q–Q plots using the mrMLM.GUI package (Wen et al., 2018).

2.5 | Colocalization with previously detected QTLs for RSA traits and identification of candidate genes

The colocations of significant QTNs with previously identified quantitative trait loci (QTLs) were identified using Sorghum QTL Atlas database (Mace et al., 2019) within the range of linkage disequilibrium (LD) decay (65 kb). Candidate genes were searched using Biomart tools (Smedley et al., 2009) in Phytozome website (Goodstein et al., 2012) with an LD decay distance 65 kb (Girma, Nida et al., 2020) of the QTN localized genomic regions. The SorghumBase online database (Gladman et al., 2022) was also searched for detailed gene descriptions.

3 | RESULTS

3.1 | Phenotypic variation

The correlation among the experimental runs for each trait was first analyzed (Figure S1) using the 20 genotypes repeated across two experimental runs. All traits were positively correlated in both experimental runs. The phenotypic diversity analysis conducted using the current study material revealed that significant genetic variation among accessions for all four traits including nodal root angle, nodal root number, root dry weight, and nodal root length (Table 1). A wide range in nodal root angle (7.82˚–21.3˚), number of nodal root (7.0–15.67), nodal root length (38.33–71.00 cm), and root dry weight (0.17–0.76 g) were observed among the sorghum accessions (Table 1; Table S2). Additionally, the result of this study shown that moderate broad-sense heritability of root dry weight (49.32%), nodal root angle (44.38%), number of nodal root (56.97%), and low heritability for nodal root length (17.33%; Table 1).

3.2 | Distribution of SNPs across sorghum genome

A total of 475,322 SNP markers were identified across 274 accessions. The dataset was filtered to exclude SNPs with MAF < 0.05, yielding a robust final dataset of 265,944 SNPs (Figure S2). Genome-wide marker density plot showed that markers from the study panel were distributed across the sorghum genome. The number of SNPs varied from 34,961 (SBI-01) to 19,506 (SBI-07). The marker density also varied from 3.36 kb (SBI-07) to 2.26 Kb (SBI-02) along the genome.

3.3 | QTNs identified by ML-GWAS

Multi-locus models Q–Q plots (Figure S3) show consistent with optimal trends, implying that the false-positive errors were controlled well and the results of the MLM model were reliable except nodal root length trait. A total of 215 QTNs were identified on all chromosomes that are significantly associated with the four RSA traits (nodal root angle, number of nodal roots, nodal root length, and root dry weight) using one or more of the six ML-GWAS models, with a LOD score threshold of ≥4 (Table 2; Table S3). Among the identified QTNs, 53, 38, 101, and 26 were found for total number of nodal roots, nodal root angle, root dry weight, and nodal root length, respectively. Among these, a total of 17 significant QTNs were identified in at least three ML-GWAS models (Table 3; Figure 1).

Out of the six models involved, polygenic-background-control-based least angle regression plus empirical Bayes (pLARmEB) detected the highest (16 QTNs), followed by ISIS EM-BLASSO (15 QTNs), whereas FASTmrEMMA and mrMLM detected the lowest number of QTNs (two QTNs). Out of the 17 significant markers, six were detected for root angle, five for number of roots, four for root dry weight, and two for root length (Table 3). Chromosome SBI-05 harbored the highest number of QTNs identified (four QTNs), followed

TABLE 2 Summary of quantitative trait nucleotide (QTN) identified for the major root traits investigated in the present study using the six analysis models.

Analysis model	QTN effect	LOD score	r^2 (%)	Number of QTNs
mrMLM	−0.041 to –0.035	5.066–6.78	2.24–5.60	66
FASTmrMLM	−0.47 to –3.197	4.07–10.64	2.41–12.64	33
FASTmrEMMA	−0.000012 to –0.0377	4.71–10.82	2.34E-08–2.109	25
pLARmEB	−0.56 to –1.99	4.036–17.46	0.041–5.63	78
pKWmEB	−0.57 to –2.64	4.06–8.98	2.62–12.68	23
ISIS EM-BLASSO	−0.4345 to –2.965	4.088–9.04	0.088–11.35	57

Note: r^2 (%) is the proportion of total phenotypic variation explained by each QTN.
Abbreviations: FASTmrEMMA, factored spectrally transformed multi-locus random-SNP-effect effecient mixed-model association; FASTmrMLM, factored spectrally transformed multi-locus random-SNP-effect MLM; ISIS EM-BLASSO, iterative sure independence screening EM-Bayesian LASSO; pLARmEB, polygenic-background-control-based least angle regression plus empirical Bayes; pKWmEB, polygenic-background-control-based Kruskal–Wallis test plus empirical Bayes; mrMLM, multi-locus random-SNP-effect MLM.

TABLE 3 List of significant quantitative trait nucleotides (QTNs) co-detected simultaneously by using three or more multi-locus genome-wide association study (GWAS) methods for sorghum root system architecture traits.

Trait	QTN	Chr	Position (bp)	LOD score	r^2 (%)	Method	Number of genes ± LD
NNR	S1_24908017	1	24,908,017	4.088–5.88	1.85–3.22	4,5,6	10
	S2_52681643	2	52,681,643	5.185–7.946	3.97–6.60	2,4,5,6	4
	S5_62001114	5	62,001,114	4.69–8.98	2.92–5.05	4.5.6	5
	S5_8994835	5	8,994,835	4.31–7.88	2.56–4.37	4,5,6	5
	S10_55702393	10	55,702,393	4.64–6.48	1.75–4.74	4,5,6	9
NRA	S1_56872999	1	56,872,999	4.20–5.66	1.86–5.99	2,4,5,6	6
	S2_47979513	2	47,979,513	4.07–6.94	2.31–4.20	2,4,6	1
	S4_6185121	4	6,185,121	4.25–5.71	1.73–3.64	2,4,5,6	11
	S6_48244728	6	48,244,728	4.66–6.17	4.96–7.43	4,5,6	7
	S7_48851050	7	48,851,050	4.23–7.78	2.04–4.65	4,5,6	3
	S9_1212069	9	1,212,069	4.63–8.78	1.85–7.22	4,5,6	15
RDW	S1_26887021	1	26,887,021	6.05–8.63	2.57–5.60	1,2,4	1
	S5_5667192	5	5,667,192	4.51–6.69	2.10–4.88	2,3,5	9
	S9_46653193	9	46,653,193	10.63–17.39	2.3406E-08–6.70	2,3,4,6	11
	S10_1788735	10	1,788,735	4.03–5.06	0.041–2.24	1,4,6	15
NRL	S2_58681356	2	58,681,356	4.06–9.04	3.59–4.75	4,5,6	4
	S5_65538924	5	65,538,924	4.85–8.78	1.39–12.68	2,4,5,6	2

Note: Methods 1–6 are as follows: 1 = mrMLM, 2 = FASTmrMLM, 3 = FASTmrEMMA, 4 = pLARmEB, 5 = pKWmEB, and 6 = ISIS EM-BLASSO. r^2 (%) is the proportion of total phenotypic variance explained by each QTN.
Abbreviations: Chr, chromosome; FATmrEMMA, factored spectrally transformed multi-locus random-SNP-effect effecient mixed-model association; FATmrMLM, factored spectrally transformed multi-locus random-SNP-effect MLM; NNR, number of nodal roots; NRA, nodal root angle; NRL, nodal root length; RDW, root dry weight.

by SBI-01 and SBI-02 (three QTNs each), and SBI-09 and SBI-10 (two QTNs each). With one QTN each, chromosomes SBI-04, SBI-06, and SBI-07 had the least number of QTNs detected. No significant QTNs were identified on chromosomes SBI-03 and SBI-08 (Table 3; Figure 1). Overall, the LOD value ranged from 4.03 to 17.46, and the proportion of phenotypic variance explained (r^2) by each QTN ranged from 2.34E-08% to 25.92% (Table 3).

3.4 | A priori QTL

The genomic locations of the new QTNs identified were compared with previously mapped QTLs for the RSA reported in Sorghum QTL Atlas database (Mace et al., 2019). Out of the 17 detected QTNs, 11 were colocated with previously reported QTLs for the RSA, while the remaining six QTNs were distinct (Table 4).

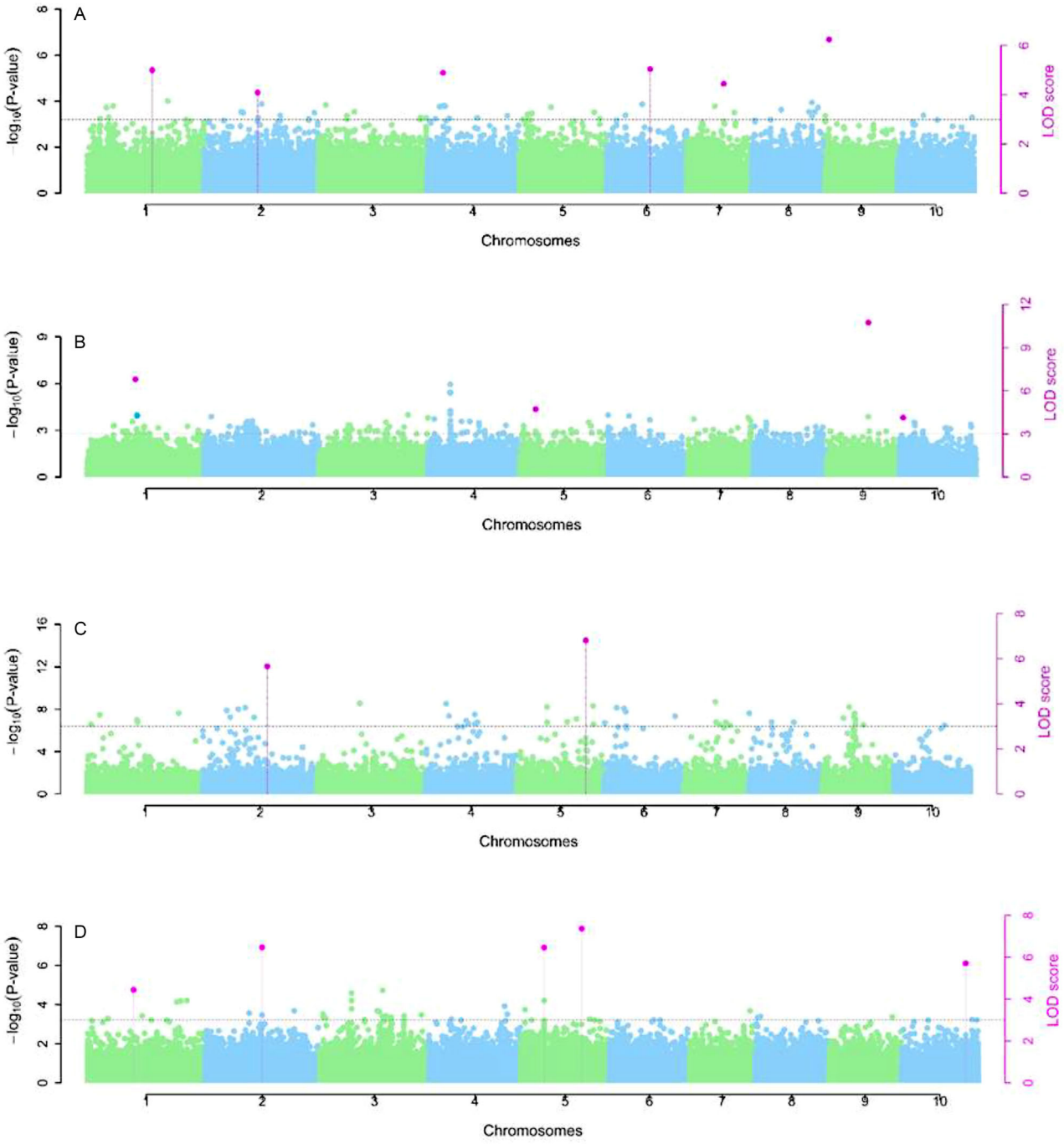

FIGURE 1 Manhattan plot showing *p*-values and genome-wide associations for root system architecture (RSA) traits (A) nodal root angle, (B) root dry weight, (C) nodal root length, and (D) number of nodal root. Each marker median of the −log10(*p*) values from the multi-locus random-SNP-effect mixed linear model (mrMLM), factored spectrally transformed multi-locus random-SNP-effect MLM (FASTmrMLM), andfactored spectrally transformed multi-locus random-SNP-effect effecient mixed-model association (FASTmrEMMA) approaches were used to draw the Manhattan. The dots are indicated by light colors quantitative trait nucleotides (QTNs); all QTNs commonly identified by three approaches are indicated by pink dots with dotted vertical lines.

3.5 | Prediction of potential candidate genes

We discovered 118 colocated genes within a 65 kb window size up- and down-streams of the 17 QTNs (Table S4) for all traits. Among the genes identified near the significant QTNs, 46 genes are uncharacterized or unknown proteins that are colocated with the number of nodal roots (10 genes), nodal root angle (21 genes), root dry weight (14 genes), and nodal root length (one gene). The rest of the genes have protein description such as

TABLE 4 List of colocated quantitative trait nucleotides (QTNs) with previously reported root system architecture (RSA) trait QTLs (quantitative trait loci) in the sorghum QTL Atlas database.

Trait	QTN	Chr	colocated QTLs	RSA traits	References
NNR	S1_24908017	SBI-01	QRTDW1.1	Root biomass; network surface area and network area	(Moghimi et al., 2019; Parra-Londono et al., 2018)
	S2_52681643	SBI-02	QRTLN2.1	Root length and root fresh weight	(Bekele et al., 2014; Wang et al., 2014)
	S5_62001114	SBI-05	QRTDW5.1	Root dry weight	(Bekele et al., 2014; Mace et al., 2012)
	S5_8994835	SBI-05	QRTLN5.1	Root length	(Bekele et al., 2014)
NRA	S1_56872999	SBI-01	QRTNW1.4	Network length distribution	(Parra-Londono et al., 2018)
	S2_47979513	SBI-02	QRTFW2.1 and QRTLN2.1	Root fresh weight and root length	(Bekele et al., 2014; Wang et al., 2014)
	S6_48244728	SBI-06	QRTFW6.1	Root fresh weight	(Bekele et al. 2014; Wang et al., 2014)
	S7_48851050	SBI-07	QRTFW7.1	Root fresh weight	(Bekele et al., 2014)
RDW	S1_26887021	SBI-01	QRTDW1.1 and QRTNW1.1	Root biomass; network surface area and network area (cm)	(Moghimi et al., 2019; Parra-Londono et al., 2018)
	S9_46653193	SBI-09	QRTLN9.1	Root length	(Bekele et al., 2014)
NRL	S2_58681356	SBI-02	QRTDW2.1	Root dry weight	(Bekele et al., 2014)

Abbreviations: Chr, chromosome; NNR, number of nodal roots; NRA, nodal root angle; NRL, nodal root length; RDW, root dry weight.

Sobic.001G240000 (similar to *putative ribosomal protein L28*), *Sobic.001G240800* (similar to *protein kinase APK1B*), and *Sobic.005G073500* (*B3 DNA binding domain containing protein*) for number of nodal roots; *Sobic.001G273200* (similar to *transferase family protein*) and *Sobic.009G013900* (*receptor-like kinase*) for nodal root angle trait. Additionally, *Sobic.010G021100* and *Sobic.010G021800* genes for root dry weight and *Sobic.002G188600* (*potassium transporter*) and *Sobic.002G188800* (*flavanone 7-O-beta-glucosyltransferase*) for nodal root length trait were colocated with QTNs regions of the traits. Some of the QTNs such as S5_8994835, S10_55702393, S1_56872999, S9_1212069, and S5_5667192 located in the intragenic regions of *Sobic.005G073101*, *Sobic.010G198000*, *Sobic.001G273000*, *Sobic.009G013600*, and *Sobic.005G054700*, respectively (Figure 2).

4 | DISCUSSION

4.1 | Three models of multi-loci analysis have identified associated genomic regions

A comparison of the six ML-GWAS methods revealed that pLARmEB (16 QTNs), ISISEM-BLASSO (15 QTNs), and pKWmEB (polygenic-background-control-based Kruskal–Wallis test plus empirical Bayes) (13 QTNs) were more useful approach than the other three models in the detection of reliable significant QTNs for RSA traits. Similarly, Ma et al. (2018) detected 160 and 130 significant QTNs for five traits using ISISEM-BLASSO and pLARmEB, respectively. Additionally, Zhang et al. (2019) reported ISISEM-BLASSO being the most powerful multi-locus approach in R package Genome Association and Pridiction Integrated Tool (GAPIT). In their ML-GWAS, Zhong et al. (2021) also reported higher numbers of significant QTNs using pKWmEB (189), ISIS EM-BLASSO (171), and pLARmEB (160). In contrast to this study, Wondimu et al. (2023) reported that among the six ML-GWAS methods, mrMLM was more advanced in detection of reliable QTNs for sorghum agronomic traits like plant height, days to flowering, grain yield, tiller number, 100-seed weight, and panicle exertion. This might be due to the traits and population panels used in the study. Reliable QTNs identified in the current study will advance the genetic improvement of the RSA traits in the future.

4.2 | ML-QTNs and colocated with previously studied QTL in different populations for RSA traits

In this study, we identified a total of 17 reliable QTNs detected at least three ML-GWAS models such as five QTNs for nodal root number, six nodal root angle, four root dry weight, and two nodal root length. Wang et al. (2016) reported that mrMLM has the highest power for QTN detection, the best

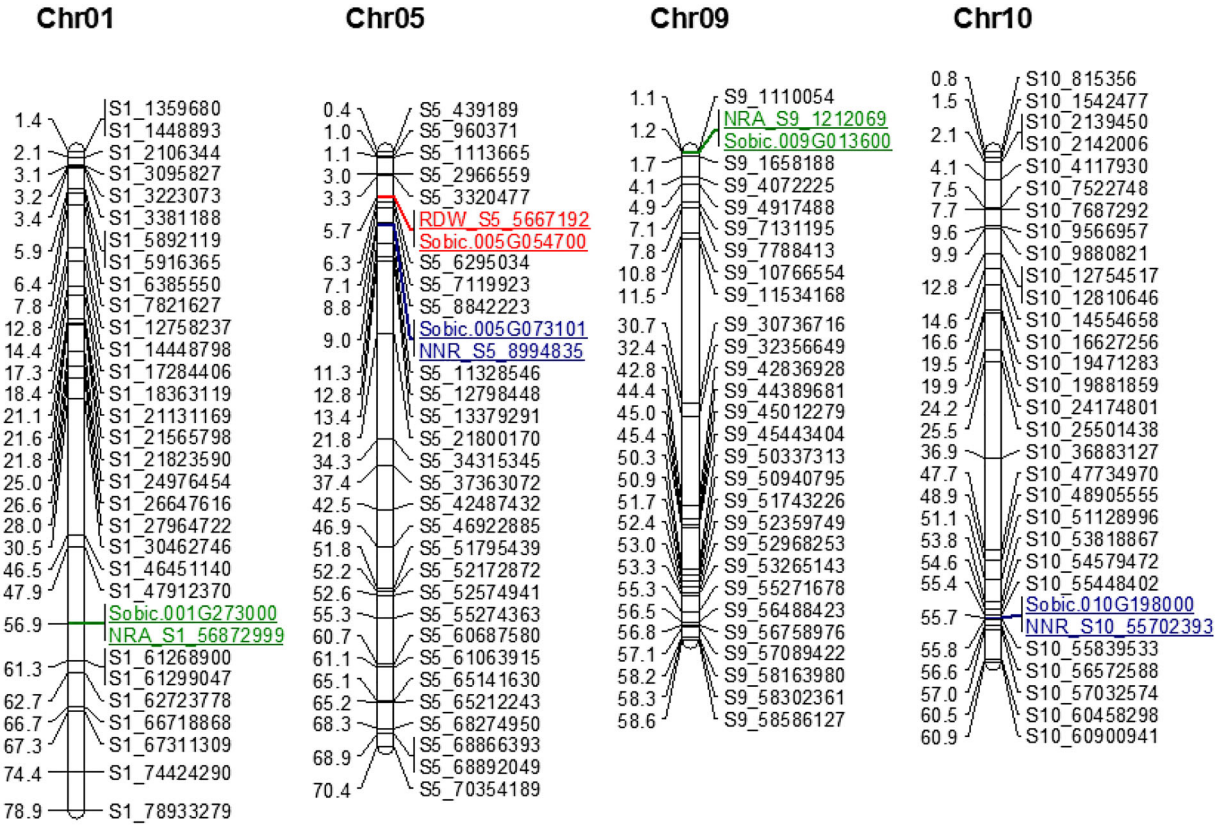

FIGURE 2 Linkage groups and chromosomal positions of significant quantitative trait nucleotides (QTNs) and intragenic genes identified for sorghum root system architecture (RSA) traits. The QTNs and genes are labeled on the right side of the chromosomes, and trait name abbreviations display different traits. QTNs and candidate genes on each chromosome are highlighted with different colors (green = nodal root angle (NRA), red = root dry weight (RDW), blue = number of nodal root (NNRs). The intervals between adjacent loci within chromosomes (numbers on the left) denote the physical distance in mega bases.

fit for genetic model, the minimal bias in the estimation of the QTN effect and the strongest robustness, as compared with the rMLM and the effecient mixed-model association. Out of 17 QTNs, 64.7% (11/17) of QTNs colocate with previously identified root traits. Out of the five nodal root number QTNs, four were found to colocate with previously reported QTLs for root biomass, network surface area, network area, root length, and root fresh weight in different populations (Bekele et al., 2014; Mace et al., 2012; Moghimi et al., 2019; Parra-Londono et al., 2018; Wang et al., 2014). Bekele et al. (2014) and Wang et al. (2014) used a recombinant inbred line population derived from the crossing between sweet sorghums (SS79 and L-Tian) and grain sorghum (M71 and Shihong137), respectively. However, Mace et al. (2012) used a backcross-nested association mapping population (BC-NAM). The nodal root angle QTNs were also shown to colocate (4/6) with previously reported QTL for root network length distribution, root fresh weight, and root length (Bekele et al., 2014; Parra-Londono et al., 2018; Wang et al., 2014). Out of the four root dry weight QTNs, two were found to colocate with previously reported QTL for root biomass, network surface area, network area, and root length (Bekele et al., 2014; Moghimi et al., 2019; Parra-Londono et al., 2018). Finally, out of the two nodal root length QTNs, one QTN was found to colocate with a QTL previously reported by Bekele et al. (2014) for root dry weight. A total of six QTNs were novel in this study, including S4_6185121 and S9_1212069 for nodal root angle, S5_5667192 and S10_1788735 for root dry weight, S5_65538924 for nodal root length, and S10_55702393 for nodal root number. These QTNs might need further verification using different populations.

4.3 | Significant QTNs colocated with RSA responsible genes

To find candidate genes, we focused on physical intervals supported by the LD decay information on Girma, Nida et al. (2020). On chromosome SBI-04, *Sobic.004G075301*, *Sobic.004G075351*, *Sobic.004G075400*, and *Sobic.004G075550* genes are colocated with S4_6185121 QTN for root angle. These genes have the protein description gamma-thionin family (gamma-thionin). Previous studies reported that *gamma-thionins* (*defensins*) have a response in roots growth performance in sorghum and are highly expressed in root when the root is treated with mycorrhiza

(Bruix et al., 1993; Watts-Williams et al., 2019). Allen et al. (2008) reported that plant defensins may also regulate plant growth and development, such as inhibition of root growth in germinating *Arabidopsis thaliana* (L.) Heyhn seeds at low micromolar concentrations.

4.4 | Intragenic QTNs found in hormones responsible genes

Intragenic QTL or QTN, known as a QTN or QTL located within the coding region of the genes, which are genetic loci or nucleotide, associated with variations in phenotypic traits. The QTNs such as S5_8994835 (number of nodal roots), S10_55702393 (number of nodal roots), S1_56872999 (nodal root angle), S9_1212069 (nodal root angle), and S5_5667192 (root dry weight) were located in intragenic regions of *Sobic.005G073101*, *Sobic.010G198000*, *Sobic.001G273000*, *Sobic.009G013600*, and *Sobic.005G054700*, respectively. These QTNs are specifically located within the boundaries of genes and can have a direct impact on the expression or function of the gene.

Sobic.005G073101 gene responsible for *F-box domain* (*F-box*) protein was reported both in Phytozome and SorghumBase databases (Gladman et al., 2022; Goodstein et al., 2012). *F-box* proteins are one of the main components of the *S-phase kinase-associated protein 1*, *Cullin-1*, and *F-box protein (SCF) complex* that belongs to the family of *ubiquitin E3 ligases*, which catalyze protein ubiquitination and maintain the balance between protein synthesis and degradation. Yan et al. (2011) reported that this protein involves in multiple signaling pathways in regulating root growth such as overexpression of the gene promotes root growth in rice. Moreover, a recent study showed overexpressing *F-box protein gene* (*TaFBA1*) promoted root length under stress conditions in tobacco plants (Abd-Hamid et al., 2020). Additionally, Boycheva et al. (2015) demonstrated the involvement of a *cyclin-like F-box protein* in root actively dividing cell development including variations in root and hypocotyl growth.

Sobic.010G198000 gene (*tryptophan synthase beta chain* [*trpB*]) also identified for RSA traits, particularly for the number of nodal roots. *Tryptophan synthase* catalyzes the biosynthesis of tryptophan from indol-3-glycerol phosphate and serine to tryptophan (Walter, 1999). Tryptophan is a precursor of indole-3-acetic acid, which is the major auxin involved in the regulation of lateral root formation (Sanada & Agehara, 2023; Taiz et al., 2015). Sanada and Agehara (2023) reported that application of *exogenous tryptophan increases* lateral root formation, root dry matter accumulation, and root-to-shoot ratio. Additionally, trpB is involved in the coordination of tryptophan and abscisic acid (ABA), thereby affecting plant growth and abiotic stress responses (Liu et al., 2022).

Sobic.009G013600 is described as *PROTEIN PHOSPHATASE 2C* both in Phytozome and SorghumBase databases. This protein has been reported as a negative regulator of ABA signaling and to have independent and overlapping functions in Arabidopsis (Nishimura et al., 2010; Saez et al., 2006). However, some studies reported that *PROTEIN PHOSPHATASE* activity is required for the normal regulation of auxin transport. Rashotte et al. (2001) reported that reduced phosphatase activity alters auxin transport and dependent physiological processes in the seedling root. These authors also mentioned that phosphatase inhibition reduced root gravity response and delayed the establishment of differential auxin-induced gene expression across a gravity-stimulated root tip, implicating this gene participation in root development through auxin induction.

5 | CONCLUSION

Exploring sorghum genetic diversity is essential in improving and conserving Ethiopian sorghum germplasm accessions grown in diverse environments. In this study, 11 novel and six colocated QTNs were identified, along with five intergenic QTNs associated with growth hormones such as ABA and auxin. These intergenic QTNs-associated candidate genes mapped to various functions associated with RSA traits, suggesting that these genetic variations may directly affect gene function and drive variations in root traits. Additionally, a total of 46 genes were described as uncharacterized proteins, and further studies could validate these descriptions to reveal the exact relevance of candidate genes in the biochemical pathway. Based on this study, Ethiopian sorghum accessions could be a valuable source of genetic material for identifying resistance genes for abiotic stress, particularly related root traits. Furthermore, establishing a core collection through the characterization and cataloging of newly discovered candidate genes for emerging traits in Ethiopian sorghum improvement is essential.

AUTHOR CONTRIBUTIONS

Masarat Elias: Conceptualization; data curation; formal analysis; writing—original draft; writing—review and editing. **Diriba Chere**: Formal analysis; writing—review and editing. **Dagnachew Lule**: Conceptualization; formal analysis; supervision; writing—review and editing. **Desalegn Serba**: Conceptualization; formal analysis; supervision; writing—review and editing. **Alemu Tirfessa**: Validation; resources; writing—original draft; writing—review and editing. **Dandena Gelmesa**: Conceptualization; validation; supervision; writing—original draft; writing—review and editing. **Tesfaye Tesso**: Validation; resources; writing–original draft; writing—review and editing. **Kassahun Bantte**: Validation; resources; writing—original draft;

writing—review and editing. **Temesgen M. Menamo**: Conceptualization; supervision; methodology; formal analysis; writing—original draft; writing—review and editing.

ACKNOWLEDGMENTS

The authors would like to thank Oda Bultum University for funding the graduate study of the first author of this paper. The authors would like to thank Haramaya University School of Plant Science for accepting the first author to its postgraduate program and helping shape the research proposal that bore this manuscript. The authors are also grateful to Purdue and Kansas State University (SMIL-team members) for developing genotypic data. The authors would like to acknowledge the Department of Horticulture and Plant Science, College of Agriculture and Veterinary Medicine, Jimma University, for granting permission to utilize high-throughput root phenotyping in greenhouse conditions. The authors give special thanks to the Ethiopian National Sorghum Improvement Program and Melkasa ARC for making the test materials available and supporting the study.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article (and its supplementary information files) and the SNP data available in the Ethiopian sorghum landrace SNP and phenotype data repository: https://purr.purdue.edu/publications/3189/1.

ORCID

Masarat Elias https://orcid.org/0000-0002-1520-911X
Temesgen M. Menamo https://orcid.org/0000-0003-4856-3147

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SUPPORTING INFORMATION

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

How to cite this article: Elias, M., Chere, D., Lule, D., Serba, D., Tirfessa, A., Gelmesa, D., Tesso, T., Bantte, K., & Menamo, T. M. (2024). Multi-locus genome-wide association study reveal genomic regions underlying root system architecture traits in Ethiopian sorghum germplasm. *The Plant Genome*, e20436. https://doi.org/10.1002/tpg2.20436