Genetic dissection of root architecture in Ethiopian sorghum landraces

Temesgen Menamo[1] · Andrew K. Borrell[2] · Emma Mace[2,3] · David R. Jordan[2] · Yongfu Tao[2] · Colleen Hunt[3] · Bantte Kassahun[1]

Abstract

Key message **This study quantified genetic variation in root system architecture (root number, angle, length and dry mass) within a diversity panel of 1771 Ethiopian sorghum landraces and identified 22 genomic regions associated with the root variations.**

Abstract The root system architecture (RSA) of crop plants influences adaptation to water-limited conditions and determines the capacity of a plant to access soil water and nutrients. Four key root traits (number, angle, length and dry mass) were evaluated in a diversity panel of 1771 Ethiopian sorghum landraces using purpose-built root chambers. Significant genetic variation was observed in all studied root traits, with nodal root angle ranging from 16.4° to 26.6°, with a high repeatability of 78.9%. Genome wide association studies identified a total of 22 genomic regions associated with root traits which were distributed on all chromosomes except chromosome SBI-10. Among the 22 root genomic regions, 15 co-located with RSA trait QTL previously identified in sorghum, with the remaining seven representing novel RSA QTL. The majority (85.7%) of identified root angle QTL also co-localized with QTL previously identified for stay-green in sorghum. This suggests that the stay-green phenotype might be associated with root architecture that enhances water extraction during water stress conditions. The results open avenues for manipulating root phenotypes to improve productivity in abiotic stress environments via marker-assisted selection.

Introduction

Sorghum is a staple food crop for millions of resource-poor farmers in the semi-arid tropics, particularly in Sub-Saharan Africa, including Ethiopia. This is due, in part, to the higher levels of abiotic stress tolerance sorghum displays in comparison to other cereals, particularly in terms of water stress (Borrell et al. 2020). Sorghum has a very broad environmental adaptation and is grown in both temperate and tropical climates, high and low altitudes, and in both water-logged

✉ Bantte Kassahun
kassahunb@gmail.com

1 College of Agriculture and Veterinary Medicine, Jimma University, P.O. Box 307, Jimma, Ethiopia

2 Queensland Alliance for Agriculture and Food Innovation (QAAFI), University of Queensland, Hermitage Research Facility, Warwick, QLD 4370, Australia

3 Agri-Science Queensland, Department of Agriculture and Fisheries, Hermitage Research Facility, Warwick, QLD 4370, Australia

and drought-stressed conditions (Borrell et al. 2021). However, in the arid and semi-arid regions where sorghum is pre-dominantly grown, it is often affected by water stress at either the vegetative and/or reproductive stages of crop development, particularly during the post-flowering stage (Kebede et al. 2001; Ali et al. 2009; Jordan et al. 2012). Sorghum has a range of phenotypic characteristics that contribute to the crop's enhanced adaptability to such harsh environments, both above ground and below ground (Borrell et al. 2020, 2021). In particular, the root system architecture (RSA) has been shown to play a critical role in productivity under such water-limited conditions, with narrow root angles increasing access to water from deep soil in cereal crops, including sorghum (Singh et al. 2012; Uga et al. 2013). A putative association between root angle and grain yield has also been reported in sorghum (Mace et al. 2012). Therefore, characterization and deployment of selection for RSA traits could accelerate the development of drought adapted sorghum varieties.

Significant progress has been made in the past decade on RSA as a selection criterion for stress adaptation in maize (Trachsel et al. 2011; Lobell et al. 2014), rice (Uga et al.

2013), wheat (Richard et al. 2018; Voss-Fels et al. 2018), barley (Robinson et al. 2018; Voss-Fels et al. 2018) and sorghum (Singh et al. 2008; Mace et al. 2012) following the development of a range of root phenotyping methods. Previous studies in sorghum have identified that significant variation for RSA exists, suggesting opportunities for selection and genetic improvement for water stress response breeding (Blum et al. 1977; Singh et al. 2011; Joshi et al. 2017; Zheng et al. 2020).

In addition to characterization of RSA variation, linkage analysis and genome-wide association study (GWAS) approaches have been used to dissect genetic control of root traits in sorghum, with 11 recent studies identifying 392 QTL for sorghum root traits (Mace et al. 2012; Fakrudin et al. 2013; Bekele et al. 2014; Hufnagel et al. 2014; Li et al. 2014; Wang et al. 2014; Chopra et al. 2017; Parra-Londono et al. 2018; Bernardino et al. 2019; Moghimi et al. 2019; Zheng et al. 2020). Among these QTL, 248 were repeatedly detected, while the remaining 144 were unique to each specific study. For example, Mace et al (2012) identified four QTL for nodal root angle in a bi-parental recombinant inbred line (RIL) population, which were subsequently also identified in other root trait GWAS studies (Bekele et al. 2014; Parra-Londono et al. 2018; Bernardino et al. 2019; Moghimi et al. 2019; Zheng et al. 2020). These four root angle QTL also co-localized with previously identified QTL for stay-green (Mace et al. 2012). Crops with stay-green exhibit a distinct green leaf phenotype during grain filling under terminal drought, resulting in enhanced grain yield under post-anthesis drought stress (Borrell et al. 2000a, b; Jordan et al. 2012; Borrell et al. 2014a, b; Borrell et al. 2022). Stay-green loci also modify root architecture (Manschadi et al. 2006; Christopher et al. 2008), which is likely linked to the increased water accessibility displayed by stay-green genotypes during grain filling under end-of-season drought (Borrell et al. 2014b).

In general, RSA is highly plastic to environmental changes and heritability varies from low to high (Singh et al. 2011; Mace et al. 2012; Joshi et al. 2017). Despite the discovery of heritable natural variation in RSA, only limited genetic-based and co-location studies with stay-green QTL of sorghum roots have been identified via high-throughput phenotyping. Recent studies have found that a stay-green phenotype involves modifications in root architecture through *PIN* genes (Borrell et al. 2022). Furthermore in barley, stay-green and greater yield have been associated with deeper roots and delayed root senescence (Williams et al. 2022). Hence, a study in genetic analysis of RSA and co-location with stay-green is needed to further understand its regulatory mechanisms. Ethiopian sorghum landraces are particularly rich in genetic diversity and thus are very valuable to dissect the genetic basis governing the variability of RSA traits. Around 80% of sorghum in Ethiopia is cultivated

in the dry and hot lowlands where abiotic factors such as inadequate and erratic rainfall are among the major production constraints (Hulluka and Esele 1992; EIAR 2014). Still there is no single root system trait or even set of traits that will confer drought adaptation in this environment. The Ethiopian Biodiversity Institute (EBI) has a collection of more than 10,000 sorghum accessions from across the country. However, the RSA of this germplasm resource has not yet been characterised. This study aims to: (a) characterise the RSA of a subset of the collection of diverse Ethiopian sorghum landraces, and (b) undertake GWAS to identify genomic regions associated with RSA to inform molecular breeding strategies in the future.

Materials and methods

## Site for root phenotyping platform

The experiment was conducted in a polyhouse at Jimma University, College of Agriculture and Veterinary Medicine (JUCAVM), Jimma, Ethiopia (7° 33′ N latitude, 36° 57′ E longitude). The mean daily air temperature during the growing season in the polyhouse ranged from a minimum of 7.8 °C to a maximum of 40.8 °C. The polyhouse allowed transmission of 60% of incident photosynthetically active radiation.

Genetic materials

In total, 1,771 Ethiopian landraces were selected for root phenotyping (Online Resource 1). The landraces were selected systematically from the EBI (791 genotypes), Melkassa Agricultural Research Center (935 genotypes) and JUCAVM (45 genotypes) to include landraces from all sorghum producing regions and ecologies of the country (Fig. 1A and B). An individual from each accession was selected and self-pollinated for one generation to reduce residual heterozygosity and to increase seed quantity. A subset of 940 landraces were genotyped and used for GWAS. This panel of 940 landraces has been described previously by Menamo et al. (2021) and used for genetic diversity and genome environment association studies.

Experimental procedure and design

The experiment was conducted using a purpose-built high-throughput root phenotyping platform (Singh et al. 2011; Joshi et al. 2017) consisting of 500 pairs of perspex plates (40 cm high, 35 cm wide and 3 mm thick; Fig. 2). The centre of the pairs of perspex sheets were drilled and screwed together to maintain a 3 mm spacing and minimize bulging of the chambers during soil filling. The void between each
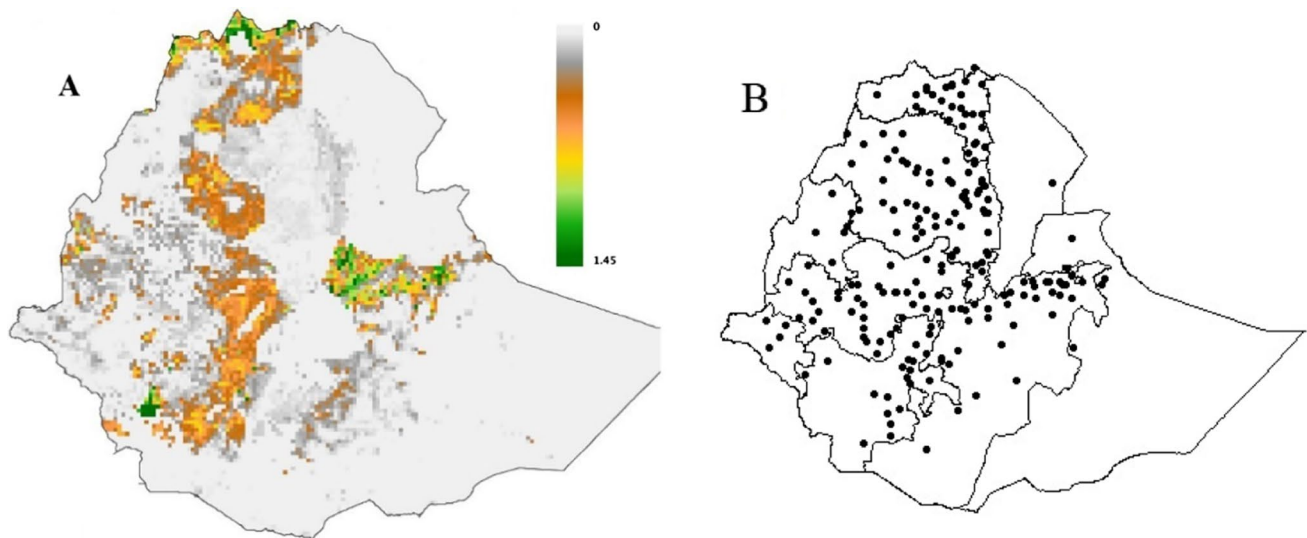
Fig. 1 Ethiopian geographical map **A** sorghum production areas from 1961 to 1990, and **B** sorghum collected from Woredas/districts (dots represent the collection sites) that were used in the current study
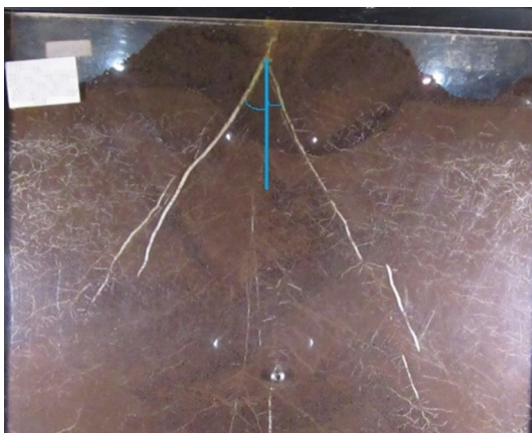
Fig. 2 Root angle was measured relative to the vertical line at the 5-leaf stage

pair of plates was filled with red soil (Online Resource 2A). Fifty root chambers were placed vertically into slots in each of 10 large metal tubs (Online Resource 2C). The tops of the chambers in each tub were covered with black polycarbonate covers to exclude light from the soil and roots, but leaving 5 cm long slits for the seedlings to emerge. Prior to planting, chambers were watered to field capacity and left until soil water saturation. Three seeds were planted in the centre of each chamber on one side of the Perspex with the embryo angled towards the base (Online Resource 2B), then thinned to one plant per chamber after emergence. The seeds were treated with ApronStar 42WS fungicide before sowing. Plants were watered one or two times per week, depending on the environmental conditions. Plant nutrients were applied in the form of diammonium phosphate (DAP, 1.5 kg) and urea (1 kg) across the 500 chambers, i.e. equivalent to 100 kg/ha urea and 150 kg DAP for a population of 50,000 plants per hectare. DAP was applied during sowing in a granular form and urea when the plants reached the 3rd leaf stage in liquid form, applied equally in each chamber.

A total of 1840 landraces were phenotyped across 13 independent experimental runs, with each run consisting of 500 chambers (10 tubs with 50 root chambers per tub). However, 1,771 landraces were used for analysis due to non-germinated accessions (69 genotypes) were removed. In each run, 160 genotypes were included, of which 140 genotypes were replicated three times and 20 check genotypes were replicated four times. The 20 check genotypes were common to all experimental runs enabling co-analysis of all entries across all runs in a single analysis. The 20 genotypes that have been included in all runs are "stable". These were tested in the first three runs to ensure their stability. The experimental runs had two blocks of ten tubs (five tubs in each block) and arranged in a row column design; columns being represented by the tubs and rows by the root chamber position within each tub.

Collection of phenotypic data

Images of the root structure were taken on both sides of each chamber using two digital cameras (CANON SX610 HS) remotely operated through Wi-Fi by tablets in order to synchronize the imaging on both sides. The images were used to determine the root angle (RA) relative to the vertical plane (Fig. 2). RA was taken from the first flush of nodal roots at a distance of 2 cm from the base of the plant (Singh et al.

2011) using *Opengelphoto.tcl* software (www.activestate.com/activetcl). The recorded root angle for each plant was the mean of four observations (left and right side of each plant for both sides of the chamber). After root imaging, each chamber was laid flat, the top perspex plate removed, and then root length (the longest 1st flush nodal root) was measured. Root numbers per plant were counted after soil was washed from the roots with tap water. Root samples were air dried on blotting paper, then oven-dried at 60 °C for 3 days to determine root dry mass (g/plant). Additionally, shoot traits were included to provide important information regarding impact on plant size. Leaf length and width (cm) were measured on fully expanded leaves (2nd, 3rd, 4th and 5th leaves) when the plants exhibited five fully expanded leaves. Leaf area (cm^2) was determined by multiplying the maximum leaf length and width, and by including a shape factor of 0.69 (Lafarge et al. 2002). The shoot length (cm) was measured from the base of the plant (soil level) to the ligule of Leaf 5. The shoot diameter of the wider side was measured 2 cm above the top of the chamber (soil surface) using digital callipers. Just before imaging the roots, shoots were cut at the base of the plant and subsequently dried. Shoot dry mass (g/plant) was determined after oven drying at 60 °C for 3 days.

Total genomic DNA extraction and genotyping

Samples of four individual leaves were collected from 15-day-old plants grown in small pots in a greenhouse and stored at −20 °C in 96 well plates for 24 h. The leaf samples were freeze-dried and sent to DArT (www.diversityarrays.com) for DNA extraction. The DNA samples were then genotyped using DArTseq as previously described in Tao et al. (2020). This involves complexity reduction of the genomic DNA to remove repetitive sequences using methylation sensitive restriction enzymes prior to sequencing on Next Generation sequencing platforms. The sequence data generated were then aligned to version v3.1.1 of the sorghum reference genome sequence (McCormick et al. 2018) to identify SNPs (Single Nucleotide Polymorphisms).

Root phenotype data analysis

The single and combined data for all experimental runs were analysed using a restricted maximum likelihood (REML) mixed model allowing for spatial variation across the experiments using "asreml-R" R package (Gilmour et al. 2006): REML mixed model: $y = X\tau + Zu + e$; where: y = the measured data for each trait and each run; τ = the fixed effects (genotypes) in the trial; X = the design matrix for the fixed effects (row and column); u = random effects for each analysis; Z = the design matrix for the random effects and e = residual error effects. Best linear unbiased estimates

(BLUEs) were analysed for each genotype and trait using linear mixed model (REML) spatial analysis by asreml-R package in R software. Repeatability (H^2) was estimated as $H^2 = 1 - uBLUE/2\sigma^2_g$ (Cullis et al. 2006), where uBLUE is the mean variance of a difference BLUE, and σ^2_g is the genotypic variance component. The BLUE for each landrace was used to perform the pair-wise Pearson's correlation analysis among the traits. The analysis was conducted using R software's corrplot package (Wei et al. 2017).

Genome wide association study and co-location

A total of 54,080 SNP markers were identified across the 940 landraces. The data set was further filtered to exclude SNPs with MAF < 0.01 or missing values > 25%, leaving a final data set of 25,634 SNP markers (Menamo et al. 2021). The remaining missing values were inferred using the Beagle 5.0 software package (Browning et al. 2018). Best linear unbiased estimates (BLUEs) for all traits were used in the genome-wide association study (GWAS) analysis using the software BLINK (Huang et al. 2018). The SNP data set was initially filtered for minor allele frequency (MAF) of 0.01. Kinship was calculated as described by Van-Raden (2008). Q–Q plots of *p*-values were examined to determine how well the models accounted for population structure and familial relatedness. The genetic type I error calculator (GEC) was used to determine significance thresholds for trait-marker associations (Li et al. 2012).

The QTL detected in this study for each RSA trait was compared with 392 RSA QTL in 12 previously identified studies. Additionally, co-located QTL was also identified between root angle and stay-green QTL studied under post-flowering water-stress conditions using Sorghum QTL Atlas database (Mace et al. 2019). The data collection for stay-green or green leaf area was considered between the flowering and maturity stage only. A 1-cM window was used to identify QTL co-location based on the genetic distances from the sorghum consensus map (Mace et al. 2009; Menamo et al. 2021). Enrichment analysis of QTL co-location was undertaken using Chi-square test with R software ("chisq.test" procedure).

Results

Substantial variation in root system architecture (RSA) traits

The correlation among the experimental runs for each trait was first analysed (Online Resource 3) using the 20 genotypes repeated across the experimental runs. In almost all cases, all traits were positively correlated across experimental runs (Online Resource 3), with root angle showing

the highest correlations ($r = 0.57$ to 0.94) among all 11 experimental runs; and root to shoot ratio showing the largest range in correlation values among the experimental runs ($r = 0.02$ to 0.76).

Significant variation was observed for all traits measured across all genotypes (Table 1) with an approximately normal distribution (Online Resource 4), indicating the quantitative nature of the traits. The nodal root angle varied from 16.6° (Fig. 3A) to 26.4° (Fig. 3B), with an average of 20.9°. Both root number and length varied considerably, with number varying from 8.0 to 12.0 (average = 9.8), and length varying from 33.3 to 56.0 cm (average = 45.0 cm). The repeatability (H^2) was generally high across traits, with nodal root angle having the highest repeatability of 78%. The phenotypic best linear unbiased estimations (BLUE) of root traits are detailed in Online Resource 5.
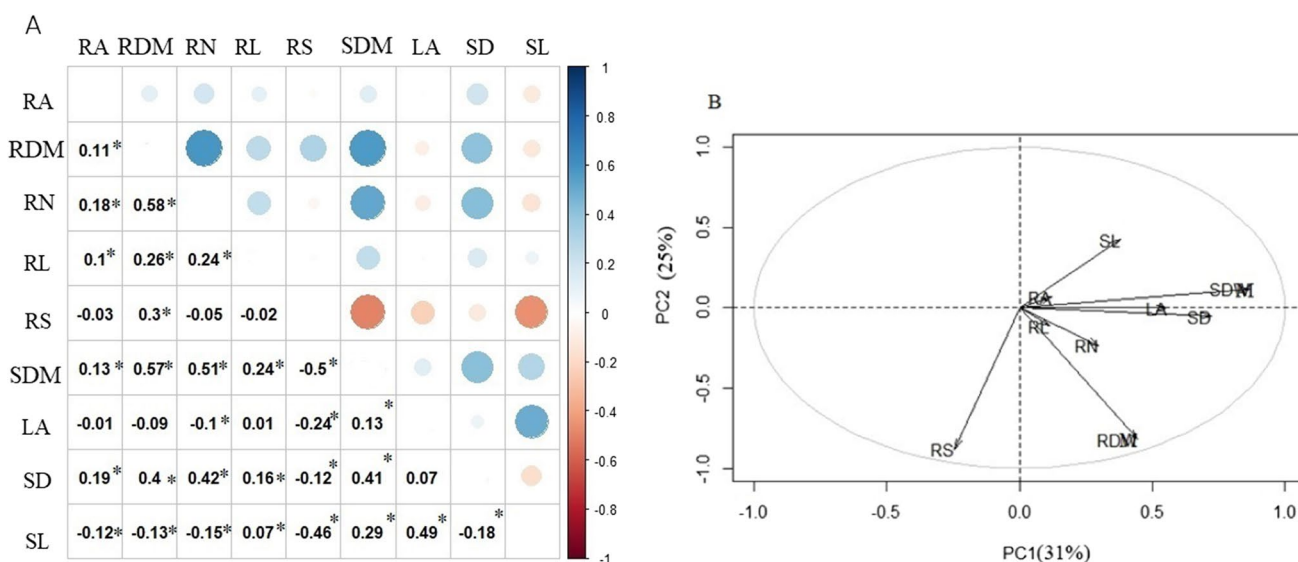
Correlation among RSA and shoot traits

Root angle (RA) was poorly correlated with the other root and shoot traits measured (Fig. 4A and 4B). In contrast, root number (RN), root dry mass (RDM) and root length (RL) grouped together (Fig. 4B), indicating a positive correlation among them, with the strongest correlation between RN and RDM. Shoot dry mass (SDM) also showed a strong positive correlation with RDM and RN, but negative correlation with root to shoot ratio. Shoot diameter was moderately correlated with SDM, RDM and RN.

Genome-wide association for RSA traits

GWAS was conducted to identify trait-marker associations, where Q-Q plots showed that the observed p-values were close to the expected p-values for all traits with minimal deviation

Table 1 Phenotypic variation of all traits based on best linear unbiased estimations (BLUE) (calculated as genotype performance across the experimental runs)

Traits	Unit	Max	Min	Mean	G. Var	Err Var	H^2	Sign
RA	degree	26.4	16.6	20.9	5.5	13.2	78.9	**
RDM	g	0.55	0.34	0.46	0.10	0.10	18.20	**
RN	number	12.0	8.0	9.8	0.6	2.0	24.9	**
RL	cm	56.0	33.3	45.0	9.1	15.7	33.7	**
RS	ratio	0.38	0.25	0.31	0.1	0.1	24.3	**
SDM	g	1.68	1.31	1.49	0.1	0.1	17.2	**
SL	cm	16.9	9.6	12.9	2.1	2.0	49.7	**
SD	mm	7.2	5.4	6.5	0.2	0.6	20.7	**
LA	cm^2	38.8	10.42	22.5	20.5	17.5	52.3	**

RA = root angle, RDM = root dry mass, RN = root number, RL = root length, RS = root shoot ratio, SDW = shoot dry mass, SL = shoot length, SD = shoot diameter and LA = leaf area.

**Significant level at $p < 0.01$

Fig. 3 Structure of nodal roots visible on the glass surface of root chambers **A** narrow root angle (JUS173657 = 16.6°) and **B** wide root angle (JUS171368 = 26.4°)

A

B

Fig. 4 Correlations of root system architecture traits with shoot traits **A** Correlation plots among key root and shoot traits. The colour indicates the correlation values and size of the circle shows the level of significance (large circle highly significant and small/non circle non-significant). **B** Bi-plot of root system architecture traits. RA = root angle, RDM = root dry mass, RN = root number, RL = root length, RS = root to shoot ratio, SDM = shoot dry mass, LA = leaf area, SD = shoot diameter and SL = Shoot length

observed (Online Resource 6). A total of 22 SNP markers were significantly (p-value ≤ 8.09E-05) associated with RSA traits (Table 2 and Fig. 5). Of the 22 significant SNPs for RSA traits, seven, seven, four and four were associated with root angle, root number, root length and root dry mass, respectively. Similar to the correlation result, none of the significant markers showed pleiotropy effects in root vs shoot traits. The significant markers were distributed over all chromosomes except chromosome SBI-10, with numbers varying from two on chromosomes SBI-04 and SBI-05 to ten on chromosome SBI-01. Of the 22 QTL associated with four RSA traits, 15 co-located with previously identified RSA QTL based on a 1-cM window, while the remaining seven were unique to this study (Online Resource 7).

Co-location of root angle QTL with previously identified stay-green QTL

Six out of the seven root angle QTL (6/7 = 85.7%) co-located with previously identified QTL for drought resistance traits (Online Resource 8), including green leaf area after anthesis (Haussmann et al. 2002; Srinivas et al. 2009; Sabadin et al. 2012) and total number of green leaves after anthesis (Reddy et al. 2014), with a significant genome enrichment (p-value = 0.011).

Table 2 Summary of significant single-nucleotide polymorphisms (SNPs) for the four RSA traits at seedling stage

Traits	QTLs	Chr	POSv3.01	p-values
RA	QERA3.1	SBI-03	70,640,280	6.5E-06
	QERA4.2	SBI-04	52,404,340	4.85E-12
	QERA5.3	SBI-05	7,621,214	1.57E-08
	QERA6.4	SBI-06	47,152,373	4.48E-10
	QERA6.5	SBI-06	49,330,161	1.4E-06
	QERA7.6	SBI-07	53,856,839	6.06E-05
	QERA8.7	SBI-08	47,689,441	1.22E-08
RN	QERN1.1	SBI-01	76,107,179	3.35E-05
	QERN2.2	SBI-02	19,789,147	3.21E-06
	QERN2.3	SBI-02	60,605,417	7.76E-05
	QERN2.4	SBI-02	73,281,144	3.91E-05
	QERN6.5	SBI-06	54,403,226	9.5E-05
	QERN8.6	SBI-08	53,766,348	2.62E-05
	QERN9.7	SBI-09	49,867,350	1.54E-07
RL	QERL1.1	SBI-01	66,615,979	9.22E-09
	QERL3.2	SBI-03	2,540,858	1.96E-06
	QERL3.3	SBI-03	10,025,278	3.64E-05
	QERL5.4	SBI-05	53,377,135	9.78E-05
RDM	QERDM1.1	SBI-01	27,268,290	1.24E-05
	QERDM1.2	SBI-01	65,740,047	9.54E-10
	QERDM3.3	SBI-03	59,395,700	1.02E-07
	QERDM3.4	SBI-03	60,530,147	1.04E-09

RA = root angle, RN = root number, RL = root length, RDM = root dry mass, Chr = chromosome, POSv3.01 = marker/QTL position on chromosome
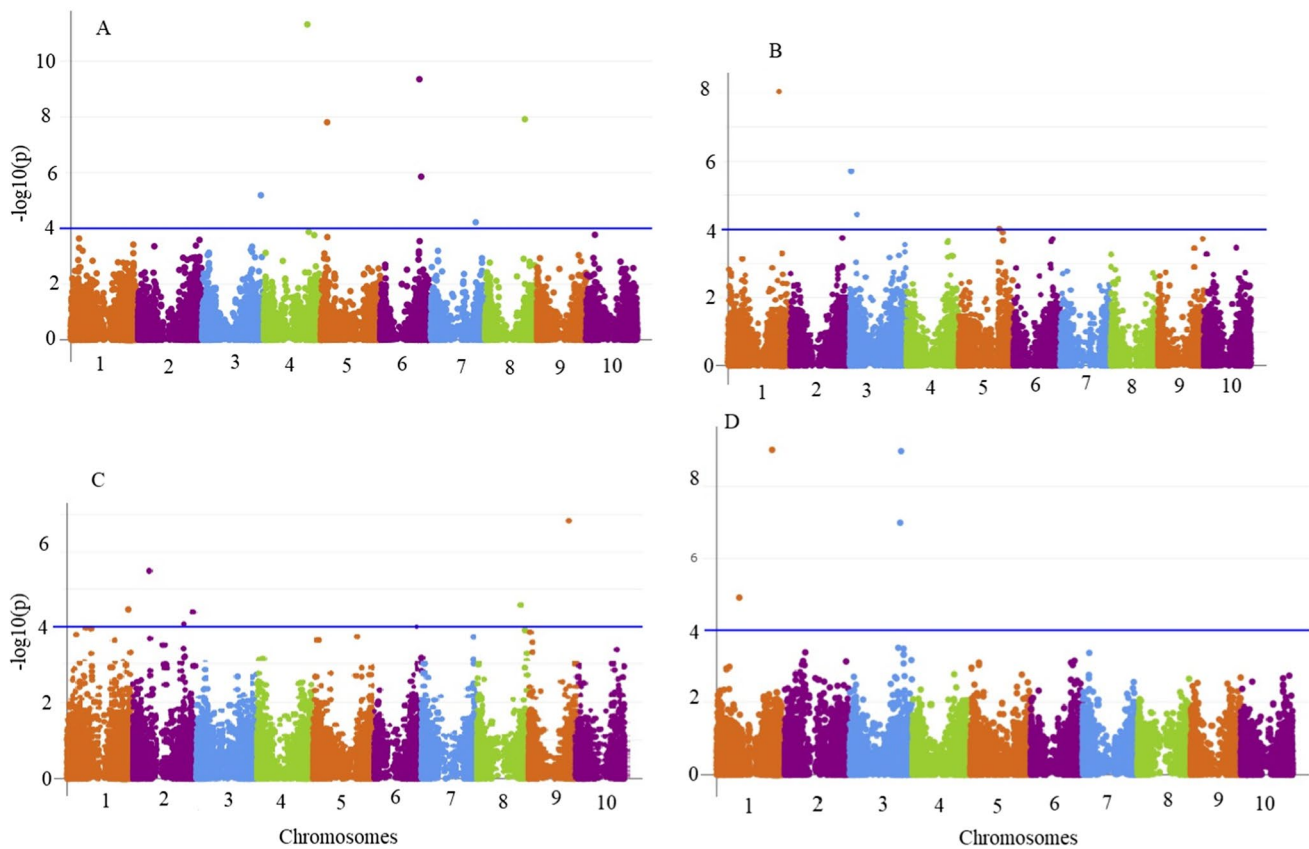
Fig. 5 Genome wide association of four key root architecture traits using 940 diverse sorghum germplasm **A** root angle, **B** root length, **C** root number, and **D** root dry mass. Horizontal line indicates significance threshold ($p = 8.09E-5$)

Discussion

Root system architecture screening in sorghum differs from other cereal crops

Individual root growth patterns, including tropism, branching and elongation rates are determined by underlying genetics, contributing to the architecture of the overall system (Rich et al. 2020). The passage of roots through the soil is also constrained by soil physical and chemical conditions, with many biotic and abiotic factors contributing to the final architecture, including gravity, temperature, water, oxygen and nutrient availability, and soil biota (Rich and Watt 2013). Changes to the mature (or near-mature) architecture will also be driven by the timing and initiation of lateral and adventitious roots under the control of nutrient and hormonal signals (Atkinson et al. 2014). The efficiency of soil water uptake is determined by the root system because roots are usually the site of the greatest resistance in the pathway for liquid phase movement of water through the soil–plant-atmosphere continuum (Kramer and Boyer 1995).

High-throughput phenotyping of RSA traits is of major importance to elucidate the underlying genetic control and to incorporate such traits in breeding programms through marker-assisted selection. Recently designed high-throughput root phenotyping platforms are an opportunity for further improvement of crop productivity, particularly in moisture-limited environments. Various high-throughput RSA phenotyping systems have been designed to screen seminal roots at the seedling stage i.e. clear pot and growth pouch methods in wheat (Richard et al., 2015) and clear-pot phenotyping method in barley (Robinson et al., 2016). However, these methods are less relevant for sorghum since seminal roots do not constitute the major proportion of the mature root system. Hence, Singh et al. (2010) developed a new root chamber-based method to screen nodal root angle in sorghum. While the assumption is that seedling screens for root architecture will be correlated with mature root systems, this is not always the case (Rich et al. 2020). However, it was reported that narrow primary root angle was found to be associated with deep field roots, providing some evidence that screening for narrow root angle at the seedling stage is worthwhile (Rich et al. 2020).

Correlations between seedling and mature root traits in the field have been shown to be inconsistent from site to site and year to year in wheat (Rich et al. 2020). This

could be due to the influence of soil physical, chemical, and edaphic factors on root traits. However, Singh et al. (2012) reported that root angle of young sorghum plants can be a useful selection criterion for specific drought adaptation. The Singh et al. (2012) study also reported that relationships between root angle screening and mature field traits were best achieved using soil rather than germination paper and agar in the initial screening. Hence, exploiting RSA variability and identifying genomic regions controlling the variability could open avenues for sorghum breeders to develop more drought adapted varieties through manipulating root architecture. In particular, the extent to which screening for root angle will help sorghum breeders to develop more drought adapted varieties will depend on the strength of the correlation between RA at seedling stage (6th fully expanded leaf stage) and the mature root system architecture. RSA may play a role in stay-green during post-flowering moisture-stress conditions. For example, narrow root angle increases access to water stored deep in the soil during the post-flowering period compared to wider root angles in wheat (Manschadi et al. 2006) and sorghum (Singh et al. 2012). Therefore, narrow root angle may contribute to a stay-green phenotype, although this will depend to some extent on genetics (e.g. canopy size), management (e.g. row spacing, fertilization) and environment (e.g. soil type, water availability).

Genetic variation for diverse RSA traits identified in Ethiopian sorghum

In this study, genetic materials were collected from all sorghum producing regions in Ethiopia to capture the genetic diversity. Hence, significant variability for RSA traits was identified. The study also revealed high repeatability and a broad range of variation for individual root traits, in particular, for root angle. Nodal root angle showed wider variation and higher repeatability than other root-related traits, suggesting that the variation observed in this trait was under genetic control. The high repeatability of root angle in this study (78%) is consistent with previous studies in sorghum (Mace et al. 2012; Joshi et al. 2017), which reported high repeatability in root angle measurements (73.7% and 96%, respectively), while Singh et al. (2011) also observed moderate repeatability (46.6%) in nodal root angle in sorghum. The presence of genetic variation with moderate to high repeatability suggests that nodal root angle could respond to selection in sorghum-improvement programmes.

Root angle is poorly associated with other traits

Drought adaptation is of particular interest because it depends upon components associated with both water supply (e.g. root traits) and water demand (e.g. canopy traits). Root structural traits are important sub-component traits for extracting water and nutrients from soil. These traits might contribute positively or negatively to drought adaptation in association with other traits. Therefore, understanding the interaction between component traits could be helpful for breeders to decide whether to make direct or indirect selection for better yield under drought conditions.

In this study, a poor association between root angle and other RSA traits was observed. Similar results have been observed previously in sorghum (Singh et al. 2011; Mace et al. 2012), wheat (Sanguineti et al. 2007) and barley (Robinson et al. 2018). The finding of weak correlations between root angle and plant size traits also agrees with the results reported by Singh et al. (2011), which found that traits associated with plant size (root mass, shoot mass, and leaf area) had a very low correlation with root angle in sorghum. These weak associations suggest that root angle and plant size traits might be controlled by different sets of genes. Furthermore, the incorporation of root angle screening into a sorghum breeding program would be helped if root angle was not associated with plant size because such an association might inadvertently result in selection for early vigour (Singh et al. 2011). Therefore, nodal root angle selection could be one strategy for selection in the improvement of drought adaptation.

Genetic control of RSA traits

GWAS has proven to be a powerful tool to understand the genetic control of quantitative traits in sorghum and other species (Huang et al. 2012; Morris et al. 2013; Tao et al. 2020, 2021). Using this approach, this study identified 22 QTL for key RSA traits (root angle, length, dry mass and number) in Ethiopian sorghum germplasm. Among RSA QTL, 15 were co-located with RSA QTL reported in previous sorghum studies using different genetic materials. These QTL were repeatedly reported with different genetic backgrounds, possibly indicating hotspot genomic regions for root development. The seven unique QTL potentially represent novel genetic loci for RSA from Ethiopian germplasm.

The root traits investigated in this study have previously been associated with grain yield under water stressed conditions in different cereal crops (Mace et al. 2012; Ali et al. 2016; Robinson et al. 2018). Ali et al. (2016) reported that total root mass and root length in maize were significantly positively correlated with grain yield under water deficit

conditions in the field. A positive significant association between narrow root angle and grain yield was reported in sorghum by Mace et al. (2012).

Root angle QTL co-located with previously reported stay-green QTL

The majority of root angle QTL (5/7) in this study co-located with previously reported stay-green QTL under water-stressed conditions. For example, QERA7.6 co-located with a previously studied QTL (green leaf area 15 days after flowering) by Haussmann et al. 2002 who conducted the experiment during the post-rainy season at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). These authors reported that the season was ideal for evaluating stay-green under terminal moisture-stress conditions and the crop was dependent almost entirely on stored soil moisture. Similarly, QERA6.4 co-located with a previously studied 'green leaf area at maturity' QTL under post-flowering moisture-stress conditions (Srinivas et al. 2009). Additionally, QERA6.5 and QERA8.7 co-located with previously studied green leaf area QTL (QGLFA6.1 and QGLFA8.1, respectively) at 45 days after flowering under post-flowering moisture-stress conditions (Sabadin et al. 2012). The authors reported that one of these QTL (QGLFA8.1) explained the green leaf area variation by 14.46% and co-located with grain yield under water-stressed conditions. This is consistent with reports that stay-green QTL co-located with QTL for nodal root angle in sorghum (Mace et al. 2012) and seminal root angle in wheat (Christopher et al. 2018). These previous studies indicate that genotypes with narrow root angle can extract water from greater depth, thereby increasing access to water under drought, resulting in a stay-green phenotype. This suggests the importance of nodal root angle during post-flowering moisture-stress conditions.

Stay-green is an important drought-adaptation feature in cereal crops, especially when they are subjected to moisture stress after flowering. Stay-green (*Stg*) loci reduce canopy growth at flowering by modifying tillering, leaf number, and leaf size (Borrell et al. 2014a, 2014b). The decreased canopy area at flowering reduces pre-anthesis water demand, moving it from pre- to post-anthesis and therefore boosting water availability during grain filling and thus grain production. However, the increased post-anthesis water use of *Stg* QTL compared to RTx7000 was not solely a consequence of changes in temporal water-use patterns, as it more than compensated for the reduced pre-anthesis water use, resulting in increased total water use of 19 mm and 10 mm in the *Stg1* and *Stg3* NILs, respectively (Borrell et al. 2014a, 2014b). As water uptake under drought stress is supply-limited, these results indicate that the *Stg* QTL could access more water than RTx7000,

achieved either by better water extraction from the soil by roots or increasing the soil volume explored by the roots via deeper rooting or greater lateral spread.

Importantly, the majority of root angle QTL identified in this study were co-located with previously identified QTL for stay-green. This suggests that the stay-green phenotype is a consequence of not only shifting water from pre- to post-anthesis (due to water savings from reduced pre-anthesis canopy size), but of increased water uptake throughout crop growth due to modification of root angle. This finding has significant implications for crop improvement programmes because it highlights that root system architecture contributes to an enhanced stay-green phenotype in sorghum, presumably by impacting elements of water supply in water-limited scenarios. This aligns with the findings of Liedtke et al. (2020) which suggest that factors other than canopy size (e.g. roots) have an important role in the expression of a stay-green phenotype in grain sorghum.

Conclusions

Ethiopian sorghum genotypes have unexploited variation in root system architecture traits which could contribute to sorghum breeding programs for drought adaptation. Of the root system architecture traits included in this study, root angle likely has an important role in shaping root architecture and root distribution in deeper soil layers, thereby enhancing water uptake from depth. Several diverse landraces with narrow and wide root angles have been identified and these landraces could be used as a source of valuable RSA alleles for varietal development under drought tolerance. Root angle had high repeatability (H^2) and weak association with other traits. Furthermore, the poor correlation of RA with shoot traits suggests that selection for RA should not inadvertently result in selection for early vigour. Root angle QTLs also co-located with previously identified stay-green QTL. Hence, further investigation is required to better understand the components of RSA that contribute to the water supply component of stay-green and genotype by management interaction strategies for target environments. The co-localization of the significant GWAS RSA traits in this study with RSA traits from previous studies (root angle and root length) could indicate the target regions for RSA. Introgressing these QTLs effectively into elite sorghum genotypes via molecular breeding strategies should enhance productivity of sorghum under moisture stress conditions. Furthermore, the root angle screening system highlighted in this paper is simple and inexpensive, indicating its utility for screening large numbers of sorghum genotypes, with potential for screening other cereal crops with further modification.

Availability of data and material All data are publicly accessible. Genotypic list with geographical information, SNP genotype and phenotypic data are available from Digital Repository and provided in Online Resource.

Code availability Not applicable.

Declarations

Conflict of interest Authors have declared that no competing interests exist.

Ethical approval Not applicable.

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