ORIGINAL PAPER

QTL for nodal root angle in sorghum (*Sorghum bicolor* L. Moench) co-locate with QTL for traits associated with drought adaptation

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Abstract Nodal root angle in sorghum influences vertical and horizontal root distribution in the soil profile and is thus relevant to drought adaptation. In this study, we report for the first time on the mapping of four QTL for nodal root angle (*qRA*) in sorghum, in addition to three QTL for root dry weight, two for shoot dry weight, and three for plant leaf area. Phenotyping was done at the six leaf stage for a mapping population (*n* = 141) developed by crossing two inbred sorghum lines with contrasting root angle. Nodal root angle QTL explained 58.2% of the phenotypic variance and were validated across a range of diverse inbred lines. Three of the four nodal root angle QTL showed homology to previously identified root angle QTL in rice and maize, whereas all four QTL co-located with previously identified QTL for stay-green in sorghum. A putative association between nodal root angle QTL and grain yield was identified through single marker analysis on field testing data from a subset of the mapping population grown in hybrid combination with three different tester lines. Furthermore, a putative association between nodal root angle QTL and stay-green was identified using data sets from selected sorghum nested association mapping populations segregating for root angle. The identification of nodal root angle QTL presents new opportunities for improving drought adaptation mechanisms via molecular breeding to manipulate a trait for which selection has previously been very difficult.

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Introduction

Sorghum (*Sorghum bicolor* L. Moench) is a dryland cereal, often grown in areas with marginal rainfall. The growing instability of seasonal rainfall patterns and temperature conditions has prompted greater attention towards the genetic improvement of traits that maximise grain yield in water-limited environments. Grain yield in such environments is closely linked to post-anthesis water use (Turner 2004) and hence to water availability at anthesis, which can be increased by either reducing pre-anthesis water use or increasing the total amount of water available to the crop (Hammer 2006; Manschadi et al. 2006). The quantum and temporal dynamics of water captured by the crop are partly determined by the ability of the root system to both gradually occupy the soil volume available and effectively

extract the available water in that volume. Genotypic differences for these traits have been reported for wheat (*Triticum aestivum* L.) (Manschadi et al. 2006) and are likely to involve traits associated with root system architecture. One such trait that has received particular interest across cereals is root angle in seedlings (Bengough et al. 2004; Hochholdinger et al. 2004; Kato et al. 2006; Manschadi et al. 2008). This trait can affect both horizontal and vertical exploration of the soil (Hammer et al. 2009; Kato et al. 2006; Oyanagi 1994), and hence potentially the ability of a crop to capture water. The importance of the root system to grain yield under drought has long been recognised (Richards 1991). However, root traits, including root angle, have had limited use in cereal crop improvement programs as selection targets for indirectly improving grain yield, partly because of the difficulty in measuring roots on a sufficiently large number of plants, particularly in field experiments, but also because of the limited information available on the genetic control of root traits.

Incorporation of root angle as a selection criterion in crop improvement programs requires a rapid and cost effective screening method with repeatable results. As sorghum produces only one seminal root and the major root system forms from nodal root axes, root angle needs to be measured on nodal roots, the first flush of which appears only when approximately five leaves have fully expanded (Singh et al. 2010). This requires screening with small soil-filled chambers to allow plants to grow for a few weeks (Singh et al. 2010, 2011). Phenotypic screening for root angle in sorghum is thus less efficient than for crops like wheat and maize (*Zea mays* L.), for which root angle can be measured at a much earlier growth stage, thus allowing the use of considerably smaller chambers (Hochholdinger et al. 2004; Hund et al. 2009; Manschadi et al. 2008). Seedling root angle tends to have medium to high heritability and is not related to plant size in both sorghum (Singh et al. 2011) and durum wheat (*Triticum turgidum* L. var. *durum*) (Sanguineti et al. 2007). Hence, seed size, which affects seedling vigour (López-Castañeda et al. 1996; Manga and Yadav 1995), is unlikely to affect root angle. The lack of association between plant size and root angle could allow screening for root angle in hybrids via screening of parental inbred lines. Such an approach, however, is complicated by the existence of significant specific combining ability (SCA) for the trait (Singh et al. 2011). This SCA, along with the logistics involved in the phenotypic screen for root angle, suggests that improved knowledge of the genetic determinants of nodal root angle, through the identification of genomic regions associated with this trait, could allow for more targeted selection approaches, such as marker-assisted selection. Such selection methods are required by breeding programs to predict genotypes with desirable variation when selecting breeding material prior to field evaluation.

The aims of this study were to map QTL for nodal root angle in sorghum at the leaf six stage and assess potential relevance of the trait for improving drought adaptation via marker-assisted selection. To achieve that, in this study we (1) identify QTL for nodal root angle and plant size using a sorghum mapping population, (2) validate these QTL across a range of diverse sorghum germplasm, (3) demonstrate that the nodal root angle QTL are associated with variation in yield performance in a subset of the mapping population, (4) project the QTL onto the sorghum consensus map to determine where they are located and identify a co-location with stay-green QTL and (5) explore the link between root angle and traits associated with the stay-green drought tolerance mechanism using a subset of sorghum nested association mapping (NAM) populations (Jordan et al. 2011) based on parental lines with contrasting root angle.

Materials and methods

Genetic material

Two different sets of germplasm were used in the experiments measuring root angle. The first set included a subset of 141 F_6 recombinant inbred lines (RILs) that were developed by single seed descent from a cross between inbred lines B923296 (narrow angle relative to a vertical plane for first flush of nodal roots) and SC170-6-8 (wide angle for first flush of nodal roots) (Fig. 1). The second set comprised 44 diverse inbred lines (ESM Table 1) that are
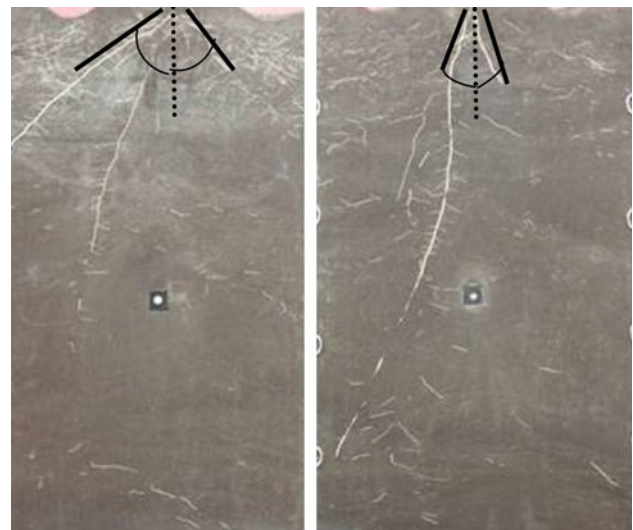
Fig. 1 Nodal roots, visible on the glass surface of root chambers, for the two parents of the RIL mapping population: SC170-6-8 (*wide angle*, *left* panel) and B923296 (*narrow angle*, *right* panel). *Thick solid lines* represent first flush of nodal roots, *dotted line* the *vertical plane*, and the *arc* the estimated root angle

used by the sorghum breeding program of the Department of Employment, Economic Development and Innovation (DEEDI). These lines included a sample of diverse germplasm originating from the USA, Africa, Asia, and Australia, including parental lines of several mapping populations and are a subset of the diverse set detailed in Mace et al. (2008).

Experimental design

Experiments on both the RIL population and the set of diverse inbred lines were conducted in a naturally lit, temperature controlled glasshouse with day/night temperature of 28/23°C at the University of Queensland, Brisbane, Australia. Individual plants were grown in specially designed root observation chambers until the sixth leaf had fully expanded (Singh et al. 2011). Each chamber was 50 cm high, 45 cm wide, and 3 mm thick (Fig. 1) and consisted of two transparent perspex sheets, held in place by fold back paper clips and separated on three sides by 3 mm thick rubber. Chambers were filled with 1,090 g of black-coloured, fine sandy soil each and stacked vertically in black tubs. Each tub contained 12 chambers, separated by wooden spacers of 9 mm and had drainage holes in the bottom. There were nine tubs (108 chambers) in total. Chambers in each tub were wrapped together with black plastic sheeting to exclude light and were watered to field capacity before planting. Seeds were pre-germinated in a petri dish at 28°C for 2 days and two germinated seeds with emerged plumule and radicle were planted in the centre of each chamber, before thinning to one plant 2 days after establishment. A complete hydroponic solution was applied once a day to all plants to provide enough nutrients for the growing plants (Singh et al. 2011). Experiments were concluded when six leaves had fully expanded.

Experiments were laid out as a partially replicated row (36) and column (3) spatial design with a number of runs. The experiment for the RIL population consisted of three runs, each run containing 68 genotypes with a single replication, two runs containing 17 genotpes with 2 replicates and a single run containing 16 genotypes with 2 replicates and 1 genotype with 3 replicates. The remaining 6 chambers were used for border plants. The 44 diverse inbred lines, along with 30 hybrids (Singh et al. 2011), were grown in two runs, which contained 52 genotypes with one replication, 22 with two replicates, and 12 border plants. In both experiments, the genotypes that were replicated and the randomisation of the genotypes differed across runs in order to optimise the trial layout. For the RIL population experiment, a total of 174 genotypes were only in a single run, 37 genotypes were in two runs and 2 genotypes were in all three runs. For the diverse set of 74 lines and hybrids, all 74 genotypes were replicated across runs.

Phenotyping of key shoot and root traits

Plants were harvested when six leaves had fully expanded. Prior to harvest, leaf area per plant was estimated by summing individual leaf size, obtained by in situ measurement of leaf length and width, multiplied by a shape factor of 0.69 (Lafarge and Hammer 2002). At harvest, the shoot of each plant was cut off at the base of the stem and shoot dry weight was determined after drying at 60°C for 3 days.

After removing the shoot, the root system that was visible through the perspex was scanned on both sides of each chamber using a flatbed scanner (HP Scanjet 4670). After scanning, roots were washed from the soil and root dry weight was determined after drying at 60°C for 3 days. The scanned images were used to determine the encompassing angle, relative to the vertical plane, of the first flush of nodal roots at a distance of 2 cm from the base of the plant. Root angle for a plant was the mean of four observations (two angles on each of two sides per chamber).

Statistical analysis of phenotypic data

Data were analysed for each of the experiments using a REML mixed model allowing for all possible sources of variation (Gilmour et al. 1997; Butler et al. 2007). Predicted values were calculated for each of the four traits, namely leaf area, shoot dry weight, root dry weight and root angle.

The standard representation for a mixed model for each trait within each experiment may be written as

$$\mathbf{y} = \mathbf{X}\tau + \mathbf{Z}\mathbf{u} + \mathbf{e}.$$

The vector $\mathbf{y}$ represents the measured data for each trait and each run, in the case of the RIL data there are three runs and for the inbred lines there are two. The vector τ represents all the fixed effects in the trial. For both experiments, there is a fixed effect for the mean value of each run and also means for each genotype and also the run by genotype interaction. Other fixed effects may be associated with any linear changes in experimental conditions along the columns or rows of each run of each experiment. The matrix $\mathbf{X}$ is the design matrix for the fixed effects. The vector $\mathbf{u}$ contains all the random effects for each analysis. These random effects are typically associated with different sources of error due to replication or blocking, they may also be used for any random effects due to the placement of the adjoining plots within each experiment and also possible column and row effects of each experiment. The matrix $\mathbf{Z}$ is the design matrix for the random effects. The vector $\mathbf{e}$ contains all the residual error effects.

The predicted values for genotype for each trait and each experiment were calculated as fixed effect Best Linear

Unbiased Estimators (BLUEs), which allow for information from all runs in an experiment as well and the run by genotype interaction. Heritabilities were calculated using a combination of the genetic variance and the average standard error.

A principal component analysis was carried out using the princomp function of the R statistical package (R version 2.12.0 2010; http://www.R-project.org). The predicted BLUEs from each of the four traits were combined into a four column matrix. This matrix was analysed with the princomp function in order to identify pairwise correlations for the four traits. Matrix decomposition was then used to create a set of loadings (eigenvectors of the correlation matrix) of length equal to the number of traits and a set of scores with length equal to the number of observations, thus enabling further investigation of the relationships between the trait observations (scores) and the trait correlations (loadings). Scores and loadings were plotted as a biplot.

DNA extraction, genetic map and QTL analysis

Total genomic DNA was extracted from the RIL population and the set of 44 diverse lines as described by DArT P/L (http://www.diversityarrays.com). The RIL population had previously been screened with DArT markers and a map consisting of 337 markers was constructed using the multipoint mapping software (http://www.multipoint.com) as described previously (Mace et al. 2009). The set of 44 diverse lines had also been previously screened with DArT markers (Mace et al. 2008).

Single marker analysis (SMA) and composite interval mapping (CIM) were conducted using QTL Cartographer for Windows v2.5 (Wang et al. 2010) on the four traits (root angle, root and shoot dry weight, leaf area) observed in the RIL population. Linkage between individual markers and each trait was initially evaluated using SMA, prior to analysis using CIM. Background markers for inclusion in the CIM model were selected by forward stepwise regression for each trait. The five most significant background markers were then used for analysis (default). The 'walking speed' was set at 2 cM and the 'window size' at 10 cM for CIM. A conservative permutation threshold at the 0.01 significance threshold was obtained for each trait using 1,000 permutations. 1-LOD and 2-LOD support intervals were determined, as described by Lander and Botstein (1989). The additive effects and percentage of variation explained (R^2) for all significant QTL were determined at their peak LOD values. QTLNetwork v2.0 (Yang et al. 2007) was used with default settings to search for epistatic effects among markers. The graphical representation of the map and identified QTL was created using MapChart software (Voorrips 2002).

In addition, 208 genome-wide DArT markers were used across the set of 44 diverse lines to identify significant associations between the DArT markers and the root angle phenotypic data using the association mapping population type in GenStat13 (VSN International, 2010). The eigen analysis relationship model was selected within the Single Trait Association Analysis and a threshold value of 1.5 on the $-\log_{10}(P)$ scale was set to identify significant marker–trait associations.

Cross species QTL homology for root angle

The homology of the nodal root angle QTL to previously identified root angle QTL in rice (*Oryza sativa* L.) and maize (*Zea mays* L.) was determined through BLAST analysis (Johnson et al. 2008) of the nucleotide sequences of flanking markers identified in rice (Norton and Price 2009) and maize (Omori and Mano 2007), using the genomics resources available at Phytozome (http://www.phytozome.net).

Single marker analysis to test the association between nodal root angle QTL and yield performance

The data for the yield analysis was extracted from a larger, analysed breeding trial from a single location (Dalby Box, QLD, 2010). A subset of lines (53) from the mapping population were selected for inclusion in the yield trials as part of the DEEDI applied sorghum breeding program based on their ability to be converted into cytoplasmic male versions and also to exclude lines with extreme height or phenology, which could confound yield results in these trials. In total, yield data for 53 individuals from the mapping population in hybrid combination with three tester lines (R986087, R993396, R995248) were analysed using SMA in R (version 2.12.0), together with DArT markers flanking the nodal root angle QTL, in order to determine whether the validated nodal root angle QTL were associated with variation in yield performance.

Cross study QTL comparison

In order to compare the location of the QTL identified in this study with QTL identified in previous studies, the individual QTL were projected onto the sorghum consensus map (Mace et al. 2009), following the projection strategy detailed in Mace and Jordan (2011).

Single marker analysis to test the association between nodal root angle QTL and the stay-green drought response

Following comparison of the location of the nodal root angle QTL with previously identified QTL for the stay-green drought tolerance response, seven backcross-derived

NAM populations were identified, which segregated for nodal root angle. A detailed description of the development of the NAM populations is provided in Jordan et al (2011). The seven non-recurrent parental lines were SC35C, Dorado, LR9198, SC108C, SC62C, RS29 and SC103-14E, all back-crossed to the elite recurrent parent R931945-2-2. All seven selected NAM populations have been previously screened with DArT markers (Jordan et al. 2011). In total, stay-green data for 339 individuals from the seven NAM populations (on average 48 individuals per population, ranging from 31 individuals from the LR9198 population to 59 from the SC35C population) were extracted from a number of larger breeding trials across years and locations. In total, stay-green data from the seven selected NAM populations were available from up to seven environments (five sites across 3 years, as detailed in Table 4), however, in any one environment only a subset of populations were grown. SMA was conducted in R (version 2.12.0), using DArT markers flanking the nodal root angle QTL in the seven selected NAM populations with the stay-green data across multiple environments.

Results

Phenotypic trait variation and correlations

The nodal root angle measured on the 141 RILs ranged from 14.5° to 32.3° (Table 1; ESM Figure 1). These extremes were close to the nodal root angle of parent lines B923296 (18.3°) and SC170-6-8 (32.3°) and indicated some minor transgressive segregation. Results for the parents compared well with values of 20.6° (B923296) and 35.7° (SC170-6-8) obtained in an earlier screening study (Singh et al. 2011), supporting the repeatability of these results. For traits related to plant size (root dry weight, shoot dry weight and total leaf area), extreme values of the observed range for the RILs were also close to the parental values (Table 1). Heritability for nodal root angle (73.7%) was considerably greater than for plant size traits, for which heritability ranged from 13.0 (root dry weight) to 20.4% (total leaf area) and 21.6% (shoot dry weight) (Table 1).

The principal components analysis of trait values determined that the first three principal components accounted for 69.9, 19.3 and 5.1% of total variation respectively for the 141 RILs (Fig. 2). Traits associated with plant size at the six leaf stage (root weight, shoot weight, total leaf area) tended to group together, indicating a high correlation among them. In contrast, nodal root angle at the six leaf stage grouped separately and was generally independent of plant size, as indicated by the near 90° angle among the directional vectors (Fig. 2).

Genetic linkage mapping

A total of 141 individuals, genotyped with 377 DArT markers, were used to construct the genetic linkage map for the RIL population. A total of 337 markers were mapped to seventeen linkage groups with a total map length of 2,259 cM. The linkage groups were assigned to sorghum chromosomes SBI-01 to SBI-10, according to recent nomenclature (Kim et al. 2005), with multiple linkage groups within chromosomes given a Roman numeral suffix to determine their order along the chromosome. This map
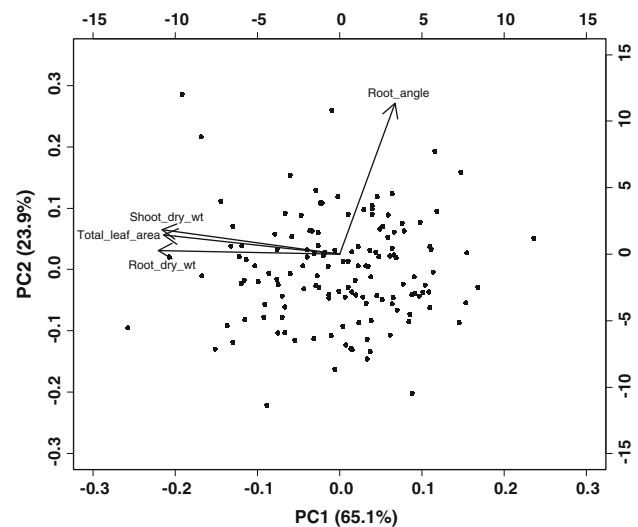
Fig. 2 Biplots generated from principal components analysis of traits measured for 141 RIL inbred sorghum lines. Directional vectors represent root and shoot traits and the points are genotype values

Table 1 Predicted mean and range of the RIL progenies, means of the two parents, genotypic variance and heritability for nodal root angle, root dry weight, shoot dry weight and total leaf area in the sorghum RIL population of B923296/SC170-6-8

Trait	RIL progenies		Parental lines		Genotypic variance	Heritability
	Mean	Range	B923296	SC170-6-8		
Nodal root angle (°)	23.14	14.60–32.30	18.30	32.30	20.60	73.7
Root dry wt (g)	0.47	0.39–0.60	0.53	0.37	0.01	13.0
Shoot dry wt (g)	0.55	0.42–0.76	0.68	0.34	0.01	21.6
Total leaf area (cm^2)	266	195–326	315	175	2335	20.4

showed strong alignment with a previously developed DArT consensus map of sorghum (Mace et al. 2009). The genome coverage of this map, in comparison to the sorghum consensus map, was 72% overall, ranging from 96% for SBI-10 to just 30% for SBI-06.

QTL analysis and validation

The results of the QTL analysis for the four traits in the RIL population are shown in Fig. 3 and the QTL statistics are summarized in Table 2. The significance levels for each trait, as determined by permutation analyses, are detailed in ESM Table 2. For nodal root angle, two significant QTL were detected. Both were located on SBI-05, with qRA2_5 identified as highly significant (LOD of 4.96) and qRA1_5 identified as significant (LOD of 3.69). In addition, two suggestive QTL were detected on SBI-08 and SBI-10. All four QTL were identified by SMA (data not shown). The four QTL explained 58.2% of the phenotypic variance for nodal root angle. Only one of these QTL (qRA1_5) showed a positive additive effect (Table 2), indicating that its alleles were derived from the narrow angled parent B923296. In contrast, the negative additive effects of the other three nodal root angle QTL (qRA2_5, qRA1_8 and qRA1_10) indicated that their alleles were derived from the wide angled parent SC170-6-8.

For root dry weight, one significant QTL was identified on SBI-05 (qRDW1_5), and two suggestive QTL were

identified on SBI-02 and SBI-08 (Table 2). SMA identified the QTL on SBI-02 and SBI-05. The three QTL for root dry weight explained 32.1% of the phenotypic variance. The significant QTL on SBI-05, qRDW1_5, co-located with a significant QTL for shoot dry weight, qSDW1_5 (Table 2). A second significant QTL for shoot dry weight was detected on SBI-01. Both QTL were identified by SMA and together explained 23.0% of the total phenotypic variance for shoot dry weight. Three QTL for total leaf area, one significant (qTLA1_8) and two suggestive (qTLA2_8 and qTLA3_8), were detected on SBI-08 (Table 2). SMA only detected the significant QTL, qTLA1_8. One of the suggestive QTL for total leaf area (qTLA2_8) co-located with qRDW1_8 (suggestive root dry weight QTL). In total, the three QTL for total leaf area explained 28.9% of the phenotypic variance. Hence, the phenotypic variance explained by the QTL detected for plant size traits (root and shoot dry weight, total leaf area) was lower than for nodal root angle, which was expected given the greater heritability of nodal root angle (Table 1). Six of these eight plant size QTL (qSDW1_1, qSDW1_5, qRDW1_2, qRDW1_5, qTLA1_8 and qTLA3_8) showed negative additive effects (Table 2), indicating that the QTL alleles were derived from SC170-6-8.

No significant epistatic interaction was identified among the main effect QTL for all four traits using CIM analysis in QTL Cartographer, or genome-wide using QTL Network.
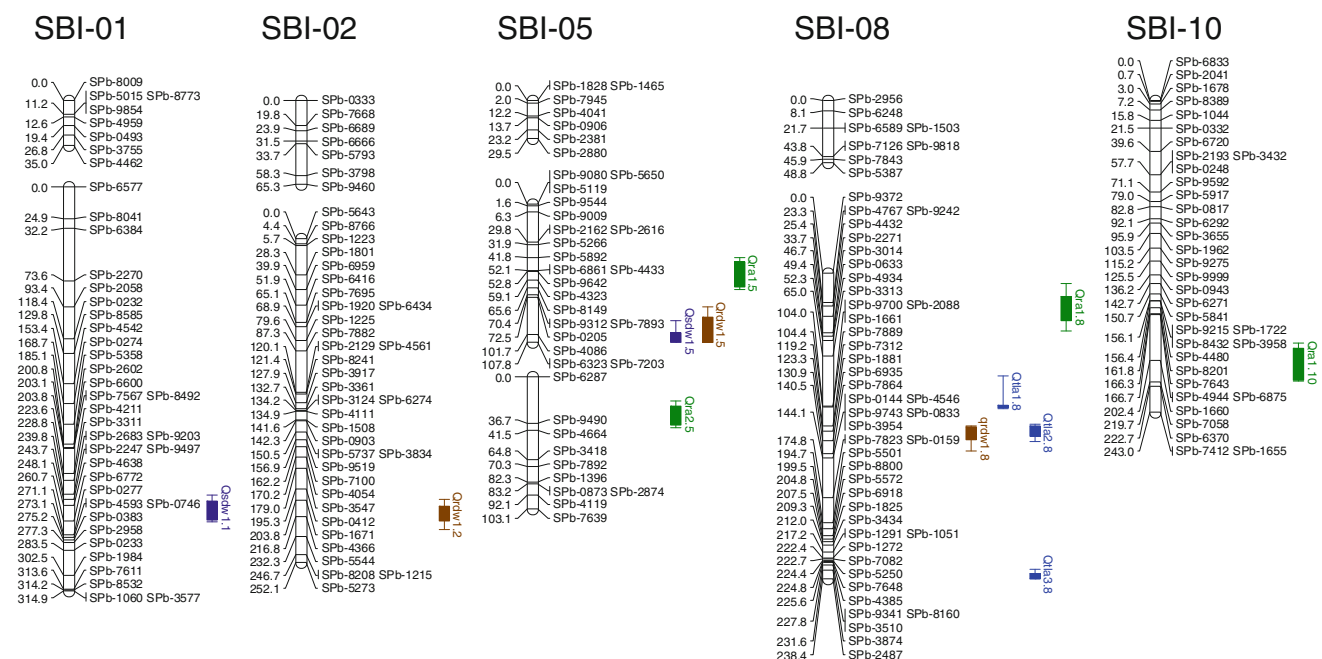
Fig. 3 Localisation of QTL for five traits measured in this study on a genetic linkage map based on the B923296/Sc170-6-8 RIL mapping population. QTL are represented by *bars* (1-LOD interval) and extended lines (2-LOD interval). QTL are colour-coded according to trait; nodal root angle *green*; root dry weight *brown*; shoot dry weight *dark blue*; total leaf area *light blue*

Table 2 Summary of QTL analyses for nodal root angle, root and shoot dry weight, and total leaf area, identified from 141 RILs, detailing the QTL location (Linkage Group, peak LOD location and the LOD-1 locations), marker interval, peak LOD score, phenotypic variation explained (R^2), additive effect and the approximate physical location in base-pairs (bp)

QTL ID	Trait	Linkage group	Peak LOD position (cM)	LOD−1 (left)	LOD−1 (right)	Marker interval	LOD	R^2	Additive effect	QTL start (bp)	QTL end (bp)
qRA1_5	Nodal root angle	SBI-05-II	51.8	45.8	65.1	SPb-5892/ SPb-4323	3.69	10.01	1.074	13,413,924	45,779,999
qRA2_5	Nodal root angle	SBI-05-III	34.0	23.0	38.7	SPb-6287/ SPb-9490	4.96	29.78	−1.825	55,169,818	55,690,833
qRA1_8	Nodal root angle	SBI-08-II	25.4	20.0	33.7	SPb-4767/ SPb-4432	2.68	6.72	−0.845	8,067,699	41,591,844
qRA1_10	Nodal root angle	SBI-10	208.4	196.7	218.4	SPb-1660/ SPb-7058	2.26	11.65	−1.064	57,494,967	58,573,866
qRDW1_2	Root dry weight	SBI-02-II	216.8	209.8	220.8	SPb-4366/ SPb-5544	2.82	13.05	−0.012	71,995,008	77,001,005
qRDW1_5	Root dry weight	SBI-05-II	101.7	88.5	107.7	SPb-4086/ SPb-6323	3.97	12.06	−0.012	50,729,356	51,538,433
qRDW1_8	Root dry weight	SBI-08-II	123.3	104.0	130.9	SPb-7889/ SPb-6935	2.63	6.97	0.011	48,269,890	50,970,340
qSDW1_1	Shoot dry weight	SBI-01-II	254.2	245.7	260.2	SPb-4638/ SPb-6772	3.54	14.59	−0.024	59,861,427	64,432,960
qSDW1_5	Shoot dry weight	SBI-05-II	107.7	100.5	107.7	SPb-4086/ SPb-6323	3.23	8.39	−0.019	50,729,356	51,538,433
qTLA1_8	Total leaf area	SBI-08-II	104.0	100.0	105.0	SPb-9700/ SPb-7889	3.96	10.56	−8.267	47,817,803	48,269,890
qTLA2_8	Total leaf area	SBI-08-II	123.3	119.2	127.3	SPb-7312/ SPb-1881	2.89	7.86	7.076	50,145,421	50,412,370
qTLA3_8	Total leaf area	SBI-08-II	237.6	234.0	238.0	SPb-3874/ SPb-2487	2.64	10.49	−7.041	54,169,320	54,249,166

Two hundred and eight genome-wide DArT markers were used in an association mapping study with the nodal root angle data measured across the set of 44 diverse lines. In total, 11 significant markers were identified within 10 genomic regions (ESM Table 3). Markers within the CI of three of the four previously identified root angle QTL were found to be significantly associated with the nodal root angle trait; qRA2_5 ($-\log_{10} P = 2.581$), qRA1_8 ($-\log_{10} P = 1.917$), and qRA1_10 ($\log_{10} P = 1.513$). Additionally, two significant marker–trait associations ($\log_{10} P = 2.33$) were identified less than 10 cM away from the fourth root angle QTL, qRA1.5. In addition to the significant marker–trait associations in, or very near, the CI of the four root angle QTL, an additional six significant associations were identified; two on SBI-01 and SBI-08, and one on SBI-03 and SBI-04.

Three of the four nodal root angle QTL detected in sorghum in the current study had homology to previously identified QTL for root angle in rice and maize, as determined through BLAST analysis (Johnson et al. 2008) of flanking markers. qRA1_5 and qRA2_5 in sorghum were homologous to a QTL for seminal root morphology on rice chromosome 11 (qSRM11) (ESM Figure 2A). A significant epistatic interaction was detected in the same rice mapping population between qSRM11 and one of two root angle QTL (qBA6 on chromosome 6) (Norton and Price 2009). Additionally, qRA1_8, was homologous to a QTL on maize chromosome 10 (Qra2nd10.03) for the angle of roots emerging from the second node (Omori and Mano 2007) (ESM Figure 2B).

Nodal root angle QTL and grain yield

To investigate a possible link between grain yield and nodal root angle, yield data for a subset of the RIL population, in hybrid combination with three different tester lines, was used to conduct SMA. Overall, markers within three of the four nodal root angle QTL were significantly associated with grain yield (Table 3) across two hybrid combinations. Markers within qRA1_5 were significantly

Table 3 Single marker analysis summary (*p* values) for the level of association between markers in the four nodal root angle QTL regions with grain yield in subsets of the RIL population in three different hybrid combinations

QTL	LG	RIL/hybrid combinations		
		R995248	R986087	R993396
qRA1_5	SBI-05-II	0.0228[+]	NS	0.0696[+]
qRA2_5	SBI-05-III	NS	NS	NS
qRA1_8	SBI-08-II	0.0602[+]	0.0780[+]	NS
qRA1_10	SBI-10	0.0087**	0.0415[+]	NS

NS not significant

$P > 0.1$; [+] $P < 0.1$; ** $P < 0.01$

($P < 0.05$) associated with grain yield in one hybrid combination (with the tester R995248) and suggestive ($P = 0.0696$) in a second hybrid combination (with the tester R986087-2-4-1). In both cases, the direction of the effect indicated that yield was increased in the presence of the narrow root angle allele of the *qRA1_5* QTL. Markers within qRA1_10 were significantly ($P < 0.05$) associated with grain yield in hybrid combination with two tester genotypes (R995248 and R986087). The same hybrid combinations showed suggestive associations with grain yield for the markers within *qRA1_8*.

Nodal root angle QTL and the stay-green drought response

The locations of the twelve QTL identified in the current study were projected onto the sorghum consensus map (Mace et al. 2009), onto which QTL from previous studies have also been projected (Mace and Jordan 2011). All four nodal root angle QTL identified in this study co-located with previously identified QTL for stay-green (Fig. 4). Specifically, the peak LOD location of all four QTL occurred within a stay-green QTL region. For *qRA1_5*, the peak location occurred within four previously identified QTL for stay-green (Crasta et al. 1999; Subudhi et al. 2000; Xu et al. 2000; Kebede et al. 2001). The peak location of *qRA2_5* occurred within one previously identified QTL for stay-green (Srinivas et al. 2009). For *qRA1_8* the peak location occurred within two previously identified QTL for stay-green (Haussmann et al. 2002), and the peak location of *qRA1_10* occurred within six previously identified QTL for stay-green (Crasta et al. 1999; Haussmann et al. 2002). Additionally, of the 6 new putative marker–trait associations identified via association mapping with the diverse set of 44 lines, 5 of these (83%) co-located with QTL for stay-green (ESM Table 3).

The putative association between nodal root angle and the stay-green drought response was further studied in

selected sorghum NAM populations (Jordan et al. 2011). Seven NAM populations were selected where the parents exhibited contrasting nodal root angle based on the results of the root angle screen across the 44 diverse lines. SMA was conducted for the stay-green trait across these populations and across multiple environments. Significant ($P < 0.05$) or suggestive ($P < 0.1$) marker–trait associations were identified in all four nodal root angle QTL regions (qRA1_5, qRA2_5, qRA1_8 and qRA1_10) in six of the seven NAM populations (Table 4) across multiple environments. For *qRA1_5*, the marker–trait association was significant or suggestive in all but one environment in which five (SC62C, Dorado, SC35C, SC103-14E, and RS29) of the populations were grown, whereas it was not significant across environments for the remaining two populations (SC108C and LR9198) (Table 4). In contrast, for *qRA2_5* the marker–trait association showed significance across all three environments for the Dorado NAM population and in one environment type for the SC35C NAM population, but showed no significance across environments for the remaining five populations. For *qRA1_8* and *qRA1_10*, the marker–trait association showed significance in four of the seven NAM populations. Two of these populations (SC103-14E and RS29) were consistently significant across environments for these two QTL.

Discussion

The difficulty of measuring root features in soil under field conditions has hindered both the genetic dissection of root traits and their use as a selection tool in crop breeding programs. The recent development of a high throughput, effective root screening method for sorghum (Singh et al. 2010) has facilitated the identification of genetic variation for nodal root angle. This study is the first in the literature to identify QTL for nodal root angle in sorghum.

Root angle phenotype is independent of plant size

This study determined that the heritability for nodal root angle (73.7%) was considerably greater than for plant size traits (ranging from 13% for root dry weight to 21.6% for shoot dry weight). Additionally, the heritability for nodal root angle as determined in this study was considerably greater than the 46.6% observed for the 44 diverse inbred lines in a previous study (Singh et al. 2011), but close to the value of 87.8% reported for a panel of 57 durum wheat accessions (Sanguineti et al. 2007). The consistency of the nodal root angle phenotype of parents across experiments and the high broad sense heritability (repeatability) indicate that nodal root angle is predominately influenced by genotype. This study also demonstrated, through principal
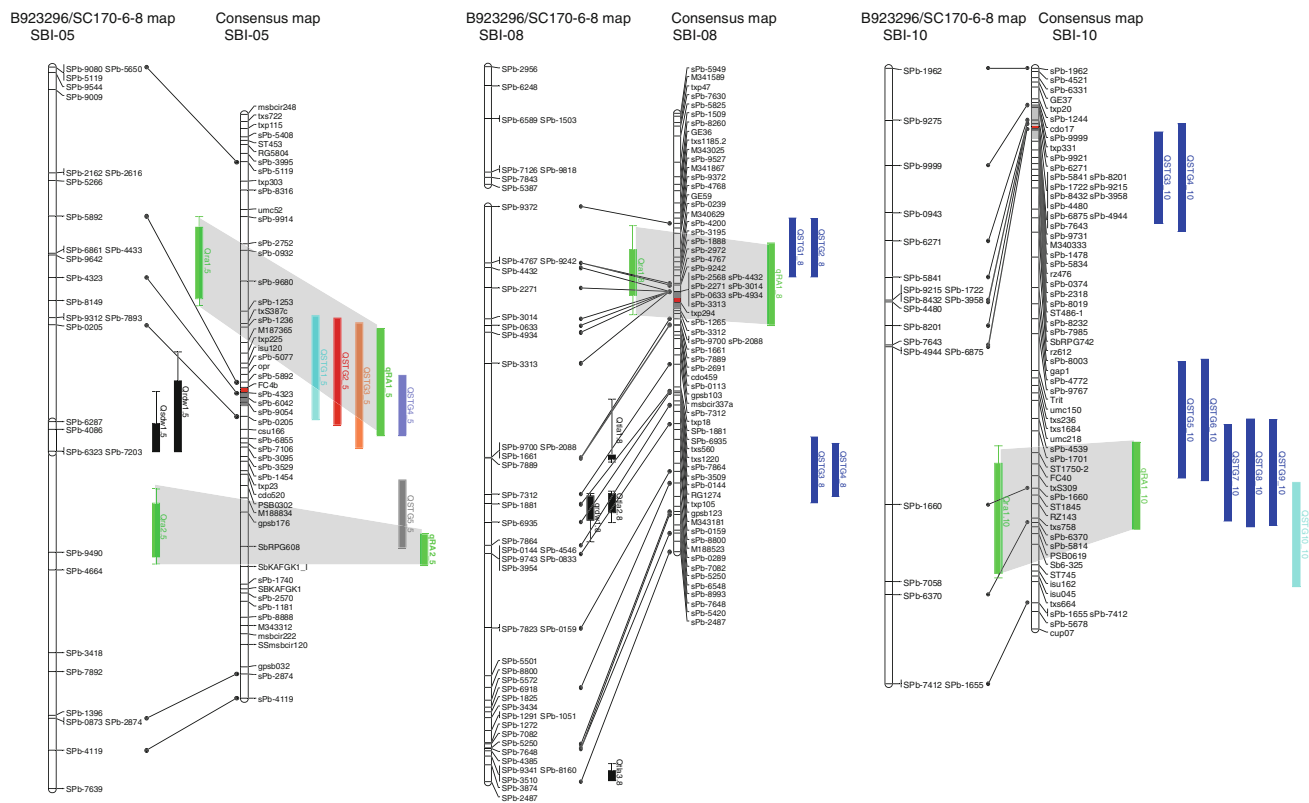
Fig. 4 Projection of the root angle QTL onto the sorghum consensus map and comparison with stay-green QTL identified in previous studies color-coded as follows; Crasta et al. (1999) *light blue*; Feltus et al. (2006) *green*; Haussmann et al. (2002) *dark blue*; Kebede et al. (2001) *purple*; Srinivas et al. (2009) *grey*; Subudhi et al. (2000) *orange*; Xu et al. (2000) *red*

component analysis and trait correlations, that nodal root angle was independent of plant size at the six leaf stage, which was consistent with results of a previous study on the 44 diverse inbred lines (Singh et al. 2011) and indicates that selection for nodal root angle will not have unforeseen consequences on plant size at this stage of crop growth.

Genetic linkage in RIL population between QTL for nodal root angle and plant size is limited

The four nodal root angle QTL identified in the mapping population, and validated in the association mapping study, explained 58.2% of the phenotypic variance for nodal root angle. In comparison, two QTL for root angle in rice explained 44.8% of the phenotypic variance (Norton and Price 2009). In maize, Omori and Mano (2007) identified five QTL for root angle emerging from the second node, explaining a total of 49.9% of the phenotypic variance, and a further five QTL for root angle emerging from the third node, explaining a total of 58.8% of the phenotypic variance. The high percentage of phenotypic variation explained by root angle QTL across species suggests that

the trait is controlled by a relatively small number of genes with large effects. Due to the low genome coverage of some chromosomes, in particular SBI-06, it is possible that there are additional QTL controlling this trait that were unable to be detected with the current map. However, it is very likely that the genomic regions not included in this map due to lack of polymorphisms are identical by descent between the parental genotypes.

Of the four nodal root angle QTL detected, two (*qRA1_5* and *qRA1_8*) were on the same linkage group as one or more plant size QTL. The 2-LOD confidence interval for the significant *qRA1_5* was within 20 cM of two co-locating, significant plant size QTL (*qSDW1_5* and *qRDW1_5*) on SBI-05 (Fig. 3). Similarly, the 2-LOD confidence interval for suggestive *qRA1_8* was within 30 cM of a significant QTL for total leaf area (*qTLA1_8*) (Fig. 3). These two root angle QTL were thus loosely linked to some of the suggestive and significant plant size QTL. In general, however, only limited genetic linkage was detected between the nodal root angle QTL and the plant size QTL. Similar results have been obtained in maize (Guingo et al. 1998), where QTL for biomass were

Table 4 Single marker analysis summary (p values) for the level of association between markers in the four nodal root angle QTL regions with stay-green in field trials from NAM populations segregating for nodal root angle

Environment		QTL	LG	Non-recurrent parent of NAM populations						
Site	Year			SC62C	Dorado	SC35C	SC108C	SC103-14E	RS29	LR9198
Biloela	2006	qRA1_5	SBI-05-II	0.0073**	–	–	–	–	–	–
Biloela	2007	qRA1_5	SBI-05-II	0.0074**	0.0579[+]	0.0656[+]	–	–	–	–
Dalby Box	2005	qRA1_5	SBI-05-II	0.0833[+]	–	–	NS	0.0545[+]	0.0057**	NS
Dysart	2007	qRA1_5	SBI-05-II	0.0087**	0.0582[+]	0.0701[+]	–	–	–	–
Hermitage	2005	qRA1_5	SBI-05-II	0.0317[+]	–	–	NS	0.0285[+]	0.0076**	NS
Hermitage	2007	qRA1_5	SBI-05-II	0.0208[+]	0.0561[+]	0.0078**	–	–	–	–
Jimbour	2005	qRA1_5	SBI-05-II	NS	–	–	–	–	0.0595[+]	NS
Biloela	2006	qRA2_5	SBI-05-III	NS	–	–	–	–	–	–
Biloela	2007	qRA2_5	SBI-05-III	NS	0.0080**	NS	–	–	–	–
Dalby Box	2005	qRA2_5	SBI-05-III	NS	–	–	NS	NS	NS	NS
Dysart	2007	qRA2_5	SBI-05-III	NS	0.0305[+]	0.0715[+]	–	–	–	–
Hermitage	2005	qRA2_5	SBI-05-III	NS	–	–	NS	NS	NS	NS
Hermitage	2007	qRA2_5	SBI-05-III	NS	0.0142[+]	NS	–	–	–	–
Jimbour	2005	qRA2_5	SBI-05-III	NS	–	–	–	–	NS	NS
Biloela	2006	qRA1_8	SBI-08-II	NS	–	–	–	–	–	–
Biloela	2007	qRA1_8	SBI-08-II	NS	NS	NS	–	–	–	–
Dalby Box	2005	qRA1_8	SBI-08-II	NS	–	–	NS	0.0071**	0.0049**	0.0780[+]
Dysart	2007	qRA1_8	SBI-08-II	NS	NS	NS	–	–	–	–
Hermitage	2005	qRA1_8	SBI-08-II	NS	–	–	NS	0.0689[+]	0.0406[+]	NS
Hermitage	2007	qRA1_8	SBI-08-II	NS	NS	0.0633[+]	–	–	–	–
Jimbour	2005	qRA1_8	SBI-08-II	NS	–	–	–	–	0.0030**	NS
Biloela	2006	qRA1_10	SBI-10	NS	–	–	–	–	–	–
Biloela	2007	qRA1_10	SBI-10	NS	0.0907[+]	NS	–	–	–	–
Dalby Box	2005	qRA1_10	SBI-10	NS	–	–	NS	0.0078**	0.0044**	NS
Dysart	2007	qRA1_10	SBI-10	NS	NS	0.0763[+]	–	–	–	–
Hermitage	2005	qRA1_10	SBI-10	NS	–	–	NS	0.0228[+]	0.0121[+]	NS
Hermitage	2007	qRA1_10	SBI-10	NS	NS	0.0098**	–	–	–	–
Jimbour	2005	qRA1_10	SBI-10	NS	–	–	–	–	0.0300[+]	NS

NS not significant

$P > 0.1$; [+] $P < 0.1$; ** $P < 0.01$

– not tested in this population/environment combination

reported to be independent of those for root angle. In durum wheat, Sanguineti et al. (2007) reported that root spread angle was characterised by a genetic control largely independent from all other investigated root or shoot traits. Our results showed no evidence to support a pleiotropic association between plant size and nodal root angle. In addition, the lack of any significant epistatic interaction among the main effect QTL for all four traits, indicates that root angle is largely independent of plant size.

Validation of the root angle QTL

The results of the association mapping study across the set of 44 diverse lines validated the four QTL for nodal root angle detected in the mapping population. Significant marker–trait associations were identified either within the CI (for *qRA2_5, qRA1_8* and *qRA1_10*) or closely linked to (*qRA1_5*) all four nodal root angle QTL. The association mapping study also indicated the potential for additional genomic regions to be involved in the genetic control of this trait. However, the additional six significant marker–trait associations identified in the association mapping study need to be treated with caution, due to the small population used.

Three of the four nodal root angle QTL detected in the mapping population also had homology to previously identified QTL for root angle in rice and maize, as determined through BLAST analysis (Johnson et al. 2008) of

flanking markers. This homology with previously identified QTL for root angle in different species further supports the results of the current study.

Link between root angle QTL and grain yield

Variation in yield performance was observed in the mapping population used in the current study. The single marker analysis conducted to investigate a possible link between grain yield and nodal root angle identified significant associations between grain yield and three of the four nodal root angle QTL. The number of individuals tested in hybrid combination with the three different testers (R986087, R993396, R995248) was relatively low (18, 19 and 16, respectively) and therefore the power to detect marker–trait associations was limited. It is therefore possible that additional yield QTL were segregating but were not detected as significant or suggestive with SMA. An association between a major QTL affecting root traits and leaf ABA concentrations, *root-ABA1*, and grain yield and other agronomic traits has recently been identified in maize (Giuliani et al 2005; Landi et al. 2007), which the authors postulate is likely due to pleiotropy. The results of the current study indicate a possible association between nodal root angle in sorghum and yield performance.

Root angle QTL co-located with stay-green QTL

The projection of the twelve QTL identified in the mapping population onto the sorghum consensus map (Mace et al. 2009) enabled comparison with QTL identified in other studies. We specifically focused on the comparison of the location of the nodal root angle QTL with locations of previously identified QTL for stay-green. Stay-green is associated with increased water availability during grain filling, possibly via greater water capture through modified root architecture. Hence, the temporal dynamics of leaf senescence is an integrated measure of water status. All four nodal root angle QTL identified in the mapping population co-located with previously identified QTL for stay-green (Fig. 4). Stay-green has been associated with grain yield under post-anthesis drought stress in Australia (Borrell et al. 2000; Jordan et al. 2003). As the amount of water used after anthesis affects grain yield under post anthesis drought stress (Turner 2004), each of the four root angle QTL thus co-located with QTL for traits that can affect grain yield under post-anthesis drought stress. The projection of the six additional significant marker–trait associations identified for nodal root angle in the association mapping study using the set of 44 diverse lines onto the consensus map identified that five out of six (83%) of these new potential QTL were also found to co-locate with regions of the genome containing previously mapped QTL

for stay-green and this is unlikely to have occurred by chance.

An observation of putative association between narrow root angle and moderate to high levels of stay-green was made in the recent study looking at the genetic diversity of nodal root angle in a set of 44 diverse sorghum genotypes (Singh et al. 2011). The putative association between nodal root angle and the stay-green drought response was further studied in 7 sorghum NAM populations (Jordan et al. 2011), which were selected as the parents exhibited contrasting nodal root angle based on the results of the root angle screen across the 44 diverse lines. The single marker analysis conducted determined that all four genomic regions associated with nodal root angle were also associated with the stay-green drought tolerance mechanism; specifically, significant or suggestive marker–trait associations were identified for stay-green in all four nodal root angle QTL regions in six of the seven NAM populations, showing a general level of consistency across environments. Although the total number of lines tested was relatively high (339 individuals overall), the population sizes of each of these sub-populations were relatively small (ranging from 31 to 59 individual per population) and therefore these populations have limited power to detect QTL. Hence, it is not unlikely that QTL segregated in some populations but were not identified as significant or suggestive for the SMA. The combination of the co-location of the QTL, as projected onto the sorghum consensus map, and the significant associations between the root angle markers and stay-green observed in the NAM provides further support for the putative genetic association between nodal root angle and the stay-green drought response.

Breeding for drought tolerance, particularly through avoidance mechanisms, is likely to involve root characteristics. The ability of the root system to both gradually occupy the soil volume available and effectively extract the available water in that volume affects the quantum and temporal dynamics of water captured by the crop. Both factors can have significant impact on grain yield and crop water productivity. The results of the present study indicate a putative association between the QTL identified for nodal root angle at the leaf six stage and both yield and the stay-green drought response.

Implications for crop improvement programs targeting drought adaptation

The importance of root characteristics as a selection criterion in a breeding program has long been recognized, but the complex nature of root architecture and interactions of roots with the surrounding rhizosphere has made it difficult to select for root-associated traits in the field during the breeding cycle (Richards and Passioura 1981; Watt et al.

2007). As breeding programs rely on high throughput strategies to select genotypes with desirable trait variation that are easy-to-apply, reliable and affordable, genetic improvement has to date largely been driven by selection for yield associated with traits that control the growth and development of above-ground plant parts.

The recent development of an effective root screening method for sorghum (Singh et al. 2010) has facilitated the identification of genetic variation for nodal root angle. Combined with the high heritability for nodal root angle observed in the current and previous studies, this could allow selection for nodal root angle in a crop improvement program. However, phenotypic selection may be hampered by complex genetic control of the trait. This includes significant interactions between male and female parents of hybrids (Singh et al. 2011), which can reduce the association between the root angle of a hybrid and the mean of its two parents. The identification and validation of genomic regions associated with nodal root angle in sorghum in the current research provides tools for plant breeders to predict genotypes with desirable variation when selecting breeding material prior to field evaluation. The apparent weak linkage between nodal root angle and plant size traits suggests these different adaptive mechanisms are likely controlled independently, indicating that selection for nodal root angle at the six leaf stage will not have unforeseen consequences on plant size at that stage. Hence, the identification of nodal root angle QTL presents new opportunities for improving drought adaptation via molecular breeding to manipulate a trait for which selection has previously been very difficult. The co-localisation of nodal root angle QTL with QTL for stay-green and the association with grain yield presents opportunities to use molecular breeding strategies to improve drought adaptation through manipulating nodal root angle.

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