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ORIGINAL PAPER

QTL for root angle and number in a population developed from bread wheats (*Triticum aestivum*) with contrasting adaptation to water-limited environments

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Abstract Root architecture traits in wheat are important in deep soil moisture acquisition and may be used to improve adaptation to water-limited environments. The genetic architecture of two root traits, seminal root angle and seminal root number, were investigated using a doubled haploid population derived from SeriM82 and Hartog. Multiple novel quantitative trait loci (QTL) were identified, each one having a modest effect. For seminal root angle, four QTL ($-\log_{10}(P) > 3$) were identified on 2A, 3D, 6A and 6B, and two suggestive QTL ($-\log_{10}(P) > 2$) on 5D and 6B. For root number, two QTL were identified on 4A and 6A with four suggestive QTL on 1B, 3A, 3B and 4A. QTL for root angle and root number did not co-locate. Transgressive segregation was found for both traits. Known major height and phenology loci appear to have little effect on root angle and number. Presence or absence of the T1BL.1RS translocation did not significantly influence root angle. Broad sense heritability (h^2) was estimated as 50 % for root angle and 31 % for root number. Root angle QTL were found to be segregating between wheat cultivars adapted to the target production region indicating potential to select for root angle in breeding programs.

Introduction

Water limitation is the greatest single production constraint in dryland cropping systems. The breeding of wheat varieties with improved yield and yield stability in water-limited environments is a high priority for improving food and feed supply, as well as security. Optimising root architecture can lead to a significant yield advantage in water-limited environments (Manschadi et al. 2010). For example, in sub-tropical northern Australia with summer-dominant rainfall, autumn sown wheat and barley crops are often heavily reliant on stored soil moisture from previous summer rainfall. Optimisation of root architecture to maximise soil moisture extraction deep in the soil, late in the season, is advantageous in such environments (Kirk-

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egaard et al. 2007; Manschadi et al. 2006). Deep soil moisture acquisition is also important in maize, rice and sorghum (Hammer et al. 2009; Hund et al. 2009; Steele et al. 2006; Uga et al. 2011; Vadez et al. 2011a, b).

The bread wheats SeriM82 and Hartog have been shown to differ significantly in their performance under water limitation and in root architecture. SeriM82 is a high-yielding line from the International Centre for Maize and Wheat Improvement (CIMMYT). It has been shown to yield, on average, 12 % more than the locally adapted cultivar Hartog in multi-environment trials in north-eastern Australia (Peake et al. 1996; Cooper et al. 1999). In this environment, due to the summer-dominant rainfall pattern, winter crops rely heavily on stored soil moisture in the deep clay soils. This can lead to severe terminal moisture stress in seasons with little in-crop rainfall. It has been reported that post-anthesis drought stress is the most common production environment in the region representing over 50 % of environments (Chenu et al. 2011). For single plants grown in large root-observation chambers, Manschadi et al. (2006) demonstrated that, compared with Hartog, SeriM82 possesses a more compact and uniform root architecture with greater root length density at depth. These traits increased access to water from the deeper soil layers during the grain-filling period (Manschadi et al. 2006).

SeriM82 also expressed a stay-green phenotype by maintaining green leaf area for longer during the grain-filling period after anthesis compared with standard varieties. However, in the absence of deep soil moisture, SeriM82 was not able to maintain green leaf area longer than Hartog (Christopher et al. 2008). Thus, the availability of deep soil moisture late in the season is important for expression of the high-yielding, stay-green phenotype (Christopher et al. 2008; Manschadi et al. 2010). SeriM82 exhibited greater grain mass due to its ability to prolong grain filling rather than an ability to accumulate biomass more rapidly under moisture limitation. Using computer simulation modelling, Manschadi et al. (2006) were able to show that for each additional millimetre of water extracted during grain filling, more than 55 kg ha^{-1} of additional grain yield can be generated; this has also been confirmed experimentally (Kirkegaard et al. 2007). Thus, the marginal water use efficiency of extra soil moisture that becomes available post-anthesis is nearly three times higher than that calculated over the whole growing season. This means that small amounts of additional moisture extracted post-anthesis can lead to relatively large differences in grain yield. Yield increases are mainly due to the fact that the extra water extracted late in the season is not required to build more structural crop biomass but is utilised solely for grain growth (Richards et al. 2010).

It is impractical to assess mature root architecture in high numbers of plants using large root-observation chambers. To be able to screen the large numbers of plants required for a robust quantitative trait loci (QTL) analysis, the measured root traits should ideally be expressed at an early stage and determine the growth and functioning of the root system later in the season. Two types of roots occur in cereals, the seminal roots coming direct from the embryo and the later, nodal roots emerging at the lower tiller nodes (Manske and Vlek 2002). The seminal roots penetrate the soil earlier and deeper than nodal roots, so are considered important for deep soil moisture extraction. It has been reported that the maximum lateral distribution of the mature wheat root system is narrower in certain genotypes with more vertical seminal root angle and greater root length density at depth compared to others with a wider seminal root angle (Manschadi et al. 2008; Nakamoto et al. 1991; Oyanagi et al. 1993, 2001). Manschadi et al. (2008) demonstrated that the angle of the first pair of seminal roots to emerge after the primary seminal root (or radical) exhibits variation between Australian wheat cultivars and best correlates with differences in mature root system distribution. SeriM82 has been shown to have a narrower seminal root angle, narrower maximum lateral root distribution and greater root length density at depth than Hartog (Manschadi et al. 2006, 2010). Seminal root angle can be screened using small gel-filled root chambers as described by Bengough et al. (2004).

Attempts to breed elite wheat varieties with superior adaptation to water-limited environments by crossing SeriM82 and Hartog have been unsuccessful to date. SeriM82 carries the T1BL·1RS rye translocation which has been associated with higher yield in certain wheat types and environments but not in others (reviewed in Peake et al. 2011). However, studies of recombinant inbred SeriM82 × Hartog lines indicated that the superior yield of SeriM82 over Hartog is not associated with the T1BL·1RS translocation (Peake et al. 1996, 2011). Further, the higher yield of SeriM82 is, in part, due to positive epistatic genetic effects (Peake 2003). These results suggest that high yield in SeriM82 is under complex genetic control (Christopher et al. 2008). By partitioning this yield advantage into smaller measurable physiological sub-traits, including root architecture, we aim to determine the genetic control of this yield advantage. By identifying the genetic regions contributing to root architecture, genetic markers could be used to select lines most likely to express the desired root architecture traits for specific target environments. A QTL for deep root ratio in hexaploid wheat has been reported by Hamada et al. (2012), although they did not identify QTL for first-pair seminal root angle per se.

The aim of the current study was to identify the genetic regions controlling seminal root angle and number in a population segregating for high yield and stay-green in the northern Australian grain region. A doubled haploid

population made by crossing SeriM82 and Hartog was used to detect QTL associated with seminal root angle and number. A validation set of 20 wheat cultivars bred in Australia or at CIMMYT was also assessed to determine whether variation for seminal root angle exists at regions identified as QTL in the SeriM82 × Hartog population and whether such regions are associated with root angle in a broader set of cultivars adapted to the target production region.

Materials and methods

Plant material

The angle and number of seminal roots was measured for 184 doubled haploids derived from the parents SeriM82 and Hartog. SeriM82 is a high-yielding drought-tolerant line from CIMMYT derived from the Veri cross (Sivapalan et al. 2001, 2003; Olivares-Villegas et al. 2007). Hartog is a locally adapted line derived from the CIMMYT cross Pavon. The 184 lines were selected from a larger population of lines based on their similarity in days to maturity and height, while retaining variation for yield. They included 77 F_1-derived doubled haploid lines and 107 BC_1-derived doubled haploid lines, with Hartog as the recurrent parent.

The validation study included 18 Australian and CIMMYT bred wheat cultivars plus SeriM82 and Hartog (Table 1). Seminal root angle measurements from a previous study were used for the validation analysis (Table 1; Manschadi et al. 2008).

Measurement of seminal root angle and number, seed mass and T1BL·1RS status

The growth angle of the first pair of seminal roots and the number of seminal roots of wheat seedlings were measured using gel-filled root observation chambers based on methods developed from Bengough et al. (2004) and

Table 1 Genotypes used for the validation study ranged in seminal root angle and in the number of markers in common with SeriM82 × Hartog map

Genotype	Pedigree	Parentage	Seminal root angle	Standard error of angle	Number of markers in common with SeriM82/Hartog map
SeriM82 (3)	Veri#5: Kavkaz/Buho//Kalyansona/Bluebird	V	36.25	1.57	69
Sunvale (1)	Cook*2/VPM1/3*Cook	C	38.19	2.03	52
Baxter (10)	QT2327/Cook//QT2804	C	39.44	1.45	72
EGA Wylie (2)	QT2327/Cook/QT2804	C	40.81	1.72	78
EGA Gregory (2)	Pelsart/2*Batavia DH	C	42.00	1.08	99
Giles (2)	Janz/Vulcan	C	42.06	1.55	89
Sunco (2)	Cook*3/WW15/4SUN9E − 27/3Ag14	C	42.56	1.73	83
Babax (2)	Bobwhite/Nacozari-//Veri/3/Bluejay/Cocoraque-75	V	44.06	1.64	24
Lang (1)	QT3765/Sunco	C	44.94	1.19	55
EGA Wentworth (1)	Janz*2/Vulcan	C	46.69	1.54	55
Rosella (1)	Farro Lunga/Heron//2*Condor/3/Quarrion sib	C	47.81	0.91	67
Chara (1)	BD225/CD87(= Beulahsib//Pavon'S/Condor)	C, P	48.19	2.03	59
Kennedy (2)	Hartog/Veri#5	P, V	48.56	1.71	80
Janz (3)	3Ag3/4*Condor//Cook	C	49.37	1.48	99
EGA Hume (2)	Pelsart/2*Batavia DH	C	49.69	1.76	99
Ventura (1)	Sunvale/Rowan	C, P	51.87	1.03	59
Hartog (4)	Pavon 'S': Vicam S 71//Ciano F 67/Siete Cerros T 66/3/Kalyan Bluebird	P	51.94	1.50	66
Rees (2)	Quarrion/3*Hartog	P	52.00	1.47	67
Leichhardt (2)	CNT2/4*Hartog	P	53.25	1.03	65
Diamondbird (1)	Vicam S 71//Ciano F 67/Siete Cerros T 66/3/Kalyan Bluebird	P	56.25	1.68	32

Lines related to the CIMMYT cross Pavon (P) tend to have wider root angle than those related to Cook (C) or to the CIMMYT cross Veri (V). Data are presented for the genotype name with the number of DArT genotypes used to create the consensus DArT genotype given in parenthesis, pedigree, angle from the vertical of the first pair of seminal roots from Manschadi et al. (2008) and number of markers in common with the SeriM82/Hartog map

Manschadi et al. (2008) (Fig. 1). Chambers were constructed from two plates of clear glass, each measuring 210 × 300 × 3 mm. Sterilised agar (Sigma Type A; 2 % w/v) was poured onto each plate and allowed to gel before the two plates were taped together with the agar surfaces inward. Surface-sterilised wheat seed was imbibed with sterile deionised water for a few hours and then placed on wet blotting paper and kept at room temperature for 2 days to allow germination. Two germinated seeds were placed into the narrow air space of approximately 2.5 mm between the agar layers, 80 mm apart, and 50 mm from the top edge of the vertically mounted chambers. The seeds were oriented vertically with the radicle facing downwards. The gel-filled chambers were then kept in a plant growth cabinet at 15 °C for 5 days in the dark until the first leaf emerged at the top of the trays, followed by growth under constant temperature of 15 °C with a 12/12-h dark/light regime. The light intensity in the growth cabinet at plant height was 220 µmol photons m^{-2} s^{-1}. Light was excluded from the root observation chambers using black PVC plastic covers, except during observations. At 8 days after seed transfer, the seminal roots visible through the clear glass were scanned using a flat bed scanner (HP Scanjet 4670). The total number of seminal roots including the primary seminal root was recorded. The growth angle from the vertical of each root in the first pair of seminal roots to emerge after the primary root was measured within the first 3 cm of root (Fig. 1). Specifically designed computer software allowed rapid measurement of seminal root angle from the digital images as described in Manschadi et al. (2008).

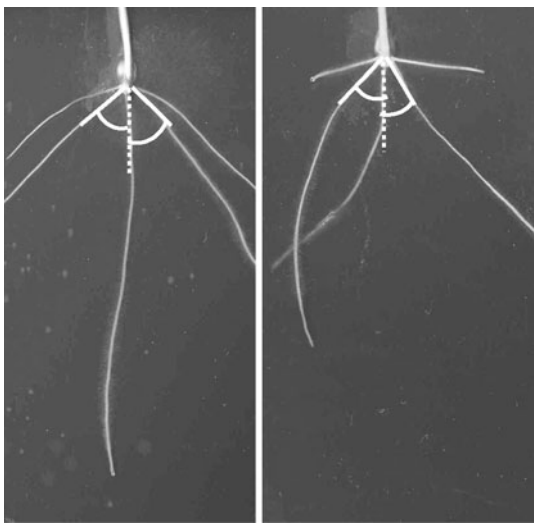

Fig. 1 Seminal roots, visible on the glass surface of root chambers, for the two parents of the DH mapping population: Hartog (*left panel*) and SeriM82 (*right panel*). *Thick solid lines* represent seminal roots, *dotted line* the vertical plane, and the *arcs*, the estimated root angle

Seed mass was measured using the average for two samples of 100 seeds. To test for correlation with seed mass, a simple linear regression was performed using the raw average for the root angle and for root number for each genotype.

The T1BL·1RS translocation was detected in the mapping population using an ELISA test (Andrews et al. 1996; Peake et al. 2011) and confirmed through rust resistance testing (Peake et al. 1996, 2011).

Experimental design

It was not possible to phenotype all 184 doubled haploid genotypes in a single batch. Eighteen test genotypes were included in each batch, requiring 11 batches to produce a single replicate of all 184 genotypes. This allowed 14 unassigned test positions in each replicate, providing space in the experiment for retesting any material compromised by poor growth, gel damage or microbial contamination. In addition to the test lines, the two parents were included in every batch. Each genotype was tested in a separate gel chamber, totalling 20 chambers per batch. An incomplete block design with three replicates of 11 batches was generated as an alpha design using the CycDesigN software package (Whitaker et al. 2002). Two seeds of each genotype were placed in a single gel chamber. The seedlings constitute the observational units while each gel chamber is an experimental unit. At least six seedlings of each genotype were used overall.

The same grade of commercial agar, Sigma grade A, was used throughout the experiment and agar with the same batch number was used within each replicate.

Statistical design and analysis

A linear mixed model was used to determine the effect of genotype on root angle. To correctly partition the randomisation restrictions of the design, the model included random terms for replicate, batch, gel chamber and seedlings within chambers. The left and the right hand side roots of each seedling constituted the lowest level strata in the final analysis. The final linear model used for analysis was: angle ~ Genotype + Replicate + Replicate:Batch + Replicate:Batch:Chamber + Replicate:Batch:Chamber:Seedling + Replicate:Batch:Chamber:Seedling:Side

This analysis model was run with genotype as a fixed effect. Best linear unbiased estimates (BLUEs), were used for further quantitative trait loci (QTL) analysis (van Eeuwijk et al. 2010). In this two-stage approach for QTL analysis, phenotypic means are used to allow adequate discrimination of the test material. Standard errors and least significant differences (LSDs) were calculated for comparison of genotype means.

To further explore the genetic variability of the material and estimate the heritability (h^2) for root angle and root number, the analysis was also conducted with genotype as a random effect. The two traits were analysed separately and best linear unbiased predictors (BLUPs) were calculated. The residual maximum likelihood algorithm (REML; Patterson and Thompson 1971) was used to provide estimates of the variance components.

Data were analysed with ASReml-R (Butler et al. 2009) using R software version 2.13.0 (R Development Core Team 2009). The second stage of the QTL analysis was performed in GenStat 14 (VSN International 2011).

Genetic map construction and QTL analysis

The same subset of 184 SeriM82/Hartog doubled haploid lines was used for genetic map construction and QTL analysis. Young leaf tissue was collected from the progeny and parents. DNA was isolated using the method recommended by Diversity Array Technology Pty. Ltd. (DArT) (http://www.triticarte.com.au/pdf/DArT_DNA_isolation.pdf).

The population was genotyped with DArT markers, using the method described in Wenzl et al. (2004) and the most recent DArT high-density Wheat PstI (TaqI) v3 array. Simple sequence repeat (SSR) markers Barc124-A, Barc124-B, Gwm372-A, Gwm372-B, Gwm459, Rht1-Wild, Rht1-Mutant, VrnA1 and Wmc149-A were scored manually according to base pair size using polyacrylamide gel electrophoresis (PAGE) or agarose gel electrophoresis (Bassam and Caetano-Anollés 1993; Eagles et al. 2009; Song et al. 2005; Zhang et al. 2006; Sanguineti et al. 2007).

The SeriM82 × Hartog map reported below was constructed using Multipoint version 2.2 Software (http://www.multiqtl.com). Initially, two genetic maps and a merged map were created, reflecting the fact that the population consisted of double haploid progeny being either F_1-derived or BC_1-derived. One map was created using data for F_1-derived DH and was analysed within Multipoint as a double haploid population. A second map (data not shown) was created using BC_1-derived double haploids and was analysed within Multipoint as an F_2 population, to match the observed genetic ratios of the progeny with those expected by the program (1:3, SeriM82:Hartog). Monomorphic markers and markers with significant segregation distortion were excluded. Linkage groups (LGs) were assigned to chromosomes on the basis of previously mapped DArT markers (Diversity Arrays Technology Pty. Ltd. http://www.triticarte.com.au; Singh et al. 2010; Lowe et al. 2011; Zwart et al. 2010; Wang et al. 2011a, 2011b; Tsilo et al. 2010; Crossa et al. 2007; Sherman et al. 2010; Uphaus et al. 2007; Grain Genes 2.0 http://wheat.pw.usda.gov/browse, accessed 31 May 2012), and a suffix added where chromosomes were split, with LG-1 starting at the distal end of the short arm. A merged map was manually created (data not shown) by aligning the two maps and discarding those markers that showed order discrepancies. As the BC_1 and merged maps differed little from the F_1 map, the F_1 map was used for further analysis.

QTL analysis was conducted based on the F_1 map using the phenotypic information for the entire population of 184 analysed using GenStat 14 (VSN International, 2011). A mixed model marker-trait association analysis was performed in GenStat 14. Population structure was accounted for using the subpopulation grouping option. A 1 % false discovery rate, ($-\log_{10}(P) = 2$), was used to declare QTL significance. The QTL interval is given from the proximal marker above the threshold to the distal marker above the threshold of ($-\log_{10}(P) \geq 2$) for minor QTL designated "q" and ($-\log_{10}(P) \geq 3$) for major QTL designated "Q".

QTL analysis of the validation set of 20 lines was conducted based on the SeriM82/Hartog F_1 map using the same software and settings as for the single trait association analysis of the mapping population given above except that no sub-groupings were used. For some genotypes, multiple DArT analyses were available (Table 1). In these cases, a "consensus DArT genotype" was created by eliminating a small number of DArT markers inconsistent between analyses. Root angle data from a previous study were used (Manschadi et al. 2008).

Results

Root angle and number

Estimated root angle for the first pair of seminal roots in the doubled haploid population ranged from 27.3° to 51.2°, with standard errors (SE) of means from 2.4° to 4.3°. The estimated population average angle was 40.6°. The estimated seminal root angle for SeriM82 was 39.6° (SE 0.9) and for Hartog was 41.3° (SE 0.9) giving a mid-parent angle of 40.5°. LSD ($p < 0.05$) for means comparison was 2.2° between parents; 6.4° between the parents and the DHs; 8.4° between the DH genotypes.

Estimated root number ranged between 3.53 and 5.43, with standard errors between 0.2 and 0.3 roots. The average predicted root number was 4.72. The predicted root number for SeriM82 was 4.76 (SE 0.09) and was not significantly different from Hartog at 4.78 (SE 0.09) or the mid parental mean of 4.77. The average LSD was 0.76.

There was no evidence for a significant genetic correlation between seminal root number and seminal root angle.

Seed mass was not significantly correlated with root angle (F probability = 0.710). Seed mass was significantly correlated with root number (F probability = 0.011, $r = 0.17$), but the effect was small, with the percentage of variance accounting for only 3.0 %.

Broad sense heritability (h^2) was estimated at 0.50 for root angle and 0.31 for root number.

T1B·1R translocation status did not significantly correlate with root angle (0.20° difference between classes, standard error of differences 0.46°). The translocation was significantly associated with root number, but the effect was small. Genotypes carrying the translocation had on average 0.144 fewer roots (LSD 0.081; $p < 0.05$).

Seri × Hartog genetic map

A total of 184 individuals were used to construct the genetic linkage map for the DH population. A total of 841 markers, consisting of 264 framework markers, were mapped to 27 linkage groups with a total map length of 1,661.1 cM (Supplementary Fig. 2).

The average distance between framework markers was 2.4 cM. The linkage groups were assigned to wheat chromosomes 1A through to 7D. These linkage groups could be assigned to 20 of the 21 wheat chromosomes on the basis of mapped DArT markers; no linkage group was assigned to chromosome 6D. Chromosomes 1A, 2A, 2B, 3B, 4A, 4B, 5B, 6A, 7A, 7B and 7D were each made up of two separate linkage groups. Chromosome 4D and 5A as well as chromosome segments 1A2, 3B1, 4A1, 5B2 and 7D1 were each represented by less than five loci. An abbreviated map chart showing chromosomes with QTL assigned is presented in Fig. 2 and a full chart in Supplementary Fig. 1.

Identification of QTL

For first pair seminal root angle, four QTL ($-\log_{10}(P) > 3$) were located on 2A, 3D, 6A2 and 6B, and two suggestive QTL ($2 < -\log_{10}(P) < 3$) were identified on 5D and 6B (Table 2). For root number, two QTL ($-\log_{10}(P) > 3$) were identified on 4A and 6A1 and four suggestive QTL on 1B, 3A, 3B and 4A ($2 < -\log_{10}(P) < 3$; Table 2).

QTL were named according to McIntosh Catalogue of Gene Symbols for Wheat (http://wheat.pw.usda.gov/ggpages/wgc/98/, accessed 22 March 2012) modified using an upper case "Q" for a QTL ($-\log_{10}(P) > 3$) and a lower case "q" for a suggestive QTL ($2 < -\log_{10}(P) < 3$). RA is used to represent seminal root angle, RN is for seminal root number, qgw for Queensland Government wheat, followed by chromosome number and then numbered from the distal end of the short arm.

The QTL with the largest effect for root angle, *QRA.qgw-2A* had an estimated effect of −1.754° which explained 7.3 % of the total root angle range of 23.9° (Table 2). The QTL with the least effect, *qRA.qgw-5D*, had an estimated effect of −1.041° which represents 4.4 % of the range in root angles.

For root number, the largest QTL explained 5.0 % of the range of 1.9 roots, the smallest explaining 3.4 % of the range. SeriM82 contributed alleles associated with both fewer and a greater number of seminal roots (Table 2).

The confidence intervals of QTL varied greatly from a single marker to large parts of chromosome segments (Fig. 2). In most cases, however, peaks for $-\log_{10}(P)$ were clear, and often coincided with, or were close to, the locus of maximum QTL effect (Table 2; Supplementary Figs. 3 and 4). The very broad QTL (41.1 cM) *QRA.qgw-6A*, however, could be interpreted as having two peaks, one at 5.3 cM (maximum $-\log_{10}(P) = 4.33$) and one at 43.7 cM ($-\log_{10}(P) = 4.19$), and thus, could be interpreted as two QTL (Supplementary Fig. 3).

Diversity for root angle in wheats adapted to northern Australia

A validation set of 18 cultivars including current cultivars adapted to the northern Australian grains region and the CIMMYT cultivar Babax, plus SeriM82 and Hartog (Table 1), were assessed in a single trait association analysis to determine, (a) whether variation for root angle exists in genetic regions identified as QTL for seminal root angle in the SeriM82 × Hartog population and, (b) whether any such QTL are significantly associated with root angle in a broader set of cultivars adapted to the target production region. Population structure is evident in the validation set with genotypes related to the CIMMYT cross Pavon tending to exhibit broader root angle compared to those related to Cook or to the CIMMYT cross Veri (Table 1; Manschadi et al. 2008). Including structure in the validation set association analysis to account for groups of genotypes with similar parentage had little effect on the result but lead to small numbers of genotypes in some sub-groupings. Thus, the analysis without sub-groupings was retained. Linkage groups 2A1, 3D and 5D where root angle QTL were identified in the SeriM82 × Hartog population were either not polymorphic or not represented in the validation set analysis. Approximately 16 regions of the genome on 10 of the 18 chromosomes represented in the validation set were associated with root angle in the analysis. Some of these associations may arise due the population structure and or population size. However, the data did clearly indicate an association with root angle at three genetic regions identified in the SeriM82 × Hartog population in the current study *QRA.qgw-6A*, *qRA.qgw-6B* and *QRA.qgw-6B.2* (Table 2). In the validation set, *QRA.qgw-6A* is represented by the markers wPt-0696, wPt-4229 and wPt-9474, with $-\log_{10}(P)$ of 3.36, 3.29 and 3.21 respectively. At wPt-0696, lines without the Seri DArT genotype exhibited an increase of +3.50° (SE 0.99) in root angle. The QTL *qRA.qgw-6B* is represented by the

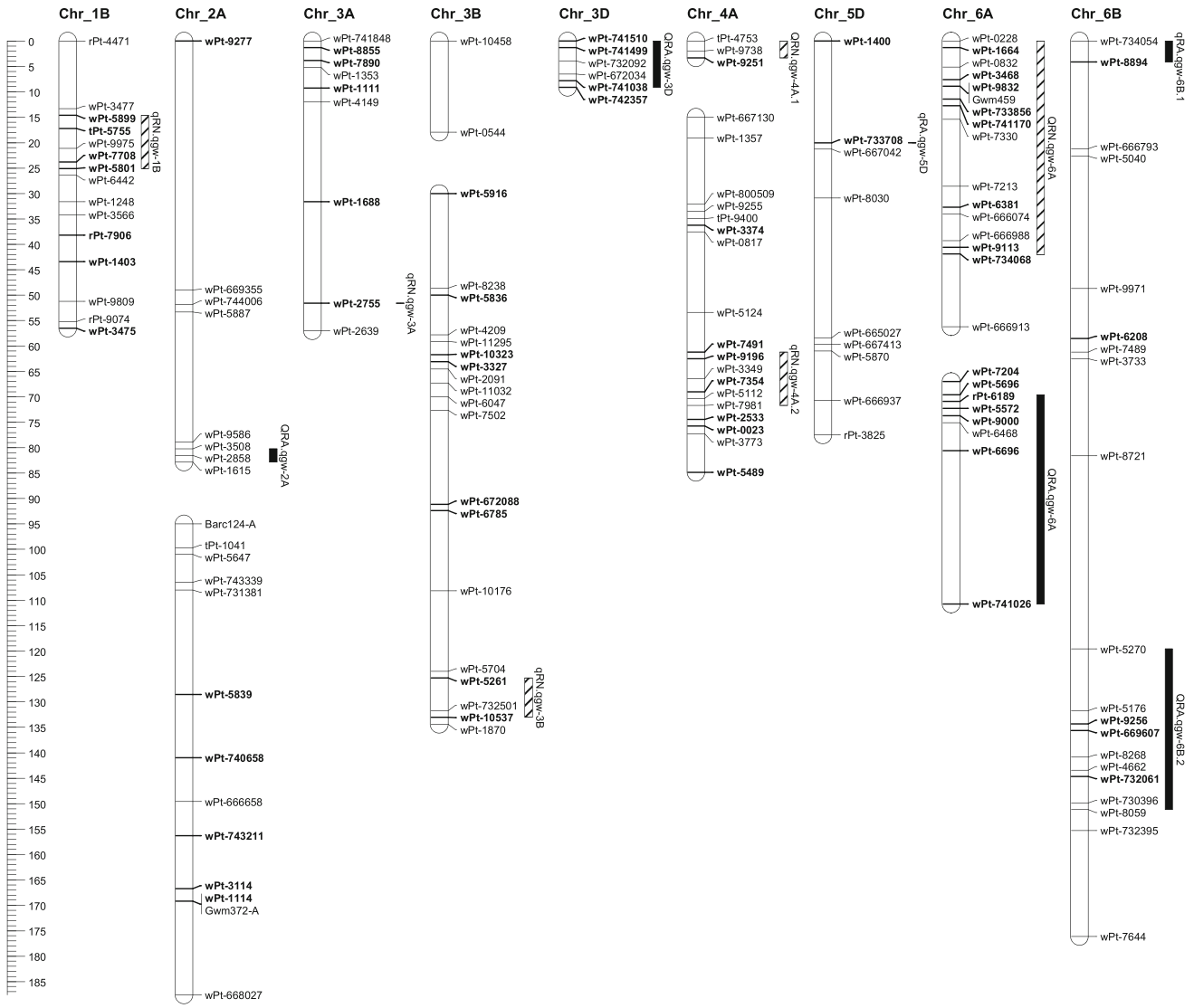

Fig. 2 Chart of SeriM82 × Hartog genetic linkage map based on the SeriM82/Hartog DH mapping population showing localisation of QTL for seminal root angle (RA; *solid bars*) and root number (RN; *hatched bars*). Markers in *bold* are delegates for multiple markers. Markers prefixed with wPt are derived from wheat genetic libraries, tPt from triticale libraries and rPt from rye libraries. The QTL interval is given from the proximal marker above the threshold to the most distal marker above the threshold of ($-\log_{10}(P) \geq 2$) for minor QTL designated "q" and ($-\log_{10}(P) \geq 3$) for major QTL designated "Q". Marker distance in centimorgan is indicated by the *scale at the left*

marker wPt-1547, with a $-\log_{10}(P)$ of 3.22 where lines not having the Seri DArT genotype had an increase of +3.77° (SE 1.10) in root angle. *QRA.qgw-6B.2* is represented by two markers wPt-8268 and wPt-4662, with $-\log_{10}(P)$ of 4.15 and 4.28, respectively. This marker accounted for an increase of +3.72° (SE 0.94) and +3.61° (SE 0.89) seminal root in those genotypes not having the Seri DArT genotype.

Discussion

The aim of the current study was to identify the genetic regions controlling seminal root angle and number in a hexaploid wheat population segregating for high yield in the northern Australian grain region. A broader set of cultivars were also examined to determine whether any identified root angle QTL could be detected in cultivars adapted to the target production region.

Multiple QTL were identified for both root angle and number

A total of six QTL each were observed for both root angle and number (Table 2). The range of root angle within the populations was 23.9° while the sum of the effects for the QTL was 7.642°. The total additive effect of all the QTL alleles contributing to narrow root angle was −6.467° while a single QTL contributed to broader root angle with an effect of

Table 2 Summary of QTL for seminal root angle and root number identified from the linkage map for DHs from SeriM82 and Hartog parentage using single trait association analysis as represented in Fig. 2

QTL ID	Root trait	Linkage group	Peak $-\log_{10}(P)$ position (cM)	Marker at max $-\log_{10}(P)$	$-\log_{10}(P) > 2$ top position (cM)	$-\log_{10}(P) > 2$ bottom position (cM)	Marker interval (cM)	$-\log_{10}(P)$ at peak	Max allele effect (° or root number)	Standard error of effect	Marker at max QTL effect
QRA.qgw-2A	RA	2A1	80.2	wPt-3508	80.2	82.8	2.6	3.29	−1.754	0.505	wPt-3508
QRA.qgw-3D	RA	3D	9.1	wPt-731357	0.0	9.1	9.1	3.67	−1.261	0.341	wPt-731357
qRA.qgw-5D	RA	5D	20.0	wPt-731945	20	20.0	0.0	2.35	−1.041	0.366	wPt-731945
QRA.qgw-6A	RA	6A2	5.3	wPt-5572	2.6	43.7	41.1	4.33	−1.311	0.322	wPt-5572
qRA.qgw-6B.1	RA	6B	0.0	wPt-734054	0.0	4.1	4.1	2.16	−1.100	0.407	wPt-734054
QRA.qgw-6B.2	RA	6B	149.9	wPt-730396	119.6	151.2	31.6	3.76	1.175	0.313	wPt-730396
qRN.qgw-1B	RN	1B	21.1	wPt-9975	14.6	25.1	10.5	2.14	−0.070	0.027	rPt-0079
qRN.qgw-3A	RN	3A	51.6	wPt-2755	51.6	51.6	0.0	2.57	0.071	0.024	wPt-2755
qRN1.qgw-3B	RN	3B	95.3	wPt-5261	95.3	103.0	7.7	2.24	−0.065	0.024	wPt-9368
QRN.qgw-4A.1	RN	4A1	3.3	wPt-9251	0.0	3.3	3.3	3.01	0.086	0.026	wPt-9251
qRN.qgw-4A.2	RN	4A2	46.2	wPt-740561	46.2	56.7	10.5	2.30	−0.069	0.025	wPt-7981
QRN.qgw-6A	RN	6A1	40.7	wPt-734004	0.0	42.0	42.0	4.34	0.096	0.024	wPt-734004

QTL identifiers (QTL ID) follow McIntosh Catalogue of Gene Symbols for Wheat. Root traits are seminal root angle (RA) and number (RN). The QTL marker interval is given from the proximal marker above the threshold to the most distal marker above the threshold of $(-\log_{10}(P) \geq 2)$ for minor QTL designated "q" and $(-\log_{10}(P) \geq 3)$ for major QTL designated "Q". Maximum allele effects are quoted as the effect of substituting the SeriM82 allele for the Hartog allele

1.175°. The 23.9° range in root angle for the Seri × Hartog population is similar to the range of 20° previously reported between 29 mainly Australian wheat genotypes (Table 1; Manschadi et al. 2008). It also encompasses most of the range reported for other wheat genotypes (Hamada et al. 2012; Nakamoto and Oyanagi 1996; Nakamoto et al. 1991; Oyanagi et al. 1991, 1993, 2001).

The range of root number within the populations was 1.9 while the sum of the effects for the root number QTL was 0.456 roots per plant.

The difference in mean seminal root angle between SeriM82 and Hartog observed in this experiment is smaller than previously reported (Table 1; Manschadi et al. 2008) and, in this instance, was not statistically significant ($p > 0.05$). The cause of the contrast between studies remains unclear. Potential contributing factors could include residual genetic heterogeneity in the parent cultivars, subtle differences in the phenotyping technique or genetic drift during seed multiplication. If genetic variation between seed samples is the cause of this difference, then it is likely that the material used in the study by Manschadi et al. (2008) may be more representative of the actual parent plants used to generate the populations. In any case, the QTL evidence indicates that transgressive segregation for root angle should be expected in the doubled haploid population (Table 2).

The observation that QTL for both narrower and broader root angle may come from one parent is consistent with transgressive segregation. Of the six seminal root angle QTL, five of the alleles for narrow angle came from SeriM82. However in one case, *QRA.qgw 6B.2*, the SeriM82 allele contributed to broader root angle (Table 2).

There was little difference in root number between the parents which is in agreement with previous results (Manschadi et al. 2008). However, as with root angle, transgressive segregation was observed in the progeny and the parents contributed QTL for both increased and decreased root number. The Seri alleles at QTL *qRN.qgw-1B*, *qRN1.qgw-3B* and *qRN-qgw-4A2* are associated with fewer roots while at *qRN.qgw-3A*, *QRN.qgw-4A.1* and *QRN-qpw-6A* the Seri alleles are associated with more roots (Table 2).

The evidence indicates complex inheritance for both traits in this population. Each trait is controlled at multiple loci of relatively small effect. The mid-parental values and population means did not differ significantly for either parameter, providing no direct support for epistatic effects. The evidence for complex genetic control contrasts with a previous report suggesting that root angle in wheat is controlled by a single dominant gene based on frequency distribution data (Oyanagi et al. 1991).

Due to the low genome coverage of some chromosomes, with some linkage groups having less than ten markers

assigned, the possibility cannot be excluded that additional QTL controlling the traits remained undetected using the current map. However, it is also likely that some regions of low polymorphism will exist in this mapping population, because they are identical by descent. The coefficient of parentage between SeriM82 and Hartog has been calculated as 0.274 (McLaren et al. 2004). The selection of the population for uniform flowering time and height, could also potentially affect map distances, coverage and/or order.

The root angle QTL identified in the current study are novel

No QTL for first pair seminal root angle have been previously reported for wheat. However, QTL for possibly related traits have been reported for both hexaploid wheat and durum (Hamada et al. 2012; Ren et al. 2012; Sanguineti et al. 2007; Sharma et al. 2009, 2011). However, few of the seminal root angle and number QTL identified here co-locate with previously reported wheat QTL. Possible exceptions to this could include the root number QTL reported on 3A and 6A by Ren et al. (2012) and on 1A by Sharma et al. 2011, discussed below. It is also possible that the 5D DRR QTL of Hamada et al. (2012) is associated with *qRA.qgw-5D*. Further work is required to clarify this.

QTL have been reported in other crop species for a range of traits related to root angle and or number. For example, QTL for nodal root angle were described in sorghum (Singh et al. 2012; Mace et al. 2012) and maize (Omori and Mano 2007). In maize, QTL have been identified for average angle of root growth direction at internode 7 (Giuliani et al. 2005; Guingo et al. 1998). In rice, QTL have been detected for seminal root morphology (Norton and Price 2009), ratio of deep to shallow roots (Uga et al. 2011), vertical root distribution (Yadav et al. 1997), and deep root dry weight per tiller (Champoux et al. 1995). Synteny has been reported between some of the chromosomes where root QTL are located in these crop species and certain Triticum chromosome groups where root QTL were identified in the current study. However, sequence data for the wheat QTL reported is not currently available to allow confirmation of any relationship with root QTL in other crop species.

Known dwarfing and phenology genes have little effect on root angle or number

Dwarfing genes can influence many aspects of the morphology and physiology of wheat (Rebetzke et al. 2012; Borrell et al. 1991). SeriM82 and Hartog are both semi-dwarf types, but SeriM82 has the Rht-B1b allele (Rht1, chromosome 4B) while Hartog has the Rht-D1b allele (Rht2, chromosome 4D). Lines with Rht1 and with Rht2 were anticipated in the mapping population. However, no QTL were detected on 4B or 4D, suggesting that neither dwarfing locus affects root angle or number in this population.

Genes controlling vernalisation and photoperiod response have been associated with yield and many other traits. Hartog and SeriM82 do not segregate for Ppd-D1 (2D), Vrn-B1 (5BL) or Vrn-D1 (5DL) genes (Eagles et al. 2009). So the suggestive QTL identified for root angle on 5D is not a pleitropic effect from known Vrn-D1 genes. Hartog and SeriM82 both have the Vrn-A1 (5A) winter-type allele, but vary in a single nucleotide polymorphism in exon 4 of this gene. However, no QTL were identified on 5A. Ppd-A1 (2AL) occurs on the same chromosome as a QTL for root angle (Table 2). A suggestive QTL for seminal root number occurs on chromosome 1A, the chromosome where the photoperiod responsive gene Ppd-B2 is located. However, it is yet to be determined whether SeriM82 and Hartog carry different alleles at Ppd-A1 and/or Ppd-B2 loci, or whether these loci are co-located with the identified QTL. Thus, we found no evidence that major height or phenology loci affect root angle or number. The T1BL·1RS has a small effect on root number. The mapping population is segregating for the T1BL.1RS rye translocation carried by SeriM82 but not by Hartog (Rajaram et al. 1983). Greater root biomass and branching compared to the recurrent spring wheat parent has been reported in T1BL·1RS translocation lines (Ehdaie et al. 2003, 2008; Waines and Ehdaie 2007; Manske and Vlek 2002). Sharma et al. (2009, 2011) reported that 1RS loci can be 'major contributors' of both additive effects and epistatic interactions for increased root length and biomass in spring wheat. No QTL were detected on the T1BL·1RS chromosome for root angle by Sharma et al. (2009, 2011) or in the current study. Sharma et al. (2009, 2011) identified three loci associated with root number. One or more of these may be associated with the suggestive root number QTL identified in the present study on chromosome 1B (*qRN.qgw-1B*, $-\log_{10}(P) = 2.14$; Table 2). The presence of the non-rye, Hartog allele correlated with a small but significant reduction in root number which is consistent with the hypothesis that the presence of T1BL·1RS is linked with a more vigorous root system.

The presence or absence of the T1B·1RS translocation had a small affect on root number but did not significantly influence root angle. Thus, we might not expect it to interact with any yield effects modulated by root angle. This agrees with previous reports that the translocation did not contribute positively to yield under water-limited conditions (Peake 2003; Peake et al. 2011; Rattey et al. 2009; Mathews et al. 2008). Root number interacts with seed mass. Seed mass was positively correlated with root number. It is possible that this may reflect higher energy content in larger seeds, allowing development of more root primordia. It is worth noting that in this study, variation in

seed mass was reduced by the selection of even-sized seed for testing. In contrast to our results, Sanguineti et al. (2007) found no significant interaction between root number and seed mass in durum wheat.

How are root angle in seedlings, root architecture in mature plants, and yield associated? Studies in a number of species have found association between narrow root angle in seedlings and narrow root architecture in adult plants (Manschadi et al. 2008; Nakamoto et al. 1991; Oyanagi et al. 1993, 2001). Others have reported a link between root architecture, soil water extraction and yield under water-limited conditions, particularly under terminal drought stress (Passioura 1972; Ludlow and Muchow 1990; Tuberosa et al. 2002a, b, 2007; de Dorlodot et al. 2007). However, a direct link between seedling root angle and crop yield has yet to be established. It has been suggested that a more vertically oriented root system is beneficial for accessing residual moisture in deeper soil layers when upper-layer soil moisture is insufficient (Manschadi et al. 2006; reviewed in Manske and Vlek 2002).

In the present study, moderate heritability was observed for root angle (50 %) and for root number (31 %). The observed heritability values for root angle and root number suggest that the genetic component determining these traits is sufficient to allow phenotypic selection. Given the challenges of root phenotyping, marker-assisted selection may be more cost effective but will require additional QTL experiments to be conducted.

Variation for and significant association with root angle were identified for the validation set at three QTL identified in the SeriM82 × Hartog population (*QRA.qgw-6A, qRA.qgw-6B* and *QRA.qgw-6B.2*; Table 2). This indicates that the variation for and association with root angle at these genetic regions are not restricted to the SeriM82 × Hartog population. Their detection also suggests that the QTL have relevance for adaptation to the target production region. Detection of these QTL further indicates that they are still segregating in cultivars adapted to the target production region and could potentially be responsive to the conventional or marker-assisted selection.

Further studies are under way to identify genetic regions associated with high yield and adaptive traits such as the stay-green phenotype in the Seri × Hartog population. Identification of QTL for root traits in the SeriM82 × Hartog population will facilitate future studies to identify and characterise the relationship between root traits, yield and stay-green phenotype.

Conclusion

Here, we report the first QTL for seminal root angle in a bread wheat population. Multiple, novel QTL were identified for both seminal root angle and root number. Root angle QTL were found to be segregating between wheat cultivars adapted to the target production region. These findings suggest that there is potential to select for root angle in order to enhance adaptation to water-limited environments, particularly environments with terminal water-limitation.

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