Genomic Regions Influencing Seminal Root Traits in Barley

Hannah Robinson,* Lee Hickey, Cecile Richard, Emma Mace, Alison Kelly, Andrew Borrell, Jerome Franckowiak, and Glen Fox

Abstract
Water availability is a major limiting factor for crop production, making drought adaptation and its many component traits a desirable attribute of plant cultivars. Previous studies in cereal crops indicate that root traits expressed at early plant developmental stages, such as seminal root angle and root number, are associated with water extraction at different depths. Here, we conducted the first study to map seminal root traits in barley (Hordeum vulgare L.). Using a recently developed high-throughput phenotyping method, a panel of 30 barley genotypes and a doubled-haploid (DH) population (ND24260 × ‘Flagship’) comprising 330 lines genotyped with diversity array technology (DArT) markers were evaluated for seminal root angle (deviation from vertical) and root number under controlled environmental conditions. A high degree of phenotypic variation was observed in the panel of 30 genotypes: 13.5 to 82.2 and 3.6 to 6.9° for root angle and root number, respectively. A similar range was observed in the DH population: 16.4 to 70.5 and 3.6 to 6.5° for root angle and number, respectively. Seven quantitative trait loci (QTL) for seminal root traits (root angle, two QTL; root number, five QTL) were detected in the DH population. A major QTL influencing both root angle and root number (RAQ2/RNQ4) was positioned on chromosome 5HL. Across-species analysis identified 10 common genes underlying root trait QTL in barley, wheat (Triticum aestivum L.), and sorghum [Sorghum bicolor (L.) Moench]. Here, we provide insight into seminal root phenotypes and provide a first look at the genetics controlling these traits in barley.

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Barley—the fourth largest cereal grain produced (in metric tonnes) worldwide—is an essential raw material for malting and beer production (Food and Agriculture Organization of the United Nations, 2014). It is also an important food source in some countries in Northern Africa and the Middle East. To date, barley breeding conducted in developed countries has focused on yield and the commercial value of improved malt quality. To ensure farmers get a greater return from barley breeding, more emphasis is needed to assemble new cultivars with increased adaptation to abiotic stress and with improved yield stability.

Actual yields are dependent on seasonal and local environmental factors, but one of the more critical factors in Australia is rainfall. Annual crop yields decline sharply during low-rainfall seasons (Gray et al., 2014), a result of drought stress and the lack of drought-adapted cultivars. Barley cultivars predominately grown in Australia are largely based on northern European...
germplasm, which was developed for high-rainfall environments. Hence, Australian barley cultivars are relatively susceptible to drought stress.

Drought adaptation is a complex trait, not only interacting with environment and management practices but also with underlying physiological mechanisms that can be partitioned into many component factors. For example, the drought adaptation trait stay-green alters canopy development, root architecture, and leaf anatomy to maintain green stems and upper leaves during grain filling in water-limiting environments (Pinheiro and Chaves, 2011; Borrell et al., 2014b). Expression of heat-shock proteins, another component trait of abiotic stress tolerance, is switched on during heat stress to improve photosynthesis and water-use efficiency (Wahid et al., 2007). Transpiration efficiency (Sinclair, 2012), relative water content, osmotic adjustment capacity (Blum, 2005), and canopy temperature (Talebi, 2011) are other target traits for the improvement of drought adaptation in small grains such as barley. Quantitative trait loci mapping studies have reported QTL for drought-adaptive traits in barley for leaf relative water content (Teulat et al., 2003), osmotic adjustment capacity, and water-soluble carbohydrate concentration (Teulat et al., 2001).

While a number of studies have examined aboveground water-use traits in barley (Diab et al., 2004; Teulat et al., 2001, 2003; Chen et al., 2010; Siahasar and Narouei, 2010), little research has been conducted for root-system traits. The root-system architecture of a crop can influence the efficiency of water capture and extraction (Kondo et al., 2000; Pennisi, 2008). The fibrous root system of cereals is broadly divided into two categories: seminal roots originating from the primordia in the embryo of the seed and nodal roots developing from the lower tillering and leaf bearing area of the stem (Hochholdinger et al., 2004). Seminal roots emerge first, while nodal roots develop once the plant reaches the tillering growth stage. For drought adaptation, the seminal roots are of interest because of their early development and association with root-system architecture of mature plants (Richard et al., 2015). For instance, in wheat, a more vertical (narrow) angle of the seminal roots and a higher number of seminal roots in seedlings has been linked to a more compact root system with more roots at depth (Manschadi et al., 2006). Therefore, seminal root traits are considered useful proxy traits for desirable root-system architecture within a breeding context (Richard et al., 2015).

Studies in wheat comparing the root architecture of a drought-adapted genotype vs. a standard genotype revealed the drought-adapted genotype to have a compact root system (maximum lateral spread of 45 cm from stem base) where the roots occupied the soil volume uniformly and allocated more root growth to the deepest soil layers, resulting in greater root length (3.8 times more than the standard) in the deepest soil layer (Manschadi et al., 2006). This root-system architecture improves the plant’s access to stored moisture deep in the soil profile. Furthermore, application of a cropping system model in the study by Manschadi et al. (2006) demonstrated that each additional millimeter of water extracted during grain filling generated an extra 55 kg of yield per hectare. Field studies have also found that 1 mm of additional water transpired during grain filling can increase grain yield by ~50 kg ha\(^{-1}\) in sorghum grown under postanthesis drought in a rain-out shelter facility (Borrell et al., 2014a). Studies of this nature are yet to be conducted in barley; therefore, the most beneficial root-system architecture for barley, grown under water-limiting conditions, is unknown.

The importance of identifying QTL for root traits in cereal crops for drought adaptation has been established by several recent QTL mapping studies conducted in wheat, rice (*Oryza sativa* L.), sorghum and barley. In wheat, a large number of QTL—each with minor effect on components for root-system architecture—have been reported, with some 31 QTL identified on chromosomes 2A, 2D, 3A, 3B, 4D, 5A, 5B, and 6A (Hamada et al., 2012; Ren et al., 2012; Bai et al., 2013; Christopher et al., 2013; Liu et al., 2013; Zhang et al., 2013). More specifically for root angle, four QTL have been identified on chromosomes 2A, 3D, 6A, and 6B with two suggestive QTL on 5D and 6B, and for root number two QTL have been detected on 4A and 6A with four suggestive QTL position on 1B, 3A, 3B and 4A (Christopher et al., 2013). The study by Christopher et al. (2013) examined a DH population; therefore, further studies on genetically diverse or elite breeding material may reveal additional QTL. Also, QTL regions identified did not colocate, suggesting that the gene × gene and gene × environment interactions may not be the only challenging obstacle faced when breeding for root traits. In rice, the ratio of deep rooting has been used to evaluate root architecture and can vary from 5 to 95% across rice genotypes. Seven QTL have been identified for rice root traits on chromosomes 2, 4, 6, 7, and 9 (Uga et al., 2011, 2012, 2013a, 2013b). Two QTL were reported as major-effect QTL for root angle: qSOR1 on chromosome 7 (Uga et al., 2012) and DRO1 on chromosome 9 (Uga et al., 2013a, 2013b). The recent cloning and characterization of DRO1 demonstrated that this gene improved deep rooting and enhanced drought adaptation by increasing yield in the field under drought conditions (Uga et al., 2013a). In sorghum, Mace et al. (2012) mapped QTL for nodal root angle (14.5–32.3°) in 141 recombinant inbred lines (RILs) and reported two major QTL, both positioned on linkage group SBI-05 and two suggestive QTL on SBI-08 and SBI-10. All four QTL appeared to colocate with previously identified QTL for stay-green expressed under drought conditions (Mace et al., 2012).

In barley, the following root traits have been mapped: root system size (RSS), root dry weight (RDW), root volume (RV), root-to-shoot ratio (RSR), and root length (RL). The RSS was measured at three time points throughout the life span of a field-grown barley DH population (Derkado [European cultivar] × B83-12/215 [European breeding line]) that was later found to
segregate for the trait (Chloupek et al., 2006). Four QTL were identified for total RSS on chromosomes 1H, 3H, 4H, and 7H and, therefore, reported as a polygenic trait (Chloupek et al., 2006). Three studies have mapped the remaining root traits, all with a focus on detecting QTL in the wild barley accession ISR42-8 and the modern cultivar Scarlet (Naz et al., 2012, 2014; Arifuzzaman et al., 2014). As a result, 37 QTL have been identified for root-related traits in barley (RDW, 16 QTL across all chromosomes; RV, seven QTL on 1H, 2H, 5H, 6H and 7H; RSR, five QTL on 1H, 3H, 5H and 7H; RL, nine QTL on chromosomes 1H–5H). Before the mapping of root traits, Linde-Laursen (1977) demonstrated that root number is under genetic control through examination of a low-rooting barley mutant. Other than this, the availability of barley mutants affecting seminal root angle and number is limited. A mutant with a highly geotropic root system was identified through a chemically mutagenized barely population; however, the population was reported to be unstable and display inconsistent phenotypes (Bovina et al., 2011). Quantitative trait loci for seminal root angle and number are yet to be reported in barley.

The lack of efficient phenotyping methods for root traits is a reason why such traits have not been the subject of study in the past. Methods have been traditionally labor intensive, potentially unreliable, and unrelatable (Zhu et al., 2011). For instance, the 2-D, gel-filled chamber system that enables noninvasive, sequential measurements of root systems preserved in natural orientation is limited when it comes to evaluating large numbers (Bengough et al., 2004). Further, the artificial anaerobic environment may not reflect soil conditions in the field. Sand- and soil-based 3-D methods such as soil coring or plant excavation are limited by their destructive and single-point measurement (Hargreaves et al., 2009). X-ray microtomography imaging is noninvasive and nonharmful, but is restricted to small sample sizes and is expensive and, therefore, inappropriate for screening large numbers (Pierret et al., 2003; Hargreaves et al., 2009). Recently, a high-throughput phenotyping method was described by Richard et al. (2015) that uses clear (transparent) pots and imaging to rapidly evaluate seminal root angle and number in wheat seedlings. This high-throughput method presents new opportunities for mapping of root traits in other cereals, particularly barley where knowledge is limited.

This study exploits the high-throughput, clear-pot phenotyping method to characterize a panel of 30 barley genotypes (comprising Australian cultivars and elite breeding lines) for seminal root angle and number. In addition, the method is employed to characterize a barley DH population (ND24260 × Flagship), which was previously genotyped with DArT markers (Hickey et al., 2011) for discovery of QTL controlling root traits: seminal root angle and root number. We aligned root trait QTL with previously reported QTL for abiotic stress tolerance in barley to help identify key genomic regions possibly underpinning drought adaptation.

### Table 1. Details for the panel of 30 barley genotypes evaluated in this study.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Pedigree</th>
<th>Breeding or commercial use</th>
<th>Commercial use</th>
</tr>
</thead>
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<td>Barke/Rawson</td>
<td>Breeding</td>
<td></td>
</tr>
<tr>
<td>NRB090885</td>
<td>GD24205-1/Grout/Dash</td>
<td>Breeding</td>
<td></td>
</tr>
<tr>
<td>NRB11077</td>
<td>Shepherd/Pinnacle</td>
<td>Breeding</td>
<td></td>
</tr>
<tr>
<td>NRB11116</td>
<td>NRB03470/2ND25389</td>
<td>Breeding</td>
<td></td>
</tr>
<tr>
<td>NRB11755</td>
<td>ND24260-1/Flagship</td>
<td>Breeding</td>
<td></td>
</tr>
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<td>Not available</td>
<td>Breeding</td>
<td></td>
</tr>
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<td>NRB091087/NRB091047</td>
<td>Breeding</td>
<td></td>
</tr>
<tr>
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<td>NRB091087/NRB091047</td>
<td>Breeding</td>
<td></td>
</tr>
<tr>
<td>NRB120742</td>
<td>CLE 245/NRB090734</td>
<td>Breeding</td>
<td></td>
</tr>
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<td>NRB080404-1/2ND25316</td>
<td>Breeding</td>
<td></td>
</tr>
<tr>
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<td>NRB080404-1/NRB08708</td>
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<td></td>
</tr>
<tr>
<td>NRB120883</td>
<td>NRB090031/NRB090326-3</td>
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<td></td>
</tr>
<tr>
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<td>Bowman*5/PI 584760/1/NRB091087</td>
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</tr>
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<td>NRB091098/NRB100285-1-1</td>
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<td>Oxford</td>
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<td>Commercial</td>
<td>Feed</td>
</tr>
<tr>
<td>Shepherd</td>
<td>Reselection from Baronesse</td>
<td>Commercial</td>
<td>Feed</td>
</tr>
<tr>
<td>Westminster</td>
<td>NS197-5547/Barke</td>
<td>Commercial</td>
<td>Malting</td>
</tr>
</tbody>
</table>

1 na, not applicable.

‡ Currently undergoing Stage 1 malting evaluation. Released as feed grade with malting accreditation decision expected 2017.

§ Pedigree confidential under exclusive licence with a third party.

### Materials and Methods

#### Plant Material

Seminal root angle and number were measured for a panel of 30 barley genotypes, comprising a selection of commercial barley cultivars and elite breeding lines (Table 1). Included in this panel are four Australian cultivars (Commander, Compass, Shepherd, and La Trobe), two European cultivars (Oxford and Westminster), and...
24 elite breeding lines from the Northern Region Barley (NRB) breeding program, Warwick, Australia. Nine of the 24 breeding lines are coded (FNDxxx) to protect confidentiality under exclusive licence with a third party.

In addition, seminal root angle and number were examined in 330 DH lines derived from the cross ND24260 × Flagship using the F1 anther culture method performed by the Cereal Double Haploid Program at the Department of Agriculture and Food, Western Australia (Hickey et al., 2011). ND24260 (ND19869-1//ND17274/ND19119), an advanced breeding line from Barley Breeding Program North Dakota State University, has superior grain quality and displays a stay-green phenotype during water deficit (Gous et al., 2013). Flagship (‘Chieftain’/‘Barque’/‘Manley’/VB9104) is an Australian malting cultivar released by the Barley Program at the Waite Campus University of Adelaide.

Characterizing Seminal Root Angle and Number

The seminal root angle and root number of barley seedlings were measured using the clear-pot method detailed by Richard et al. (2015). Grains were sown vertically with the embryo pointing toward the base of the pot at a depth of 2 cm with a 2.5-cm space between kernels and 24 grains per pot against the wall of the transparent ANOVApot (ANOVApot Pty. Ltd.) pot (200-mm diam., 190-mm height, 4 L) in pine bark potting media (pH 6.35, EC = 650 mg kg⁻¹, nitrate = 0, ammonia <6 mg kg⁻¹, and P = 50 mg kg⁻¹). After sowing, the clear ANOVApot pots were placed in black ANOVApot pots (200-mm diam., 190-mm height, 4 L) to exclude light from the developing roots. Seedlings were watered once after sowing and no other nutrients were supplied. Seedlings were grown in a climate-controlled growth facility, where a diurnal (12 h) artificial light and temperature setting of 22 and 17°C (day vs. night) was adopted.

Five days postsowing, roots were imaged using a Canon D500 camera and image analysis was performed using ImageJ software (http://rsb.info.nih.gov/ij/). Seminal root angular spread was defined as the deviation angle from the first vertical root to the first pair of seminal roots (Fig. 1), as outlined by Christopher et al. (2013) and Richard et al. (2015). The first pair of seminal roots was measured at a point 3 cm below the embryo of the grain.

Six days postsowing, seedlings were manually removed from pots by the initial removal of excess soil in the center of the pot. Individual seedlings were then carefully removed along with their roots intact. Excess soil was removed by hand and the individual root axes of each seedling were then counted to determine total seminal root number for each seedling.

Experimental Design

Characterization of the panel of 30 barley genotypes used eight replicates corresponding to a design of 10 pots in 10 rows of a single column on one bench where each pot contained 24 barley seeds. The 30 barley genotypes were allocated to the 24 positions within a pot using a nonresolvable incomplete block design where pots formed the incomplete blocks.

Characterization of the 330 DH lines (including the two parent lines) used eight replicate seeds for each DH line and 32 replicates for each parental line. The experiment included 117 pots, 24 genotypes per pot, with genotypes allocated to the positions within the pots using an optimal resolvable design (Butler et al., 2008). Pots were placed across three benches in the growth facility, with 44 pots per bench aligned in 2-D array of four columns by 11 rows on benches one and two. Bench three contained the remaining 29 pots arranged with 11 rows for columns one and two and seven rows in column three. The eight replicates of the 330 DH lines were aligned with benches, where benches one and two each contained three replicates and bench three contained two replicates. The two parent lines were also distributed across the pots and benches with 13 to 14 replicates of each parent on benches one and two and five replicates of each on bench
three. Thus, benches formed complete replicates and pots formed the incomplete blocks.

**Analysis of Phenotypic Data**

A linear mixed model was fitted to the data for each experiment, where spatial location was accounted for in the design model allowing for bench, column, and row positions in the growth facility. For the panel of 30 barley genotypes, a fixed term for genotype and a random term for pot were used. For the DH population, a fixed term for genotype and a random term for bench, pot, and the positional effect of pot using rows and columns of the design array. Variance components were estimated using residual maximum likelihood (REML) and best linear unbiased estimators (BLUEs) were formed for the fixed genotype effects. Variance components of the analysis were used to calculate the heritability of each trait (root angle and root number) in the panel of 30 genotypes and the DH population. The model was fitted in ASReml-R (Butler et al., 2008).

Linear regression analysis was performed in Genstat 17 (VSN International, 2014) to determine the correlation coefficient between root angle and root number trait means (in each experiment), where a significant correlation was deemed as having a p value <0.05. Linear regression analysis was also used to determine the correlation coefficient between root traits (root angle and root number) and average plant height across four field environments (i.e., Bithramere, Brookstead, Walgett, and Warwick). Summary statistics (population means, standard deviation and 95% confidence intervals) were calculated using Genstat 17 for each experiment.

**Linkage Map and Quantitative Trait Loci Analysis**

The linkage map for the ND24260 × Flagship DH population reported by Hickey et al. (2011) was used for initial marker order and mapping in this study. The map comprises 605 polymorphic (DArT) markers for the 330 DH lines.

The QTL analyses were performed using BLUEs for seminal root angle and root number in Genstat 17. The QTL analysis for single environment with single trait means using composite interval mapping was performed, where seminal root angle and root number were analyzed and mapped individually. The −log10(P) values >3.6 were considered preliminary candidate QTL. This QTL significance level was calculated based on the Bonferroni-based multiple-test control threshold that corrects the experiment-wide error rate for the number of tests performed (Malosetti et al., 2006). A REML variance-components analysis was performed to select the final QTL.

**Collation of Published Quantitative Trait Loci Studies**

Previously reported QTL for traits underpinning drought adaptation in barley were collated from six discovery studies (Teulat et al., 2001; Diab et al., 2004; Chloupek et al., 2006; Chen et al., 2010; Siahsar and Narouei, 2010; Arifuzzaman et al., 2014). From each study, information on the population pedigree, population type (i.e., DH or RIL), population size, observed traits, marker platform, QTL positioning, and the amount of the variation explained by the QTL (R2) was collected.

Across the six discovery studies, 11 different traits related to drought adaptation were analyzed with a total of 62 QTL reported. The location of individual QTL were projected onto the DArT consensus map (Wenzl et al., 2006) along with the QTL identified in this study using the projection strategy detailed by Mace and Jordan (2011). A confidence interval of 4 cM (i.e., 2 cM above and below the peak marker location) was implemented for display purposes. The DArT consensus marker data and QTL positions were visually displayed using MapChart v2.2 (Voortrips, 2002).

**Across Species Analysis of Genes underlying Root Trait Quantitative Trait Loci**

Barley QTL detected in the current study, seminal root angle and number QTL reported in wheat (Christopher et al., 2013), and QTL identified for root traits in sorghum (Mace et al., 2012; Rajkumar et al., 2013; Bekele et al., 2014; Hufnagel et al., 2014; Li et al., 2014; Phuong et al., 2014; Wang et al., 2014) were used for the across-species comparison. A BLAST analysis was performed to identify the physical location of the QTL using QTL flanking markers against the respective genome (i.e., sequence for barley DArT marker BLAST against barley genome), and results filtered by E < 0.0005. The Ensembl genome browser and the genome assembly versions barley 082214v1, wheat IWGSC1.0+popseq, and sorghum 2.1 were used to perform BLAST analyses (http://www.ensembl.org/). Genes underlying each QTL confidence interval were identified using the genome annotations available for each species, and BLASTp was performed to determine the locations of the genes underlying the root confidence interval in wheat and sorghum on the barley genome.

The physical positions of the wheat and sorghum QTL were used to project wheat and sorghum root QTL onto the barley ND24260 × Flagship map. A confidence interval of 10 cM was applied to the projected QTL for display purposes based on the average confidence intervals reported in the original QTL mapping studies. MapChart v2.2 (Voortrips, 2002) was used to visually display the marker locations and QTL.

**Results**

**Root Trait Expression in the Panel of Barley Genotypes**

A high degree of variation in phenotypes for root angle and root number was observed in the panel of 30 barley genotypes (Fig. 2). Seminal root angle ranged between 13.5 and 82.2° with a mean of 49.4° and SD of 16.6°. Seminal root numbers varied from 3.6 to 6.9 roots with a mean of 5.5 and SD of 0.7. Seminal root angle and seminal root number were not significantly correlated (R2 = 0.004). The heritability of the differences observed
for seminal root angle was 0.64 and root number was 0.99. FND004 displayed the narrowest root angle for the breeding lines (13.5°) and La Trobe displayed the narrowest root angle for the commercial cultivars (37.4°). NRB120834-4 and Shepherd displayed the widest root angles of 82.2 and 71.8°, for commercial cultivars and breeding lines respectively (Fig. 2). Breeding lines NRB090885 and NRB11077 had the lowest and highest root numbers, respectively (Fig. 2).

**Root Trait Expression in the Doubled-Haploid Population**

The phenotypic distribution for root traits in the segregating ND24260 × Flagship DH population ranged...
from narrow to wide root angles (Fig. 3a) and low to high root numbers (Fig. 3b). The BLUEs for seminal root angle ranged from 16.4 to 70.5°, with a population mean of 40.2° and SD 9.2°. Root number varied from 3.6 to 6.5 roots, with a population mean of 5.5 roots and a SD of 0.4. In comparison with means obtained by parental lines, Flagship (seminal root angle 34.8° and 5.7 roots) and ND24260 (seminal root angle 45.6° and 5.1 roots), the DH population, displayed transgressive segregation for seminal root traits. Transgressive segregation was depicted by the population exceeding the 95% confidence intervals of both parents (Fig. 3a, 3b). Seminal root angle and number were weakly correlated in the DH population ($R^2 = 0.08, P < 0.001$). High heritabilities were obtained for both traits in the DH population: 0.63 and 0.95 for root angle and number, respectively. Seminal root angle and root number were not significantly

Fig 4. Linear regression between root angle and plant height means and root number and plant height means (across four field environments: Bithramere, Brookstead, Walgett, and Warwick) for the ND24260 × ‘Flagship’ doubled-haploid population. Plant height measurements were collected at spike emergence (stage 5 on the Zadoks growth scale). Positions of the parental lines (ND24260 and Flagship) are represented by blue arrows in Bithramere and Walgett environments where plant height data was available. Correlation coefficient represented by $R^2$ value for each linear regression analysis.
Table 2. Quantitative trait loci (QTL) for seminal root traits in the ND24260 × ‘Flagship’ doubled-haploid (DH) population.

<table>
<thead>
<tr>
<th>QTL</th>
<th>Linkage group</th>
<th>Peak marker†</th>
<th>Position at peak marker cM</th>
<th>−log10(P)‡</th>
<th>Confidence interval§</th>
<th>Flanking markers at peak</th>
<th>Source¶</th>
<th>Variation explained#</th>
</tr>
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<td>226.9</td>
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<td>7.6</td>
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</table>

† Peak position of QTL region on genetic linkage map of the ND24260 × Flagship DH population.
‡ −log10(P) score for QTL peak position derived from composite interval mapping, where a QTL significance threshold −log10(P) of 3.6 was applied based on the Bonferroni threshold.
§ Confidence interval of QTL calculated by the two logarithm of odds drop method.
¶ Parental allele source for wide root angle and high root number for each QTL derived from composite interval mapping.
# Percentage of phenotypic variation for root angle or root number explained by the QTL.

Table 2. Quantitative trait loci (QTL) for seminal root traits in the ND24260 × ‘Flagship’ doubled-haploid (DH) population.

The QTL on chromosome 5HL (RAQ2/RNQ4) were in close proximity to two QTL influencing WSC and leaf relative water content (RWC; Diab et al., 2004) (Fig. 5). The majority of previously identified QTL for drought adaptation traits were located on chromosomes 2H, 3H, 5H, and 7H (Supplemental Table S1). This study is the first to map a QTL for a drought adaptation trait to chromosome 6H (Fig. 5).

Across Species Analysis of Genes Underlying Root Trait Quantitative Trait Loci

The across-species genetic analysis identified 10 genes underlying root QTL across barley, wheat, and sorghum. The 10 genes identified underlie three QTL in barley (RNQ1, RNQ2, and RAQ2/RNQ4), four QTL in wheat (QRA.qgw-2A, QRA.qgw-3D, qRN.qgw-3B, and qRA.qgw-5D), and 10 QTL in sorghum (QRTWgt9.1, QBr.cRt6.1, QRtShtR3.2, QRTWgt4.1, QRTShtR3.1, QRtAng10.1, QRtShtAng7.1, QTAng5.1, QRTWgt6.1, and QRTShtR4.1) (Supplemental Table S2). The gene annotations for the 10 common genes are inexplicit, except for one gene that belongs to the expansin gene family.

Projection of wheat and sorghum QTL onto the barley ND24260 × Flagship map (Supplemental Table S3) shows the overlap of root QTL across all three species. Furthermore, chromosome 5HL and 6H appear to have key regions where root QTL from all three species collocate.

Discussion

This is the first study to measure both seminal root angle and root number in barley. Importantly, this root phenotyping was completed in <7 d, thus highlighting the high-throughput capacity of this phenotyping system. As a result, seven novel genomic regions influencing barley seminal root traits have been detected in the ND24260 × Flagship DH population. Four major effect QTL were identified: one for root angle (RAQ2 located on 5HL) and three for root number (RNQ1, RNQ2, and RNQ4 located on 1H-1, 3H, and 5H, respectively (Fig. 5). Although significant, the effects associated with these QTL were minor, where RAQ1 explained 3.8% of the phenotypic variation and RNQ3 and RNQ5 explained 3.2 and 4.1%, respectively (Table 2). RAQ1 and RAQ2 collectively explain 13.4% of the phenotypic variation observed for seminal root angle, leaving 86.6% unexplained. The five QTL detected for root number collectively explain 30% of the phenotypic variation observed for root number, leaving 70% unexplained. The two parents, ND24260 and Flagship, both contributed positive and negative QTL for seminal root angle and root number.

Of the seven QTL identified in this study, three QTL (RAQ2, RNQ3, and RNQ4) collocate with previously reported genomic regions influencing drought adaptation traits in barley (Fig. 5). RNQ3 positioned on chromosome 4H collocated with QTL influencing water-soluble carbohydrate (WSC; Diab et al., 2004). The QTL on chromosome 5HL (RAQ2/RNQ4) were in close proximity to two QTL influencing WSC and leaf relative water content (RWC; Diab et al., 2004) (Fig. 5). The majority of previously identified QTL for drought adaptation traits were located on chromosomes 2H, 3H, 5H, and 7H (Supplemental Table S1). This study is the first to map a QTL for a drought adaptation trait to chromosome 6H (Fig. 5).
The colocation of previously reported QTL for drought adaptation traits (i.e., RWC and WSC; Diab et al., 2004) on the barley consensus map suggests the genomic region identified on chromosome 5HL (bPb-34072, bPb-5053, bPb-1217, and bPb-2689) may be an important region for abiotic stress tolerance in barley. Furthermore, this genomic region on 5HL is identical to the major QTL previously detected for grain dormancy (qSDND) in the ND24260 × Flagship DH population (Hickey et al., 2012), with the allele for both QTL (grain dormancy and RAQ2/RNQ4) donated by ND24260. It is possible that seed harvested from a plant with a root system that allows increased access to soil moisture may have less abscisic acid as a result of less stress during grain filling and thus reduced dormancy. It is probable that underlying mechanisms for grain dormancy expressed in mature grain may also influence seminal root growth characteristics in barley.

Phenotypic Expression of Root Traits

Previous studies of root traits in wheat, rice, and sorghum have identified narrow root angle and high root number (expressed in seedlings) as a precursor for deep rooting and greater branching at depth. These traits were reported to be particularly beneficial under terminal drought conditions with evidence of water stored at depth (Manschadi et al., 2006; Uga et al., 2011; Mace et al., 2012; Christopher et al., 2013). Based on these trends across cereal crops, such root-system architecture might be desirable in barley cultivars. However, these root traits are yet to be validated as beneficial for barley drought adaptation. Field studies are required to better understand the root architecture of barley and how it contributes to drought adaptation. Further evaluation of the ND24260 × Flagship population in water-limited and irrigated field trials should further our understanding of root-system architecture in barley and its influence on drought adaptation.
While root traits may influence access to water, there are many physiological traits influencing water use and, thus, yield under water-limited conditions, that is, plant height, maturity, tiller production, and early vigor (Gavuzzi et al., 1997; Gonzalez et al., 2010; del Pozo et al., 2012; de Mezer et al., 2014). In rice, no significant difference was observed for shoot traits in the DROI-NIL and its respective standard even though root distribution and drought adaptation differed between the genotypes (Uga et al., 2013a). Similarly, in this study, root angle and root number were not correlated with plant height in the DH population across four field environments (Fig. 4). Across these four field environments in Queensland and New South Wales, postanthesis drought stress is commonly observed. Postanthesis drought stress has less effect on plant height, as plants tend to reach their maximum height at anthesis before the stress. Preanthesis drought stress, on the other hand, is more likely to affect plant height, as the stress occurs during early plant growth. Therefore, in postanthesis drought environments, it is unlikely that root traits and plant height will be correlated, and it is more likely that grain filling will be affected.

Root length has been associated with some semi-dwarf genes in barley. The semidwarfinning gene *ari-e.GP* aligns with QTL detected for root length and is thought to be associated with a reduced root length in barley (Chloupek et al., 2006). The Australian cultivar La Trobe carries this semidwarf gene. Therefore, while La Trobe displays a narrow seminal root angle, the effects associated with the semidwarfinning gene *ari-e.GP* may hinder root length of mature plants and restrict the depth of roots for this cultivar. Flagship, as with many European-derived cultivars, carries the *sdw1* semi-dwarfing gene, which segregates in the ND24260 × Flagship DH population. The lack of correlation between plant height and root traits across four environments (Fig. 4) suggests that *sdw1* does not influence seminal root angle and root number. Similarly, the study by Chloupek et al. (2006) also found no relationship between *sdw1* and root length. The *sdw1* gene was also projected onto the DARt consensus map (Fig. 5) using the 3HL chromosome position reported by Chloupek et al. (2006) to investigate the alignment between *sdw1* and QTL for root traits detected in the current study (RAQ1 and RNQ2). Projection of *sdw1* showed no alignment between the gene and the root trait QTL, with RAQ1 in closest proximity to *sdw1*.

Stay-green is an important trait influencing drought adaptation in cereal grains (Jordan et al., 2012; Mace et al., 2012; Borrell et al., 2014a,b). In other genetic studies using the ND24260 × Flagship DH population, it has been observed that ND24260 contributes the stay-green phenotype in the cross (Gous et al., 2013). In sorghum, four QTL identified for nodal root angle were reported to collocate with stay-green QTL and a putative association was established between nodal root angle and stay-green (Mace et al., 2012). Such genetic relationships are yet to be reported for seminal root angle and stay-green in wheat and barley.

In our study, ND24260 displayed a wider seminal root angle than Flagship and was found to donate the allele for wide root angle and increased root number at the major QTL on chromosome 5HL (RAQ2/RNQ4). ND24260 is a breeding line that was bred and selected under a short summer season in Fargo, ND. ND24260 has good levels of heat stress tolerance but has reduced tiller number and yield in some environments. The soil environment in Fargo consists of black silty clays with high water-holding capacity, similar to southeast Queensland (Natural Resources Conservation Service Soils, 2015; Queensland Government, 2015). When grown in Queensland, ND24260 displays the stay-green drought adaptation phenotype, which is normally a consequence of the improved balance between the supply and demand of water during the grain-filling phase (Borrell et al., 2014a). It is possible the heat stress tolerance of ND24260 is a key factor maintaining this water balance to enable the plant to remain green. In wheat, field experiments have shown that root number, length, and diameter are reduced under high temperatures, especially during the grain-filling phase (Batts et al., 1998). Thus, the heat stress tolerance of ND24260 could act as a protective mechanism for root traits, allowing roots to extract more water during heat stress.

Alternatively, it is possible that the architecture of wide root angle and high root number (displayed by ND24260) enhances the plant’s ability to capture water stored in the soil, resulting in the expression of stay-green. In sorghum, a narrow root angle was associated with the stay-green genotype, which improved access to soil water at depth in the profile (Singh et al., 2012). Similarly, in wheat, the stay-green genotype, SeriM82, extracted more water from depth after anthesis on vertosol soils in northeastern Australia (Christopher et al., 2008). On the other hand, wheat genotypes with wider root angles might be better equipped to use in-crop rainfall as a result of their denser, but shallower, root systems (Liao et al., 2006). It is likely such trends stretch across cereal species; however, field and modeling studies are required to assess the interactions between various barley root architectures, target environments and management strategies (i.e., genotype × environment × management strategy interactions) to identify optimum root angle phenotypes for breeders to target.

**Novel Quantitative Trait Loci for Seminal Root Traits in Barley**

Seven genomic regions influencing seminal root traits were identified in the ND24260 × Flagship DH population in this study. Two QTL were detected for seminal root angle and five QTL for seminal root number. Based on the colocation of the two major QTL on chromosome 5HL (RAQ2 for root angle and RNQ4 for root number), it is highly possible a single gene could underpin both root traits within this genomic region.

Interestingly, the RAQ2/RNQ4 region on 5HL is identical to the major QTL previously detected for grain dormancy (i.e., qSDND) in the ND24260 × Flagship DH population (Hickey et al., 2012), with the allele for both QTL (i.e., grain dormancy and RAQ2/RNQ4) donated by ND24260. In the study by Hickey et al. (2012), spikes were sampled from the field at the point of physiological
maturity, dried, grain threshed by hand, and stored at −20°C to preserve grain dormancy before germination testing. On the other hand, grain used in the current study was sourced from long-term seed storage and lacked grain dormancy, and rapid and synchronous germination was observed for all lines. This suggests the underlying mechanisms (e.g., accumulation of hormones in the grain) that are responsible for expression of grain dormancy in harvest-ripe grain may also influence seminal root growth characteristics during the early stages of germination in barley. To further investigate this key genomic region on 5HL and to identify other genomic regions influencing seminal root traits in barley, experimentation on a barley population with a greater genetic diversity would be desirable. Quantitative trait loci identified in the current study are based on phenotypes assessed at early seedling growth stage, which has yet to be correlated with adult root trait phenotypes in barley. Further experimentation is required to validate the assumption that root angle and number phenotypes observed in early seedlings are representative of these traits at adult growth stage. This could be determined by phenotyping adult plants using large root chambers commonly used for nodal root angle phenotyping in sorghum (Singh et al., 2011). These chambers allow the root system of a plant to be visualized throughout its entire lifecycle, and therefore, comparison between early seedling root phenotypes and adult phenotypes is possible.

Across Species Analysis of Genes underlying Root Trait Quantitative Trait Loci

The comparative genomics analysis identified 10 common genes underlying root trait QTL confidence intervals in barley, wheat, and sorghum (Supplemental Table S2). This suggests that the genetics influencing root traits, more specifically root angle and number, may be similar across the three cereal crops. Of the 10 genes identified, seven of the genes underlie the key barley root trait QTL identified on 5HL in the current study (RAQ2/RNQ4). The same seven genes underlie a minor root angle QTL identified in wheat positioned on chromosome 5D (qRA.agw-5D). Projection of this wheat QTL onto the barley ND24260 × Flagship map (Supplemental Table S3) shows that the two barley root QTL (RAQ2 and RNQ4) and the wheat root QTL (qRA.agw-5D) are in close proximity. For sorghum, three of the seven common genes identified for root angle QTL in barley and wheat underlie a sorghum root angle QTL position on chromosome 5 (QRAAng5.1).

The sorghum gene annotation is the most detailed of the three cereal species and, therefore, is the annotation used in the Supplemental Table S2. Of the 10 common genes identified, one gene has a descriptive annotation and is most likely a member of the expansin gene family. Expansin genes function as principle regulators of cell wall expansion in plants throughout their growth (Lee et al., 2003; Li et al., 2015). Expansin genes have been correlated with the initiation of root growth and root elongation in soybean (Lee et al., 2003) and reported to enhance root growth and improve water stress tolerance in tobacco (Nicotiana tabacum L.) (Li et al., 2015). The gene identified as a member of the expansin gene family would be a key root trait gene to target for any gene specific investigations.

Quantitative Trait Loci for Seminal Root Traits Colocate with Quantitative Trait Loci for Drought Adaptation

RNQ3 mapped to chromosome 4H colocated with QTL for WSC reported by Diab et al. (2004). Water-soluble carbohydrate stored in the stems and the leaf sheaths provide essential nutrients required during grain filling; however, the WSC concentration of a plant is under complex genetic control (McIntyre et al., 2012). The colocation of the genetic control for WSC and root number could suggest that improved WSC concentration may be due to improved root number and greater access to nutrients stored in the soil. Interestingly, the key QTL controlling both seminal root angle and number (RAQ2/RNQ4) on chromosome 5HL is positioned within only 3 cM of QTL influencing WSC and RWC. Relative water content is an indicator used to assess the water status of a plant (Saura-Mas and Lloret, 2007), whereby a high RWC in the leaf during grain filling indicates that the plant is accessing sufficient water to keep cells turgid during this developmental phase. Therefore, it is likely that a more efficient root architecture (root angle and number) enhances the plants ability to access water stored in the soil and improve the plant’s RWC and WSC for vital development. Based on the current literature, this is the first study to map a possible drought adaptation trait (root number) to chromosome 6H. However, the current understanding of genomic regions influencing drought adaptation in barley is far from comprehensive. Future knowledge in these areas should evolve with the increased affordability of genotyping and the development of high-throughput phenotyping methods.

Conclusions

Barley breeders have focused on aboveground traits and have indirectly selected for drought adaptation via selection for yield per se in target environments. Here we present the first study to phenotype root system architecture in barley, in particular, seminal root angle and number, using the high-throughput and inexpensive clear-pot method. A high degree of diversity for seminal root traits was observed in the panel of 30 barley genotypes and the DH population evaluated in this study. The genomic regions identified in this study provide a first look at the genetics of seminal root traits (angle and number) in barley. We have flagged regions on chromosomes 1H, 3H, 4H, 5H, and 6H as influencing seminal root traits, with a region influencing both root angle and root number positioned on 5HL. This key genomic region was found to colocate and share seven common genes with a wheat root angle QTL. Alignment of previously reported drought adaptation QTL with regions identified in the current study highlight the colocation of aboveground (WSC and RWC) and belowground traits (seminal root angle and root number) related to drought adaptation in barley, particularly on chromosomes 4H.
and 5HL. Further investigation is required to determine the desirable barley root system architecture that imparts drought adaptation for specific environments.

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References