At the root of it all: a QTL analysis of root distribution in perennial ryegrass

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Abstract. Perennial ryegrass is the key forage component in pasture systems in New Zealand, Australia and Europe, and is typically characterised by a shallow root system. Breeding for a deeper root profile is desirable, as root depth influences drought tolerance, anchoring of plants against removal by grazing animals, and nutrient capture. Difficulties associated with conventional selection for root traits may be alleviated by developing marker-assisted selection (MAS) tools, the first stage of which is the identification of quantitative trait loci (QTL). The objective here was to detect QTL influencing vertical root distribution in ryegrass. A root distribution ratio, *RTD*, was measured in a replicated mapping population (n=188) grown in sand-filled tubes. Six QTL for *RTD* were detected on five linkage groups, individually accounting for 4 – 18% of phenotypic variation in this trait. *In silico* comparative analysis indicated co-linearity between ryegrass *RTD* QTL and QTL for root traits in the rice genome. To enrich marker density specifically at *RTD* QTL, comparative information was used to develop sequence-tagged site (STS) markers from rice genome-aligned ryegrass expressed sequence tags, with, thus far, STS markers ppt014 and ppt019 successfully targeted to QTL qRTD-3.2 and qRTD-6, respectively. This investigation represents the first step towards developing a MAS strategy for improved root distribution and, subsequently, perennial ryegrass cultivars with superior persistence and environmental performance.

Introduction

Perennial ryegrass (*Lolium perenne* L.) is the dominant pasture species grown in pastoral systems in New Zealand, Australia and Europe. Persistence may be improved through enhanced drought tolerance and resistance to whole plant removal (“pulling”) by grazing animals, both of which are influenced by root system architecture. Root mass in ryegrass is typically shallow, with approximately 80% of the roots concentrated in the top 10-15 cm of soil (Troughton 1957). A root profile penetrating deeper into the soil is expected to enhance drought tolerance by improving the ability of the plant to access water at depth (Huang *et al.* 1997), and is a factor in anchoring the plant against “pulling” (Thom *et al.* 2003). In addition to persistence, increased root density deeper in the soil profile may enhance the interception of leached nitrate (Bowman *et al.* 1998).

Heritable differences between ryegrass families in root system size and root/shoot ratio have been established (Bonos *et al.* 2004, Crush *et al.* 2006), indicating potential to breed for deeper root systems in this species. The relative difficulty associated with conventional selection for root traits may be mitigated by a marker-assisted selection (MAS) approach, with molecular marker assays employed as an adjunct to conventional selection. The merit of such a strategy has seen considerable effort in QTL (quantitative trait locus) discovery for root traits in major crops including maize (Tuberosa *et al.* 2003) and rice (Price and Tomos 1997, Li *et al.* 2005), and introgression of QTL for root traits using MAS has been demonstrated with some success in rice (Shen *et al.* 2001, Steele *et al.* 2005).

Our objective was to use QTL analysis to identify genetic factors influencing vertical root distribution in perennial ryegrass, using data from a mapping population grown in a root tube system. This represents the first step towards developing a MAS strategy for improved root system characteristics, and thereby enhanced persistence and environmental performance, in perennial ryegrass.

Materials and methods

Population

Mapping population IxS is the F₁ full-sibling progeny (n=198) of a pair cross between heterozygous parents from cultivars 'Grasslands Impact' and 'Grasslands Samson'.

Measurement of vertical root distribution

Single tillers from three clonal replicates of each F₁ and parental genotype were planted in sand-filled 1m deep x 0.09m wide rigid plastic tubes, and arranged in a randomised complete block design. Tubes were kept in a temperature-controlled glasshouse and irrigated daily with low-ionic strength nutrient solution (Crush *et al.* 2005). After 60 days growth, sand profiles were divided into 11 depth increments (0-5 cm, 5-10 cm, and 10 cm thereafter), washed, and roots dried and weighed. Data used for QTL analysis were *RTD*, the ratio of root dry weight in the 10-20 cm depth/ root dry weight in the 0-10 cm depth.

Genetic linkage analysis and comparative mapping

A genetic linkage map was constructed using JoinMap 3.0 (<http://www.kyazma.nl/>), with 165 EST-SSR markers (simple sequence repeat markers derived from expressed sequence tags) for population IxS. Marker PCR and genotyping were conducted as in Faville *et al.* (2004). The macro-syntenic relationship of the ryegrass genome map to the physical rice genome was established *in silico*, as described previously (Faville 2005).

QTL analysis

QTL analysis of *RTD* in population IxS was conducted using MQM in MapQTL 4.0 (<http://www.kyazma.nl/>). Permutation testing (n=1000) established a logarithm-of-odds (LOD) threshold of 3.42 to declare a QTL at genome-wide significance of $\alpha=0.05$.

STS marker development and mapping

Sequence-tagged site (STS) markers were developed from perennial ryegrass ESTs aligned by GMAP (Wu and Watanabe 2005) to positions in the TIGR rice pseudomolecule assembly proximal to rice markers for root trait QTL (root dry weight, root weight, rooting depth, root length, and deep root dry weight) identified at Gramene (<http://www.gramene.org>). STS primers (18-24 bp long, targeted product length 120 – 380 bp) were designed with Primer 3 (Rozen and Skaletsky 1998). Primer synthesis, PCR and genotypic analysis were performed as for EST-SSR analysis.

Results and discussion

Distribution of root mass in the IxS population was consistent with previous observations for perennial ryegrass (Troughton 1957, Crush *et al.* 2005), with approximately 70% of root dry weight in the top 20 cm of the root tubes after 60 days growth (data not presented). Mean *RTD* values were normally distributed (Figure 1), ranging from 0.138 to 1.010 across 190 genotypes (overall mean = 0.470). Values of 0.245 and 0.313 were obtained for the 'Impact' and 'Samson' parents, respectively, and ANOVA confirmed significant differences in *RTD* amongst genotypes ($p<0.0001$).

Figure 1. Distribution of mean *RTD* values (ratio of root dry weight in the 10-20 cm depth/ root dry weight in the 0-10 cm depth) amongst 188 IxS population genotypes, plus values for ‘Impact’ (I) and ‘Samson’ (S) parents indicated by arrows.

Figure 2. Perennial ryegrass genetic linkage map showing QTL (qRTD-2 to qRTD-7) detected for the root distribution trait *RTD*. Linkage groups LG 1 to LG7 show marker names at left of bars, QTL positions at right of the bars, and length of linkage groups in centimorgans (cM) at the foot of the bars. The position of STS markers ppt014 and ppt019 are shown at right of LG 3 and LG 6, respectively.

Table 1. QTL detected for parameter *RTD*. LOD = LOD score at peak of the QTL, %PVE = percentage of phenotypic variation explained by QTL. Marker position in rice = rice genome position (Mb, megabase pairs) of putative rice orthologue identified for the ryegrass marker underlying the QTL.

QTL	LOD	%PVE	Marker position in rice (chromosome: Mb)
qRTD-2	4.16	5.9	4: 20.2
qRTD-3.1	8.67	17.6	1: 22.7
qRTD-3.2	6.51	15.1	1: 35.6
qRTD-4	4.09	6.3	3: 0.1
qRTD-6	3.49	4.0	2: 8.7
qRTD-7	3.69	5.6	6: 26.2

Linkage analysis located 164 EST-SSR loci on seven linkage groups (Figure 2) with assignments consistent with other ryegrass maps (Faville *et al.* 2004). The IxS map is 588 Kosambi cM long, with mean density of 3.6 cM/locus and eight intervals > 10 cM. Six genomic regions affecting RDT were identified (Figure 2) by QTL analysis, with single QTL of moderate effect on each of linkage groups LG 2, LG 4, LG 6 and LG 7, and two of large effect on LG 3 (Table 1).

A previously-established comparative framework (Faville 2005) was used to identify rice root trait QTL in positions potentially colinear to ryegrass *RTD* QTL (Table 2). Particularly close correspondence was identified for qRTD-3.1, -6 and -7. Co-location may reflect conservation of genetic control of root distribution between these species, although this comparison may be influenced by ambiguities associated with genetic linkage analysis.

Anchoring of ryegrass *RTD* QTL to rice syntenic positions provides access to the rice genome as a template for further marker development. Enrichment of marker density specifically at *RTD* QTL sites will facilitate development of markers with greater MAS potential and provide a starting point for future QTL fine-mapping activities. STS markers were developed from ryegrass ESTs aligned to rice BAC/PACs carrying root trait QTL markers, with STS markers ppt014 and ppt019 targeted and successfully mapped to the QTL regions occupied by qRTD-3.2 and qRTD-6, respectively (Figure 1). Development of markers targeted to other *RTD* QTL is in process.

QTL discovery is the first step in the longer process of developing marker assays that may be deployed in a MAS strategy. The next phase of this project will entail testing and further development of QTL-targeted markers, and the analysis and validation of QTL in different genetic backgrounds and environments. This will include establishing the significance of data derived from the root tube system to plant performance in field plots.

Table 2. Comparison of perennial ryegrass (RG) *RTD* QTL with rice root trait QTL recorded at Gramene (<http://www.gramene.org>). RG and rice marker positions refer to the marker underlying the QTL, or nearest flanking marker, and data shown are rice chromosome and position in Mb. Rice QTL map: 1=Aberdeen Bala/Azu QTL 2002, 2=CNHZAU Zh97/Ming63 RI QTL 2002, 3=CNZU Azu/IR1552 RI QTL 2003, 4=Cornell IR64/Azu DH QTL 2001, 5=CTIR CT9993/IR6226 QTL 2000, 6=IGCN, 7=IRRI IR64/Azu DH QTL 2003, 8=TTU IR64/Orufi RI QTL 2003. BAC = bacterial artificial chromosome, PAC = P1-derived artificial chromosome.

RG QTL	RG marker position in rice	Rice QTL map	Rice QTL marker (BAC/PAC)	Rice marker position
qRTD-03-2	4: 20.2	1, 8	RZ467 (AL606615)	4: 22.2
qRTD-03-3.1	1: 22.7	8	bcd134 (AP003075)	1: 21.6
qRTD-03-3.2	1: 35.6	1, 2, 3	C86 (AP003235)	1: 37.7
qRTD-03-4	3: 0.1	3, 4, 5, 7	RZ329 (AC107225)	3: 3.5
qRTD-03-6	2: 8.7	6	RM452 (AP005428)	2: 9.6
qRTD-03-7	6: 26.2	3, 5	RZ140 (AP003517)	9: 26.4

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