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A quantitative trait locus analysis of root distribution in perennial ryegrass (Lolium perenne L.)

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Introduction Root system architecture impacts perennial ryegrass performance, with deeper roots potentially contributing to drought tolerance, nutrient interception, and anchoring of plants. Root mass in a perennial ryegrass sward is typically shallow, concentrated in the top 10 cm of soil (Troughton 1957). Phenotypic selection for deeper root systems in breeding programmes is limited by the inaccessibility of underground plant components. We aim to use quantitative trait locus (QTL) analysis to discover genetic factors influencing root architecture traits, including vertical root distribution, in perennial ryegrass. Ultimately, markers linked to root architecture QTL may be used in a marker-assisted selection strategy that would alleviate the limitations of conventional selection, and lead to ryegrass cultivars with improved production and environmental performance.

Materials and methods A bi-parental genetic linkage map was constructed (JoinMap 3.0) using 165 EST-SSRs (simple sequence repeat markers derived from expressed sequence tags) in population IxS, a full-sib F_1 (n=198) developed by pair-crossing individual heterozygous genotypes from cv. 'Grasslands Impact' and cv. 'Grasslands Samson'. Tillers from three clonal replicates of each F_1 and parental genotype were planted in sand-filled 1m deep x 0.09m wide rigid plastic tubes. Tubes were irrigated daily with low-ionic strength nutrient solution, in a temperature-controlled glasshouse. Root dry weight (RDW) (g) in 11 depth increments was recorded after 60 days of treatment. Data analysed were the ratio of RDW in the 10-20 cm depth to RDW in the 0-10 cm depth. ANOVA indicated significant differences amongst genotypes for the RDW ratio (F value = 2.86). Mean RDW ratio for each genotype was used for QTL analysis by MQM mapping implemented in MapQTL 4.0 software. Permutation testing (n=1000) established a logarithm-of-odds (LOD) threshold of 3.60 to declare a QTL at a genome-wide significance of α =0.05.

Results Mean RDW ratio varied from 0.138 to 1.010 across 198 genotypes (overall mean = 0.470). Values of 0.245 and 0.313 were measured in the Impact and Samson parents, respectively. Linkage analysis located 160 EST-SSR loci on seven linkage groups (LG1 – 7) with assignments consistent with other ryegrass maps (Faville *et al.*, 2004). The IxS map is 462 Kosambi cM long, with mean density of 2.9 cM/marker and only 8 intervals > 10 cm. Five genomic regions affecting RDW ratio were identified by MQM QTL analysis, with two exceeding the significance threshold of LOD 3.60 - one on LG3 (LOD 6.07, peak 49 cM, 11% variance explained) and another on LG6 (LOD 3.61, peak 18 cM, 6% variance explained). *In silico* analysis suggests the QTL occur in genomic regions syntenic with regions of the rice genome (chromosomes 1 and 2) where QTL for root morphological traits have been identified (Yadav *et al.*, 1997; Hemamalini *et al.*, 2000).

Conclusions We have detected two QTL affecting vertical root distribution in perennial ryegrass, the first reported for root architecture in this species. The results demonstrate that the experimental system is suitable for detecting genetic variation in root architecture of perennial ryegrass, and for investigation of QTL underlying that variation. This system will be used for validation and expansion of the current experimental results, and will serve as a vehicle for further investigation of the genetic basis of root architecture in perennial ryegrass.

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