Approaches for Associating Molecular Polymorphisms with Phenotypic Traits Based on Linkage Disequilibrium in Natural Populations of *Lolium Perenne*

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Introduction Association mapping relies on linkage disequilibrium (LD) between haplotypes and quantitative trait loci (QTL). The level of LD in a genome determines the resolution of this approach. In out-breeding species, LD is expected to decay rapidly, thus allowing for high-resolution mapping. It has been most extensively used in human genetics, but recent work with maize populations has demonstrated its potential in plants (Thornsberry *et al*., 2001; Wilson *et al*., 2004), and used in *L. perenne* to identify AFLP markers associated with a major QTL for heading date on linkage group 7 (Skøt *et al*., 2004). The objective of the present work is to associate allelic variation in candidate genes for heading date and water soluble carbohydrates (WSC) in natural populations of *L. perenne* with phenotypic variation. Both these traits are important breeding targets in ryegrass.

Materials and methods 100 genotypes from each of 9 *L. perenne* populations with a wide range of variation in heading date, were grown in pots, vernalised during the winter, prior to determining the heading date in days after March 1st for emergence of the third inflorescence. The plants were then cut back before taking tillers for planting two replicates of each genotype as spaced plants in the field in a completely randomised design. The above ground biomass was later harvested from the remaining tillers in the pots, and ground for analysis of water soluble carbohydrates, total nitrogen and dry matter digestibility using Near Infrared Spectroscopy. DNA was extracted using a QIAGEN kit, and sequencing performed with an ABI 3100 Genetic Analyzer.

Results In *L. perenne* the orthologue of *Hd1* in rice is a potential candidate gene for involvement in the control of photoperiod dependent flowering. A 7.3 kb region containing the putative *Hd1* orthologue was sequenced from a BAC clone, and identification of single nucleotide polymorphisms (SNP) in this region is in progress. An alkaline invertase gene located on linkage group 6 co-locates with a QTL for WSC and is thus a candidate for involvement in this phenotypic trait. A 6238 bp genomic clone of this gene was sequenced (Figure 1), and SNPs were identified in 24 genotypes representing the variation of the populations being investigated. One SNP per 28 nucleotide was found. The average nucleotide diversity was 0.0039, approximately 5 times higher than in *Sorghum*, but similar to maize. LD decays to 0.1 within 2-3 kb (Figure 2). These data indicate a high level of diversity. Association analysis of the SNPs identified in all the genotypes for both the alkaline invertase and the *Hd1* orthologue is being related to phenotypic data for WSC and heading date.

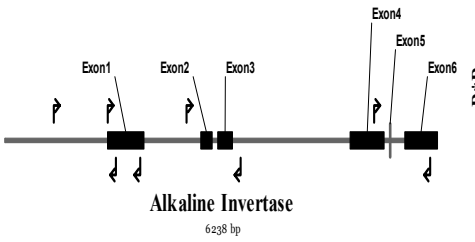

Figure 1 Structure of alkaline invertase gene in *L. perenne*

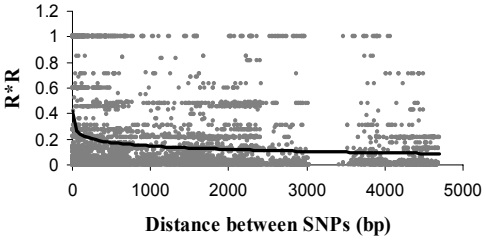

Figure 2 Pattern of LD within alkaline invertase

Conclusions The high level of nucleotide diversity, and the rapid decay of LD within the alkaline invertase gene is consistent with *L. perenne* being a self-incompatible species. Association mapping has potential value in candidate gene approaches for identifying allelic variants contributing to phenotypic variation.

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