

SNP discovery and haplotypic variation in full-length herbage quality genes of perennial ryegrass (*Lolium perenne* L.)

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Introduction The development of forages with enhanced nutritive value through improvements of herbage quality (digestibility, carbohydrate content) is potentially capable of increasing both meat and milk production by up to 25%. However, the expense and time-consuming nature of the relevant biochemical and biophysical assays has limited breeding improvement for forage quality. The development of accurate high-throughput molecular marker-based selection systems such as single nucleotide polymorphisms (SNPs) permits evaluation of genetic variation and selection of favourable variants to accelerate the production of elite new varieties.

Materials and methods SNP marker discovery in perennial ryegrass has been based on PCR amplification and sequencing of multiple amplicons designed to scan all components of the transcriptional unit. Full-length genes with complete intron-exon structure and promoter information corresponding to well-defined biochemical functions are ideal for the determination of complete SNP haplotype data. The gene classes involved in this study were the lignin biosynthetic genes *LpCCR1* (cinnamoyl CoA-reductase, 12.1 kb, AY061889), *LpCAD2* (cinnamyl alcohol dehydrogenase, 7.2 kb, AF472592) and the fructan biosynthetic genes *LpFT1* (= *Lp6SFT* =sucrose:fructose 6-fructosyltransferase, 9.98 kb, AF481763) and *Lp1-SST* (sucrose:sucrose 1-fructosyltransferase, 4.63 kb, AY245431).

Results Multiple SNPs located at regular intervals across the transcriptional unit were detected within and between the heterozygous parents of the F₁(NA₆ x AU₆) genetic mapping family, and were validated in the progeny set. The total numbers of putative SNPs identified were 54 (*LpCAD2*), 212 (*LpCCR1*), 265 (*LpFT1*) and 240 (*Lp1-SST*). The *Lp1-SST* gene locus was assigned for the first time to a genetic map location on LG7 of perennial ryegrass through SNP mapping (Fig 1). Haplotype structures in the parental genotypes were defined and haplotypic variation was assessed in diverse germplasm. This data was used to determine the average rate of decay of linkage disequilibrium (LD) over distances comparable with gene length. This analysis will provide crucial information for the validation of strategies to correlate haplotypic variation and phenotypic variation.

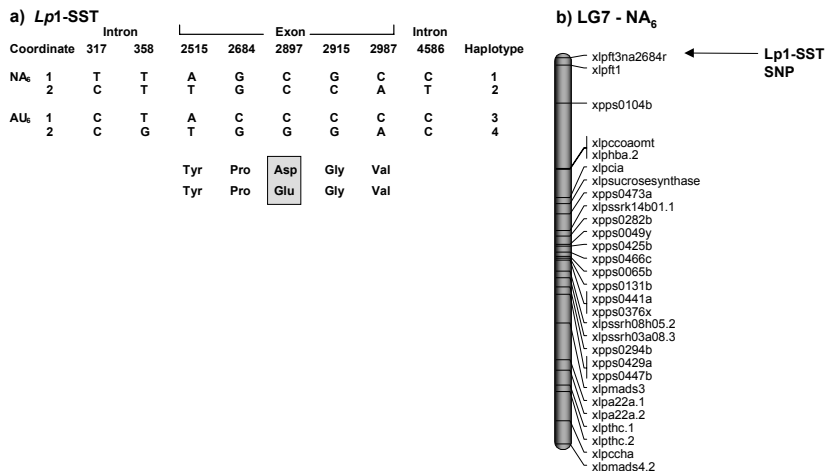


Figure 1 a) Partial SNP haplotype structures for the *Lp1-SST* gene and b) genetic map location of a validated *Lp1-SST* SNP locus on LG7 of the NA₆ parental genetic map derived from the perennial ryegrass F₁(NA₆ x AU₆) genetic mapping family