

Genetic analysis of the interaction between perennial ryegrass and the fungal endophyte *Neotyphodium lolii*

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Introduction The fungal endophyte *Neotyphodium lolii* is widely distributed in perennial ryegrass pastures, especially in Australia and New Zealand. The presence of the endophyte is associated with improved tolerance to water and nutrient stress and resistance to insect pests, but is accompanied by reduced herbivore feeding. The molecular mechanisms responsible for these endophyte-related traits are in general poorly understood. Comparisons of different grass-endophyte associations show that endophyte-related traits are affected by both endophyte and host genotype, and environmental interactions.

Materials and methods To investigate the role of host genetic factors, preliminary research has focused on quantitative trait loci (QTL) analysis of a full-sib mapping family with a single resident endophyte. This F₁ (Northern African₆ x Aurora₆) population is based on a two-way pseudo-testcross structure for which two genetic parental maps have been generated, populated by genomic DNA-derived simple sequence repeat (SSR), expressed sequence tag-derived SSRs (EST-SSRs) and EST-RFLPs. The F₁(NA₆xAU₆) family also provides the resource for *in vitro* gene-associated single nucleotide polymorphism (SNP) discovery. A semi-quantitative enzyme-linked immunosorbent assay (ELISA) test was used to detect endophyte in the progeny of this family and ELISA scores in the field were examined using simple interval mapping (SIM) and composite interval mapping (CIM). The development of a quantitative real-time (RT) PCR assay to measure endophyte content *in planta* in the F₁(NA₆xAU₆) family is described.

Results SIM and CIM mapping of the variation in ELISA scores assessed in the field for endophyte-positive progeny has led to the identification of several genomic regions on the maps of both parents that significantly affect the trait. The ELISA score may be potentially correlated with endophyte incidence, efficiency of colonisation and associated metabolic traits. Candidate symbiosis-associated genes in perennial ryegrass have been identified based on sequence annotation and gene expression data, and mapping of these candidate genes will allow evaluation of co-location with endophyte-specific trait QTLs. Strategies for the identification of sequence variation in key symbiosis-related genes of the endophyte, such as the *dmaW* gene that plays a determinate role in ergovaline biosynthesis, will be described.

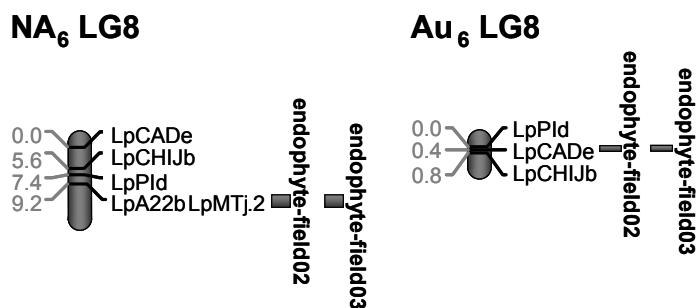


Figure 1 Identification of an endophyte-associated QTLs based on semi-quantitative ELISA scores derived from field-grown progeny of the F₁(NA₆xAU₆) genetic mapping family. Measurements were taken in two successive seasons (summer 2002 and summer 2003).