Controlled Flowering Project for Lolium Perenne at Agresearch: an Overview

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Introduction Ryegrass (*Lolium perenne*) is an important forage crop in New Zealand. The work presented here has the goal of developing a system for complete and arbitrary control of the transition from vegetative to floral development. For this, we have pursued an integrated approach utilising genomics with both forward and reverse genetics. Like other model plants, photoperiodic and vernalization pathways are presumed to be operating in ryegrass and control the activity of the meristem identity/floral patterning genes. The candidate gene approach targeting the photoperiodic pathway is described in an accompanying abstract (Gagic *et al*.). Other candidate genes include the meristem identity gene LEAFY and a range of the MADS box transcription factors. Relevant expression profiles are established for these genes, i.e. vernalization time course at weekly intervals, and daily and circadian collections during the secondary induction. A detailed genetic map of ryegrass has been developed at AgResearch (see abstract by Faville *et al*.) which we are using to map candidate genes. We are also conducting detailed phenotypic analysis of the flowering behaviour variation within this population in an effort to isolate relevant QTLs. Ryegrass transformation has been used to ascertain functions of the candidate genes and to manipulate flowering time control directly. We are developing a universal switch to turn on the flowering that consists of a cassette of the arabidopsis genes under a control of a chemically inducible promoter.

Materials and methods For the candidate gene isolation, a combination of approaches involving screening of our ryegrass EST library and direct amplification using degenerate oligonucleotides derived from conserved sequences, followed by additional screening to obtain full-size cDNA and genomic sequences (5' and 3'-RACE, genome walking) have been used. Gene expression was assayed using real-time RT-PCR analysis from samples of cloned material grown in a controlled environment. To map the genes, SNPs were identified and their segregation scored using direct sequencing of PCR products from genomic DNA. For the QTL mapping, sets of clones of the mapping population were exposed to variable vernalization and then either induced under artificial long days, or naturally. Dates of the 1st and 3rd head emergence were scored and the number of total and floral tillers counted at the end of the experiment. Traits reflecting flowering time, uniformity of induction and percentage of induced tillers were derived from these measurements, as well as the effect of vernalization on the traits across treatments. For transformation, micro projectile bombardment of leaf explants/young inflorescences was used with subsequent callus formation and plant regeneration in tissue culture. For the floral induction switch, we used a derivative of the pHTOP/LhG4::GR (Moore *et al.,* 1998) system in which expression is controlled with dexmethasone.

Results Candidate genes were isolated that are putative orthologues of the arabidopsis GI, CO, LFY, FT, TFL, MFT, AP1, SOC1, SVP, and wheat VRN1. GI, FT and CO expressions have circadian cycles (see Gagic *et al*.). LFY, GI, SVP, FT and CO have been mapped and the others are in progress (see Faville *et al*.); the VRN1 mapping has been published (Jensen *et al.,* 2004). Significant QTLs have been found on LG1, 2 and 4. Some of these may overlap with other published QTLs (Armstead, *et al.,* 2004) and the position of candidate genes. Direct over-expression of the *Arabidopsis* FT gene in ryegrass led to an expected acceleration of flowering. The inducible flowering switch works very efficiently in *Arabidopsis* and its utility in other species including ryegrass is being investigated.

Conclusion A complex approach toward understanding the genetics of flowering in ryegrass is an efficient way to isolate relevant genes and provides a foundation for the manipulation of flowering behaviour using both transgenic and conventional breeding strategies.

References

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