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Flowering in ryegrass and conservation of the photoperiodic response

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Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Biological Sciences, University of Auckland, August 2007

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ABBREVIATIONS

CO, CO-like, TOC1 domain
Complementary DNA
Cultivar
Derived cleaved amplified polymorphic sequences
Continuous darkness
Deoxyribonucleic acid
Deoxyribonucleoside triphosphate
Expressed sequence tag
Gibberellin
Genomic DNA
Green fluorescent protein
Kilobase
Long day
Continuous light
Minutes
Megabases
Messenger RNA
Nearly isogenic line
Nuclear localization sequences
Polymerase chain reaction

QTL	Quantitative trait loci
Pfr	Far-red light absorbing form of the photochromic protein
Pr	Red light absorbing form of the photochromic protein
RACE	Rapid amplification of cDNA ends
RFLP	Restriction fragment length polymorphism
rpm	Revolution per minute
RT-PCR	Real time PCR
rt-PCR	Reverse transcription PCR
S	Seconds
SAM	Shoot apical Meristem
SD	Short day
SNP	Short nucleotide polymorphism
SSR	Simple sequence repeat
UTR	Untranslated region
wt	Wild type
ZT	Zeitgeber time

ABSTRACT

Grasslands account for almost one quarter of the world's cover of vegetation. Almost three quarter of the world's milk, beef and veal are produced from temperate grasslands. In New Zealand, ryegrass (*Lolium perenne*) is the main pasture constituent with more than half of the total export revenue coming from grass-related products. Much of ryegrass production and quality depends on the timing of flowering through seasonal progression. In many plants, day length is the critical environmental parameter that controls when plants begin to flower. In *Arabidopsis* the *CONSTANS* (*CO*) gene mediates day length response. Upstream of *CO* is the *GIGANTEA* (*GI*) gene which is associated with the circadian clock mechanism and is required to promote *CO* expression. The *FT* gene is the immediate downstream genetic target of *CO* and is a direct promoter of flowering.

In this study, cDNA libraries, sequence alignment and genome walking were used to sequence and describe three putative orthologues from the ryegrass photoperiod pathway: LpGI, *LpCOL1*, and *LpFT3*. All three behaved in a true photoperiod manner characterised by cycling expression patterns under continuous light conditions and differential expression patterns in LD and SD conditions. Different photoperiods brought about differences in expression of these genes measured either by the phase shift change (LpGI and LpCOLI) or by the change of the transcript level (*LpFT3*). Gene expression changes over a vernalisaton time course were also analysed and results indicated that LpFT3 acts as the flowering integrator. The role of *LpGI*, *LpCOL1*, and *LpFT3* as putative photoperiod genes was further confirmed by genetic mapping, which placed them on linkage groups 3, 6, and 7, respectively. The syntenic positions in rice contain major heading date quantitative trait loci. The function of LpFT3 was examined by over-expressing the gene in Arabidopsis under control of the cauliflower mosiac virus (CaMV) 35S promoter. Substantially higher expression of the endogenous Arabidopsis AtFT transcript was observed in the mutated ft-1 line overexpressing *LpFT3*, suggesting a positive feedback loop either directly or through upstream intermediaries. Overexpression of the LpGI and LpFT3 genes restored rapid flowering to the respective gi-3 and ft-1 Arabidopsis mutants while overexpression of LpCOL1 did not accelerate flowering either in co-2 or wild type Arabidopsis plants. However, overexpression

of *LpCOL1* completely restored the late flowering phenotype of the *gi-3* mutant indicating the existence of another important link outside the well established hierarchy of *GI-CO-FT* in the photoperiod pathway.

This study revealed that the ryegrass photoperiod pathway genes show high similarity to their wheat, rice and *Arabidopsis* counterparts. Exploring ways to modulate flowering time in ryegrass could provide major benefits to the agricultural industry by increasing forage quality, controlling seed and pollen production, and addressing potential problems linked with climate change.