## Towards understanding photoperiodic response in grasses

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**Keywords:** flowering, photoperiod candidate gene orthologues

**Introduction** In many plants, day length is the critical environmental parameter that controls flowering time. In long day plants, such as Arabidopsis and ryegrass (*Lolium perenne*), increasing day length in spring signals flowering, while in short day plants like rice, flowering is accelerated when days become shorter. Recently, significant progress has been made in understanding the molecular genetic mechanisms that govern this response. Most results have been obtained in the model plant *Arabidopsis* where *CONSTANS (CO)* is a critical candidate gene. Upstream of it is the *GIGANTEA (GI)* gene which is associated with the circadian clock mechanism (1). The *FT* gene is the immediate downstream genetic target of *CO*, and is a direct promoter of flowering (2). Characteristically, all three genes show circadian expression, albeit in different phases, and both the *CO* and *FT* genes are up-regulated under long-day (inductive) conditions. Work in ryegrass should help reveal both the conserved and divergent segments of the photoperiod response between different plant species.

**Material and methods** Putative orthologues of *Arabidopsis* (At) GI, CO, and FT genes were isolated and sequenced using a combination of different methods including a ryegrass EST library screen, multiple alignment and degenerate primers, 5' and 3' RACE-PCR, and gene walking. Gene expression was carried out using real time RT-PCR on clones of Grasslands Impact ecotype grown in a controlled environment.

**Results** The full-size *LpGI* gene was found to contain 11 introns and encoded a 1149 amino acid (aa) protein. Expression analysis of GI showed diurnal oscillation and different expression patterns under long day (LD) and short day (SD) conditions with the peaks in LD coinciding with the light period of the day. The *LpCO* gene encoded a 365 aa protein whose sequence contained two zinc-finger domains, a feature specific for the *CO*–like genes, as well as a CCT region near the carboxy domain similar to that observed in the barley *CO* and rice *Hd1* gene. In addition, the intron region contained a Dof2 transcription factor binding domain whose role is yet to be elucidated. Phylogenetically, the *LpCO* groups align closely with *OsHd1*, *HvCO1*, and *AtCO* (Fig 1). We have also isolated 3 ryegrass FT-like genes with *LpFT3* showing the highest similarity with rice *Hd3a*. Expression pattern analysis using real-time RT-PCR revealed a 30-fold increase under LD conditions which is consistent with previous findings in *Arabidopsis*.

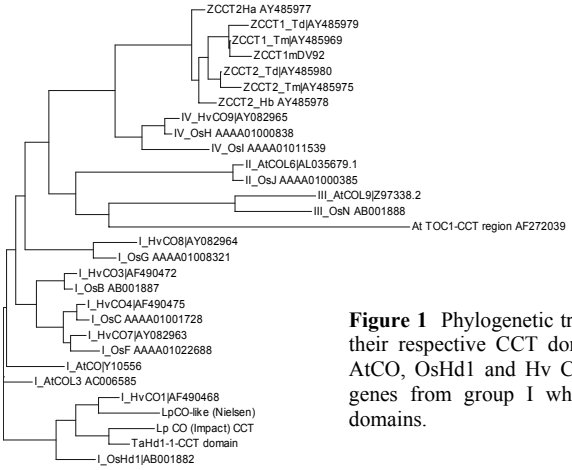


**Figure 1** Phylogenetic tree of the CO and COL genes deduced from their respective CCT domains. The LpCO groups closely with the AtCO, OsHd1 and Hv CO1 genes as well as the rest of the COL genes from group I which all contain two functional zinc-finger domains.

**Conclusion** Initial results reveal that the ryegrass photoperiod pathway genes show high similarity to their wheat and rice orthologues. The CO gene contains two complete B-box regions as indicated by the presence of His and Cys residues with conserved positions in agreement with the general consensus. Expression analysis under LD and SD conditions showed that GI and FT cycle in the same manner as the *Arabidopsis* orthologues.

**References**

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