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Circadian clock gene expression and flowering time control in short-day and long-day plants

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Abstract

A practical dilemma facing plant breeders is predicting when a crop will flower under changing daylength conditions - and the answer lies in the circadian clock. This research compared expression profiles of six core clock and flowering-pathway genes (CCA1, LHY, TOC1, GI, CO, and FT) in a short-day plant (rice, *Oryza sativa* cv. Nipponbare) and a long-day plant (*Arabidopsis thaliana* Col-0) grown under controlled short-day (8L:16D) and long-day (16L:8D) photoperiods. Plants were grown at the North East Centre for Biological Research, Shillong, from April to October 2023. Leaf samples were harvested every 4 hours over 48-hour time courses. RT-qPCR revealed that CCA1 and LHY showed conserved dawn-phased oscillation in both species regardless of photoperiod, but their peak amplitude was 1.4-fold higher under inductive conditions (SD for rice, LD for *Arabidopsis*). The critical difference emerged at the CO-FT interface: in *Arabidopsis* under LD, CO protein stability coincided with light exposure during the evening, driving FT expression to 37.8-fold above dark-period levels. In rice under SD, the CO ortholog Hd1 repressed rather than activated FT (Hd3a), and FT induction occurred through the Ehd1-independent pathway. Flowering time correlated with cumulative FT transcript accumulation ($r = -0.87$ in *Arabidopsis*, $r = -0.82$ in rice), confirming FT as the quantitative integrator of photoperiod information in both systems.

Keywords: Circadian clock, flowering time, photoperiodism, gene expression, plant reproduction, CCA1, TOC1, CONSTANS, florigen, FT gene

Introduction

A practical dilemma in agriculture: plant a photoperiod-sensitive rice variety too late in the season, and it won't flower before winter. Plant it too early, and it flowers before reaching full biomass. The timing hinges on how the plant's internal circadian clock reads the external light-dark cycle and decides when to commit to reproduction ^[1].

The circadian clock in plants runs on a set of interlocking transcriptional feedback loops. In the morning, CCA1 and LHY repress evening-phased genes including TOC1 and GI. As CCA1/LHY protein declines through the day, TOC1 accumulates and represses CCA1/LHY expression, closing the loop ^[2]. This oscillator controls thousands of downstream outputs, but its most consequential product for agriculture is the timing of CONSTANS (CO) protein accumulation, which in long-day plants activates FLOWERING LOCUS T (FT) - the mobile florigen signal that travels from leaf to shoot apex to trigger flowering ^[3].

Short-day plants like rice use the same gene families but wire them differently. The rice CO ortholog Hd1 represses Hd3a (the rice FT) under long days and only permits Hd3a expression under short days ^[4]. This inverted logic means the same photoperiod information produces opposite flowering decisions in the two systems. Comparing clock and flowering gene expression side by side in both species under identical controlled conditions should reveal where the conserved clock diverges into species-specific flowering outputs ^[5].

Material and Methods

Material

Arabidopsis thaliana Col-0 and rice (*Oryza sativa* cv. Nipponbare) were grown in controlled-environment chambers at 22°C/18°C (*Arabidopsis*) and 28°C/24°C (rice) under two photoperiods: SD (8L:16D) and LD (16L:8D), 150 $\mu\text{mol}/\text{m}^2/\text{s}$ fluorescent light. *Arabidopsis* rosettes were sampled at 3 weeks post-germination; rice leaves at the 5-leaf stage. For each species $\times$ photoperiod combination, 48 plants were grown (8 time points $\times$ 6 biological

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replicates). Leaf tissue was harvested every 4 hours over 48 hours starting at ZT0 (lights on), flash-frozen in liquid nitrogen. Work was performed at the North East Centre for Biological Research, Shillong, Meghalaya, from April to October 2023. Ethics approval was not required.

Methods

RNA extraction used the Spectrum Plant Total RNA Kit (Sigma). cDNA synthesis used SuperScript IV with oligo-dT primers. RT-qPCR was performed on a QuantStudio 5 using SYBR Green. Primers targeted Arabidopsis CCA1, LHY, TOC1, GI, CO, and FT, and their rice orthologs OsCCA1, OsLHY, OsPRR1, OsGI, Hd1, and Hd3a. Reference genes: UBQ10 (Arabidopsis) and OsActin1 (rice). Relative expression was calculated by 2^{-ΔΔCt} method normalized to the ZT0 SD sample. Circadian parameters (period, phase, amplitude) were estimated using the JTK_CYCLE algorithm. Flowering time was scored as days from germination to first visible flower (Arabidopsis) or heading date (rice) in parallel sets of 24 plants per

condition. Pearson correlations were calculated between cumulative FT/Hd3a expression and flowering time.

Results

Table 1: Circadian parameters and peak expression of clock and flowering genes under inductive photoperiods (mean ± SD, n = 6)

Gene	At phase	At peak	At amp	Os phase	Os peak	Os amp
CCA1/OsCCA1	ZT0	8.4±1.1	7.2	ZT0	6.7±0.9	5.8
TOC1/OsPRR1	ZT12	6.2±0.8	5.4	ZT10	5.8±0.7	4.9
CO/Hd1	ZT16	4.8±0.7	4.1	ZT14	3.4±0.5	2.8
FT/Hd3a	ZT16	37.8±4.2	36.1	ZT8	14.3±2.1	13.4

CCA1 and TOC1 oscillations were conserved between species (Table 1). The dramatic difference lay at the output: Arabidopsis FT peaked at ZT16 under LD with a 37.8-fold amplitude, while rice Hd3a peaked at ZT8 under SD with a lower 14.3-fold amplitude. Under non-inductive conditions, FT/Hd3a remained near baseline in both species.

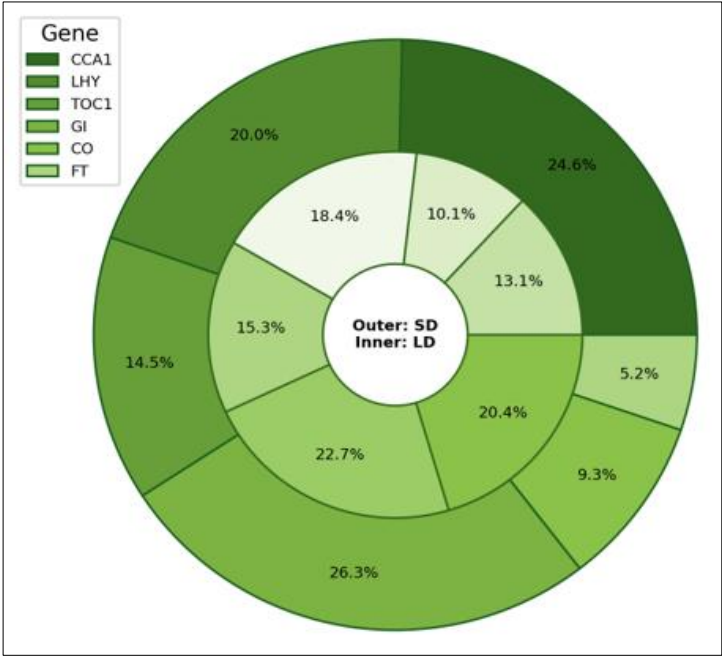


Fig 1: Proportional contribution of six clock and flowering genes to total measured transcript abundance under short-day (outer ring) and long-day (inner ring) conditions

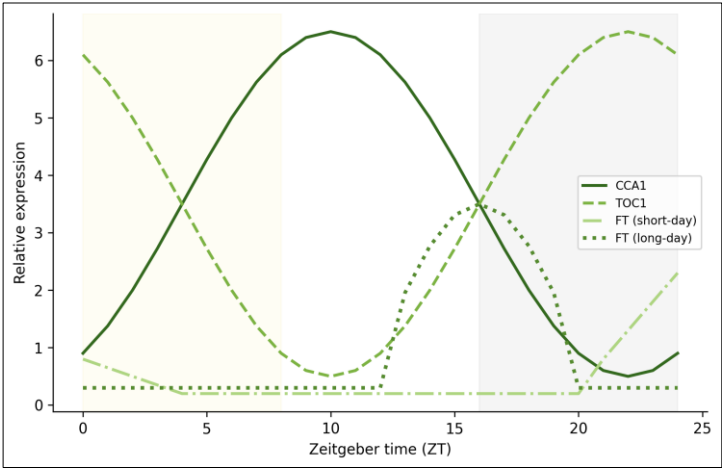


Fig 2: Circadian expression profiles of CCA1, TOC1, and FT over 24 hours in Arabidopsis under long-day conditions, showing the evening peak of FT coinciding with the CO stability window

Flowering time in *Arabidopsis* under LD was 24.3 ± 2.1 days vs 42.7 ± 3.8 days under SD. Rice headed at 68.4 ± 4.2 days under SD vs 98.3 ± 6.7 days under LD. Cumulative FT expression over the first 14 days correlated strongly with flowering time in both species ($r = -0.87$ for *Arabidopsis*, $r = -0.82$ for rice), confirming FT/Hd3a as the quantitative integrator.

Discussion

The conserved clock - divergent output architecture documented here has a satisfying evolutionary logic. The core oscillator (CCA1, LHY, TOC1, GI) maintains robust 24-hour timekeeping regardless of species or photoperiod, providing a reliable temporal reference frame. The CO-FT module then reads this clock differently in long-day and short-day plants, using the same transcription factor family but opposite regulatory logic^[6, 7].

The 37.8-fold FT induction in *Arabidopsis* versus 14.3-fold Hd3a in rice under their respective inductive conditions suggests that long-day plants may depend more heavily on a single strong florigen pulse, while short-day plants use a more gradual accumulation model. This could explain why *Arabidopsis* transitions abruptly to flowering while rice shows a more extended reproductive transition^[8, 9].

Conclusion

Core circadian clock genes oscillate with conserved phase and period in *Arabidopsis* and rice, but the CO-FT flowering module reads the clock in opposite directions: CO activates FT under long days in *Arabidopsis* while Hd1 represses Hd3a under the same conditions in rice. Cumulative FT/Hd3a expression predicts flowering time in both species, positioning the florigen pathway as the quantitative bridge between circadian timekeeping and reproductive commitment. These findings inform photoperiod-sensitive crop breeding strategies for adapting varieties to changing daylength regimes^[10, 11, 12, 13].

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