SUPPLEMENTAL MATERIALS

Materials and Methods

Phylogenetic Analyses

Public databases, including TIGR and JGI, were searched using various *Arabidopsis* and rice flowering time genes as queries. Gene names and their nucleotide accessions can be found in Supplemental Table 1. OsFTL5 was not included in this study as it has been identified as being a pseudogene. Nucleotide sequences were aligned based on a protein sequence alignment generated using Muscle 3.52 (Edgar, 2004). The alignments were edited using GeneDoc (http://www.psc.edu/biomed/genedoc/) to remove non-aligning regions from the alignment (Supplemental Figure 4). Neighborjoining trees were constructed using the computer program MEGA3.1 (Kumar et al., 2004). The Kimura 2-parameter substitution model was used and a consensus tree was built from 1000 bootstrap replicates (Supplemental Figure 2). In addition, Bayesian phylogenetic analyses were performed using MrBayes 3.0B4 (Huelsenbeck and Ronquist, 2001). Searches were run using four different Markov chains for 3,000,000 generations using the GTR model with a substitution rate that varies according to the gamma distribution and allows for a proportion of sites to be invariable (GTR + IG). Sampling occurred every 100 trees and the first 10,000 trees were discarded to build a consensus tree (Supplemental Figure 3).

In situ mRNA Hybridization

Petiole and stem tissues excised from 6-week-old *C. moschata* PI441726 plants, grown under both LD and SD conditions, were immediately fixed, dehydrated and paraffin-embedded as described (Ruzin, 1999). A 200 bp DNA fragment, encoding for a common region in the Cmo_ *FTL1* and Cmo_*FTL2* gene was obtained by PCR using the following primer pairs: F-5'-ATGCCGAGAAATCGTGACCCTCTA-3' and R-5'-CAAAGTGAAGAAGGTGCGAAGGTCAG-3' for Cmo_*FTL1*, and F-5'-ATGCCGAG-AGACCGTGACCCTTTG-3' and R-5'-CAAAGTGAAGAAGGTGCGAAGGTCAG-3' for Cmo_*FTL2*. The resultant fragment was cloned into the TOPO II dual promoter vector (Invitrogen, Carlsbad, CA). Linearized vector with *Xho* I or *Hin*d III were used to transcribe digoxigenin-labeled sense or antisense mRNA, using SP6 or T7 promoters, respectively, using the MAXIscript kit (Ambion, Austin TX). *In situ* hybridization was performed as described (Ruiz-Medrano et al., 1999). Digoxigenin-labeled RNA was detected with the anti-Dig antibody conjugated to alkaline phosphatase (Boehringer, Mannheim) and developed using NBT and BCIP from BioRad. Images were recorded on an Axioskop 2 Plus using phase contrast microscopy and Axiovision 5 software (Zeiss, Germany).

References

Edgar, R.C. (2004). MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics **5,** 119.

Huelsenbeck, J.P., and Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogeny. Bioinformatics **17,** 754-755.

Kumar, S., Tamura, K., and Nei, M. (2004). MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics **5,** 150-163.

Ruiz-Medrano, R., Xoconostle-Cázares, B., and Lucas, W.J. (1999). Phloem long-distance transport of CmNACP mRNA: implications for supracellular regulation in plants. Development **126,** 4405-4419.

Ruzin, S.E. (1999). Plant Microtechnique and Microscopy. Oxford University Press. USA.