Studies of the Molecular Basis of Flowering in Longan (*Dimocarpus longan* Lour.)

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Problem statement

Longan (*Dimocarpus longan* Lour.) is a commercial fruit crop belonging to the Sapindaceae family. In Thailand, longan flowers from late December to late February following dry and cool (<18ºC) climatic conditions during the natural period of flower induction from mid November to mid December.

Although plant species show vast variation in flowering behaviour; recent findings suggest that there is a common possibly universal protein, termed FLOWERING LOCUS T (*FT*) which was originally identified in *Arabidopsis thaliana* (Koornneef *et al*., 1991). It accumulates in leaves under flowering inducing environmental conditions and moves via the phloem to the meristem (Corbesier *et al.*, 2007) where it meets and interacts with the FD transcription factor (Abe *et al*., 2005). Together the proteins activate transcription of several genes required for flowering. Some of the molecular basis in this flowering process has been shown to also play a similar role in perennial crops (Endo *et al*. 2005).

Working hypothesis

We hypothesize that specific genes coding for flowering will be up-regulated, temporarily or spatially, with the transition to flowering in this fruit crop. We suggest that the temporal increase in *FT* expression can be triggered by environmental stimuli.

Material and methods

Plant material

- 3-year-old mature 'Daw' longan trees at Mae Jo University, Thailand, were used in 2008.
- Terminal buds and leaves were collected from selected trees at 2-day intervals over a 3 week period prior to flower buds becoming visible.

Methods

1. RNA extraction and cDNA synthesis
2. Primer design and PCR analysis
3. Cloning and transformation of PCR product
4. Screening for positive colonies by PCR
5. DNA purification from positive colonies for sequencing

Results and discussion

1. cDNA synthesis and PCR analysis

cDNA was successfully synthesized from all tissue samples and at all sampling times. Samples will be used in future gene expression studies.

2. Primer design and PCR analysis

Four designed degenerate primer pairs had different positions on the flowering locus T cDNA according to their amplicon size between 150-500 bp (Fig. 1). Consequently, the four *FT* primers amplified had varying fragment sizes.

Two pairs of *FT* degenerate primers (F2/R2; F4/R4) successfully amplified bulk cDNA sample by PCR (Fig. 2). The bands corresponded to the expected product sizes of 305 bp and 165 bp, respectively.

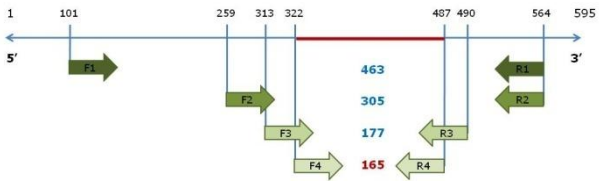

Figure 1. Location and length of the degenerate primer pairs on the flowering Locus T (*FT*) cDNA.

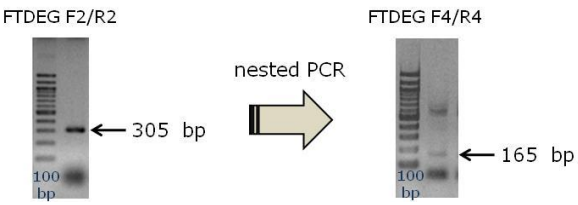

Figure 2. Nested PCR using FTDEG F2/R2 and FTDEG F4/R4.

3. Screening for positive colonies by PCR

The success of the transformation was evident by sizes of the amplified products . Size of band (413 bp) constituted the original distance between primers (248bp) together with the size of the insert (165bp). Six colonies out of the 60 colonies tested had the correct fragment sizes and were selected for sequencing.

4. Sequence analysis

Sequencing revealed to distinct clones (Longan #1 and #2) each encoding an FT-like protein. Alignment with other FT-like proteins showed high homology (Fig 3).

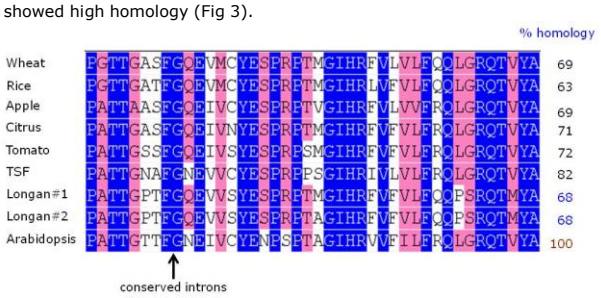

Figure 3. Alignment of FT-like proteins including longan.

Conclusions

Our results suggest that we have successfully cloned two cDNAs from longan encoding FT-like proteins. Our future work will use the sequences isolated to design specific primers for RT-PCR analysis of these FT-encoding genes and to test if expression of these genes is affected by flower inducing treatment. We will also study expression of other genes that mark the floral transition such as those encoding proteins similar to Arabidopsis *LEAFY, APETALA1* and *PISTILLATA*.

Literature cited

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Acknowledgement
This research was funded by the Deutsche Forschungsgemeinschaft (DFG) through the Uplands Program (SFB 564) in Southeast Asia.