

## ARTICLE

# Improved Heat *FT* Induction Leads to Earlier and More Prolific Flowering in Poplar

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### ABSTRACT

Trees have a long juvenile phase before reproductive onset. This makes their breeding and studying floral development difficult. Precocious flowering using FT technology has shown promise. However, transgenic FT overexpression has significant negative pleiotropic effects. Hence, there has been interest in inducible FT expression for flower induction. Previously reported heat inducible expression of FT in poplar successfully induced flowering. However, flowering was sporadic and took up to 6 weeks. Here we report improvements in the protocol, which led to faster and more prolific flowering. Specifically, we increased the once to three times daily heat treatment. The repeated heat inductive treatments led to nearly five times higher FT expression, compared to the single daily treatment. The highly increased FT expression led to significant acceleration and abundance of flowering.

## 1. Introduction

The long juvenile phase before reproductive onset in perennial trees is a major obstacle in breeding and studies of flower development. To overcome this challenge various methods were developed to induce early flowering such as pruning, girdling, water stress and growth regulator paclobutrazol<sup>[1,2]</sup>. More recently transgenic up-regulation of floral meristem identity genes like *LEAFY* and *Flowering Locus T (FT)* were successfully used to induce early flowering in many trees<sup>[3-5]</sup>. However, ectopic expression of floral meristem identity genes produces severe pleiotropic phenotypes which renders transgenic plants unusable for breeding purposes and studying gene function. To overcome this problem inducible system driving three FT homologs, two from poplar (FT1 and FT2) and one

from Arabidopsis (FT), was developed in poplar<sup>[6]</sup>. The system employed the heat inducible promoter from soybean heat shock protein and tested in a male (353) and female (717) poplar clones<sup>[7]</sup>. Surprisingly, among the three FT homologs, the Arabidopsis FT showed best results in inducing flowering in both clones, although the male clone typically produced earlier and more prolific flowers<sup>[7]</sup>. Although the system was successful, flower inflorescences were sporadic and needed weeks of treatments (3 weeks for the male and 6 weeks for the female clone). The induction protocol employed a daily single 37°C treatment. We hypothesized that this treatment is insufficient to mount sufficient FT expression and thus the lengthy and inefficient flower production. The major objective of this study therefore was to modify the existing protocol to increase the *FT* expression and ac-

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celerate and improve flower abundance.

## 2. Methodology

We used 353 male and 717 female poplar clones transformed with the Arabidopsis *Flowering Locus T* (*FT*) regulated by a heat-inducible promoter<sup>[7]</sup>. These clones were kindly provided by Dr. Steven Strauss at Oregon State University.

### 2.1 Heat Induction Experiment

Approximately 5-6 weeks old plants, grown in a greenhouse (16-hour light, 20-22°C) were used for the heat induction experiment. The 353 and 717 plants were 40-45cm and 45-50cm in height respectively at the time of heat treatment initiation. The plants were transferred to growth chamber (set at 16-hour light, 20°C, except during heat induction period). Heat shock (37°C for 90 min) was administered once daily at same time each day<sup>[7]</sup>. To increase *FT* expression, we used three cycles of heat induction of 90 min at 3h intervals: First heat cycle 4.30am-6:00am, second 9:00am-10.30am and third cycle 1:30-3:30pm). The rest of the time growth chamber setting was 16h light (4am-8pm), 20°C.

### 2.2 RNA Isolation and Quantitative Real-time PCR (qRT-PCR) Analysis

Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen) from leaf samples. 500ng of the total RNA from each sample was used to generate cDNA using an iScript cDNA synthesis kit (BioRad). Selected ACT7 reference genes were validated using GeNorm Software<sup>[8]</sup>. qRT-PCR analyses were carried out with StepOnePlus Real-Time PCR System (Applied Biosystems, Life Technologies) using Maxima SYBR Green qPCR master mix (Thermo Scientific Co.), and relative expression values were calculated using the  $\Delta$ -CT-method, as previously described<sup>[9]</sup>. A complete list of primers used in RT-PCR analysis is presented in Table 1.

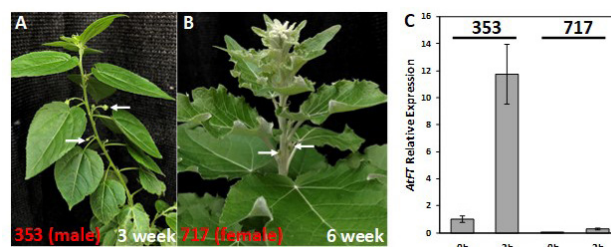
**Table 1.** List of primers used in this study

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
<i>ACT7</i>	TGGCCGATGCCGAGGA-TATTCAAC	ATCACCTGCAAACCCAG-CCTTCAC
<i>FT</i>	CAGGAATTCATCGTGTG-CGTG	AGCCACTCTCCCTCTGA-CAA

## 3. Results

Using the previously described protocol and transgenic clones, we found similar flower induction as previously described<sup>[7]</sup>. We used transgenics transformed with Ara-

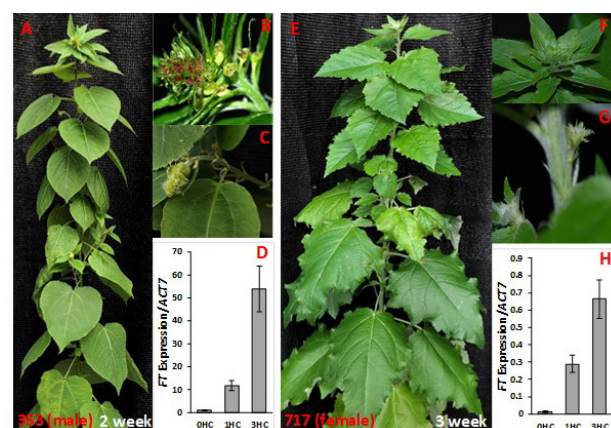
bidopsis *FT*, because they showed the best results in both clones. The daily, 90 min of heat treatment (37°C) did induce flowering in both clones but flowering was sporadic and took up to 6 weeks (Figure 1).



**Figure 1.** Floral development in transgenic poplar harboring the heat inducible *FT* construct ( $P_{HSP}::FT$ )

**Note:** Plants of approximately 40 cm height were exposed to 37°C of 90 min per day for 3-6 weeks, (A) 353 (male) clone initiated flowering after 3 weeks of heat induction (B) 717 (female) clone took more than 6 weeks of heat induction to initiate flowering. White arrows indicates the emerging inflorescences (C) comparison of *FT* expression at 0h, and 2h after the heat indicative treatments in both genotypes, actin used as internal control to normalize gene expression, error bars  $\pm$  SE.

Specifically, flowering initiated in 3 weeks in the male 353 genotype and 6 weeks in the female 717 genotype (Figure 1A-B). We studied the *FT* expression in leaf samples at 2h and 4h after heat induction in both clones. We found higher expression in male genotype 353, as compared to female genotype 717 (Figure 1C), which took twice longer to induce floral development. Based on this expression pattern of *FT*, we hypothesized that insufficient *FT* expression may be the limiting factor to induce early flowering. To increase *FT* expression, we used three cycles of heat induction of 90 min at 3h intervals. The repeated heat inductive treatment resulted in an almost five-fold increase in *FT* expression compared to normal once daily heat induction and earlier and more uniform flowering (2-3 weeks after heat induction) (Figure 2 A-H).

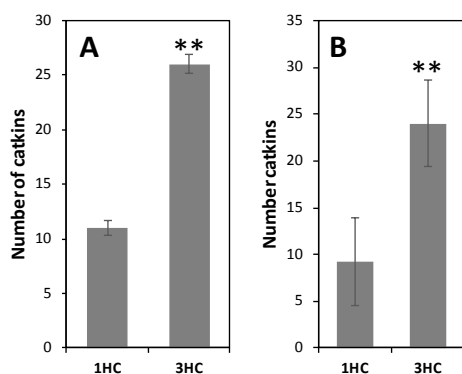


**Figure 2.** Three cycles of heat induction per day induce much early flowering in transgenic poplar ( $P_{HSP}::FT$ )

**Note:** Plants of approximately 40 cm of height were exposed daily three heat cycles (3HC) of 37°C for 90 min with a 2 hour breaks for 2-3 weeks

(see Methodology for more details). (A) 353 (male) clone start flowering only after 2 weeks of heat treatment (B, C, F, G) close-up of terminal and axillary catkin in 353 and 717 clones (D, H) expression of *FT* at 0h (OHC), 2h after the one heat cycle (1HC) treatment and 2h after the three heat cycles (3HC) treatment; actin was used as internal control to normalize gene expression; error bars  $\pm$  SE (E) 717 (female) clone takes less than 3 weeks of heat induction to initiate flowering.

The repeated heat inductive treatment also resulted in a greater number of catkins in both clones (Figure 3 A, B). These results indicate that *FT* is a limiting factor during heat induction methods used previously, and our method of three cycles of heat induction is a more efficient to induce much earlier and more prolific flowering.



**Figure 3.** Three cycles of heat induction induce more flowering in transgenic poplar ( $P_{HSP}::FT$ )

**Note:** (A) Number of catkins in 353 (male) clone after one heat cycle (1HC), and three heat cycles (3HC) (B) Number of catkins (nascent fluorescence) developed in 717 (female) clone. Data shown are mean values from 10 plants of each genotype, error bar  $\pm$  STDEV and asterisks over bar indicate significance differences at  $P < 0.001$  with corresponding one heat cycle treatment.

#### 4. Discussion

*FT* is well studied flowering time gene and ectopic expression induces early flowering across plant species including trees<sup>[10,11]</sup>. However constitutive upregulation causes major negative pleiotropic effects<sup>[10]</sup>. Previously developed *FT* inducible system in poplar showed promise to address this challenge but also had some significant limitations<sup>[7]</sup>. Specifically, up to 6 weeks of the inductive treatments were needed and flowering was sporadic<sup>[7]</sup>. We show here that the likely cause for these problems was insufficient induction of *FT*. Male genotype 353 flowers twice as fast as compared to the female 717 genotype<sup>[7]</sup>. *FT* induction at the same time points and conditions was much higher in 353 compared to 717 (Figure 1A-C). Increase in the once to three times of heat induction daily, resulted in almost five-fold expression increase of *FT* in both genotypes. This led to significant acceleration of flowering, particularly in the female 717 clone, which as mentioned earlier has a significantly lower *FT* induction compared to the

male 353 clone. In addition to acceleration of flowering, the modified treatment and higher *FT* expression also led to increase in number of catkins (Figure 3A, B). Despite earlier flowering and increased number of catkins, the inflorescence development in the female clone was not complete and catkins aborted within 3-4 weeks after treatment termination. The most likely cause was the much lower expression of *FT* in the female background. Even after the improvement in administrating the heat treatments, *FT* expression was nearly 10-fold lower in the female genotype (Figure 2D, H). The cause for the lower induction of *FT* in the female background is unknown. It could be a result of a position effect due to insertion of the construct in a less active chromatin, overall less effectiveness of the heat induction system in this background or methylation and other chromatin modifications of the transgene. Further investigation of these potential causes will lead to strategies that can overcome the low *FT* induction.

#### 5. Conclusions

We report improvements to the heat induction protocol of *FT* in poplar, which leads to faster and more prolific flowering. The primary cause for the observed improvements is likely the significant increase in *FT* induction, caused by the repeated administration of the inductive treatments. The improvements in the protocol can be also applied to other inducible technologies employing the same promoter.

#### References

- [1] Flachowsky, H., et al., A review on transgenic approaches to accelerate breeding of woody plants. 2009, 128(3): 217-226. DOI: 10.1111/j.1439-0523.2008.01591.x
- [2] Meilan, R.J.N.F., Floral induction in woody angiosperms. 1997, 14(3): 179-202. DOI: 10.1023/a:1006560603966
- [3] Weigel, D. and O. Nilsson, A developmental switch sufficient for flower initiation in diverse plants. Nature, 1995, 377(6549): 495-500. DOI: 10.1038/377495a0
- [4] Klocko, A.L., et al., *FT* overexpression induces precocious flowering and normal reproductive development in Eucalyptus. Plant Biotechnology Journal, 2016, 14(2): 808-819. DOI: 10.1111/pbi.12431
- [5] Tang, M., et al., An ortholog of *LEAFY* in *Jatropha curcas* regulates flowering time and floral organ development. Sci Rep, 2016, 6: 37306. DOI: 10.1038/srep37306
- [6] Borghi, L., Inducible gene expression systems for

- plants. *Methods Mol Biol*, 2010, 655: 65-75.  
DOI: 10.1007/978-1-60761-765-5\_5
- [7] Zhang, H., et al., Precocious flowering in trees: the FLOWERING LOCUS T gene as a research and breeding tool in *Populus*. *J Exp Bot*, 2010, 61(10): 2549-60.  
DOI: 10.1093/jxb/erq092
- [8] Vandesompele, J., et al., Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology*, 2002, 3(7): research0034.1.  
DOI: 10.1186/gb-2002-3-7-research0034
- [9] Azeez, A., et al., A tree ortholog of APETALA1 mediates photoperiodic control of seasonal growth. *Curr Biol*, 2014, 24(7): 717-24.  
DOI: 10.1016/j.cub.2014.02.037
- [10] Kardailsky, I., et al., Activation tagging of the floral inducer FT. *Science*, 1999, 286(5446): 1962-5.  
DOI: 10.1126/science.286.5446.1962
- [11] Kobayashi, Y., et al., A pair of related genes with antagonistic roles in mediating flowering signals. *Science*, 1999, 286(5446): 1960-2.  
DOI: 10.1126/science.286.5446.1960