

**Project Title:** Characterization of gene expression in transgenic plants with modified floral gene expression

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Transgenic plants are genetically modified through the use of recombinant DNA techniques to create plants with desirable or scientifically informative characteristics. Conventional breeding generally makes use of the genes found within the plant species itself or its close relatives, whereas genetic engineering allows for the insertion of advantageous genes that have either been modified by scientists or that are found in distant species. Through genetic engineering, the diversity of plant characteristics available to breeders can be substantially expanded beyond what is accessible through conventional breeding methods. Transgenic plants have already been widely adopted in many countries for use in agriculture and have led to major economic benefits for countries that produce and market them. The plant species of interest for this study was poplar, an important source of lumber and a major focus of efforts to produce sustainable, perennial biofuel crops.

Despite the possible benefits provided by the use of transgenic plant species, the release of transgenes into the environment can pose ecological, ethical, and economic problems. Moreover, there are strict regulations on field research and commercial applications of transgenic species, a major component of which is concerned with preventing or managing the dispersal of transgenic material. In order to be able to use transgenic plants for many purposes—particularly species like poplar whose pollen and seed can move widely and become incorporated into natural populations—it is likely that a containment method will be needed. Because poplars are vegetatively propagated, one option for containment is to produce trees that do not flower and therefore cannot produce viable pollen or seed.

The technique RNAi is used to silence specific genes by breaking down molecules responsible for the synthesis of the proteins associated with these genes. Due to their contribution to floral development, the poplar genes *LEAFY* and *AGAMOUS* are targeted using RNAi techniques. In addition to RNAi transgenes, the gene *HSP:FT* is inserted into the poplar trees as a tool to study the effects of RNAi. Through controlling the temperature of the poplar's environment, one can induce HSP to turn on FT, which triggers the plant to flower early. Once *HSP:FT* is inserted into the poplar trees, they are placed in a greenhouse at a controlled temperature in order to induce HSP to turn on FT. The successful insertion of *HSP:FT* is critical to studies that analyze the efficacy of RNAi, as flowering would take years to occur without it.

Before one can study the effects of RNAi techniques that target the *LEAFY* and *AGAMOUS* floral developmental genes, they must ensure that all of the plants with *HSP:FT* insertion, but without RNAi transgene insertion, successfully flower. Prior to this study it was found that not all poplars with *HSP:FT* insertion produce flowers—this finding is problematic because one needs the poplars to reliably form flowers so that following RNAi transgene insertion, an absence of flowering can be attributed directly to the RNAi techniques used. Thus the goal of this study was to determine if there is a threshold level of FT required to induce flowering in poplar trees.

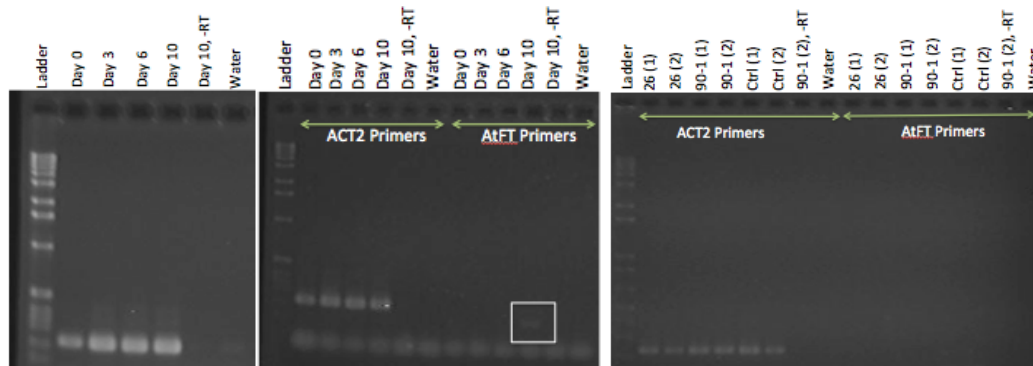
Three different poplar constructs were used in this study—a non-transgenic poplar control, a poplar with *HSP:FT* insertion that demonstrated flowering, and a poplar with *HSP:FT* insertion that did not demonstrate flowering. Tissue samples from the non-transgenic poplar control construct and *HSP:FT* flowering construct were taken at days 0, 3, 6, and 10 of heat induction, and tissue samples from the *HSP:FT* non-flowering construct were taken at day 10 of heat induction. The gene actin, present in both non-transgenic and transgenic poplars, served as the control gene in this study. Unlike the actin gene, FT expression was expected to be seen only in the constructs with *HSP:FT* insertion. Therefore, expression of the actin gene was analyzed in all of the tissue samples, and expression of FT was analyzed in only the constructs with *HSP:FT* insertion.

The first step to analyze gene expression was to extract RNA from each tissue sample using a Qiagen RNeasy kit and the Qiagen protocol Purification of Total RNA from Plant Cells and Tissues and Filamentous Fungi. To remove any DNA from the RNA samples, the Ambion protocol Turbo DNase Removal of Contaminating Genomic DNA from RNA Samples was used. The purified RNA was then used as a template for the Invitrogen protocol First-Strand cDNA Synthesis, which uses the enzyme reverse transcriptase to synthesize complementary DNA (cDNA). Following cDNA synthesis, polymerase chain reaction (PCR) including *ACT2* and FT primers specific to the actin and FT genes, respectively, was used to amplify the amount cDNA. The final step was agarose gel electrophoresis of the PCR product to determine the expression of the actin and FT genes. Both

negative reverse transcriptase and water control samples were included to ensure that the cDNA synthesis protocol ran efficiently and that there was no contamination.

Because FT is only expressed in the poplar constructs with HSP:FT insertion, only actin gene expression was analyzed in the non-transgenic poplar control construct. The primer ACT2, which is specific to the actin gene, was used in the PCR, and strong bands were present for each sample, indicating that the actin gene was expressed in each sample. As expected, no bands were present for the negative reverse transcriptase and water control samples (Figure 1a). For the non-flowering and flowering poplar constructs with HSP:FT insertion, both the ACT2 primer and FT primer, which is specific to FT, were used in separate PCRs. For the poplar construct with HSP:FT insertion that demonstrated flowering, all of the non-control samples that underwent PCR using the ACT2 primer had bands showing actin expression, and the day 10 sample that underwent PCR using the FT primer had a faint band showing FT expression (Figure 1b). For the poplar construct with HSP:FT insertion that did not demonstrate flowering, all of the non-control samples that underwent PCR using the ACT2 primer had bands showing actin expression, and none of the samples that underwent PCR using the FT primer showed bands that would indicate FT expression (Figure 1c).

The gel electrophoresis results showed that FT was expressed only in the day 10 tissue sample of the poplar construct with HSP:FT insertion that demonstrated flowering. Based on these results, there appears to be a correlation between the expression of FT and flowering in poplar trees. This is supported by the lack of FT expression seen in the poplar construct with HSP:FT insertion that did not demonstrate flowering. The method used in this study was qualitative—the gel electrophoresis results only indicated whether or not FT was expressed, and did not provide information on the amount of FT present in the flowering construct. The next step in determining the threshold level of FT required for floral development is to quantify the amount of FT present in flowering poplar constructs using quantitative PCR (qPCR) methods. Once the threshold level of FT required for flowering is known, FT expression can be quantified in poplar constructs with both HSP:FT insertion and RNAi transgene insertion to determine if the poplar has enough FT expression to flower—if the poplar has enough FT expression to flower, but does not flower, this absence of flowering can then be attributed to RNAi.



**Figure 1.** Gel electrophoresis results. **(a)** Agarose gel electrophoresis results for the tissue samples of the non-transgenic poplar control construct. Bands indicate the expression of the gene actin. **(b)** Agarose gel electrophoresis results for tissue samples of the poplar construct with HSP:FT insertion that demonstrated flowering. Bands for the samples that underwent PCR using the ACT2 primers indicate actin expression, and bands for the samples that underwent PCR using the FT primers indicate FT expression. All of the non-control samples demonstrated actin expression, and only the day 10 tissue sample expressed FT. **(c)** Agarose gel electrophoresis results for tissue samples of the poplar construct with HSP:FT insertion that did not demonstrate flowering. Bands for the samples that underwent PCR using the ACT2 primers indicate actin expression, and bands for the samples that underwent PCR using the FT primers indicate FT expression. All of the non-control samples demonstrated actin expression, and none of the samples expressed FT.