AtFT accelerates flowering and leads to viable seed production in hybrid Eucalyptus

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Abstract

Eucalyptus is an economically important hardwood tree that is widely planted for pulp, energy, and timber in several countries. There are many active breeding programs for Eucalyptus focused on improving specific traits, such as wood or fiber qualities, insect resistance, and cold tolerance. However, as with most trees, it takes several years for the flowering events to take place. This long generation time limits the rate at which breeding in this process. Physiological methods for floral induction, such as application of paclobutrazol, can accelerate Eucalyptus flowering onset but results are highly variable, environment and genotype specific, and generally do not produce flowers for one to several years. Speeding up the generation time would allow for faster incorporation of desirable traits, testing of new breeding approaches such as genomic selection, and would allow new varieties to be tested and marketed in less time. To obtain rapid flowering, we transformed a Eucalyptus grandis x urophylla hybrid (SP7) with a variety of constructs to express FLOWERING LOCUS T (FT) from Arabidopsis thaliana (AtFT). While FT gene overexpression is known to induce precocious flowering in a variety of tree species (including plum, citrus, apple and poplar), to the best of our knowledge ours is the first test of the constitutive 35S:AtFT events. In this study we evaluated transgenic control trees.

Flowering events had higher AtFT expression than non-flowering events. As a result, flowering events yielded a shorter, branchier, and had reduced apical dominance. However, these trees did not produce many flowers. We found that flowering 409S:AtFT (H) trees were short, branchy and had reduced apical dominance. However, their average stem diameter was significantly smaller than control trees. These trees had the most severe vegetative alterations.

Flower buds opened when trees were tiny. Typically, Eucalyptus trees flower after multiple years when the trees are meters tall. By contrast, the flowering AtFT events had open flowers at less than a year of age. Below are representative images of trees from the three constitutive AtFT transgenic constructs at the time of initial bud burst. The initial flowers were single or small clusters. Note the flowers originating off of the main stem of the 409S-AtFT (K) tree (arrow box and enlarged event). The flowering events had higher AtFT expression than non-flowering events. All bars present standard error

Tested different FT constructs in Eucalyptus

409S:AtFT (H) flowering events had the highest AtFT expression. Overall, AtFT-induced flowering in Eucalyptus may be a valuable means for accelerating breeding and genetic studies as the transgene can be segregated away in progeny for further evaluation.

AtFT expression reduced apical dominance

Different constitutive AtFT constructs resulted in different percentages of flowering events, with 35S:AtFT giving the highest rate of floral induction (64.7% of events) and 409S:AtFT (K) giving the lowest rate (3.6%, see Table 2). We also evaluated transgenic control trees. These flowering trees showed robust growth and continual flowering. The flowering 35S:AtFT (K) trees were similar in form to control trees, with no significant differences in height, stem diameter, branching, or apical dominance. However, these trees did not produce many flowers.

Constitutive AtFT led to precocious floral bud formation

We found that flowering 409S:AtFT (H) trees were short, branchy and had reduced apical dominance. However, their average stem diameter was the same as control trees. These flowering trees had robust growth and continual flowering. The flowering 409S:AtFT (K) trees were similar in form to control trees, with no significant differences in height, stem diameter, branching, or apical dominance. These trees also did not produce many flowers.

AtFT induced flowers had normal floral organs and viable pollen grains

AtFT induced flowers had normal floral organs and viable pollen grains. AtFT induced flowers contained the four expected whorls of floral organs (outer perianth, inner perianth, stamens, stigma). Both female (pistil) and male (pollen grains) gametophytes were also present. Images from 409S-AtFT (H) flowers are shown, other constitutively AtFT constructs gave similar results.

AtFT induced flowers produced viable seeds

The 409S:AtFT (H) and 409S:AtFT (K) flowering events underwent two types of pollination to assess floral fertility. These trees were allowed to open-pollinate (self) in the greenhouse, which did not contain any other flowering trees, leading to the formation of self-seeds. However, flowers were often hand-pollinated with g. grandis pollen to obtain outcrossed offspring. These cross-pollinated flowers were emasculated and tented with plastic wrap to reduce the likelihood of self-seed. Overall, cross-pollination of the 409S:AtFT (H) events resulted in a much higher percentage of seeds formed from cross-pollinations than the 409S:AtFT (K) events (see Table 3). Germination testing of self and cross-pollinated flowers showed that cross-pollination led to a higher percentage of germinated seeds than self-pollination (see Table 4).

AtFT was inherited in progeny and led to early flower bud formation

We genotyped a set of the open-pollinated progeny of the flowering 409S:AtFT (H) and 409S:AtFT (K) events. Off the 6 tested, 409S:AtFT (K) self-seedlings, all 6 inherited AtFT. In addition, all 6 of these seedlings had viable floral buds. Floral buds were visible within 2-3 months of transplanting, which is similar to the parental trees. We genotyped 9 progeny of open-pollinated 409S:AtFT (H) trees. All 9 offspring inherited AtFT. To date, 2 of these trees had floral buds, these were visible within 2 months, which is much faster than the 5 month time span displayed by the parent tree. The remaining 6 seedlings are younger, we will continue to monitor these progeny for floral bud formation over time.

Summary

- Constitutive expression of AtFT successfully induced early flowering in hybrid Eucalyptus
- AtFT led to an reduction in apical dominance
- AtFT-induced flowers were morphologically normal and produced viable seed
- Cross-pollination of AtFT-induced flowers led to a higher percent of viable seeds than self-pollination
- AtFT was inherited by progeny and led to early floral bud formation in these plants

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