FAST-TRACK TRANSFORMATION AND FLOWERING FOR RAPID-CYCLE BREEDING IN POPLAR

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Forest trees have a juvenile phase of several years to several decades. The long generation time is a major impediment to breeding and a bottleneck to fundamental research involving reproductive traits, transgenerational monitoring, and genetic containment. Several regulators of floral development, such as LEAFY and FLOWERING LOCUS T (FT), have been used to induce precocious flowering in annual models. Translating these findings into the poplar system has met with various challenges, including dwarfism and sterile flowers. While the use of heat-inducible promoters for floral gene expression has alleviated many of the developmental anomalies, tedious heat treatments over multiple weeks and months are detrimental to microsporogenesis. The efficacy is also season- and genotype-dependent, limiting widespread adoption of this method. For dioecious species like *Populus*, early flowering male and female genotypes must be available for cross-pollination. Building on the recent discovery of a single female-specific cytokinin response regulator (FRR) underlying sex determination in *Populus*, we reasoned that early flowering and sex switch can be engineered simultaneously to produce male, female and/or hermaphrodite individuals from the same genetic background to accelerate poplar breeding and research. We used CRISPR/Cas9 to knock out a negative regulator of floral initiation in a female hybrid aspen *Populus tremula* x *alba* INRA 717-1B4. *In vitro* flowering was readily observed within 3-4 months of *Agrobacterium*-mediated transformation. When *FRR* was also targeted, transgenic plants developed male and sometimes hermaphrodite flowers in tissue culture. With further development of an efficient early flowering and sex switch system like this, the promise of CRISPR to accelerate rapid-cycle breeding or rapid-cycle genomic selection via controlled crosses is finally within reach for long-lived woody perennials.