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FLOWERING NEWSLETTER REVIEW

Precocious flowering in trees: the *FLOWERING LOCUS T* gene as a research and breeding tool in *Populus*

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Abstract

Expression of *FLOWERING LOCUS T* (*FT*) and its homologues has been shown to accelerate the onset of flowering in a number of plant species, including poplar (*Populus* spp.). The application of *FT* should be of particular use in forest trees, as it could greatly accelerate and enable new kinds of breeding and research. Recent evidence showing the extent to which *FT* is effective in promoting flowering in trees is discussed, and its effectiveness in poplar is reported. Results using one *FT* gene from *Arabidopsis* and two from poplar, all driven by a heat-inducible promoter, transformed into two poplar genotypes are also described. Substantial variation in flowering response was observed depending on the *FT* gene and genetic background. Heat-induced plants shorter than 30 cm failed to flower as well as taller plants. Plants exposed to daily heat treatments lasting 3 weeks tended to produce fewer abnormal flowers than those in heat treatments of shorter durations; increasing the inductive temperature from 37 °C to 40 °C produced similar benefits. Using optimal induction conditions, ~90% of transgenic plants could be induced to flower. When induced *FT* rootstocks were grafted with scions that lacked *FT*, flowering was only observed in rootstocks. The results suggest that a considerable amount of species- or genotype-specific adaptation will be required to develop *FT* into a reliable means for shortening the generation cycle for breeding in poplar.

Key words: Biotechnology, grafting, reproduction, transgenes.

Introduction

The multiple-year delay in onset of flowering in trees presents a substantial obstacle to research and breeding. Breeders often avoid complex breeding methods such as inbreeding and introgression breeding because of the long delays in progress toward commercial goals. Researchers often avoid detailed inheritance analyses and fine mapping of underlying genes because their experiments do not fit within institutional timelines and grant programmes. As a consequence, there has been extensive research over many decades to devise methods to speed the onset of tree flowering (Meilan, 1997; Flachowsky *et al.*, 2009). These have included physical manipulations such as root pruning and girdling; the application of growth regulators such as paclobutrazol; intensive management of plant nutrition; imposition of plant stress; the use of grafting onto specialized rootstocks; selection of naturally precocious breeding stocks; and, most recently, the use of flower-promoting transgenes.

Arabidopsis LEAFY (*LFY*), a floral meristem identity gene, was the first transgene noted to cause a major acceleration of flowering in a tree (Weigel and Nilsson, 1995), as well as in other plant species. It caused poplars to initiate flowering in weeks to months after regeneration of transgenic shoots, whereas comparable wild-type poplars generally require several years to a decade to begin flowering. A large number of other floral genes, of widely varying

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functions, also accelerate flowering in one or more annual plant species (Komeda, 2004). Most, however, fail to affect flowering consistently in trees; this includes for poplar it's own *LFY* gene (*PTLF*), *APETALA1* (*AP1*), *CONSTANS* (*CO*), *AGAMOUS-LIKE20* (*AGL20*), and rice (*Oryza sativa*) *MADS1* (Strauss *et al.*, 2004). Exceptions are *AP1*, which stimulated flowering and normal seed production in *Citrus* (Cervera *et al.*, 2009), and the acceleration of flowering time by birch (*Betula pendula*) *MADS4* in apple (Flachowsky *et al.*, 2007). However, few other similar cases are known (reviewed in Flachowsky *et al.*, 2009). Even in cases where early onset of flowering was achieved in trees, the structure and development of floral organs and/or fruits were often altered. For example, overexpression of *LFY* in poplar appears to cause vegetative meristems to skip the stage of inflorescence meristem development and thus spurs a direct transition to floral meristems. This results in the production of single flowers rather than the usual inflorescence, a catkin. The single flowers have never, to our knowledge, been shown to be able to sustain fully normal reproductive development and support production of fertile male or female gametes. In addition, *LFY* and its poplar homologue *PTLF* were found to be highly variable in their effects, depending on tree genotype and gender (Rottmann *et al.*, 2000).

As a result of inconsistent floral induction responses with *LFY* and other transgenes in poplar, the observation that *FLOWERING LOCUS T* (*FT*) appeared to stimulate normal flowering was greeted with considerable enthusiasm (Araki *et al.*, 1998). In addition, the putative 'florigen-like' function of *FT* increased the possibility that not only could *FT* stimulate flowering of transgenic shoots, but that it might be used to transmit flowering signals to grafted non-transgenic tissues, as it has done in some species of annual plants (discussed below). This could avoid difficult and costly regulatory and other social obstacles to field research and breeding with transgenic, flowering trees.

FT *biology*

The *FT* gene promotes the transition from vegetative to reproductive phase under photoperiodic regulation (Kardailsky *et al.*, 1999; Kobayashi *et al.*, 1999). Flowering is delayed in *ft* mutant plants, whereas overexpression of *FT* causes early flowering and a determinate inflorescence structure. When light signal is perceived by leaves of the long-day plant *Arabidopsis* and the short-day plant rice, CO protein activates *FT* in leaf phloem (An *et al.*, 2004; Ayre and Turgeon, 2004). Then, FT protein moves to the shoot apical meristem where it forms a complex with FLOWERING LOCUS D (FD) protein, which up-regulates the floral meristem identity gene *AP1* to induce reproductive development (Abe *et al.*, 2005; Wigge *et al.*, 2005; Corbesier *et al.*, 2007; Mathieu *et al.*, 2007; Tamaki *et al.*, 2007).

FT is also regulated by the vernalization pathway. In cold-requiring accessions of *Arabidopsis*, the vernalization pathway mediates low temperature signals that induce reproduction by reducing the levels of the repressor *FLOWERING LOCUS C* (*FLC*), which up-regulates the expression of *FT* (Michaels and Amasino, 1999, 2001; Sheldon *et al.*, 1999, 2000, 2002; Bastow *et al.*, 2004; Searle *et al.*, 2006). Thus, *FT* serves as an important integrator of the photoperiod and vernalization signals.

Movement of the FT signal: When the tomato homolog of *FT*, *SINGLE-FLOWER TRUSS* (*SFT*), was overexpressed in day-neutral tomato and tobacco, it induced early flowering and rescued the late flowering phenotype of the *sft* mutant tomato (Lifschitz *et al.*, 2006). Grafting shoots from the *SFT*-overexpressing tomato plants (donor scion) to *sft* plants (receptor rootstock) rescued the late flowering phenotype of *sft* plants, suggesting that a systemic floral signal was transmitted from the donor to the receptor. The *sft* receptor shoots produced normal inflorescences, flowers, and a sympodial architecture 3–5 weeks following grafting. Unlike the *SFT*-overexpressing donor, wild-type donors failed to rescue the late flowering phenotype of the *sft* receptor, suggesting that the amount of *SFT* signal transmission is perhaps not sufficient. A persistent emission of *SFT*-generated floral signal was required from the donor to the receptor. Removal of the donor resulted in reappearance of *sft* features, indicating lack of an *SFT*-dependent autoregulation loop for the systemic regulation of flowering. *SFT* mRNA did not cross the graft union from the donor to the receptor, nor was *SFT* RNA present at the shoot apex, suggesting that *FT* mRNA does not appear to be a component of the mobile floral signal in tomato. A similar grafting experiment was performed in *Arabidopsis* (Corbesier *et al.*, 2007). In this experiment, transgenic donors that expressed the FT:GFP (green fluorescent protein) fusion protein under control of the *SUCROSE TRANSPORTER 2* promoter in phloem companion cells of the late flowering *ft-7* mutant plants were grafted onto *ft-7* receiver plants. The receiver plants flowered earlier than controls. FT:GFP protein, but not mRNA, was detected across the graft union, suggesting that FT protein is a mobile floral signal transmitted from leaves. These results were confirmed via independent research (Jaeger and Wigge, 2007; Mathieu *et al.*, 2007; Notaguchi *et al.*, 2008). The fact that homologues of FT protein are normally present in the phloem sap of *Brassica napus*, *Cucurbita maxima*, and rice provides further evidence that FT protein is part of the phloem translocated signal (Giavalisco *et al.*, 2006; Lin *et al.*, 2007; Aki *et al.*, 2008).

FT biology in trees: *FT*-like genes have been identified from *Populus* (Böhlenius *et al.*, 2006; Hsu *et al.*, 2006; Igasaki *et al.*, 2008) and *Citrus* (Endo *et al.*, 2005). The *Populus FT1* and *FT2* are in the same gene family with 91% amino acid sequence similarity, and are major players in first-time and seasonal sexual reproduction (Böhlenius *et al.*, 2006; Hsu *et al.*, 2006). The amount of *FT1* and *FT2* mRNAs in leaves increased from the juvenile to reproductive developmental phases, suggesting that these genes might play a role in juvenile to mature transition. Based on this observation, when constitutive overexpression constructs driving *FT1*

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and *FT2* were independently inserted into juvenile *Populus*, flowers were observed on trees within several months (Böhlenius *et al.*, 2006; Hsu *et al.*, 2006). Wild-type trees generally produce first flowers after 5–10 years. Similar results were found when *Citrus FT* was overexpressed in trifoliate orange (*Poncirus trifoliata*) (Endo *et al.*, 2005). The *Populus* trees harbouring the *FT1* construct did not cease primary growth under short days or cold temperatures, whereas wild-type trees ceased primary growth under short days within 6 weeks as part of the perennial growth habit (Böhlenius *et al.*, 2006). This result suggests that the onset of sexual reproduction and primary shoot growth are intertwined in trees.

In mature *Populus deltoides* trees, the abundance of *FT2* transcript in leaf 11 (from the base of a shoot) was low from February to April, but was high in mid-May (Hsu *et al.*, 2006). During this time, leaves developed from a primordial pre-formed leaf to a fully expanded leaf (Yuceer *et al.*, 2003). Beginning in mid-May, *FT2* transcript was abundant in bud 11 which formed an inflorescent shoot and floral meristems on its flanks. Potential factors involved in the increase of *FT2* transcript in leaves include temperature, development, and photoperiod. *Populus* trees were treated under two temperature regimes (23 °C and 38 °C) to determine if this affected *FT2* transcript abundance. No change, however, was observed in the expression pattern of *FT2* under either temperature regime, suggesting that temperature is not a factor controlling the expression of *FT2* (Hsu *et al.*, 2006). When poplar trees were grown under long (14 h) and short (8 h) days for 14 d, *FT2* transcripts were abundant under long days throughout the experiment, whereas they were either at background levels (first 7 d) or undetectable after 14 d under short days (Hsu *et al.*, 2006). Similar patterns were observed for *FT1* (Böhlenius *et al.*, 2006). These results suggest that long days promote the abundance of *FT1* and *FT2* transcripts, thus the formation of flower buds in *Populus* once reproductively mature.

Although the basic outline of *FT* function is becoming clear, there are many details that remain to be fleshed out. In addition, there has been little effort to date on how to use *FT* as a tool to advance flowering in trees. The goal of this paper is to present the results of a number of experiments whose aim is to define a system capable of providing reliable and abundant *FT*-induced flowers, and ultimately pollen and seeds, to speed research and breeding in poplar.

Materials and methods

Heat-inducible FT constructs

A DNA containing the *Arabidopsis FT* gene driven by a heat shock promoter was provided by M Fladung (Federal Research Centre for Forestry and Forest Products, Institute for Forest Genetics and Forest Tree Breeding, Germany). This construct (Supplementary Fig. S1 available at *JXB* online) includes a soybean heat-inducible promoter [*GmHsp17.6-L* (Severin and Schoffl, 1990), abbreviated as *HSP*] ligated to *FT*, and was contained in the Gateway binary vector pK2GW7 (Karimi *et al.*, 2002). The vector includes the plant-selectable marker *NPTII* driven by the *NOS* promoter. Two additional *FT* constructs were created by replacing the *FT* sequence (Supplementary Fig. S1) with *FT1* and *FT2* sequences from *Populus trichocarpa*. Forward and reverse primers (Supplementary Table S1 at *JXB* online) were selected from gene model fgenesh4_pg.C_LG_ VIII000671 (http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html) for *FT1*, and from gene model eugene3.14090001 for *FT2*. *FT1* and *FT2* coding sequences were amplified from cDNA prepared from a local (Corvallis, Oregon, USA) *P. trichocarpa* tree and directionally cloned using the restriction sites indicated in Supplementary Table S1.

Plant material, transformation, and regeneration

All plant materials were derived from either of two hybrid aspen clones: INRA 353-53 (male, *Populus tremula*×*P. tremuloides*) and INRA 717-1B4 (female, *P. tremula*×*P. alba*). Methods for *Agrobacterium* (strain AGL1) and plant transformation as well as plant regeneration were as described in Filichkin *et al.* (2005), except that the culture medium for transformed *Agrobacterium* contained a mix of 50 mg l^{-1} rifampicin, 50 mg l^{-1} spectinomycin, and 60 mg l^{-1} carbenicillin. All putative plant transformants, along with non-transgenic control plants, were tested using PCR (Filichkin *et al.*, 2005) to verify transgene presence using a common forward primer (5′-AGTGAAGGCATCGTATCAAGC-3′) coupled with a reverse primer specific for *FT* (5′-CGCGGGATATCACCACTTTG-3′), *FT1* (Supplementary Table S1, 5′-TCAGATATCTTATCGCCTCCTACC-3′), or *FT2* (5′-ATGGCCGCGGGATCCTTCAA-3′). Approximately 30 independent events were identified for each of the three *FT* transgenes.

Plant cultivation and heat induction

After shoots and roots were produced *in vitro*, 50- to 60-day-old PCR-positive plantlets were transferred to soil in 5.7×8.3 cm Rose pots (Meilan and Ma, 2006) and grown for 8–12 weeks in a greenhouse. For some experiments, plants were moved into larger pots (10–15 cm) and grown for another 4–8 weeks. The greenhouse was maintained at 25 °C (day, 16 h) and 21 °C (night, 8 h) with supplemental lighting provided by metal halide bulbs at a photon flux density of 45 E m^{-2} s^{-1}. Plants were watered daily and fertilized once a week with an aqueous solution of 500 ppm 20:10:20 (N:P:K). To stimulate gene expression driven by the soybean HSP, potted plants in a given experiment were moved into a growth chamber (Percival Scientific, Model AR75L, 196×195×90 cm) that was set at 37 °C (except, as described below, when 40 °C was used). Heat was applied for 60– 90 min daily for 10–28 d, depending on the experiment. Heat treatments were applied at the same time each day, and plants were immediately moved back to the greenhouse at the end of each daily treatment. In all cases, timing of the heat induction treatment began after the growth chamber's internal temperature had stabilized at the designated 37 °C or 40 °C setting, which usually took ~10 min. Lighting within the growth chamber was provided by a combination of fluorescent (Philips TL70 E32T8/TL735) and incandescent bulbs (60 W soft white), with a light intensity of 110 μmol m^{-2} s^{-1}. For all experiments involving treatment groups, ramets (i.e. genetically identical plants, for the purposes of this paper derived from a single transgenic event) were randomly assigned to each group. In some instances, plants were subjected to multiple cycles of heat induction treatments over several months. In such cases, plants were typically pruned back to a height of 20–30 cm and then allowed to recover for 4–6 weeks before receiving a second heat treatment. The elapsed time between subsequent heat treatments was ~12 weeks. Because non-transgenic potted plants of the study genotypes have never been observed to flower in earlier studies, and a number of transgenic plants also failed to flower in every experiment, non-transgenic (non-flowering) controls were not included in any of the documented experiments.

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Grafting

Grafting was done using a cleft graft with succulent shoots ~3–6 mm in diameter. Greenhouse-grown potted plants, including *FT* transgenic plants, were used as rootstock, with scions from other potted plants or field-grown trees. Shoots used as scions were collected immediately prior to grafting, or were stored in moistened plastic bags in an ice chest and grafted later the same day. Donor shoots could yield several scions, each trimmed to a length of 5–10 cm with 1–3 internodes and 2–3 lateral buds. To prevent desiccation, graft unions were wrapped in Parafilm®.

Observing plant growth and flower development

Plant heights were recorded at the beginning and end of the heat induction treatments. Plants were observed daily for several weeks following the cessation of the treatments. The date of flower initiation was noted when the first floral buds were clearly visible with the naked eye. Catkin (inflorescence) length was recorded after they had fully elongated. Several types of reproductive structures were observed (floral buds, catkins, individual flowers) and, as appropriate, these structures were categorized and counted for each plant.

Quantitative analysis

Basic statistical analyses included simple χ^2 contingency tests of categorical traits such as flowering occurrence, and analyses of variance (ANOVA) for quantitative traits such as catkin number. ANOVA was implemented using JMP ver. 4.0.4 (SAS Institute), as described for the relevant experiments.

Results

Observations of FT-induced flowering

The earliest observations of flowering after heat-induced expression of *FT* transgenes were made with four goals: (i) to screen plant materials from multiple transformation events to assess the general level of variation in response to *FT* among events; (ii) to select the most responsive and consistently flowering events for subsequent experiments; (iii) to evaluate alternative heat induction procedures for strong floral induction; and (iv) to learn to recognize, monitor, and quantify floral development. Consequently, to accelerate these studies, we generally worked with groups of transgenic plants as they emerged directly after transformation, micropropagation, transplantation, and acclimation, rather than waiting to assess the entire transgenic populations at a single point in time.

Absence of non-induced flowering: Approximately 180 independent transformation events representing three heat-inducible *FT* transgenes (P_{HSP}:*FT*, P_{HSP}:*FT1*, and P_{HSP}:*FT2*) were screened in the two poplar study clones. Despite the large number of transgenic plants that were assessed in multiple experiments (described below), no leaky expression of the *HSP* promoter that is of sufficient intensity to induce flowering in plants receiving only ambient conditions was observed.

Efficacy of FT *gene constructs varied among poplar genotypes*: For the majority of experiments, transgenic plants were evaluated by observing 4–5 independently treated ramets per event. Screening trials (Fig. 1) and associated error bars suggest that P_{HSP}:*FT*, followed by P_{HSP}:*FT1*, was more effective in stimulating flowering than was P_{HSP}:*FT2*, particularly for poplar clone 353. No flowering was observed for P_{HSP}:*FT2* in clone 717. These and most of the subsequent evaluations were based on the frequency of flowering among events, rather than among ramets. This was done because it was expected that this would be a more sensitive indicator of the genetic potential for flowering in any given event; it should tend to average out variation due to individual plant physiology, environment, and date of evaluation.

Plant size was correlated with induced flowering: It was observed that larger plants appeared to flower more readily than did smaller plants. Examining this more closely, Fig. 2 illustrates variation in the rate of flowering among groups of ramets in different size classes. The smallest plants (<20–30 cm) did not flower, whereas the larger plants (>40 cm) were considerably more likely to flower (χ^2 test, P <0.001 for A and B, P=0.0125 for C). Because of this tendency, plants for later experiments were grown to a height of at least 30–40 cm before being subjected to heat induction treatments.

Selection of the most prolific events: Because it was desirable to focus future efforts on the most promising of the many transgenic events that were generated, other factors associated with flowering were also assessed. For P_{HSP}:*FT* in clone 353, at least two of five ramets flowered for six of 33 events. Catkin numbers also varied, so the best four events 17, 30, 32, and 42 in the poplar clone 353 were selected

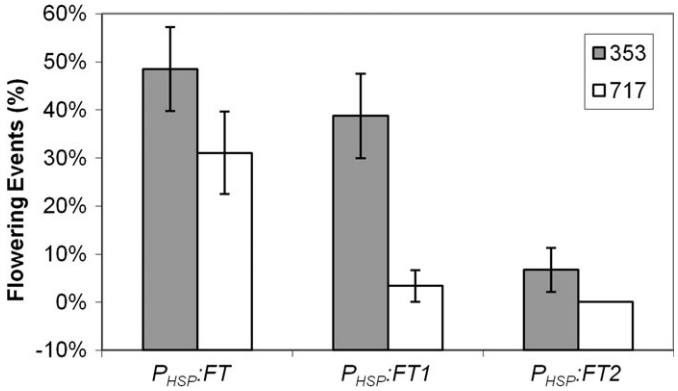

Fig. 1. Frequency of flowering upon heat treatment at 37 °C among transgenic events originating from two poplar clones (353 and 717) containing one of three *FT* constructs (P_{HSP}:*FT*, P_{HSP}:*FT1*, and P_{HSP}:*FT2*). The clone/construct combinations depicted by each vertical bar represent 29–33 independent transgenic events, most with five ramets each. An event was considered to have flowered if at least one ramet developed flowers following heat treatments of 37 °C. Results shown were pooled from individual tests over a period of 6 months as plant materials became available (vertical lines indicate standard error bars).

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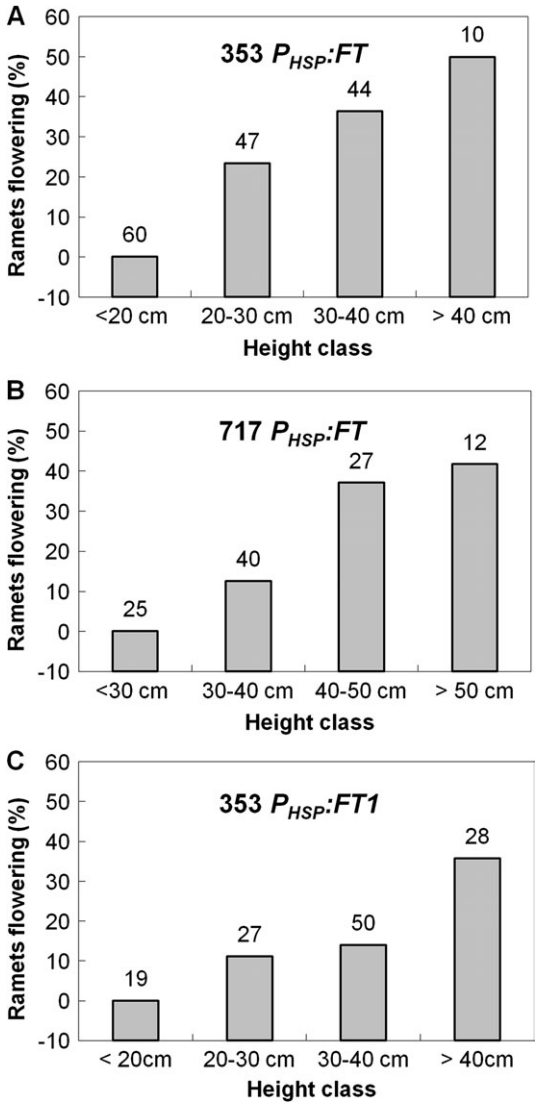

Fig. 2. Frequency of flowering in relation to initial plant height. Flowering rates (percentage of all ramets) are shown by height class as measured when 37 °C heat treatments began. (A) P_{HSP}:*FT* in clone 353 (♂); (B) P_{HSP}:*FT* in clone 717 (♀); (C) P_{HSP}:*FT1* in clone 353. Each clone/construct combination is represented by 29–33 transgenic events with a total of 104–161 ramets. The number of ramets within each height class is shown by the numbers above each bar. Note that ramets of clone 717 (B) are larger than those of clone 353 (A and C), hence the different height classes.

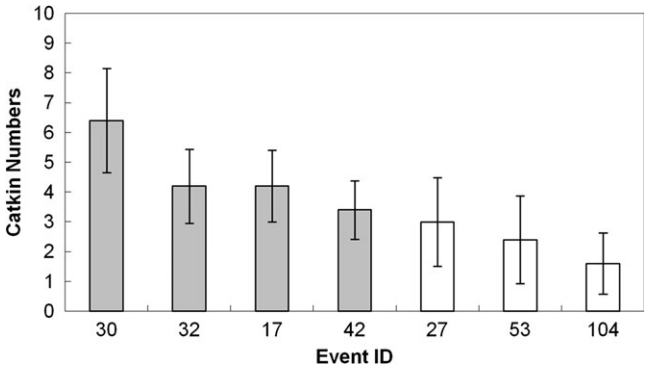

Fig. 3. Mean number of catkins per ramet for poplar clone 353 (♂) containing P_{HSP}:*FT*. *FT* expression was stimulated by subjecting plants to 37 °C heat treatments for 60 min daily for 2–4 weeks. These plants were drawn from a subset of the plants described in Fig. 1; these results depict the distribution of 32 flowering ramets (of 161 total) representing 16 of 33 transgenic events. Mean counts (and standard error bars) of catkins per flowering ramet are shown for seven events in which two or more ramets flowered. Events 30, 32, 17, and 42 (shaded bars) were selected for further study.

based on both flowering incidence and catkin numbers per plant (Fig. 3). For transgenic ramets of clone 353 containing P_{HSP}:*FT1*, at least two of five ramets flowered for five of 33 events. In contrast to ramets containing P_{HSP}:*FT*, these plants generally produced only a single catkin on their terminal shoot; thus a figure was not included. Nevertheless, the plants exhibited subtle differences in catkin quality, assessed by catkin size and maturity, so four of the better events were selected considering both flowering rate and catkin quality.

Variation in inflorescence, flowers, and pollen production: In addition to the incidence of flowering, considerable variation was observed in development and morphology among the floral structures produced (Fig. 4). For clone 353 containing P_{HSP}:*FT*, terminal catkins (Fig. 4A, B) were nearly ubiquitous among flowering ramets, and lateral catkins (produced in leaf axils) were commonly observed as well. Another type of floral structure that was also relatively common included single staminate flowers borne in leaf axils (Fig. 4C). The general organization of the flower itself resembles an individual flower on an intact catkin—with a cluster of anthers borne on a cup-like base (Boes and Strauss, 1994). Other developmental anomalies were observed, including a tendency for catkins to resume vegetative development (Fig. 4D), which were termed 'reverted catkins'. Female reproductive structures (e.g. Fig. 4E) developing on staminate catkins were also observed. These are illustrated here (Fig. 4D, E) on transgenic plants of clone 353 containing P_{HSP}::*FT1*, but such anomalies were also observed on 353 plants containing P_{HSP}::*FT*.

In some instances, staminate catkins matured to a point nearing anthesis, at least as evidenced by the apparent release of limited quantities of pollen (Fig. 4F). Pistillate catkins developed on ramets of female clone 717 containing P_{HSP}:*FT* (Fig. 4G, H), but they were less numerous and tended to be less well developed—some apparently frozen in a state of arrested development (Fig. 4I). This state of arrested development was even more pronounced for 717 plants containing P_{HSP}:*FT1*. Numerous examples were observed, as depicted in Fig. 4J, in which these apparent floral buds simply failed to develop any further, only eventually to shrivel and fall off. Plants containing P_{HSP}:*FT2* rarely flowered; for clone 353, flowers developed

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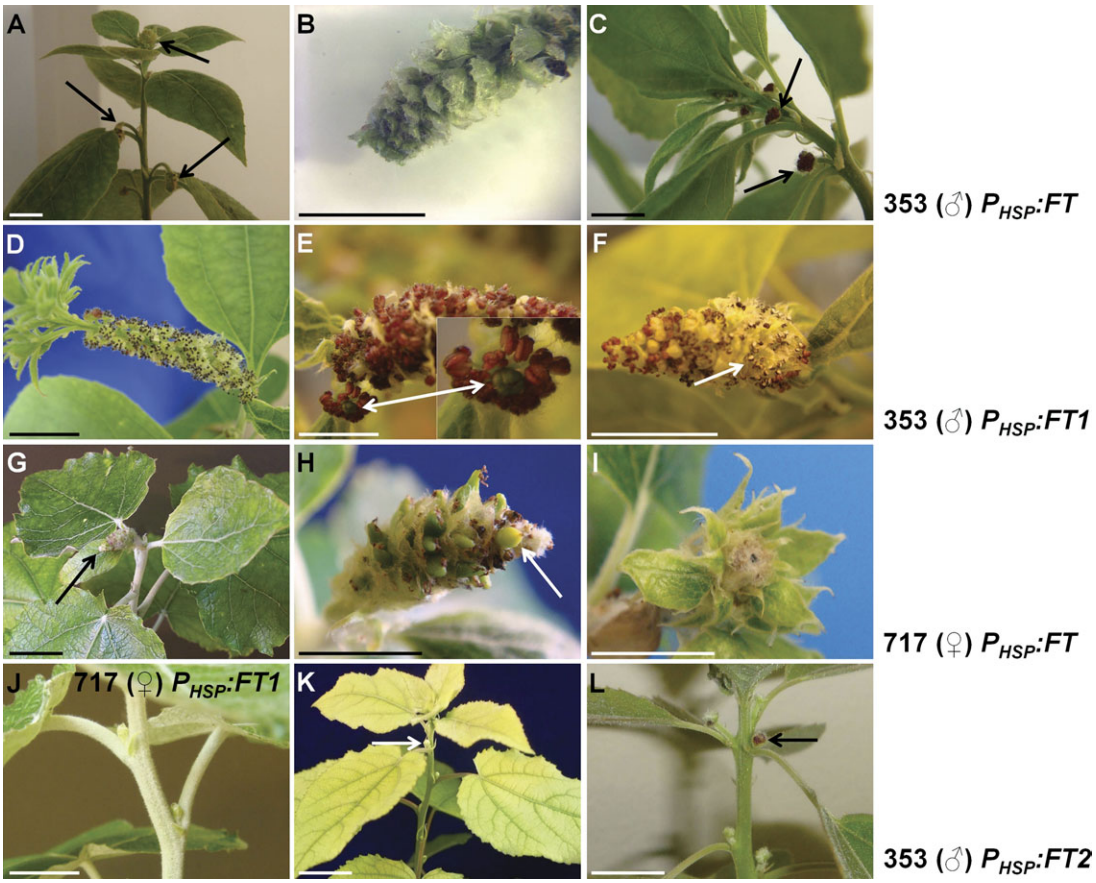

Fig. 4. Variation in floral development in 4-month-old transgenic poplars containing one of three heat-inducible *FT* contructs. *FT* expression was stimulated by exposing plants to 37 °C heat treatments of 60–90 min daily for 10–28 d (Fig. 1). Poplar clones, gender, and transgene constructs are indicated in bold. (A–C) 353 (♂) P_{HSP}:*FT*. (A) Flowering plant with staminate catkins: the upper arrow points to a terminal catkin; lower arrows point to lateral catkins. (B) Close-up of a normal staminate catkin. (C) Single staminate flowers borne in leaf axils. (D–F) 353 (♂) P_{HSP}:*FT1*. (D) Terminal staminate catkin on which the distal end has resumed vegetative development. (E) Staminate catkin with a bisexual flower (arrow and inset). (F) Staminate catkin with some locules releasing pollen (arrow). (G–I) 717 (♀) P_{HSP}:*FT*. (G) Terminal pistillate catkin (arrow). (H) Pistillate catkin with carpels (white arrow). (I) Pistillate catkin displaying arrested development. (J) 717 (♀) P_{HSP}:*FT1* early-stage floral buds in leaf axils. (K–L) 353 (♂)P_{HSP}:*FT2*. (K) Floral buds in leaf axils (arrow). (L) Single flowers (arrows) in leaf axils. Bars are ~1 cm.

on only five of 141 ramets (from two events), and no flowering was observed on transgenic plants of clone 717. Furthermore, only single flowers—never catkins—were observed.

Further testing of selected events

Completing the initial screening of transgenic events, the focus was on the most promising four events for each of two constructs, P_{HSP}:*FT* and P_{HSP}:*FT1* in poplar clone 353. During this phase, ramets of each event were exposed to alternative heat induction treatments. Given the above observations on flowering rates and plant size (Fig. 2), plants were allowed to reach a larger size (35–50 cm) before heat induction treatments were initiated.

Improvement of flowering and reversion rates: Overall rates of flowering (Fig. 5) were substantially improved relative to the initial screening trials (Fig. 1). Among plants containing P_{HSP}:*FT*, ⩾60% of all ramets flowered for each event (Fig. 5A). Among plants containing P_{HSP}:*FT1*, the frequency of flowering ramets was lower overall (Fig. 5B), but >60% of tested ramets flowered for two events. Plants in Fig. 5 include ramets from each event exposed to alternative heat treatments, varying from 60 min to 90 min daily for 2–3 weeks. Individual treatment groups are broken down in Fig. 6, showing that for neither transgene did the frequency of flowering ramets (total height of the bars) vary dramatically among the heat treatments. Nevertheless, for both constructs (Fig. 6A, B), the incidence of reverted catkins (which had resumed vegetative development) appeared lower in those groups in which the heat treatments had lasted 3 weeks rather than 2 weeks. To overcome statistical problems with small samples, flowering data from both constructs were pooled before analysis; χ^2 test showed that the lower reversion rates approached statistical significance (P=0.058). The tendency towards a reduced frequency of reverted catkins to be induced by longer duration heat

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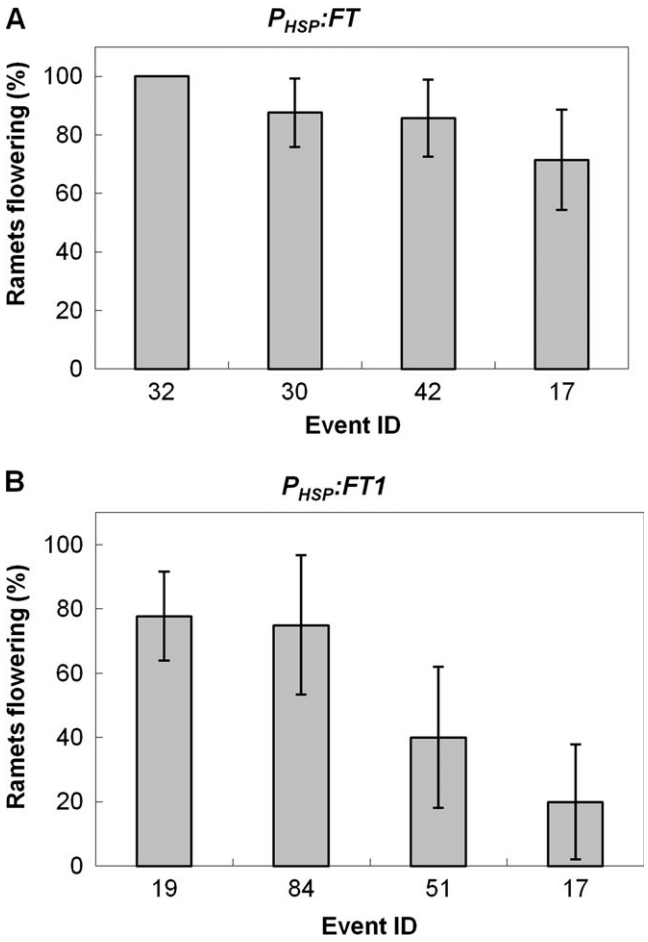

Fig. 5. Frequency of flowering ramets (percentage of total) among selected transgenic events of poplar clone 353 (♂) containing (A) P_{HSP}:*FT* or (B) P_{HSP}:*FT1*. Each bar represents 4–9 ramets pooled across four 37 °C heat treatments administered to stimulate FT expression. Heat treatments lasted 60–90 min daily for 2–3 weeks at 37 °C. Additional details are shown in Fig. 6. Vertical lines indicate standard errors among all ramets of each event, pooled over treatments.

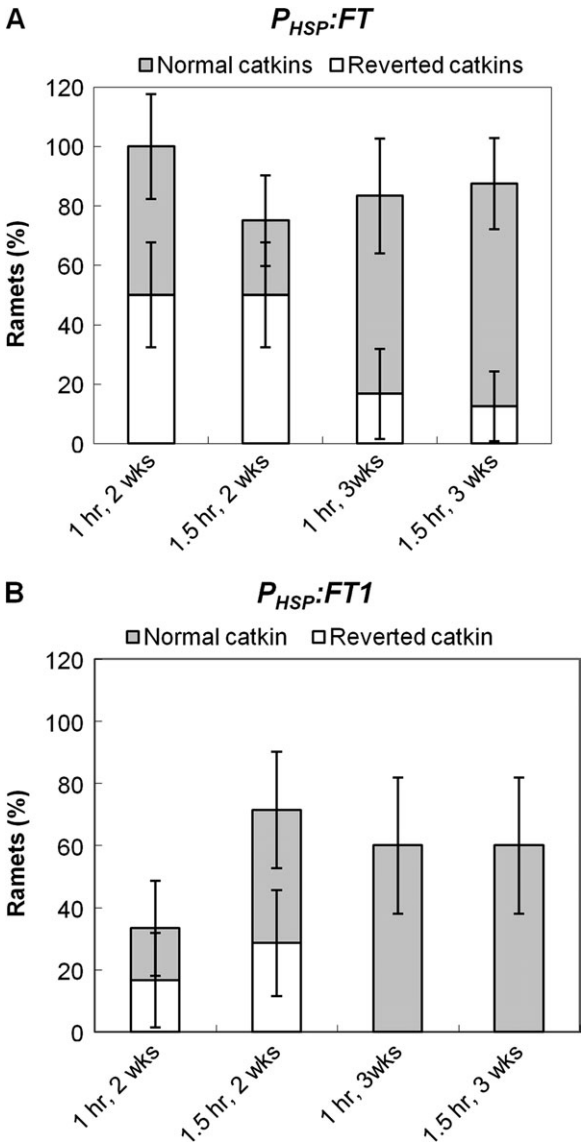

Fig. 6. Frequency of flowering and tendency to resume vegetative development after cessation of heat treatments used to stimulate FT expression. Terminal catkins (borne on terminal shoots) were classified as either 'normal' (Fig. 4B) or 'reverted' (Fig. 4D, with emerging leaves). Four 37 °C heat treatments were tested, lasting 60–90 min daily for 2–3 weeks as shown. Frequencies (percentage of ramets) indicated by each bar represent 5–8 ramets pooled across four selected events of (A) P_{HSP}:*FT* or (B) P_{HSP}:*FT1* transgenes in poplar clone 353 (♂). Catkins were scored as normal (grey shading) or reverted (no shading) 65 d after initiating the heat treatments (44 d or 51 d after heat treatments had ended). Vertical lines indicate standard errors among all ramets of each treatment, pooled over events.

treatments suggested that the examination of flowering behaviour in plants exposed to higher levels of heat treatment should be considered. For example, Severin and Schoffl (1990), working with the same soybean *HSP* promoter, used daily heat treatments of 40 °C in their studies with tobacco.

Improved catkin production with increased induction temperature: Using the same four selected events for each of the two transgenes (P_{HSP}:*FT* and P_{HSP}:*FT1*), it was observed that increased temperature had only a small effect on the frequency of flowering ramets for most events (Fig. 7A). Flowering had been scored on presence of floral buds, but from prior experience it was realized that not all floral buds mature. To better understand how temperature treatments might affect subsequent flower development, the number of catkins retained 2 weeks after observing floral buds was also counted. In contrast to rates of flowering, the average number of catkins per flowering ramet (reflecting flower development on flowering ramets) was substantially greater among ramets subjected to the higher temperature treatments (Fig. 7B). For each construct, ANOVA verified that differences among treatment means were significant (P <0.01). For many events, the average number of catkins per ramet increased ⩾3-fold.

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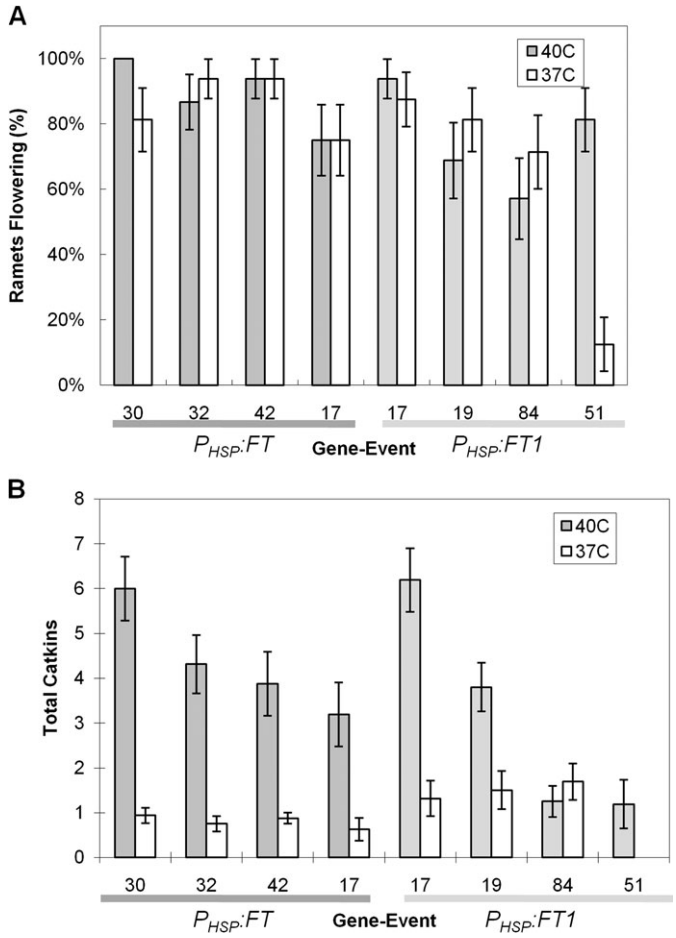

Fig. 7. Flowering behaviour of poplar clone 353 (♂) following two heat treatments to stimulate *FT* expression. Heat treatments were applied daily for 60 min for 3 weeks at temperatures of 37 °C (open bar) or 40 °C (shaded bar). Two *FT* constructs (P_{HSP}:*FT* and P_{HSP}:*FT1*) are represented by four selected transgenic events each, with 16 ramets in each treatment. Vertical lines represent standard errors. (A) Proportion of ramets (percentage of total) developing flowers. (B) Mean number of catkins per flowering ramet.

Extensive variation in inflorescence and fruit structure: When older transgenic poplars were induced to flower and their development studied in more detail, extensive variation in structure was observed, similar to that observed earlier (Fig. 4), including in pistillate flowers formed on male plants that proceeded toward fruit development (Fig. 8). Both normal and reverted catkins that resumed vegetative development at the distal end are visible (Fig. 8A, B), as well as catkins with large leaf-like bracts (Fig. 8C), and female flowers on otherwise male catkins (Fig. 8D–F). Other staminate flowers can be seen in Fig. 8G, and bisexual flowers can be seen in Fig. 8H. Young seeds and associated fibres in a female flower are visible in Fig. 8I–L.

Some of the oldest (9 months) and largest (85–110 cm, growing in 20 cm pots) plants from the experiments—all of which had been through at least a single round of prior *FT*-induced flowering—were used to study the distribution and structure of *FT*-induced flowers in the canopy. On such plants, typical floral architecture is illustrated in Fig. 9. Catkins occurred at the tips of both terminal and lateral shoots, with lateral shoots developing from leaf axils along the main shoot (Fig. 9A). Single flowers tended to be located in leaf axils along the main shoot immediately beneath the terminal catkin (Fig. 9B), whether or not they are unisexual or bisexual (Fig. 9C).

Grafting, pruning, and flowering

Grafting success was evaluated using scion survival 1 month after grafting. Using cleft grafts to join succulent scion and rootstock proved to be highly successful, as survival rates of 85–90% were routinely achieved in the greenhouse. The *FT* signal moved across graft junctions in other species (e.g. tomato, Lifschitz *et al.*, 2006), so it was of interest to determine whether *FT* in transgenic poplar rootstock could also stimulate flowering in scions lacking *FT* transgenes. Thus far, the results from experiments involving grafted scion exclusively used rootstock containing P_{HSP}:*FT*. Newly grafted plants were allowed to recover for 7 weeks before subjecting them to inductive heat treatments (60 min daily at 37 °C for 14–21 d).

A total of 128 plants were studied, which consisted of non-*FT* scions grafted onto 96 rootstocks (24 ramets from four events that were selected for high rates of heat-induced flowering) and 32 non-grafted ramets as controls (eight ramets from the same four events). All plants were maintained in the greenhouse, grew well, and appeared healthy. No flowering was observed on any scion, however, even though flowering was observed on 21 of the 32 (66%) non-grafted control *FT* plants. Following previous success in flowering of plants that had been previously heat induced to flower, scions and rootstocks were pruned and then exposed to a second round of inductive treatments; 60 min daily heat treatments were imposed for 21 d. Again, no scions were observed to flower, although >90% of non-grafted *FT* control plants flowered. In addition, flowers also developed on 45% of the rootstock (from branches beneath the graft union).

Discussion

A large number of genetic and environmental factors that affect the intensity and structure of *FT*-induced flowering in poplar were studied. Plant size often has been reported to affect flowering in trees (Longman and Wareing, 1959; Stanton and Villar, 1996; Braatne *et al.*, 1996; Chalupka and Cecich, 1997), though usually when they are much larger. The present finding suggests that, similar to the size-dependent effects of many other floral genes, including *LEAFY* (Weigel and Nilsson 1995), there is a steep maturation gradient in the meristems of all plants, including small trees. In juvenile meristems the response to floral signals is blocked or greatly attenuated. In addition, very small trees appear less capable of supporting full inflorescence meristem activity and development, often resulting in single

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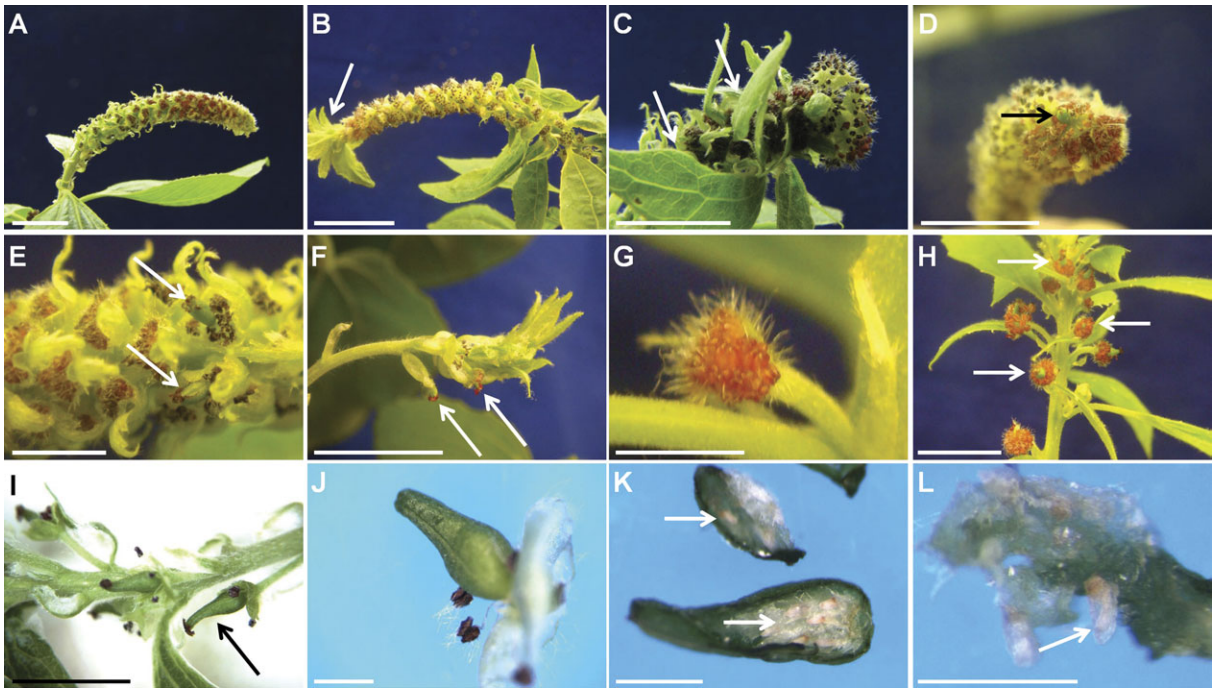

Fig. 8. Variation in floral development among 9-month-old transgenic poplars stimulated by expression of P_{HSP}:*FT* following heat induction. Ramets of clone 353 (♂) were exposed to 37 °C for 60 min daily for 21 d. (A) Normal catkin. (B) Reverted catkin resuming vegetative development at the distal end (arrow). (C) Catkin with large leaf-like bracts (arrows). (D and E) Female flowers (arrows) on otherwise normal staminate catkins. (F) Female flowers near the base of a poorly developed catkin. (G) A single staminate flower (without an inflorescence) emerging from the axil of a small leaf. (H) Numerous bisexual flowers (arrows point to female organs) borne in the axils of small leaves. (I–L) Excision of young seeds from a female flower, as follows: (I) female flower attached to plant; (J) intact female flower after removal from plant; (K) section of carpal removed to reveal immature seeds; (L) excised immature seeds. Bars are ~1 cm in A–I; and ~1 mm in J–L.

flowers, short catkins, and incomplete flower and gamete/fruit development. With respect to using *FT* to reduce generation times in research and breeding, these results suggest a balance must be reached between plant size and timing of floral induction. Premature induction may impair development of normal flowers, gametes, and fruits.

There appeared to be a threshold quantity of *FT* gene product needed for floral induction. The considerable 'leaky' expression (i.e. expression that is non-specific or non-induced), and that is commonly seen in populations of primary transgenic plants with many different types of promoters, was not sufficient to induce any flowering in the present studies. Such 'permissive' transgene expression is common in transgenic poplar based on years of experience in the Strauss laboratory. It was also noticed that a substantial proportion of the induced transgenic events did not flower or flowered to only a small degree, probably a result of variable position effect of the transgene insertion in the genome (i.e. influence of positive and/or negative elements located around the insertion site, and thus varied intensity/specificity of expression). Such a phenomenon is often observed in poplar (Kumar and Fladung, 2001) and other species (Beaujean *et al.*, 1998; Miki *et al.*, 2009; Silicheva *et al.*, 2010). Finally, unlike in *Arabidopsis* and tomato (Lifschitz *et al.*, 2006; Corbesier *et al.*, 2007; Notaguchi *et al.*, 2008), the present grafting experiments in poplar did not produce flowers on receptor scions. The lack of flowers on the grafts might be due to a dilution or attenuation of *FT* product when moving across graft junctions and into receptor scions. Thus, it may not be transmitted at a level above the required threshold for induction. Alternatively, unlike FT in other model species such as *Arabidopsis* and rice (Corbesier *et al.*, 2007; Tamaki *et al.*, 2007; Notaguchi *et al.*, 2008), it is possible that the FT protein might not be transported from the source leaves to the shoot apex in poplar. Thus, it is important to determine when and where poplar *FT* genes are expressed. This is particularly important for *FT1*, which the present results show was as efficient as *Arabidopsis FT* in inducing flowering after heat shock. In addition, experiments using fusion proteins such as *FT1:GFP* will help to determine where poplar FT proteins are targeted.

In addition to a threshold dose for initiation of flowering, the results suggest that increasing the dose or duration of *FT* synthesis beyond the threshold level may further enhance floral development. Increasing the duration of daily heat treatments (i.e. time per day or number of days) resulted in a higher incidence of normal catkins. Likewise, increasing the induction temperature from 37 °C to 40 °C resulted in the production of more catkins, particularly in

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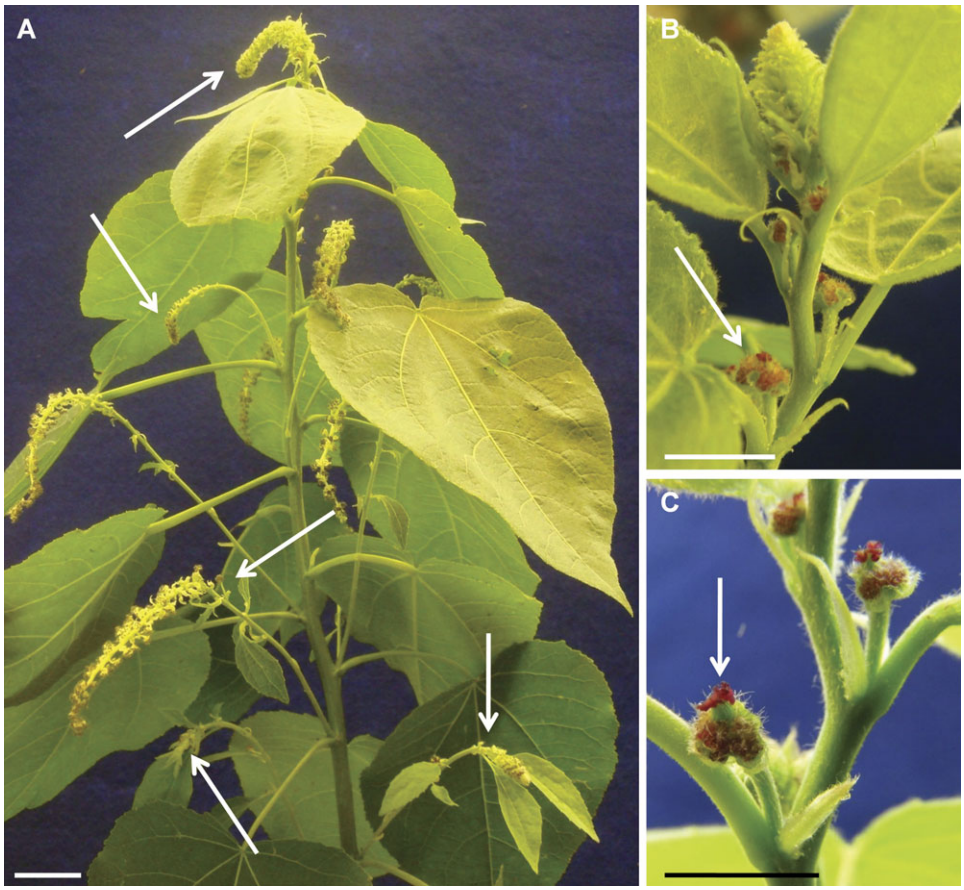

Fig. 9. Floral architecture and organization in 9-month old ramets of poplar clone 353 (♂) following expression of P_{HSP}:*FT* stimulated by 60 min daily exposure to 37 °C for 21 d. (A) A catkin on terminal and axillary shoots. (B) Single flowers basal to catkins (male). (C) Single flowers bearing both male and female organs. Bars are 1 cm.

some events. A persistent *SFT*-generated floral signal was also required for graft stimulation of flowering in tomato (Lifschitz *et al.*, 2006).

The variability observed in basic inflorescence and floral structure contrasts with the modest variation seen in the normal flowering of field-grown trees. There, even when reproductive tissues occur in different parts of trees, in different environments, and at different ages, the basic structure and shape are often conserved. Some deviations of floral structure have been reported in *Populus* (Santamour, 1956; Pauley and Mennel, 1957; Maini and Coupland, 1964; Ronald, 1978); however, these are mainly due to variations in gender identity of catkins or individual flowers with respect to crown position or genotype (Santamour, 1956; Pauley and Mennel, 1957). Even in these cases, catkin form and function are highly conserved. The extensive variation in structure seen in the experiments suggests that more consistent control of environment, physiology, plant size, and developmental state—possibly in combination with additional gene(s) that modify juvenility or other elements of competence to flower—might be needed for a system that provides routine shortening of generation time in poplar. Additional fundamental discoveries about comparative gene and genome function in poplar, especially in comparison with other perennial plants that require different environmental signals for floral induction (Yuccer *et al.*, 2003; Groover, 2005; Tuskan *et al.*, 2006), will be essential to identify new paths forward.

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. Forward and reverse primers for amplifying and cloning P_{HSP}:*FT1* and P_{HSP}:*FT2* from *Populus trichocarpa*. Bases corresponding to poplar *FT* sequences are non-italicized, whereas linker and restriction sequences for cloning are italicized.

Fig. S1. Schematic map of the T-DNA region containing heat-inducible *FT* coding sequences. The segment labelled *FT sequence* represents coding sequence from the *FLOWERING LOCUS T* (*FT*) in each of three DNA constructs. The original construct contains *FT* from *Arabidopsis thaliana* (*FT*). In two other constructs, *FT* was replaced with coding sequences from *Populus trichocarpa* (*FT1* and *FT2*). Other components in the constructs include: LB and RB, left and right T-DNA borders; *GmHsp*, *Glycine max* heat shock 17.6-L gene promoter; *NOSp* and *NOSt*,

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nopaline synthase promoter and terminator; *NPTII*, neomycin phosphotransferase II; *35St*, CaMV *35S* terminator.

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References

Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T. 2005. FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science* **309,** 1052–1056.

Aki T, Shigyo M, Nakano R, Yoneyama T, Yanagisawa S. 2008. Nano scale proteomics revealed the presence of regulatory proteins including three FT-like proteins in phloem and xylem saps from rice. *Plant and Cell Physiology* **49,** 767–790.

An H, Roussot C, Suarez-Lopez P, *et al*. 2004. CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis. *Development* **131,** 3615–3626.

Araki T, Kobayashi Y, Kaya H, Iwabuchi M. 1998. The flowering-time gene FT and regulation of flowering in Arabidopsis. *Journal of Plant Research* **111,** 277–281.

Ayre BG, Turgeon R. 2004. Graft transmission of a floral stimulant derived from CONSTANS. *Plant Physiology* **135,** 2271–2278.

Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C. 2004. Vernalization requires epigenetic silencing of FLC by histone methylation. *Nature* **427,** 164–167.

Beaujean A, Sangwan RS, Hodges M, Sangwan-Norreel BS. 1998. Effect of ploidy and homozygosity on transgene expression in primary tobacco transformants and their androgenetic progenies. *Molecular and General Genetics* **260,** 362–371.

Boes TK, Strauss SH. 1994. Floral phenology and morphology of black cottonwood. *Populus trichocarpa(Salicaceae). American Journal of Botany* **81,** 562–567.

Böhlenius H, Huang T, Charbonnel-Campaa L, Brunner AM, Jansson S, Strauss SH, Nilsson O. 2006. CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. *Science* **312,** 1040–1043.

Braatne JH, Rood SB, Heilman PE. 1996. Life history, ecology, and conservation of riparian cottonwoods in North America. In: Heilman PE, Hinckley TM, eds. *Biology of Populus*. Ottawa, Canada: NRC Research Press, 57–85.

Cervera M, Navarro L, Peña L. 2009. Gene stacking in 1-year-cycling APETALA1 citrus plants for a rapid evaluation of transgenic traits in reproductive tissues. *Journal of Biotechnology* **140,** 278–282.

Chalupka WC, Cecich RA. 1997. Control of the first flowering in forest trees. *Scandinavian Journal of Forest Research* **12,** 102–111.

Corbesier L, Vincent C, Jang S, *et al*. 2007. FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. *Science* **316,** 1030–1033.

Endo T, Shimada T, Fujii H, Kobayashi Y, Araki T, Omura M. 2005. Ectopic expression of an FT homolog from Citrus confers an early flowering phenotype on trifoliate orange (Poncirus trifoliata L. Raf.). *Transgenic Research* **14,** 703–712.

Filichkin SA, Meilan R, Busov VB, Ma C, Brunner AM, Strauss SH. 2005. Alcohol-inducible gene expression in transgenic *Populus*. *Plant Cell Reports* **25,** 660–667.

Flachowsky H, Peil A, Sopanen T, Elo A, Hanke V. 2007. Overexpression of *BpMADS4* from silver birch (*Betula pendula* Roth.) induces early-flowering in apple (*Malus*× *domestica* Borkh.). *Plant Breeding* **126,** 137–145.

Flachowsky H, Hanke M-V, Peil A, Strauss SH, Fladung M. 2009. A review on transgenic approaches to accelerate breeding of woody plants. *Plant Breeding* **128,** 217–226.

Giavalisco P, Kapitza K, Kolasa A, Buhtz A, Keh J. 2006. Towards the proteome of *Brassica napus* phloem sap. *Proteomics* **6,** 896–909.

Groover AT. 2005. What genes make a tree a tree? *Trends in Plant Science* **10,** 210–214.

Hsu CY, Liu YX, Luthe DS, Yuceer C. 2006. Poplar FT2 shortens the juvenile phase and promotes seasonal flowering. *The Plant Cell* **18,** 1846–1861.

Igasaki T, Watanabe Y, Nishiguchi M, Kotoda N. 2008. The FLOWERING LOCUS T/TERMINAL FLOWER 1 family in Lombardy poplar. *Plant and Cell Physiology* **49,** 291–300.

Jaeger KJ, Wigge PA. 2007. FT protein acts as a long-range signal in *Arabidopsis*. *Current Biology* **17,** 1050–1054.

Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, Chory J, Harrison MJ, Weigel D. 1999. Activation tagging of the floral inducer FT. *Science* **286,** 1962–1965.

Karimi M, Inze D, Depicker A. 2002. GATEWAY™ vectors for Agrobacterium-mediated plant transformation. *Trends in Plant Science* **7,** 193–195.

Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T. 1999. A pair of related genes with antagonistic roles in mediating flowering signals. *Science* **286,** 1960–1962.

Komeda Y. 2004. Genetic regulation of time to flower in *Arabidopsis thaliana*. *Annual Review of Plant Biology* **55,** 521–535.

Kumar S, Fladung M. 2001. Gene stability in transgenic aspen (Populus). II. Molecular characterization of variable expression of transgene in wild and hybrid aspen. *Planta* **213,** 731–740.

Lifschitz E, Eviatar T, Rozman A, Shalit A, Goldshmidt A, Amsellem Z, Alvarez JP, Eshed Y. 2006. The tomato FT ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. *Proceedings of the National Academy of Sciences, USA* **103,** 6398–6403.

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Lin MK, Belanger H, Lee YJ, *et al*. 2007. FLOWERING LOCUS T protein may act as the long-distance florigenic signal in the cucurbits. *The Plant Cell* **19,** 1488–1506.

Longman KA, Wareing PF. 1959. Early induction of flowering in birch seedlings. *Nature* **184,** 2037–2038.

Maini JS, Coupland RT. 1964. Anomalous floral organization in *Populus tremuloides*. *Canadian Journal of Botany* **42,** 835–839.

Mathieu J, Warthmann N, Kuttner F, Schmid M. 2007. Export of FT protein from phloem companion cells is sufficient for floral induction in Arabidopsis. *Current Biology* **17,** 1055–1060.

Meilan R. 1997. Floral induction in woody angiosperms. *New Forests* **14,** 179–202.

Meilan R, Ma C. 2006. Poplar (Populus spp.). *Methods in Molecular Biology* **344,** 143–151.

Michaels SD, Amasino RM. 1999. FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering. *The Plant Cell* **11,** 949–956.

Michaels SD, Amasino RM. 2001. Loss of FLOWERING LOCUS C activity eliminates the late-flowering phenotype of FRIGIDA and autonomous pathway mutations but not responsiveness to vernalization. *The Plant Cell* **13,** 935–941.

Miki B, Abdeen A, Manabe Y, MacDonald P. 2009. Selectable marker genes and unintended changes to the plant transcriptome. *Plant Biotechnology Journal* **7,** 211–218.

Notaguchi M, Abe M, Kimura T, Daimon Y, Kobayashi T, Yamaguchi A, Tomita Y, Dohi K, Mori M, Araki T. 2008. Long-distance, graft-transmissible action of Arabidopsis FLOWERING LOCUS T protein to promote flowering. *Plant and Cell Physiology* **49,** 1645–1658.

Pauley SS, Mennel GF. 1957. Sex ratio and hermaphroditism in natural population of quaking aspen. *Minnesota Forestry Notes* No. 55.

Ronald WG. 1978. Irregular flowering bud conformation in a hybrid poplar. *Canadian Journal of Botany* **56,** 369–370.

Rottmann WH, Meilan R, Sheppard LA, Brunner AM, Skinner JS, Ma CP, Cheng SP, Jouanin L, Pilate G, Strauss SH. 2000. Diverse effects of overexpression of LEAFY and PTLF, a poplar (Populus) homolog of LEAFY/FLORICAULA, in transgenic poplar and Arabidopsis. *The Plant Journal* **22,** 235–245.

Santamour FS. 1956. Hermaphroditism in *Populus*. Paper read at the 3rd Northeastern Forest Tree Improvement Conference.

Schultz RP. 1997. *Loblolly pine: the ecology and culture of loblolly pine (Pinus taeda L.)*. Washington, DC: USDA Forest Service Agricultural Handbook 713.

Searle I, He YH, Turck F, Vincent C, Fornara F, Krober S, Amasino RA, Coupland G. 2006. The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. *Genes and Development* **20,** 898–912.

Severin K, Schoffl F. 1990. Heat-inducible hygromycin resistance in transgenic tobacco. *Plant Molecular Biology* **15,** 827–833.

Sheldon CC, Burn JE, Perez PP, Metzger J, Edwards JA, Peacock WJ, Dennis ES. 1999. The FLF MADS box gene: a repressor of flowering in Arabidopsis regulated by vernalization and methylation. *The Plant Cell* **11,** 445–458.

Sheldon CC, Conn AB, Dennis ES, Peacock WJ. 2002. Different regulatory regions are required for the vernalization-induced repression of FLOWERING LOCUS C and for the epigenetic maintenance of repression. *The Plant Cell* **14,** 2527–2537.

Sheldon CC, Rouse DT, Finnegan EJ, Peacock WJ, Dennis ES. 2000. The molecular basis of vernalization: the central role of FLOWERING LOCUS C (FLC). *Proceedings of the National Academy of Sciences, USA* **97,** 3753–3758.

Silicheva M, Golovnin A, Pomerantseva E, Parshikov A, Georgiev P, Maksimenko O. 2010. *Drosophila* mini-white model system: new insights into positive position effects and the role of transcriptional terminators and gypsy insulator in transgene shielding. *Nucleic Acids Research* **38,** 39–47.

Stanton BJ, Villar M. 1996. Controlled reproduction of *Populus*. In: Stettler RF, Bradshaw HD Jr, Heilman PE, Hinckley TM, eds. *Biology of Populus*. Ottawa, ON, Canada: NRC Research Press, 113–138.

Strauss SH, Brunner AM, Busov VB, Ma C, Meilan R. 2004. Ten lessons from 15 years of transgenic Populus research. *Forestry* **77,** 455–465.

Tamaki S, Matsuo S, Wong HL, Yokoi S, Shimamoto K. 2007. Hd3a protein is a mobile flowering signal in rice. *Science* **316,** 1033–1036.

Tuskan GA, DiFazio S, Jansson S, *et al*. 2006. The genome of black cottonwood, Populus trichocarpa (Torr. & Gray). *Science* **313,** 1596–1604.

Weigel D, Nilsson O. 1995. A developmental switch sufficient for flower initiation in diverse plants. *Nature* **377,** 495–500.

Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D. 2005. Integration of spatial and temporal information during floral induction in Arabidopsis. *Science* **309,** 1056–1059.

Yuceer C, Land SB, Kubiske ME, Harkess RL. 2003. Shoot morphogenesis associated with flowering in *Populus deltoides* (Salicaceae). *American Journal of Botany* **90,** 196–206.

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