Chapter 11

FLORIGEN AND COTTON: MANIPULATING PLANT ARCHITECTURE TO IMPROVE PLANT PRODUCTIVITY

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INTRODUCTION

Plant architecture and the timing and distribution of reproductive structures are fundamental agronomic traits. The functions of members of the phosphatidylethanolamine binding protein (PEBP) family, specifically FLOWERING LOCUS T (FT), are important for regulating plant architecture, and manipulating FT expression has consequences for agriculture. Ectopic expression of FT in perennial, photoperiodic cotton increases determinate plant growth and overcomes photoperiodism, facilitating crosses with domesticated accessions. Thus, judicious manipulation of FT expression in cotton provides new tools for cotton breeding programs and crop management.

PLANT ARCHITECTURE IS THE PRODUCT OF MERISTEMATIC ACTIVITIES

The architecture of each plant species is uniquely specified through the activities of indeterminate and determinate meristems (Sussex and Kerk, 2001). Indeterminate meristems are replenishing reservoirs of undifferentiated plant cells needed for continued plant growth. In aerial tissues, these indeterminate meristems establish the placement of leaves, position of nodes and branches, and internode distances. This reiterative vegetative growth arises from a single point, and is referred to as monopodial growth. Cells of determinate meristems differentiate to form the reproductive structures of inflorescences and flowers. Because the apical meristem terminates in this case, the most proximal axillary bud must be released from apical dominance to continue the species-specific body plan. This is referred to as sympodial growth. Plant architecture then is a basic agronomic trait, and, not surprisingly, architecture regulation has a major impact on the agronomic success of crop plants. For example, the Green Revolution brought dramatic increases in crop yields as a result of introducing semi-dwarf varieties of wheat and rice (Borlaug, 2000; Peng et al., 1999).

Cotton (Gossypium spp.), the world’s most important textile crop, is grown primarily for fiber, which are the cell wall remains of individual cells that develop on the epidermal surface of the seed coat. The remainder of the seed is predominantly embryo and is a valuable source of oil and protein (Ruan et al., 2005; Stewart and Mauney, 1986). The entire seed is therefore a valuable
commodity, and enhancing yield would have great impact on producers and subsistence farmers alike. Historically, cotton yield increases per acre have paralleled advances in technology and production practices (http://www.ers.usda.gov/Briefing/Cotton/; Meyer et al., 2007). However, further investment in developmental biology and biotechnology is required to enhance production for an expanding world population and an increasingly competitive world market. In this chapter, we will discuss how the principles of plant architecture gleaned from model systems can be translated to cotton to further improve yields. Specifically, we will address how manipulating the timing and position of floral meristems have the potential to increase yields, reduce producer inputs, and benefit crop management.

**COTTON PLANT ARCHITECTURE: THE TRANSITION TO FLOWERING**

In cotton, the apical meristem of the main stem is indeterminate and monopodial, meaning that it remains meristematic and produces vegetative structures (nodes, internodes, leaves and axillary buds) for the life of the plant. In domesticated, day-neutral cultivars, the axillary buds of the first four nodes may remain dormant or may form monopodial vegetative branches that reiterate the main stem. Axillary buds of later-forming nodes grow out as fruiting branches and node of first fruiting branch (NFFB) is a measure of a variety’s ‘earliness’ (Guo et al., 2008). A fruiting branch is a sympodial, cymose inflorescence. The apical meristem of a fruiting branch (inflorescence apical meristem, IAM) produces a single node, internode, leaf and two axillary buds, and then transitions from a vegetative meristem to a floral meristem, forms a flower, and ultimately a boll. The leaf produced is a subtending leaf (subtends the flower); one of the axillary buds usually becomes dormant while the second axillary bud grows out to form the next sympodial unit. It in turn produces a node, internode, leaf and axillary buds, and transitions to a floral meristem. This pattern repeats for the life of the plant, giving fruiting branches a ‘zig-zag’ appearance instead of being straight like main-stems and vegetative branches (Gore, 1935; Oosterhuis, 1990).

Once the signal to flower is received by the meristem, the meristem can differentiate into a terminal flower, but commonly forms an inflorescence. Inflorescence architecture is controlled by the distribution of indeterminate inflorescence meristems (IM) and determinate floral meristems (FM). Prusinkiewicz et al. (2007) presented an elegant, unifying model to explain inflorescence architecture (Prusinkiewicz et al., 2007). An inflorescence has an inflorescence apical meristem (IAM) which produces lateral meristems from its flanks (inflorescence lateral meristem, ILM). The fate of these meristems is determined by a quantitative character called ‘vegetativeness’ (veg). Veg, is not a compound or a gene, but a ‘state of being’. If veg is high, IAMs will produce new growth with new ILMs, which may themselves produce new growth and more ILMs. If veg drops below a threshold, the IMs convert to determinate FMs and form flowers. In a young plant, veg is initially high but drops with age. In panicles such as mountain ash, veg drops uniformly throughout the inflorescence, resulting in relatively synchronized flowering and termination of growth. If veg drops quickly after formation of an inflorescence, a simple panicle is formed, but if veg drops slowly, a compound panicle is formed because the
lateral meristems are able to reiterate the inflorescence pattern before switching to floral identity. During the formation of cymes and racemes, loss of veg is not uniform. In racemes, veg stays high in the IAM and drops in the ILMs so that the IAM continues growth and the ILMs form flowers (monopodial inflorescence; Arabidopsis thaliana, snapdragon, Antirrhinum majus). In cymes, veg drops in the IAM but remains high in ILMs, such that the ILMs form a flower and growth continues from the ILMs (sympodial inflorescence; tomato, Solanum lycopersicon, cotton) (Prusinkiewicz et al., 2007) (Fig. 1).

Figure 1. Simplified representation of (A) a panicle, (B) a raceme, and (C) a cyme, after (Prusinkiewicz et al. 2007). In arrowheads, vegetativeness (veg) is above a threshold, and meristem retains an indeterminate identity (e.g. continued vegetative growth); in circles veg has dropped below a threshold and the meristem has converted to a determinate fate (e.g., a flower). In panicles (A), veg drops uniformly in all buds resulting in a synchronized transition. In racemes (B), veg stays high in the apical meristem and drops in the lateral buds to give a monopodial main axis. In cymes (C), veg drops in the apical meristem and remains high in the axillary / lateral buds, resulting in a sympodial axis.

Experiments in Arabidopsis, snapdragon, various Solanaceae and other model systems have illuminated paradigms for controlling veg levels. Floral meristem identity genes LEAFY (LFY) and APETALA1 (AP1) suppress veg to specify a flower. When either is over-expressed in Arabidopsis, the transition to flowering is accelerated and the IAM of the raceme loses its indeterminate character and terminates as a single flower. Conversely, lfy and ap1 mutants have excessive veg phenotypes: flowering is delayed, inflorescences have more branches and bract leaves, and flowers that do form have stem-like characteristics and form late on the inflorescence, consistent with the model that veg reduces with age. On the contrary, TERMINAL FLOWER 1 (TFL1) maintains veg. tfl1 mutants have solitary flowers where inflorescence branches would normally be and the IAM terminates as a solitary flower (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992). This phenotype is nearly identical to LFY over-expression. TFL1 over-expression
results in late flowering and a phenotype similar to *lfy* mutants (Benlloch et al., 2007). The tomato paralog of *TFL1* is SELF PRUNING (*SP*). The *sp* mutant of tomato has accelerated termination of sympodial growth, and results in a more compact, determinate plant with nearly homogeneous fruit set. Identifying the *sp* phenotype “was the single most important genetic trait in the development of modern agrotechniques for this crop plant because the ‘determinate’ growth habit facilitates mechanical harvest” (Rick, 1978). Consequently, appreciating how to control or manipulate *veg* levels in IMs can directly impact plant architecture and productivity.

**FLORIGEN AND PHOTOPERIODISM**

For over seventy years, the flowering factor, termed florigen, was the elusive “Holy Grail” of plant biology (Zeevaart, 2008). Abundant physiological data characterized florigen as a substance perceived by leaves and transmitted to the shoot apex to stimulate flowering yet the nature of that signal remained unknown (Chailakhyan, 1968). Extensive genetic and biochemical research, largely in model plants such as Arabidopsis, identified a number of genes involved in different flowering response pathways, and from these, *FLOWERING LOCUS T* (*FT*) emerged as a common element. The *FT* gene product is recognized as florigen (Turck et al., 2008; Zeevaart, 2008).

The Arabidopsis *FT* is part of a small gene family whose gene products share similarity with mammalian phosphatidylethanolamine binding proteins (PEBP; (Kardailsky et al. 1999; Kobayashi et al., 1999). The other members of the gene family include TWIN SISTER OF *FT* (*TSF*), TERMINAL FLOWER 1 (*TFL1*), CENTRORADIALIS (*ATC*), MOTHER OF *FT* AND *TFL1* (*MFT*), and BROTHER OF *FT* AND *TFL1* (*BFT*). *TSF* is a paralog of *FT* and also promotes flowering (Jang et al., 2009; Yamaguchi et al., 2005). *TFL1*, on the other hand, encodes a protein of similar sequence yet antagonistic function to *FT* (Kardailsky et al. 1999; Kobayashi et al., 1999), and a single amino acid change can convert *FT* into a functional *TFL1*-like molecule (Hanzawa et al., 2005). While *FT* and *TSF* promote flowering at meristems, *TFL1* maintains the indeterminate state of the meristem, effectively repressing flowering. Appreciating the antagonistic activities encoded by these two flowering genes has strong implications for understanding and manipulating plant architecture, as reviewed by McGarry and Ayre (2012).

Changes in day length, or photoperiod, have long been recognized to impact flowering among different plant species (Garner and Allard, 1920). The “external coincidence model”, the genetic basis of photoperiodic flowering, was proposed from research in Arabidopsis (Abe et al., 2005; Ayre and Turgeon, 2004; Corbesier et al., 2007; Turck et al., 2008) and is supported by research in tomato and rice (Kojima et al., 2002; Lifschitz et al., 2006; Tamaki et al., 2007). As a facultative long-day plant, Arabidopsis initiates reproductive development when grown in long days (16 hour photoperiod), but will also flower when grown for an extended time under a short 12 hour photoperiod. *CONSTANS* (*CO*) mRNA accumulates in leaves late in the day (Liu et al., 2008; Suarez-Lopez et al., 2001). In short days, *CO* mRNA accumulates after dusk but the encoded *CO* protein is degraded in the absence of light. In long days, the *CO* mRNA accumulates while plants are still illuminated, and light signaling complexes stabilize the *CO* protein (Jang et al., 2008). The *CO* protein is a transcription factor which turns on the expression of *FLOWERING LOCUS T*
FT in the companion cells of leaves (Abe et al., 2005; Wigge et al., 2005). The FT protein enters the phloem, moving from mature leaves to the meristematic regions of the plant, where it forms a heterodimer with the transcription factor FD (Abe et al., 2005). In the nuclei of apical cells, the FT/FD complex turns on the expression of two meristem identity genes, APETALA 1 (AP1) (Abe et al., 2005; Wigge et al., 2005) and LEAFY (LFY) (Schultz and Haughn, 1991; Weigel and Nilsson, 1995), and the activities of these gene products yield a flower (Fig. 2).

Figure 2. Coincidence model for a generic long-day plant. CONSTANS (CO) is expressed with a circadian rhythm, and begins accumulating late in the day. CO protein is stabilized in the light, but rapidly degraded in the dark. Under long-day conditions, when circadian expression of CO and light stabilization coincide (top left), CO protein accumulates to promote expression of FLOWERING LOCUS T (FT), encoding florigen. In short day conditions, CO expression and light stabilization do not coincide (bottom left), and CO protein does not accumulate to activate FT. FT protein is phloem mobile and migrates entirely through the symplasm (presumably) to reach the meristem (right) to interact with FD and promote flowering by activating APETALA1 (AP1).

The FT signal appears to be conserved among flowering plants (Kojima et al., 2002; Lifschitz et al., 2006; Mathieu et al., 2007). Indeed, FT orthologs from an array of monocots and eudicots, such as poplar (Populus spp.), tomato, citrus (Poncirus trifoliata L. Raf), and wheat (Triticum aestivum), have been expressed in heterologous species and induced early flowering (Bohlenius et al., 2006; Endo et al., 2005; Hsu et al., 2006; Lifschitz et al., 2006; Yan et al., 2006; Zeevaart, 2008). Furthermore, expression from FT orthologs over-rides the endogenous photoperiod of the host plant (Kojima et al., 2002).

COTTON IS A PERENNIAL, SHORT-DAY PLANT

Two allotetraploids (AADD), Gossypium hirsutum (Upland Cotton, ~90% of USA cultivation) and G. barbadense (Pima or Extra-Long Staple Cotton), are cultivated in the USA. Wild accessions have diverse morphologies, but 6000 years of independent domestication has led to convergent traits that allow these tropical, short-day photoperiodic perennials to be grown and harvested as compact, day-neutral annual crops (Lubbers and Chee 2009; Percy 2009; Wendel et al., 2009).
Perennials and annuals have fundamentally different life strategies: annuals focus end-of-season resources on reproduction to ensure the success of the next generation while perennials will compromise reproductive growth to ensure survival of the parent to the next season. *Gossypium* species experience repeated, yearly cycles of vegetative growth in long-day seasons with reproductive development triggered by short-day photoperiods. Despite its inherent perennial nature, cotton varieties domesticated for temperate climates have been bred for day-neutrality and are cultivated and harvested as an annual crop (Oosterhuis, 1990): Seed is planted each spring, plants flower early in their life cycle and bolls are harvested late in the season before cold temperatures terminate the crop. This management strategy is well-suited to highly mechanized production practices but is at odds with the plant’s natural growth habit and can complicate breeding and crop management, and reduce the quantity and quality of yields (Oosterhuis, 1990). In addition, flowering and fruit set in both ancestral and modern lines are not synchronous but continue throughout the season, encouraging producers to extend the growing season to maximize yield. But the highest quality fibers are from bolls that form at the first fruiting position of the first 10 fruiting branches, and poor quality fiber from later-forming bolls can discount value despite contributing to yield (Kerby *et al.*, 2010; Oosterhuis, 1990). Extending the growing season also increases producer costs for irrigation, fertilization, pesticides and herbicides (Jost *et al.*, 2006). Further still, both modern and ancestral lines continue vegetative growth after initiating reproductive growth. This perennial trait diverts resources away from fiber and seed production, and late season rain can complicate harvest by causing a flush of vegetative growth (Oosterhuis, 1990). To control growth habit, growth inhibitors are used during the growing season to make the crop pseudo-determinate and defoliants are used at the end of the season in preparation for mechanical harvest (Cothren and Oosterhuis, 2010; Jost *et al.*, 2006; Shurley *et al.*, 2004). These treatments further increase producer costs and also have negative environmental consequences (2009 Georgia Cotton Production Guide, http://www.ugacotton.com).

Because breeding has focused primarily on fiber yield and quality among domesticated, day-neutral lines, modern cultivated cotton suffers from restricted genetic diversity (Paterson *et al.*, 2004). This highly vulnerable gene pool is in fact attributed to several domestication bottlenecks. For instance, polyploid cottons arose from only two of eight extant diploid genomes, and only a small subset of wild genotypes was domesticated (Paterson *et al.*, 2004). Moreover, tetraploid genotypes were trafficked from their center of diversity in Mexico and central America to the USA, Australia, China and other countries (Paterson *et al.*, 2004). Ancestral accessions, however, including heirloom cultivars, landraces, natural *G. hirsutum* and *barbadense* isolates and their diploid progenitors, are a rich but generally untapped source of natural variation (Iqbal *et al.*, 2001) affecting fiber quality and yield, and resistance to biotic and abiotic stresses (Guo *et al.*, 2008; Robinson, 2007; Saha *et al.*, 2006).

One solution to counter genetic vulnerability is to introduce exotic germplasm (Myles *et al.*, 2011). Introgressing the diversity exhibited among ancestral accessions into elite lines has potential for crop improvement; however, ancestral lines are photoperiodic short-day plants and do not flower until the short days of fall. Domesticated day-neutral cultivars, on the other hand, flower early in their life cycle irrespective of day length, and have already reached cutout (*i.e.*, the point at which the resource demand of existing bolls ostensibly prevents new growth) by autumn. These differences in the onset of flowering complicate crossing and increase costs by
necessitating growth in greenhouses or tropical territories and limit breeding to annual cycles unless photoperiod is artificially shortened in specialized growth facilities (Paterson et al., 2004; Robinson, 2007; Saha et al., 2008). Furthermore, some accessions require additional environmental cues, such as specific temperatures, to initiate reproductive growth and the specific conditions required for flowering are difficult to replicate. Therefore, any practical introduction of exotic germplasm requires a mechanism to uncouple desired parent lines from photoperiodism.

In addition, the cotton research community is interested in generating nested association mapping populations, in which numerous ancestral lines are crossed to a single domesticated line, and progeny of these crosses are then backcrossed to the domesticated parent to obtain recombinant inbred lines (Bergelson and Roux, 2010; Kump et al., 2011; Yu et al., 2008). The goal is to develop a population of lines homozygous for stretches of ancestral DNA in an otherwise modern genome and associate traits to these segments. This effort is hampered by photoperiodism in the ancestral lines: either the populations need to be created under short-day conditions, or homozygous regions linked to photoperiodic QTLs will be lost from the population. The former will be demanding in time and resources, and the latter will compromise the value of the population. A mechanism to promote flowering and accelerate the life cycle of ancestral lines would alleviate these limitations.

**MANIPULATING FLOWERING TIME: A TRANSGENIC APPROACH**

Enhancing plant productivity is intimately linked with improving the time to flower. Trees are perennial plants that often experience an extended juvenile phase, sometimes years, before becoming competent to flower, and this delay poses a significant challenge for biotechnology and breeding programs. In aspen, for example, the onset of reproductive growth usually requires between 8 - 20 years. However, when the Arabidopsis floral meristem identity gene LEAFY was introduced in aspen, the transgenic plants flowered within months (Weigel and Nilsson, 1995). This was an excellent demonstration of how manipulating a heterologous gene could dramatically shorten generation time, a boon for breeding and trait introgression programs in crop species.

With the subsequent identification of FT as the mobile floral signal (Corbesier et al., 2007), this gene became a target for manipulating flowering time. Over-expression of an FT ortholog in transgenic poplar induced juvenile trees to produce inflorescences (Bohlenius et al., 2006) instead of solitary flowers (Weigel and Nilsson, 1995). Interestingly, functionally diverged paralogs FT1 and FT2 work in contrasting seasons to coordinate cycles of reproductive and vegetative growth in perennial poplar (Hsu et al., 2011). Thus, FT determines flowering time, even in an adaptive perennial with a duplicated genome (Hsu et al., 2011). Consequently, flowering time could be accelerated in plants amenable to transformation which held particular promise for biotechnological applications in species with long life cycles.

**VIRUS-INDUCED FLOWERING IN COTTON**

Generating transgenic cotton is a time-consuming labor that requires extensive tissue culture (Wilkins et al., 2004). A significant drawback to transformation of cotton is that, while cotton species can be infected with Agrobacterium tumefaciens (the standard method for introducing
foreign DNA into plant cells to generate stable transgenics, the subsequent regeneration from callus to fertile plants through tissue culture is very limiting (John and Stewart, 2010). Indeed, consistent regeneration has been observed only among Coker varieties (Trolinder and Goodin, 1987). Thus, ectopically expressing *FT* in transgenic ancestral and/or diploid photoperiodic lines of cotton may require herculean effort.

Because some plant species remain recalcitrant to transgenic approaches, virus-derived technologies offer a practical alternative. Virus-derived vectors are most commonly used for virus-induced gene silencing (VIGS) (Robertson, 2004), which in cotton, has particular promise because the major cultivated lines are allotetraploids: VIGS would be expected to silence both homoeologs (unless the silencing sequence was specifically designed not to), whereas loci disrupted by mutagenesis would likely be complemented by the homoeolog. Both *Cot**ton leaf crumple virus* (*CLCrV*) (Idris et al., 2010; Tuttle et al., 2008) and *Tobacco rattle virus* (*Gao et al., 2011*) have been adapted for VIGS in cotton.

*CLCrV* is a whitefly-(*Bemisia tabaci*) transmitted *Begomovirus* (family Geminiviridae) endemic to the southwestern United States and northwestern Mexico with benign infection symptoms (Idris and Brown, 2004). In the disarmed *CLCrV* (*dCLCrV*) system, a multiple cloning site replaces sequences between the start and stop codons of the gene encoding the coat protein (Tuttle et al., 2008). Deleting the coat protein gene sequence disarms the vector since the coat protein is required for whitefly transmission (Azzam et al., 1994; Briddon et al., 1989) and whiteflies are the only natural vector for transmission. In addition, the virus is not transferred through the pollen or egg (Mink, 1993; Sudarshana et al., 1998) and seeds are thus free of virus. Tuttle and colleagues (2008) cloned up to 500 nt of sequence antisense to the *G. hirsutum* magnesium chelatase subunit 1 (*Chl1*) or phytoene desaturase (*PDS*) gene into *dCLCrV* and delivered these by biolistic bombardment to cotton seedlings. Infected plants demonstrated systemic and sustained silencing of *Chl1* or *PDS*, clearly visualized as sectors of chlorotic tissues (Tuttle et al., 2008). Virus-based vectors can also be used for gain-of-function analysis in cotton; however, geminiviruses such as *dCLCrV* have size constraints, and sequences larger than the deleted coat-protein gene (~800 nucleotides) tend to be quickly lost (Timmermans et al., 1994). Notwithstanding, *dCLCrV* was engineered to express the green fluorescent protein marker to visualize viral movement through the plant vasculature (Idris et al., 2010; Tuttle et al., 2008).

“Virus-induced flowering” (VIF) is an emerging tool to promote transient flowering and obviates the time and labor of generating stable transformants (Mccarry and Ayre, 2012; Yamagishi et al., 2011). Recently, the arabidopsis *FT* gene was cloned into *dCLCrV* and used to infect cotton (McGarry and Ayre, 2012), and into *Apple latent spherical virus* (*ALSV*) and used to infect apple (Yamagishi et al., 2011) and soybean (Yamagishi and Yoshikawa 2010) varieties. When *FT* was expressed from *ALSV* in apple, it reduced the juvenile phase such that plants flowered within months after infection instead of the usual span of several years to reach reproductive maturity (Yamagishi et al., 2011). When the same virus was used to infect indeterminate varieties of soybean, VIF yielded early flowering and reduced vegetative growth among indeterminate short-day soybean plants (Yamagishi and Yoshikawa 2010). Because the function of *FT* is demonstrated to be highly conserved across angiosperms, VIF does not require isolating florigen from non-model plants. Since almost all
viruses and the FT protein move through the phloem vasculature (Corbesier et al., 2007), VIF further amplifies the florigenic signal from infected regions of the plant to meristems where the transition from vegetative to reproductive growth occurs (Corbesier et al., 2007).

We engineered dCLCrV to express the arabidopsis FT gene, and used this to infect cotton varieties (McGarry and Ayre, 2012). The day-neutral cultivar DeltaPine 61 (DP61) and photoperiodic line Tex 701 (USDA GRIN accessions PI 607174 and PI 165329, respectively) were chosen for initial experiments because they were previously used to map QTLs related to photoperiodic flowering (Guo et al., 2008). AtFT cDNA was cloned downstream of the viral coat protein promoter in dCLCrV, generating dCLCrV::FT, and, along with control constructs dCLCrV (i.e., empty-vector control) and a vector containing antisense sequence to the G. hirsutum magnesium chelatase subunit 1 gene (dCLCrV::αChl1; Tuttle et al., 2008), were used to inoculate DP61 and Tex 701 seedlings by biolistic bombardment. Although not transmitted plant to plant, dCLCrV can move throughout the whole plant, and systemic Chl1 silencing was observed as chlorosis 14 days after bombardment and was still active in 90 d old plants. Plants bombarded with dCLCrV (i.e., no insert) showed mild symptomology as expected, demonstrating that dCLCrV delivering foreign DNA does not overtly impact plant growth. Our bombardment protocol was optimized to achieve 80% infection efficiency.

When grown in a greenhouse with supplemental light (16/8 hr day/night, non-inductive long-day conditions), the photoperiodic dCLCrV::FT-infected Tex 701 plants transitioned to reproductive growth as early as 33 days post-germination (dpg) at node 5 and the first flowers reached anthesis at 71 dpg, showing successful VIF (Fig. 3).

This compares favorably to uninfected, day-neutral DP61 plants which produced fruiting branches at node 5 with flowers reaching anthesis by 64 dpg. None of the uninfected Tex 701, nor dCLCrV- or dCLCrV::αChl1-infected Tex 701 flowered under these non-inductive conditions.

**Figure 3.** “Virus-induced flowering” (VIF) in photoperiodic cotton accession TX701. Both plants were grown under long-day conditions (16 hr light) in a greenhouse with supplemental lighting. The plant on the left was infected with a disarmed cotton leaf crumple virus carrying FT from arabidopsis in place of the coat protein gene, and arrows point to a few of the many reproductive structures on the plant. The plant on the right was not infected with an FT-carrying virus, and is complete vegetative.
FT-induced Tex 701 flowers were used as pollen donors in crosses with uninfected DP61 (McGarry and Ayre, 2012). The cross-pollinated flowers formed healthy bolls with good seed yields (21.3 ± 11.0 seeds per boll, n = 20 bolls compared to 30 ± 3.9 seeds per boll of self-pollinated DP61 plants, n = 9 bolls). The F1 generation was scored for three traits: leaf shape, node of first fruiting branch, and presence/absence of floral spots. All 46 F1 seedlings had leaf shape intermediate between the extreme lobing or “okra leaf” phenotype of the Tex 701 and normal cotton leaves of DP61. NFB among the F1 (14.7 ± 2.2, n = 46) was intermediate between day-neutral DP61 (5.1 ± 0.9, n = 10) and photoperiodic Tex 701 (no floral buds detected by node 24, n = 8). Finally, F1 flowers had floral spots characteristic of the Tex 701 pollen donor rather than the absence of spots characteristic of the DP61 pollen recipient. Importantly, the F1 did not harbor viral sequences when screened by PCR. Thus, VIF is an effective technology for facilitating crosses between ancestral and modern accessions, and the progeny of these crosses do not carry viral DNA and should not be derisively labeled as “genetically modified organisms”.

We demonstrate that VIF can convert vegetative meristems to floral meristems in cotton. Occasionally, dCLCrV::FT-infected Tex 701 fruiting branches ceased vegetative growth and terminated in floral clusters (Fig. 4).

We interpret these morphologies as ILMs that have transitioned to floral identity prior to forming a new sympodial unit (i.e., node, internode, subtending leaf and axillary bud with a new ILM), or to describe this phenomenon in terms of the Prusinkiewicz model (Prusinkiewicz et al., 2007), veg in the ILM decreased rapidly such that the IAM and ILM transitioned to a determinate floral fate at roughly the same time. Furthermore, we found that dCLCrV::FT infection phenocopied the effect of inductive short days on leaf growth in Tex 701. Leaves from fruiting branches of Tex 701 assume a determinate lanceolate shape instead of the characteristic lobing of main-stem

**Figure 4.** VIF in wild accession TX701 frequently caused fruiting branches to terminate in a floral cluster rather than continue sympodial reiterations. (A) A schematic of canonical flowering in cotton is shown. White circles are determinate floral buds and white arrows are the terminal axillary buds forming the next sympodial reiteration of the fruiting branch; black arrows are the monopodial main stem apical bud. (B, C, D) Schematics and pictures of fruiting branches that terminated with a floral structure or floral cluster rather than continuing sympodial growth. (B) Floral structure directly on the main stem in lieu of a fruiting branch. (C) Two floral buds in the same bract whorl. (D) A cluster of three independent flowers (arrows). In (C) and (D) the fruiting branch and petiole of the subtending leaf are labeled.
leaves. Tex 701 plants infected with dCLCrV::FT similarly demonstrated this determinate leaf shape transition along fruiting branches whereas dCLCrV-infected or untransfected Tex 701 grown under long days maintained the heavily-lobed "okra" leaf shape (McGarry and Ayre, 2012). In addition to these determinate features, our work with VIF in day-neutral cotton accession DeltaPine 61 showed that \( FT \) promoted determinate growth distinct from flowering. While dCLCrV::FT-infected DP61 flowered slightly earlier than uninfected controls (NFB 3 ± 0, \( n = 3 \) vs 5.1 ± 0.9, \( n = 10 \), respectively), dCLCrV::FT-infected DP61 plants exhibited fewer and shorter sympodial units per fruiting branch than uninfected or mock-inoculated controls (McGarry and Ayre, 2012). Our findings suggest that over-expression of \( FT \) accelerates determinate growth to yield a more compact plant architecture.

The maize (\( Zea mays \)) \( FT \) ortholog, \( ZCN8 \), also exhibits pleiotropic functions in plant growth (Danilevskaya et al., 2011). Down-regulating \( ZCN8 \) expression with an artificial microRNA not only delayed the floral transition, but the same transgenic plants had larger leaves and stems and more tassels (Danilevskaya et al., 2011). Conversely, over-expression of \( SFT \) and \( FT \) in day-neutral tomato and tobacco caused early flowering, and plants displayed fewer leaflets per compound leaf, shorter internodes, and thinner stems (Lifschitz et al., 2006). Taken together, these data extend the function of \( FT \) from "flowering gene" to more generally promoting the transition from indeterminate (vegetative) to determinate (floral) plant growth.

**FUTURE CONSIDERATIONS**

Although VIF provides valuable results, we cannot control the timing, duration or strength of the floral signal and the dCLCrV vector is not completely without symptomology. An inducible system for controlling \( veg \) levels would permit more meaningful analysis of the potential of manipulating plant architecture to increase yields and synchronize the crop. Alternatively, identifying the \( GhFT \) orthologs and manipulating the expression of the native genes may also reduce pleiotropic effects.

In plants with significantly larger genomes, the PEBP family is substantially expanded from that of Arabidopsis, and the functions of the gene family members are more complex. The \( FT \) family in pea and other legumes has been classified into three subclades, with members demonstrating differences in expression patterns and tissue specificity, timing of flowering, and response to photoperiod (Hecht et al., 2011). Indeed, the cooperative activities of several different pea \( FT \) members are required for floral induction (Hecht et al., 2011). In the biennial \( Beta vulgaris \) (beet), flowering time is controlled by two \( FT \) paralogs: one is essential for flowering while the other is a repressor of flowering necessary for the vernalization response (Pin et al., 2010). This finding was in contrast to work in sunflower (\( Helianthus annus \)) in which a frame-shift mutation in \( HaFT1 \), an allele that experienced selection during early domestication, delays flowering by interfering with the action of \( HaFT4 \) (Blackman et al., 2010). More recently it was shown that divergent \( FT \) paralogs in poplar, \( FT1 \) and \( FT2 \), determined the annual cycles of reproductive and vegetative growth in this woody perennial (Hsu et al., 2011). In conclusion, control of flowering time is of critical importance to plants, and the strategies employed by annuals and perennials may invoke different regulatory points. The redundancy observed among
the PEBP gene family raises questions about their functional diversification. Further focus on
the identification and functional characterizations of the cotton PEBP family may elucidate
aspects of indeterminate and determinate growth regulation in perennial cotton. Such insight
could prove invaluable for enhancing cotton productivity and improving crop management.

SUMMARY

Manipulating expression of FT in cotton holds promise for modifying cotton plant architec-
ture by reducing indeterminate and vegetative growth and promoting flowering and determinate
plant growth. These alterations in growth habit may have tangible consequences for cotton
production and management. Moreover, we demonstrate the utility of VIF, virus-induced flow-
ering, as a tool for cotton breeding to facilitate the introgression of desirable germplasm from
ancestral cotton accessions into domesticated lines without genetically modifying the germline.

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