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 'zigzag' 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 IAMs form a flower and growth continues from the ILMs (sympodial inflorescence; tomato, *Solanum lycopersicon*, cotton) (Prusinkiewicz *et al.*, 2007) (Fig. 1).

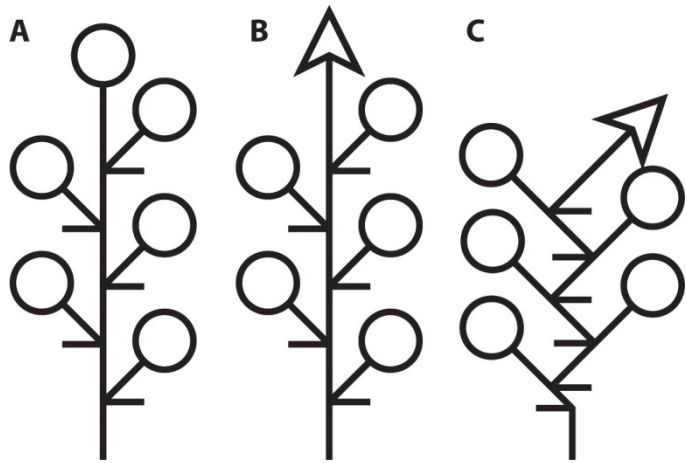

Figure 1. Simplified representation of (A) a panicle, (B) a raceme, and (C) a cyme, after (Prusinkiewicz *et al.* 2007). In arrowheads, *vegetativeness* (*veg*) is above a threshold, and meristem retains an indeterminate identity (e.g. continued vegetative growth); in circles *veg* has dropped below 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).

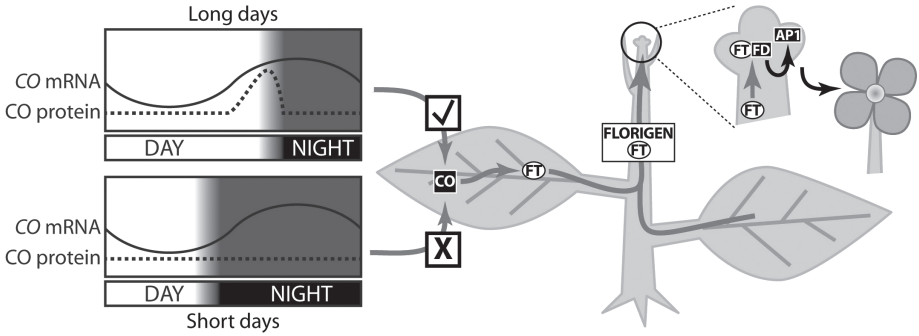

Figure 2. Coincidence model for a generic long-day plant. *CONSTANS* (*CO*) is expressed with a circadian rhythm, and begins accumulating late in the day. CO protein is stabilized in the light, but rapidly degraded in the dark. Under long-day conditions, when circadian expression of *CO* and light stabilization 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 *Cotton 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 (McGarry 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 F_1 generation was scored for three traits: leaf shape, node of first fruiting branch, and presence/absence of floral spots. All 46 F_1 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 F_1 (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, F_1 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 F_1 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

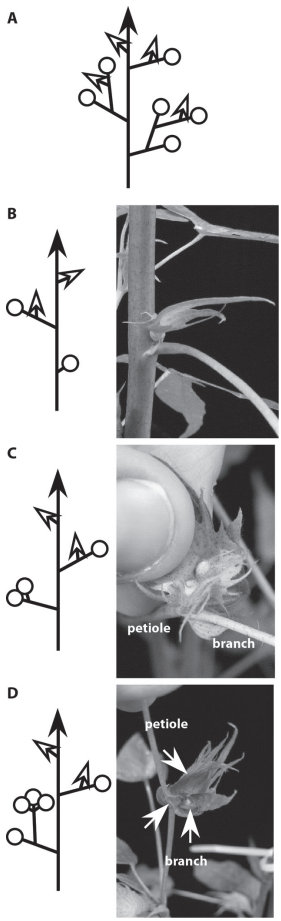

Figure 4. VIF in wild accession TX701 frequently caused fruiting branches to terminate in a floral cluster rather than continue sympodial reiterations. (A) A schematic of canonical flowering in cotton is shown. White circles are determinate floral buds and white arrows are the terminal axillary buds 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 frameshift 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 architecture 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 flowering, 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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