Plant Signaling & Behavior 8:4, e23602; April 2013; © 2013 Landes Bioscience

Overexpression of *FT* in cotton affects architecture but not floral organogenesis

Roisin C. McGarry, Sarah Prewitt and Brian G. Ayre*

Department of Biological Sciences; University of North Texas; Denton, TX USA

Keywords: balance model, cotton, *Cotton leaf crumple virus*, flowering, FLOWERING LOCUS T, FT, *Gossypium hirsutum*

Flowering marks the change from indeterminate to determinate plant growth, and this developmental transition involves the activity of the *Arabidopsis FLOWERING LOCUS T* (*FT*) gene product and its orthologs. We demonstrated that when *FT* is ectopically expressed from a viral vector in cotton, a process referred to as virus induced flowering (VIF), it uncouples flowering from photoperiodic regulation and promotes determinate growth in aerial organs. The accelerated switch to determinate growth affected cotton floral buds and sympodial growth, but did not disrupt floral organogenesis. These results can be interpreted in the context of the balance model, which argues that the balance of indeterminate and determinate growth is influenced by the relative abundance of indeterminate and determinate factors in the growing apices. These results emphasize the expanding role of FT in affecting general determinate growth.

Cotton (*Gossypium hirsutum*) is naturally a photoperiodic perennial that does not flower until the shorter days of late summer or fall. Domesticated varieties, on the other hand, are compact, day-neutral plants, and, despite persistent perennial growth habits, are managed as annual row-crops. Cultivated cotton suffers from a restricted genetic base which increases the vulnerability of the crop and limits yields.[1] Wild varieties and land races are rich stores of exotic germplasm that can introduce tolerance/resistance to diverse abiotic and biotic stresses to inbred cultivated varieties, but incompatible photoperiods restrict breeding opportunities.[2]

Virus induced flowering (VIF) was developed to uncouple flowering from photoperiod in ancestral cotton and create a unique breeding opportunity with cultivated cotton.[3] The *Arabidopsis FT* cDNA, which encodes florigen,[4,5] was expressed from a disarmed *Cotton leaf crumple virus* (dCLCrV)[6] and delivered to wild and modern cotton accessions, causing both to shift to a more determinate growth habit in aerial organs.[3] Ectopic expression of *FT* in photoperiodic, ancestral accession Texas 701 (TX701) resulted in early flowering when plants were grown under non-inductive long days.[3] In addition, VIF TX701 had deeply lobed leaves transition to a simpler, lanceolate shape coincident with flowering; and several fruiting branches terminated in a floral cluster instead of continuing sympodial growth, suggesting that both apical and lateral buds transition to floral identity prior to forming a new sympodial unit.

These data support the balance model proposed in tomato which suggests that the ratio of the FT ortholog SINGLE FLOWER TRUSS (SFT) to its antagonist SELF-PRUNING (SP) specifies determinate or indeterminate growth in an organ-specific and age-related manner.[7-9] *SP* is expressed in growing tips,[10] and when SP levels are high, as in young plants, the meristem maintains indeterminate growth.[8] Like FT, SFT is phloem-mobile: the gene is expressed in mature tomato leaves and SFT protein moves into tissues where the gene is also expressed,[7,8,11] thus amplifying the signal that reaches the meristem. When the relative contribution of SFT in the meristem exceeds that of SP, determinate growth prevails. This model implies that more florigen is produced as plants mature.

Consistent with this hypothesis, the earliest floral buds forming on TX701 plants infected with dCLCrV::FT were often aberrant in appearance. Typically, a floral bud terminates a sympodial unit and is surrounded by three bracts, forming a characteristic pyramidal enclosure called a square (**Fig. 1A**). Some of these atypical floral buds had prominent leaf-like bracts (**Fig. 1B and C**) or irregular numbers of bracts (**Fig. 1D**). On several plants, a single flower, flanked by a single prominent bract, arose directly from the main stem in place of the fruiting branch (**Fig. 1E and F**). Some squares harbored two adjacent flowers (**Fig. 1G**). In other instances, apical and axillary buds simultaneously converted to floral fates (**Fig. 1H**). Most of these aberrant floral buds failed to develop further (**Fig. 1E**); however, floral buds terminating subsequent sympodial units or from higher-node fruiting branches of VIF TX701 plants developed normally and had more characteristic appearances (**Fig. 1I**). Taken together, these phenotypes can be interpreted as the result of the precocious transition to determinate floral structures mediated by high levels of FT relative to indeterminate factors, and as the plant aged, more uniform and consistent determinate growth was observed.

*Correspondence to: Brian G. Ayre; Email: bgayre@unt.edu
Submitted: 11/27/12; Revised: 01/11/13; Accepted: 01/14/13
http://dx.doi.org/10.4161/psb.23602
Citation: McGarry RC, Prewitt S, Ayre BG. Overexpression of *FT* in cotton affects architecture but not floral organogenesis. Plant Signal Behav 2013; 8: e23602

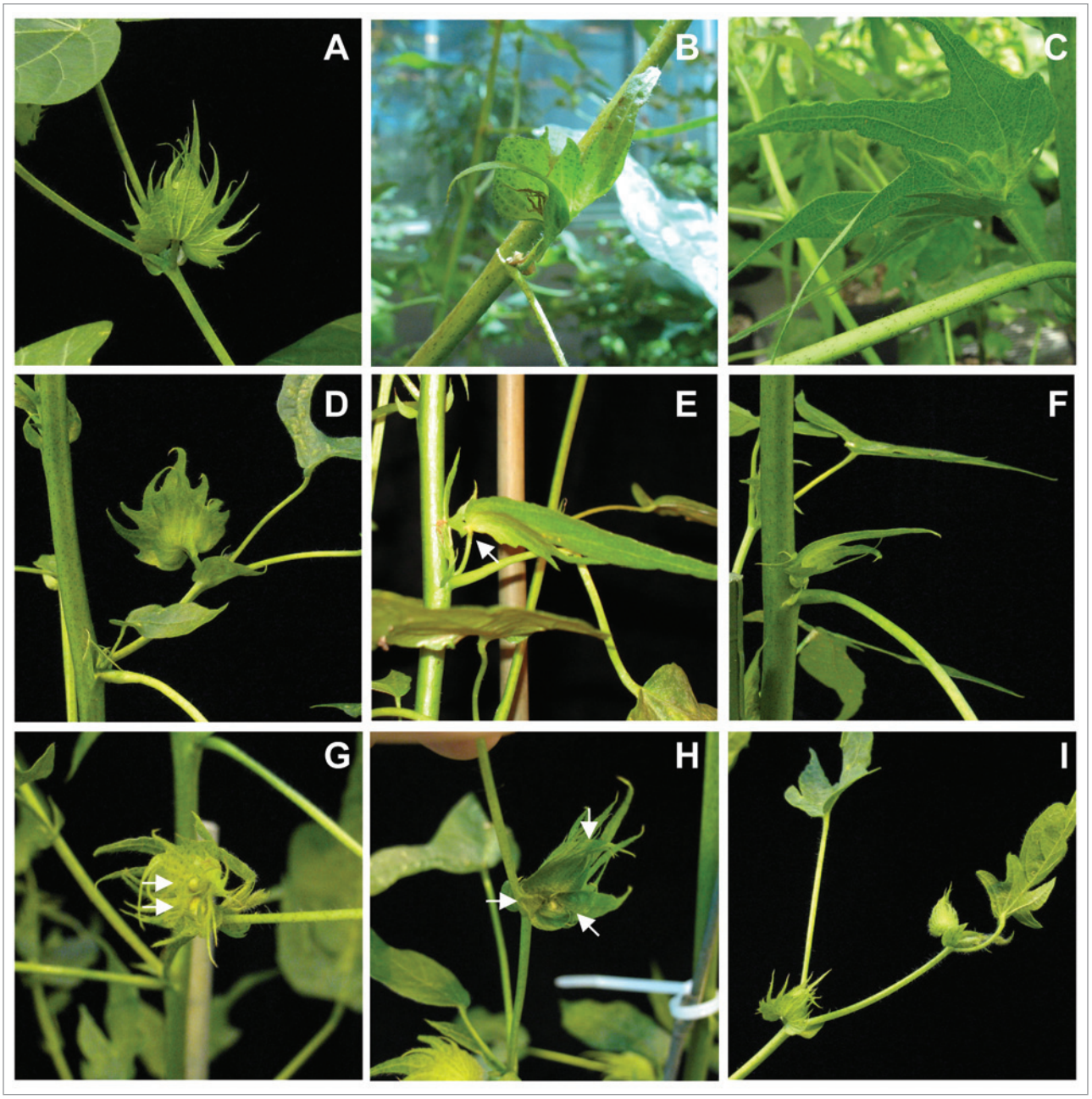

Figure 1. Early-forming floral buds on VIF TX701 plants were often atypical in appearance. (**A**) A wild type floral bud (or "square") from a domesticated accession, Delta Pine 61, has three bracts surrounding the developing flower. Sympodial growth continues from a subtending axillary meristem. (**B and C**) A reduced number of very prominent leaf-like bracts surround the floral buds in VIF TX701 plants. (**D**) The floral bud is encased by only two bracts of similar size. (**E and F**) Floral buds arise directly from the main stem instead of from a fruiting branch. Both floral buds have fewer and more leaf-like bracts than expected. In (**E**), the floral bud is exposed and senesces. (**G**) Two floral buds (arrows) develop within a single square, and this event terminates sympodial growth. (**H**) A cluster of three floral buds (arrows) terminate the sympodial unit. (**I**) Characteristic sympodial growth and floral bud formation resumes on later-forming fruiting branches of VIF TX701 plants.

The abundance of floral buds reaching anthesis and setting fruit varied among dCLCrV::FT-infected TX701 plants (**Fig. 2**, compare panels **A** and **B**). VIF TX701 flowers were used as pollen donors in successful crosses with domesticated accession Delta Pine 61, but despite the fertility of the pollen, few self-pollinated VIF TX701 flowers resulted in mature bolls.[3] While shedding of cotton buds, flowers, and bolls is not uncommon and may be affected by environmental stresses,[12] we questioned whether flower abscission resulted from *FT* overexpression.

To test if VIF affected cotton flower development and if morphological defects could be correlated with low yields among self-pollinated flowers, we examined floral bud morphology from FT-induced ancestral TX701 plants and from an untransfected day-neutral accession, Acala Maxxa. To increase

the probability of identifying differences between VIF TX701 and Acala Maxxa, "aberrant" dCLCrV::FT-induced floral buds, including those enclosed by unusual leaf-like bracts, buds positioned on the main stem instead of a fruiting branch, and buds arresting subsequent sympodial growth, were examined along with buds of more typical appearance. Floral buds were harvested, immediately frozen with liquid nitrogen, and lyophilized. When freeze-dried tissues were sectioned and visualized using scanning electron microscopy, floral buds from VIF TX701 plants were indistinguishable from the untransfected controls (**Fig. 3A–F**). No defects were observed in floral organ identity or organization of floral whorls in "aberrant" or characteristic buds from VIF plants compared with untreated Acala Maxxa. These findings suggest that VIF cotton flowers develop normally.

While the approach used to observe floral bud development precluded further observation in planta, the data suggests that ectopic *FT* expression does not adversely affect flower organ development. This finding is in contrast with reports from other species experiencing high levels of florigen. Transgenic poplar ectopically expressing *AtFT* from a heat-shock inducible promoter yielded catkins with large, leaf-like bracts; pistillate flowers on male catkins or on male plants; bisexual flowers; single staminate flowers borne in leaf axils; and pronounced bud abscission.[13] When *AtFT* was expressed from *Apple latent spherical virus* (*ALSV*) in infected apple seedlings, 50% of induced flowers lacked a pistil.[14] It is important to consider the age-related impact of these reports. That is, poplar and apple experience juvenile phases extending over years, and ectopic expression of *FT* accelerated the transition to reproductive growth within months after germination with the earliest flowers often aberrant in appearance. Perhaps flowers developing later in these transgenic poplar and VIF apples would assume more characteristic development as *FT* levels balanced throughout the plants.

In summary, VIF uncouples flowering from environmental regulation in ancestral cotton varieties. Early-forming flowers were atypical on VIF TX701 plants, with unusual bracts and disrupted sympodial growth. However, characteristic flowers and sympodial growth resumed later in VIF TX701, underscoring the age-related aspects of the balance model. It is intriguing to consider the events occurring subsequent to FT activity in forming such atypical flowers, particularly those arising from the non-meristematic main stem, and further expression analyses are warranted. Collectively, these findings suggest that judicious manipulation of *FT* and related genes holds promise for enhanced cotton production.

Figure 2. The abundance of floral buds and bolls vary among VIF TX701. (**A**) A VIF TX701 plant produced a single floral bud (arrow) which was used in successful crosses,[3] but the flower did not mature into a boll. (**B**) A VIF TX701 plant produced a flush of floral buds, many of which self-pollinated to yield mature bolls (arrows). Floral buds forming from earlier sympodial branches were removed for SEM analyses. Pots are the same size in (**A**) and (**B**).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This research is supported by Cotton Inc. Cooperative Agreement No. 10-768 and BARD Research Project US-4535-12 awarded to B.G.A.

References

1. Paterson AH, Boman RK, Brown SM, Chee PW, Gannaway JR, Gingle AR, et al. Reducing the genetic vulnerability of cotton. Crop Sci 2004; 44:1900-1; http://dx.doi.org/10.2135/cropsci2004.1900.
2. Guo YF, McCarty JC, Jenkins JN, Saha S. QTLs for node of first fruiting branch in a cross of an upland cotton, *Gossypium hirsutum* L., cultivar with primitive accession Texas 701. Euphytica 2008; 163:113-22; http://dx.doi.org/10.1007/s10681-007-9613-1.
3. McGarry RC, Ayre BG. Geminivirus-mediated delivery of florigen promotes determinate growth in aerial organs and uncouples flowering from photoperiod in cotton. PLoS ONE 2012; 7:e36746; PMID:22615805; http://dx.doi.org/10.1371/journal.pone.0036746.
4. Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T. A pair of related genes with antagonistic roles in mediating flowering signals. Science 1999; 286:1960-2; PMID:10583960; http://dx.doi.org/10.1126/science.286.5446.1960.
5. Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, et al. Activation tagging of the floral inducer FT. Science 1999; 286:1962-5; PMID:10583961; http://dx.doi.org/10.1126/science.286.5446.1962.
6. Tuttle JR, Idris AM, Brown JK, Haigler CH, Robertson D. Geminivirus-mediated gene silencing from *Cotton leaf crumple virus* is enhanced by low temperature in cotton. Plant Physiol 2008; 148:41-50; PMID:18621976; http://dx.doi.org/10.1104/pp.108.123869.
7. Lifschitz E, Eviatar T, Rozman A, Shalit A, Goldshmidt A, Amsellem Z, et al. The tomato *FT* ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. Proc Natl Acad Sci USA 2006; 103:6398-403; PMID:16606827; http://dx.doi.org/10.1073/pnas.0601620103.
8. Shalit A, Rozman A, Goldshmidt A, Alvarez JP, Bowman JL, Eshed Y, et al. The flowering hormone florigen functions as a general systemic regulator of growth and termination. Proc Natl Acad Sci USA 2009; 106:8392-7; PMID:19416824; http://dx.doi.org/10.1073/pnas.0810810106.
9. McGarry RC, Ayre BG. Manipulating plant architecture with members of the CETS gene family. Plant Sci 2012; 188-189:71-81; PMID:22525246; http://dx.doi.org/10.1016/j.plantsci.2012.03.002.
10. Pnueli L, Carmel-Goren L, Hareven D, Gutfinger T, Alvarez J, Ganal M, et al. The *SELF-PRUNING* gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of *CEN* and *TFL1*. Development 1998; 125:1979-89; PMID:9570763.
11. Navarro C, Abelenda JA, Cruz-Oró E, Cuéllar CA, Tamaki S, Silva J, et al. Control of flowering and storage organ formation in potato by FLOWERING LOCUS T. Nature 2011; 478:119-22; PMID:21947007; http://dx.doi.org/10.1038/nature10431.
12. Ritchie GL, Bednarz CW, Jost PH, Brown SM. Cotton Growth and Development. University of Georgia Cooperative Extension Service Bulletin 2004; 1252:1-14.

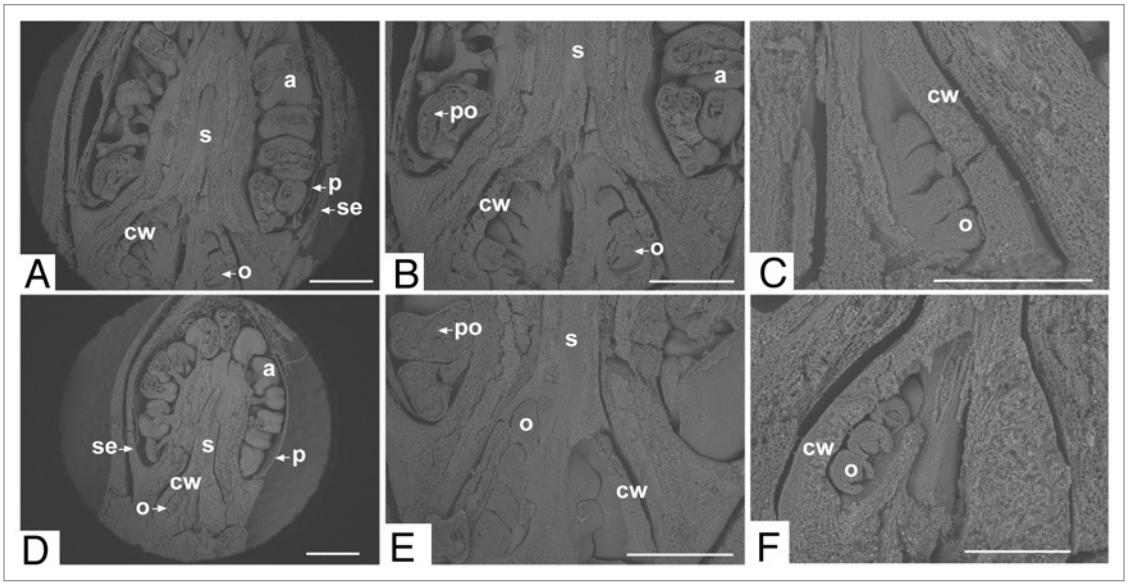

Figure 3. VIF does not affect flower formation. (**A–C**) Longitudinal sections through lyophilized floral buds isolated from the domesticated accession Acala Maxxa. (**B**) is a higher magnification of (**A**). (**D–F**) Longitudinal sections through lyophilized VIF TX701 floral buds. (**F**) is a higher magnification of (**D**). Scale bars in (**A, B, D and E**) are 1 mm and in (**C and F**) are 500 μm. Abbreviations: a, androecium; cw, carpel wall; o, ovules; p, petal; po, pollen; s, style; se, sepal.

13. Zhang HL, Harry DE, Ma C, Yuceer C, Hsu CY, Vikram V, et al. Precocious flowering in trees: the *FLOWERING LOCUS T* gene as a research and breeding tool in *Populus*. J Exp Bot 2010; 61:2549-60; PMID:20406786; http://dx.doi.org/10.1093/jxb/erq092.
14. Yamagishi N, Sasaki S, Yamagata K, Komori S, Nagase M, Wada M, et al. Promotion of flowering and reduction of a generation time in apple seedlings by ectopical expression of the *Arabidopsis thaliana FT* gene using the *Apple latent spherical virus* vector. Plant Mol Biol 2011; 75:193-204; PMID:21132560; http://dx.doi.org/10.1007/s11103-010-9718-0.