Virus-mediated Strategies for Transient Gene Expression and Silencing in Cotton

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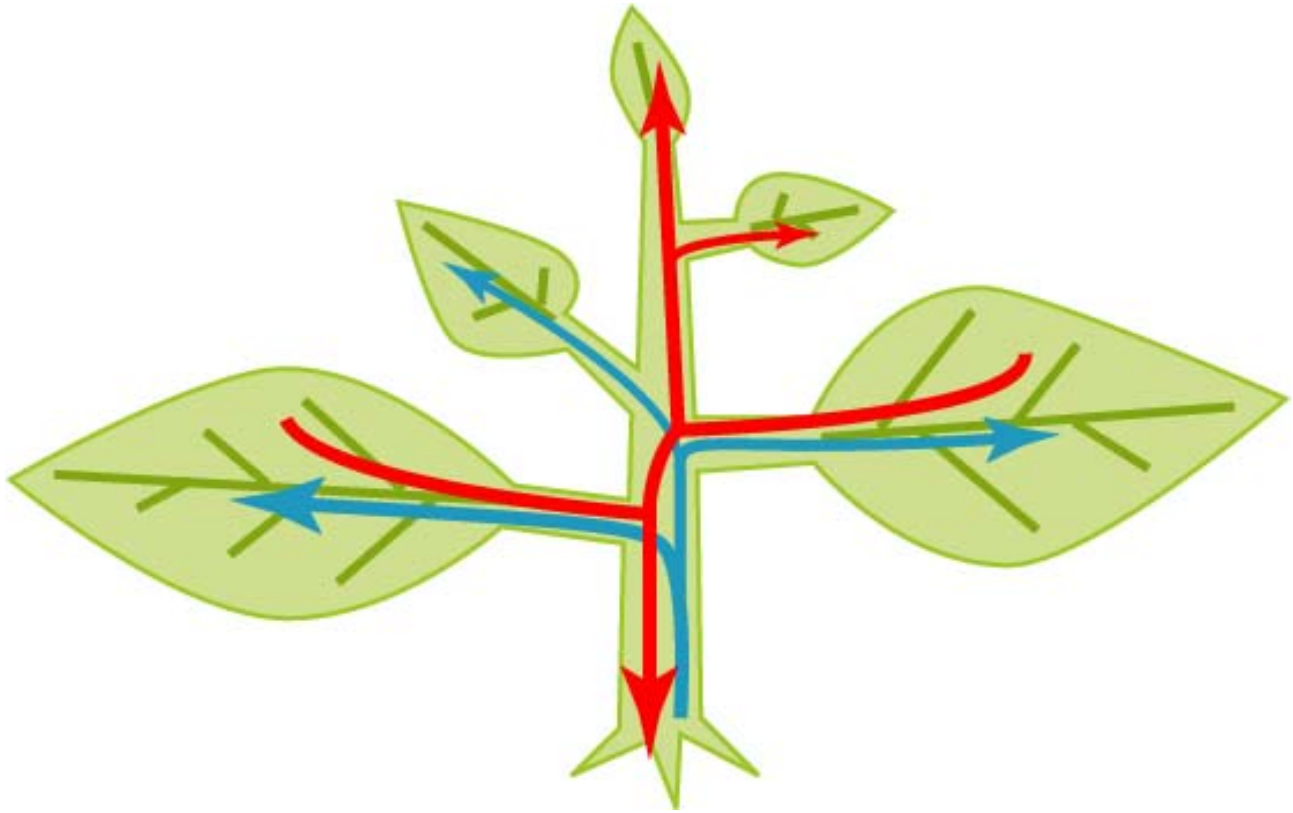

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Trends in Plant Science

Phloem-mobile signals, flowers and breeding

Cell PRESS

Overview

Virus mediated gene delivery and silencing is suitable for high-throughput analysis of gene function in systems recalcitrant to stable transformation, and also has immediate applications in biotechnology

A. Two virus systems are available for VIGS in cotton:
   i. Tuttle et al (2008): Cotton Leaf Crumple Virus (Plant Physiol. 148: 41-50)
   ii. Gao et al (2011): Tobacco Rattle Virus (Plant J. 66: 293-308)

B. Both can also be used for gene delivery:
   i. Virus Induced Flowering with Florigen (FT gene)
   ii. Transient protoplast assay and GFP

Dissecting Functions of *KATANIN* and *WRINKLED1* in Cotton Fiber Development by Virus-Induced Gene Silencing[1[C][W][OA]]

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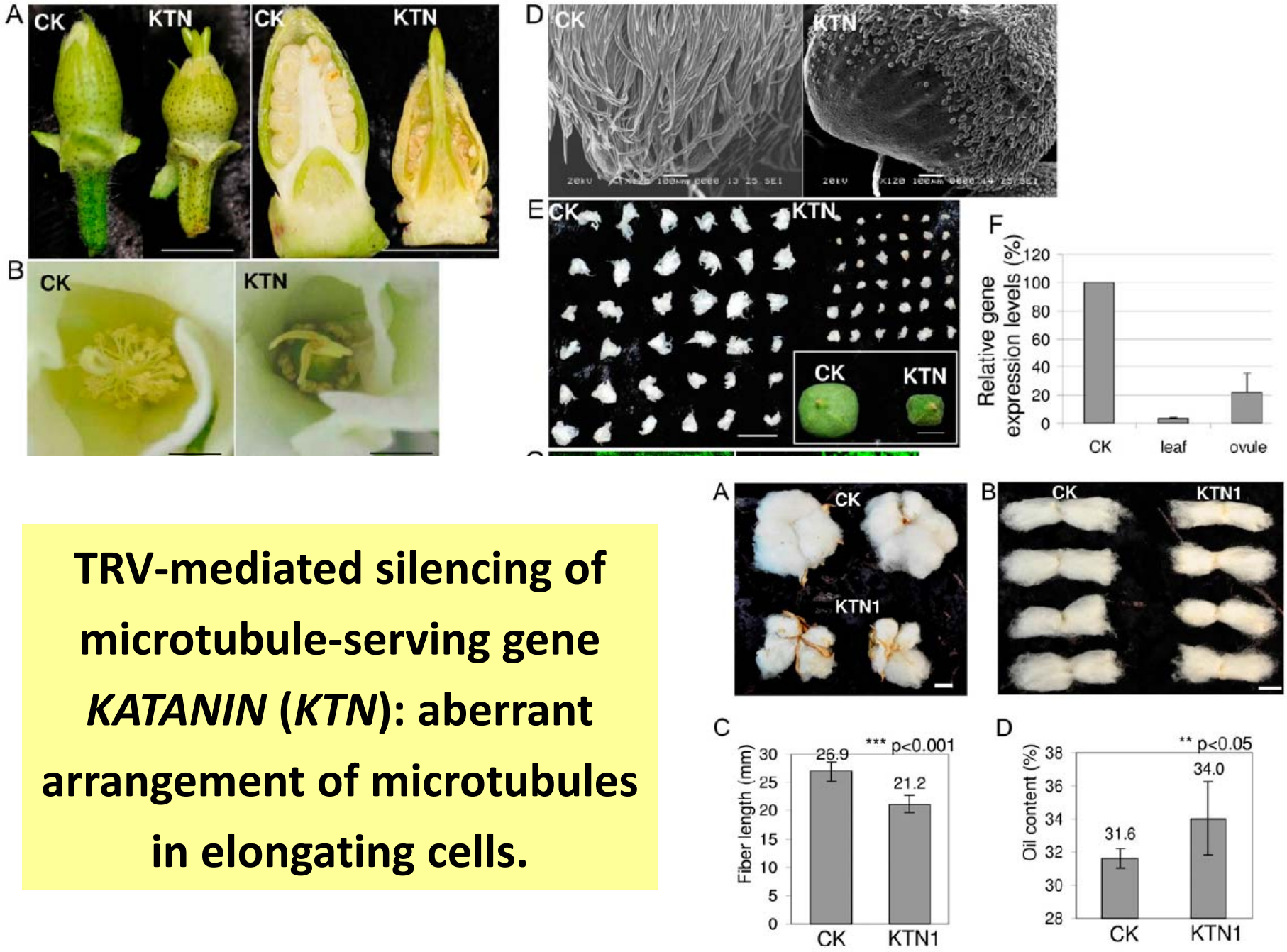
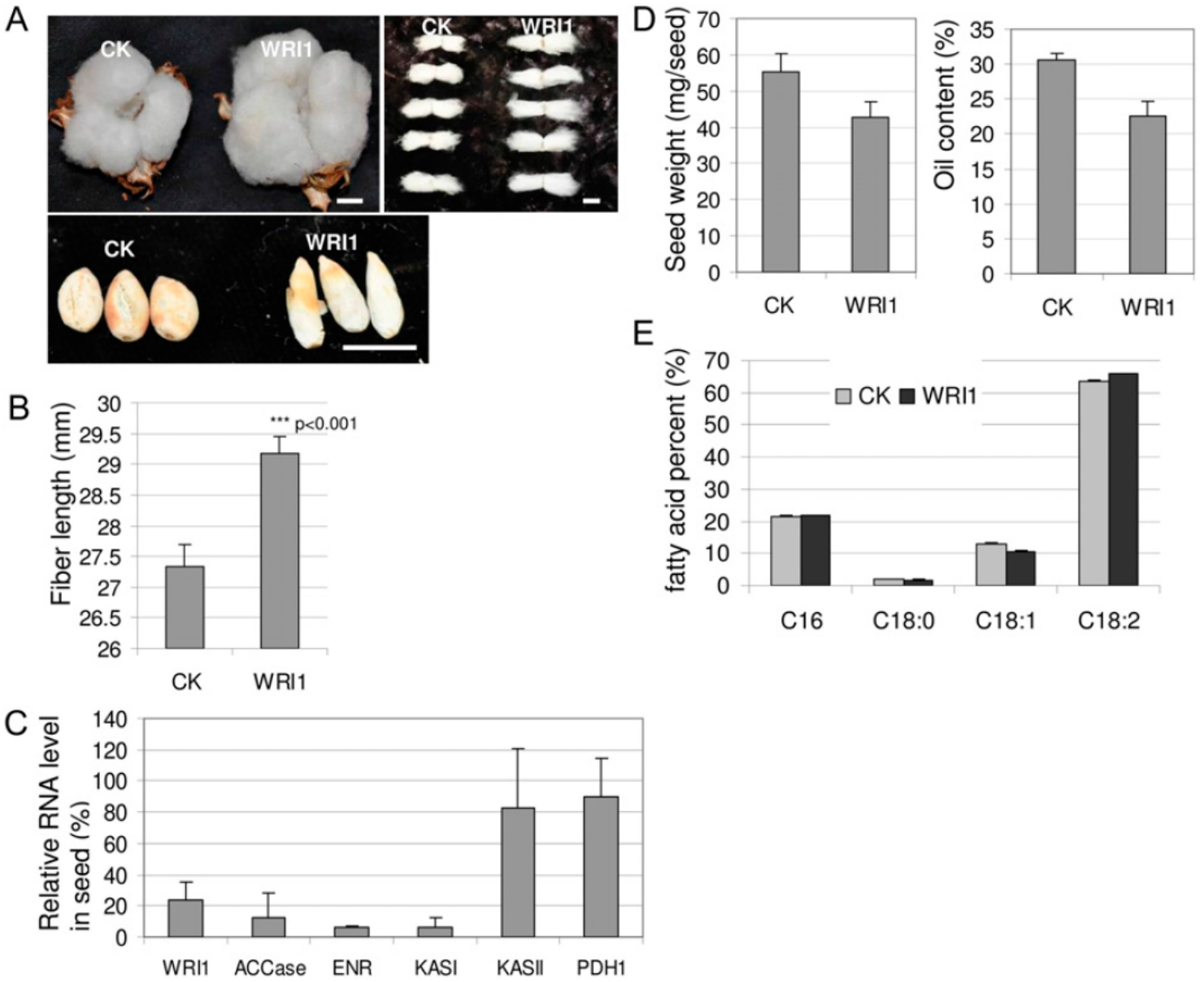
Temasek Life Sciences Laboratory, National University of Singapore, 117604 Singapore (J.Q., J.Y., Y.-F.G., Y.-W.S., S.-Q.G., B.-P.Z., W.C.); and Laboratory of Plant Molecular Biology, Rockefeller University, New York, New York 10021 (N.-H.C.)

Plant Physiology®, October 2012, Vol. 160, pp. 738–748,

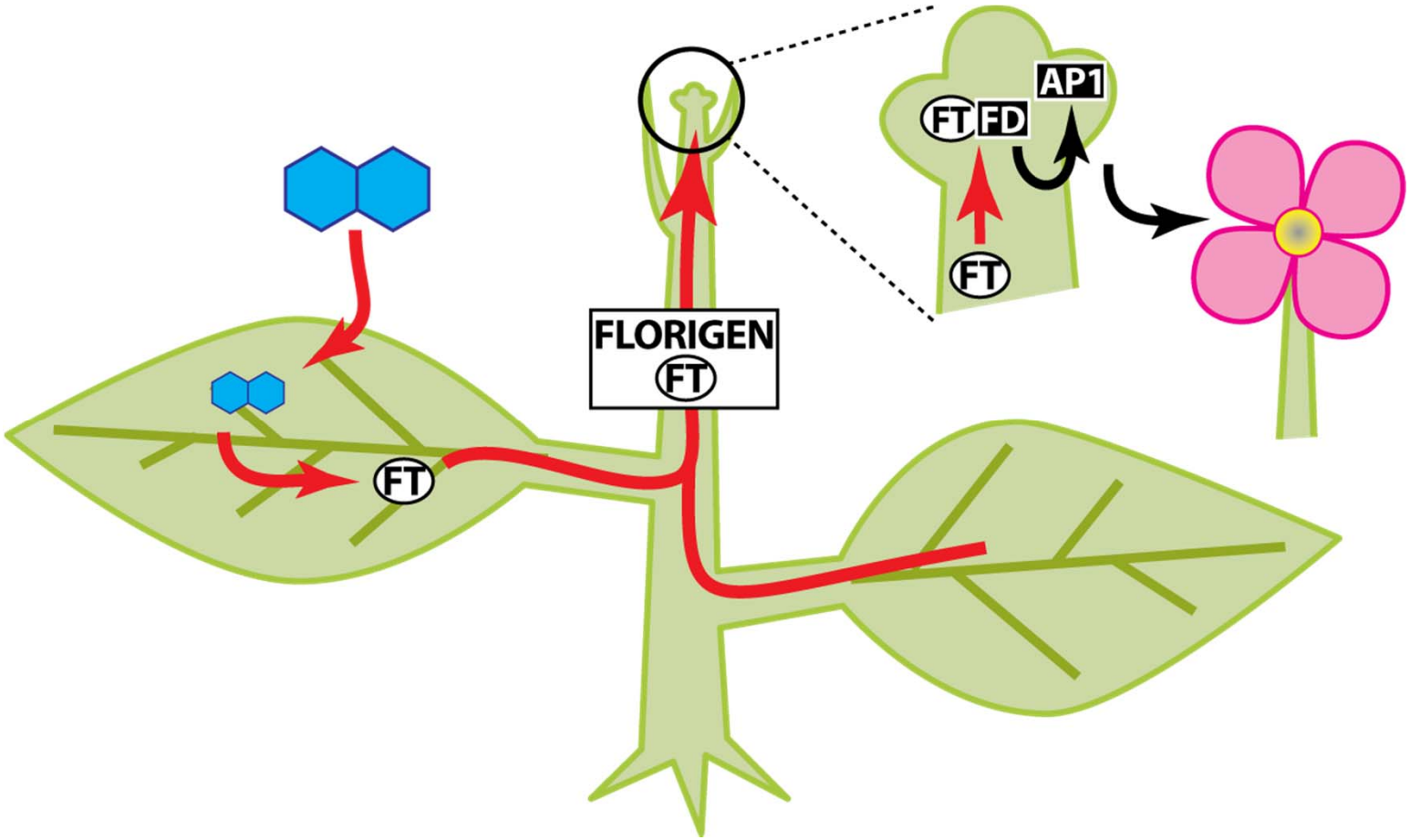


TRV-mediated silencing of microtubule-serving gene *KATANIN* (*KTN*): aberrant arrangement of microtubules in elongating cells.

TRV-mediated silencing of *WRINKLED1*, a positive regulator of oil biosynthesis: more fiber / less oil



Geminivirus-mediated delivery of *FLOWERING LOCUS T* (florigen) uncouples flowering from photoperiod and promotes determinate growth



McGarry RC, **Ayre BG** (2012) PLoS ONE 7(5): e36746

Cotton is a photoperiodic short-day perennial; Modern lines are day-neutral and grown as annuals

- Domesticated lines flower through the summer and are highly inbred
- Ancestral accessions – a rich gene pool – flower when modern lines are finished
- This complicates crosses and promotes further inbreeding

Flowering in Modern Cotton

JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC

Flowering in Ancestral Cotton

Modern *vs.* ancestral cotton, greenhouse grown, 16 hr days

Modern line: DeltaPine 61

Bushy growth

All fruiting branches above 5th node

Many flowers

Broad leaves

Ancestral line: TX701

Tall growth

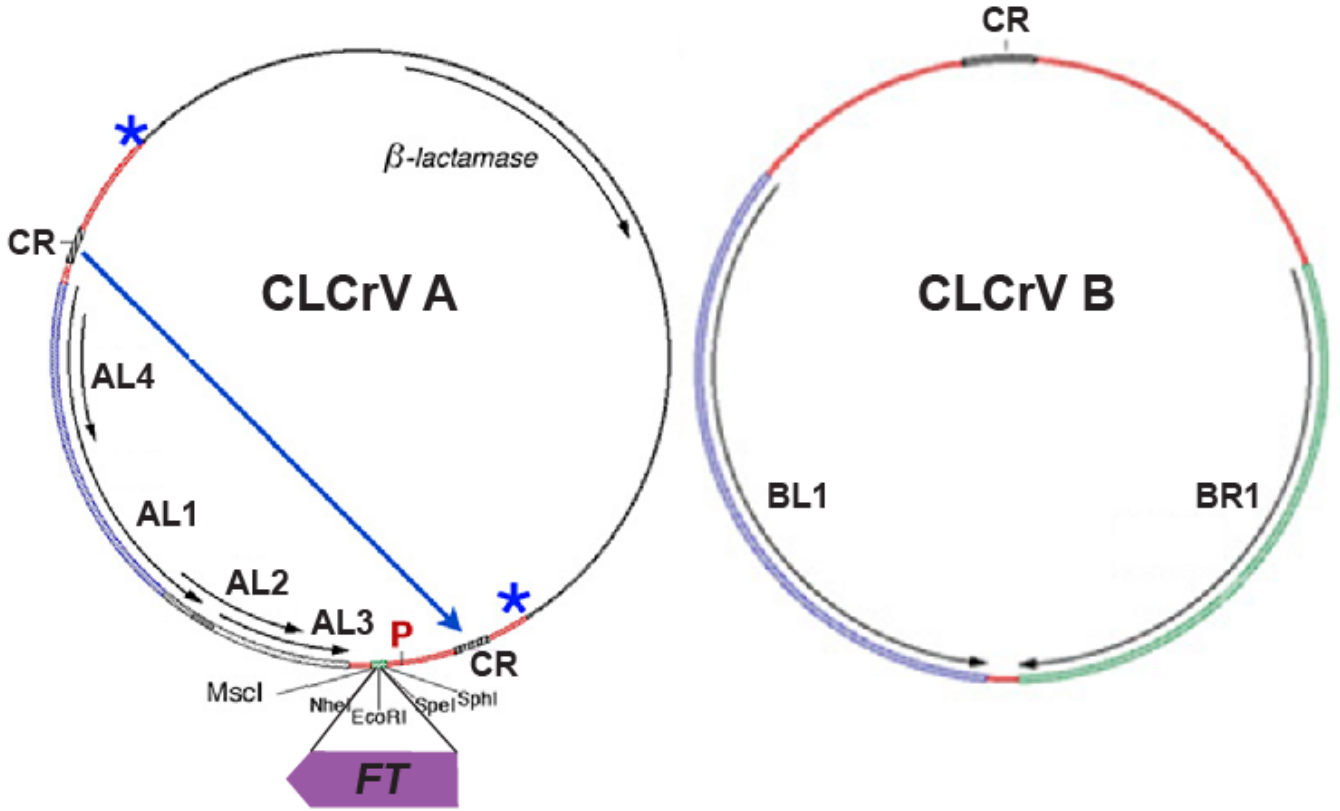
All vegetative

No fruiting branches

'Okra' leaves

Virus-Induced Flowering (VIF)

- Cotton Leaf Crumple Virus: Geminivirus endemic to the Southwest
- Developed by Rich Tuttle and colleagues for cotton VIGS
- Can deliver genes ≤ 800 bp (*FT* is 528 bp)



Tuttle JR, Idris AM, Brown JK, Haigler CH, Robertson D (2008) Geminivirus-mediated gene silencing from cotton leaf crumple virus Is enhanced by low temperature in cotton. Plant Physiol. **148**: 41-50

Virus-induced flowering: *CLCrV:FT* promotes flowering under non-inductive photoperiods in ancestral cotton

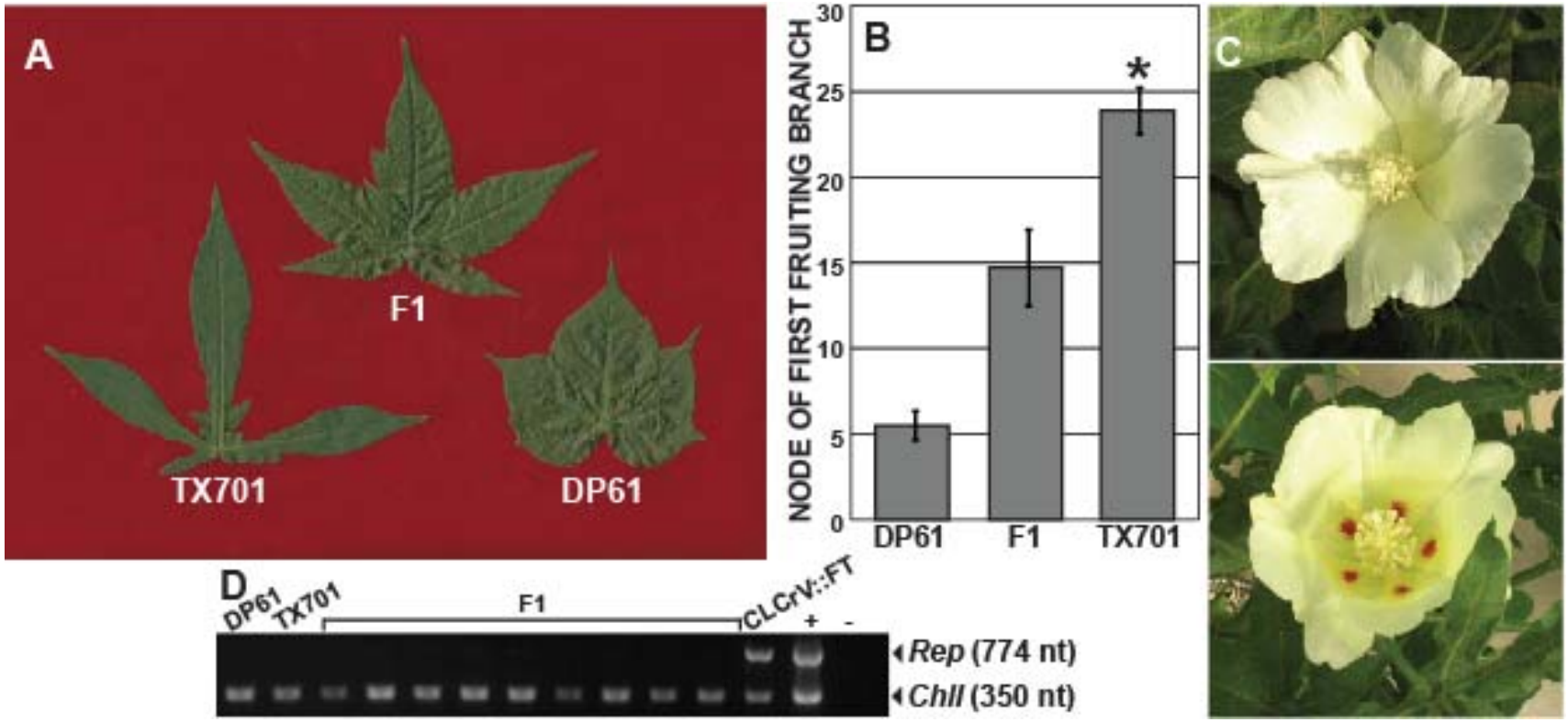
120 d TX701, 16 hr days

Left: infected with *pCLCrV:FT* showing flowers (arrows)

Right: Uninfected, only vegetative growth.

CLCrV:FT infected plants as pollen donors for crosses with modern lines

F_1 progeny have intermediate phenotypes and are virus free: Suitable for organic breeding programs



A. Intermediate leaf lobing; B. Intermediate flowering time; C. petal spots characteristic of the male parent; E. Virus does not pass through the germ line, and is not detected in the F_1 progeny

McGarry RC, Ayre BG (2012) PLoS One

Plant Biotechnology Journal

Plant Biotechnology Journal (2013), pp. 1–9 doi: 10.1111/pbi.12116

Reduced generation time of apple seedlings to within a year by means of a plant virus vector: a new plant-breeding technique with no transmission of genetic modification to the next generation

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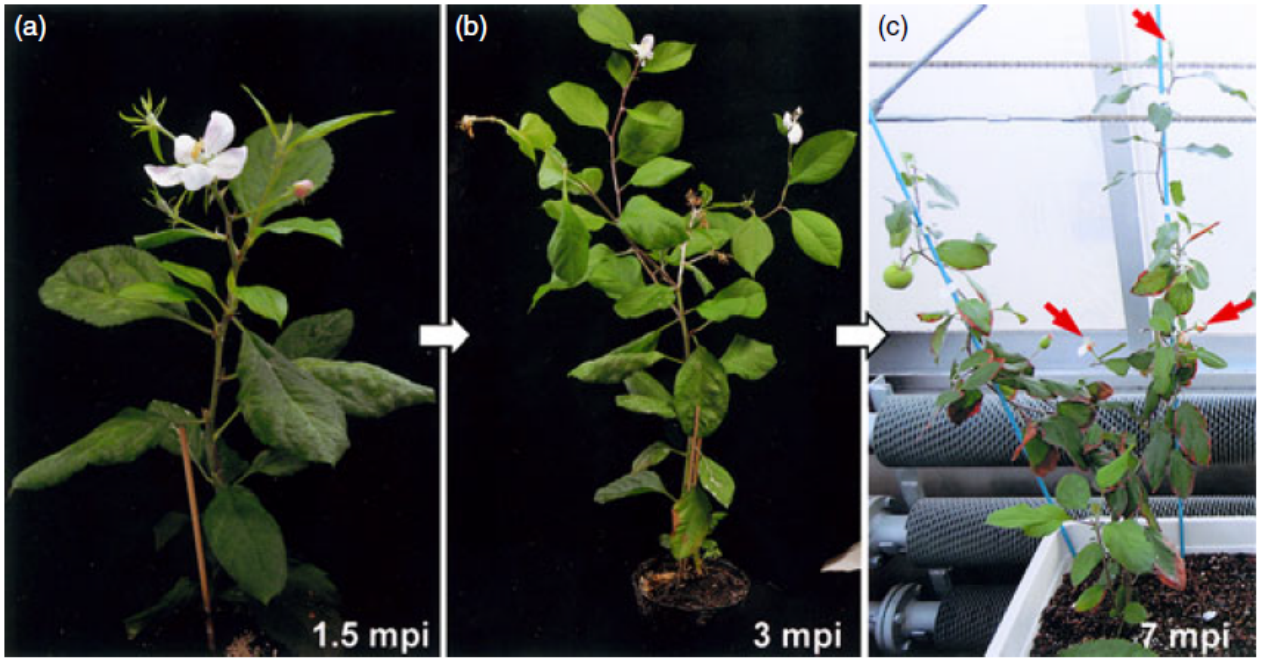
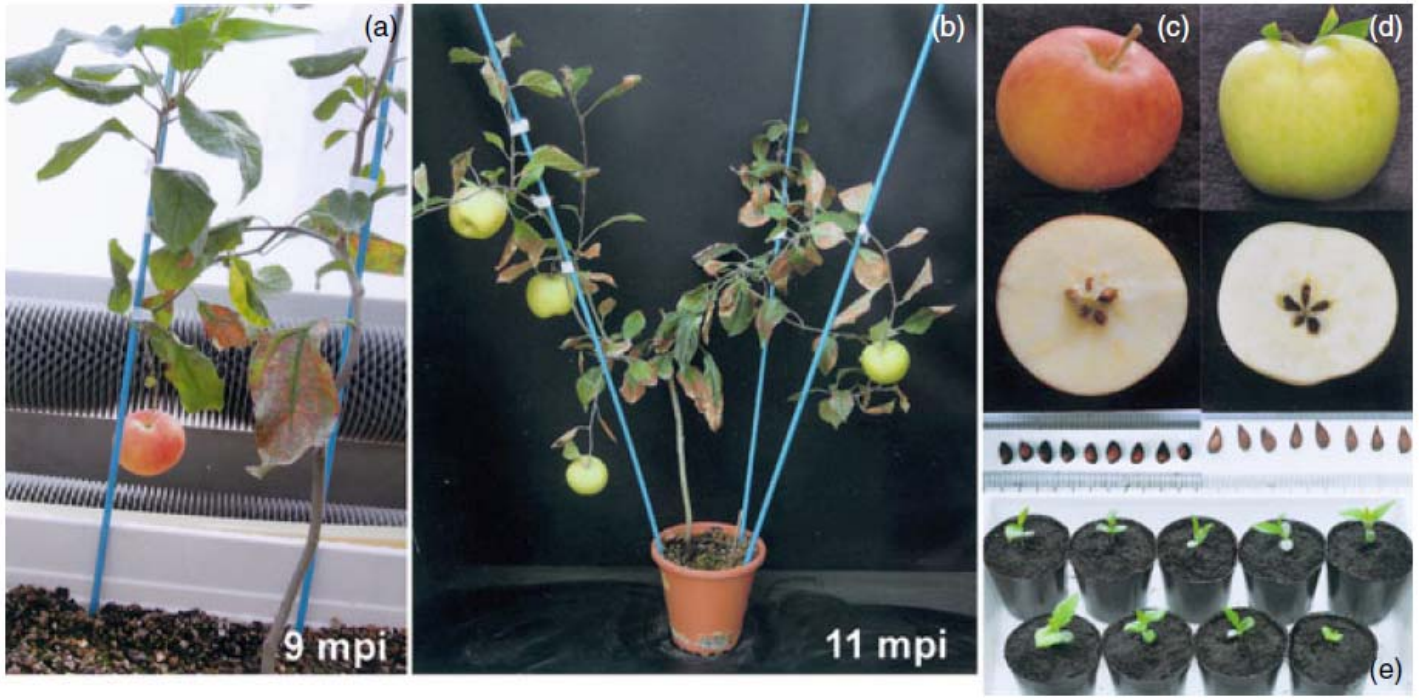
Summary

Fruit trees have a long juvenile phase. For example, the juvenile phase of apple (*Malus* × *domestica*) generally lasts for 5–12 years and is a serious constraint for genetic analysis and for


(a)
1.5 mpi
(b)
3 mpi
(c)
7 mpi


(a)
9 mpi
(b)
11 mpi
(c)
(d)
(e)

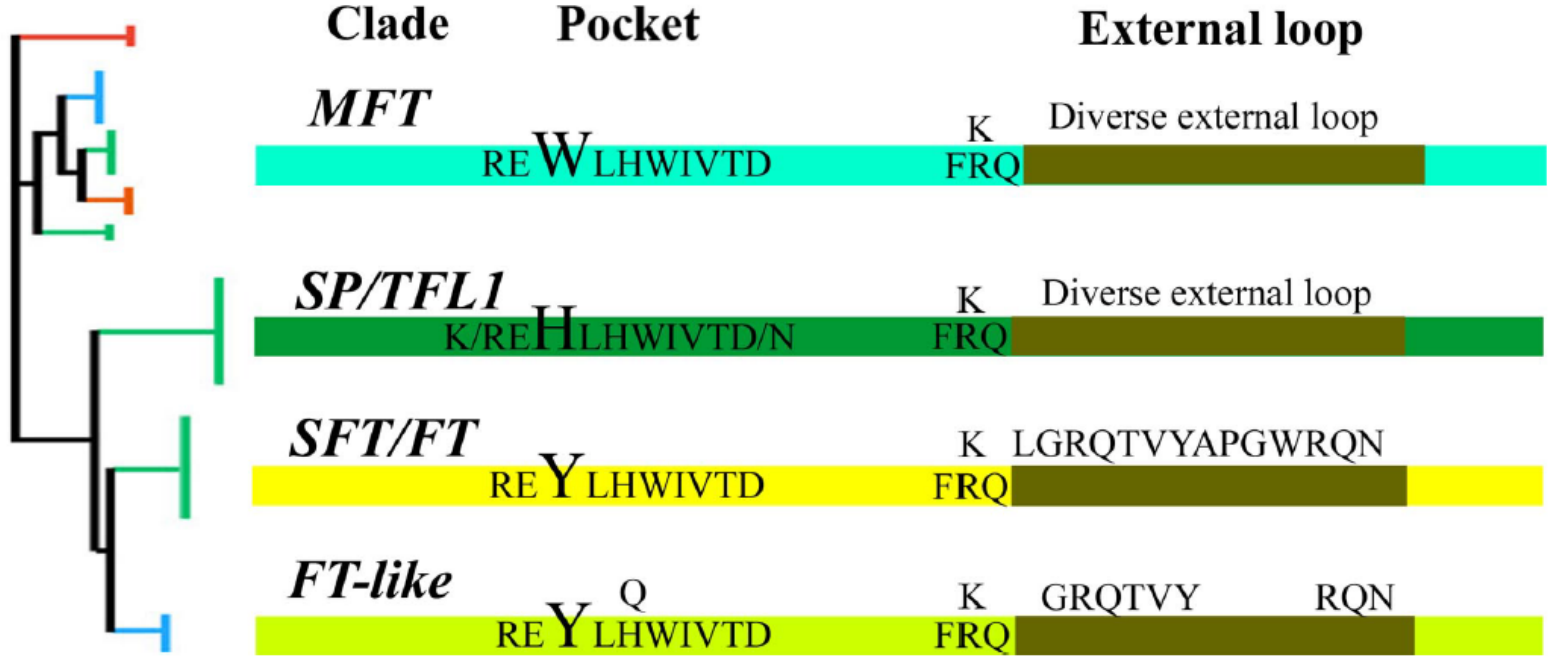
FT belongs to the *CETS* gene family

*CE*NTRORADIALIS (*Antirrhinum*) / *T*ERMINAL FLOWER 1 (*Arabidopsis*) / *S*ELF-PRUNING (tomato)

MFT: ancestral gene, poorly characterized

SFT/FT: determinacy factors; terminate growth; antagonizes *TFL1*

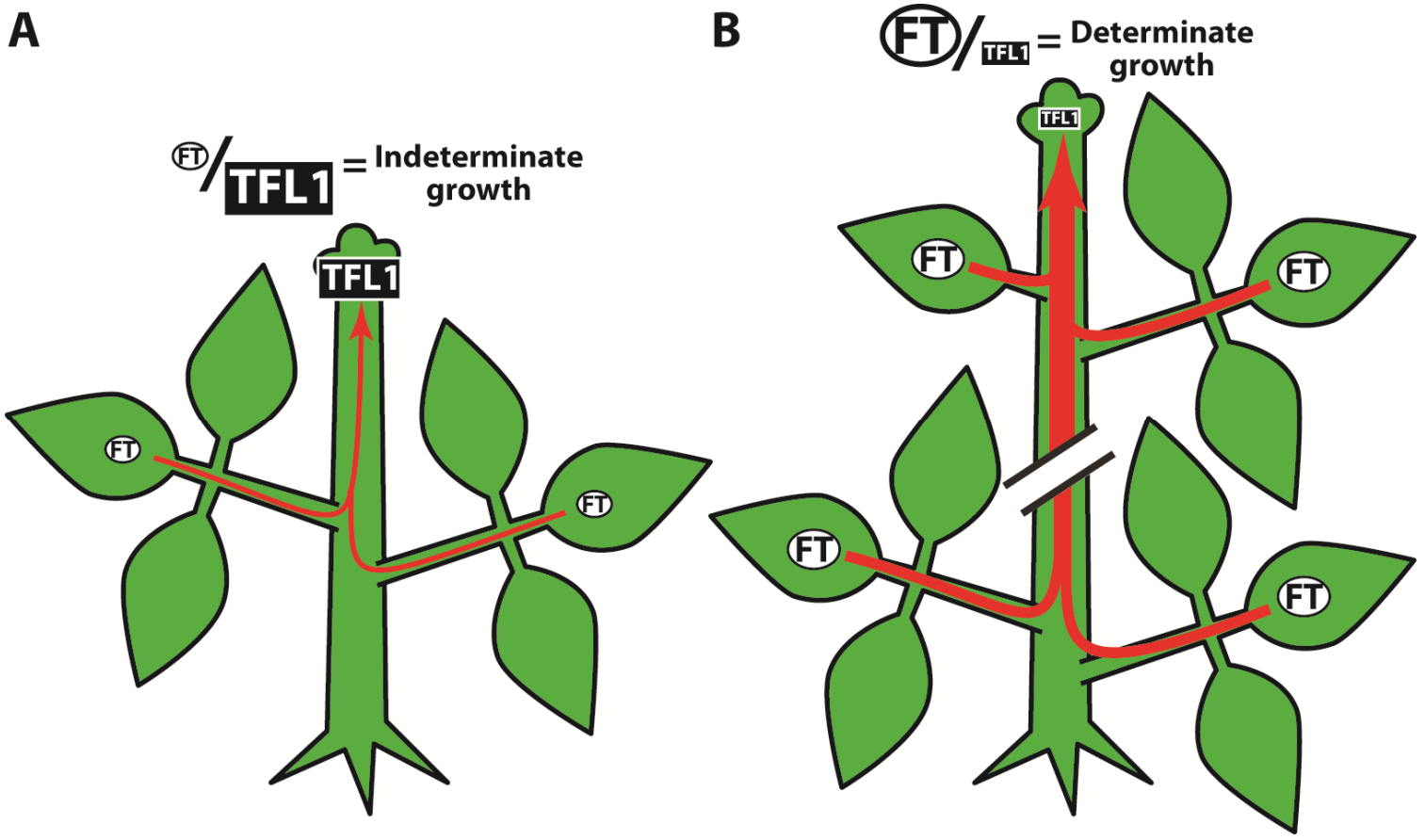
SP/TFL1: indeterminacy factors; promote growth; antagonizes *FT*



Shalit A, et al. (2009) Proc Natl Acad Sci USA 106:8392-7.

The Model: Ratio of FT-like / TFL1-like activities establish the balance of determinate / indeterminate growth

- TFL-like activities decrease with plant age
- FT-like activities are induced (i.e., photoperiod) and are mobile
- FT-like determinacy factors accumulate in meristems



McGarry RC, Ayre BG (2012) Manipulating plant architecture with members of the *CETS* gene family. Plant Science **188**: 71-81

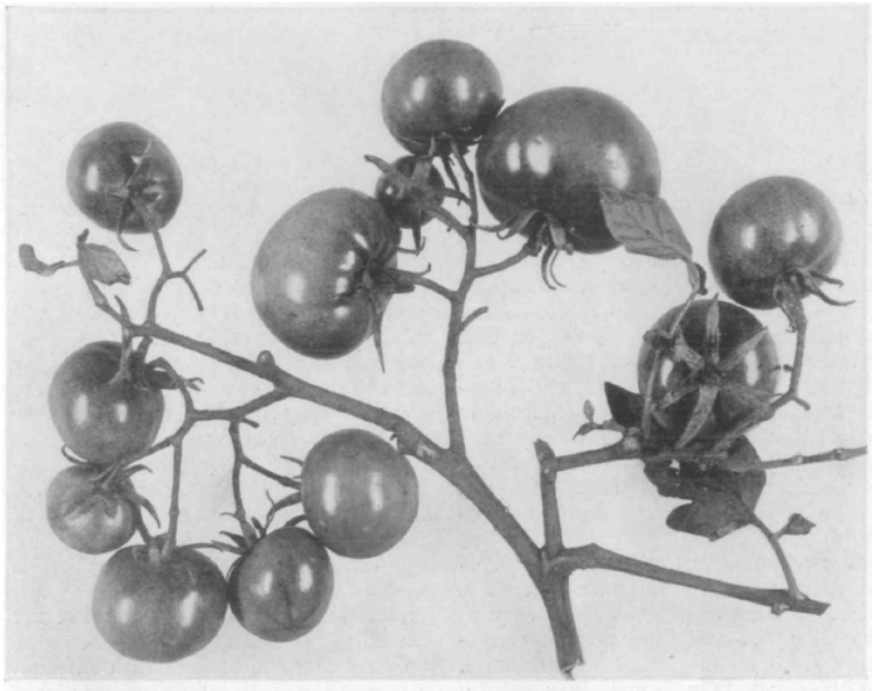
SELF PRUNING; *sp* mutant (high FT/TFL ratio) makes tomato determinate and synchronized

USUAL HABIT OF GROWTH OF TOMATO

DETERMINATE GROWTH IN THE TOMATO

A. F. YEAGER

North Dakota Experiment Station

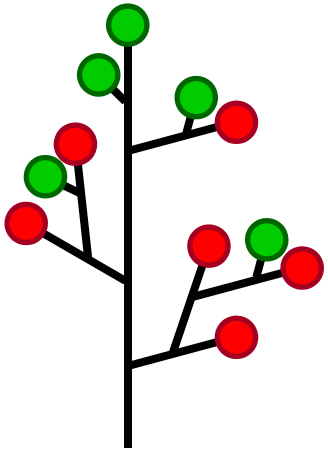


EARLY SELF-PRUNING SEGREGATE

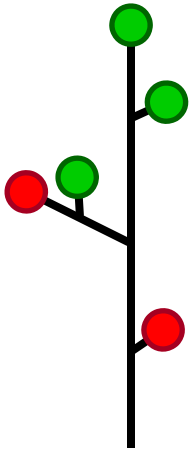
Yeager AF, 1927. Determinate growth in the tomato. *Journal of Heredity* **18**, 263-5.

Pnueli L *et al.*, 1998. The *SELF-PRUNING* gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of *CEN* and *TFL1*. *Development* **125**, 1979-89.

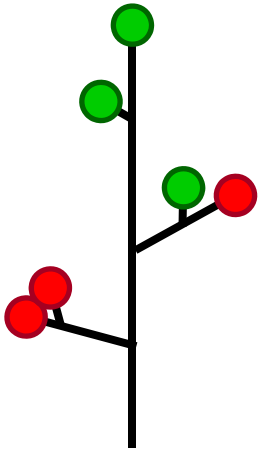
dCLCrV:FT fruiting branches have accelerated determinate habits



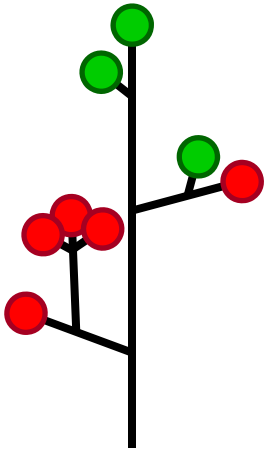
Uninfected: apical meristems become determinate flowers and axillary meristem remains indeterminate



Flower directly on mainstem (no fruiting branch)



Two terminal flowers in the same bract whorl



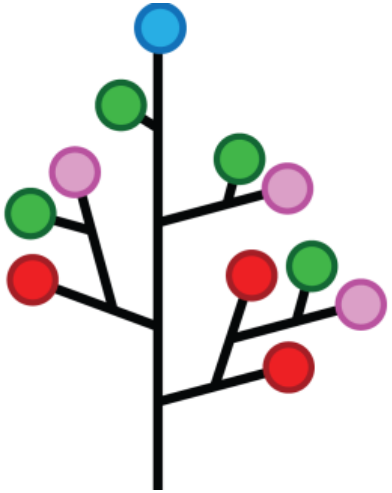
Three terminal, independent flowers

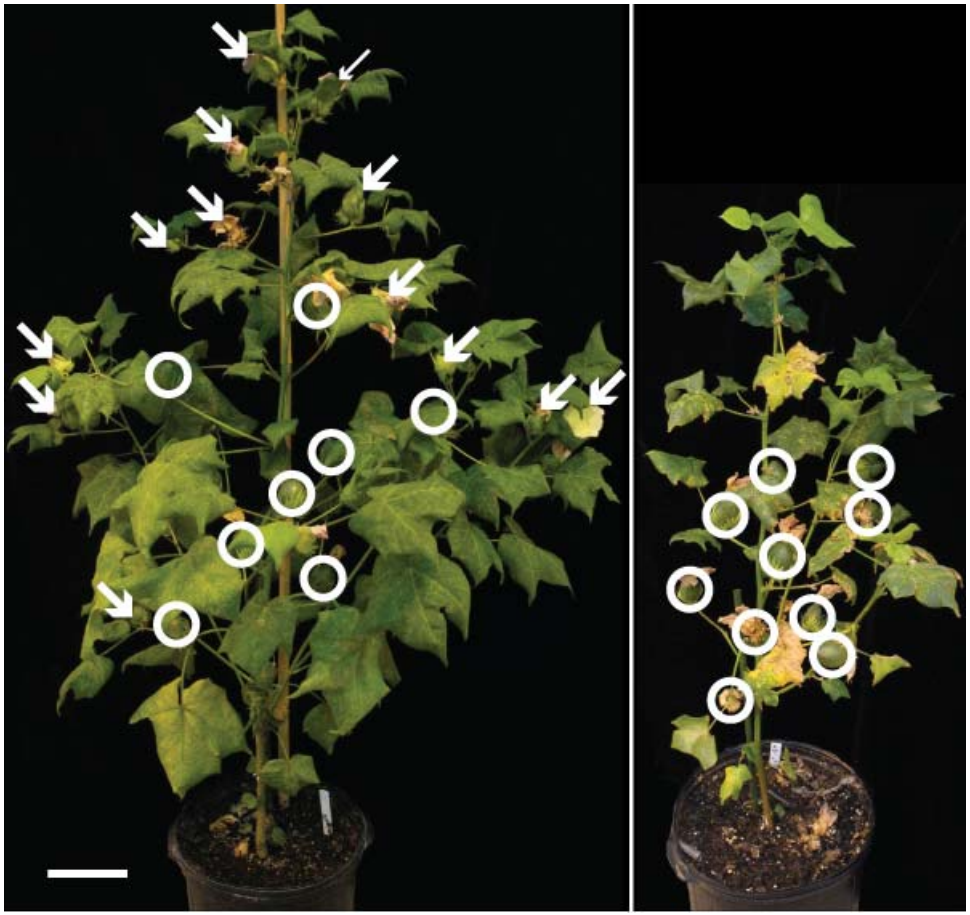
McGarry RC et al. (2013) Plant Signal and Behavior

FT overexpression impacts architecture

Axillary meristems convert to flowers instead of forming new sympodial units

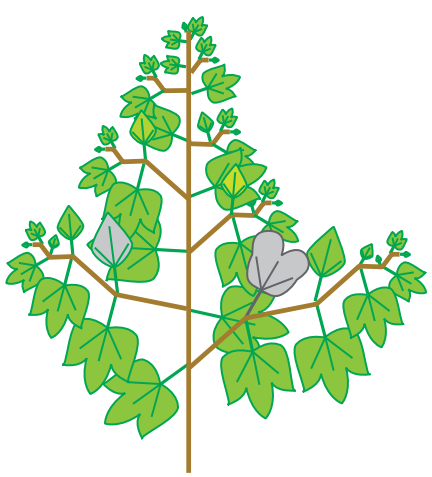
Fruiting branches terminate with a floral cluster; Flowering & fruiting is more synchronized; Plant architecture is more compact

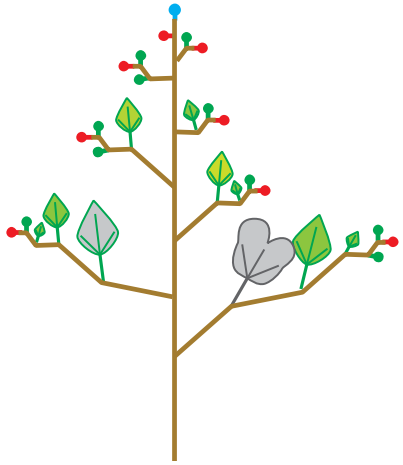




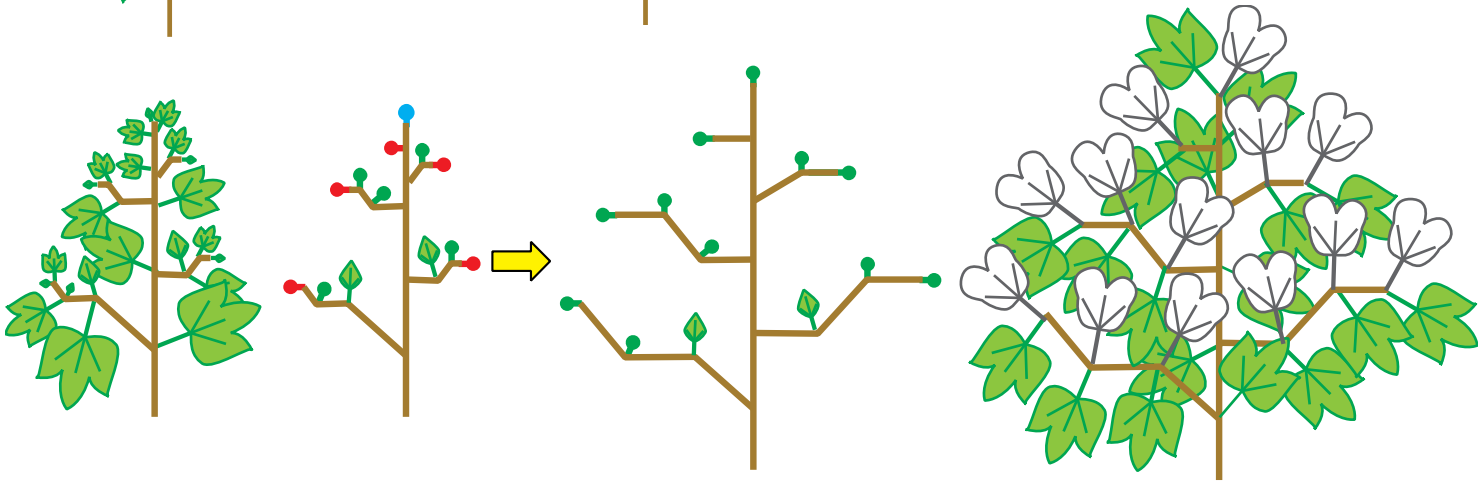
McGarry RC, **Ayre BG** (2012) PLoS ONE 7(5): e36746

Fantasy Cotton-Boll League: Control of plant architecture and synchronized flowering for specific environments





Domesticated cotton:
Asynchronous flowering
Inputs throughout the season
Inconsistent fiber quality



With inducible *"florigen"*: convert vegetative meristems to flowers for synchronized maturation, focused application of inputs, and homogeneous fiber quality

Acknowledgements

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- **McGarry RC, Ayre BG** (2012) Florigen and cotton: manipulating plant architecture to improve plant productivity. *In* DM Oosterhuis, ed, *Flowering and Fruiting in Cotton*. Springer, New York
- **McGarry RC, Ayre BG** (2012) Geminivirus-mediated delivery of florigen promotes determinate growth in aerial organs and uncouples flowering from photoperiod in cotton. *Public Library of Science (PLoS) One* **7:** e36746
- **McGarry RC, Ayre BG** (2012) Manipulating plant architecture with members of the *CETS* gene family. *Plant Science* **188–189:** 71-81
- **McGarry RC, Ayre BG** (2013) Using Virus Induced Flowering to Manipulate Cotton Shoot Architecture. *Plant Signaling & Behavior* **8:** e23602
- **McGarry RC, Kragler F** (2013) Phloem-mobile signals affecting flowers: applications for crop breeding. *Trends in Plant Science (In Press): http://dx.doi.org/10.1016/j.tplants.2013.01.004*