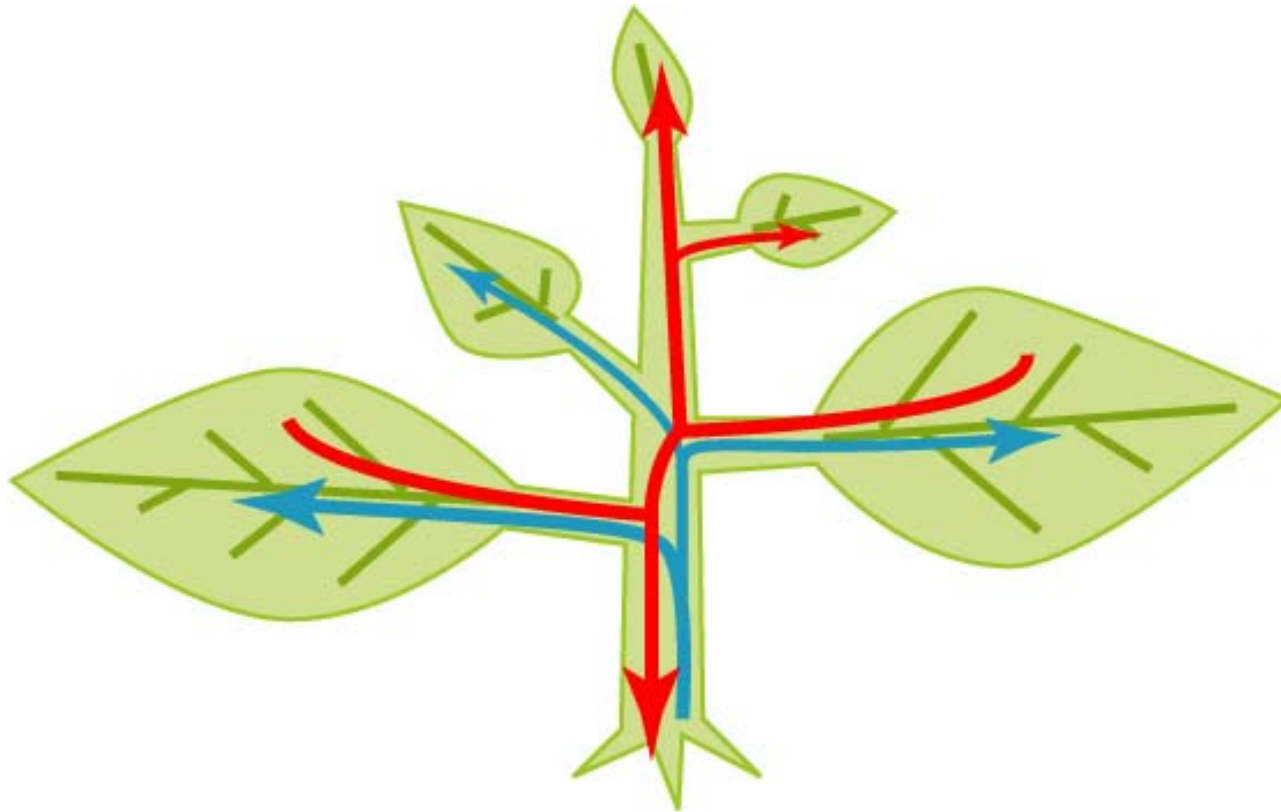


Virus-mediated Strategies for Transient Gene Expression and Silencing in Cotton

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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:

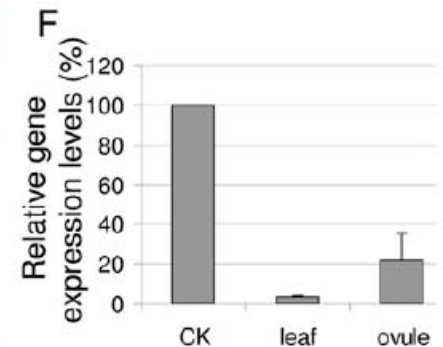
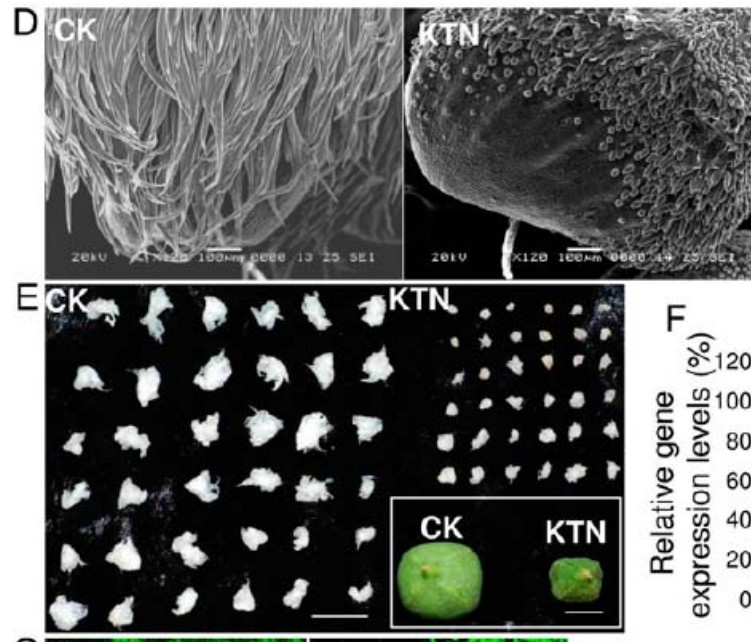
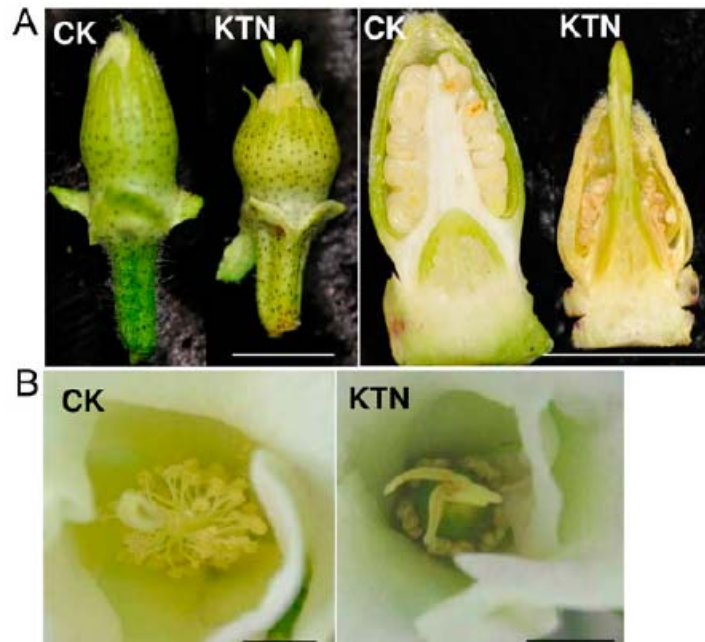
- i. Virus Induced Flowering with Florigen (FT gene)
- ii. Transient protoplast assay and GFP

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

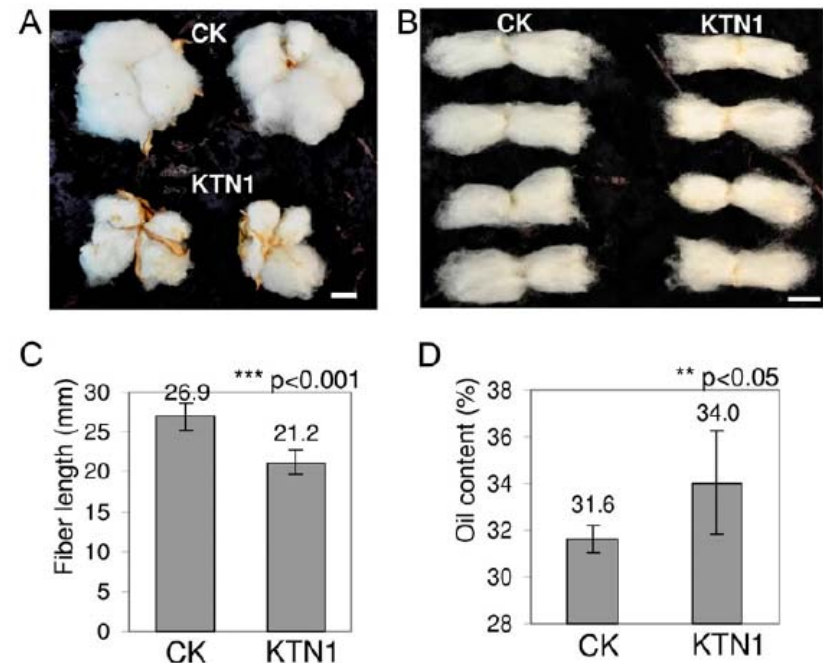
Jing Qu², Jian Ye², Yun-Feng Geng, Yan-Wei Sun, Shi-Qiang Gao,
Bi-Pei Zhang, Wen Chen, and Nam-Hai Chua*

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.)

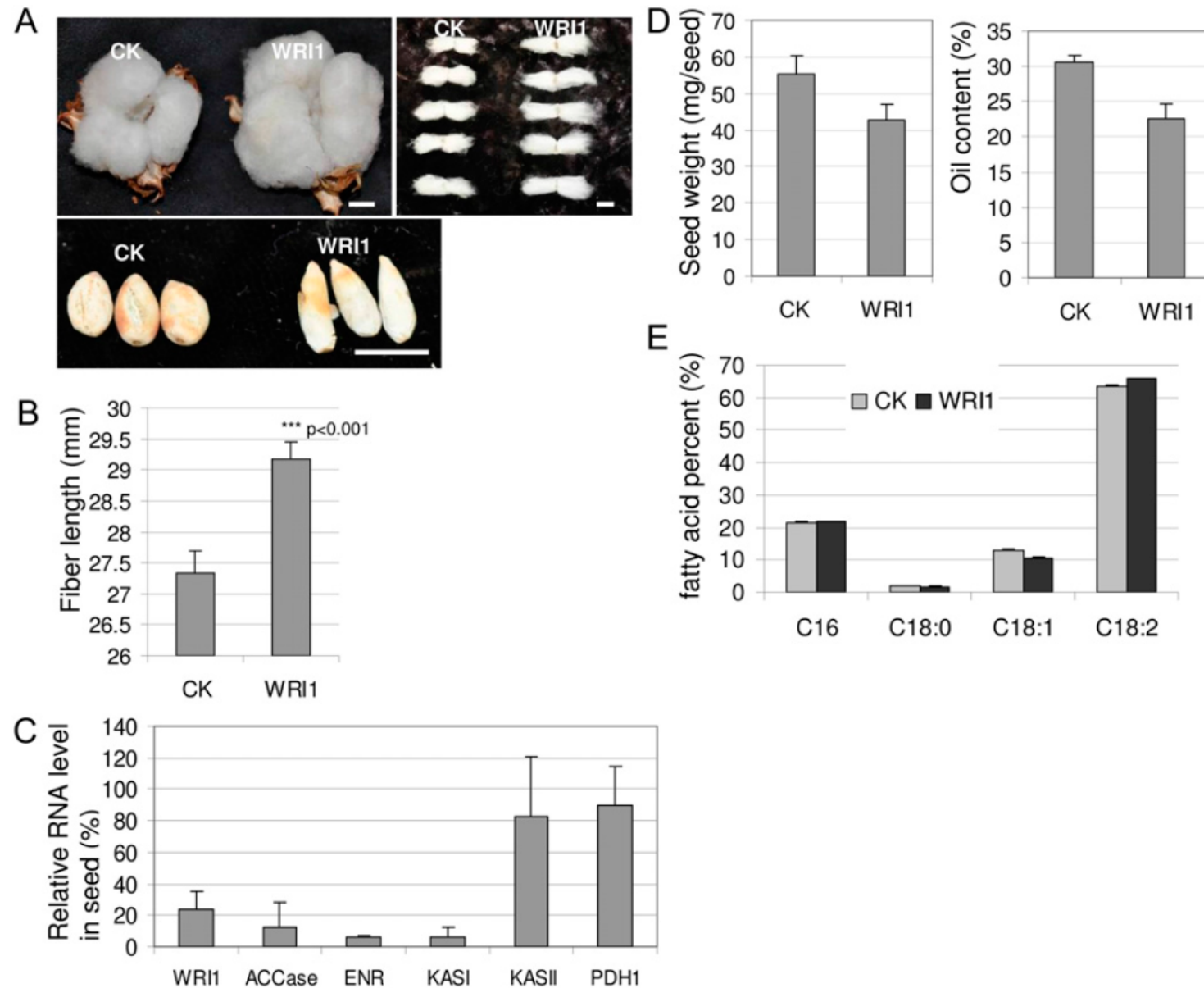
Plant Physiology[®], October 2012, Vol. 160, pp. 738–748,



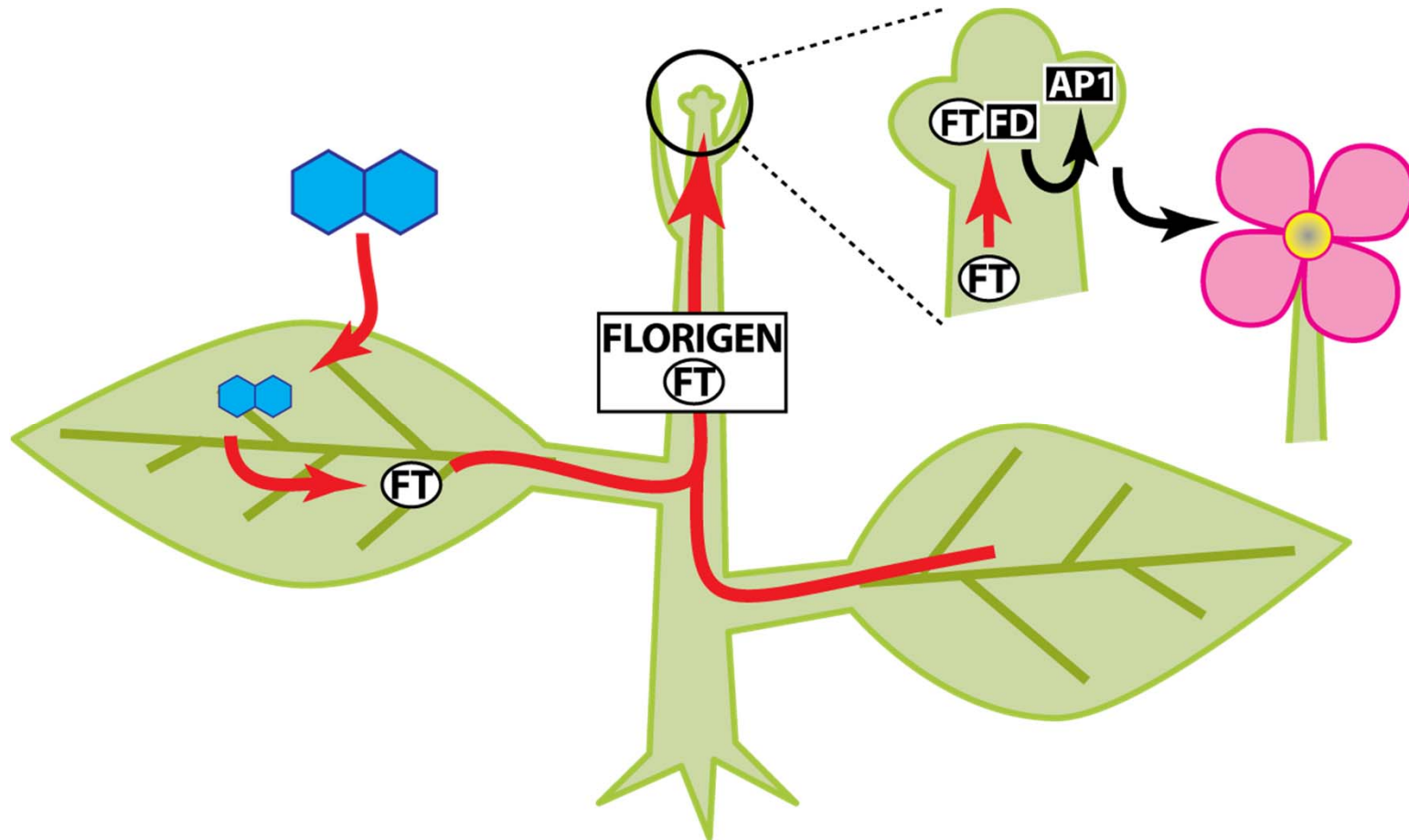
TRV-mediated silencing of microtubule-serving gene *KATANIN* (*KTN*): aberrant arrangement of microtubules in elongating cells.



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

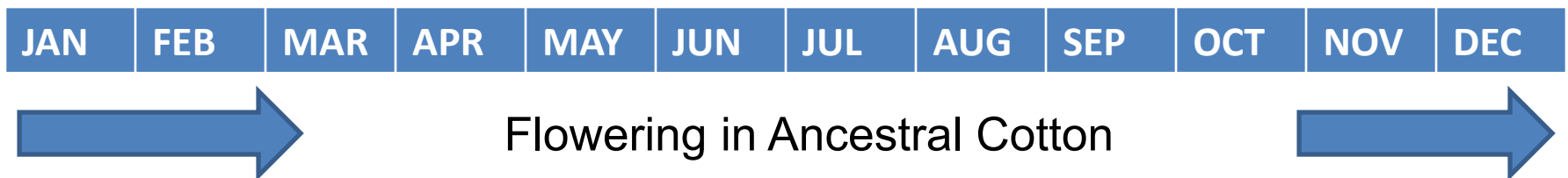


**Cotton is a photoperiodic short-day perennial;
Modern lines are day-neutral and grown as annuals**

- 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



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

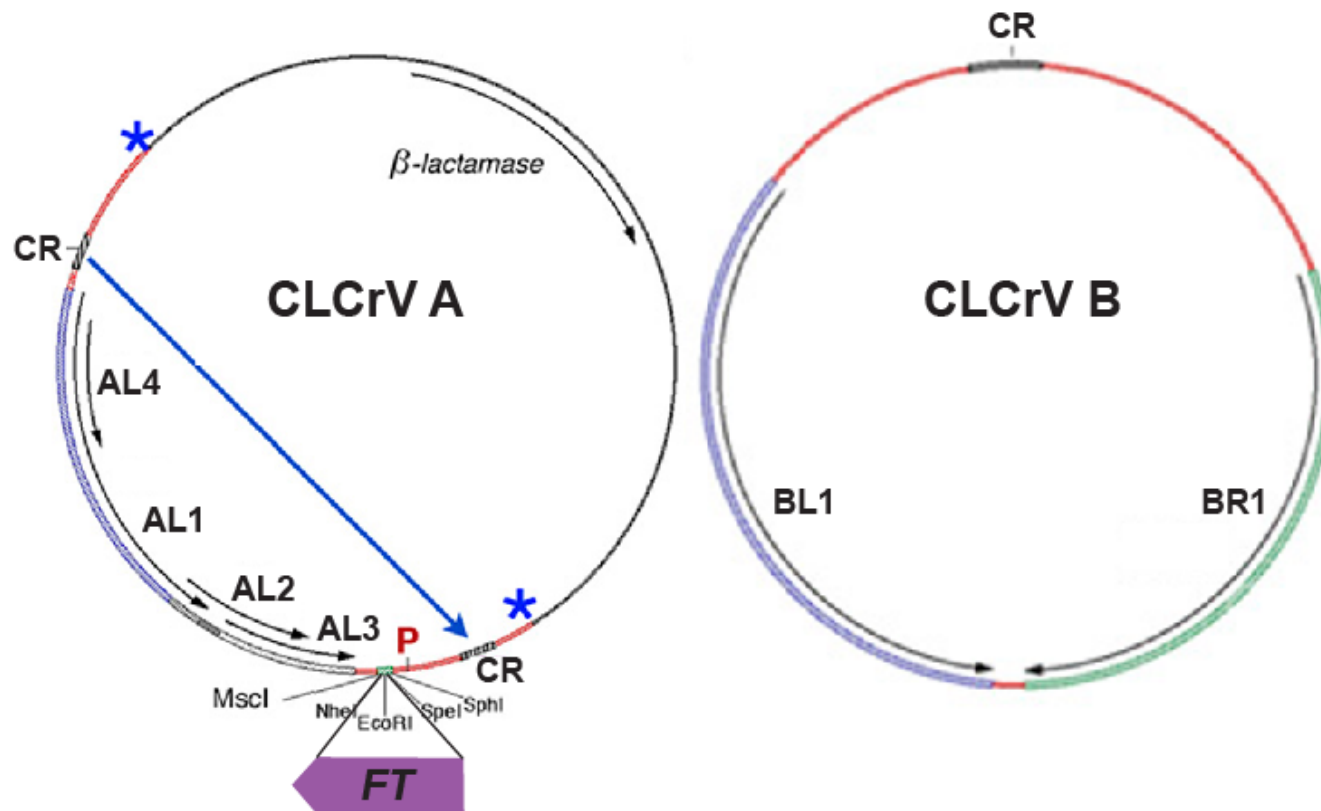


Ancestral line: TX701

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)

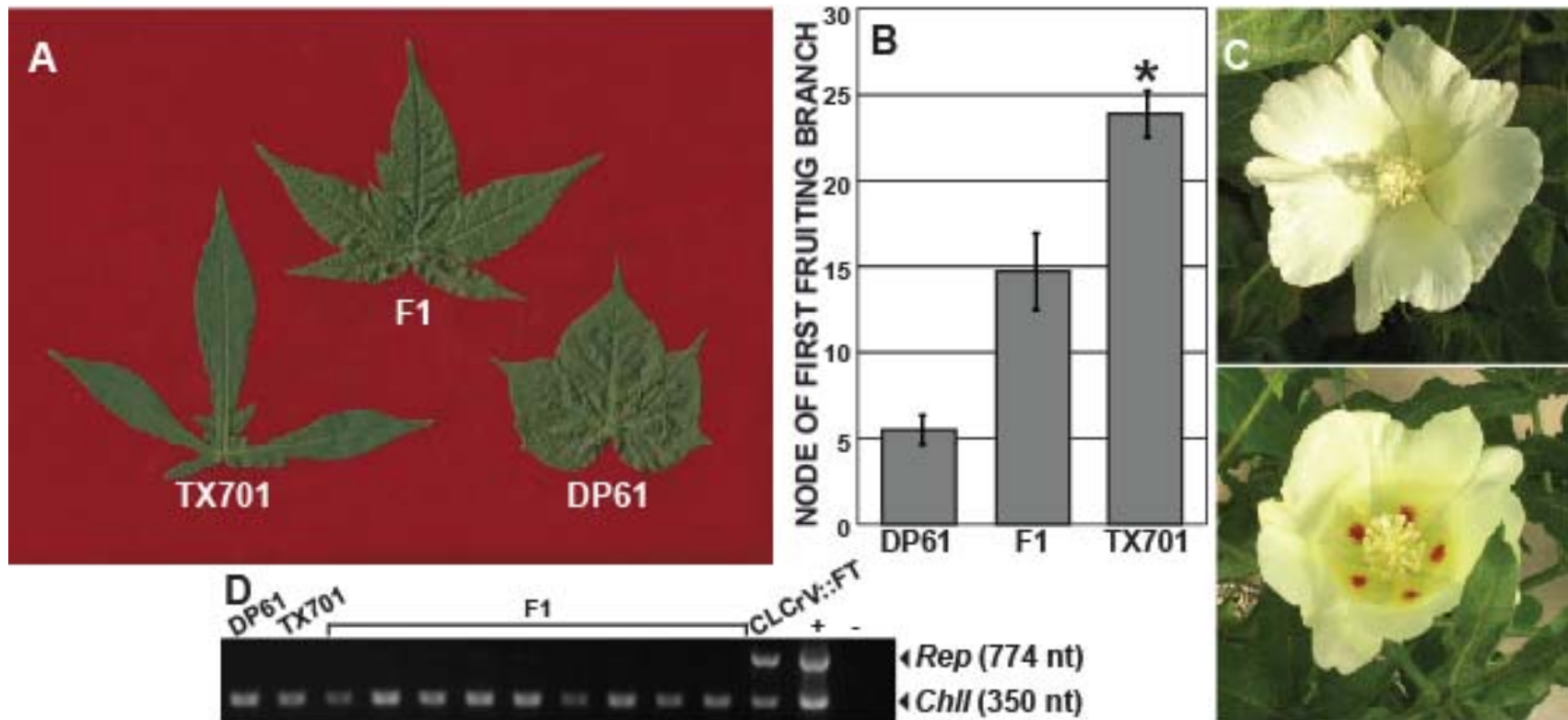
Right: Uninfected, only vegetative
growth.



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



F₁ 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 F₁ progeny

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

Noriko Yamagishi, Ryusuke Kishigami and Nobuyuki Yoshikawa

Faculty of Agriculture, Iwate University, Morioka, Japan

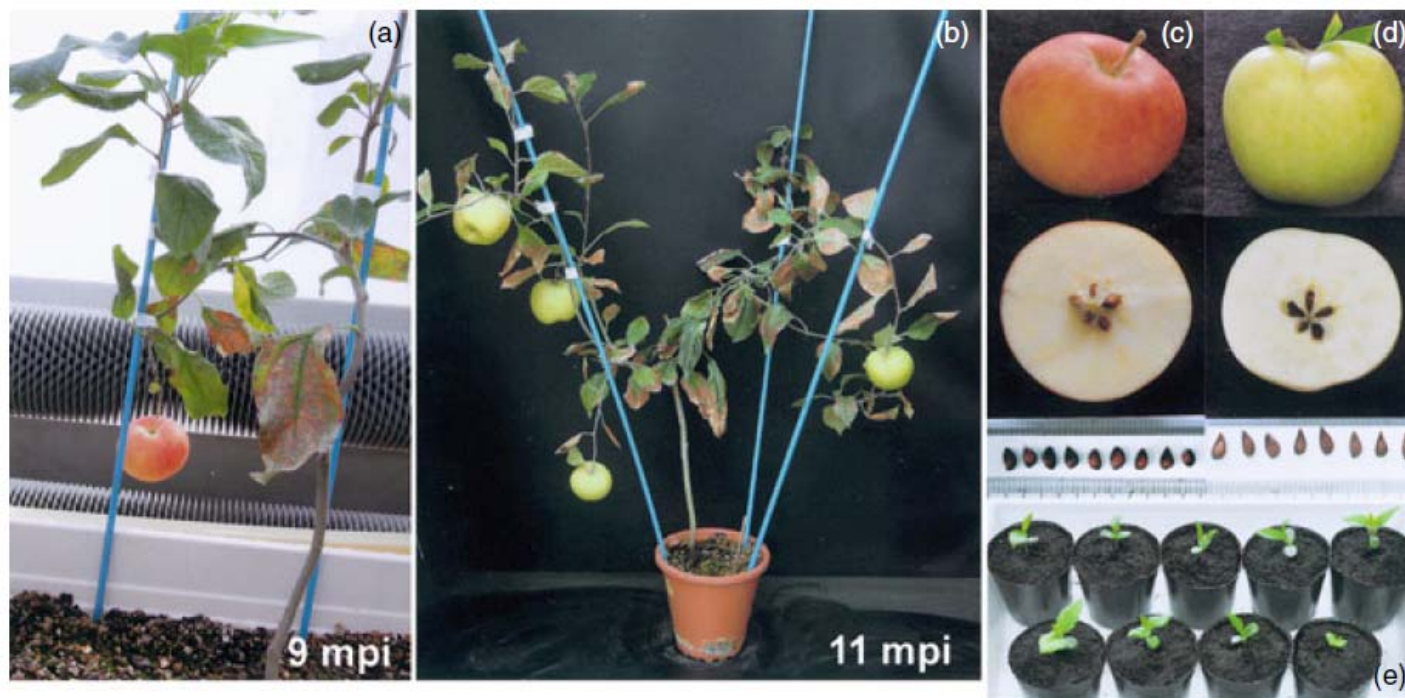
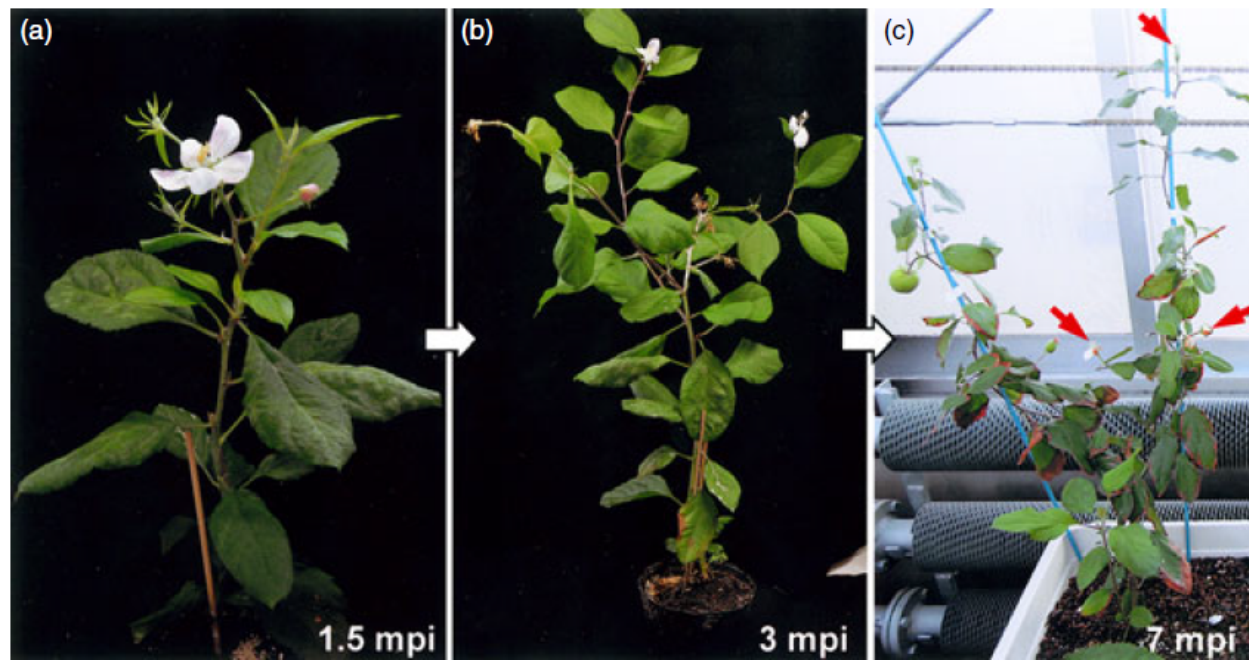
Received 30 May 2013;

revised 8 July 2013;

accepted 11 July 2013.

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



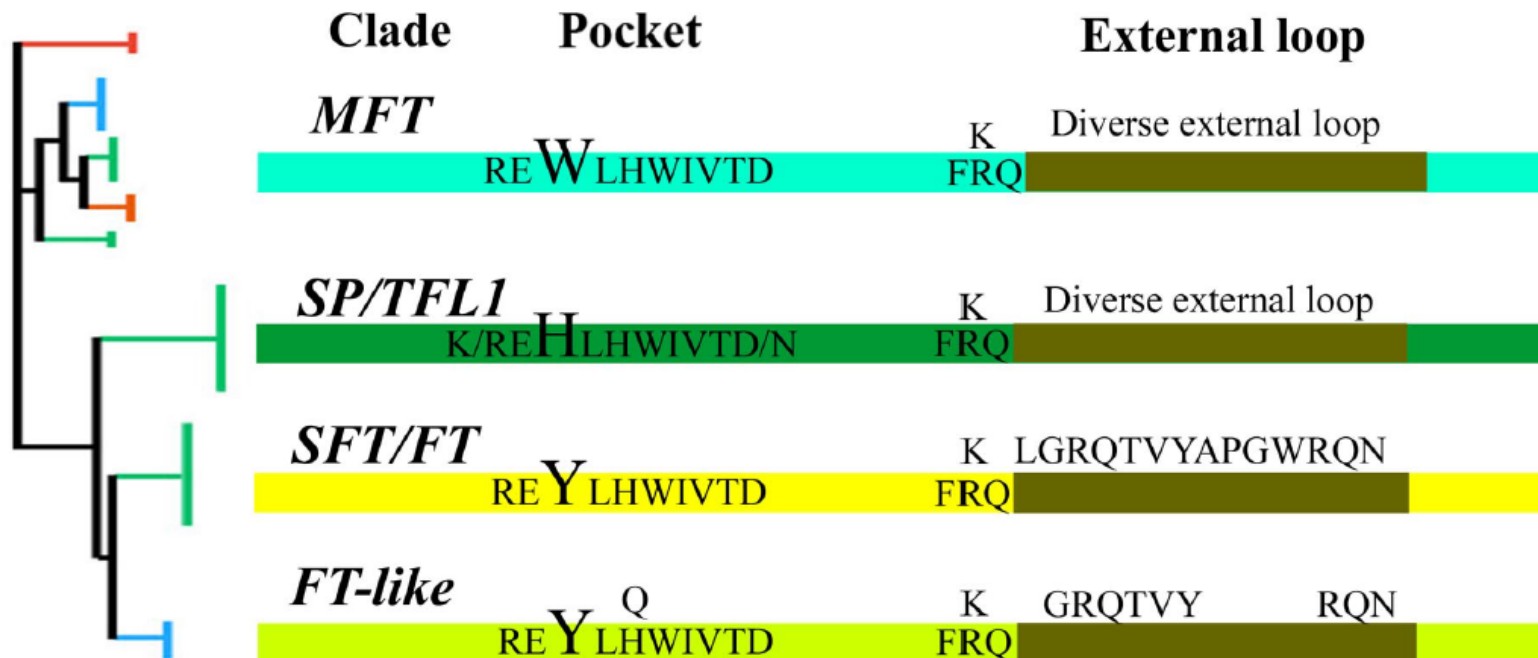
***FT* belongs to the *CETS* gene family**

CENTRORADIALIS (*Antirrhinum*) / *TERMINAL FLOWER 1* (*Arabidopsis*) /
SELF-PRUNING (tomato)

MFT: ancestral gene, poorly characterized

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

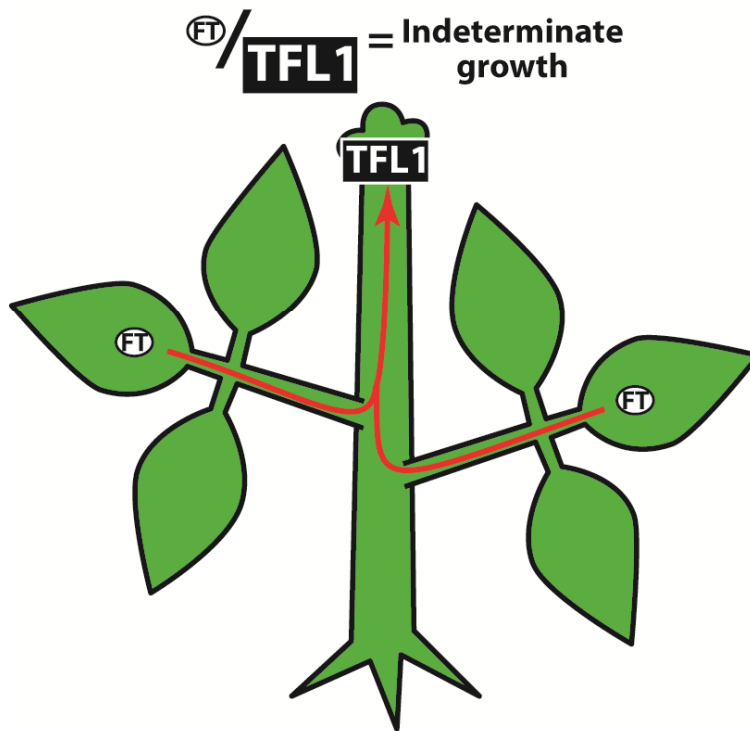
SP/TFL1: indeterminacy factors; promote growth; antagonizes *FT*



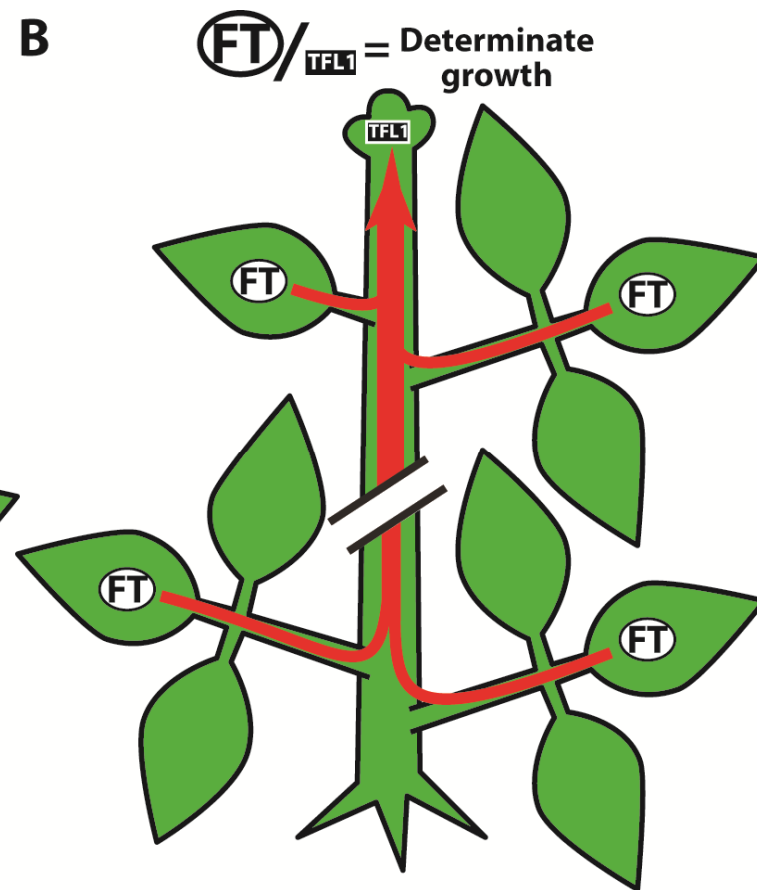
The Model: Ratio of FT-like / TFL1-like activities establish the balance of determinate / indeterminate growth

- 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

A



B



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

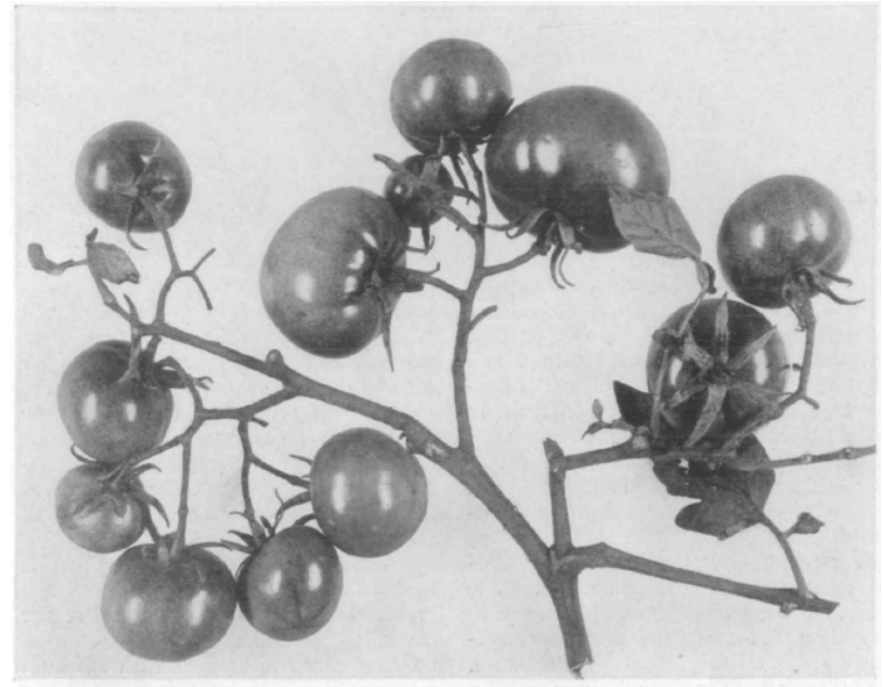


USUAL HABIT OF GROWTH OF TOMATO

**DETERMINATE GROWTH IN THE
TOMATO**

A. F. YEAGER

North Dakota Experiment Station

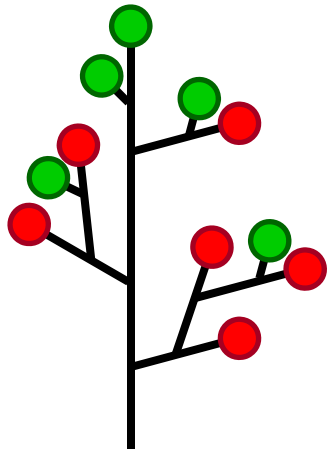


EARLY SELF-PRUNING SEGREGATE

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.

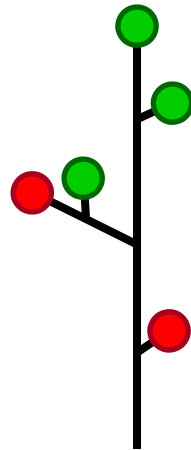
dCLCrV:FT fruiting branches have accelerated determinate habits



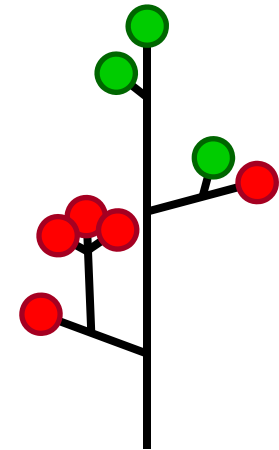
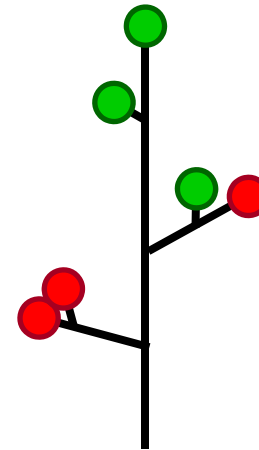
Uninfected: apical meristems become determinate flowers and axillary meristem remains indeterminate



Flower directly on mainstem (no fruiting branch)



Two terminal flowers in the same bract whorl



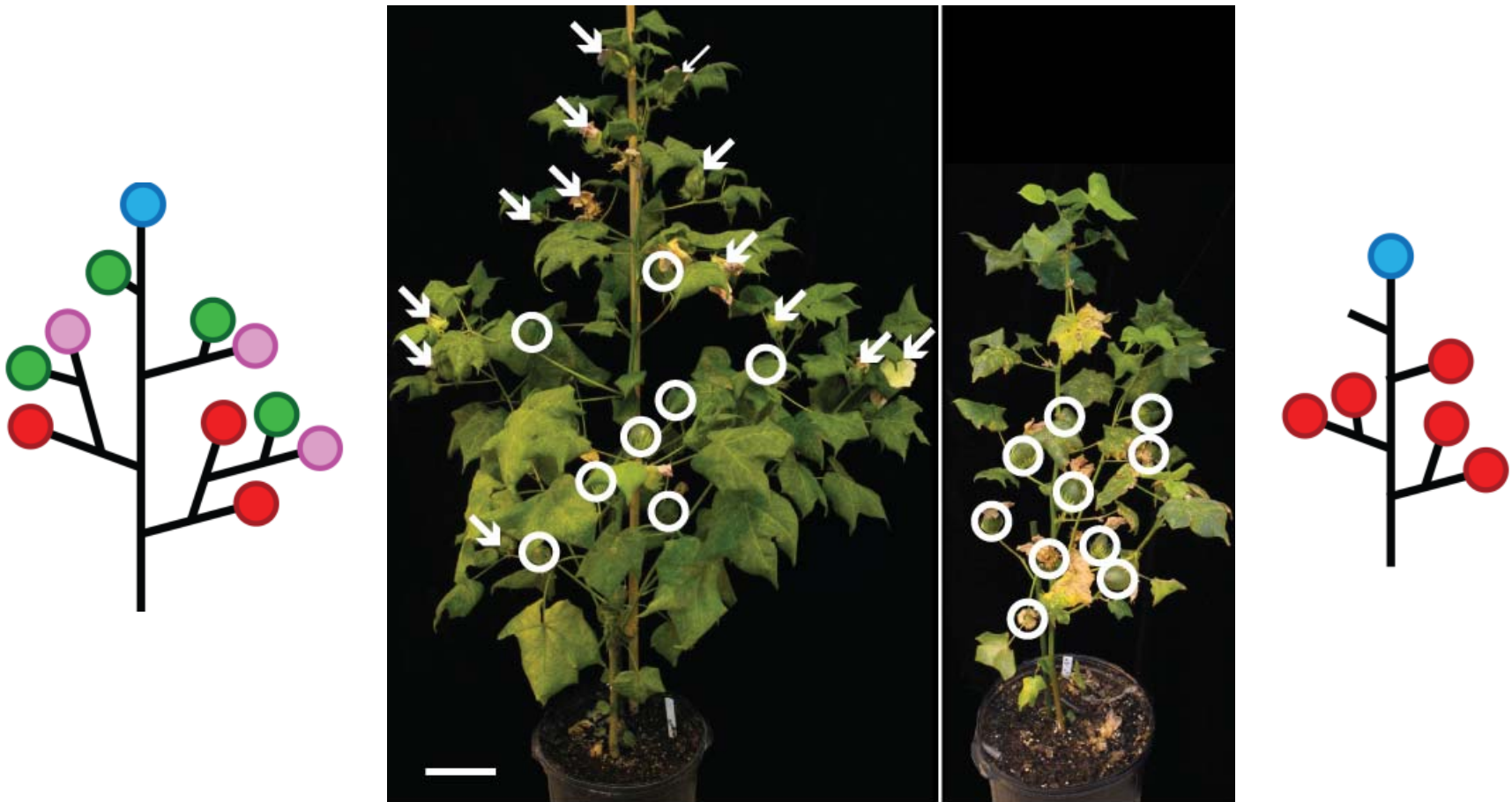
Three terminal, independent flowers



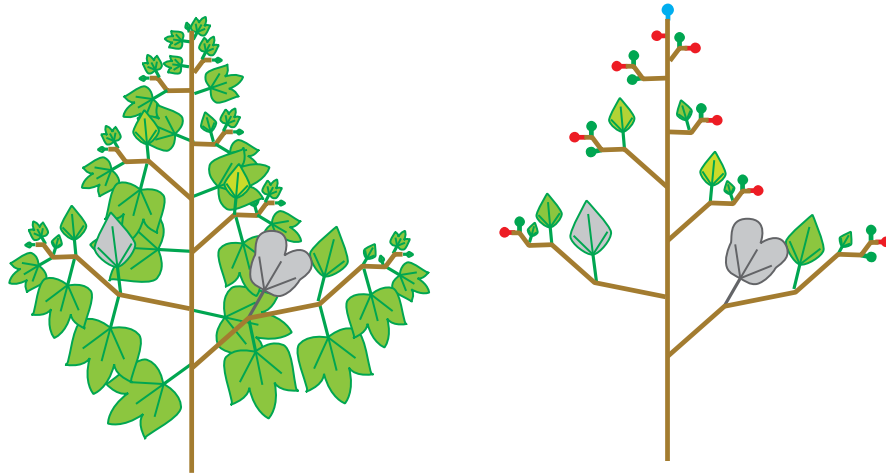
***FT* overexpression impacts architecture**

Axillary meristems convert to flowers instead of forming new sympodial units

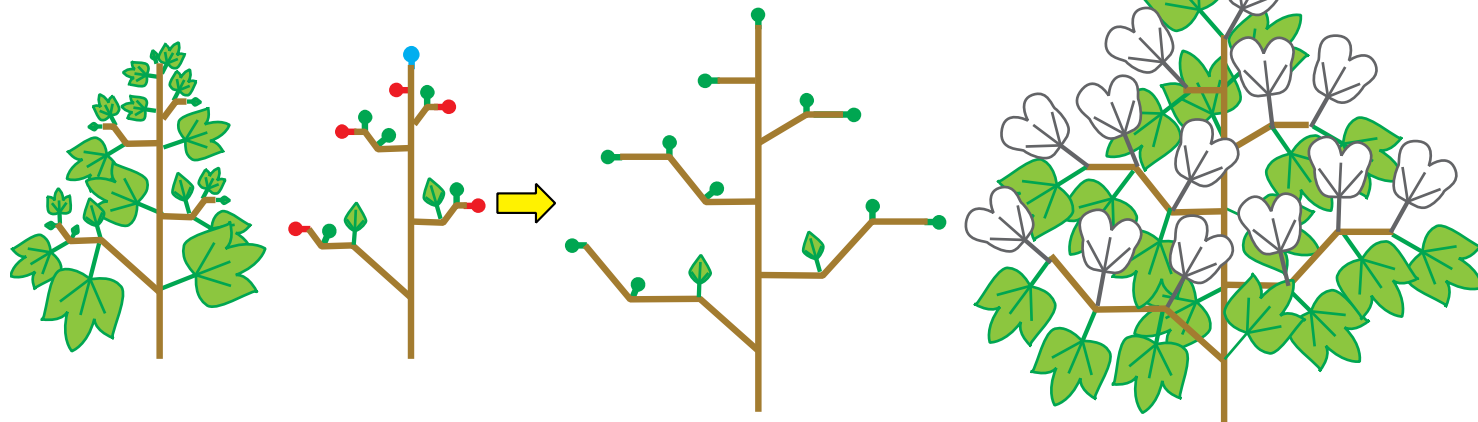
Fruiting branches terminate with a floral cluster; Flowering & fruiting is more synchronized; Plant architecture is more compact



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

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Ayre lab cotton group:

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Sarah Prewitt, MSc



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Kater Hake (Cotton Inc)
Jack McCarty (USDA ARS)

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Cotton Incorporated
The Cotton Foundation



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- **M^cGarry 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
- **M^cGarry RC, Ayre BG** (2012) Manipulating plant architecture with members of the *CETS* gene family. *Plant Science* 188–189: 71-81
- **M^cGarry RC, Ayre BG** (2013) Using Virus Induced Flowering to Manipulate Cotton Shoot Architecture. *Plant Signaling & Behavior* 8: e23602
- **M^cGarry 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>