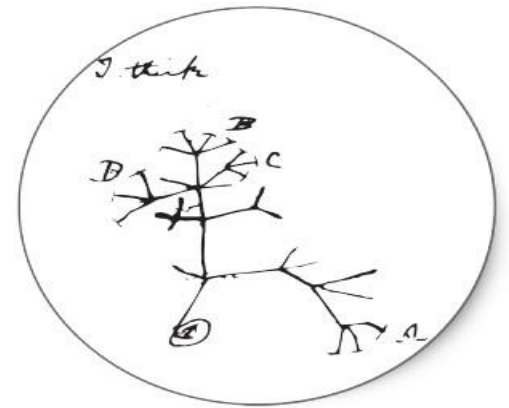


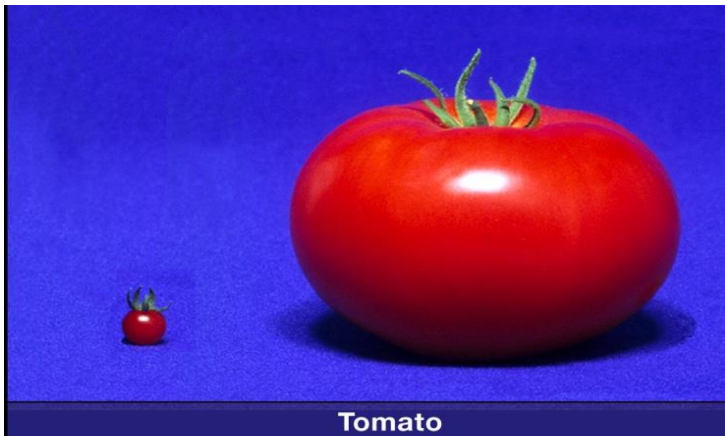
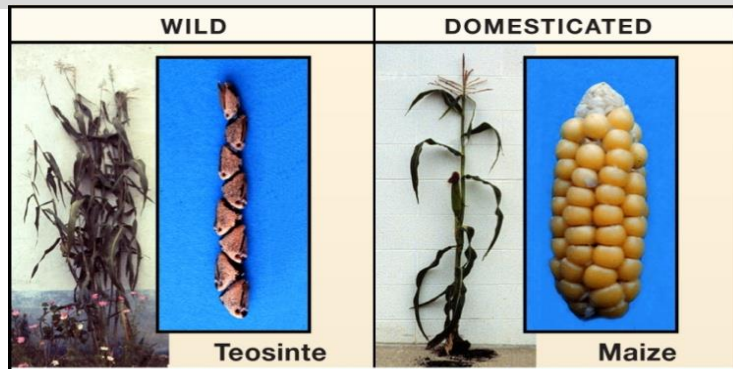
# breeding for increased yields – the epigenetic approach

**David Baulcombe**  
**[dcb40@cam.ac.uk](mailto:dcb40@cam.ac.uk)**  
**@dcb40**



**SCI - Breeding Plants for the Future**  
**Reading UK - May 15<sup>th</sup> 2014**

# crop domestication vs diversification



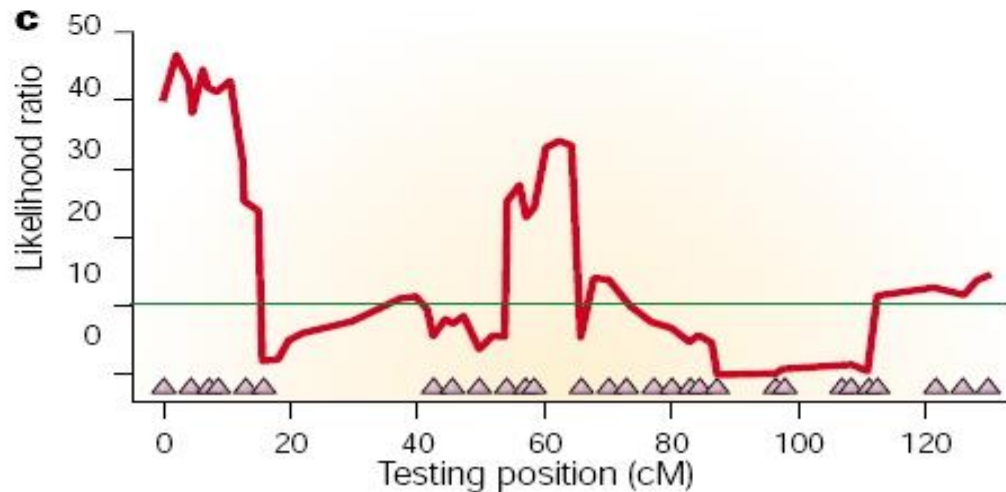
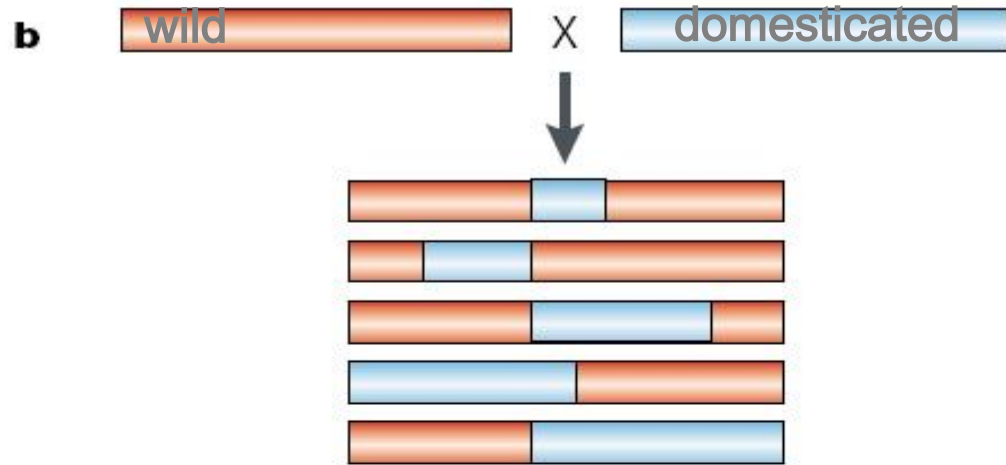
## domestication

associated in some examples with selection of few key genes affecting apical dominance, seed shattering, fruit or seed size, Most examples known affect regulators/transcription factors rather than enzymes

## diversification

associated with many genes normally associated with changes in coding sequence including enzymes

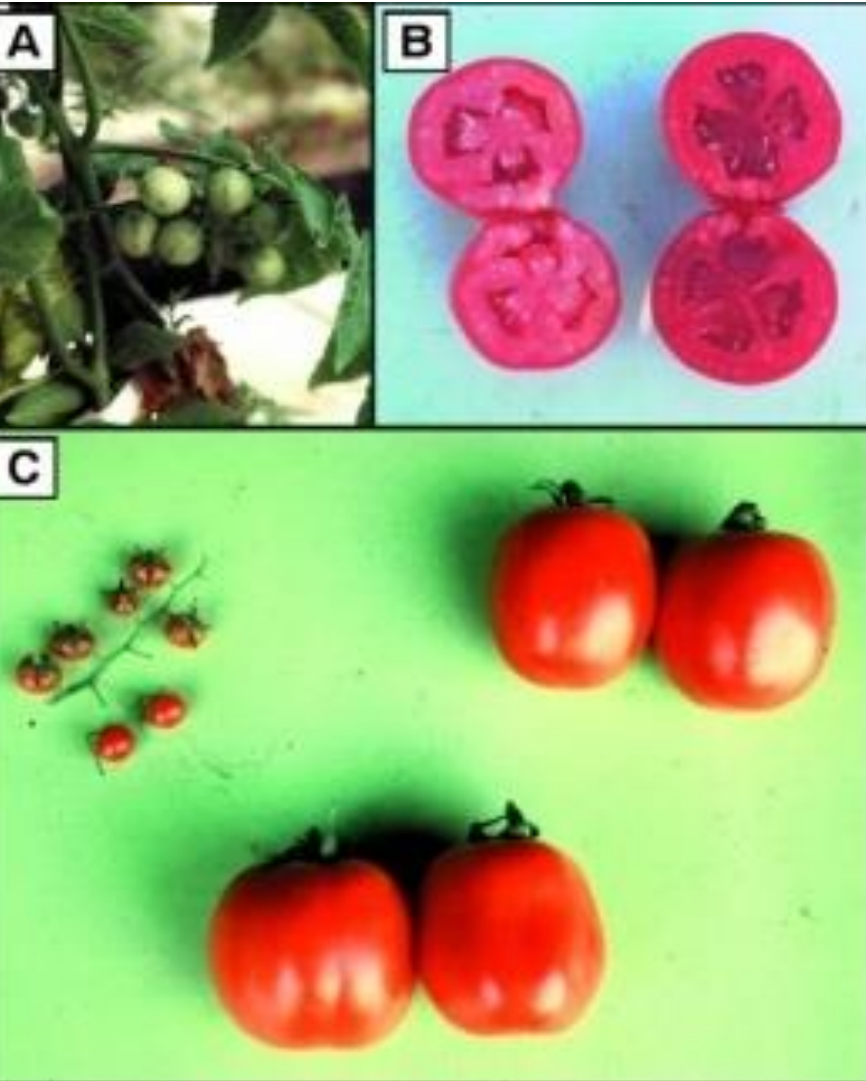
# genetic determinants of crop phenotypes



## Quantitative traits:

- several genes contribute independently to the trait
- OR
- a single gene accounts for most of the trait but its expression is influenced by other loci or by environmental factors
- QTL analysis requires genetic markers to differentiate the parental chromosomes
  - single nucleotide polymorphism
  - Insertion deletions
  - microsatellites
- Segregation is revealed in the F2
  - Likelihood ratio assesses probability that a marker is associated with a QTL affecting the trait

# the mysteries of crop diversification (= breeding = artificial evolution)



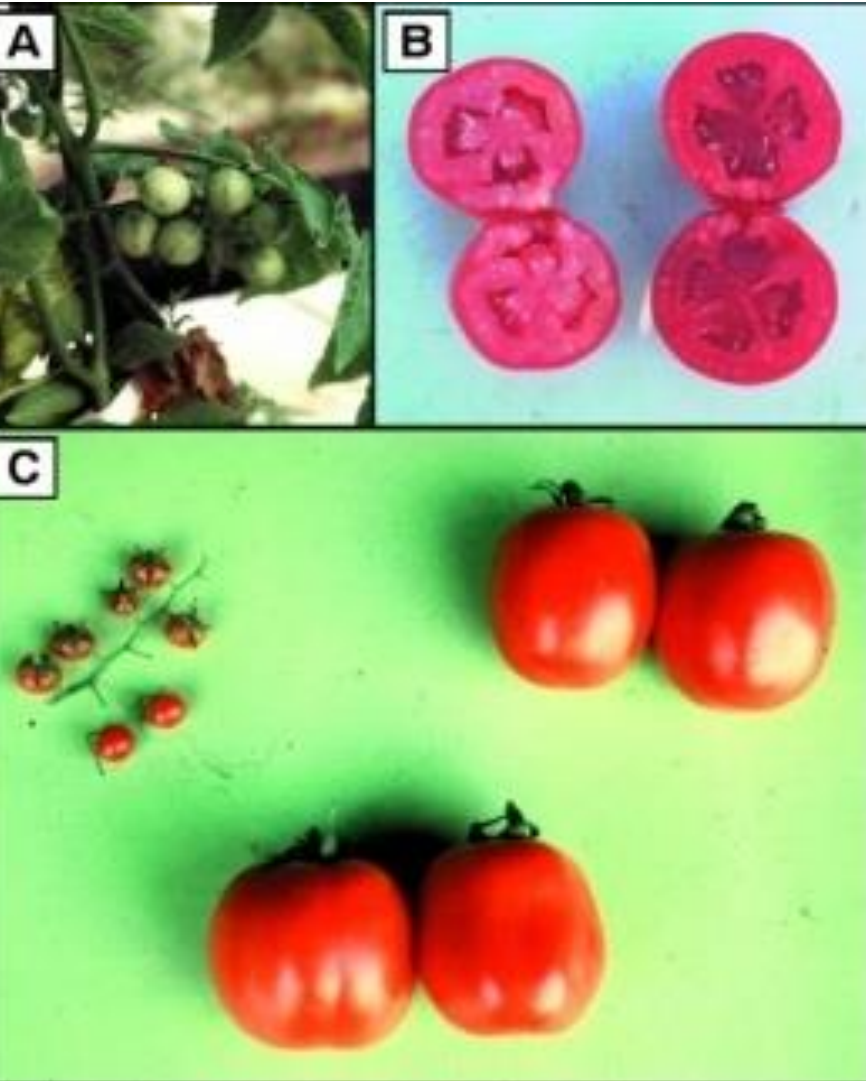
**progressive improvement  
beyond the range of the  
parents**

- transgressive segregation
- epistasis

**inability to account for all  
variation at QTL**

- multiple minor loci
- ????

# epigenetics ?????



**progressive improvement  
beyond the range of the  
parents**

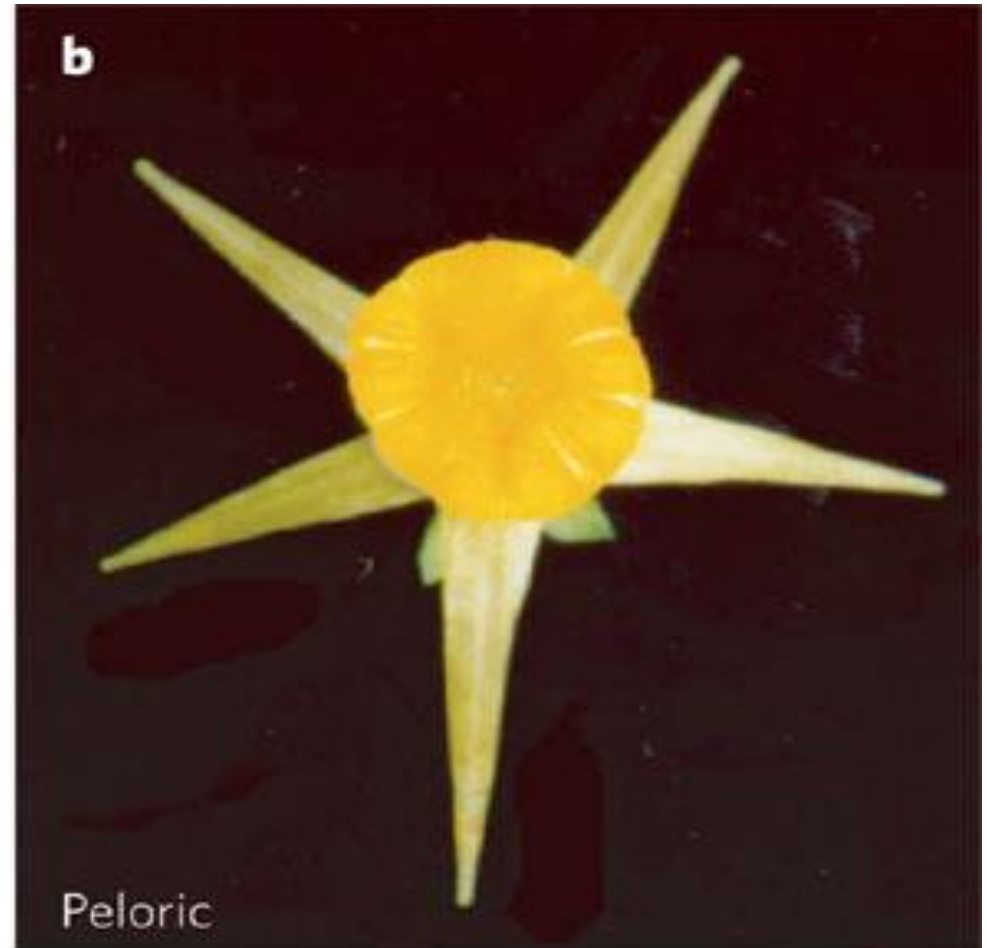
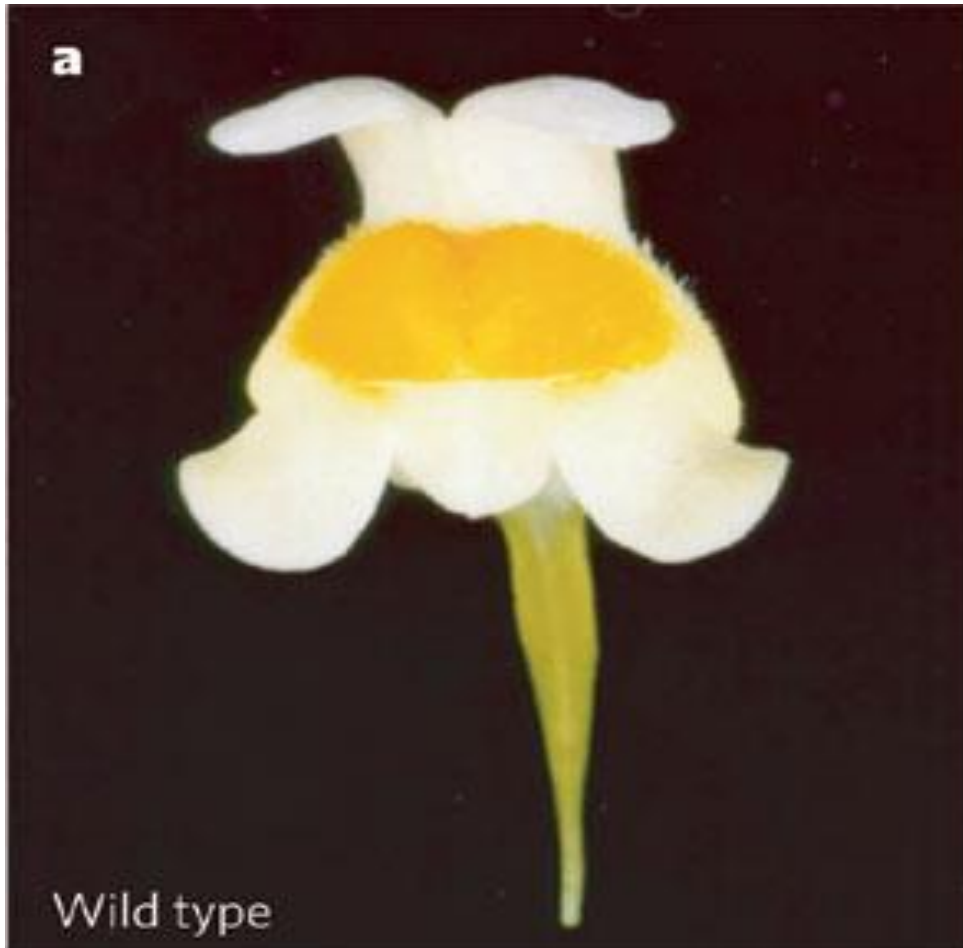
- transgressive segregation
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**inability to account for all  
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- multiple minor loci
- ?????

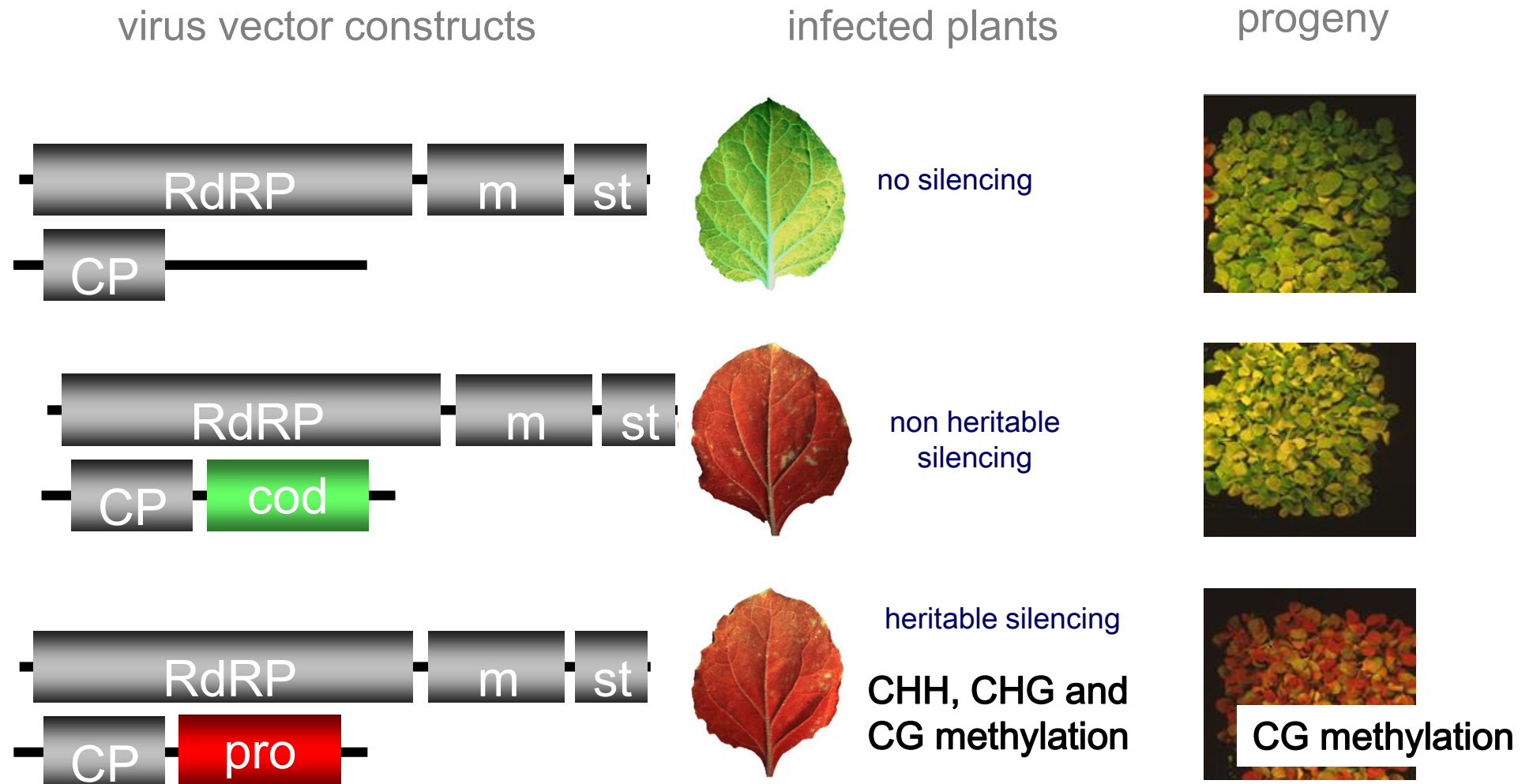


# ***peloria/cycloidea* affects the shape of toadflax flowers (*Linaria*)**



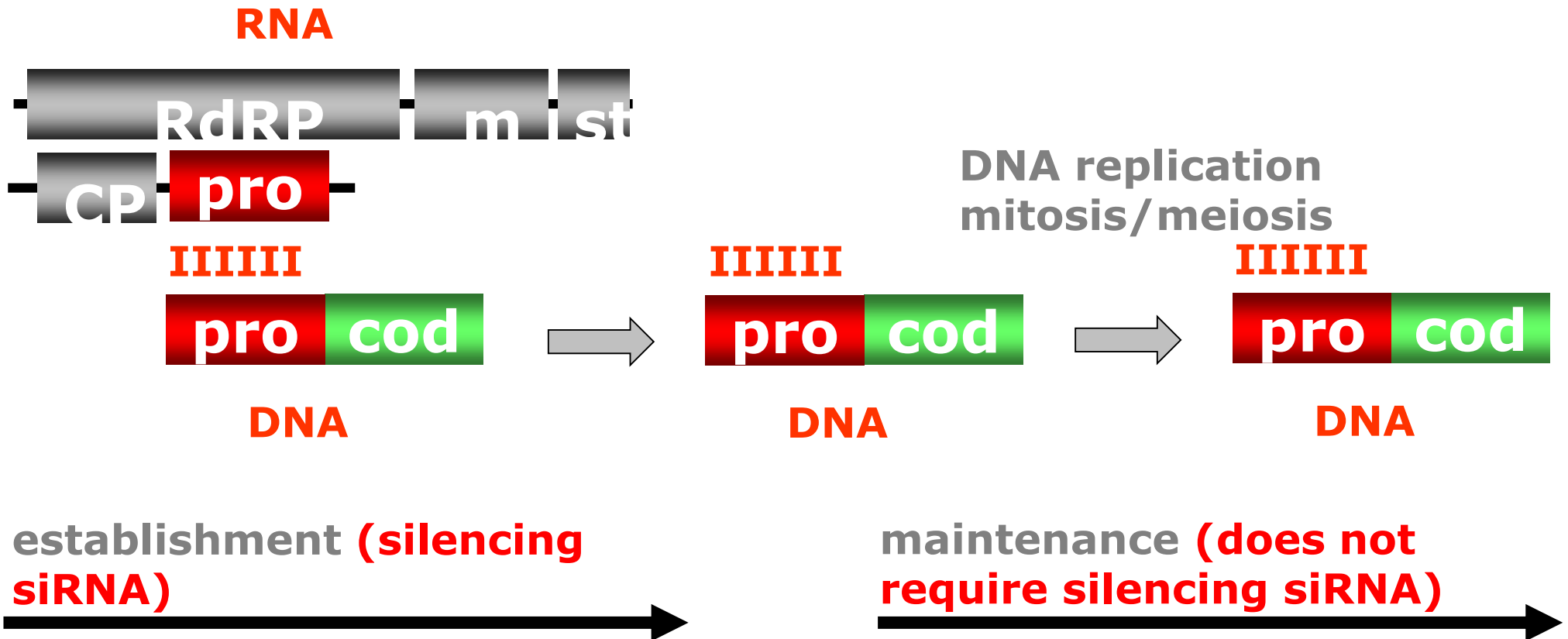
flower shape affected by *cycloidea*  
phenotype changes without differences in DNA sequence

# a very clear transgenerational epigenetic effect: virus-induced gene silencing = VIGS



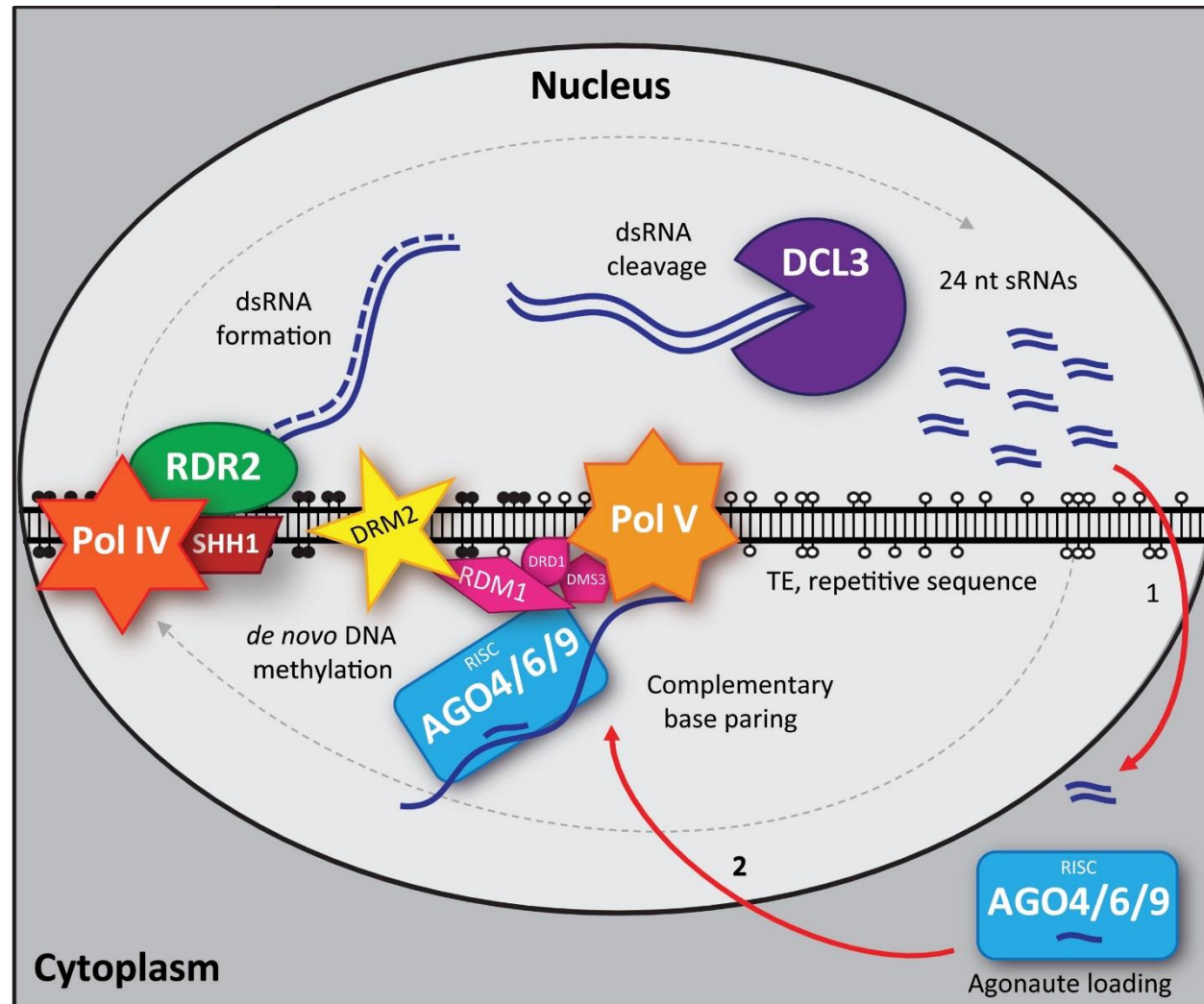
**heritable promoter silencing is dependent on promoter DNA methylation**

# separate establishment and maintenance in heritable epimutation

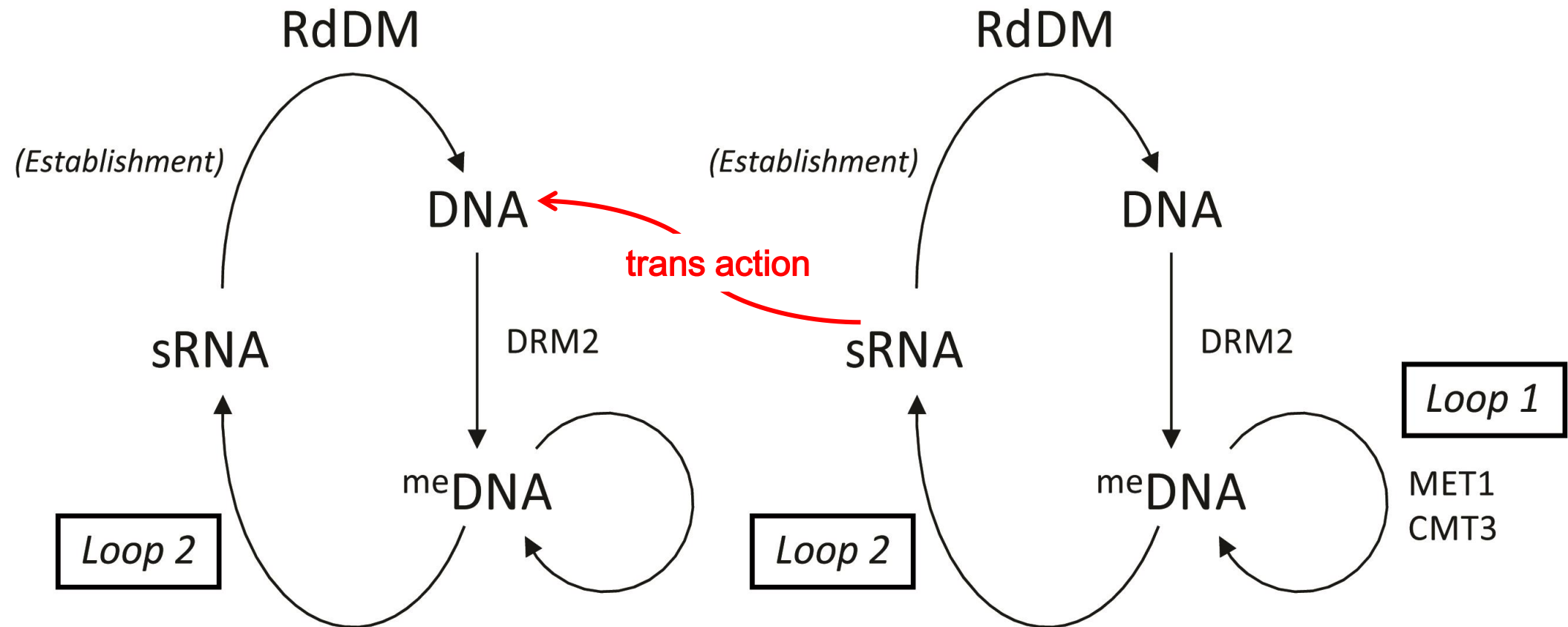




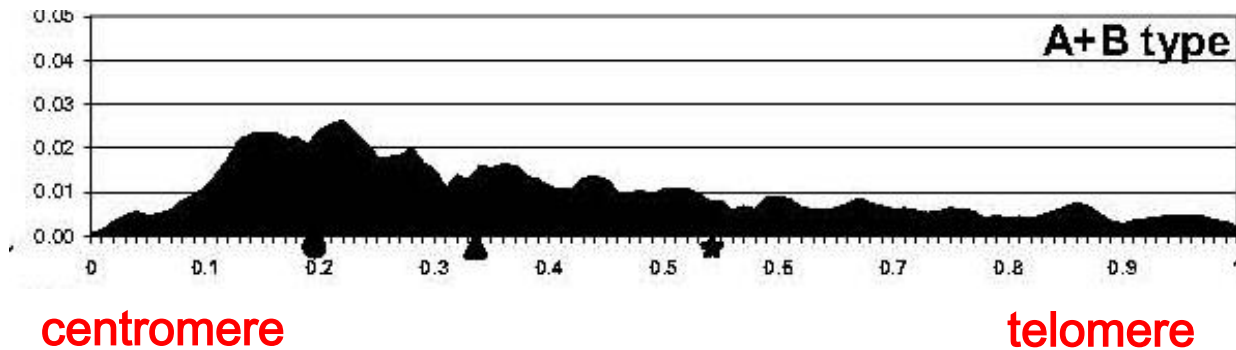
# establishment – a self reinforcing feedback system – RNA dependent amplification of silencing



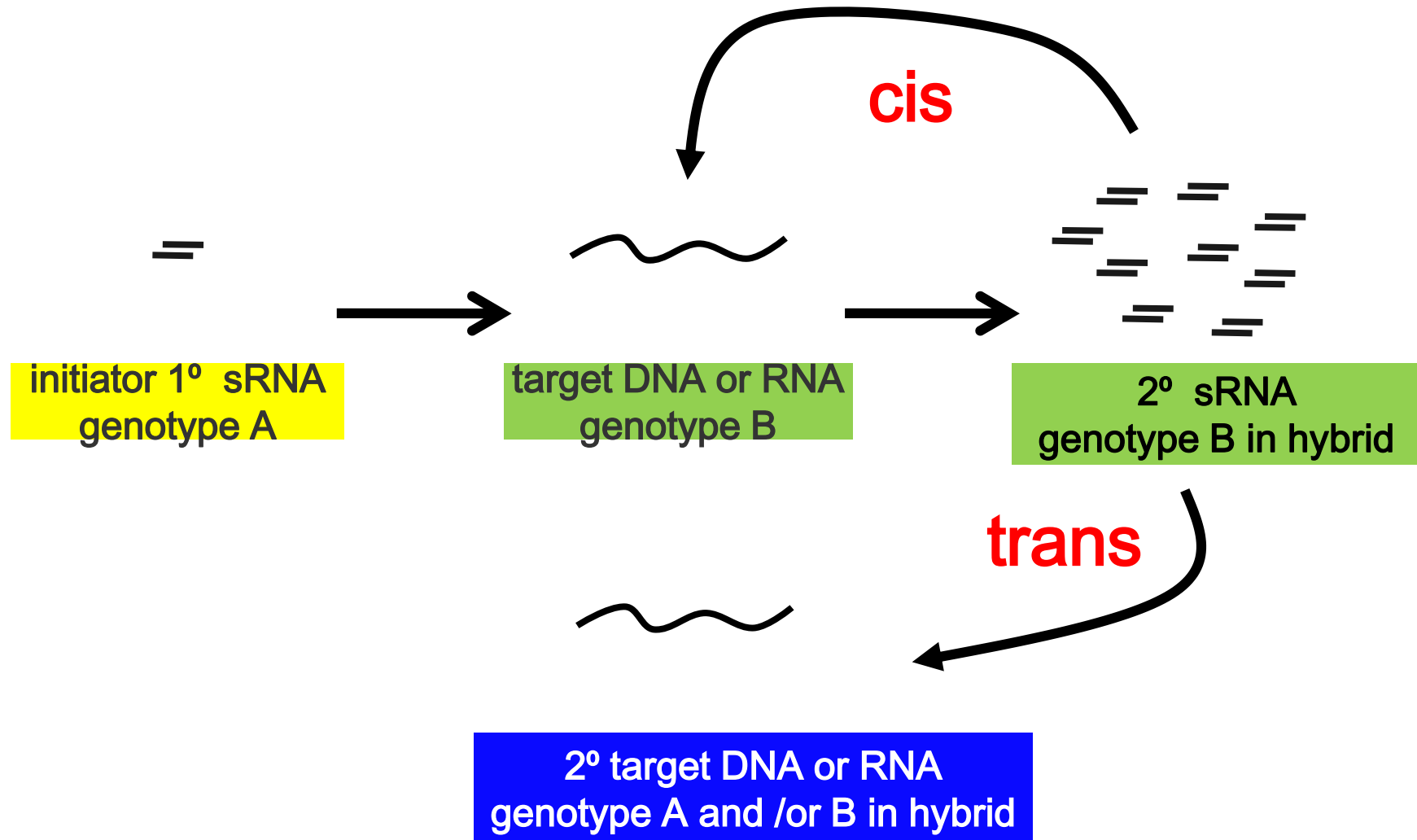
# maintenance of RdDM – a self reinforcing system with amplification and heritability



**thousands of sRNA loci are distributed throughout the chromosomes of Arabidopsis and associated with transposable elements**



# sRNA and genome interactions ?

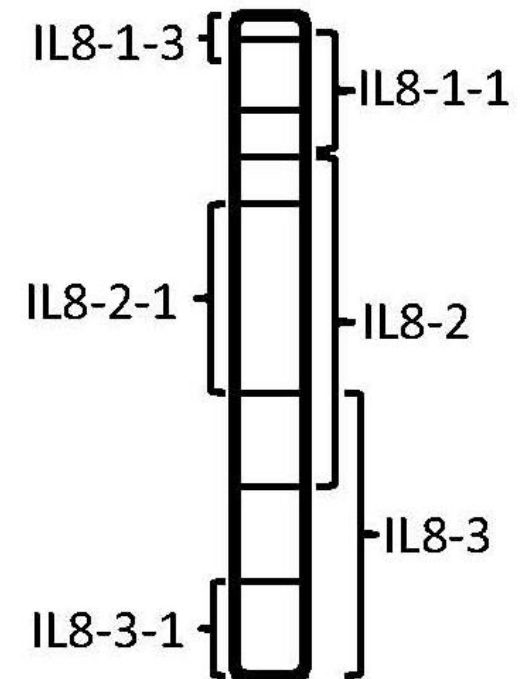


# sRNA and genome interactions in tomato and relatives

*S. lycopersicum*

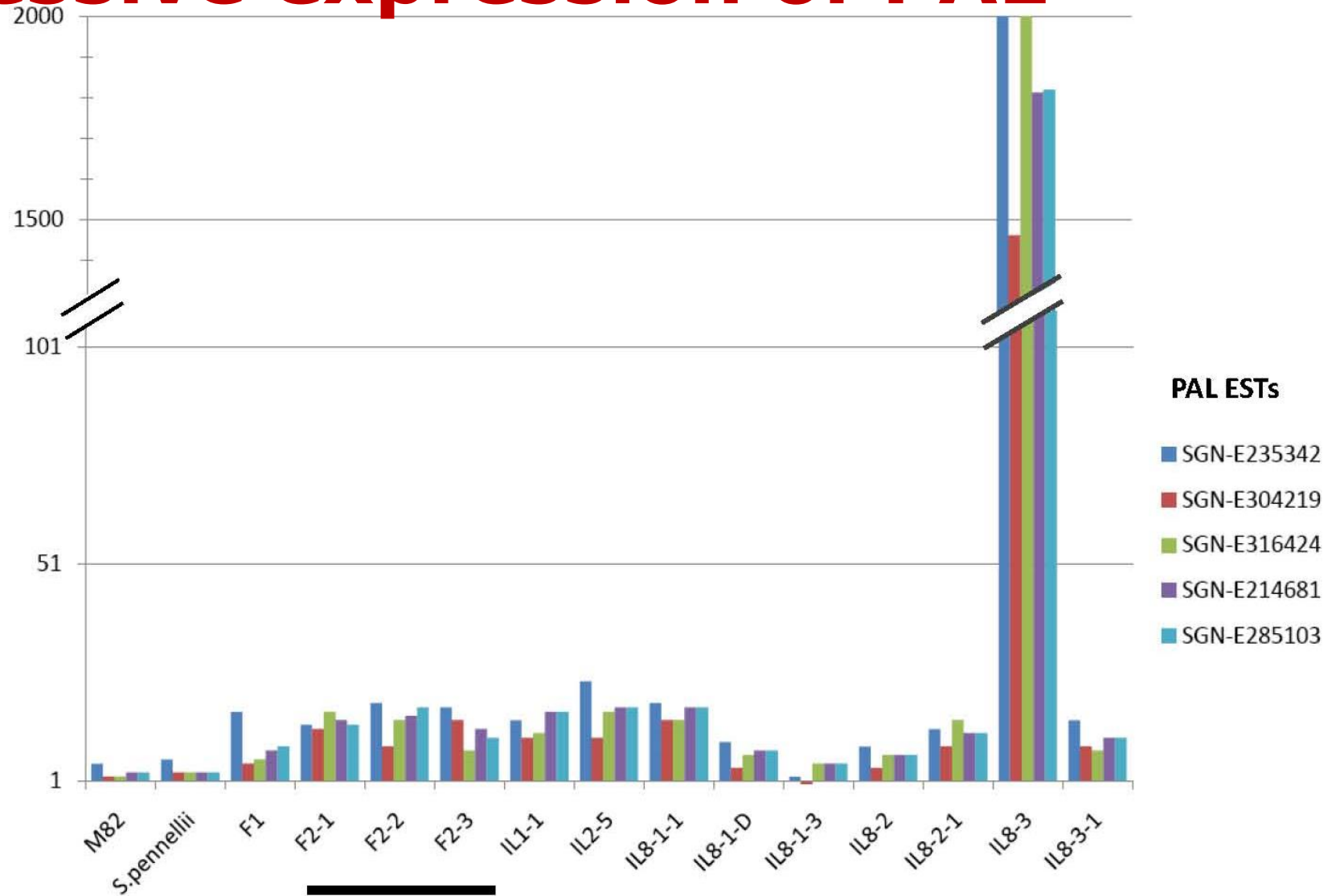


*S. pennellii*





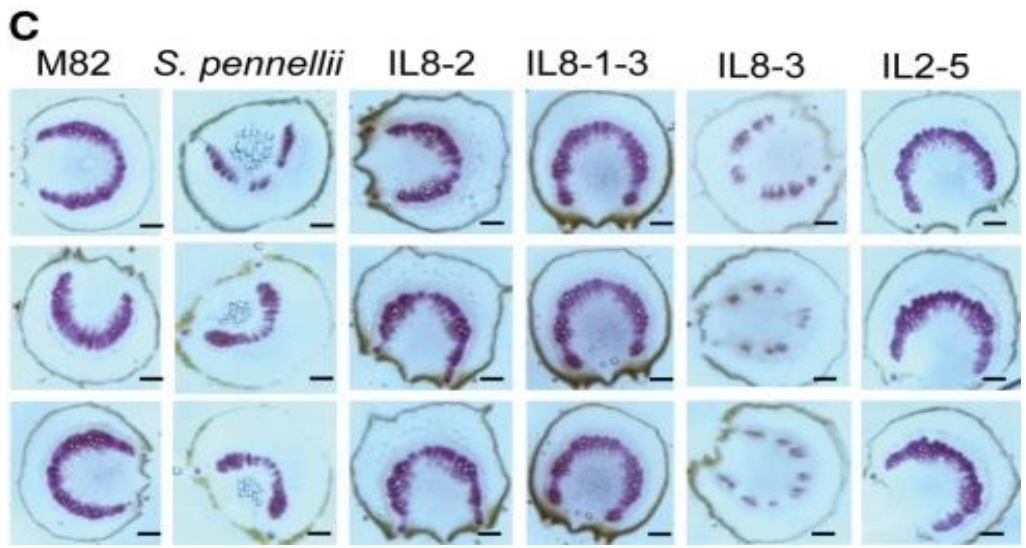
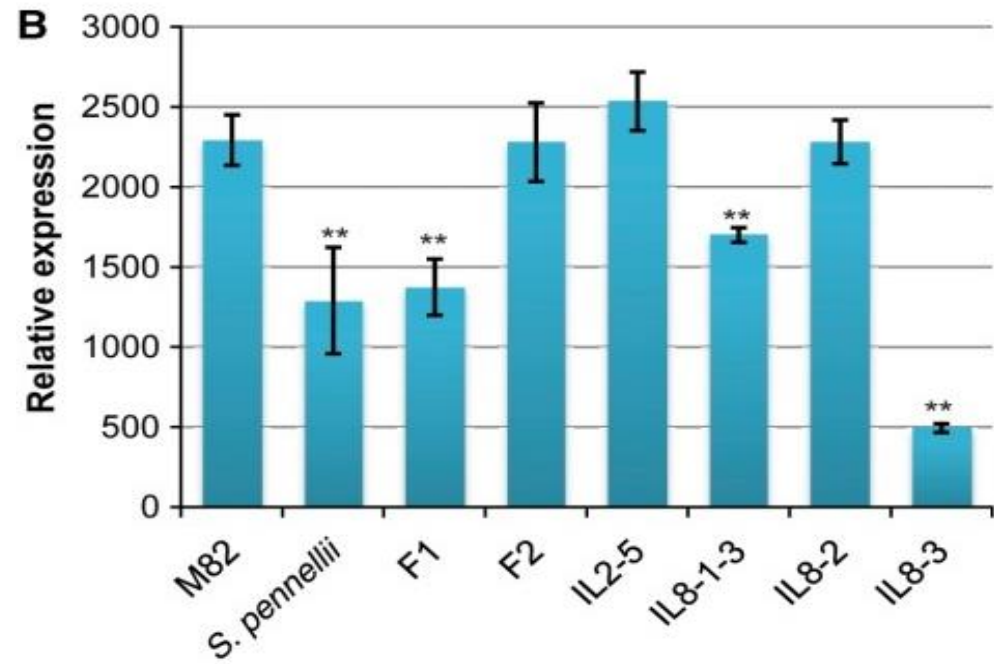
# transgressive expression of PAL siRNAs



representation of of EST siRNA in Illumina datasets – phenylalanine ammonia lyase  
in particular

Padubidri Shivaprasad

# .....and transgressive silencing of PAL

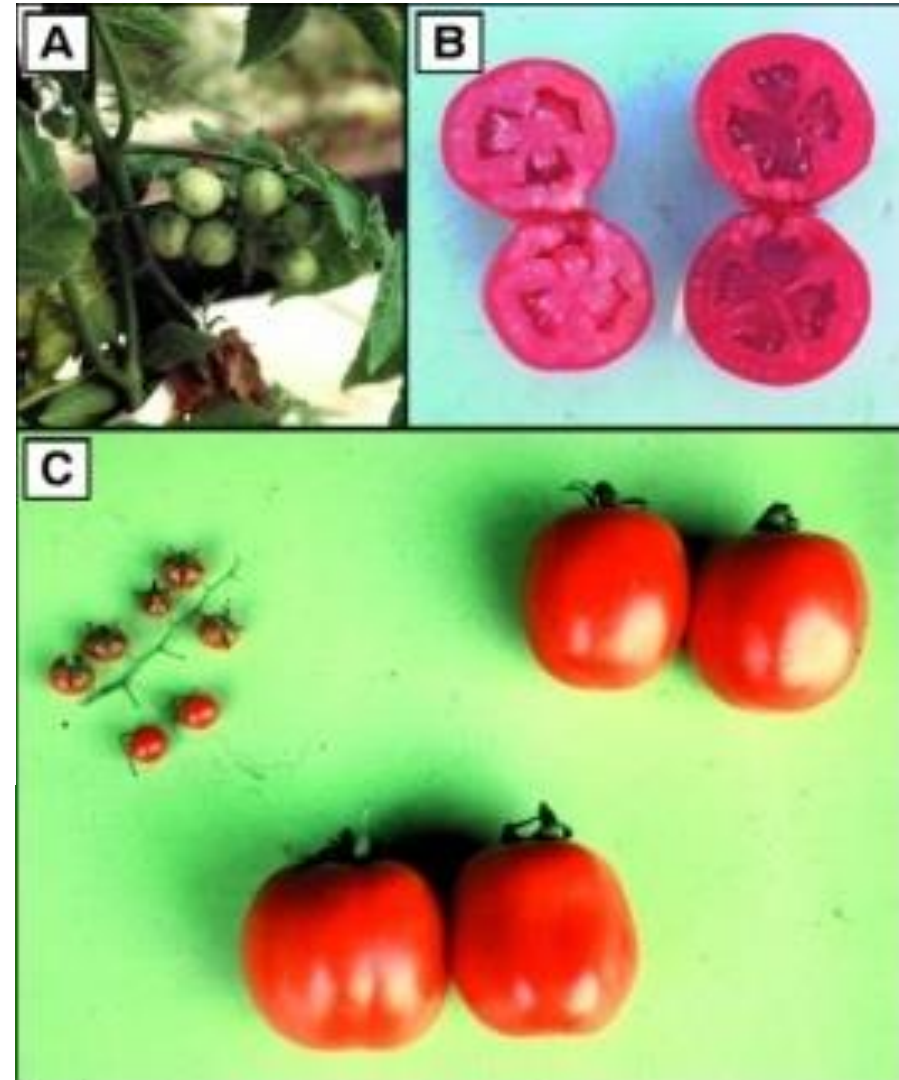


Phloroglucinol staining for total lignin

# epigenetics in (interspecific) hybrids

Tanksley and McCouch  
Science 277 (1997) 1063-1066

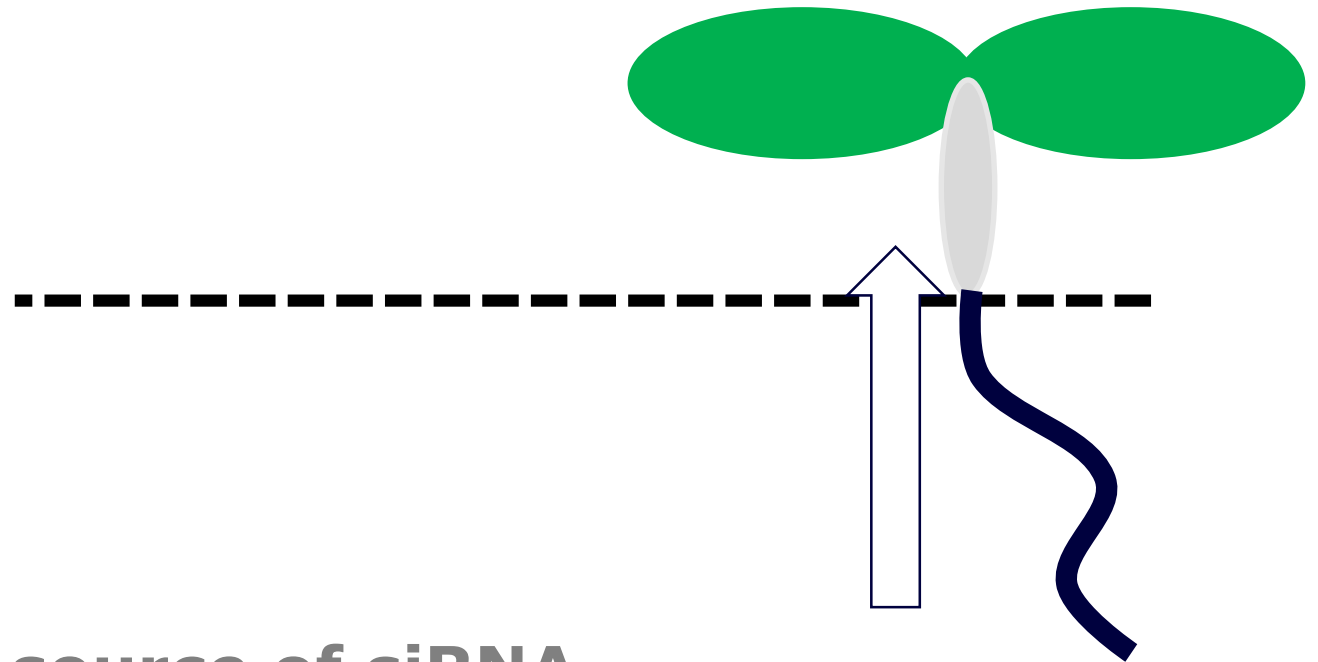
- transgressive gene expression in interspecific *Solanum* hybrids associated with RNA and epigenetics
- hybridisation may be important not only because it initiates new heritable patterns of gene expression
- heritable variation between varieties or individuals will be due to epigenetic as well as genetic differences ←



# siRNA mobility for initiation of epigenetic change

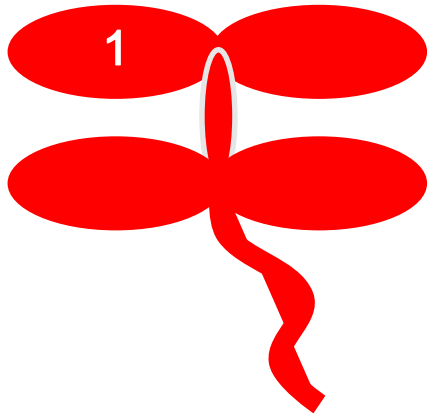


recipient of siRNA

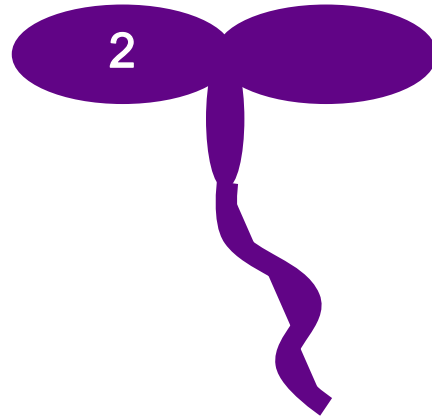


source of siRNA

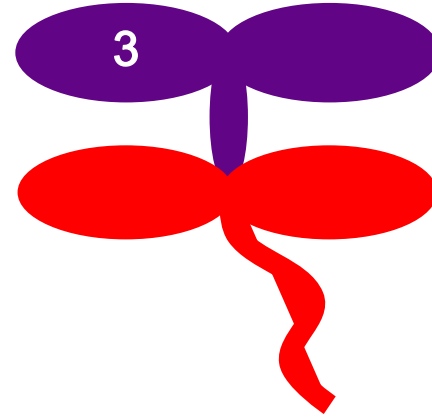
# how to make an epigenetically modified plant



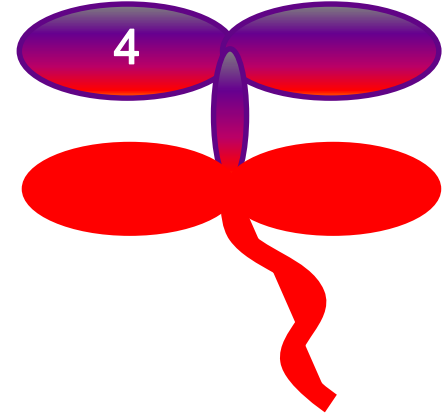
variety producing silencing signal: target gene affecting seed quality



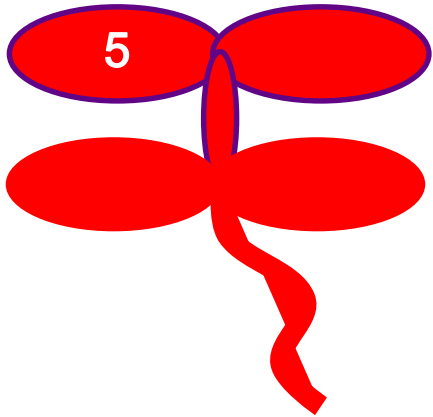
variety with reduced seed quality due to target gene overexpression



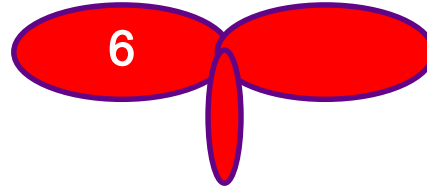
grafted chimaera



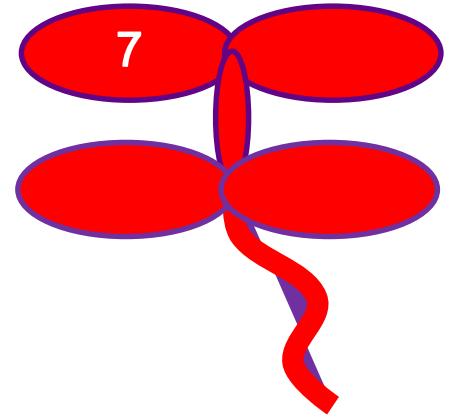
spread of silencing signal across graft union



Full silencing of target gene in grafted plant



removal of stock

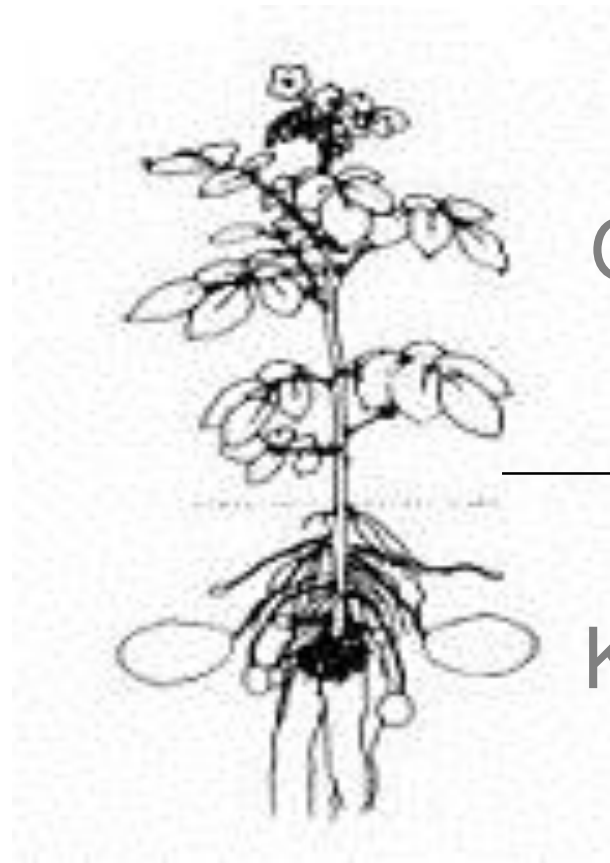
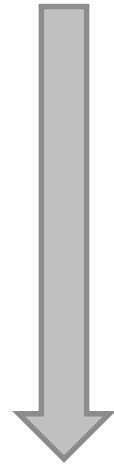


regeneration of root and persistent silencing



# TGS of GBSSI in wild type tuber by mobile sRNAs using a GBSSI silenced transgenic scion as a grafting partner

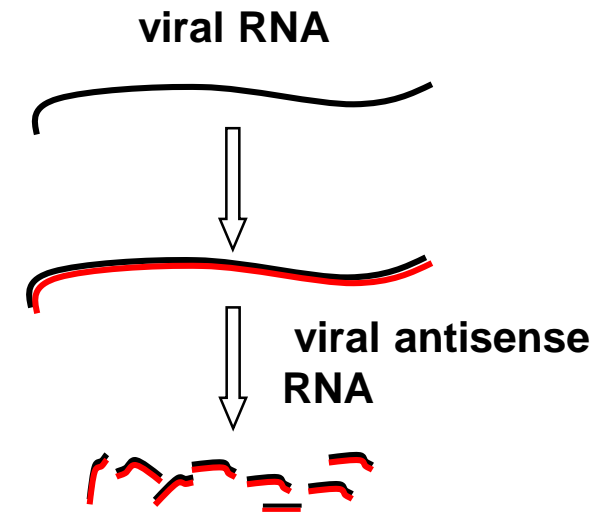
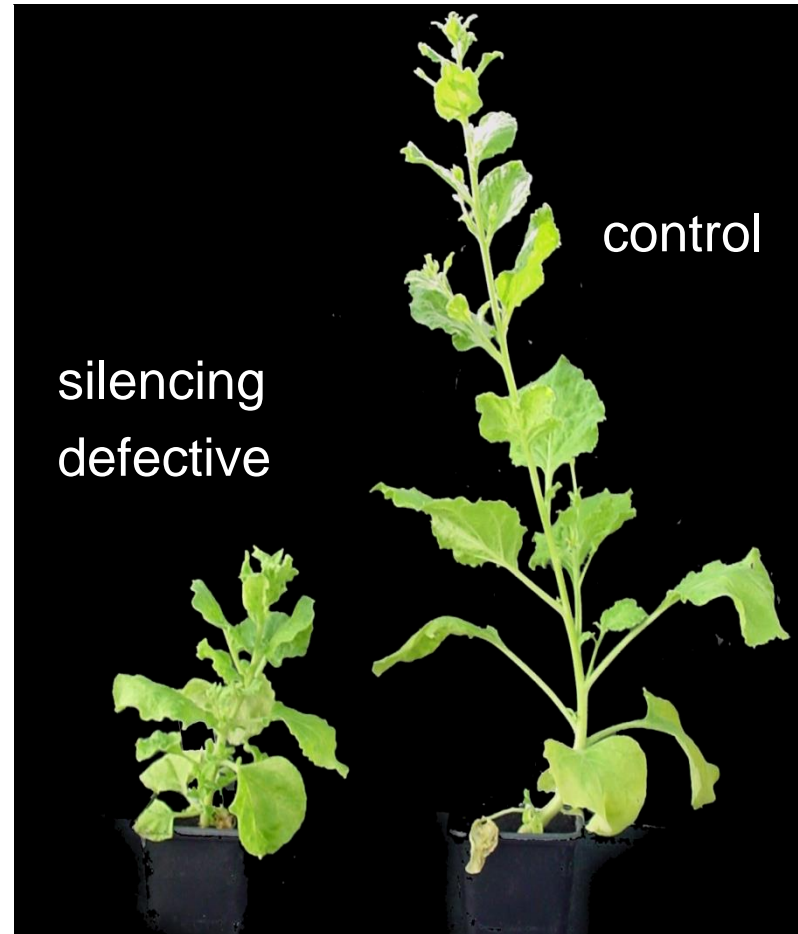
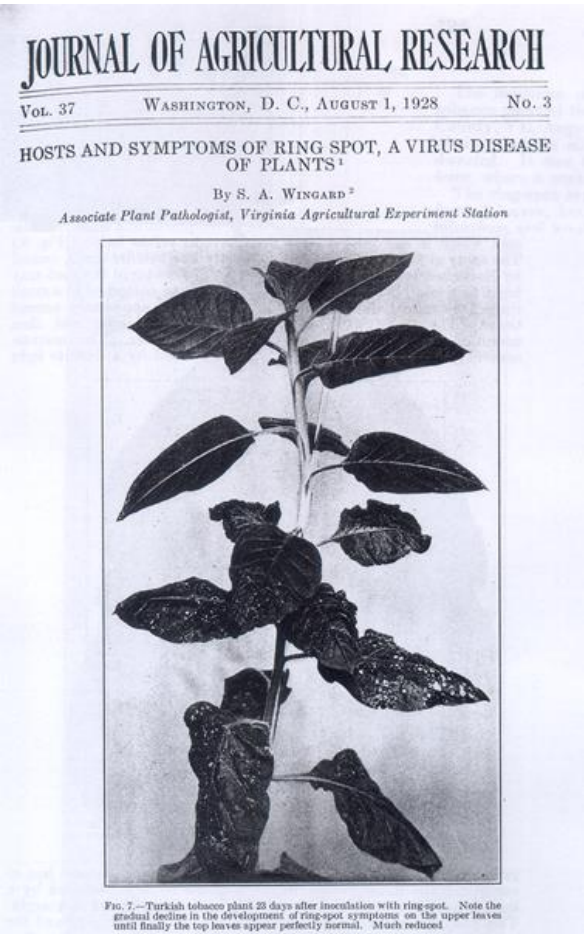
movement of  
the silencing  
signal



GBSSI silencer (s)

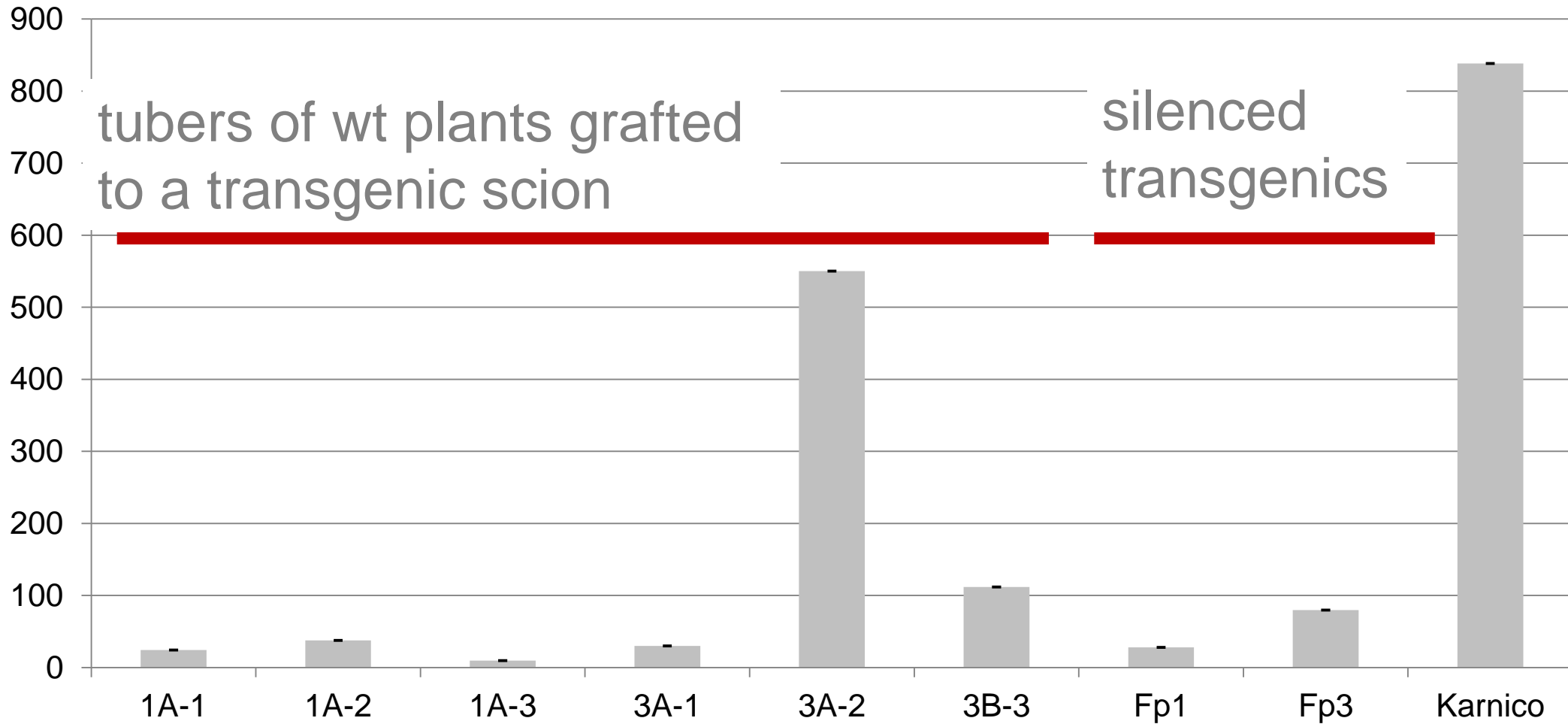
Karnico (wt)

# RNA silencing is a virus defense mechanism



# TGS of GBSS by grafting

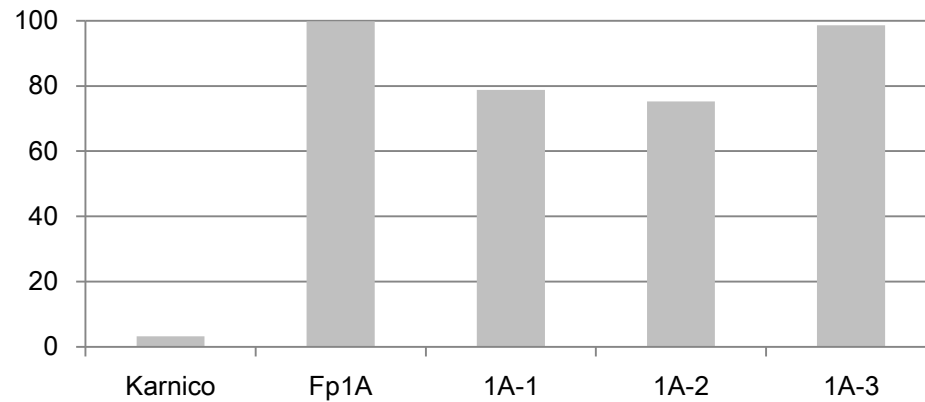
GBSSI expression level normalized to ubiquitin



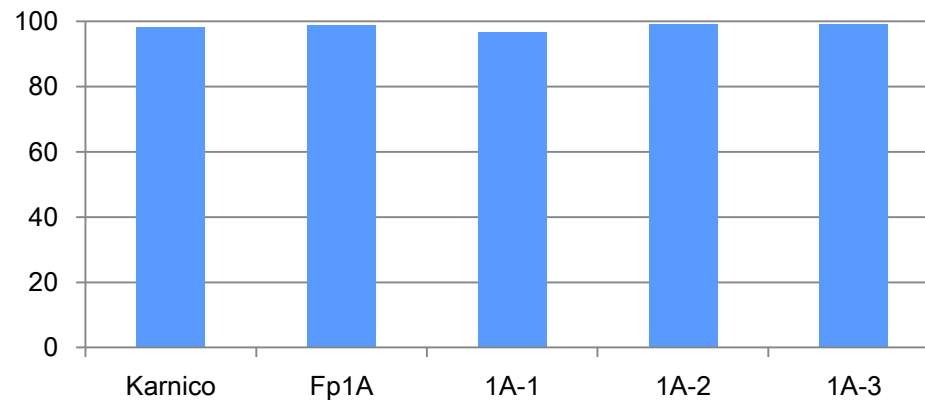
# GBSS promoter methylation by grafting

- + tubers of wt plants grafted to a transgenic scion

Methylation %-Potato GBSSI promoter



Methylation % - Potato Mite (+ Control)



# targeted epimutation

- **epigenetically modified plants**
- **delivery method for siRNAs – grafting, virus, transgene**
- **factors affecting targeting of promoter (including cis and trans-acting factors)**
- **factors affecting stability of silenced state**



# **epigenetics in plant breeding**

- **epigenetic marks affect gene expression**
  - heritable
  - induced by hybridisation
  - other types of genome shock
- **plant breeders may have been unwittingly selecting for epiQTL**
- **understanding of epigenetics may allow efficient exploitation of epiQTL in conventional breeding and Epigenetically Modified Plants**

Attila Molnar  
Charles Melnyk  
Donna Bond



Padubidri Shivaprasad  
Quentin Gouil

SIROCCO

Silencing RNAs: organizers and coordinators of complexity in eukaryotic organisms

Krys Kelly  
Tom Hardcastle

The  Charitable Foundation



UNIVERSITY OF  
CAMBRIDGE

Department of  
Plant Sciences



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[www.plantsci.cam.ac.uk/research/davidbaulcombe.html](http://www.plantsci.cam.ac.uk/research/davidbaulcombe.html)

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