

*Insights into the genetic control of tomato fruit ripening.....
and how they might be leveraged for grape improvement???*

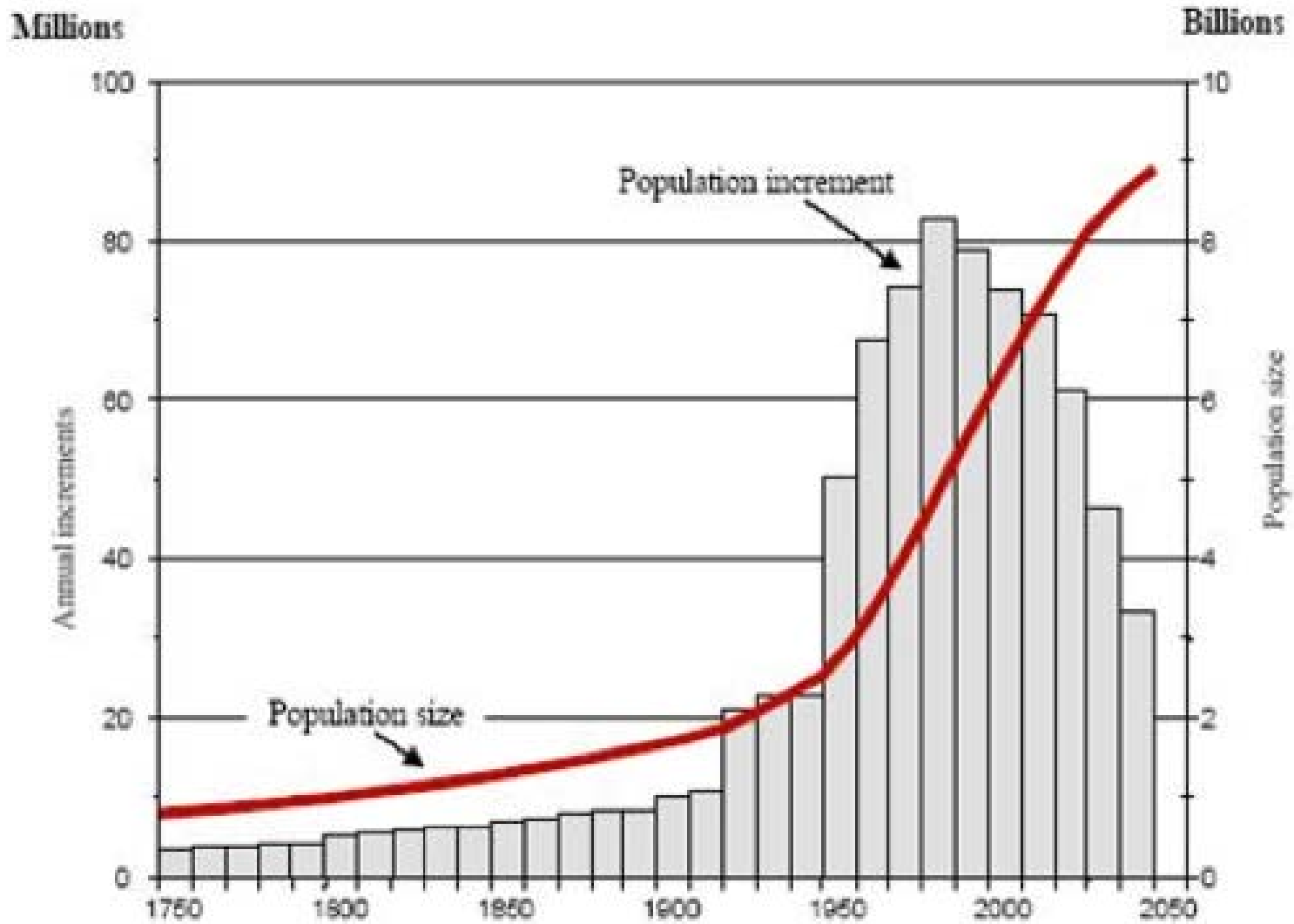


Dry Fruit



Fleshy Fruit





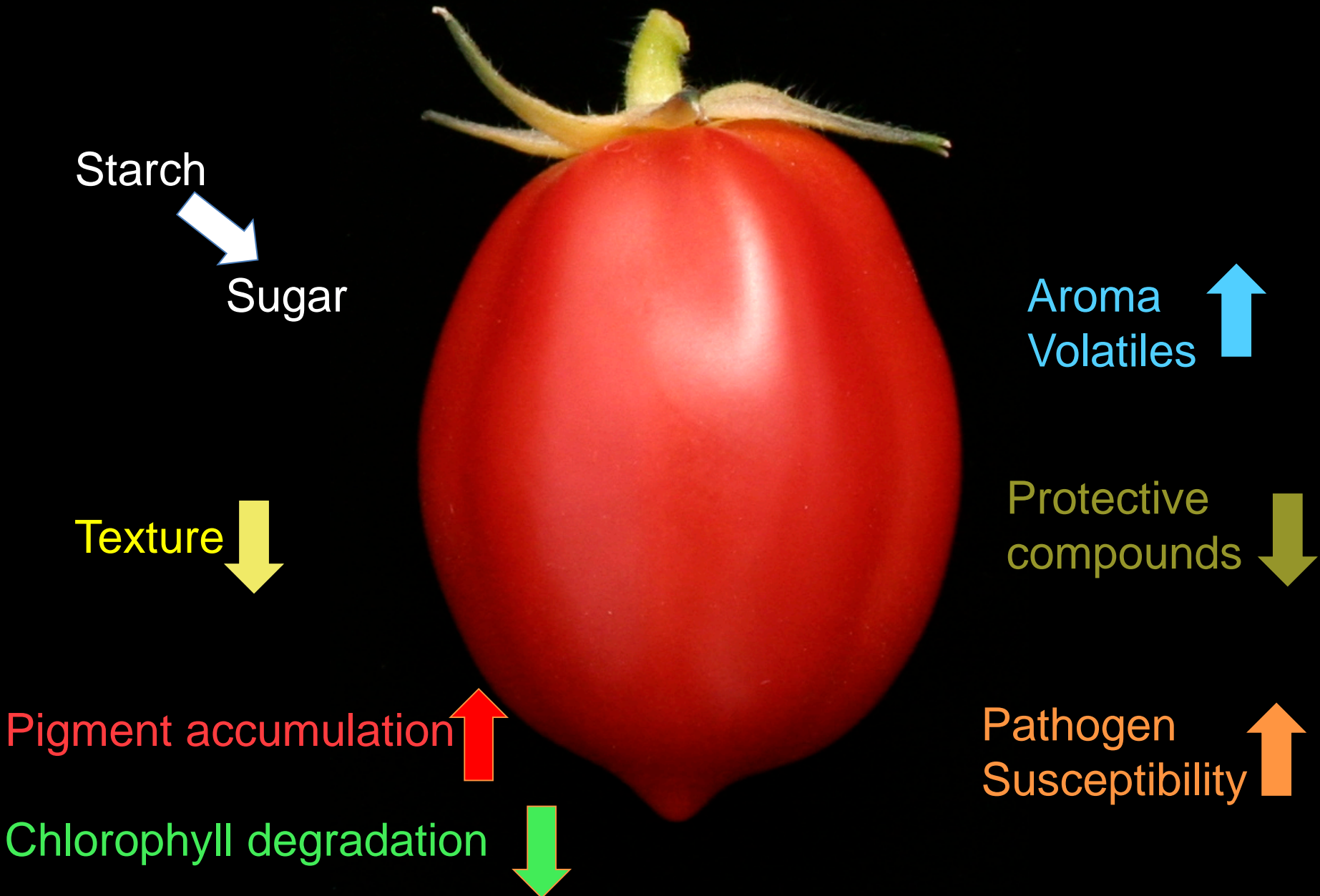


Food security is a function of production, quality and decay





Ripening is the coordination of numerous biochemical processes and their underlying pathways



Ripening is the coordination of numerous biochemical processes and their underlying pathways

Starch
↓
Sugar



Texture
↓

Aroma
Volatiles ↑

Protective
compounds ↓

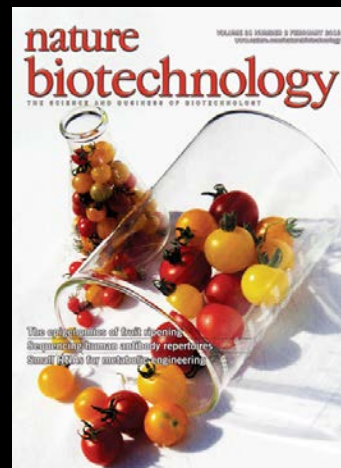
Pigment accumulation ↑

Chlorophyll degradation ↓

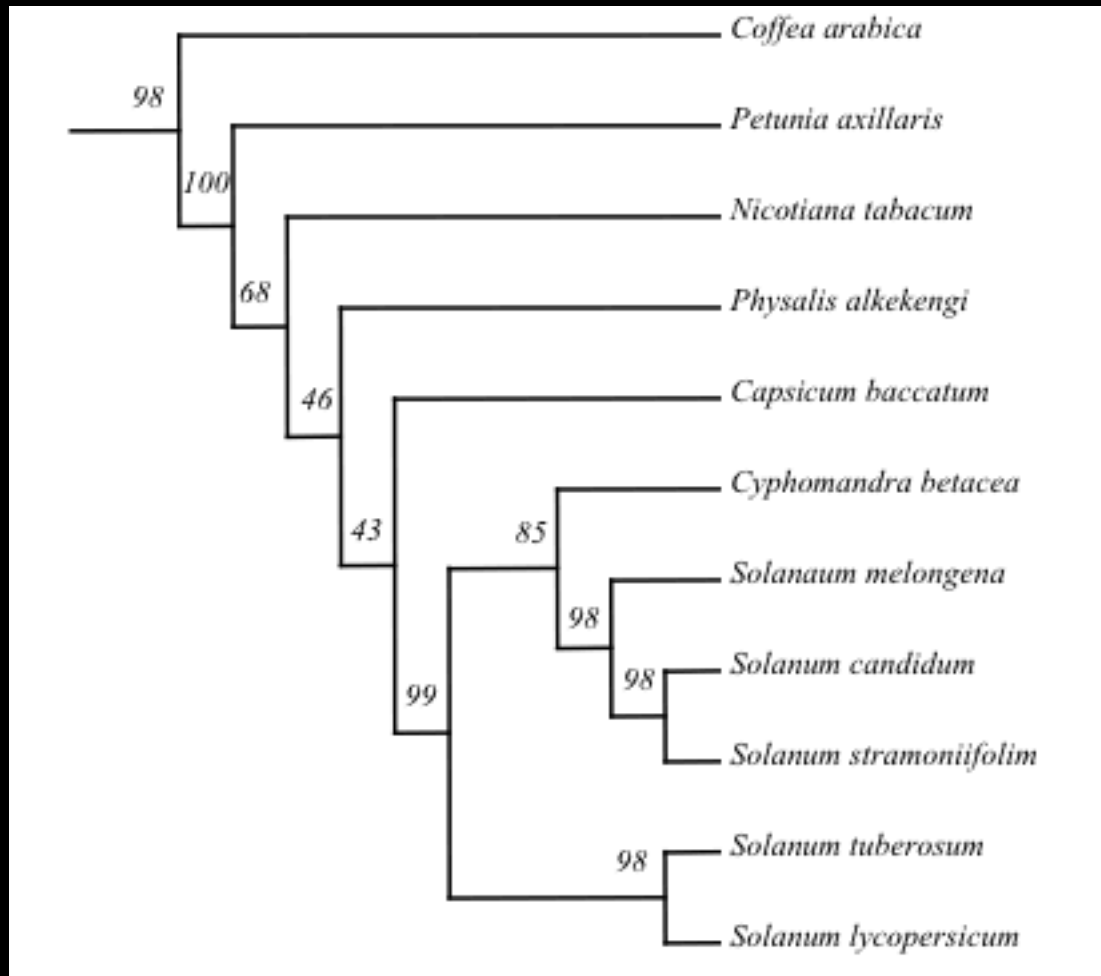
Pathogen
Susceptibility ↑

Tomato as a Model System

- genetically well characterized
- self-pollinating and easily out-crossed
- easily propagated and maintained
- large selection of compatible germplasm
- easily transformed
- numerous ripening mutants
- qualitatively and quantitatively dramatic ripening phenotype
- high marker density genetic map
- proliferation of genomics tools
- genome sequence
- Methylome dynamics



Tomato and its relatives.....



← System 1 C₂H₄ → ← System 2 C₂H₄ →



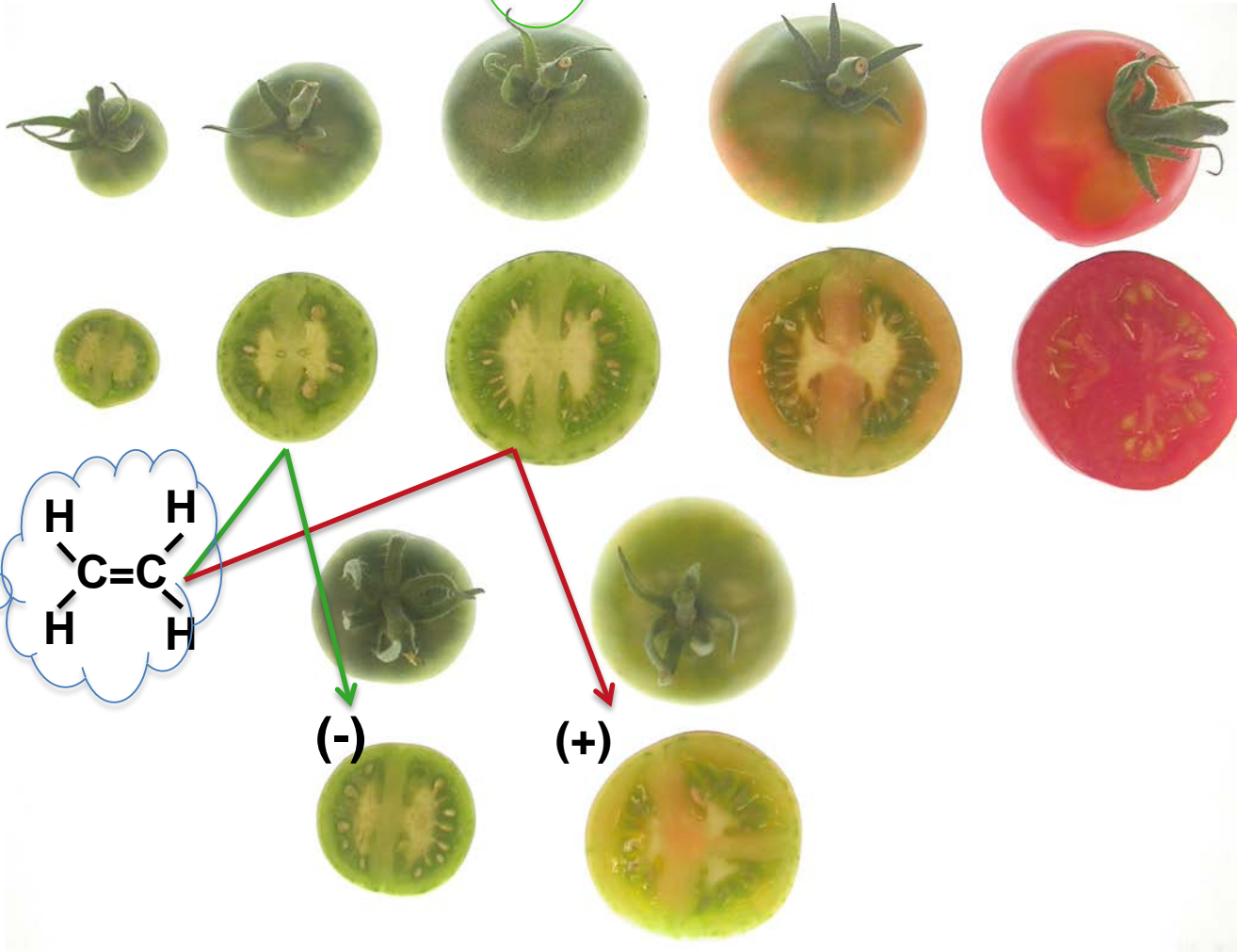
IM

LIM

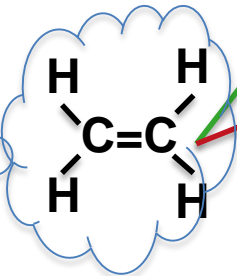
MG

BR

RR



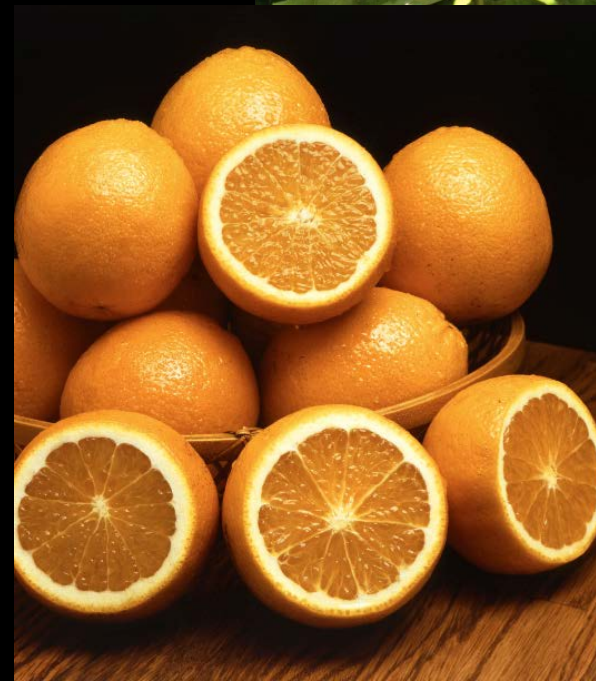
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(-)

(+)

Seed development is not required for maturation/ripening



Parthenocarpic fruit ripen but.....

- tomato *pat* mutants ripen more slowly**
- seeds deploy abscisic acid (ABA) as a regulator of dormancy**
- ABA increases in tomato and other fruit prior to ethylene**
 - exogenous ABA promotes ripening**
 - tomato ABA mutants ripen more slowly**
- there may be an evolutionary advantage for individual fruit to ripen even if pollination was not successful**

First ripening transcription factor cloned was RIN-MADS



Prof. Henry Munger
1916 - 2010



ripening-inhibitor (rin)

Hybrid *Rin/rin* tomatoes are the basis of much extended shelf-life fresh market tomato production, are seeing some use in processing varieties and are the basis of many concerns regarding fresh tomato quality and flavor.

- they simply are not fully ripe.



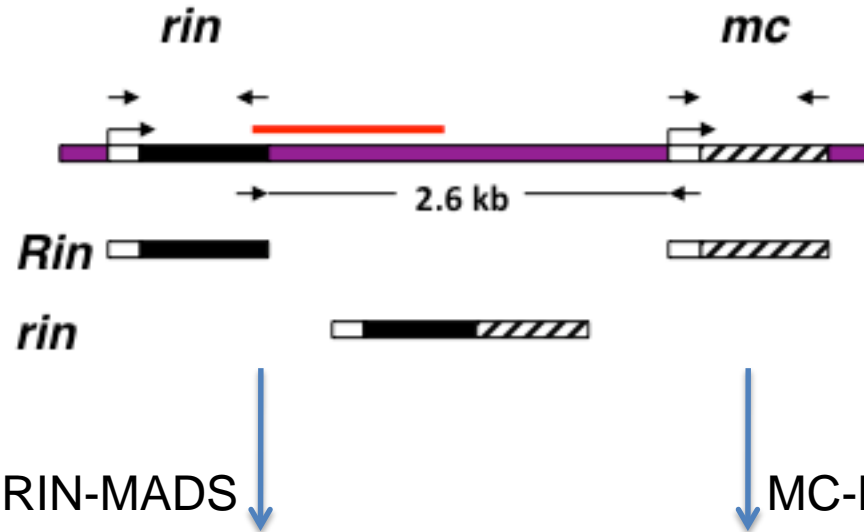
Randy Gardner, NC State

The *rin* locus encodes a *SEPELATA* clade MADS-box protein

rin/rin

Rin/Rin

(transcription factor)



(*SEP*)



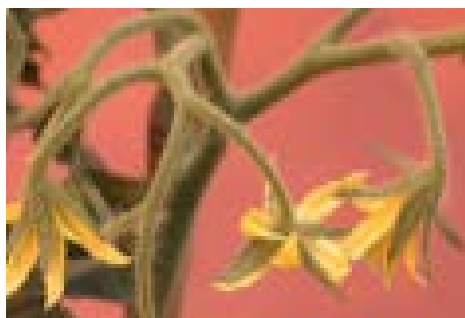
(*AP1*)



Tomato MADS-BOX genes provide insight into inflorescence, flower and fleshy fruit development



J1



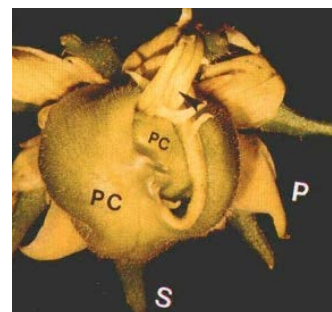
J?



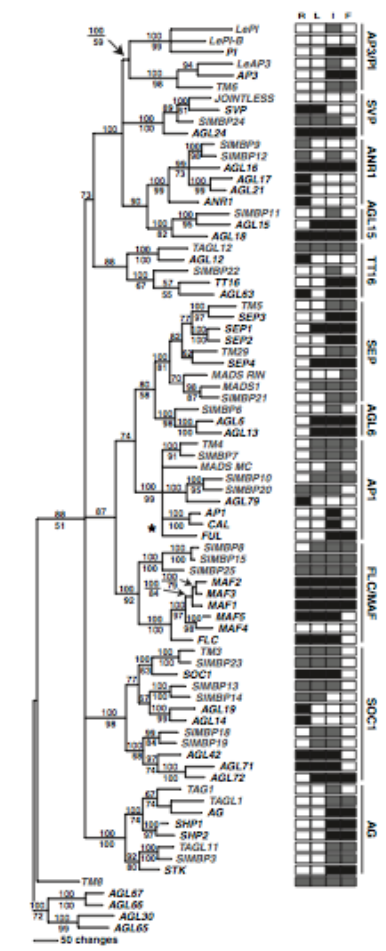
TM29



RIN



TAG1



TAGL1



FF



MC



SEP



FUL1/2



RIN-like MADS-box genes are widely conserved.



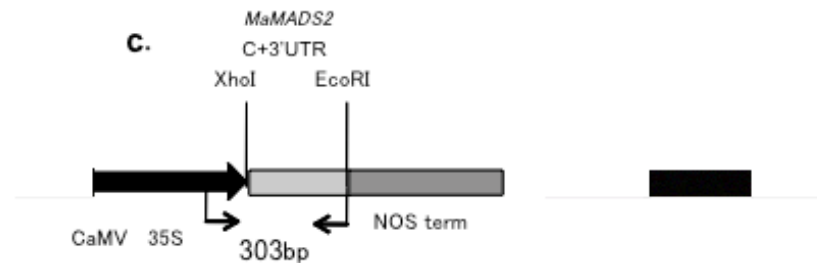
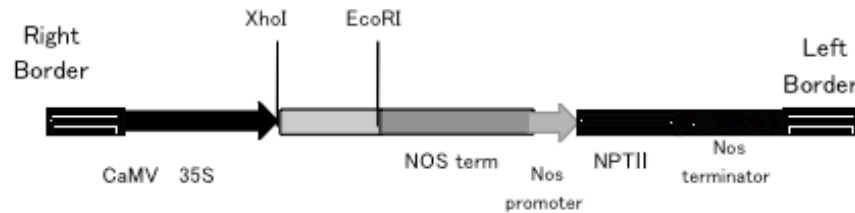
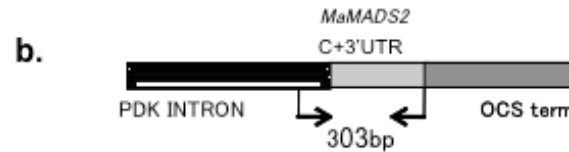
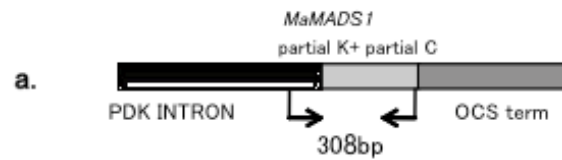
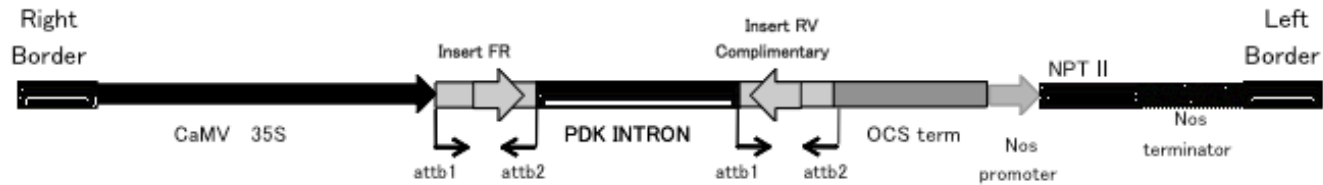
Monocots



Dicots



Constructs for *MaMADS1* and *MaMADS2* repression



Repression of *MaMADS1* or *MaMADS2* inhibits banana ripening

A.



C.



B.



D.



Control

AS-MaMADS2

RNAi-
MaMADS2

Control

RNAi-
MaMADS1

DNA-binding specificity, transcriptional activation potential, and the *rin* mutation effect for the tomato fruit-ripening regulator RIN

Yasuhiro Ito^{1*}, Mamiko Kitagawa², Nao Ihashi¹, Kimiko Yabe¹, Junji Kimbara², Junichi Yasuda¹, Hiroataka Ito², Takahiro Inakuma², Seiji Hiroi² and Takafumi Kasumi³

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The Tomato MADS-Box Transcription Factor RIPENING INHIBITOR Interacts with Promoters Involved in Numerous Ripening Processes in a COLORLESS NONRIPENING-Dependent Manner^{1[W][OA]}

Catherine Martel, Julia Vrebalov, Petra Tafelmeyer and James J. Giovannoni*

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Planta (2012) 235:1107–1122

DOI 10.1007/s00425-011-1561-2

ORIGINAL ARTICLE

Direct targets of the tomato-ripening regulator RIN identified by transcriptome and chromatin immunoprecipitation analyses

Masaki Fujisawa · Yoko Shima · Naoki Higuchi · Toshitsugu Nakano · Yoshiyuki Koyama · Takafumi Kasumi · Yasuhiro Ito

Single-base resolution methylomes of tomato fruit development reveal epigenome modifications associated with ripening

Silin Zhong, Zhangjun Fei, Yun-Ru Chen, Yi Zheng, Mingyun Huang, Julia Vrebalov, Ryan McQuinn, Nigel Gapper, Bao Liu, Jenny Xiang, Ying Shao & James J Giovannoni

[Affiliations](#) | [Contributions](#) | [Corresponding authors](#)

Nature Biotechnology 31, 154–159 (2013) | doi:10.1038/nbt.2462

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Research article

Highly accessed

Open Access

Identification of potential target genes for the tomato fruit-ripening regulator RIN by chromatin immunoprecipitation

Masaki Fujisawa, Toshitsugu Nakano and Yasuhiro Ito*

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National Food Research Institute, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan

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BMC Plant Biology 2011, 11:26

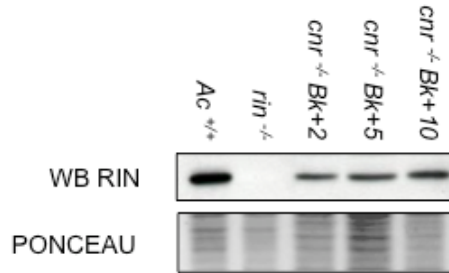
doi:10.1186/1471-2229-11-26

Plant Mol Biol

DOI 10.1007/s11103-013-0071-y

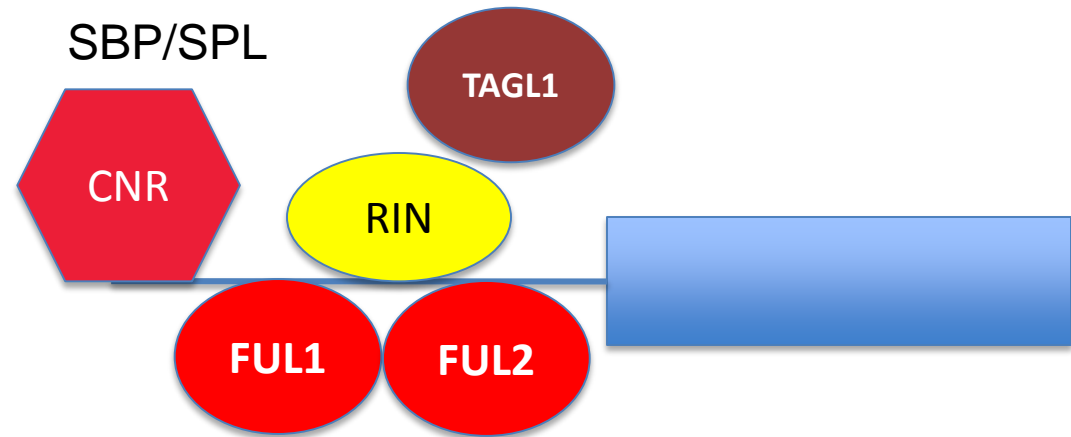
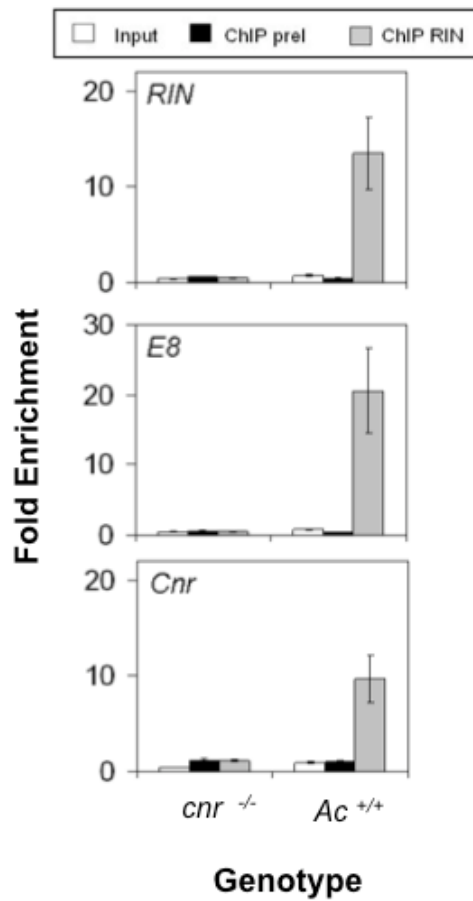
Tomato FRUITFULL homologues act in fruit ripening via forming MADS-box transcription factor complexes with RIN

Yoko Shima · Mamiko Kitagawa · Masaki Fujisawa · Toshitsugu Nakano · Hiroki Kato · Junji Kimbara · Takafumi Kasumi · Yasuhiro Ito

A

CNR is necessary for RIN promoter binding

FUL1/2 and TAGL1 interact with RIN

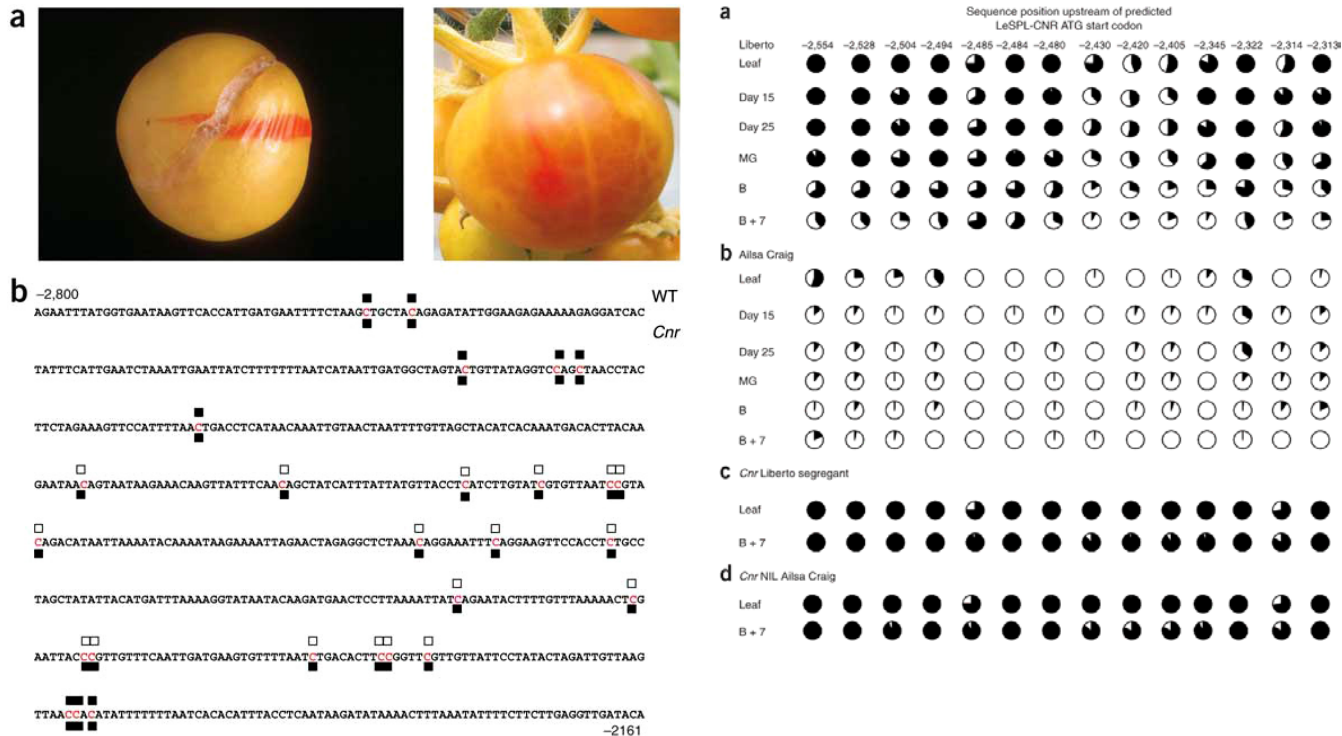
B

CNR is an epi-allele

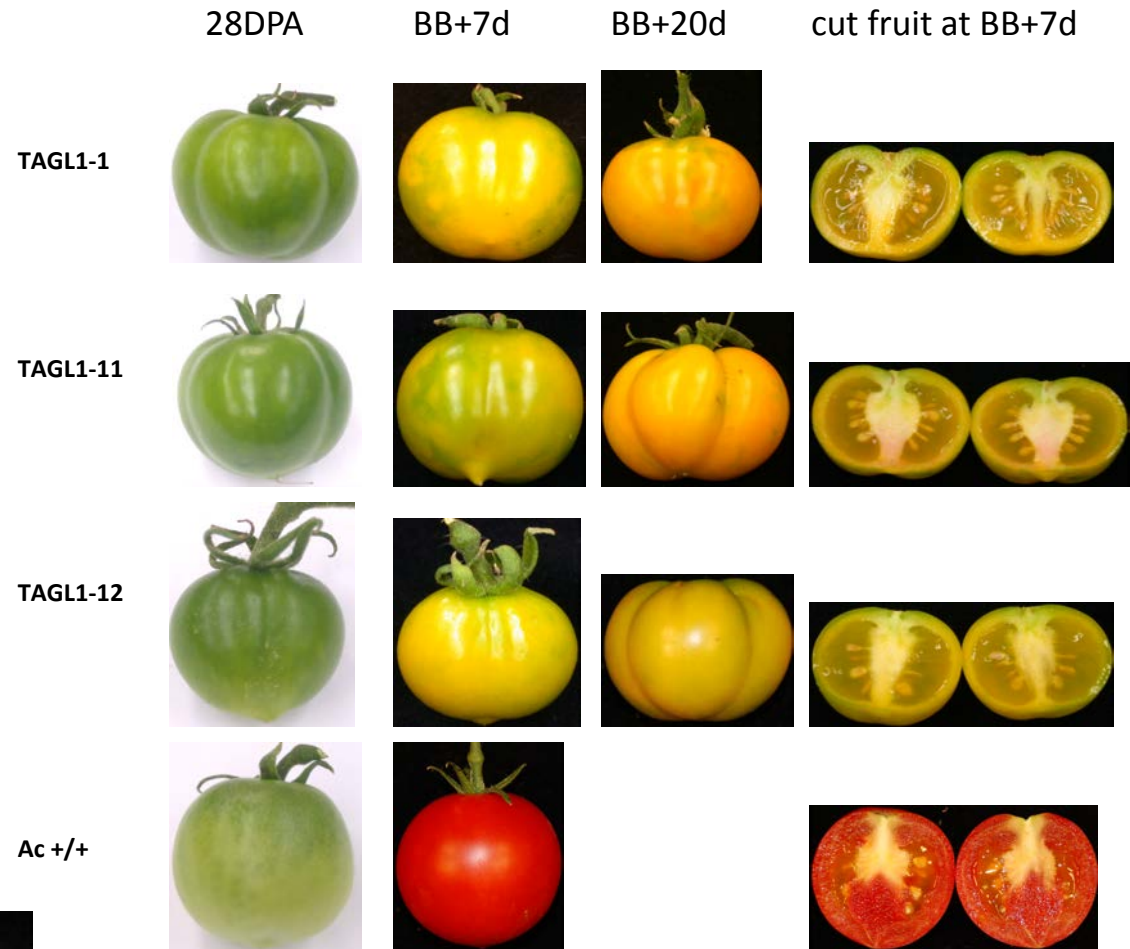
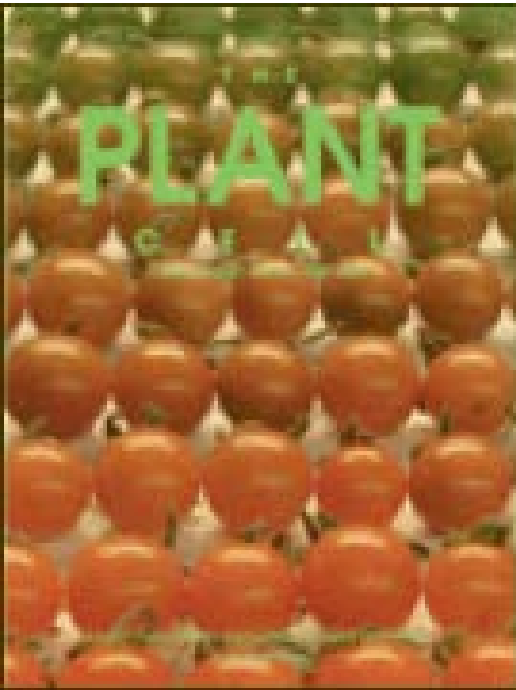
A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening

www.nature.com/naturegenetics

Kenneth Manning¹, Mahmut Tör¹, Mervin Poole², Yiguo Hong¹, Andrew J Thompson¹, Graham J King³, James J Giovannoni⁴ & Graham B Seymour²



The tomato *TAGL1* (*SHP*) MADS-box gene is necessary for fleshy fruit expansion and ripening



At least 3 NACs are expressed in kiwi fruit ripening NOL3 complementation line in NOR background



NOL3-1



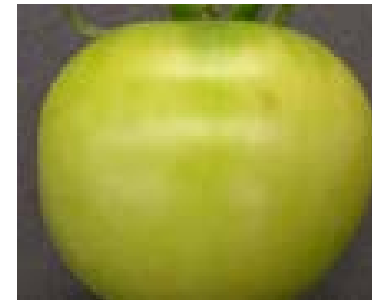
NOL3-2



NOL3-3



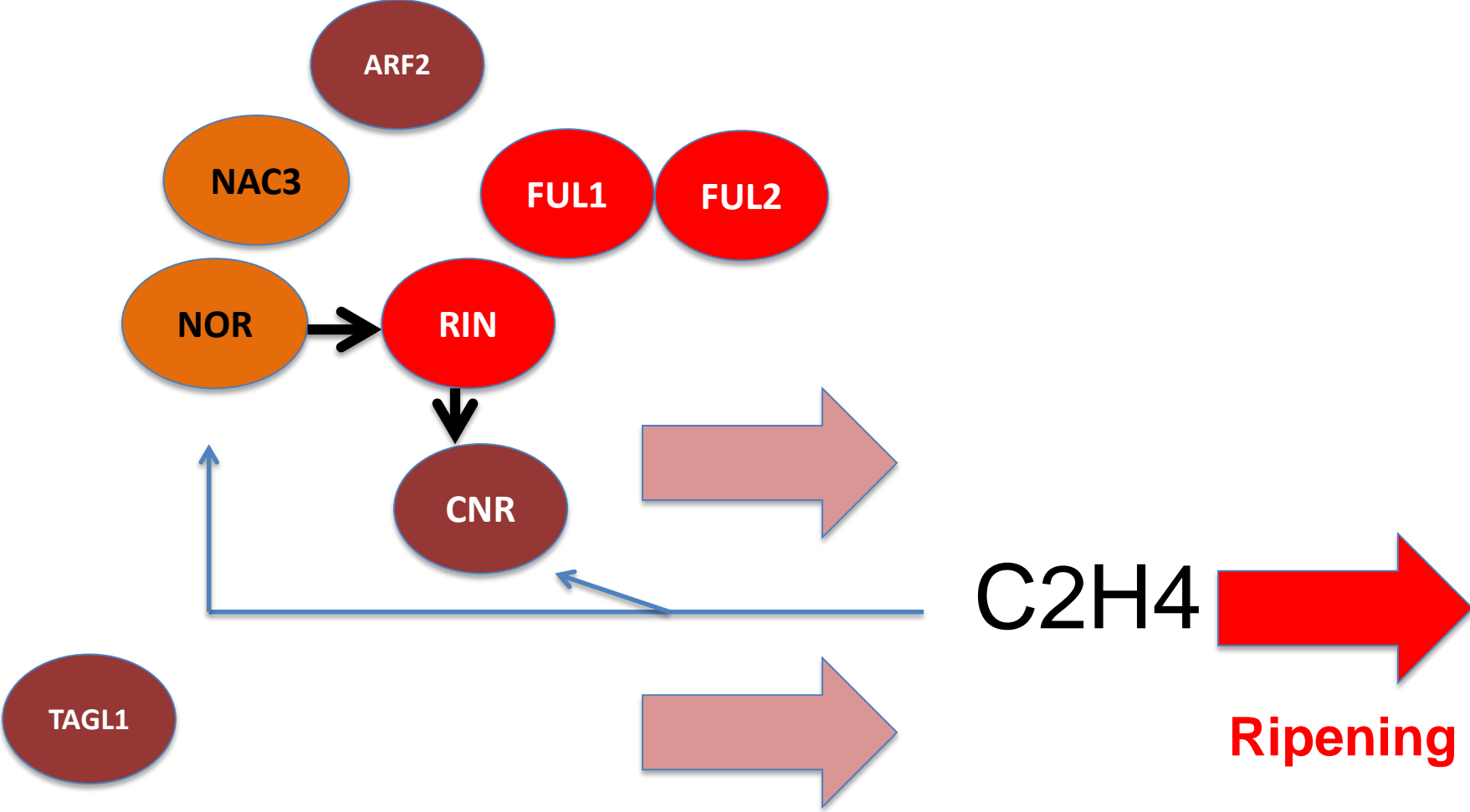
WT (AC)



NOR (AC)



Multiple ripening regulators upstream of ethylene



Tomato production is highly dependent upon the *uniform* mutation



U/U



U/U

u/u

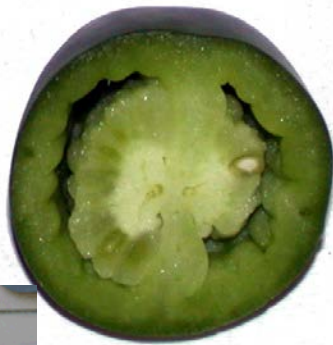


U is a Golden-like 2 (GLK) transcription factor

35S::U/GLK2 in u/u



**35S::UNIFORM in
*u/u***



Control *u/u*



**35S::UNIFORM in
*u/u***



Control *u/u*



Cuong Nguyen

Transcription Factors



ripening inhibitor

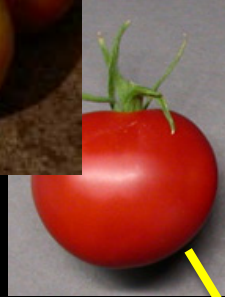
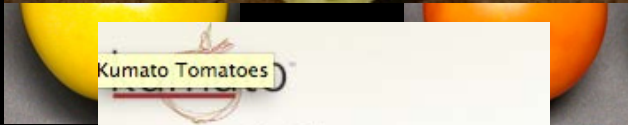
non-ripening

Colorless non-ripening

Uniform ripening



ction C



Green
(G)
RT



green-flesh
(gf)

ripe

high-pigment 1
(hp-1)DD

Plastid Develo

Never-ripe 2

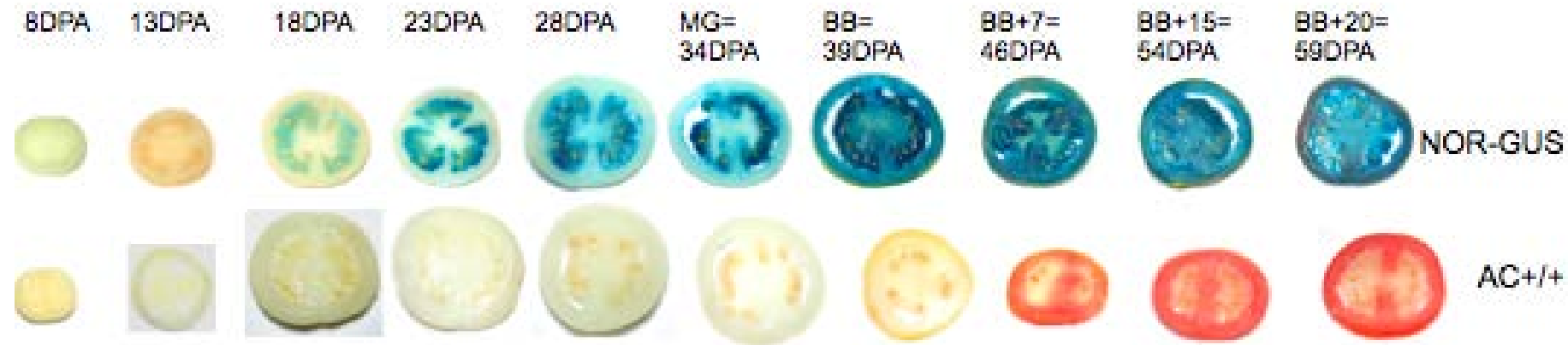
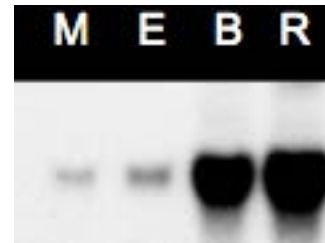
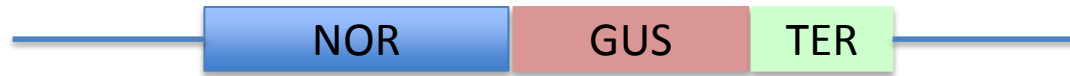
Non-ripening
ylene
sensitive
(nei)
EIN2



Much ripening molecular biology focuses on the pericarp, excluding seeds and locule.



NOR::GUS Reporter expression reveals early locule expression



← System 1 C₂H₄ → ← System 2 C₂H₄ →



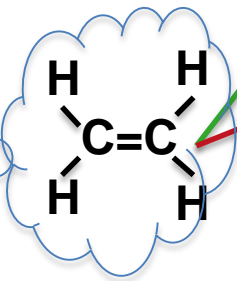
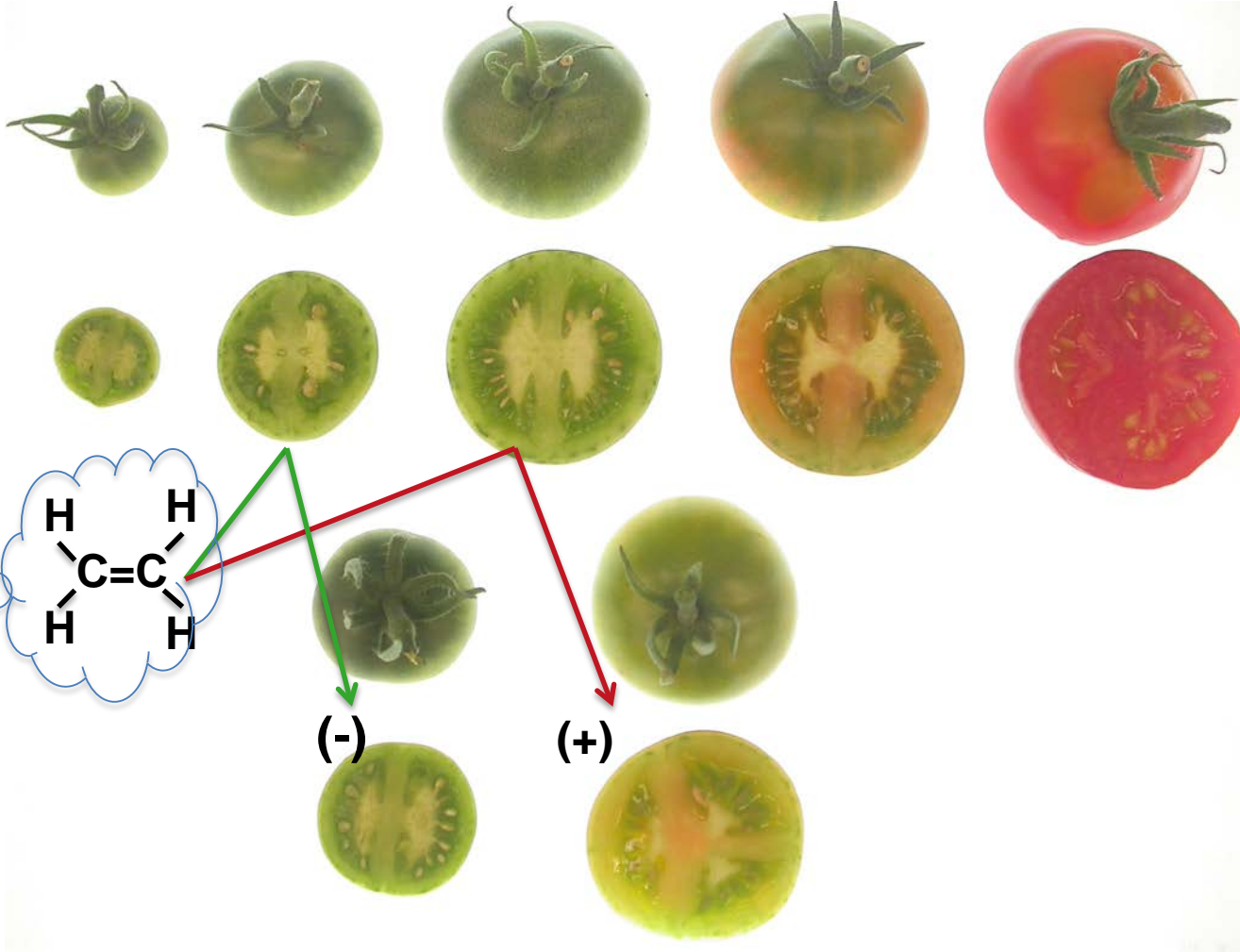
IM

LIM

MG

BR

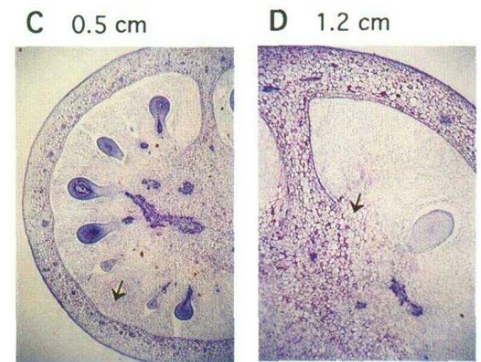
RR



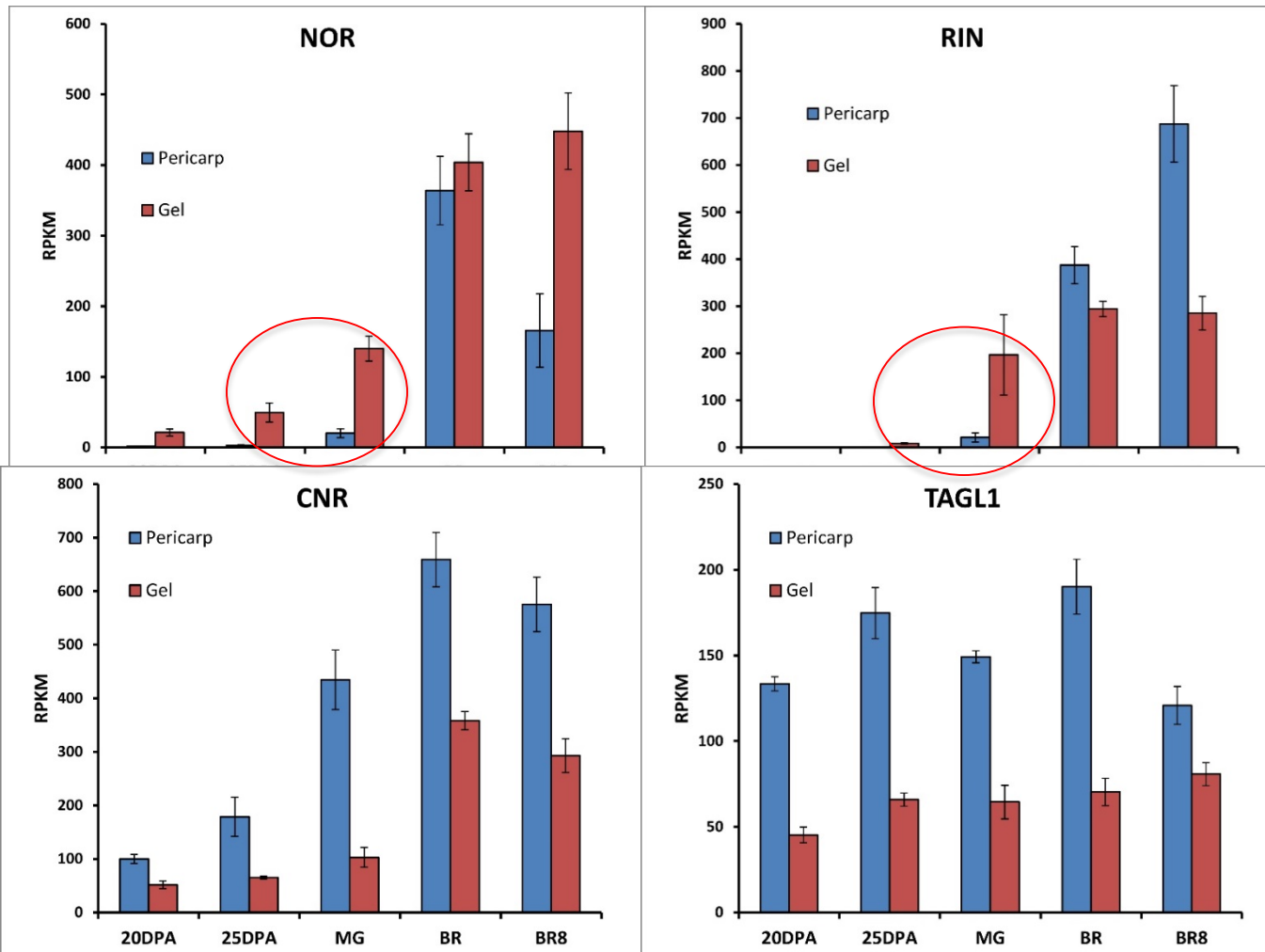
E
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RNA-seq transcriptome profiling of locule and pericarp tissue

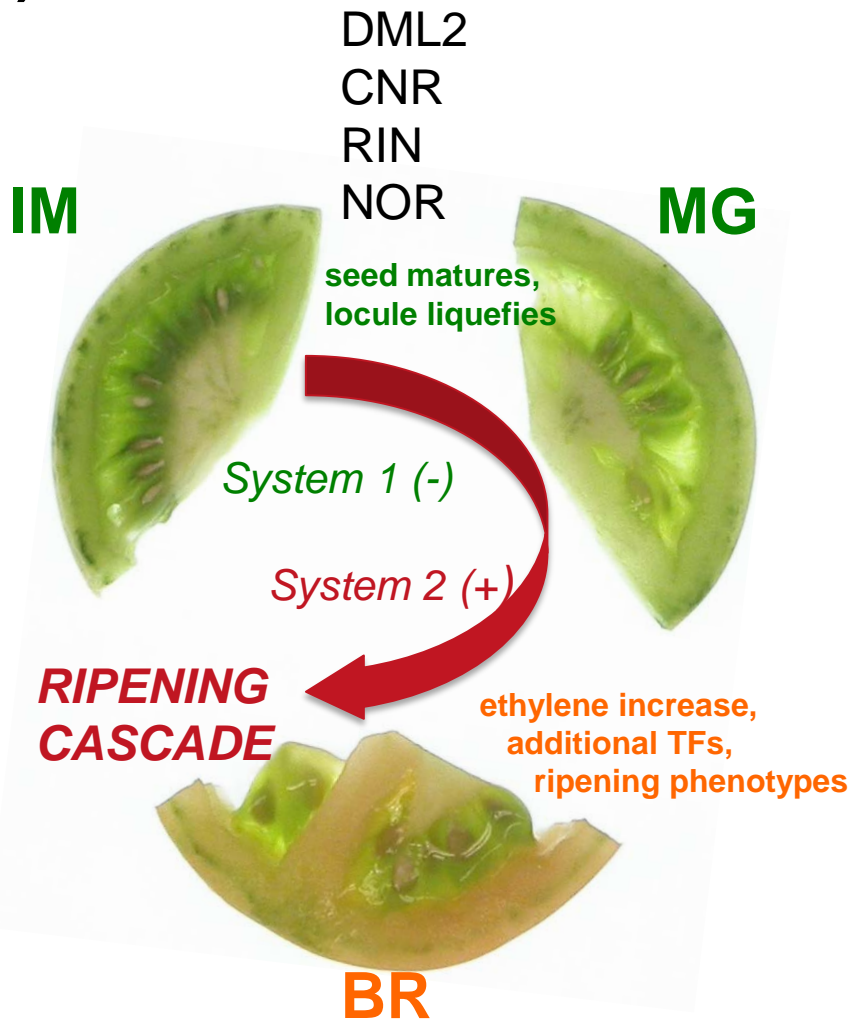
- *Most prior studies on fruit development focus on the pericarp tissue.*
- The locular tissue differentiates from the placenta and gradually changes to jelly tissue during fruit development and ripening.
- Locular gelling is an early sign of ripening preceding climacteric respiration and ethylene induction.



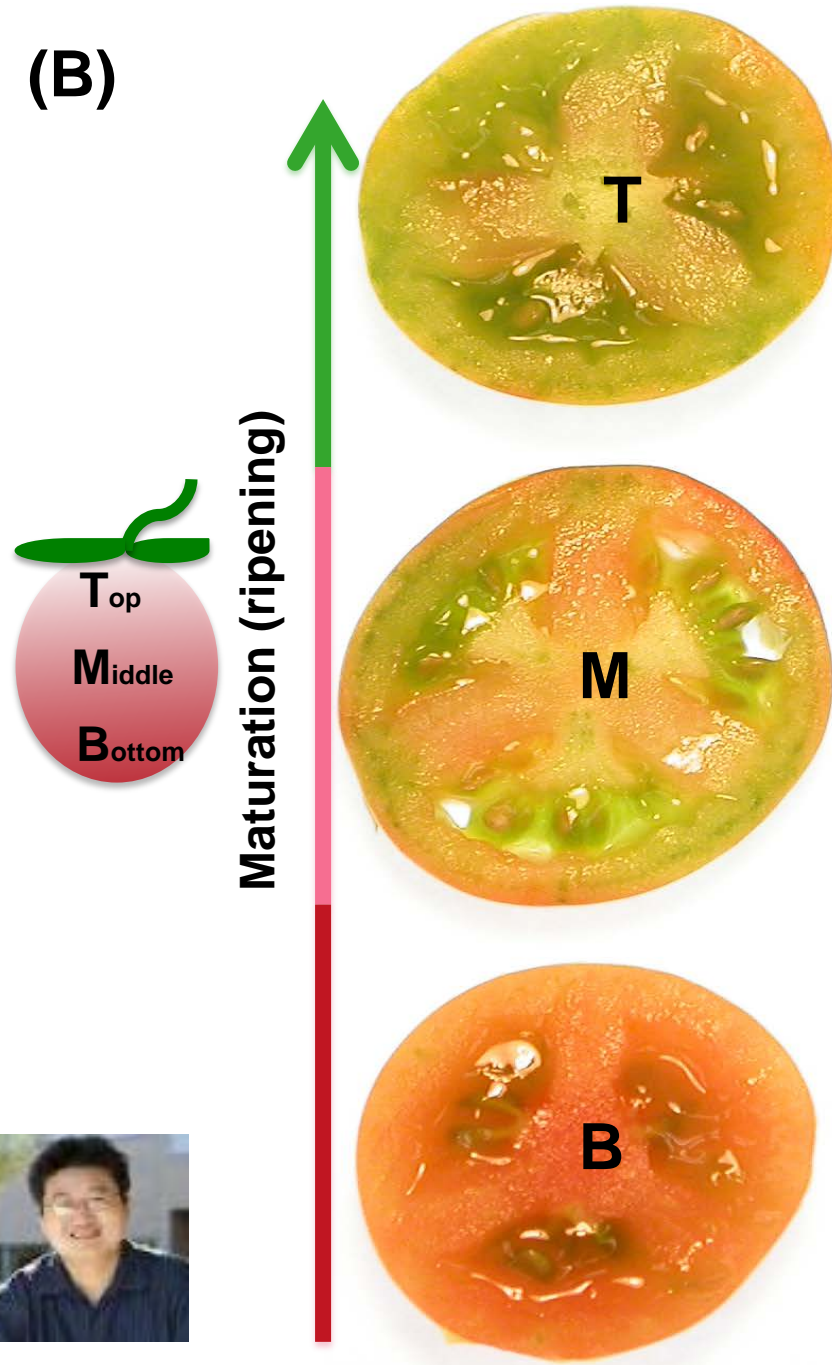
Ripening control gene expression in pericarp versus locule



(A)



(B)



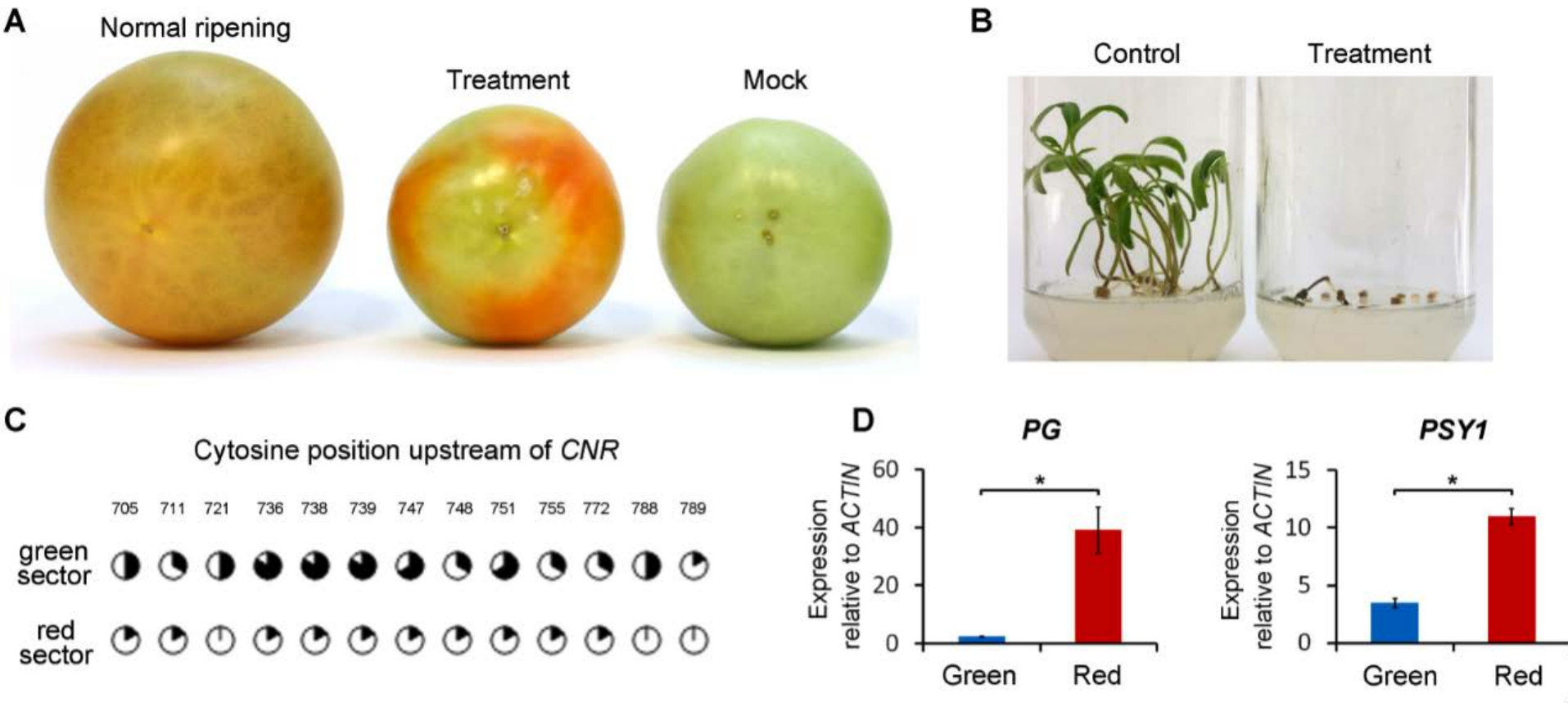
Cuong Nguyen



Zhangjum Fei

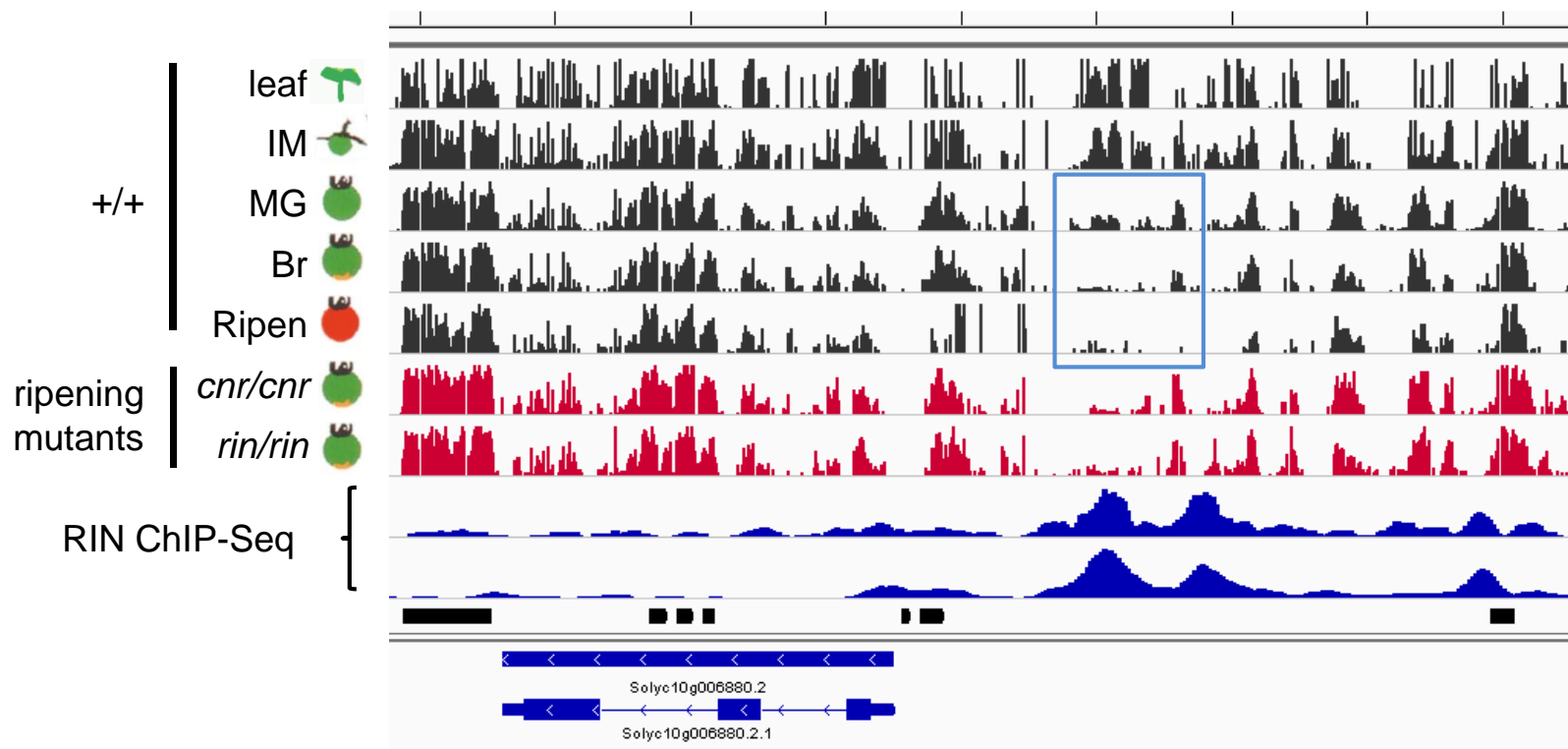
Ripening can be achieved through genome demethylation

5-azacytidine treatment of immature (17 dpa) tomato fruit



RIN-MADS bind near DMRs

nor locus



Tomato Epigenome Database

Home DNA methylation Tools Help Methods Contacts

Quick Start

DNA methylation: Search DNA methylation | Plot methylation profiles

Tools: Blast | Gene keyword search

Small RNAs: sRNA data on Genome browser

Methods | Help

News

Strand-specific RNA sequencing paper - Aug. 2011

A paper describing High-Throughput Illumina Strand-Specific RNA Sequencing Library Preparation was published on Cold Spring Harbor Protocols

Tomato Shotgun Genome Sequence released - Apr. 2010

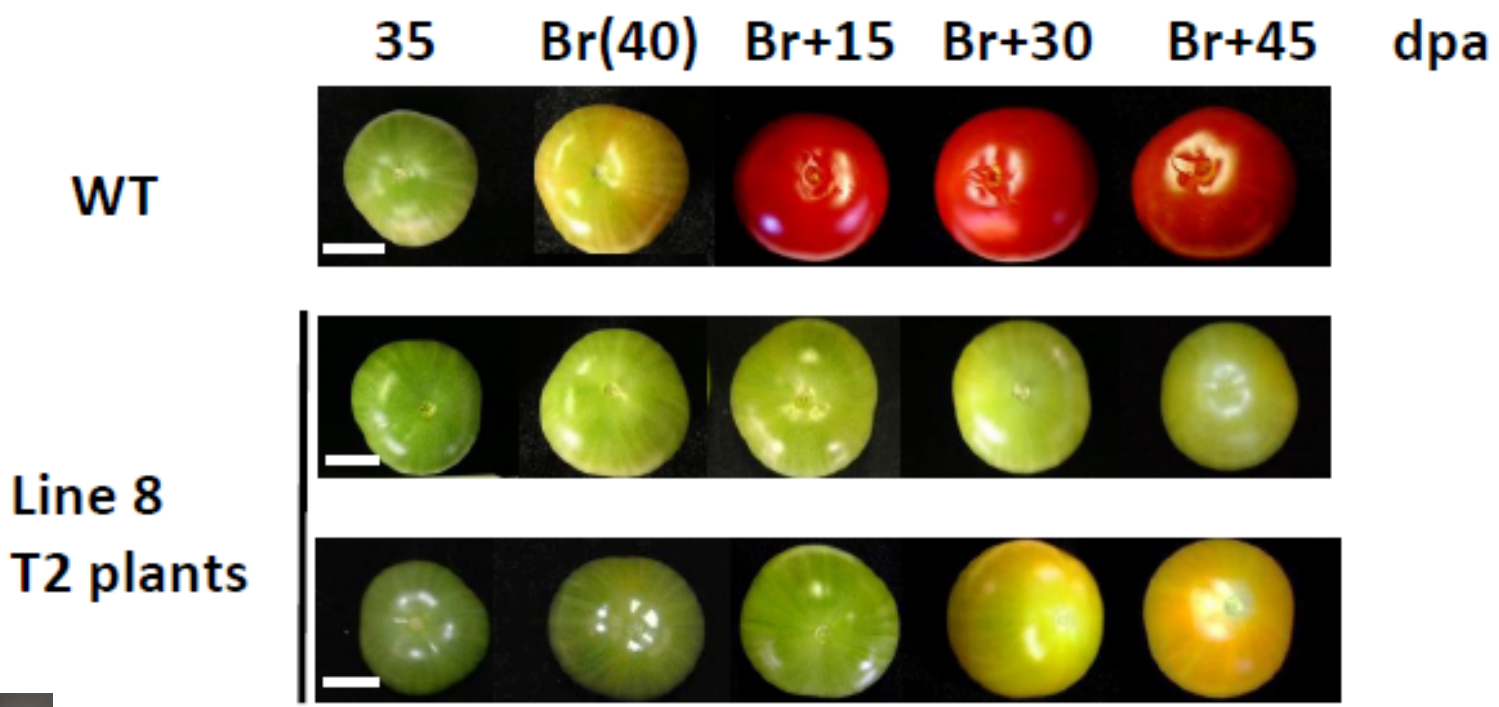
A release of the tomato shotgun genome sequence ITAG2.3 is available at SGN. Details...

See all the news...

<http://ted.bti.cornell.edu/epigenome/>



Ripening of transgenic DNA demethylase (DML) RNAi fruits is delayed



Home

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Links

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Tomato Expression Atlas

A high resolution map and search tool for
tomato genes and their products

tea.solgenomics.net



Joss Rose



Lukas Mueller



Zhangjum Fei



Carmen Catalá



Overview

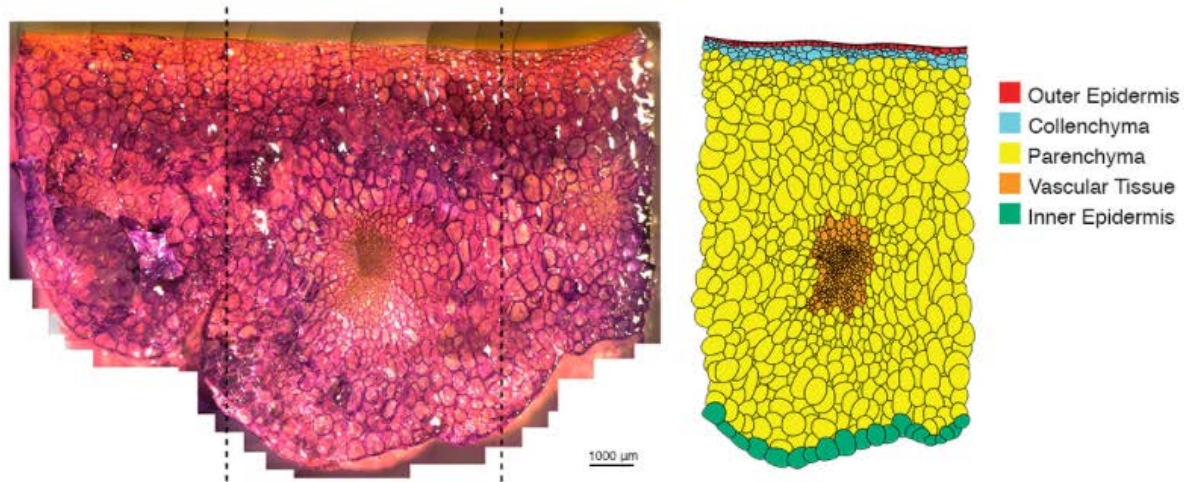


Expression Viewer



Anatomy Viewer

Project information and figures



Learn more about the samples from each project on the [Tomato Expression Atlas](#) and browse detailed drawings from photographs and light microscopy images of tomato fruit development.

Computed Tomography images & videos



Explore our high resolution CT images and videos of tomato fruit development.



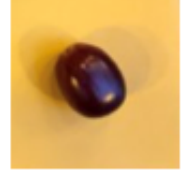
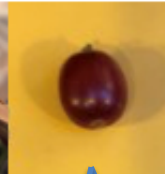
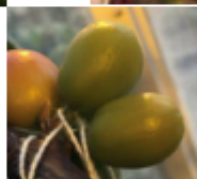
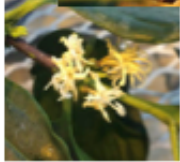
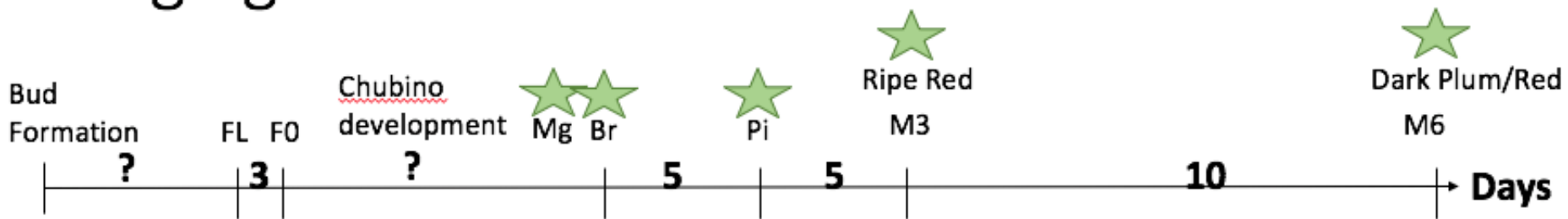
PRS

ALI



Coffee staging for seed and fruit transcriptome analysis

Staging

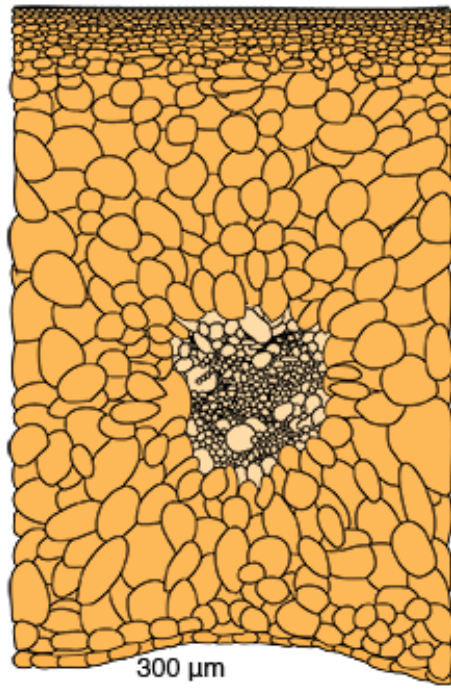


Dynamic TAGL1 expression in maturing pericarp tissues

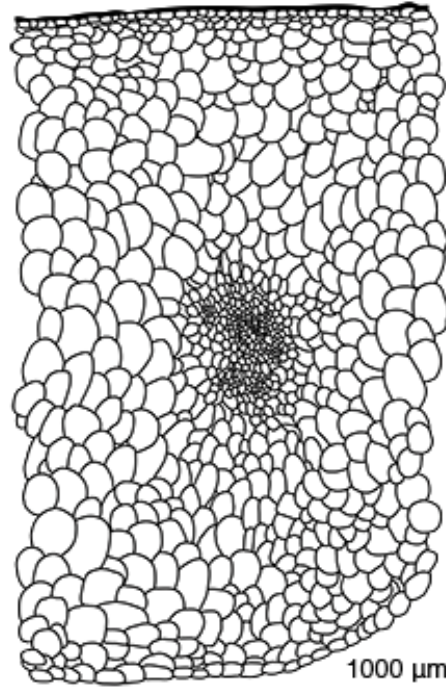


TAGL1 repression

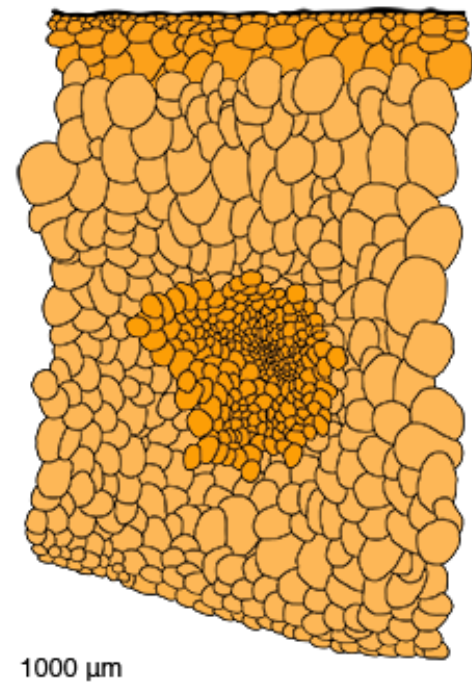
10 Days Post Anthesis
Equatorial Region



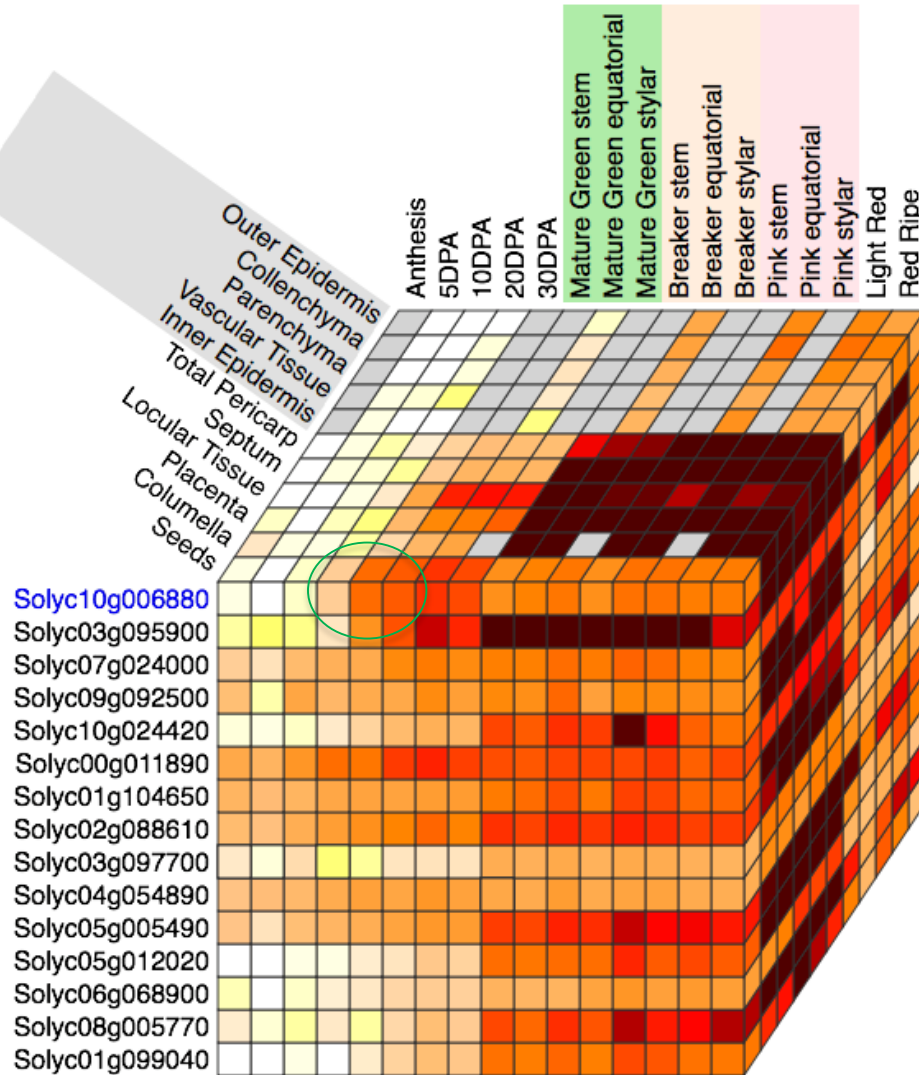
Mature Green
Equatorial Region



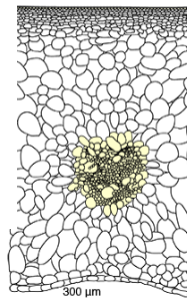
Pink
Equatorial Region



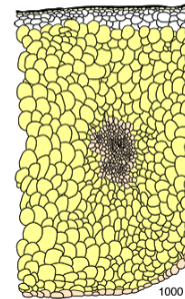
Tissue expression of early ripening regulator NOR



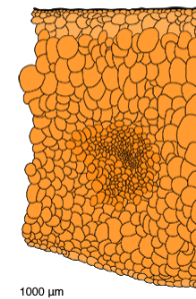
0 Days Post Anthesis
Equatorial Region



Mature Green
Equatorial Region



Pink
Equatorial Region



Outer Epidermis: 49.93
Collenchyma: 34.82
Parenchyma: 62.74
Vascular Tissue: 81.75
Inner Epidermis: 41.47

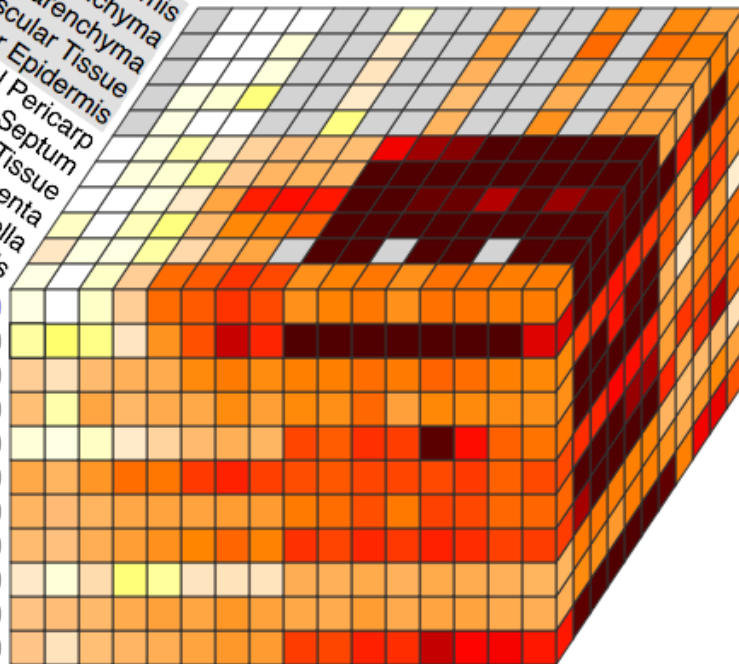
NOR

Solyc10g006880
 Solyc03g095900
 Solyc07g024000
 Solyc09g092500
 Solyc10g024420
 Solyc00g011890
 Solyc01g104650
 Solyc02g088610
 Solyc03g097700
 Solyc04g054890
 Solyc05g005490

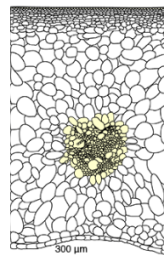
Outer Epidermis
 Collenchyma
 Vascular Tissue
 Inner Epidermis
 Total Pericarp
 Locular Septum
 Placenta
 Columella
 Seeds

Anthesis
 5DPA
 10DPA
 20DPA
 30DPA

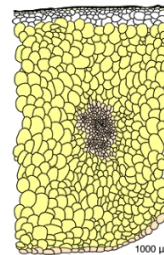
Mature Green stem
 Mature Green equatorial
 Mature Green stylar
 Breaker stem
 Breaker equatorial
 Breaker stylar
 Pink stem
 Pink equatorial
 Pink stylar
 Light Red
 Red Ripe



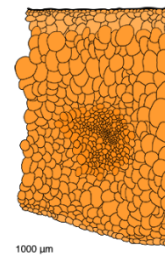
10 Days Post Anthesis
Equatorial Region



Mature Green
Equatorial Region



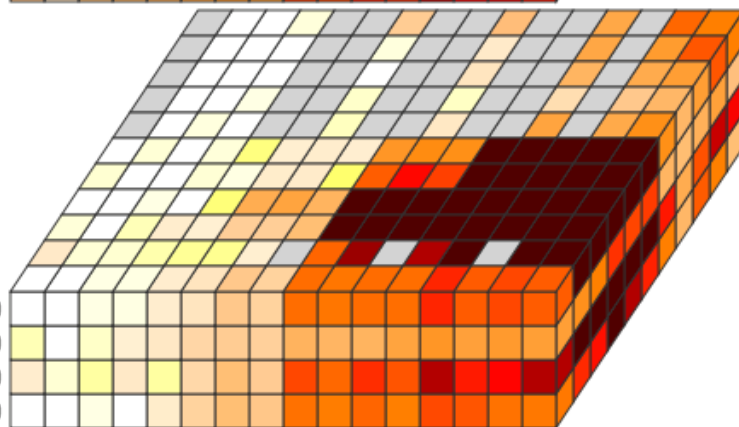
Pink
Equatorial Region



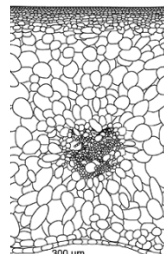
Outer Epidermis: 49.93
 Collenchyma: 34.82
 Parenchyma: 62.74
 Vascular Tissue: 81.75
 Inner Epidermis: 41.47

RIN

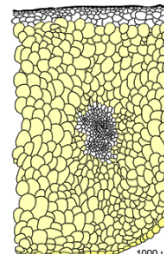
Solyc05g012020
 Solyc06g068900
 Solyc08g005770
 Solyc01g099040



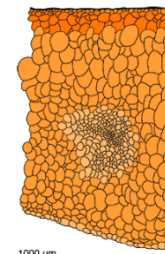
10 Days Post Anthesis
Equatorial Region



Mature Green
Equatorial Region

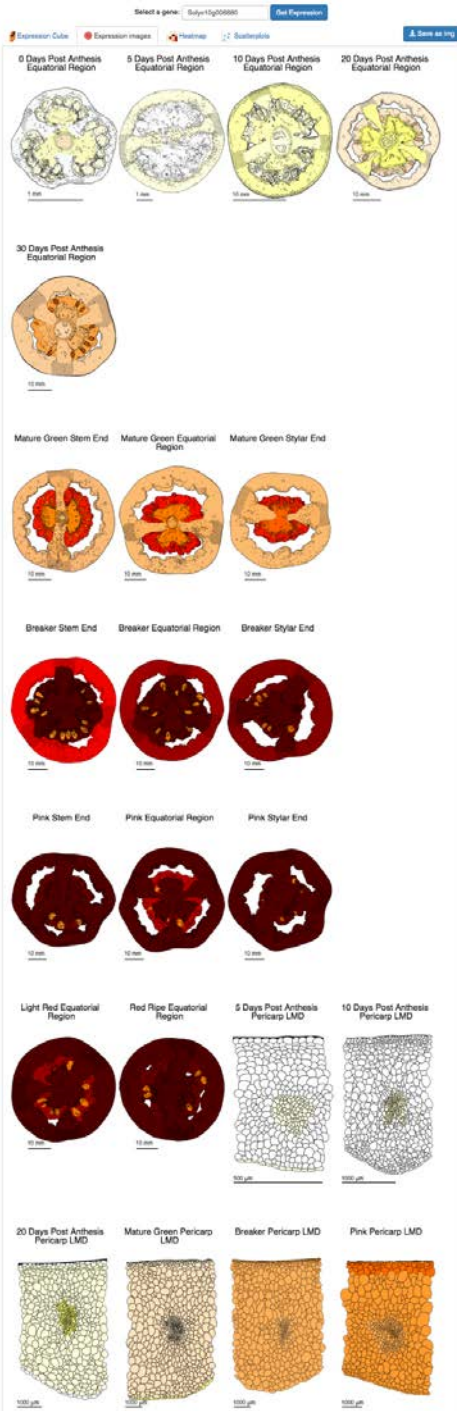


Pink
Equatorial Region

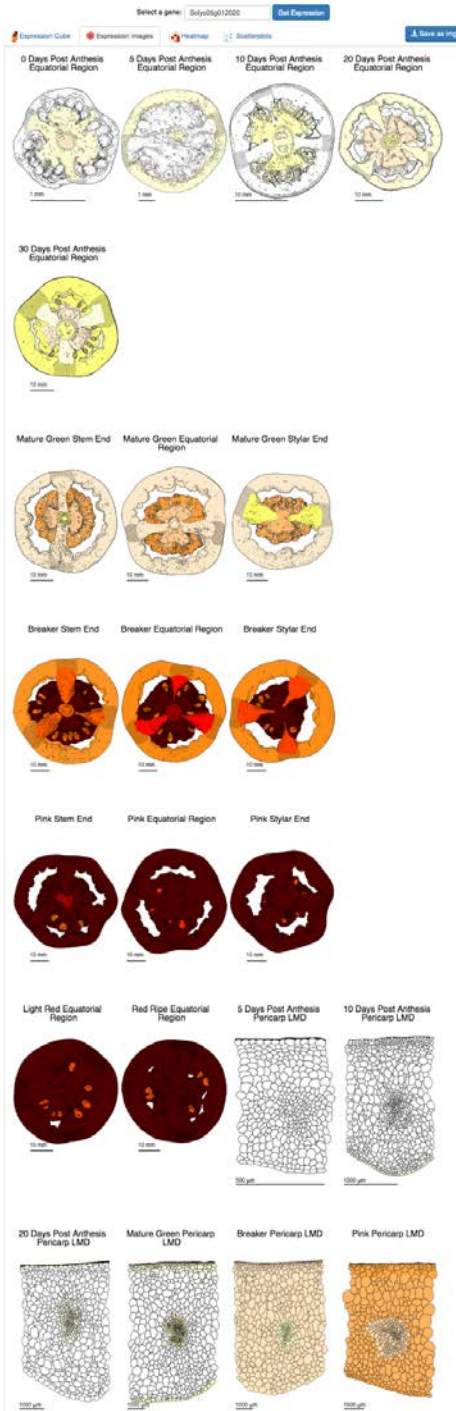


Outer Epidermis: 81.58
 Collenchyma: 117.57
 Parenchyma: 61
 Vascular Tissue: 17.04
 Inner Epidermis: 17.54

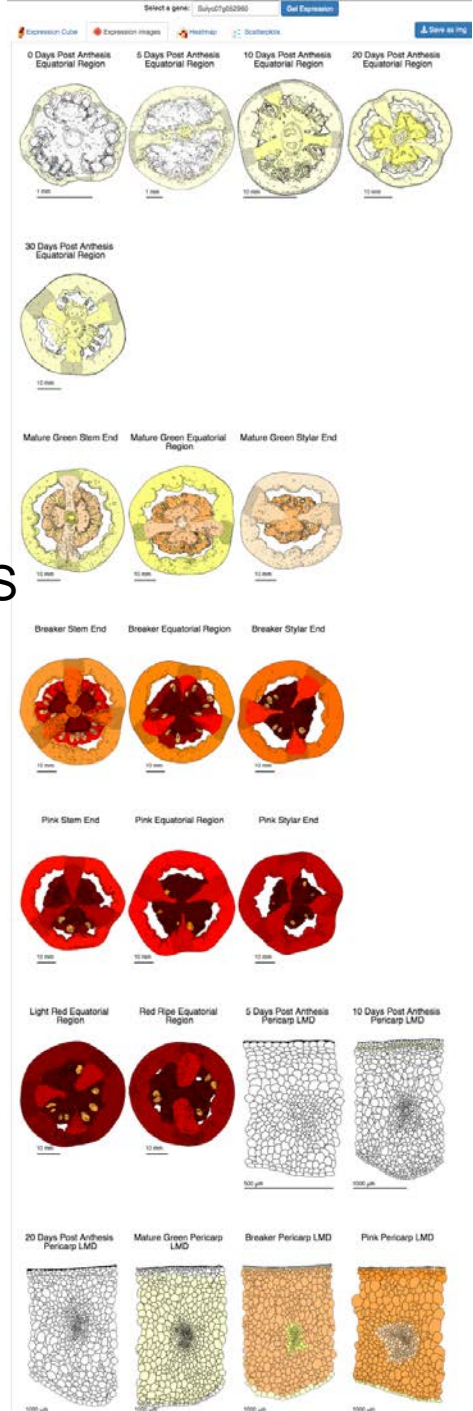
NOR



RIN

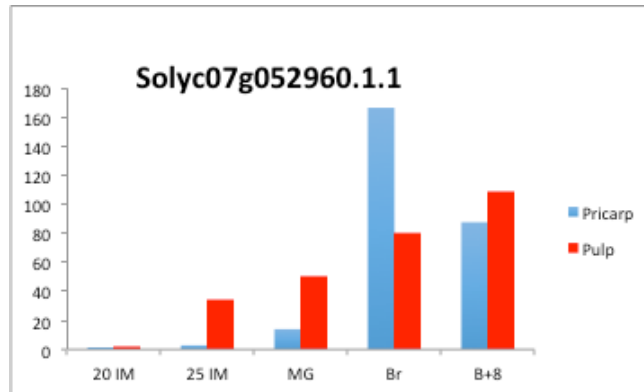
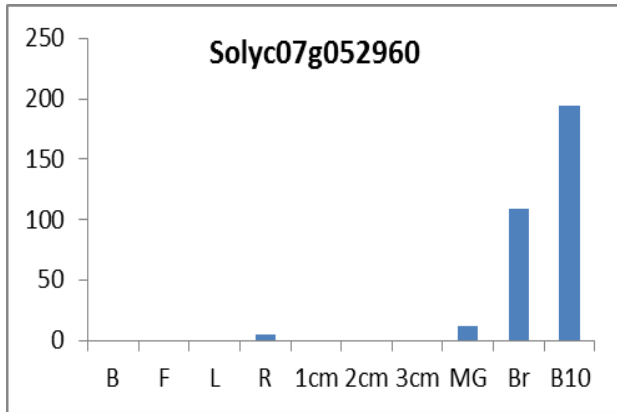


GRAS

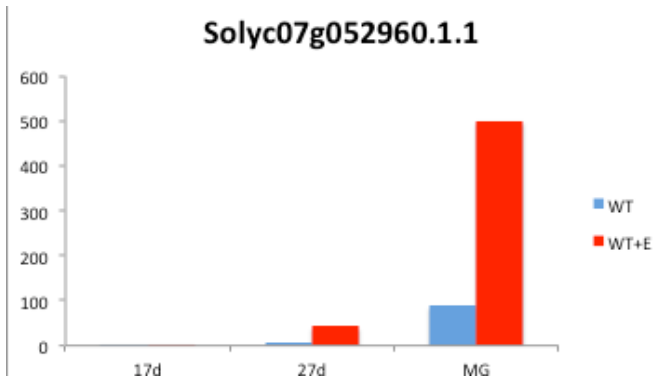


GRAS

Scarecrow-like transcription factor

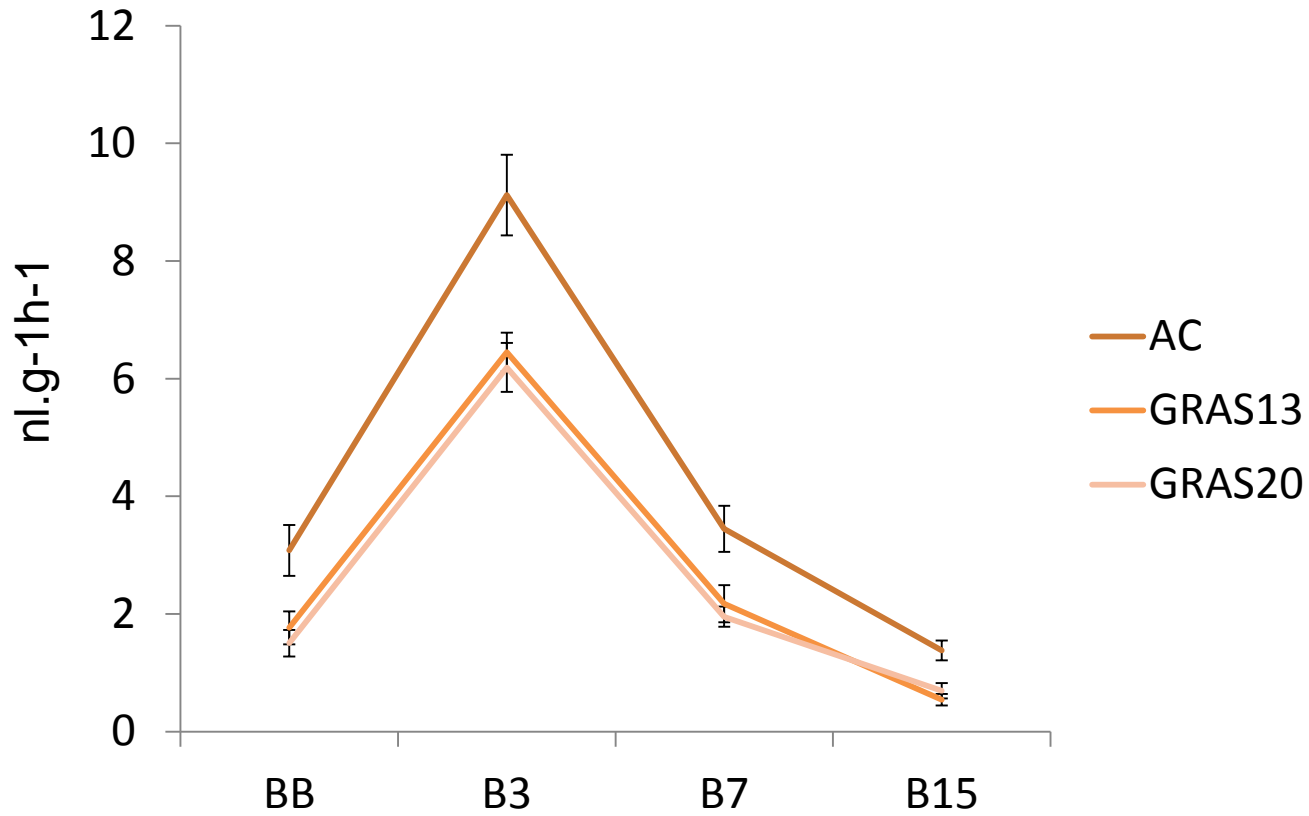


***rin* 42DPA/WT 42DPA=0.099**
***Cnr* 42DPA/WT 42DPA=0.007**



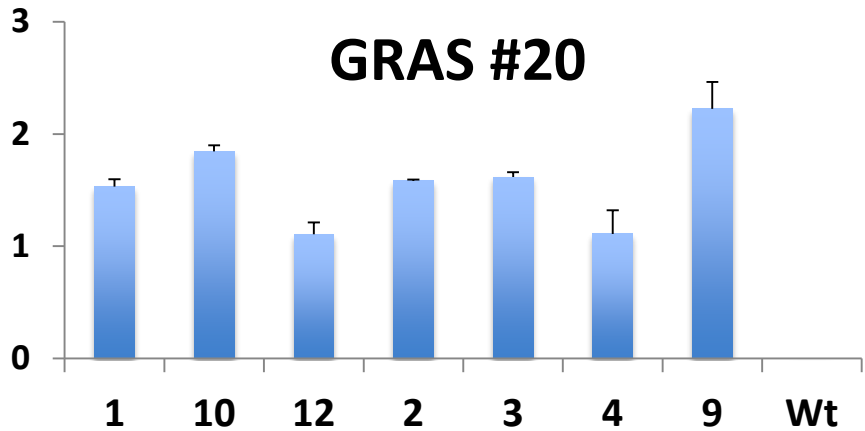
- Ripening induced
- RIN regulated
- Ethylene responsive
- Accumulates first in locule

Ethylene production



GRAS mRNA reduced ripening delay is maintained in T1 generation

GRAS #20



Line	1	10	12	2	3	4	9	Wt
Days from 1cm to breaker	32.3	29.2	30.2	30.8	31.5	30.3	30.2	26.5
	± 0.8	± 1.0	± 0.7	± 0.6	± 0.8	± 1.0	± 0.6	± 0.4
fruits	7	5	12	4	2	4	14	21

T1 fruits (B+15)



Longer self life

**T2 B+35D 33d after post harvest
3/16 photo**

AC

GRAS 13

**T2 B+60D 58d after post harvest
3/16 phto**

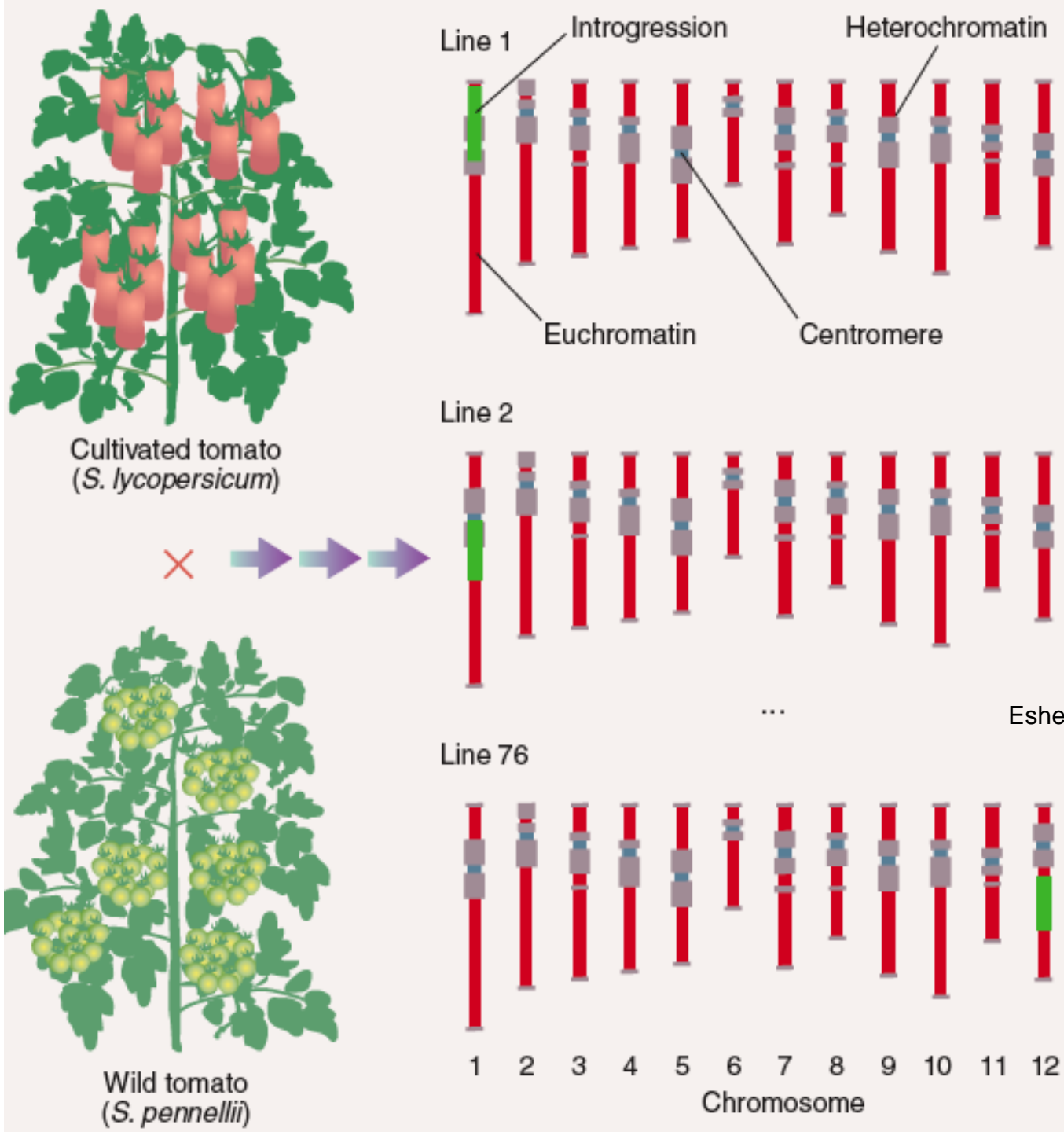
AC

GRAS 20

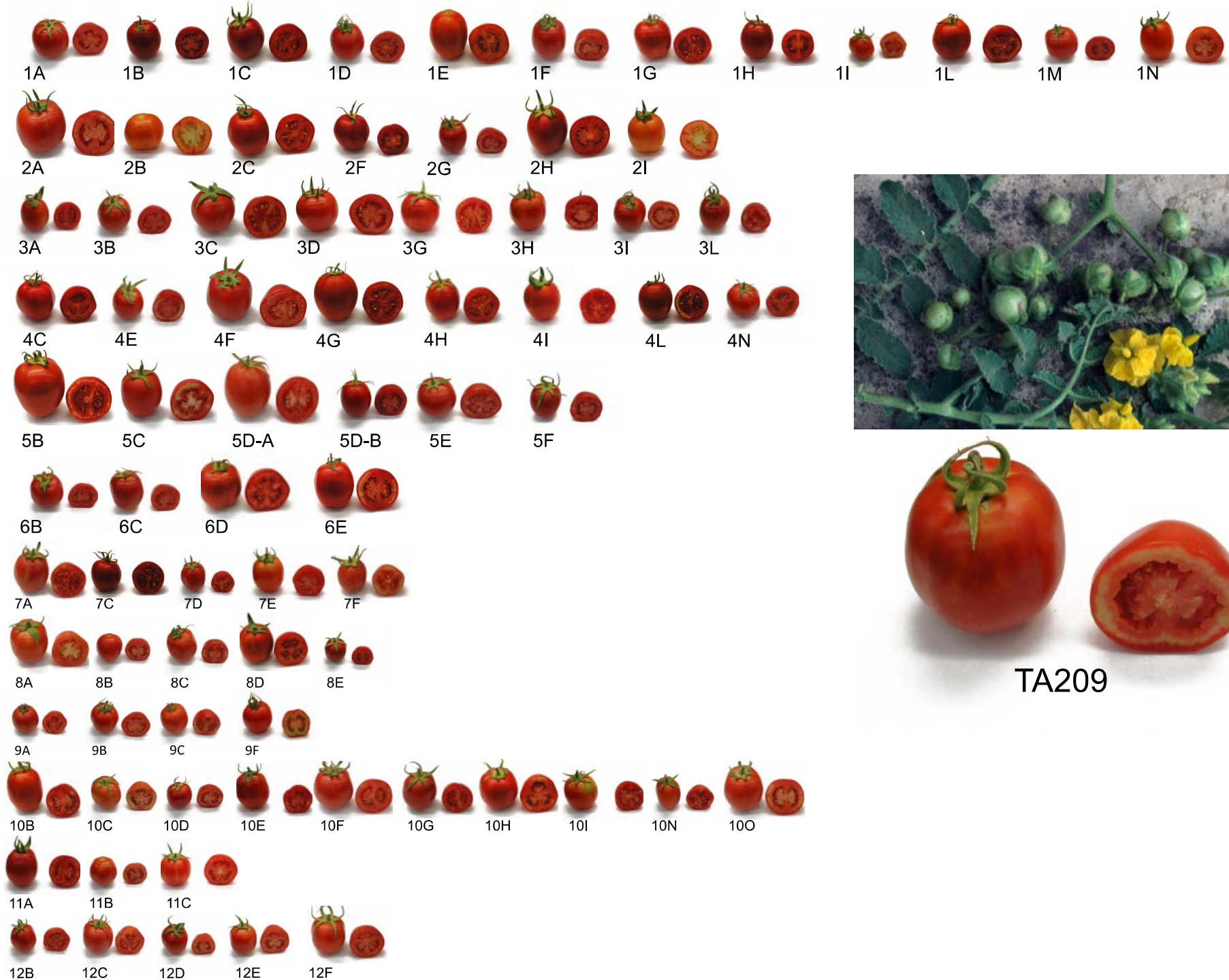
Considerable genetic diversity among wild tomato species.....



.....but less than 5% is represented in cultivated tomatoes

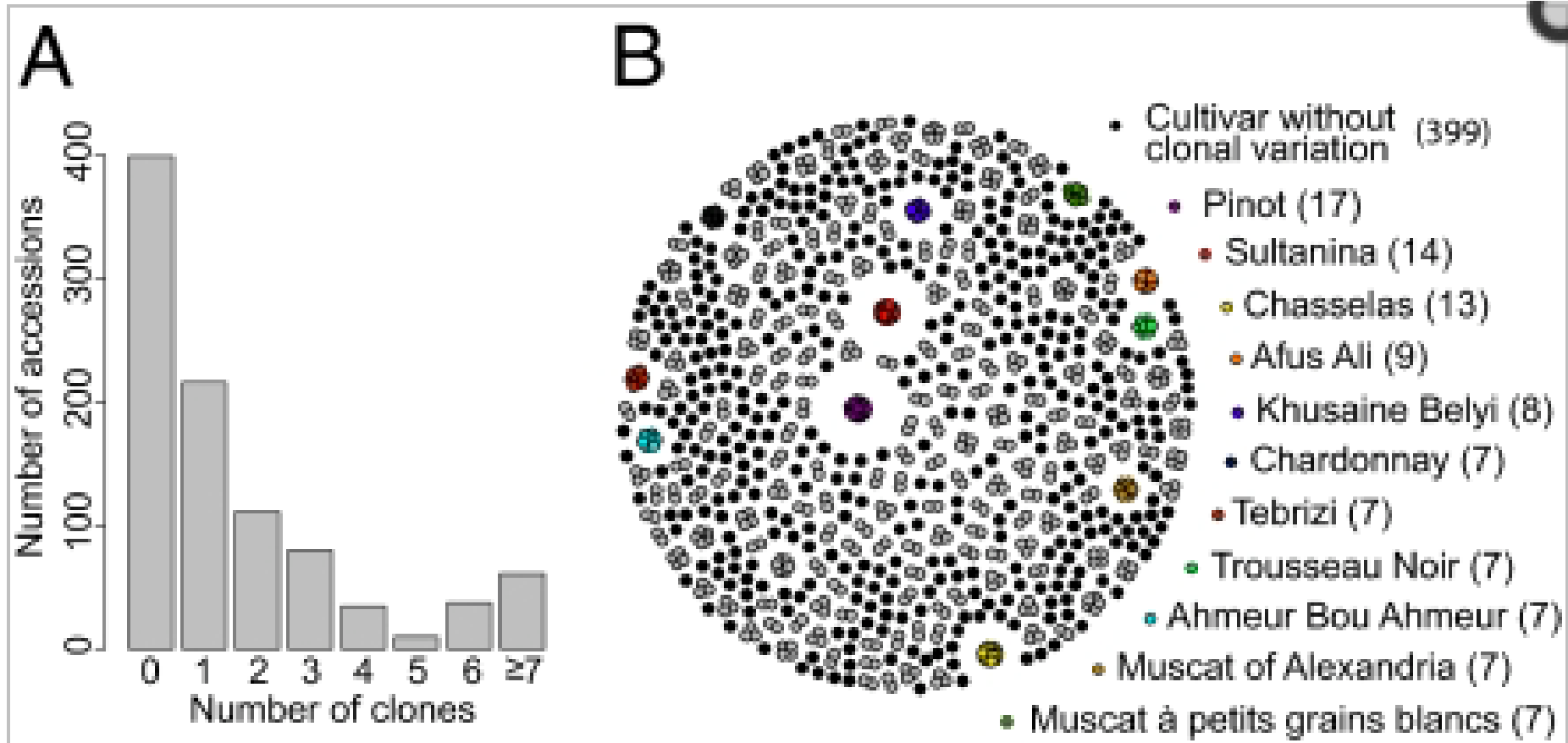


Eshed and Zamir, 1995

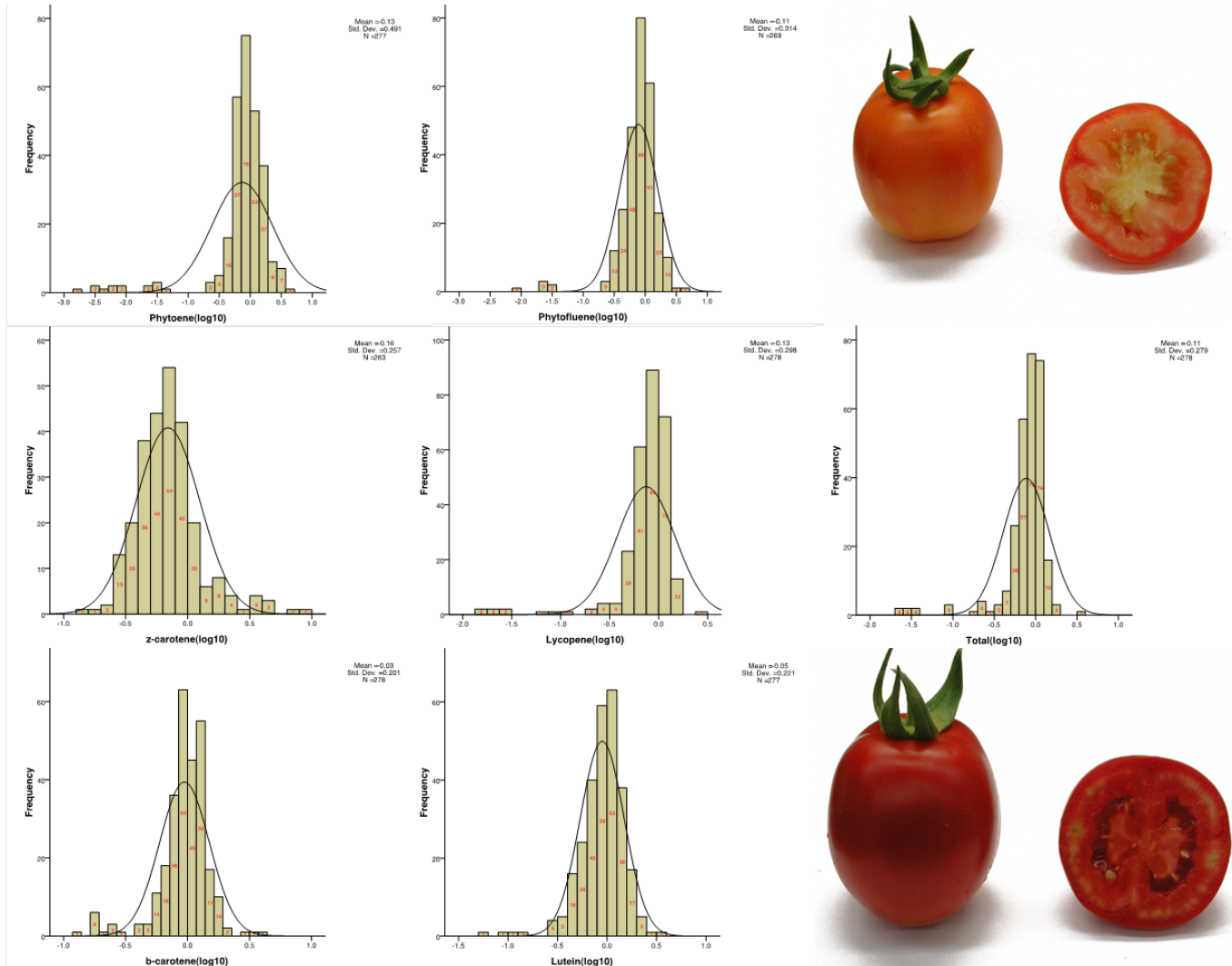


TA209

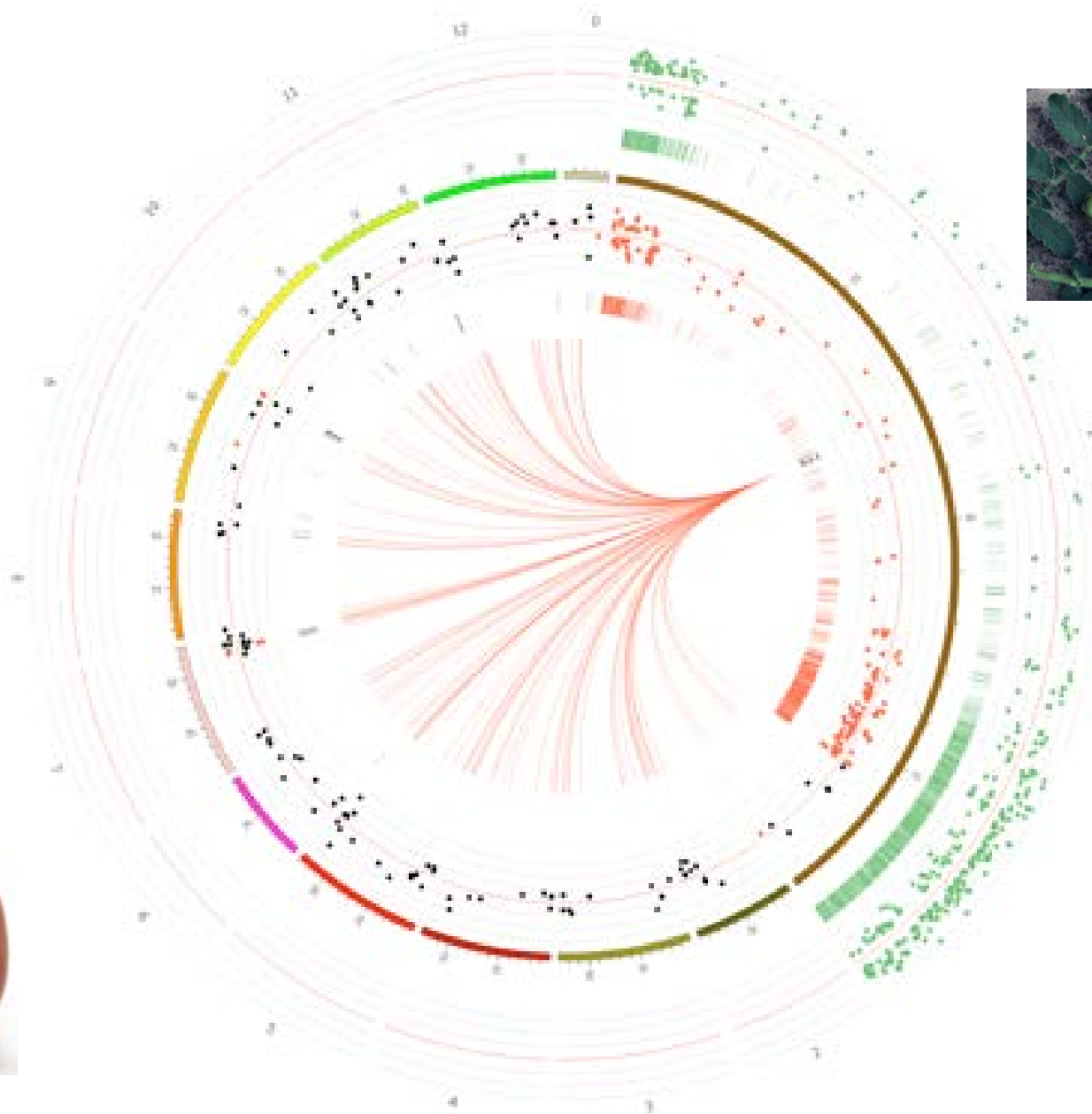
Genotyping of USDA-ARS grape collection (Davis, CA and Geneva, NY)

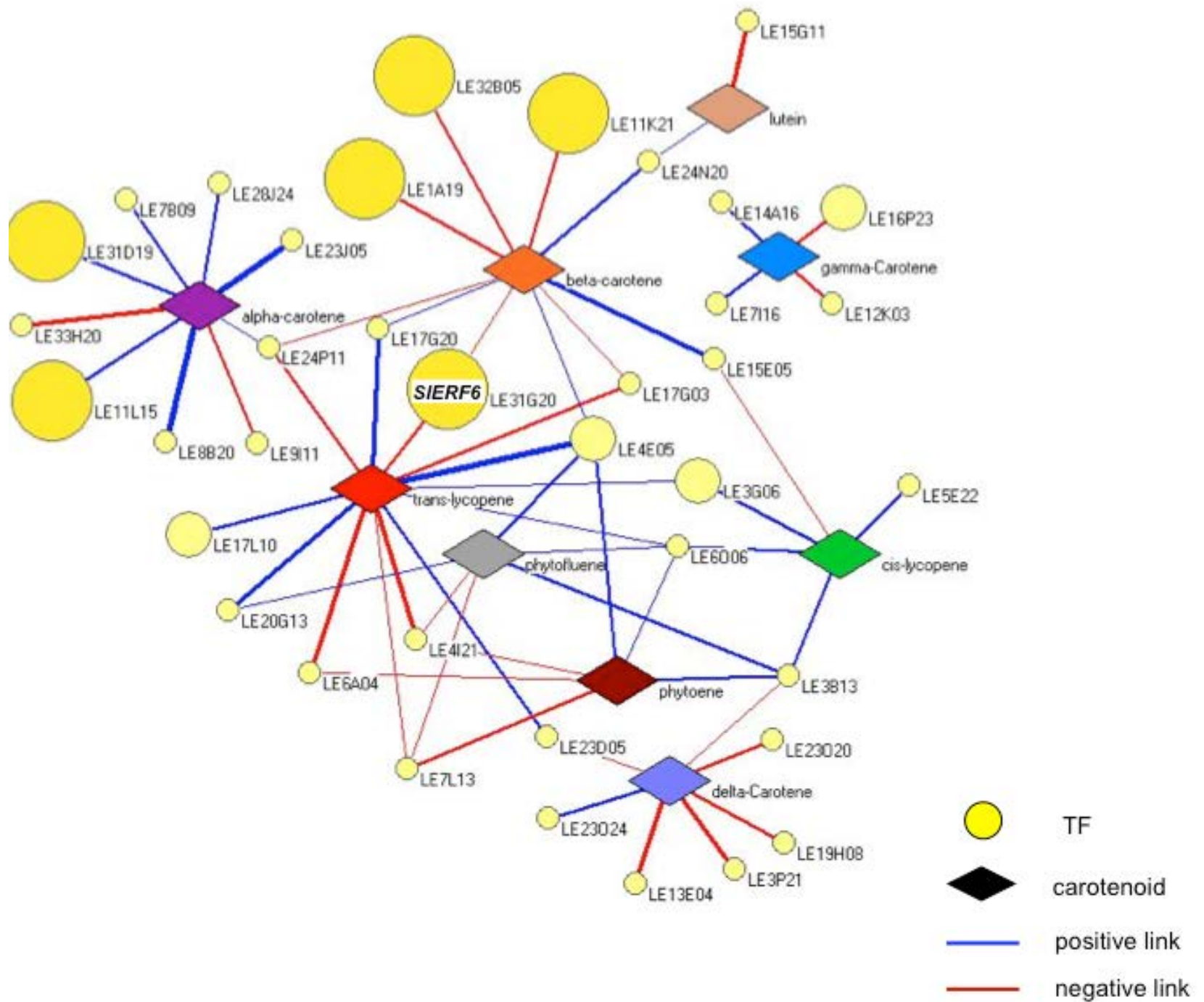


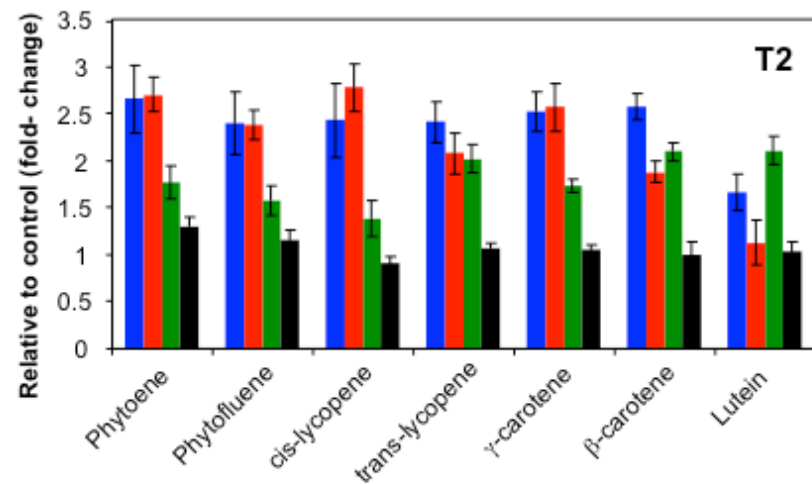
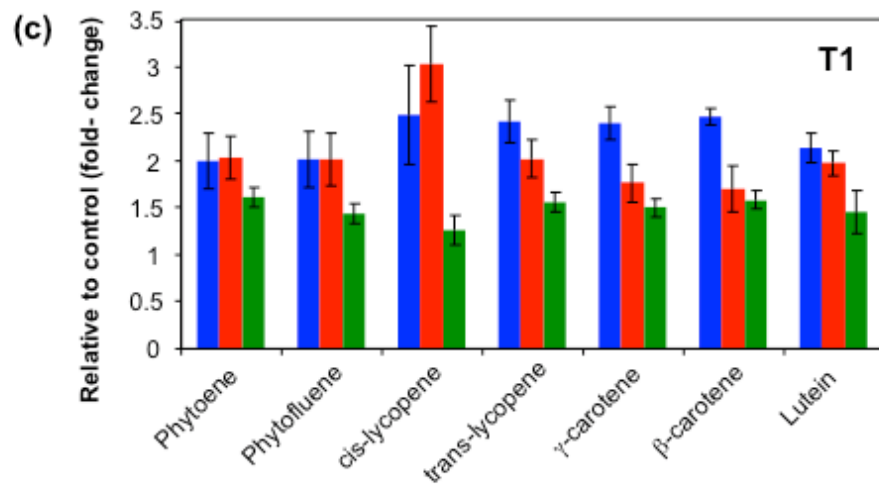
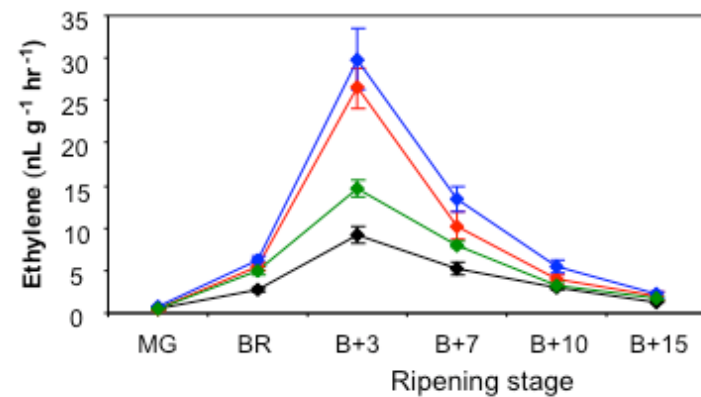
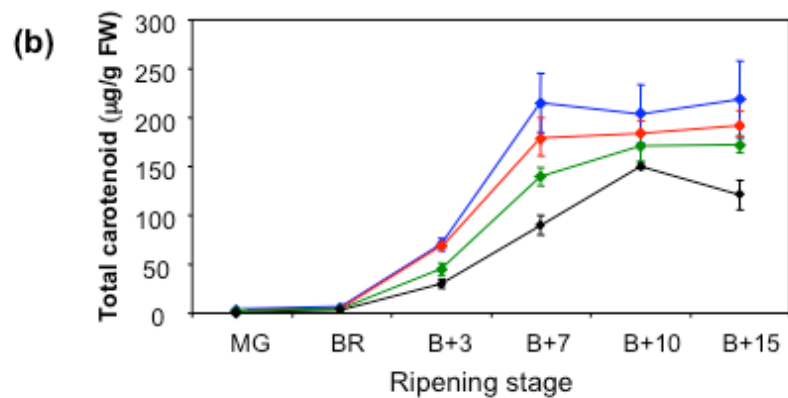
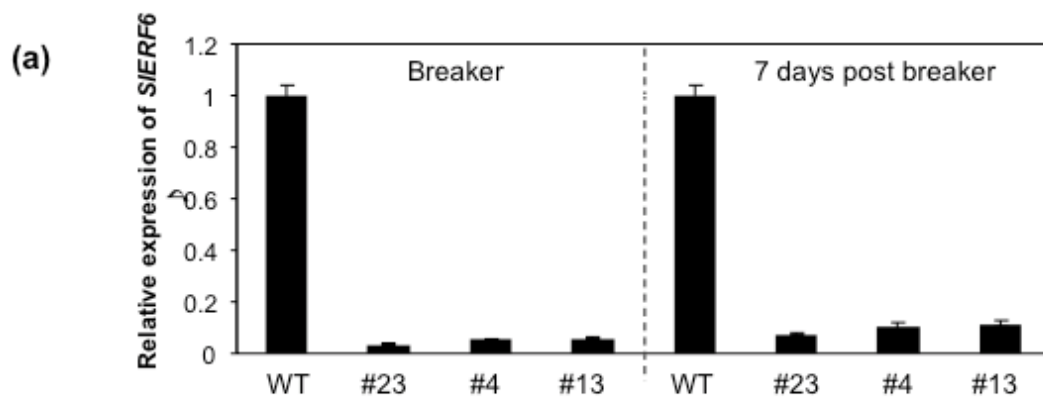
The range of carotenoid levels within the *S. habrochaites* IL population and loci influencing them provides opportunities for discovery of numerous carotenoid synthesis and regulatory loci.



eQTL mapping using a tomato introgression population







SUMMARY

- **The ripening transition is regulated by a cascade of genetic events initiating with transcription factors and epigenome changes.**
- **The MADS-box family is a rich source of fruit development and maturation regulators and evolution has tailored them for specific fruit development and morphology contexts.**
- **Ripening events do not occur uniformly throughout fruit tissues and more careful examination of tissue-specific responses is needed to fully understand ripening phenomena.**
- **Genetic diversity provides opportunities to explore the molecular basis of ripening control and fruit quality and presents natural allelic variants that can be tested and deployed in practical breeding.**
- **Primary genetic regulation influencing maturation, quality and self-life characteristics in tomato is likely conserved across diverse species....including grape.**
- **Not only tomato genes, but tomato infrastructure (e.g. TEA) can be leveraged for additional fruit species.....including grape.**

The people who do the work.....



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Ryan Mcquinn



Julia Vrebalov



Jemin Lee



Betsy Ampofo



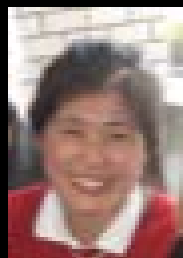
Yimin Xu



Rob Alba



Cuong Nyugen



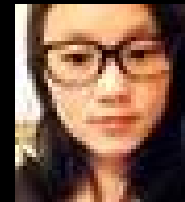
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Ari Feder



Itay Gonda



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Tomato Genome Consortium



Zhangjum Fei



Lukas Mueller



Silin Zhong



Joss Rose Harry Klee

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