A gene-editing approach to improve tomato shelf-life while ensuring fruit quality

INNOVATIONS IN FOOD LOSS AND WASTE MANAGEMENT
COST FoodWaStop
Ancona 24-25 January 2024

Bárbara Blanco-Ulate
Associate Professor – Department of Plant Sciences
Co-Director - UC Postharvest Research and Extension Center
University of California, Davis

Blanco Lab
Fruit Biology & Quality

POSTHARVEST
RESEARCH AND EXTENSION CENTER

UC DAVIS
DEPARTMENT OF PLANT SCIENCES
College of Agricultural and Environmental Sciences

POSTHARVEST
RESEARCH AND EXTENSION CENTER
UNIVERSITY OF CALIFORNIA

https://postharvest.ucdavis.edu

Over 45 years supporting California and the international produce industry through education and extension

The **Postharvest Center Goal**: increasing the economical sustainability of producers, reducing postharvest food losses, and providing high quality, safe and nutritious produce to consumers.

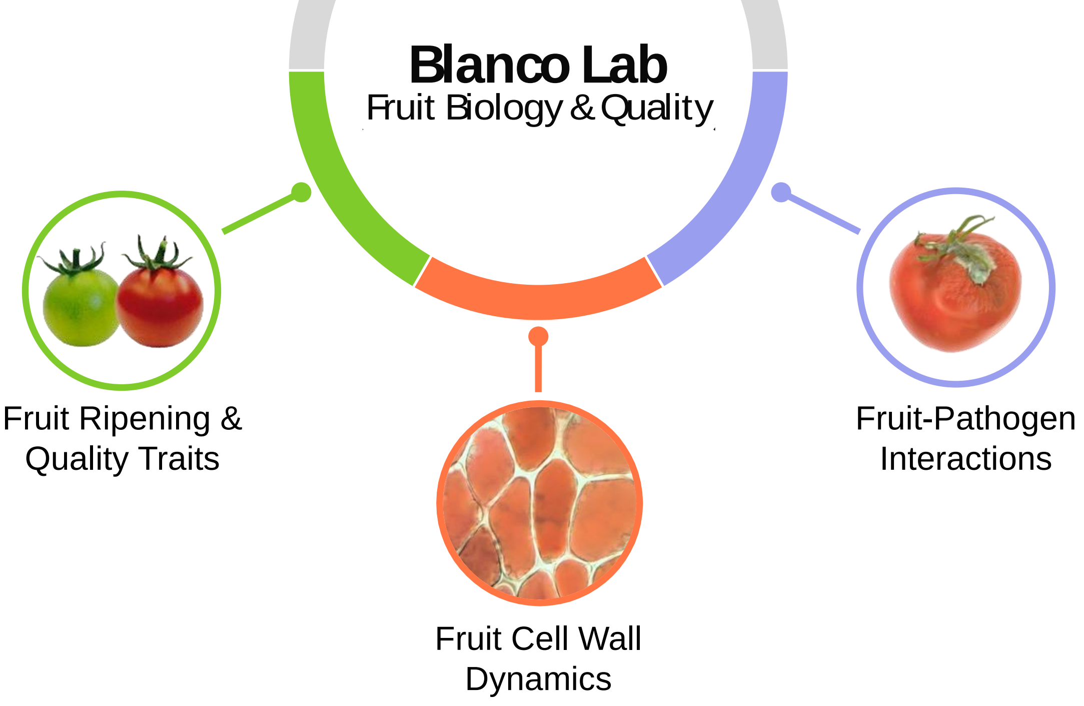
Co-Directors

Bárbara Blanco-Ulate
Associate Professor - Plant Sciences

Irwin R. Donis-González
Associate Professor of CE -
Biological and Agricultural Engineering

Scan me to join the newsletter!

Our team studies **fruit biology** to develop new strategies for improving the **availability**, **quality**, and **marketability** of fruit products



California crops

Tomato	Table grape
Strawberry	Berries
Pistachio	Olive
Stone fruit	Apple…
Citrus	
Melon	

Approaches

Field trials → Physiology → Biochemistry → Systems biology → Functional genetics

Big Challenges in Postharvest
Opportunities for Research and Innovation

Improve and maintain crop quality

Fresh, nutritious, safe, and affordable food are not always available

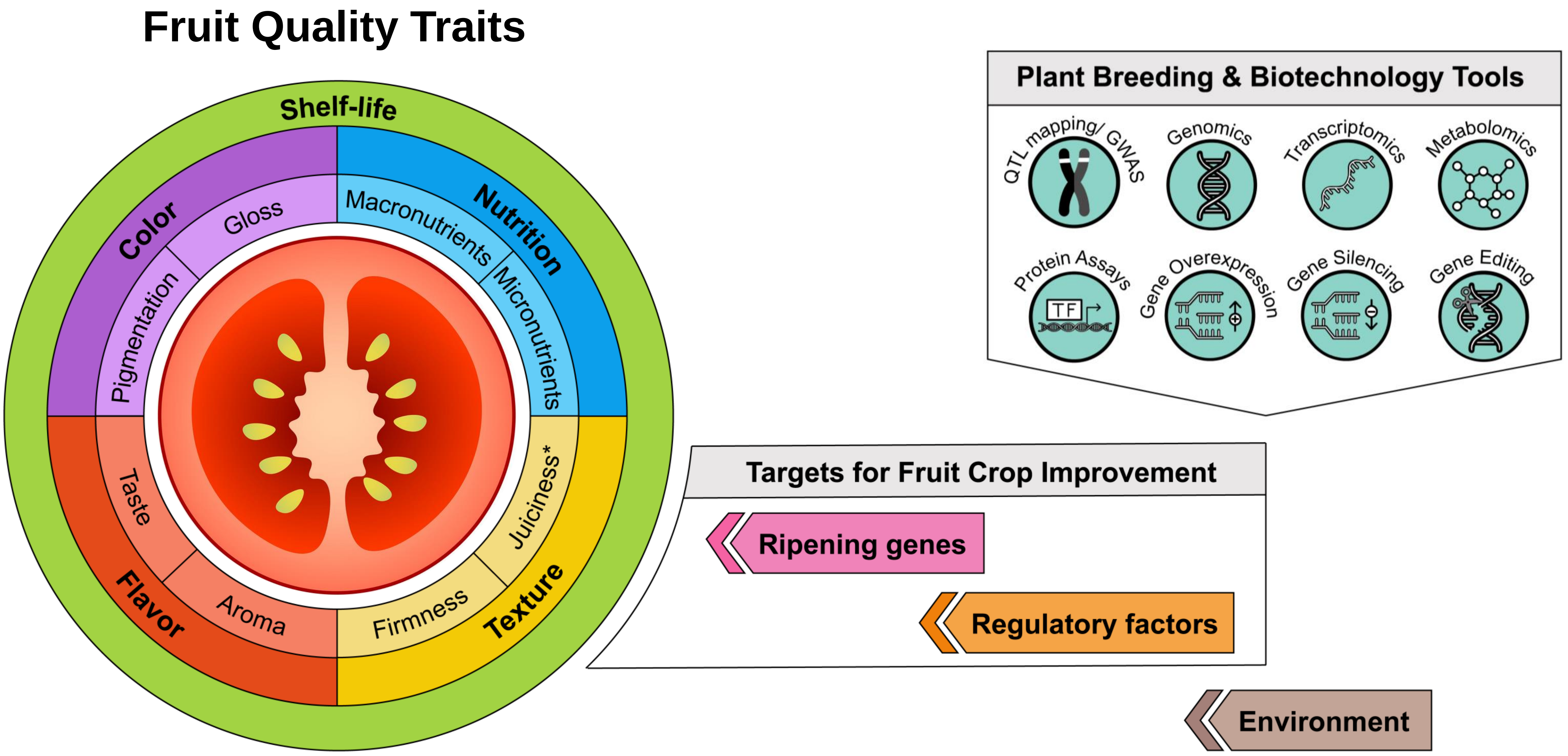
Reduce crop losses and waste

40-50% of fruits and vegetables are lost after harvest

Fruit Gain Quality During Ripening

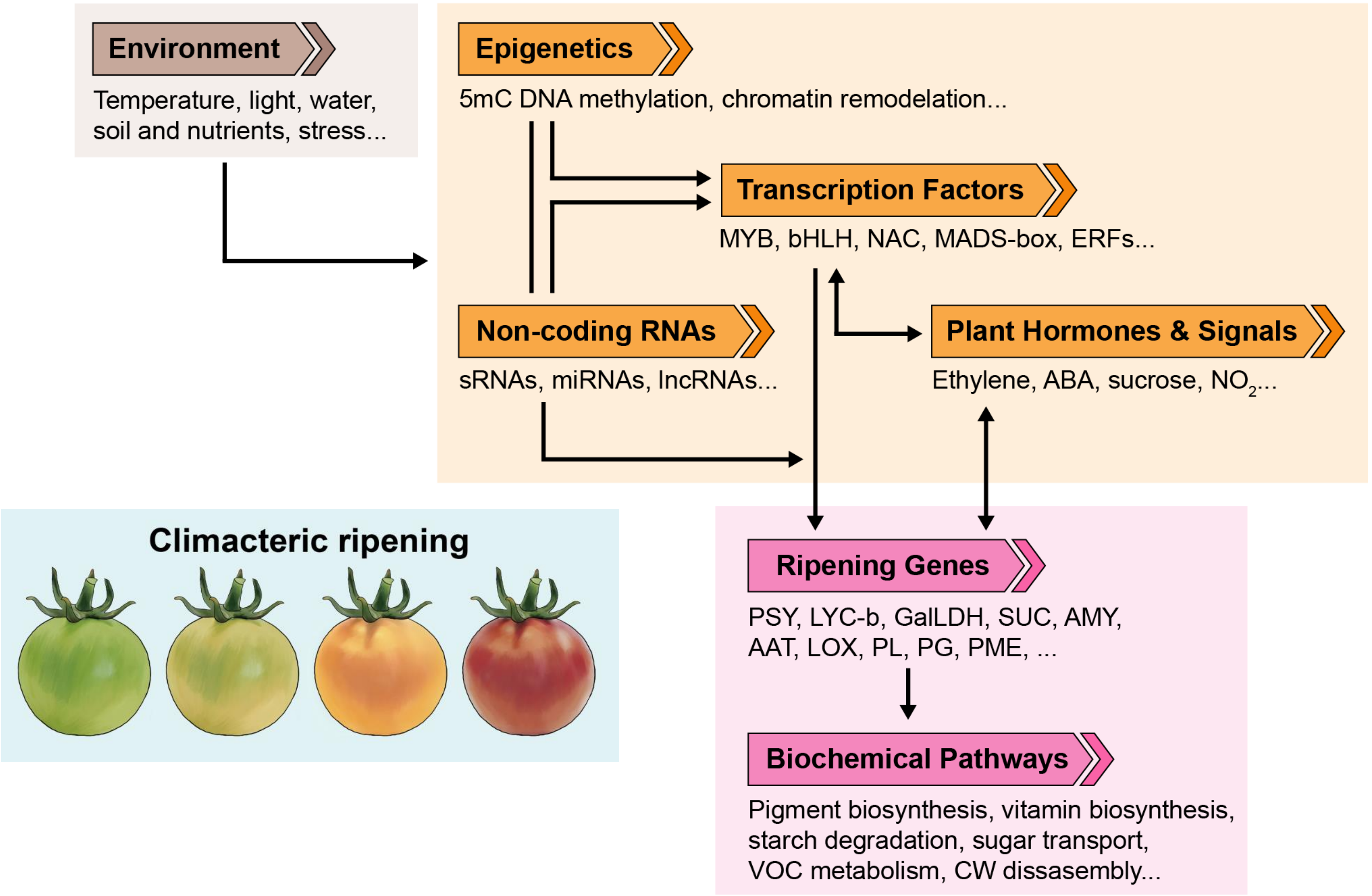
Green	Chlorophyll
Hard	Compacted cell wall
Sour	Low sugars
Mealy	Starch

Colorful	Pigments
Soft	Cell wall disassembly, turgor loss
Sweet	High sugar to acid ratio
Aromatic	Volatiles
Nutritious	Macro and micronutrients

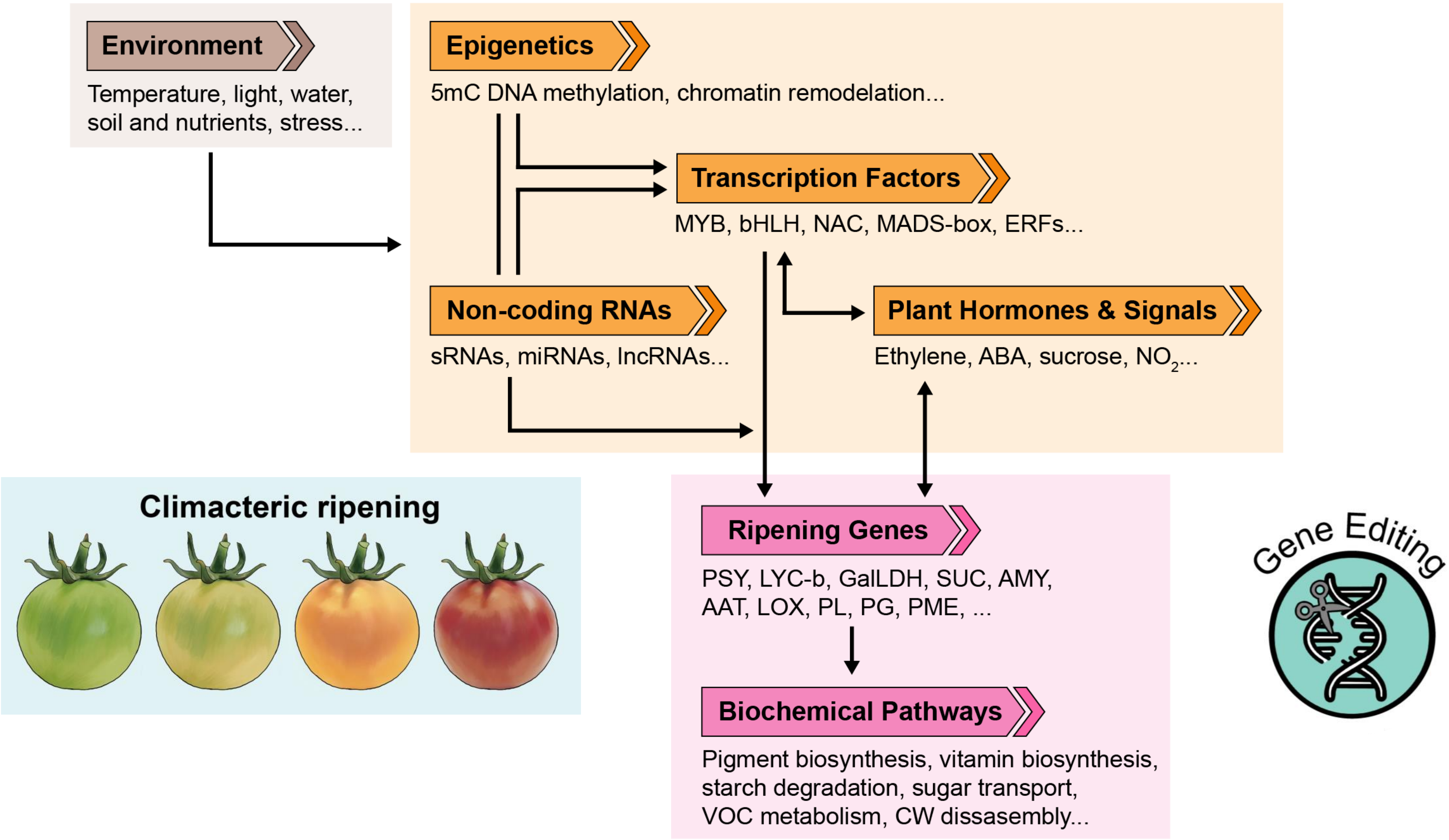


Adaskaveg & Blanco-Ulate.
2022. *Curr Opin Biotechnol*

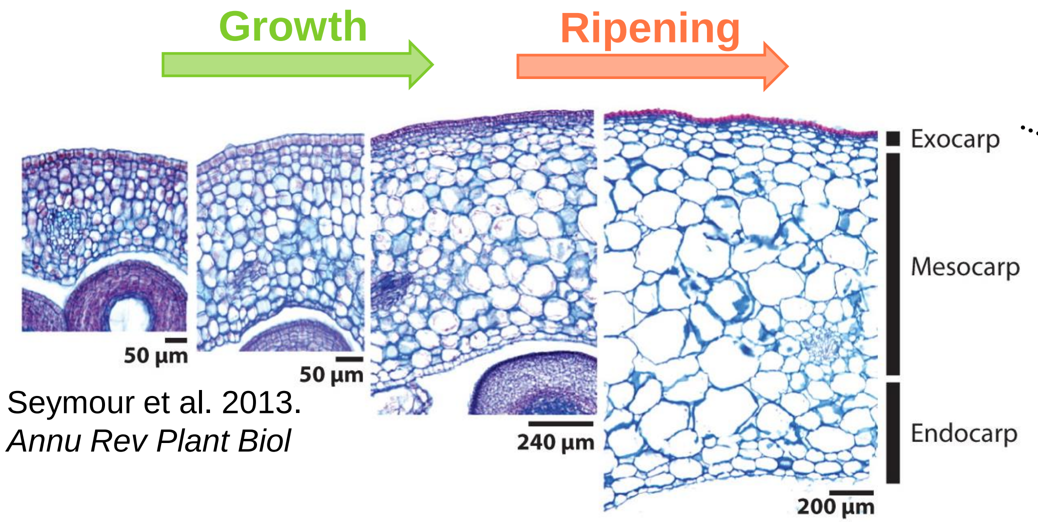
Fruit Ripening Regulators and Pathways



Fruit Ripening Regulators and Pathways

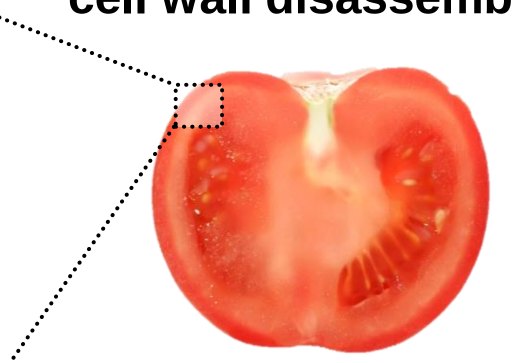


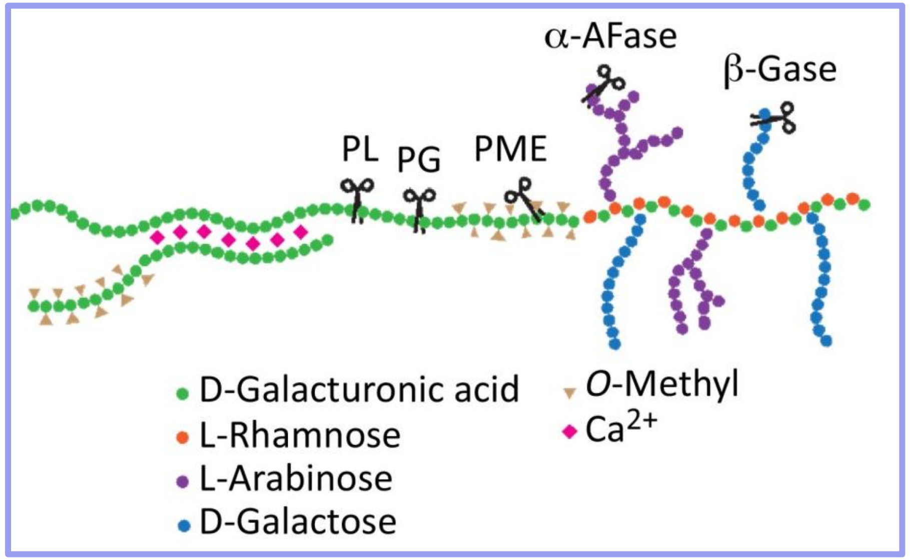
Fruit Softening is A Ripening Event Associated with Quality



Seymour et al. 2013. *Annu Rev Plant Biol*

Cell expansion and cell wall disassembly



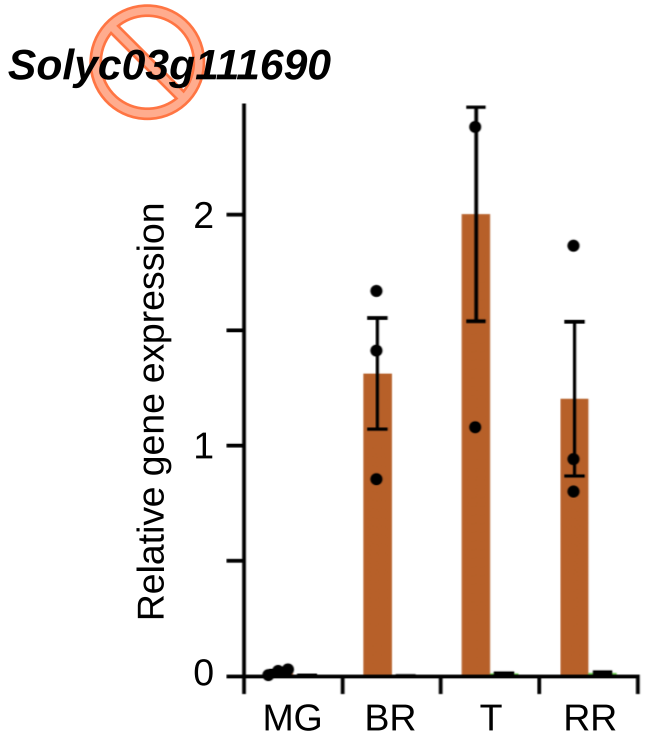


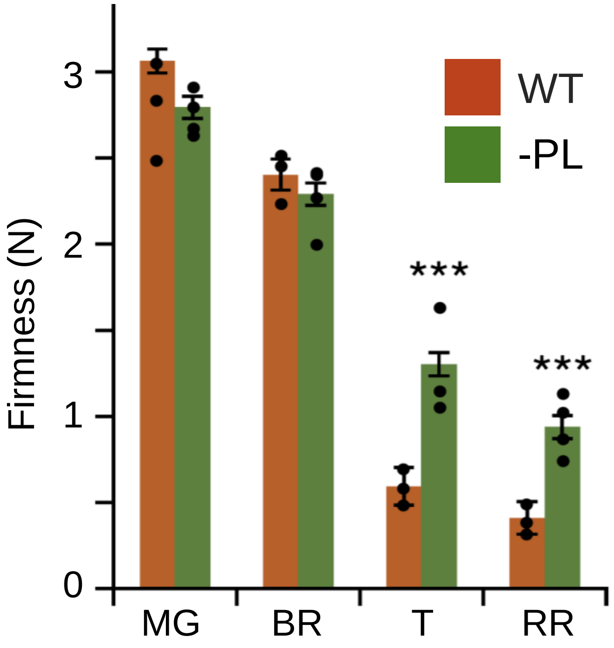
Key pectin degrading enzymes

Pectate lyase (PL) - SlPL
Polygalacturonase (PG) – SlPG2A
Pectin methyl esterase (PME)
α-arabinofuranosidase (α-AFase)
β-galactanase (β-Gase)

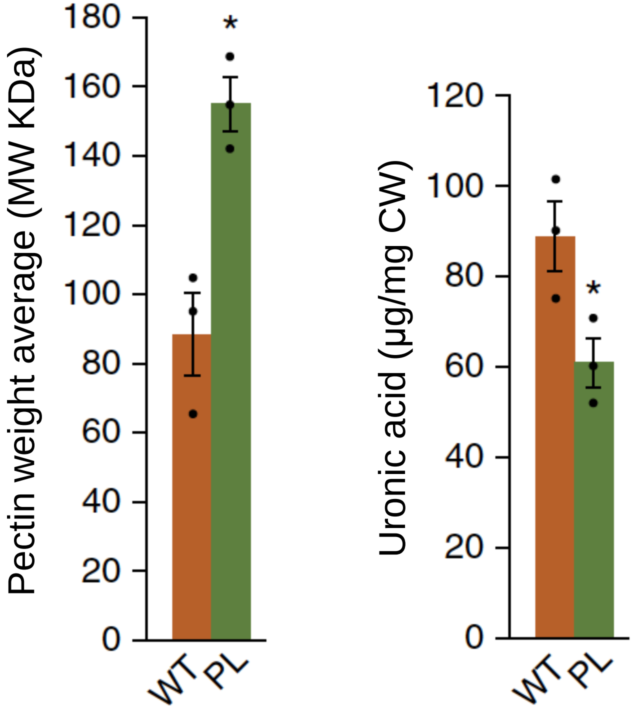
Wang et al. 2018. *Trends Plant Sci*

Pectate Lyase (PL) Significantly Impacts Fruit Softening

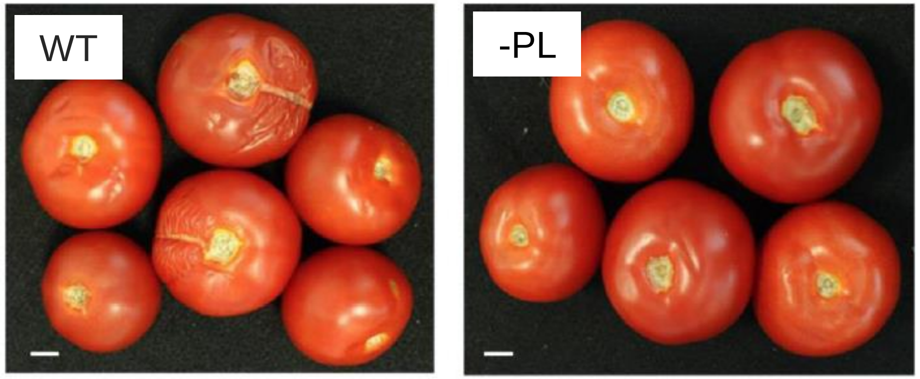




MG Mature Green
BR Breaker
T Turning
RR Red Ripe

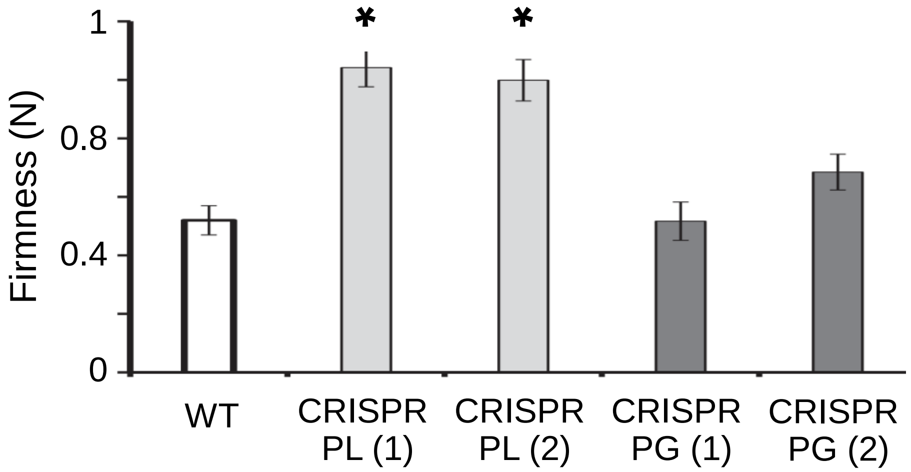


Ripe -PL tomato fruit have reduced cell wall disassembly and are firmer than WT fruit

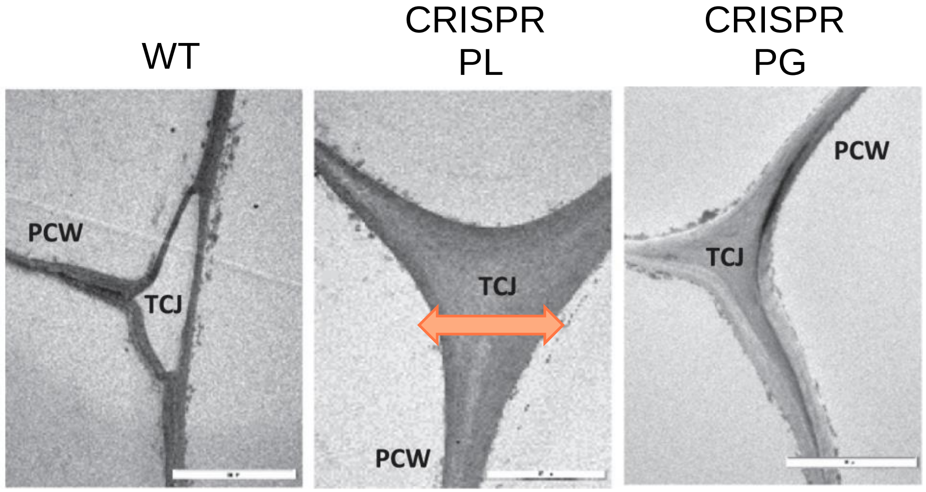


Uluisik et al. 2016. *Nature Biotechnol.*

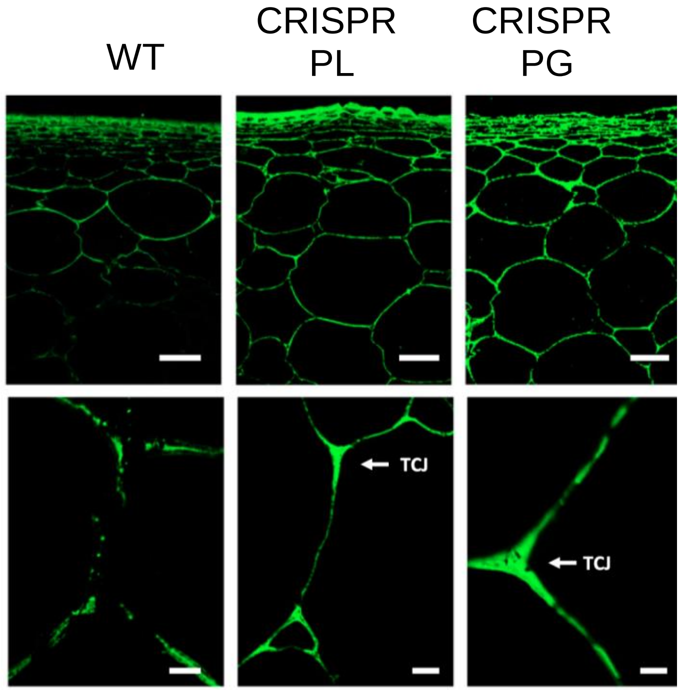
Single CRISPR-Cas9 KO of Tomato *SlPL* and *SlPG2a*



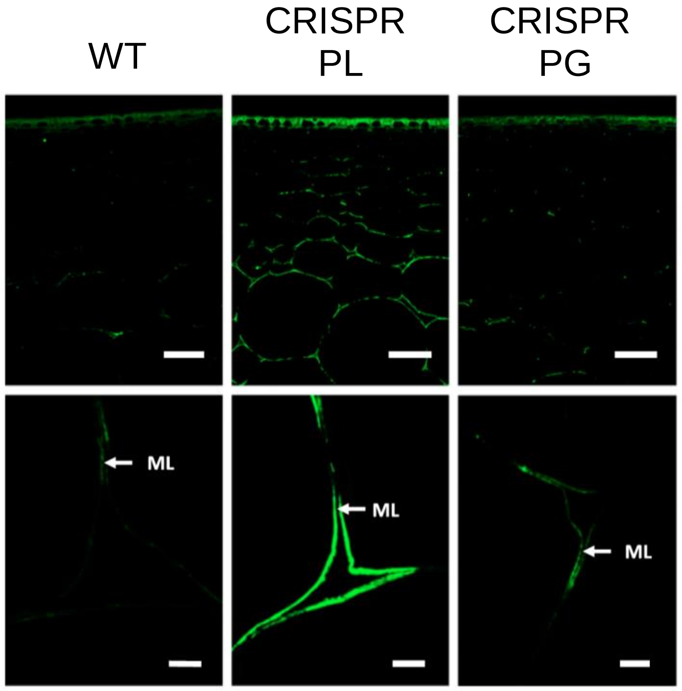
Transmission electron micrographs of cell junctions from the pericarp



Immunolocalization of de-esterified pectin



Immunolocalization of pectin-associated β-galactan



PCW, Primary Cell Wall
TCJ, Tricellular junction
ML, Middle lamella

Wang et al. 2019. *Plant Phys*

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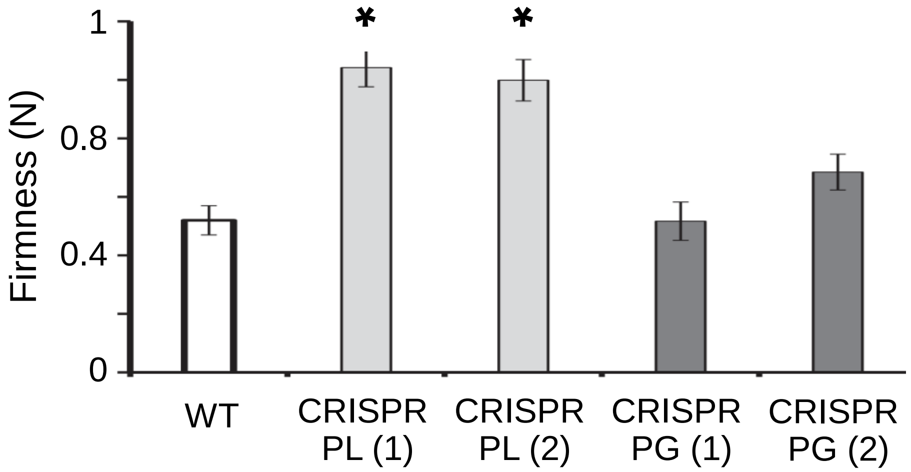

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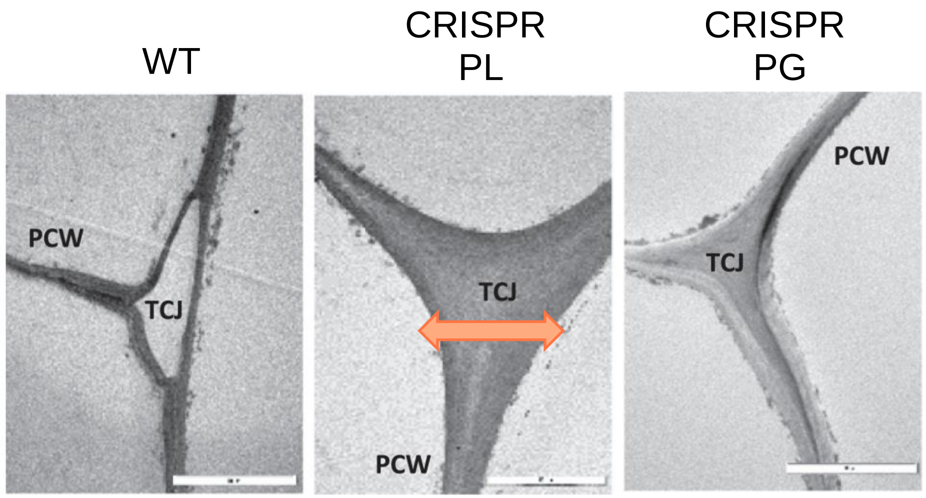
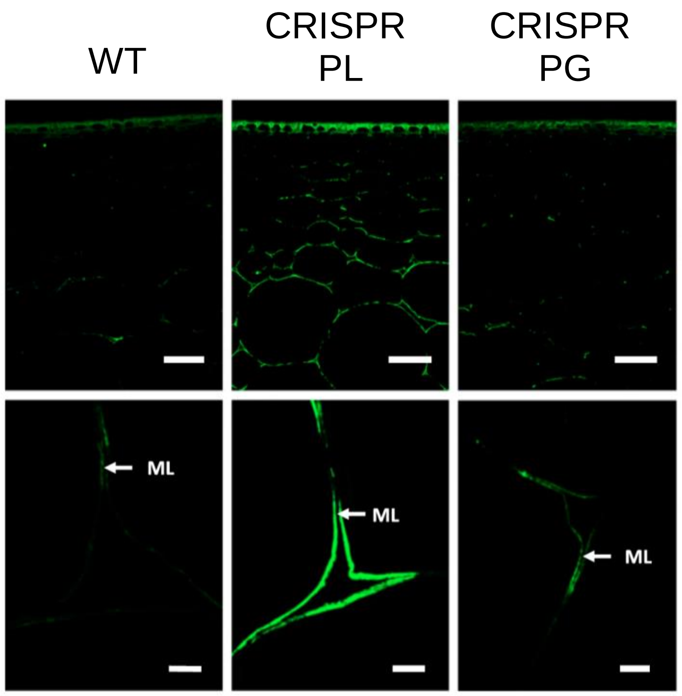


Pectin localization and distribution are altered in CRISPR PL lines

Immunolocalization of pectin-associated β-galactan



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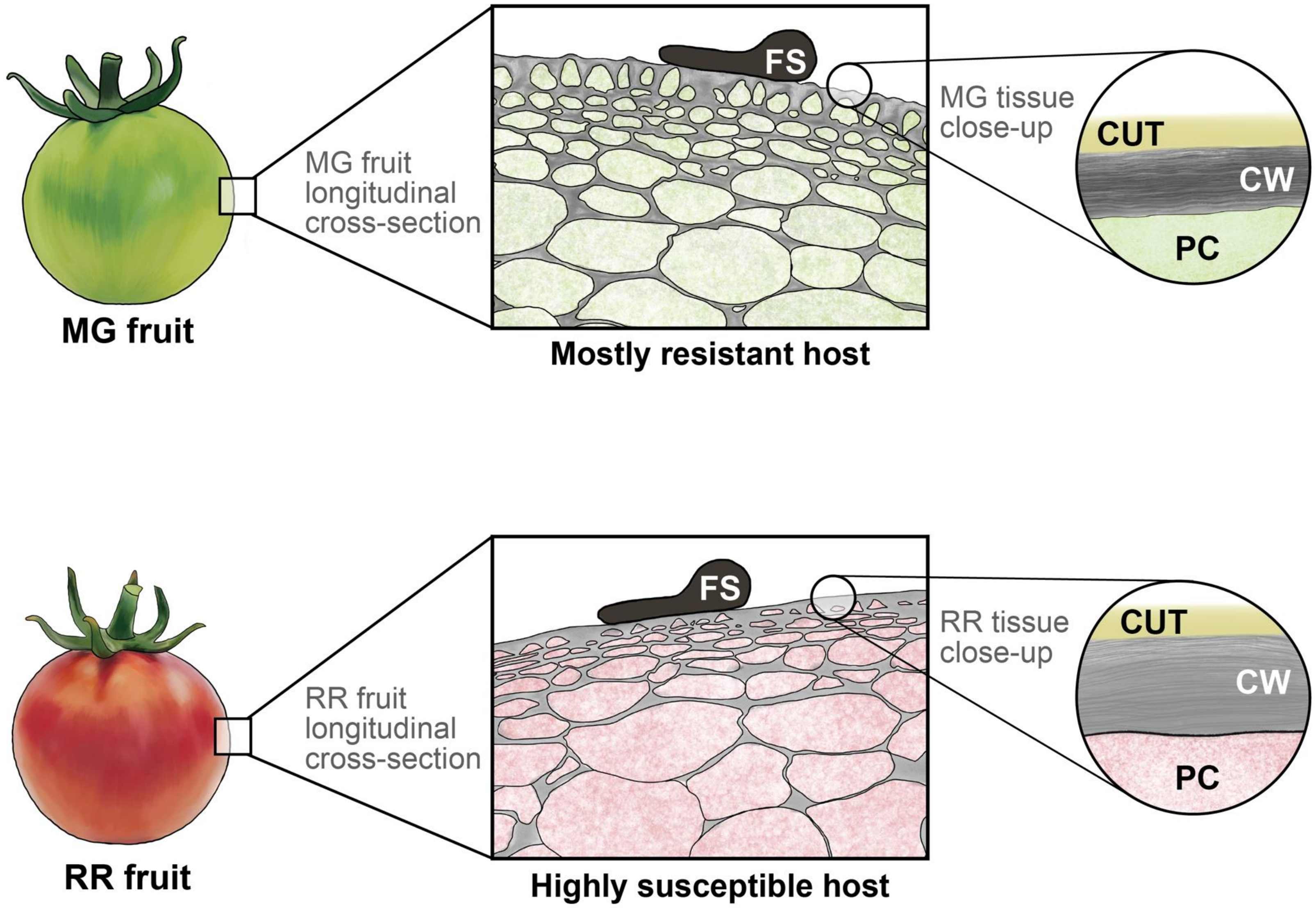
Wang et al. 2019. *Plant Phys*

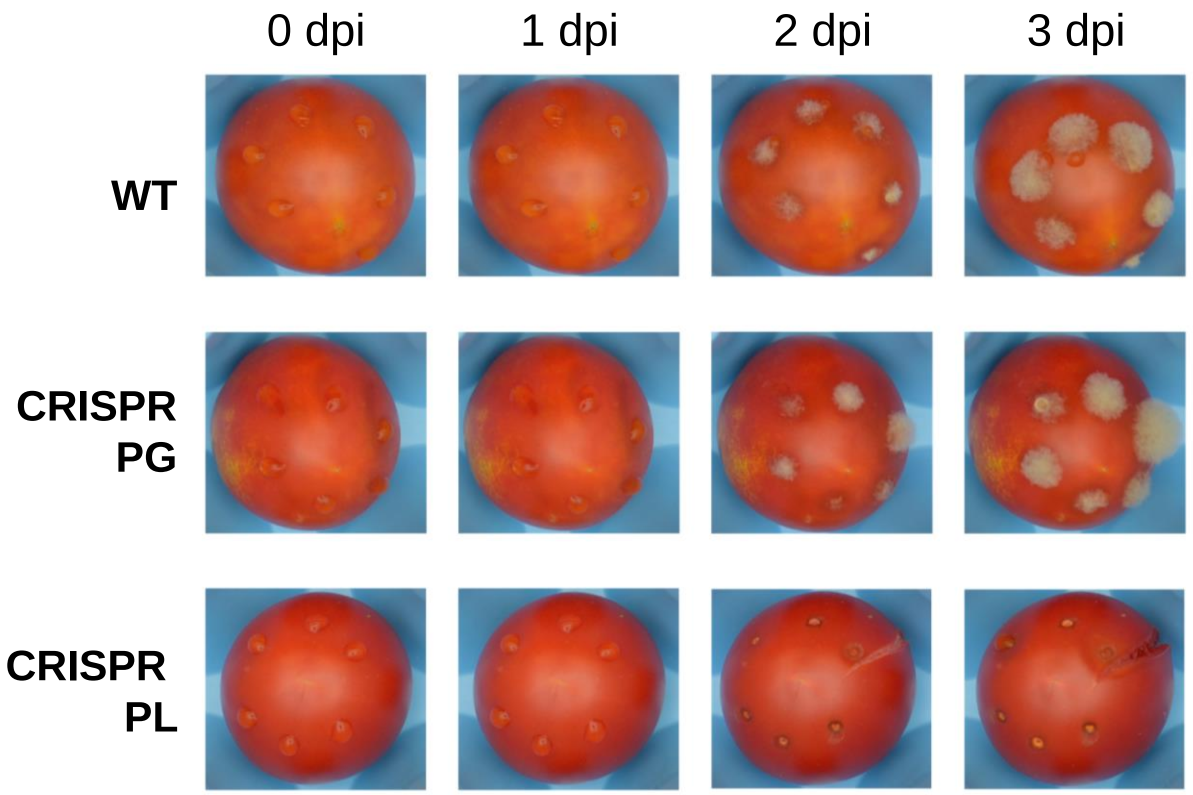
Cell Wall Integrity in Fruit-Pathogen Interactions



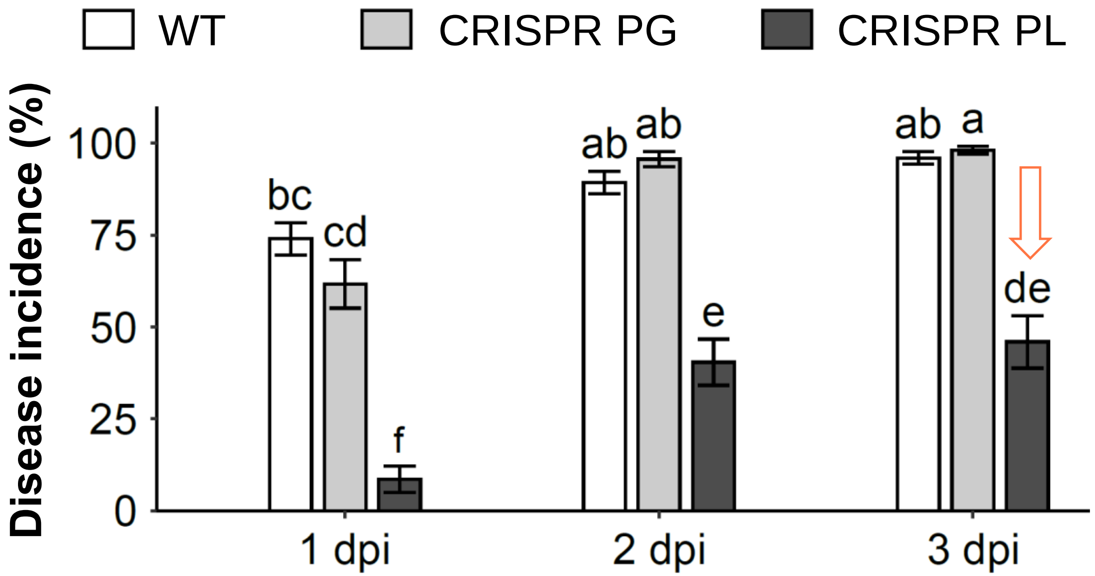
FS: Fungal spore
CUT: Cuticle
CW: Cell wall
PC: Plant cell
CWI: CW integrity

Wang et al. 2022. *Ann. Plant Reviews*

SlPL is a Major Susceptibility Factor for Fruit Rotting



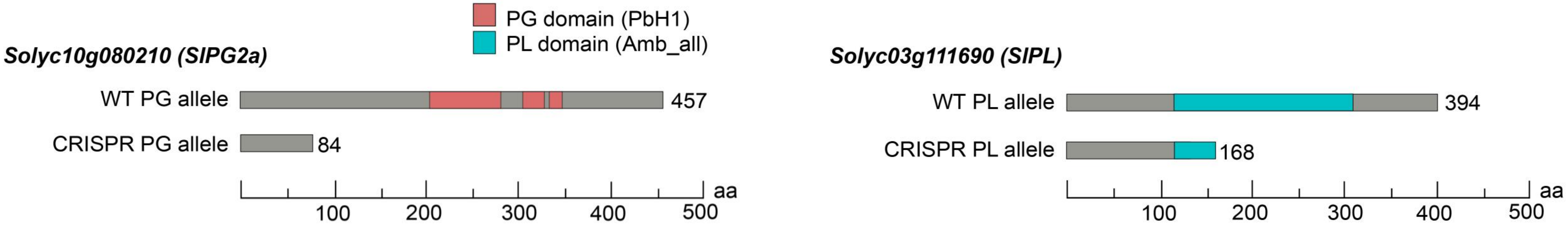
dpi = days post-inoculation

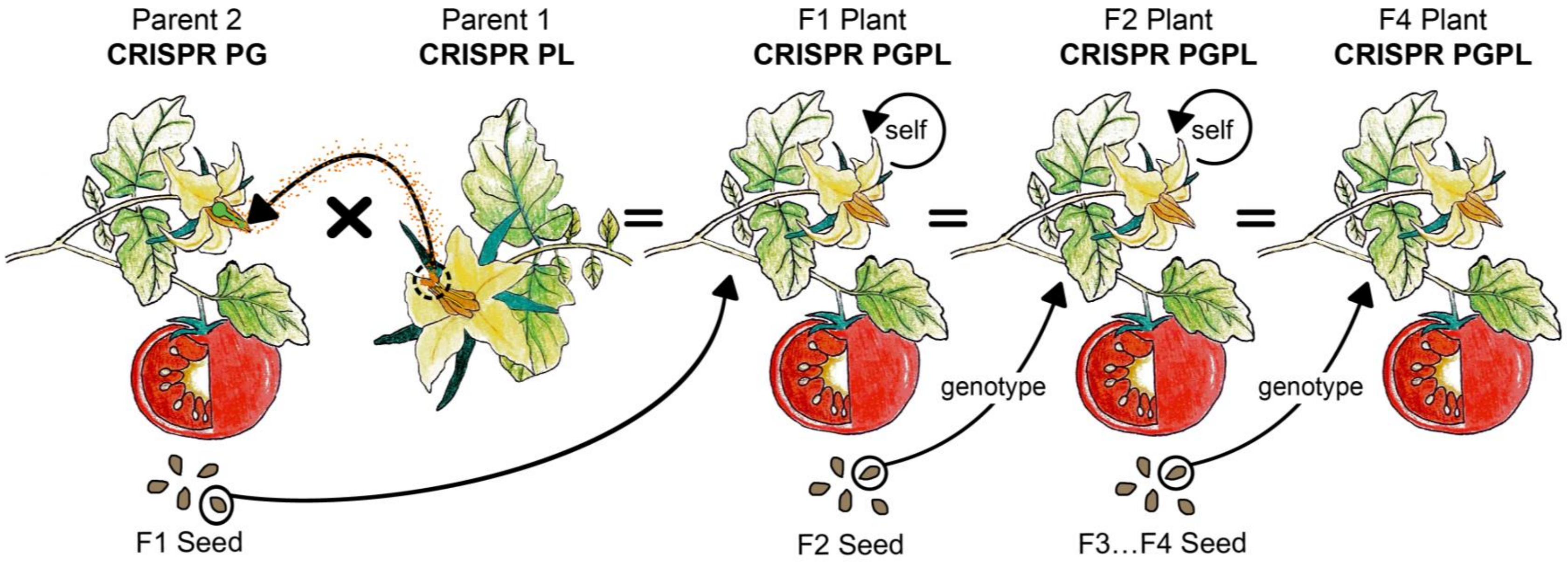


Susceptibility to *B. cinerea* in ripe CRISPR-PL fruit is reduced by ~50%!

Silva et al. 2021. *J Ex Bot*

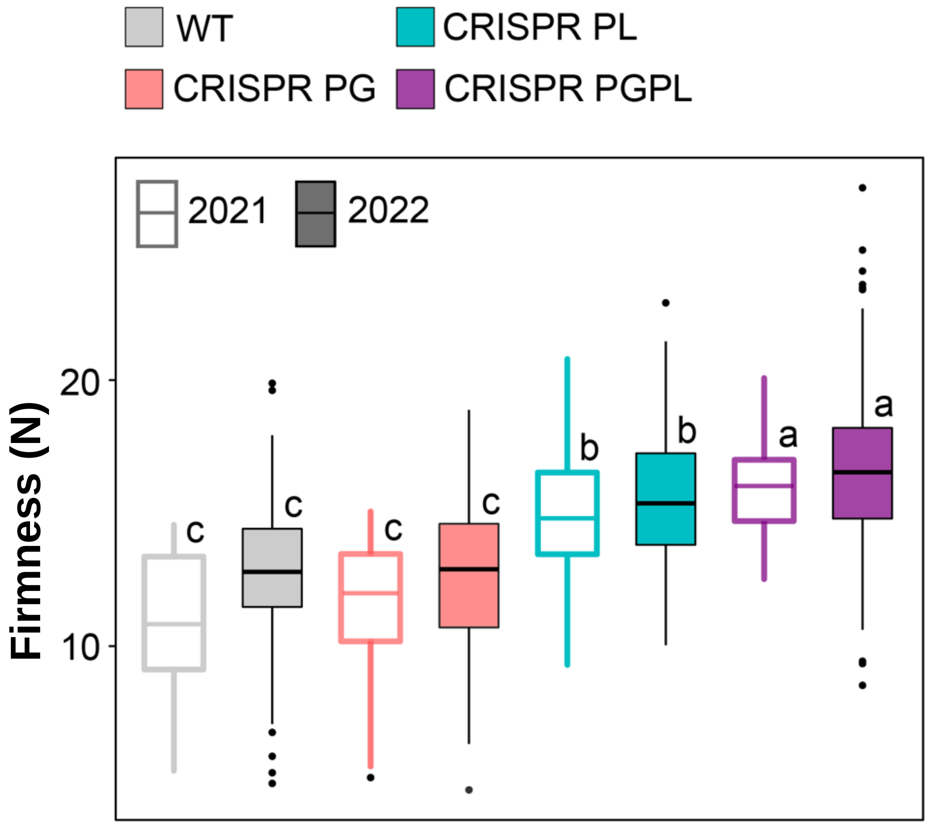
Double CRISPR-Cas9 KO of Tomato *SlPL* and *SlPG2a*





Ortega-Salazar et al. 2023. *PPP*

Double CRISPR-Cas9 KO of Tomato *SlPL* and *SlPG2a*



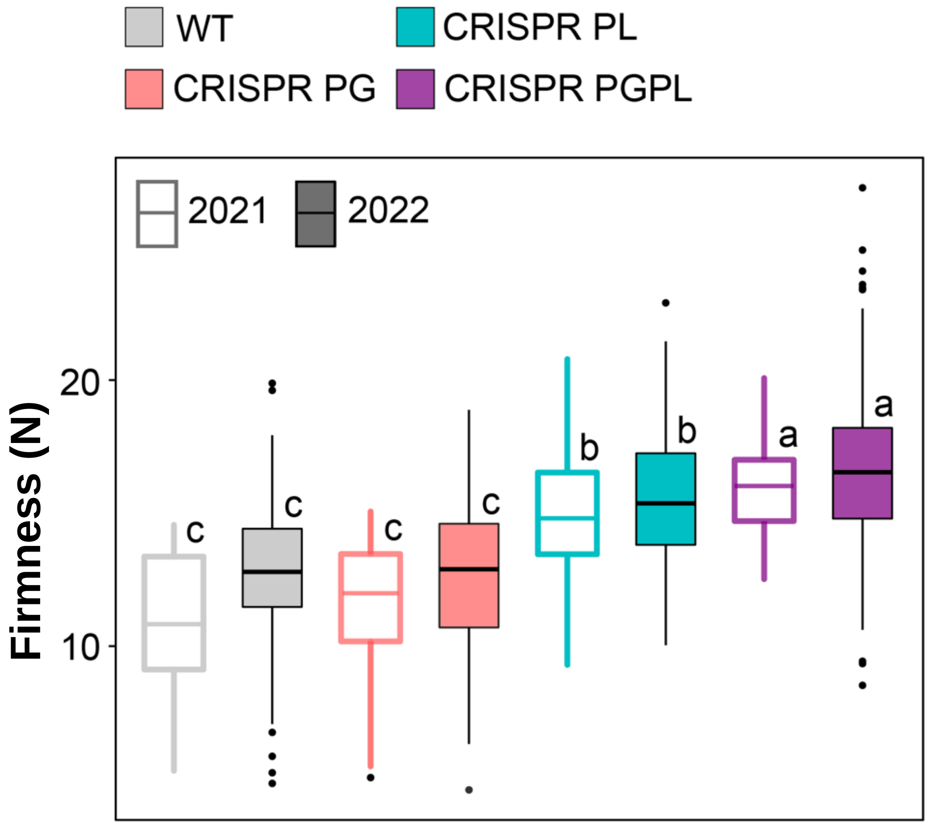
Double CRISPR PGPL shows an additive phenotype in firmness

Double CRISPR PGPL shows no differences in sugar:acid ratio to the WT

Taste related attributes

Line	Total Soluble Solids (%Brix)	pH	Titratable Acidity (%)	TSS: TA
WT	6.23 a	4.49 a	0.50 a	12.55 a
CRISPR PG	6.27 a	4.58 a	0.47 a	13.58 a
CRISPR PL	6.48 a	4.50 a	0.50 a	12.96 a
CRISPR PGPL	6.39 a	4.46 a	0.51 a	12.57 a

Double CRISPR-Cas9 KO of Tomato *SlPL* and *SlPG2a*



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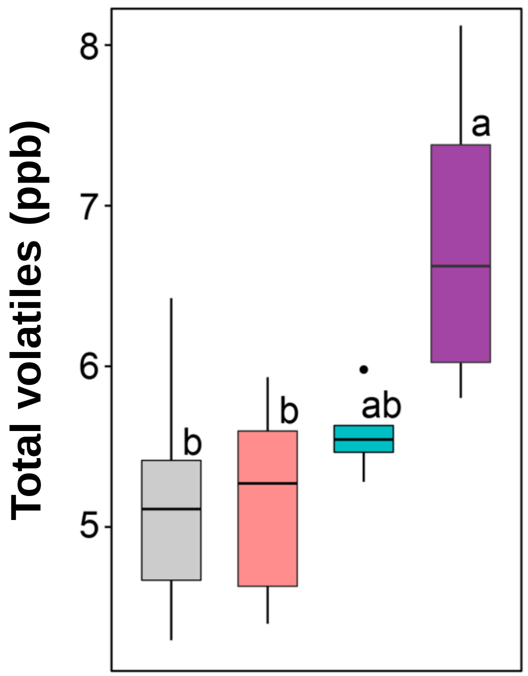
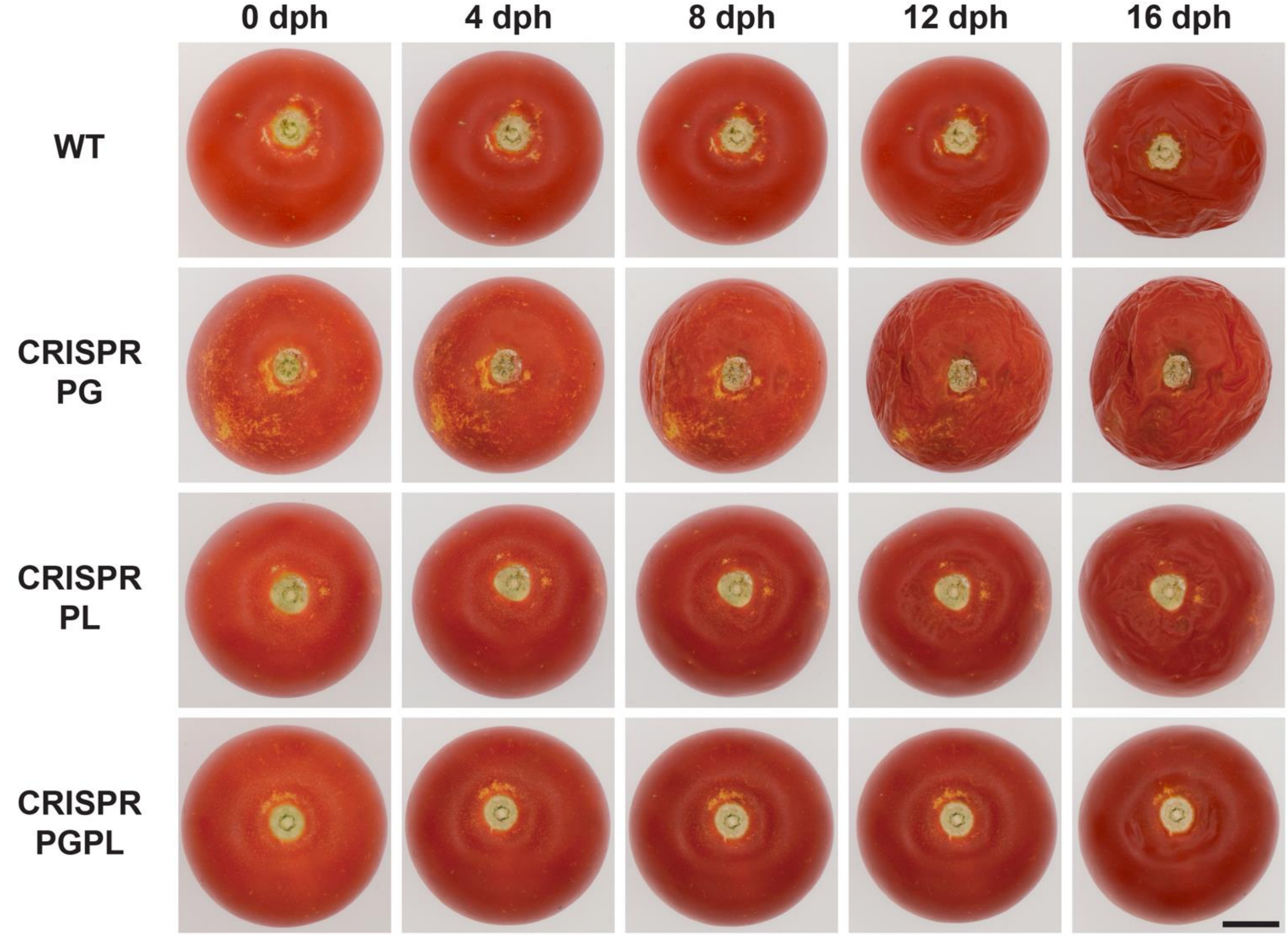


Double CRISPR PGPL has more volatiles than the single CRISPR lines and the WT

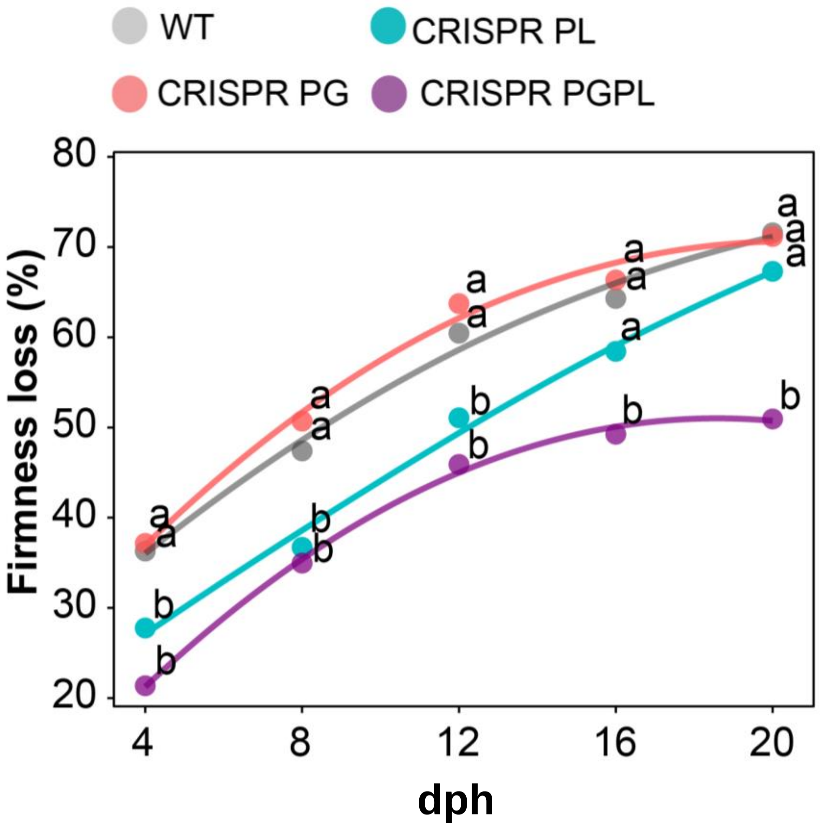
Volatiles more abundant in the CRISPR PGPL fruit

heptanal
3-methyl-2-butenal
2- hexenal, benzaldehyde
trans-beta-ionone
2-pentenal
trans-2-heptenal
2,4-heptadienal
benzeneacetaldehyde
methyl salicylate

Double CRISPR-Cas9 KO of Tomato *SlPL* and *SlPG2a*

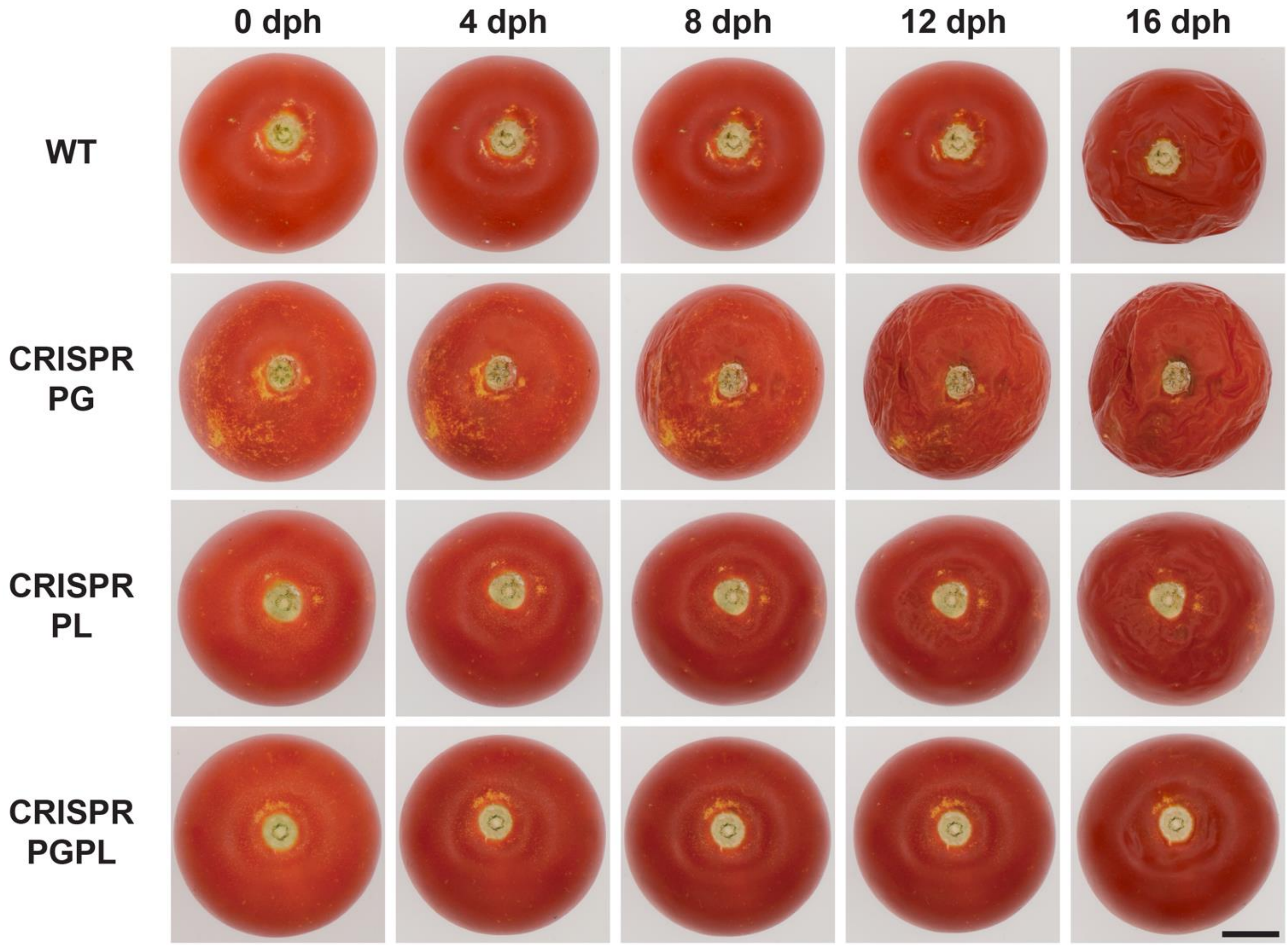


dph = days posthavest



Double CRISPR PGPL
and single CRISPR PL have
improved shelf-life

Double CRISPR-Cas9 KO of Tomato *SlPL* and *SlPG2a*



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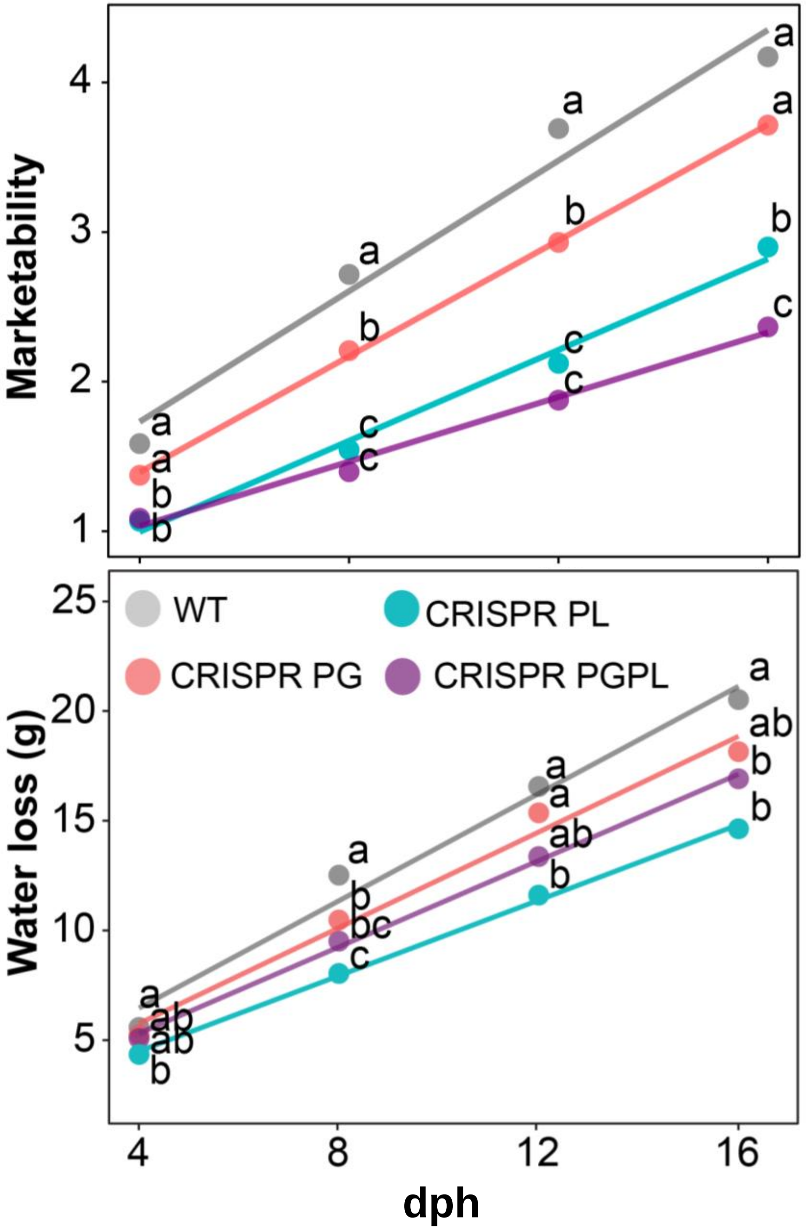

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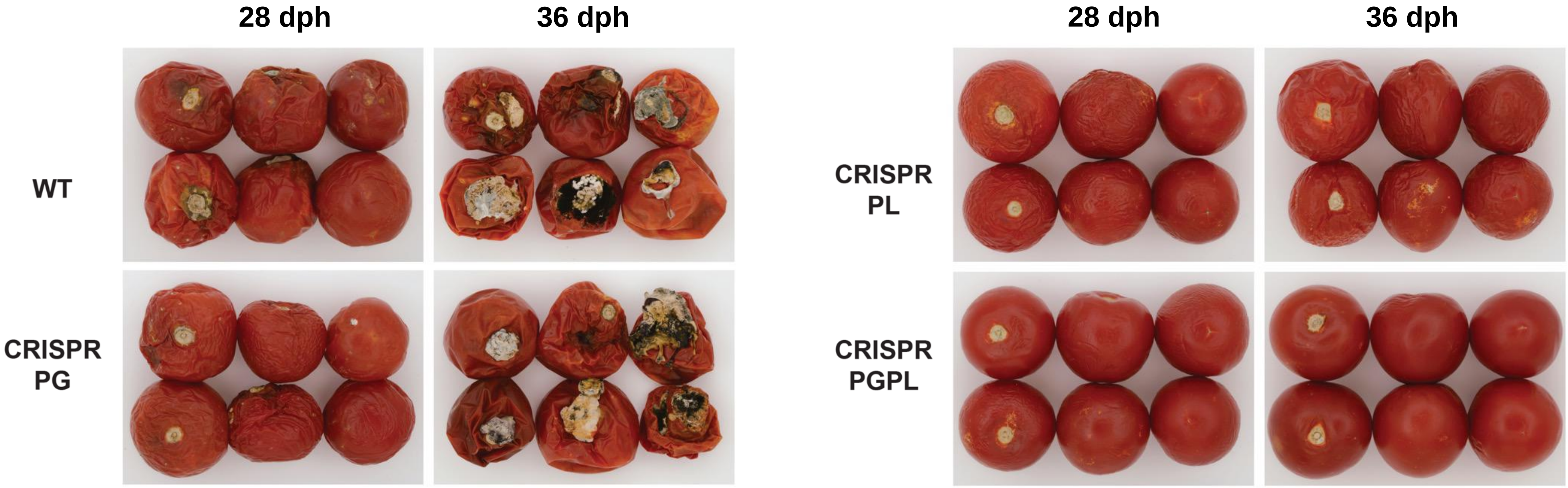
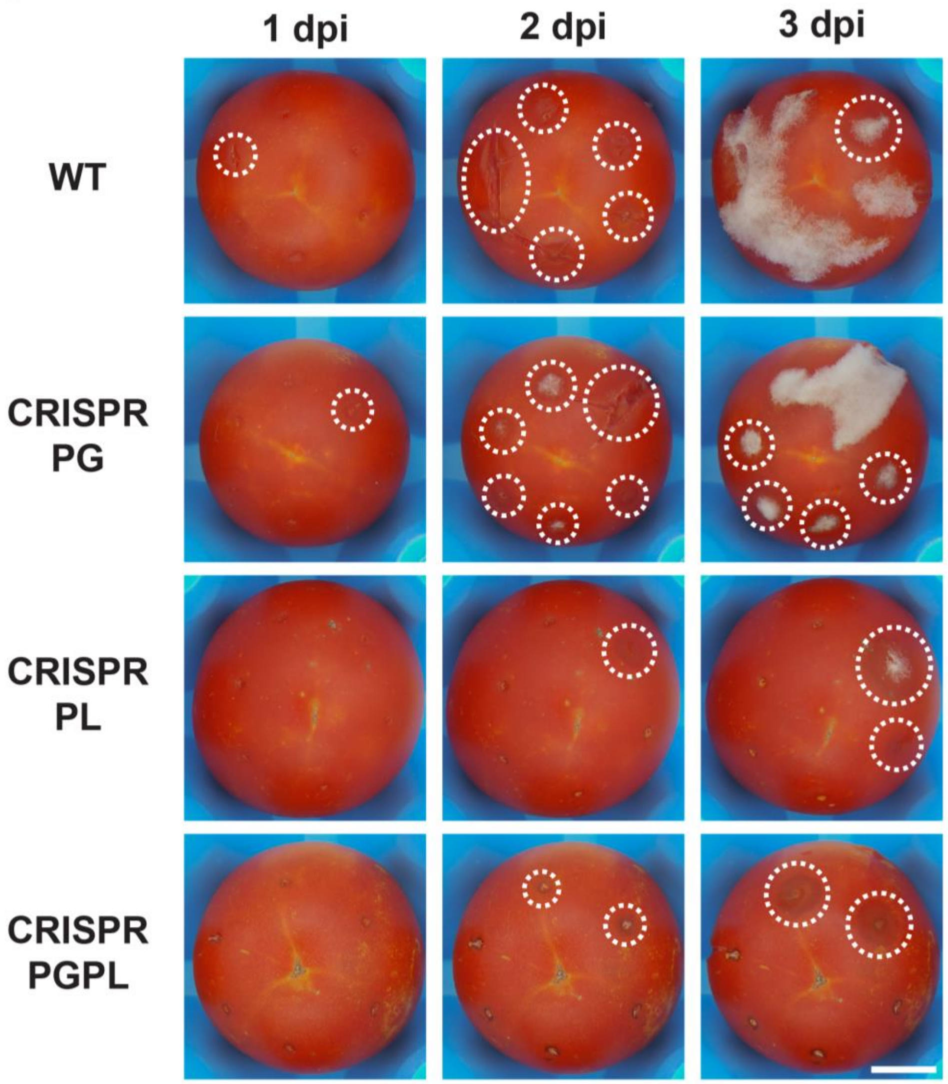


dph = days posthavest

- Double CRISPR PGPL shows longer shelf-life than WT and single CRISPR PL or PG
- No impacts on flavor attributes in the double CRISPR on flavor

Ortega-Salazar et al. 2023. *PPP*

Double CRISPR-Cas9 KO of Tomato *SlPL* and *SlPG2a*

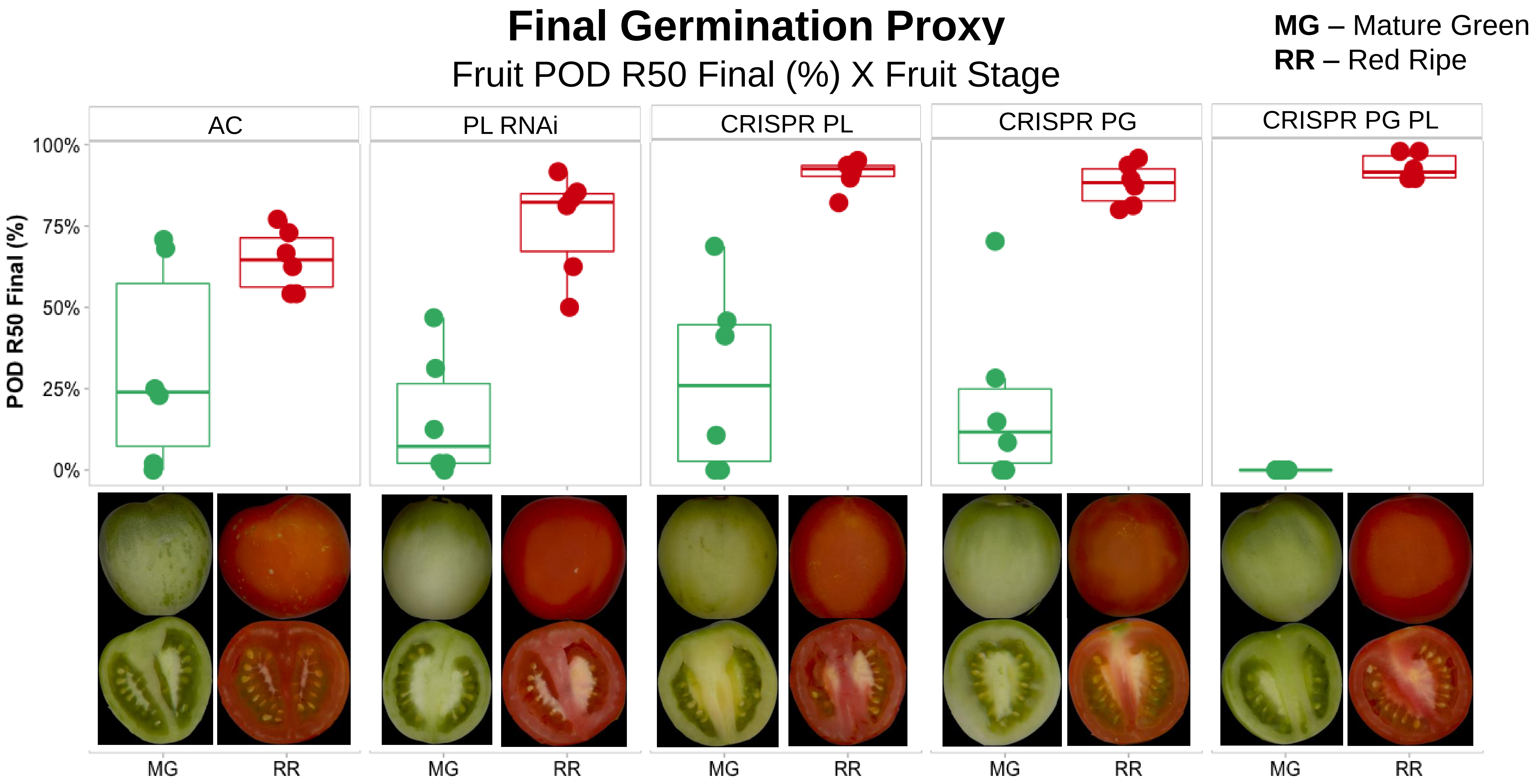


Line	Disease incidence (%) 1 dpi	2 dpi	3 dpi
WT	3.76 *b*	82.58 *a*	88.61 *a*
CRISPR PG	11.88 *a*	85.86 *a*	89.09 *a*
CRISPR PL	3.25 *b*	53.48 *b*	63.62 *b*
CRISPR PGPL	6.51 *b*	52.51 *b*	65.95 *b*

Double CRISPR PGPL and single CRISPR PL have the same improved fruit resistance to fungal infections

Ortega-Salazar et al. 2023. *PPP*

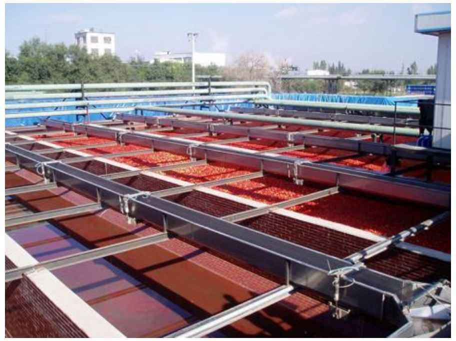
Double CRISPR-Cas9 KO of Tomato *SlPL* and *SlPG2a*

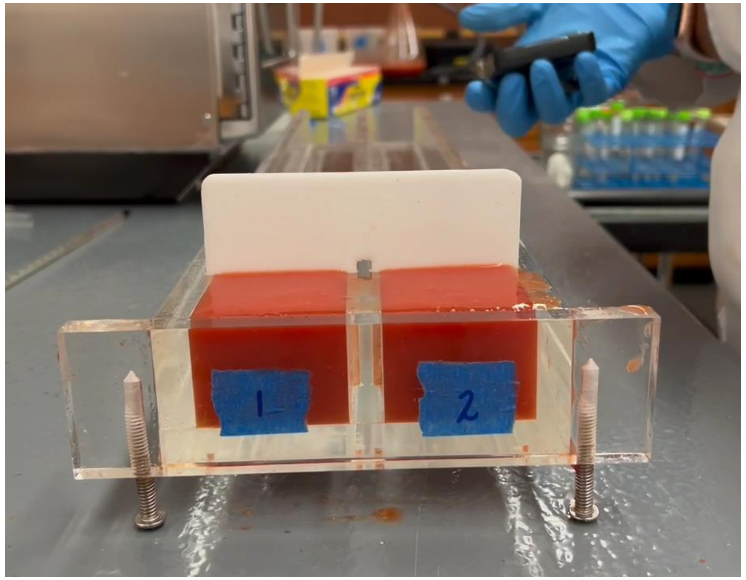


Potential Benefits to Processing Tomato Quality

Tomato processing quality traits

- ✓ **Consistency (Viscosity)**
- ✓ **Extended Field Harvest**
- ✓ Color
- ✓ Total soluble solids
- ✓ Titratable acidity and pH
- ✗ **Mold presence**





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