Evaluation of CRISPR/cas9 disease-resistant tomatoes for the prevention of *Salmonella* colonization

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INTRODUCTION

- **Salmonellosis is a common food-related illness in the US** caused by several *Salmonella* strains.
- Tomato fruit can be contaminated upon *Salmonella* exposure to the plant's roots, stems, and flowers**. 31 cultivars tested in FI have shown internal colonization.** [1,2]
- **FL8000 tomato variety was CRISPR/cas9 mutated for increased resistance** by knocking out key SAR down-regulators.
- **The CRISPR mutated tomatoes are not regulated as a genetically engineered crop.**[8]
- The **FL8000 mutants will be used to study whether *Salmonella enterica* Typhimurium 14028 can successfully colonize and persist in the mutated plants compared to their wild-type.**
- ***Salmonella* presence will be determined by extracting bacteria from the stem, petiole, roots, and rhizosphere.**
- Results from the study will provide knowledge to **improve food security and public health, pest and disease management, and sustainability and the environment.**
- Additionally, endophytic and rhizobial microbiomes will be studied.

SIGNIFICANCE

PROs

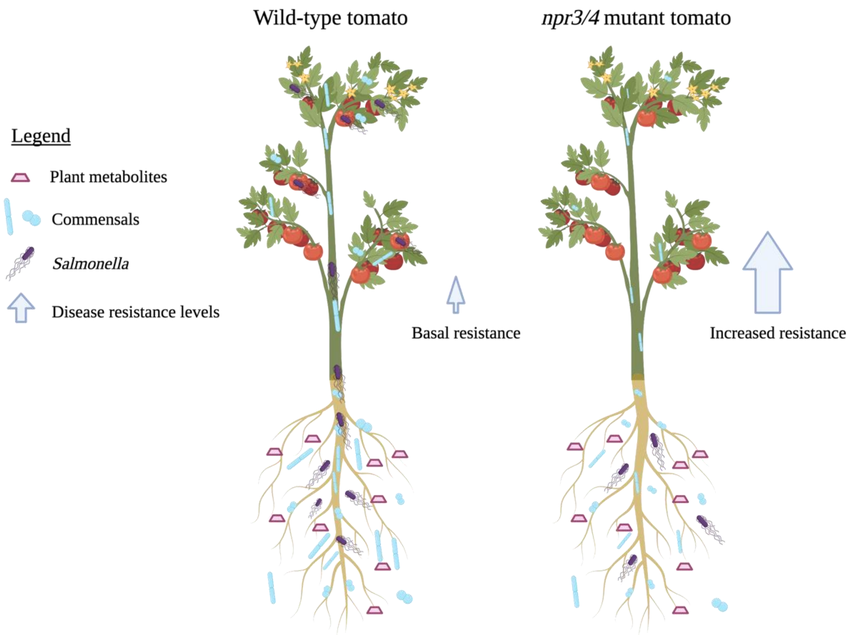
- Not a transgenic process = USDA doesn't regulate
- Built-in resistance is good for plant health and food safety
- Reduce broad-spectrum pesticides
- Promote soil diversity
- This CRISPR mutation can be used in other crops
- Can be advantageous for small farmers and organic growers

CONs

- Not studied in the field yet
- May have neg. consequences for commensals
- May have neg. results in plant yield and health in the field

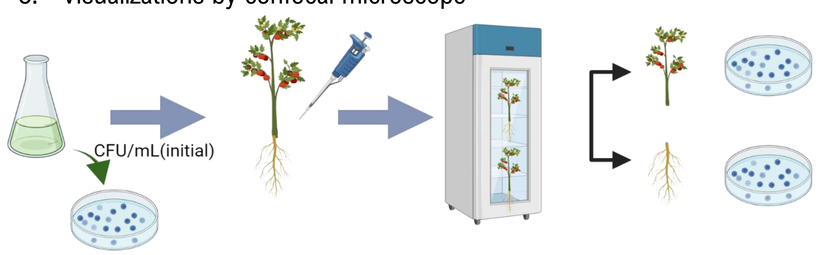
HYPOTHESIS

CRISPR/Cas9 mutated tomato for increased plant defenses will detect MAMPs and prevent bacterial colonization and persistence compared to the unmutated wild-type parent



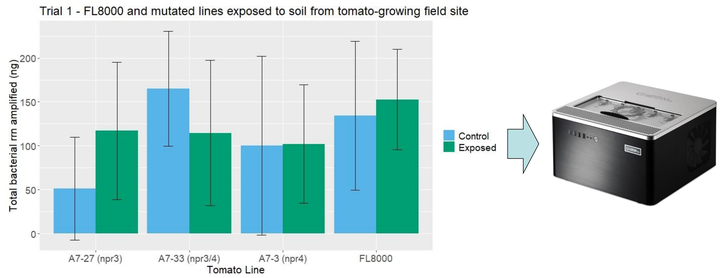
METHODS

1. Trial 1: FL8000 and mutated lines A7-27(*npr3*), A7-3 (*npr4*), A7-33 (*npr3/4*) transferred to soil collected from a tomato field site in Balm, FI.
   1. Bacterial DNA extractions from apoplast to sequence endophytes.
   2. Compare endophytes in FL8000 wild-type tomato and mutated lines for differential uptake.
2. Trial 2: FL8000 and the mutated lines inoculated with GFP-*Salmonella* to determine differential uptake and colonization.
   1. Inoculations in the roots and by clippings at different concentrations.
   2. Apoplast and roots harvested. Initial and final CFU/mL determined by agar plating.
   3. Visualizations by confocal microscope



PROGRESS AND RESULTS

Trial 1 – pending sequencing results



Trial 2 – pending extracted CFU/mL results

Inoculation Type	Initial CFU/mL	Final CFU/mL
Soil Drench 1	10^8	Late March '22
Soil Drench 2	10^5	
Soil Drench 3	10^1	
Clipping 1	10^8	
Clipping 2	10^5	
Clipping 3	10^1	

Upcoming work

Confocal microscope imaging → Commensal studies → Field trials

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Graphical hypothesis and methods created with BioRender.com

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