

Progress Developing New Citrus Varieties Using CRISPR and Other Biotech Approaches

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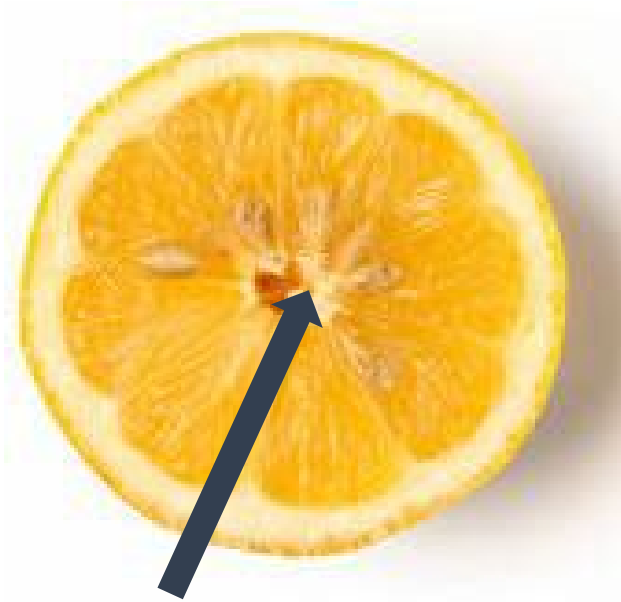


Setting Realistic Expectations

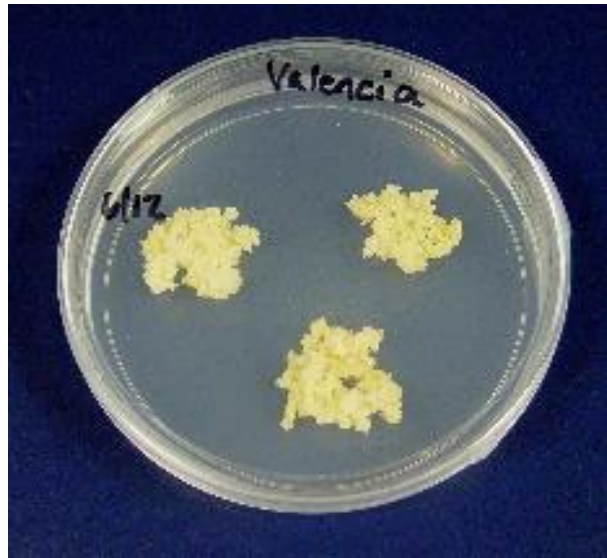
How long does it take to develop a new variety using CRISPR?

Where are we now in this process...how much longer?

CRISPR Process



Extraction of unfertilized ovules

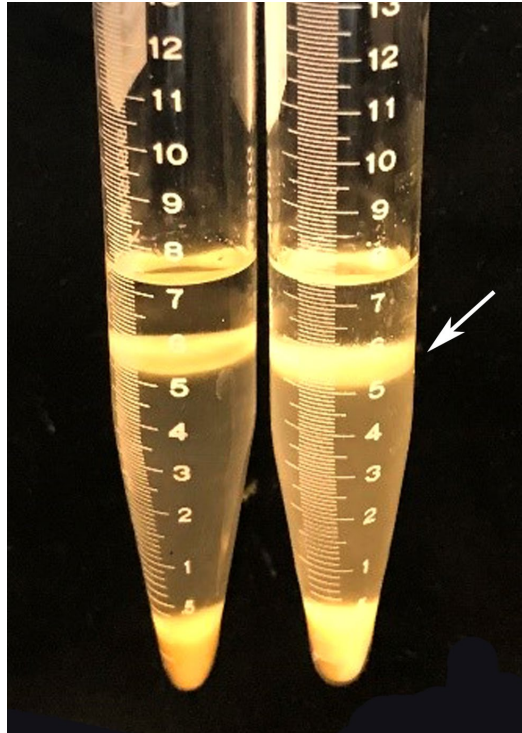


Cells obtained from unfertilized ovules

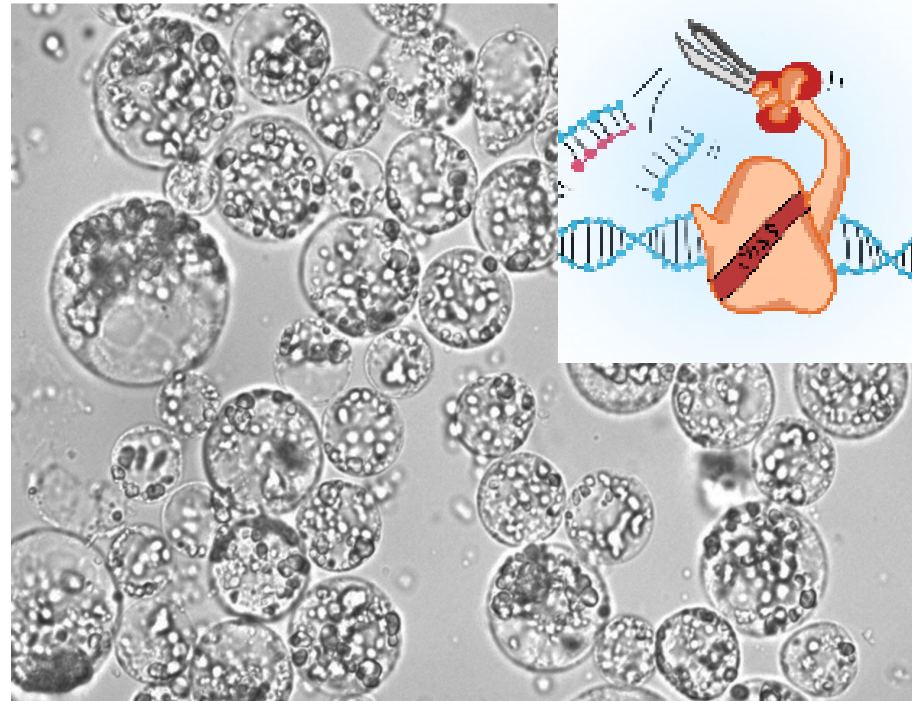


Mass production of cells in liquid medium

CRISPR Cell Editing



Protoplast obtained from cells



CRISPR used to knock out specific DNA in protoplast cells

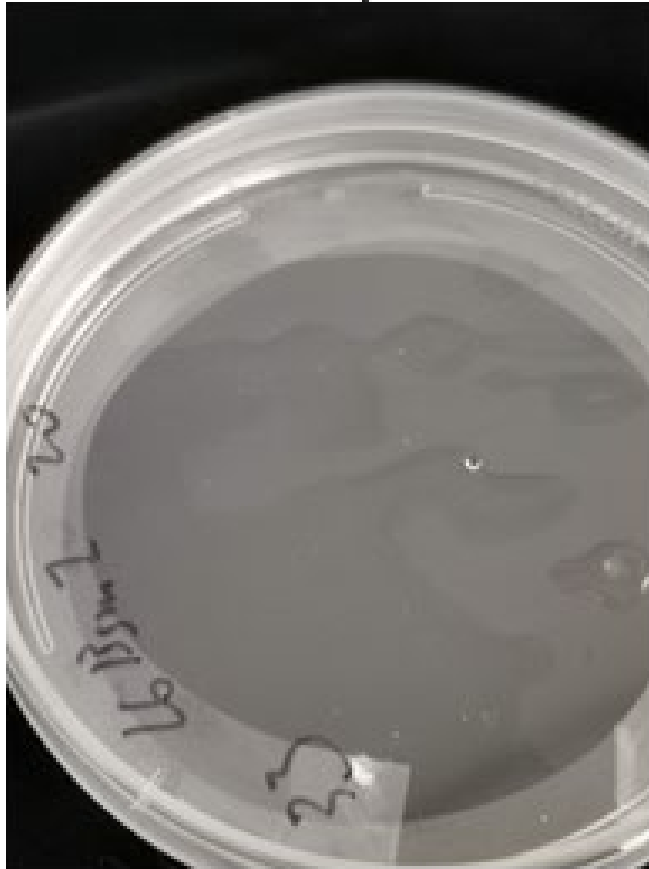
CRISPR Process – Protoplast Development

After genome editing
using citrus embryogenic
protoplast cells



A

3-4 weeks
Protoplast



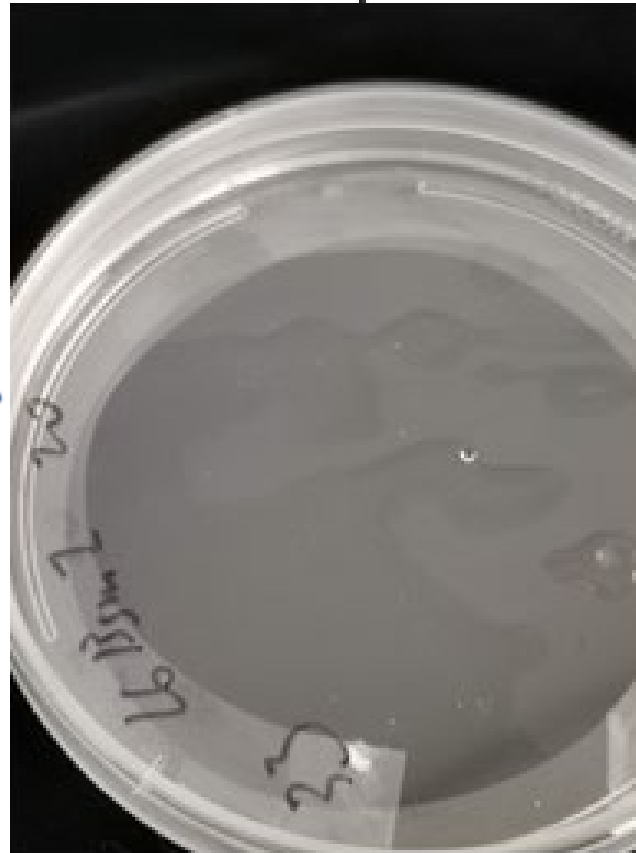
CRISPR Process – Callus Formation

After genome editing
using citrus embryogenic
protoplast cells



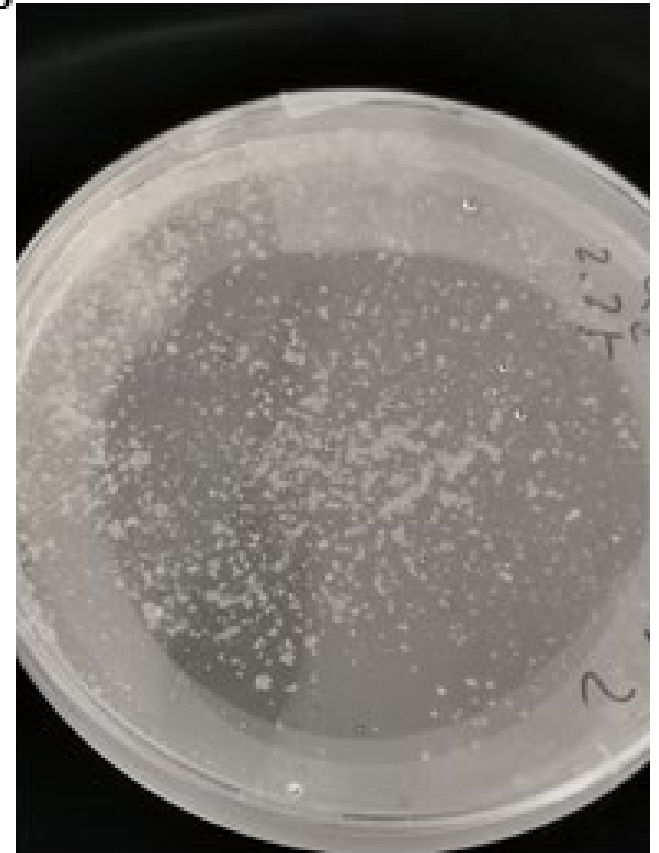
A

3-4 weeks
Protoplast



B

3-4 weeks
Callus



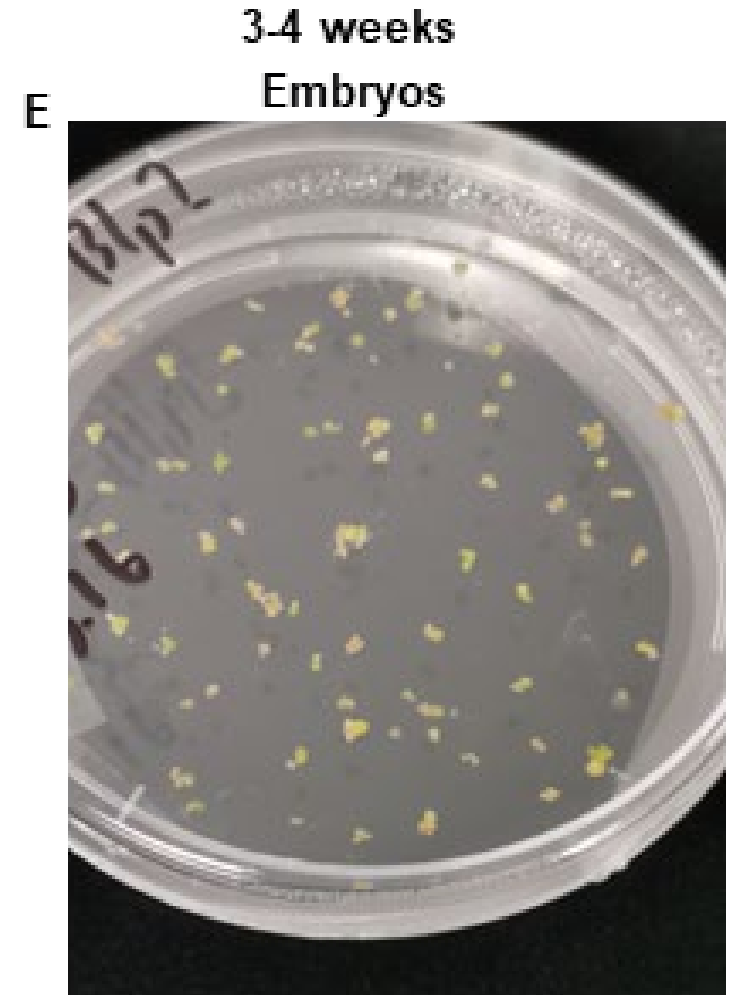
CRISPR Process – Callus Embryos

3-4 weeks



CRISPR Process – Callus Embryos, 3 to 4 weeks

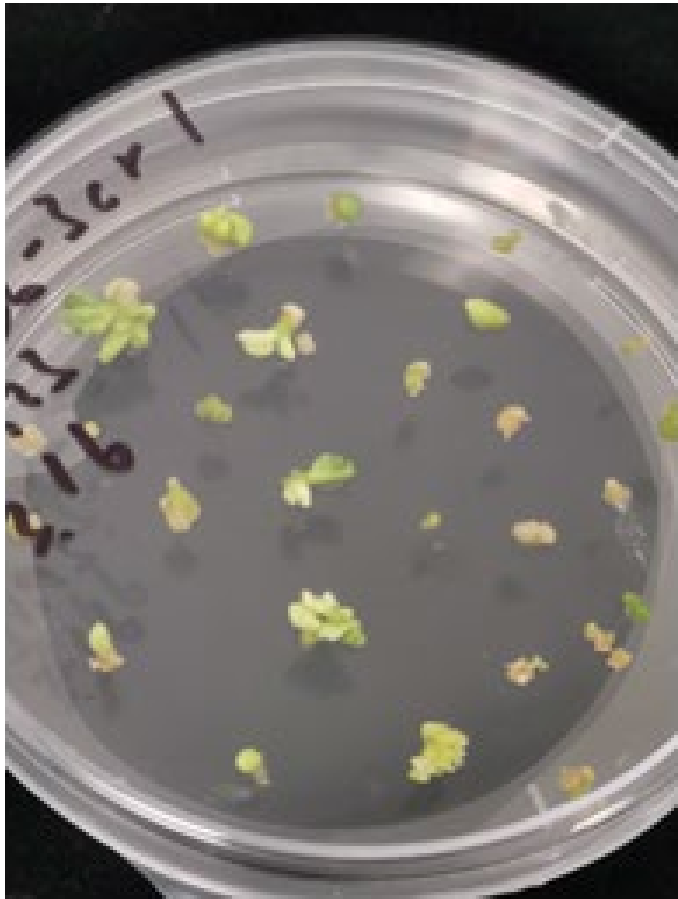
3-4 weeks



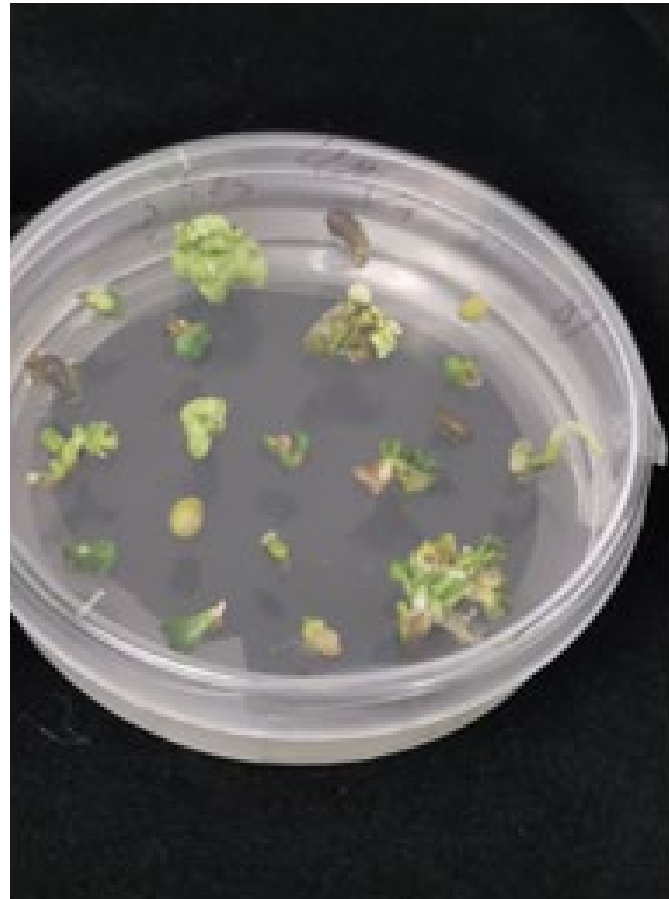
CRISPR Process – Callus Shoot Development

3-4 months

F Embryos



G Embryos

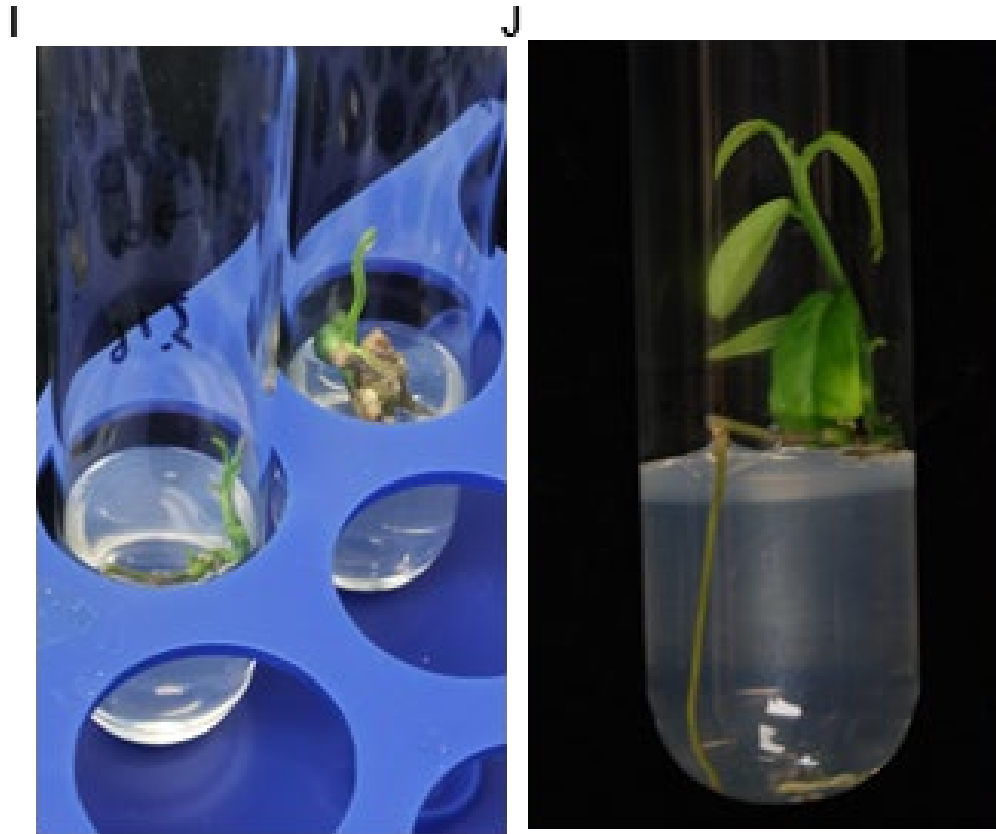


H Embryos generating shoots



CRISPR Process – Shoot generation and Micro-graft

Shoot generation



Solid medium
Rooting medium

3-4 weeks

Micro-graft



Solid medium
Rooting medium

3-4 weeks

CRISPR Process – Plants

L



Rockwool/Stonewool
Grow Cubes In Ports

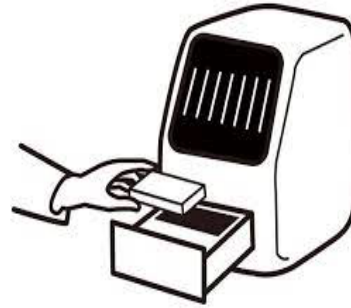
1-2 months

M



Plants

12-18 months



Evaluating Plants for HLB Resistance in Field Trials

Gene-edited plants



Liners budded



Field-ready plant



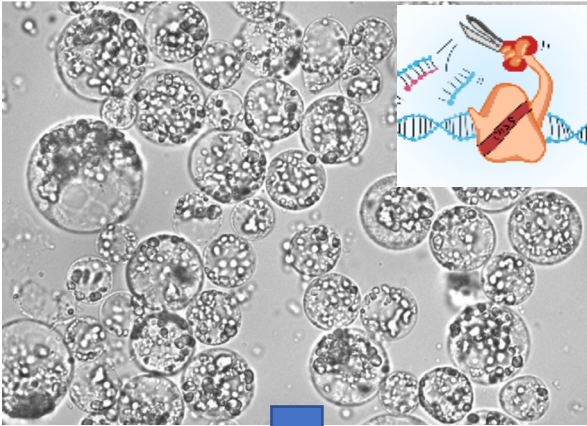
Field Trials



**3 years
(meaningful data)**

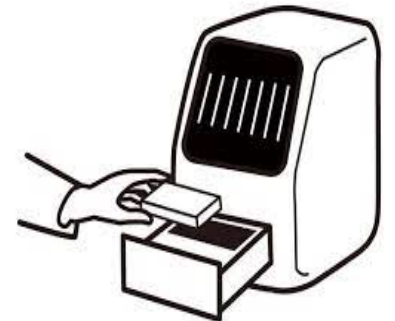
6 months

CRISPR Process Timeline



CRISPR editing → plant testing

- Process takes over 2 years
- Lots of trial and error to modify CRISPR for use in citrus
- Current success rate for generating plants with all the correct edits is **<1%**



1st gene edited (non-transgenic) citrus lines developed

Canker-resistant 'Hamlin' orange

- Performance in the field TBD
 - Never been in field or produced any fruit

Plans for 2023/24 season

- Plant replicated field trials with these new lines
 - Evaluate for both HLB and canker tolerance/resistance
- Plants were entered into DPI program for cleanup earlier this year (2023)



New Citrus Variety Development (Biotechnology)



Deng



Dutt



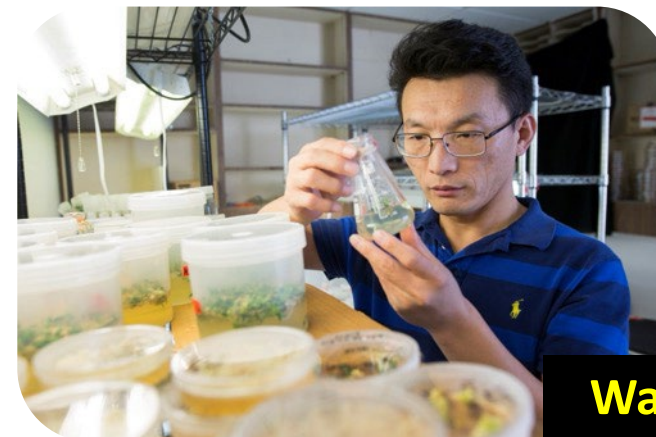
Gmitter



Grosser



Mou



Wang

Field trials

- 1st group of CRISPR (non-GMO) plants going to field this year for HLB testing
 - Many additional gene-edited lines in early stages of growth (2+ years before they can move to field)
-
- Transgenic rootstocks trials (2023) with non-transgenic sweet orange scions
 - Dozens of transgenic lines (GMOs) with robust tolerance to canker and/or HLB (greenhouse tests) are moving to field trials this year.

Transgenic HLB-tolerant varieties (Mou)

‘Hamlin’ orange (5 lines); ‘Duncan’ grapefruit (1 line)

- Field trials planted in 2019 and 2021
- Do become infected with HLB-causing bacterium
- Very little if any HLB symptoms
- Fruit quality data being collected over next two field seasons
- Request in process to deregulate and release these lines (Mou & Triplett)
 - Best guess: 2+ years for approval (if successful)

While we wait...

What can you do now?

Citrus Varieties (better HLB tolerance)

- Presentation by Dr. John Chater 11:20 AM tomorrow
“Sweet Oranges from the UF/IFAS Citrus Breeding Team”



Grove Management

- IPC's work, What comes next? – Fernando Alferez
- Optimizing Irrigation & Fertilization... – Davie Kadyampakeni
- Employing Plant Growth Regulators to improve canopy health – Tripti Vashisth
- The latest on Trunk Injection HLB Therapy – Ute Albrecht
- Psyllid Research & Management Update – Lukasz Stelinski