

# Disease Resistant, novel citrus cultivars generated through breeding citrus with Australian limes.



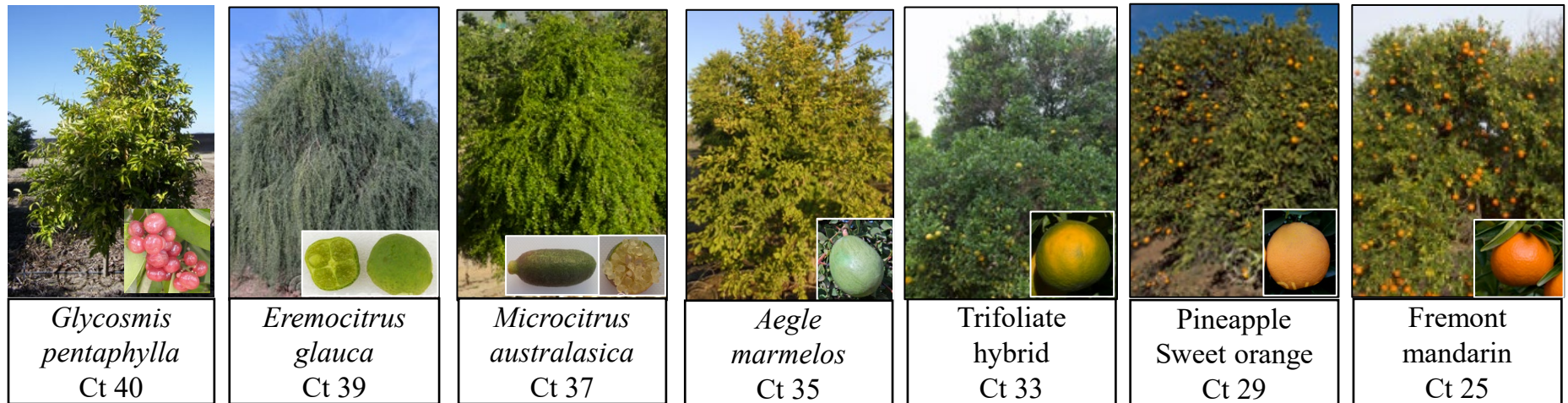
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# Citrus huanglongbing

- No known cure. Diseased trees die a slow death.
- Management practices help in delaying spread.
- Early detection of HLB can lead to removal of inoculum. Methods are being developed.
- Control of psyllid vector (best method for early detection and management – if we can keep up with this process).
- Antibacterial sprays are being developed.
- Millions of dollars spent on research for disease management.

# The Search for HLB Resistance using Germplasm From the Citrus Variety Collection at UCR



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Immune

Resistant

Tolerant

Susceptible

Australian limes

Citrus

Australian limes have resistance/tolerance to HLB.

Long term field evaluation of 886 trees (100 accessions belonging to 21 genera of Aurantioideae). Ramadugu et al. 2016.

All images from Citrus Variety Collection website (<https://citrusvariety.ucr.edu>)

- ❖ Disease resistant citrus cultivars can provide a solution to HLB.
- ❖ No HLB resistance in the genus citrus; but Australian limes have resistance and field tolerance.
- ❖ We identified 8 types of responses to HLB challenge:

1. Immunity: complete absence of the pathogen. No symptoms.

2. Resistance: Absence of pathogen colonization; transient replication seen.

3,4,5: Different types of tolerance:

Recovery from infections, seedling variations, delayed infections.

6,7,8: Susceptible: Most citrus types

6: Get HLB but survive-Citrons, limes, lemons. (Miles et al 2017).

# Why is breeding essential?

- ❖ **Genetic vulnerability:** Majority of the cultivars of citrus are derived from a few selected genotypes. Example: all grapefruit cultivars are derived from 'Duncan'. Millions of acres of citrus resulting from a few cultivars leads to '**monoculture**'. Introduction of new pathogens is detrimental when there is genetic vulnerability.
- ❖ **Genetic Enhancement:** Broadening the genetic base can be achieved by **incorporation** of new germplasm. Often wild relatives of crops are utilized. Recurrent cycles of recombination (during meiosis) and selection for the traits of interest will generate new germplasm.
- ❖ **Prebreeding:** Generating an intermediate set of plants with desirable traits (like resistance to HLB) for later use in further rounds of breeding.
- ❖ **Introgression:** Back crossing to standard cultivars to transfer desired genes (ex. resistance genes).
- ❖ **Evaluation:** Phenotypic assessment of genetic potential of the novel hybrids is needed in multiple environments. Genotype by environment interactions important.
- ❖ **Cultivar Development:** Once the essential traits are present in the new hybrids, we need to evaluate field performance in different locations, assess horticultural data and develop cultivars.

# Citrus breeding: Opportunities and **Limitations**

- Citrus is a tree crop with long generation times (4-9 years). Breeding is slow.
- Biological peculiarities like i). Nucellar embryony. ii) Sterility issues (pollen/ovule). iii) Many sexually imperfect varieties are grown that are not suitable in breeding. These can only be vegetatively propagated. iv) Inbreeding depression.
- Juvenility in citrus: No flowers or fruits for 4-9 years. Breeding cycle is determined by the availability of desired pollen.
- Confirming tolerance or resistance is time consuming. In CA where there are many quarantine regulations, testing is very challenging - a bottleneck in the whole process. Limited space in BSL3 facilities.
- APHIS-certified greenhouse space needed to maintain hybrids.
- Regulations to move citrus restrict transportation and evaluations in other regions – this is essential to confirm disease tolerance.
- Breeding is considered to be an arduous task. Very few researchers want to take this approach (long experiments, few publications... not desirable in a University environment).

## Citrus breeding: **Opportunities** and Limitations

- ❖ We can incorporate HLB resistance from citrus relatives. With backcrosses, we can introgress resistance traits into cultivated citrus.
- ❖ Time factor: a gene edited plant starting from a protoplast stage may take as long as a newly developed hybrid for proper evaluation.
- ❖ Resistant hybrids will provide economically feasible, ecologically acceptable, long-term, sustainable method for cultivation of citrus in presence of the pathogen and the psyllid vector.
- ❖ Public acceptance of new varieties is not an issue.
- ❖ Acceptance by FDA, EPA and other regulatory agencies is not needed.



# Australian limes



'Desert lime'  
*Eremocitrus glauca*



'Finger lime'  
*Microcitrus australasica*



'Australian  
Round lime'  
*Microcitrus  
australis*





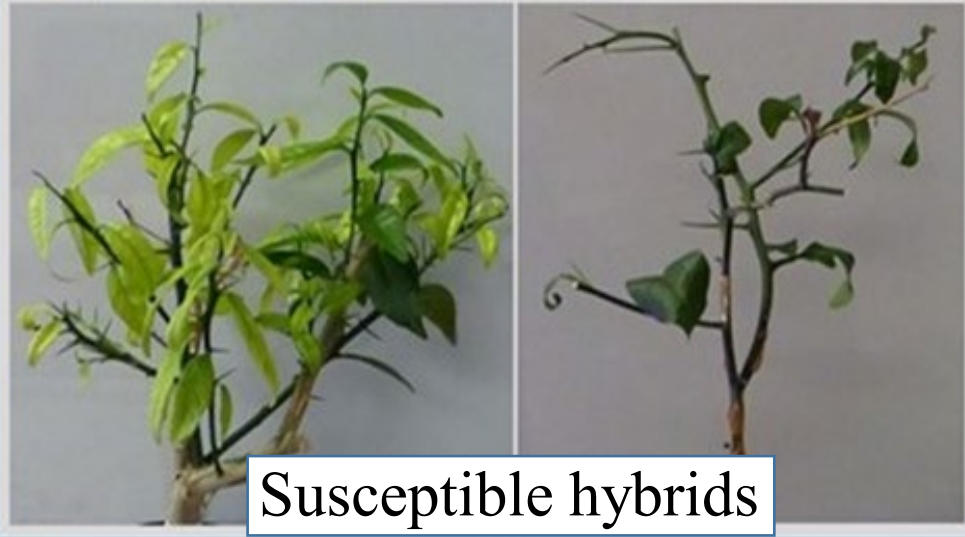
# Hybrids generated by crossing citrus and Australian limes have resistance/tolerance

Resistant hybrids



- Hybrids generated from breeding trials evaluated by exposure to psyllids carrying HLB pathogen
- Hybrids are also graft challenged
- Pathogen expts conducted in Florida or in UC Davis Contained Research Facility

Susceptible hybrids





Hybrid evaluation by three methods:

1. Exposure to free flying psyllids (FL)
2. No-choice feeding (FL)
3. Graft challenge (UC Davis)



# Evaluation of HLB resistance - greenhouse and field.

HLB susceptible hybrids – graft and no-choice psyllid challenge.



HLB resistant hybrids – challenged in GH, later validated in FL fields.

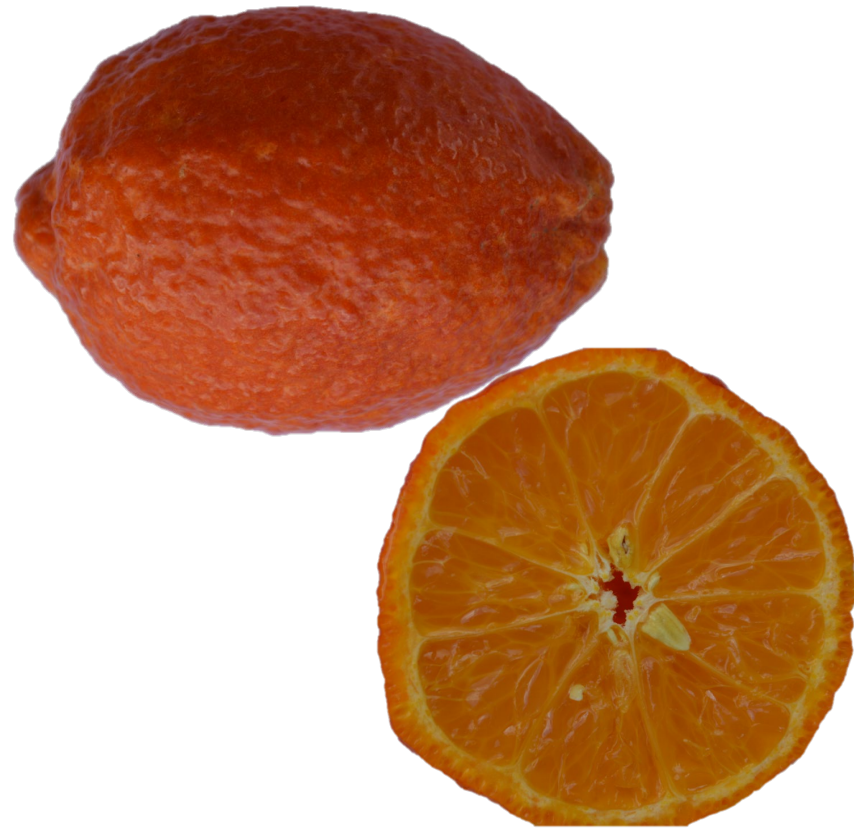


# Sequencing the genomes of Australian limes

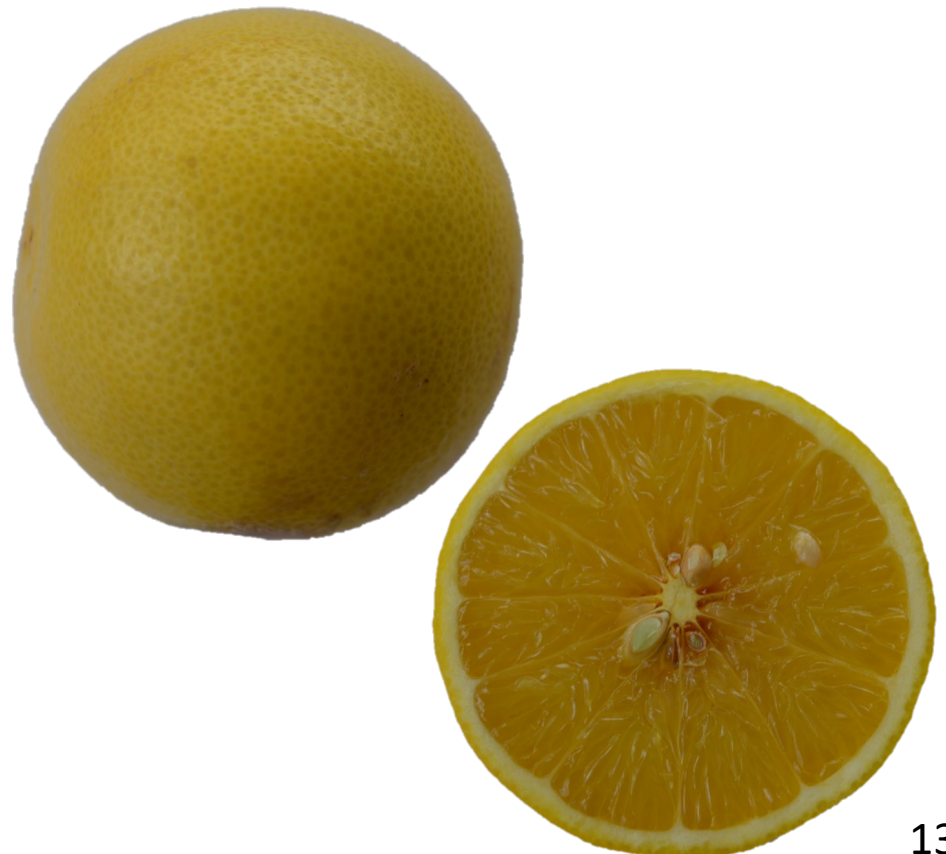
- ❖ *Microcitrus* and *Eremocitrus* diverge from *Citrus* by about 3 million years.
- ❖ Genes associated with resistance in the Australian limes may be novel.
- ❖ Sequencing provides information on the genetic makeup of these citrus relatives.
- ❖ We have draft genome sequences of : *Eremocitrus glauca*, *Microcitrus australasica* and *Microcitrus inodora*. Final scaffolding is in progress.
- ❖ Annotation of the genomes, identification of resistance genes and differences between resistant and susceptible hybrids - in progress.
- ❖ Development of information for resistance markers will be useful in identifying putatively valuable plants in the seedling stage.
- ❖ Use in other processes like plant transformations, targeted gene editing – to introduce resistance into elite citrus cultivars.

F1 hybrids with mandarin and *Microcitrus* parentage.

HLB-resistant in greenhouse studies. Being used as parents in advanced crosses.



Size: 5X6 cm; BRIX: 9.6; Juice: 38%; seeds: 7-8. Lemon-like flavor; Slightly astringent. No detectable off-flavor.

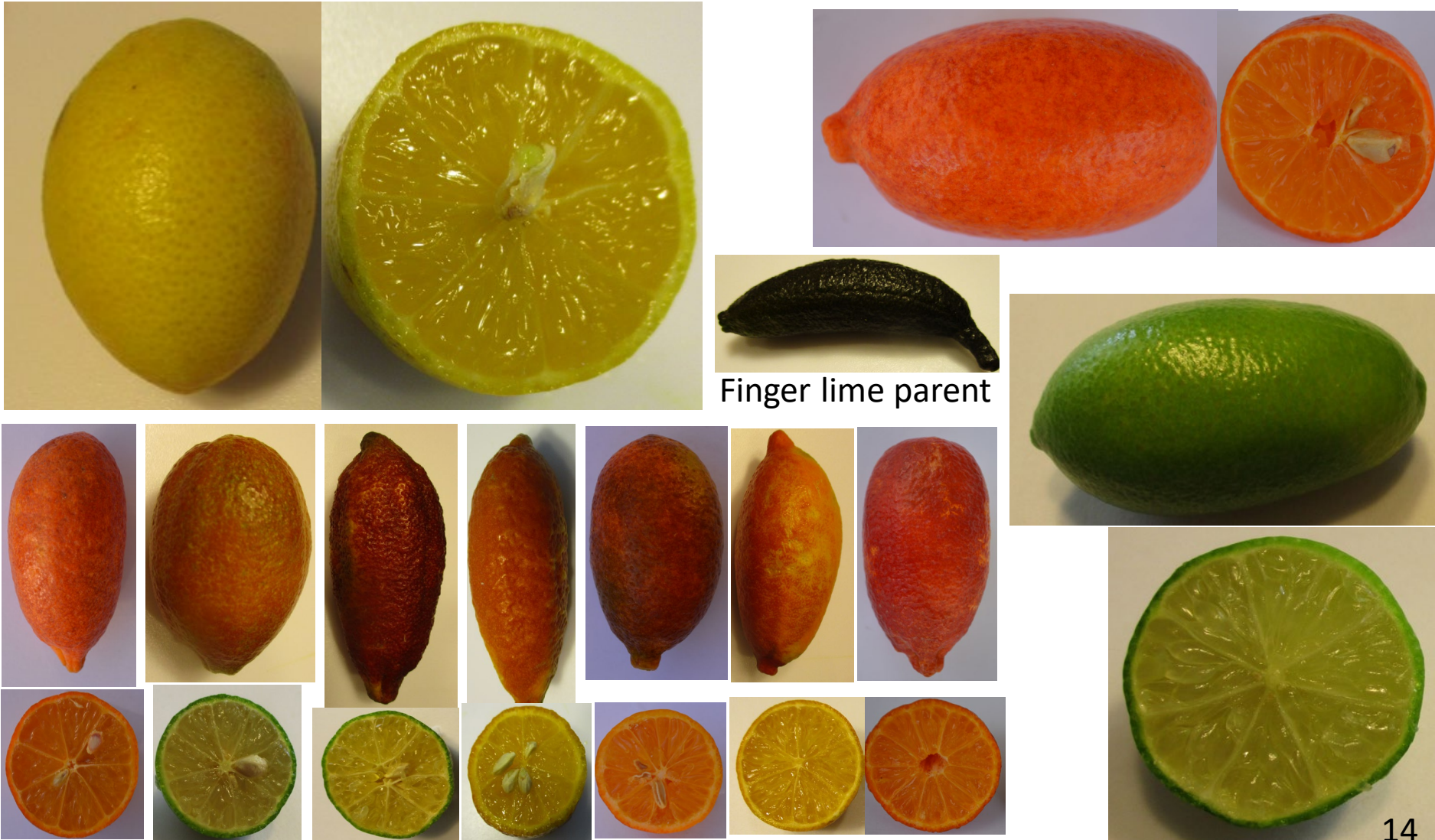


Size: 8X4.7cm; BRIX:16.3; Juice 38%; seeds: 3-5. Mandarin-like flavor with slight off-flavor from *Microcitrus*.



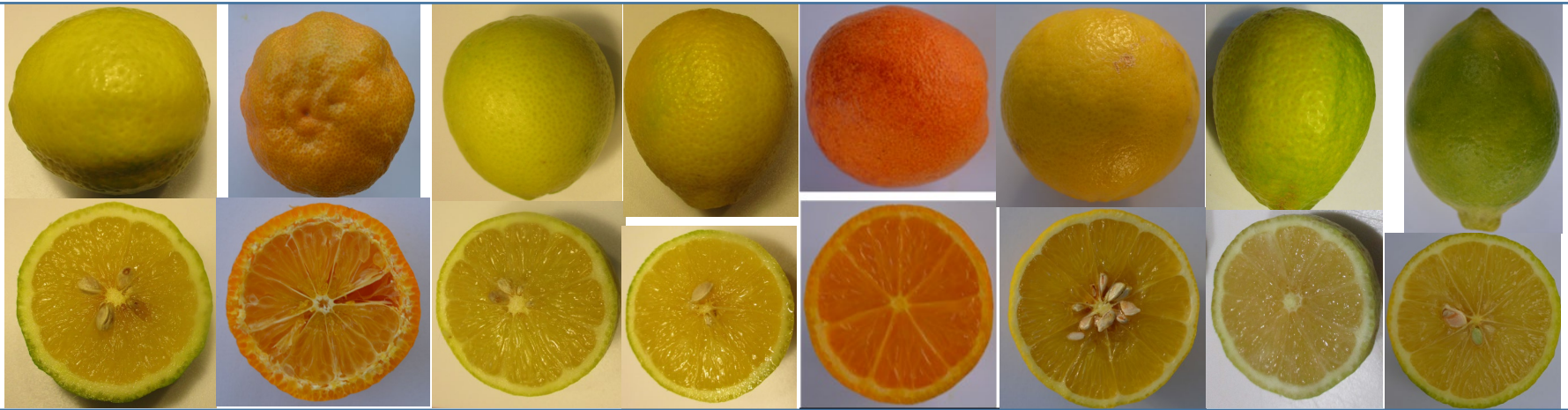
# Finger lime – like F1 hybrid fruits

Some are HLB resistant, some susceptible. Many have not been evaluated yet. Size: 2-8 cm long and 2-5 cm wide; BRIX: 10-19; Juice: 17-31%; seeds: 0-8. Many have finger lime flavor; some have off-flavor. Selected hybrids are being used as parents in advanced crosses.





# Lemon – like F1 hybrid fruits



HLB-resistance evaluated in greenhouse studies for some. Size: 4.5 to 8 cm length and 3.5-7 cm width; BRIX: 8-16; Juice 13-46%; some without any juice; seeds: 0-10. Citrus-like flavor with slight off-flavor from *Microcitrus*. Selected hybrids are being used as parents in advanced crosses.



# Advanced hybrids (backcross, BC1)

- Linkage drag: Along with disease tolerance traits, hybrids will also inherit undesirable traits. Generating advanced hybrids by backcrossing with some other type of citrus is done to get hybrids with predominantly citrus-like, edible fruits.

Wiling  
mandarin  
[4.4x5 cm]



X



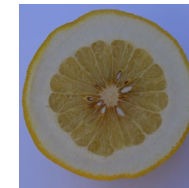
*Microcitrus  
inodora*  
[4.4x3 cm]



F1 hybrid  
[5x4 cm]



X



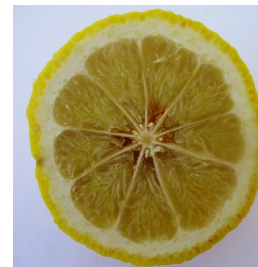
Siamese  
pummelo  
[11x10 cm]



Prebreeding



Cultivar development



BC1 (184).  
[9x8 cm]  
deformed seeds



## Fruit from advanced (backcross hybrids)

- ❖ Will taste more like citrus fruits (average 75% citrus DNA; varies from 54-88%) - based on genotyping.
- ❖ Some selections may retain HLB resistance/tolerance.
- ❖ Evaluations are in progress.







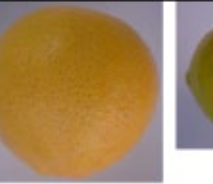





# Multi-state field trials of four HLB-tolerant F1 hybrids.

- ❖ Field evaluation of promising hybrids in different locations is required to test disease tolerance (HLB), horticultural performances and susceptibility to other relevant pathogens.
- ❖ Field trial locations: Alico Trial – Near Arcadia, De Soto county, FL; Peace River field trial – near Fort Meade, Polk county, FL; Univ. of CA Riverside experimental fields – Riverside county, CA; South Coast Research and Expt. station, Irvine, Orange county, CA. TX field trials in the citrus center and a private farm.

Arcadia field trial is in bad shape after hurricane IAN.



Field trial of four F1 hybrids (*Citrus* × *Microcitrus*) near Arcadia, FL). Plants were staked to support vertical growth and to encourage early flowering.

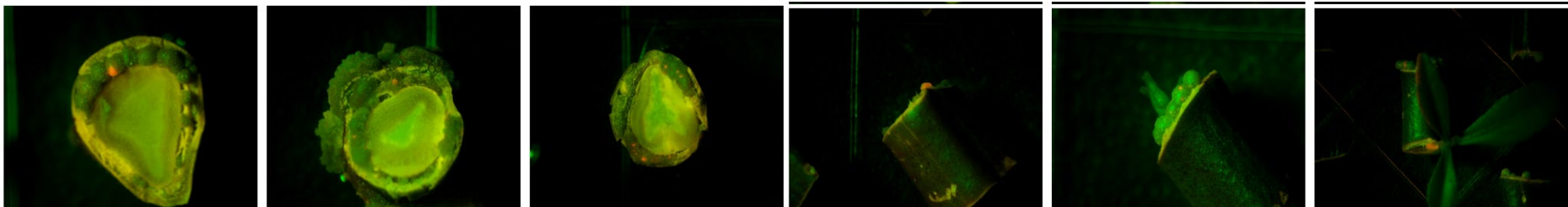
|                                                                                      |                                                                                      |                                                                                      |                                                                                      |
|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
|   |   |   |   |
|  |  |  |  |
| Hybrid 1<br>10.6 BRIX<br>28% juice<br>3-6 seeds                                      | Hybrid 2<br>18.3 BRIX<br>31% juice<br>Seedless                                       | Hybrid 3<br>11.8 BRIX<br>41% juice<br>2-4 seeds                                      | Hybrid 4<br>11.9 BRIX<br>31% juice<br>2-4 seeds                                      |

F1 hybrids selected for field trials.



# Induction of Early Flowering

- ❖ Citrus has a long juvenility period. It can take from 3-8 years for flowers to appear and for fruit set. To expedite the breeding process, we need to induce early flowering.
  - ❖ Many Australian limes have precocious flowering. Some can flower in 2-3 years.
  - ❖ Resistant hybrids that flower early can be used as parents for further crosses in breeding trials.
  - ❖ Availability of fruits will help preselect hybrids from the population.
- ❑ Two approaches to encourage early flowering and fruiting:
1. FT gene (*Flowering locus T*). Sean Cutler's team is trying to introduce the FT into certain hybrids to encourage early flowering. We transform selected hybrids with a chemically inducible flowering construct (iFT). Transformed plants can be encouraged to flower after treatment with an agrochemical, mandipropamid (Revus).



# Induction of Early Flowering

2. Field planting & cultural practices: some hybrids flower early if grown as tall field plants with a single stem. We can get flowering, fruiting within 3 years in some *Microcitrus* hybrids.

A. Hybrid made in 2018. Field planting of seedling tree in Nov 2020. Trained to grow tall, with a single stem. Flowered in May 2021. This cultural practice will work for some hybrids only.

B. Pollen can be collected and used for advanced breeding. Fruit development may occur after another year.



C, D: Field planting of F1 citrus hybrids in Riverside. Grafted hybrids planted in the field during Oct 2021. Flowering and fruit set in some plants –July 2022.

**FT approach required**; only certain hybrids can flower early in the field by altering cultural practices.



# Genotyping of Advanced Hybrids

- ❖ SNP information available from Citrus genome database and from Roose. Axiom SNP array with 56,000 markers used to genotype hybrid populations (only 46 markers shown in the example below). Genotyping with KASP assays.
- ❖ The colored regions in the picture show genome fragments derived from the F1 hybrid (*Citrus* X *Microcitrus*) or from *Citrus*. Non-colored SNPs are from citrus.
- ❖ The method helps to identify diverse hybrids from one population. If certain genomic fragments are associated with resistance traits, the putative hybrids can be selected at the seedling stage.

|    |                    |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |
|----|--------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| 1. | Chromosome         | 1            | 1            | 1            | 1            | 1            | 1            | 2            | 2            | 2            | 2            | 3            | 3            | 3            | 3            | 3            | 3            | 3            | 4            | 4            | 4            | 5            | 5            | 5            | 5            | 5            | 6            | 6            | 6            | 6            | 6            | 6            | 7            | 7            | 7            | 7            | 7            | 8            | 8            | 8            | 8            | 8            | 9            | 9            | 9            | 9            |              |
| 2. |                    | AX-160961574 | AX-160828127 | AX-159962297 | AX-160122489 | AX-160202455 | AX-160943433 | AX-160643185 | AX-160443010 | AX-160156327 | AX-160100969 | AX-160095234 | AX-160579731 | AX-159943987 | AX-160674012 | AX-160063188 | AX-160232075 | AX-159993863 | AX-160399651 | AX-160048938 | AX-159869216 | AX-160428648 | AX-160832021 | AX-161045325 | AX-160731116 | AX-161092079 | AX-160787753 | AX-160830398 | AX-161083977 | AX-160623796 | AX-159959694 | AX-161042663 | AX-160177958 | AX-159906134 | AX-159915937 | AX-161101678 | AX-160108071 | AX-160960694 | AX-159964646 | AX-160257918 | AX-160924751 | AX-159901714 | AX-160453108 | AX-161024779 | AX-160005109 | AX-160466701 | AX-159879873 |
|    | Marker name        |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |              |
| 3. | Citrus parent      | A:A          | A:A          | C:C          | T:T          | A:A          | A:A          | C:C          | A:A          | A:A          | T:T          | A:A          | G:G          | T:T          | T:T          | T:T          | A:A          | A:A          | T:T          | G:G          | G:G          | A:A          | A:A          | G:G          | C:C          | G:G          | C:C          | C:C          | C:C          | C:C          | C:C          | A:A          | G:G          | A:A          | C:C          | G:G          | G:G          | C:C          | A:A          | C:C          | C:C          | G:G          | G:G          | C:C          | G:G          | A:A          |              |
| 4. | Microcitrus parent | G:G          | C:C          | A:A          | G:G          | G:G          | G:G          | T:T          | C:C          | G:G          | C:C          | C:C          | A:A          | C:C          | G:G          | C:C          | G:G          | G:G          | C:C          | A:A          | A:A          | G:G          | G:G          | A:A          | T:T          | T:T          | T:T          | T:T          | T:T          | T:T          | T:T          | T:T          | C:C          | A:A          | G:G          | T:T          | A:A          | A:A          | T:T          | C:C          | T:T          | T:T          | A:A          | A:A          | T:T          | A:A          | G:G          |
| 5. | F1 hybrid          | A:G          | C:A          | C:A          | G:T          | G:A          | A:G          | C:T          | C:A          | G:A          | T:C          | A:C          | G:A          | T:C          | T:G          | T:C          | A:G          | A:G          | C:T          | G:A          | G:A          | A:G          | A:G          | A:G          | C:T          | G:T          | C:T          | T:C          | C:T          | C:T          | C:T          | C:T          | A:C          | G:A          | A:G          | C:T          | G:A          | A:G          | C:T          | C:A          | C:T          | C:T          | G:A          | G:A          | C:T          | G:A          | G:A          |
| 6. | BC1 hybrid 1       | A:G          | C:A          | C:A          | G:T          | G:A          | A:G          | C:C          | A:A          | G:A          | T:C          | A:C          | G:G          | T:T          | T:T          | T:T          | A:G          | A:G          | T:T          | G:G          | G:A          | A:A          | A:A          | G:G          | C:C          | G:G          | C:C          | C:C          | C:T          | C:T          | C:T          | C:T          | A:C          | G:G          | A:A          | C:C          | G:G          | A:G          | C:T          | C:A          | C:T          | C:T          | G:A          | G:A          | C:C          | G:G          | A:A          |
|    | BC1 hybrid 2       | A:G          | C:A          | C:A          | G:T          | G:A          | A:G          | C:C          | A:A          | A:A          | T:C          | A:C          | G:A          | T:C          | T:T          | T:C          | A:A          | A:A          | T:T          | G:G          | G:G          | A:A          | A:A          | G:G          | C:C          | G:G          | C:C          | C:C          | C:T          | C:T          | C:T          | C:T          | A:C          | G:A          | A:G          | C:T          | G:G          | G:G          | C:C          | A:A          | C:C          | C:C          | G:A          | G:G          | C:C          | G:A          | G:A          |
|    | BC1 hybrid 3       | A:G          | C:A          | C:A          | G:T          | G:A          | A:G          | C:T          | C:A          | G:A          | T:C          | A:A          | G:G          | T:T          | T:T          | T:T          | A:A          | A:A          | T:T          | G:G          | G:G          | A:A          | A:G          | G:G          | C:C          | G:G          | C:T          | T:C          | C:T          | C:T          | C:T          | C:T          | A:A          | G:G          | A:A          | C:T          | G:A          | A:G          | C:T          | ?            | C:C          | C:T          | G:G          | G:G          | C:T          | G:A          | G:A          |
|    | BC1 hybrid 4       | A:G          | C:A          | C:A          | G:T          | G:A          | A:G          | C:T          | C:A          | G:A          | T:C          | A:C          | G:A          | T:C          | T:G          | T:C          | A:G          | A:G          | T:T          | G:G          | G:G          | A:A          | A:A          | G:G          | C:C          | G:G          | C:T          | T:C          | C:T          | C:T          | C:T          | C:T          | A:C          | G:A          | A:G          | C:T          | G:G          | G:G          | C:C          | A:A          | C:C          | C:C          | G:G          | G:A          | C:C          | G:G          | A:A          |
|    | BC1 hybrid 5       | A:G          | C:A          | C:A          | G:T          | G:A          | A:A          | C:T          | C:A          | A:A          | T:C          | A:C          | G:A          | T:C          | T:G          | T:C          | A:A          | A:G          | C:T          | G:A          | G:A          | A:A          | A:A          | A:G          | C:T          | G:G          | C:T          | T:C          | C:T          | C:T          | C:T          | C:T          | A:A          | G:G          | A:A          | C:C          | G:G          | G:G          | C:C          | C:A          | C:T          | C:T          | G:G          | G:A          | C:C          | G:A          | G:A          |
|    | BC1 hybrid 6       | A:G          | C:A          | C:A          | G:T          | G:A          | A:A          | C:T          | C:A          | A:A          | T:C          | A:C          | G:G          | T:C          | T:G          | T:C          | A:A          | A:A          | C:T          | G:A          | G:A          | A:A          | A:G          | A:G          | C:T          | G:T          | C:C          | C:C          | C:C          | C:C          | C:C          | C:C          | A:C          | G:A          | A:G          | C:T          | G:A          | G:G          | C:C          | C:A          | C:T          | C:T          | G:G          | G:A          | C:C          | G:G          | A:A          |
|    | BC1 hybrid 7       | A:A          | A:A          | C:A          | G:T          | G:A          | A:A          | C:T          | C:A          | G:A          | T:C          | A:C          | G:A          | T:C          | T:G          | T:C          | A:A          | A:A          | C:T          | G:A          | G:A          | A:A          | A:G          | G:G          | C:T          | G:T          | C:T          | T:C          | C:T          | C:T          | C:T          | C:T          | A:A          | G:G          | A:G          | C:T          | G:A          | G:G          | C:C          | A:A          | C:C          | C:C          | G:G          | G:G          | C:C          | G:G          | A:A          |
|    | BC1 hybrid 8       | A:G          | C:A          | C:A          | G:T          | G:A          | A:A          | C:C          | A:A          | A:A          | T:T          | A:A          | G:A          | T:C          | T:G          | T:C          | A:A          | A:A          | C:T          | G:A          | G:A          | A:A          | A:G          | G:G          | C:C          | G:G          | C:T          | T:C          | C:T          | C:T          | C:T          | C:T          | A:C          | G:G          | A:A          | C:T          | G:A          | G:G          | C:C          | A:A          | C:C          | C:T          | G:A          | G:G          | C:C          | G:G          | A:A          |
|    | BC1 hybrid 9       | A:A          | A:A          | C:A          | T:T          | A:A          | A:A          | C:C          | A:A          | A:A          | T:T          | A:C          | G:G          | T:T          | T:T          | T:T          | A:G          | A:G          | C:T          | G:A          | G:A          | A:A          | A:A          | A:G          | C:C          | G:G          | C:C          | C:C          | C:T          | C:T          | C:T          | C:T          | A:A          | G:G          | A:A          | C:C          | G:A          | G:G          | C:C          | A:A          | C:C          | C:C          | G:G          | G:G          | C:C          | G:G          | A:A          |
|    | BC1 hybrid 10      | A:A          | A:A          | C:C          | T:T          | A:A          | A:A          | C:T          | C:A          | G:A          | T:C          | A:C          | G:A          | T:C          | T:G          | T:C          | A:G          | A:G          | T:T          | G:G          | G:G          | A:A          | A:G          | G:G          | C:C          | G:T          | C:T          | T:C          | C:T          | C:T          | C:T          | C:T          | A:A          | G:A          | A:G          | C:T          | G:A          | G:G          | C:C          | C:A          | C:T          | C:T          | G:G          | G:A          | C:C          | G:G          | A:A          |
|    | BC1 hybrid 11      | A:A          | A:A          | C:A          | G:T          | G:A          | A:A          | C:T          | A:A          | G:A          | T:C          | A:A          | G:G          | T:T          | T:T          | T:T          | A:G          | A:G          | C:T          | G:A          | G:A          | A:G          | A:A          | A:G          | C:C          | G:G          | C:T          | T:C          | C:T          | C:T          | C:T          | C:T          | A:A          | G:G          | A:A          | C:C          | G:G          | A:G          | C:T          | C:A          | C:T          | C:C          | G:G          | G:A          | C:C          | G:G          | A:A          |
|    | BC1 hybrid 12      | A:G          | C:A          | C:A          | G:T          | G:A          | A:A          | C:T          | C:A          | G:A          | T:C          | A:A          | G:G          | T:T          | T:T          | T:T          | A:G          | A:A          | C:T          | G:A          | G:A          | A:A          | A:G          | G:G          | C:T          | G:T          | C:T          | T:C          | C:T          | C:T          | C:C          | C:C          | A:C          | G:A          | A:G          | C:T          | G:A          | A:G          | C:T          | C:A          | C:T          | C:T          | G:G          | G:A          | C:C          | G:G          | A:A          |

- Chromosome number for the genetic markers.
- Identity of the marker (green line).
- Genotype expected in the citrus parent of the hybrid.
- Genotype expected in the Microcitrus parent of the hybrid.
- Genotype of the F1 hybrid (*Citrus* X *Microcitrus*).
- Genotype of the advanced backcross hybrids 1-12 (derived from one pollination event. Marked with a red line). Shows diversity observed in backcross hybrids. Markers on chromosomes 1-9 are color coded.

# Analyzing advanced hybrids

- 1. HLB resistance/tolerance testing in greenhouses.
- 2. Does the hybrid have acceptable fruits? Taste evaluations.
- 3. Use as parents for future crosses.
- 4. Determine disease response in multiple field environments. Challenge with different isolates of CLas.
- 5. Analyze genome data by PacBio sequencing, skim sequencing of a population (progeny derived from specific crosses). Identify genomic fragments associated with resistance.
- Metabolomic analysis of fruit juice. Associate 'off-flavor' with presence of certain compounds. Selection will be for the fruits that lack these compounds.

# Array for Simultaneous Detection of Multiple Citrus Pathogens

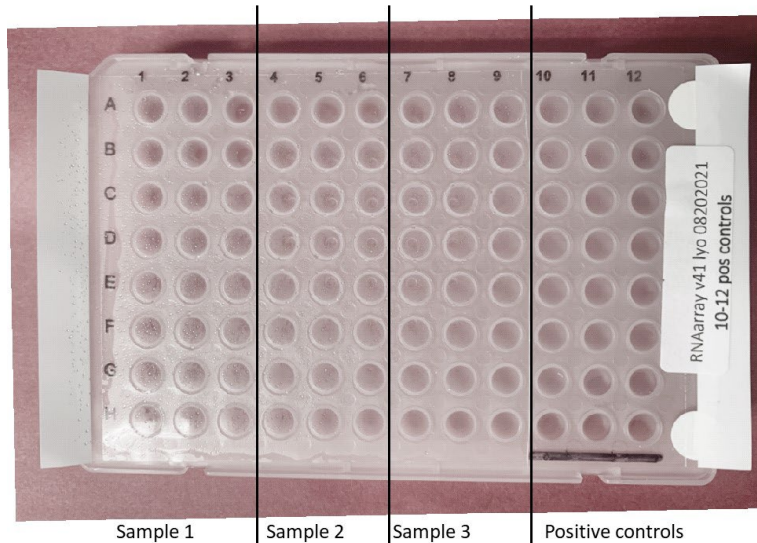
- ❖ Citrus hybrids generated in Riverside need to be tested in different locations to confirm HLB tolerance.
- ❖ Since movement of citrus budwood is regulated, it is essential for the regulatory agencies to test budwood for pathogens before they can be transported.
- ❖ To facilitate faster and safer exchange of germplasm (hybrids) between CA, FL and TX, we developed a pathogen testing array in collaboration with the USDA lab in Riverside.

## Advantages of the array:

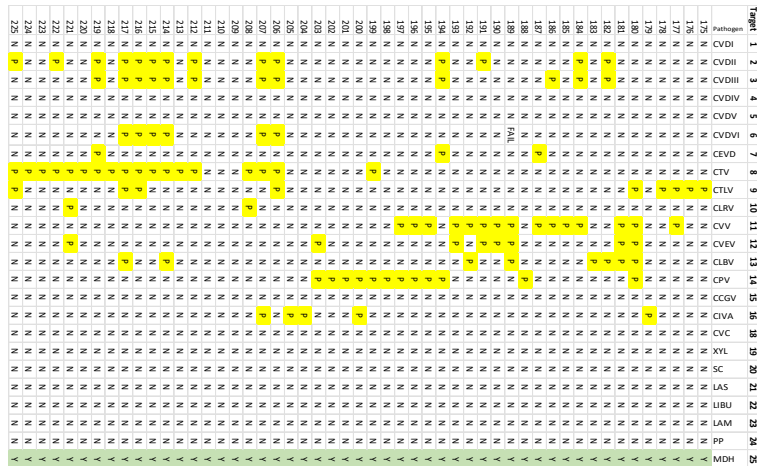
- A single array plate can detect 26 citrus pathogens from three trees in about 90 minutes (Starting from RNA). Can detect two targets each for 20 pathogens, one target each for six pathogens, two reference genes (total of 48 targets).
- Array is **modular**. Can add/delete detection suitable reagents as needed.
- Prepared array plates are stable at room temperature for at least one year.
- Uniform testing methods across different laboratories.
- Simple work flow with a two step reaction, each with just three reagents.
- Results obtained from a specific sample in different labs, conducted by multiple researchers, using different equipment and mastermixes can be compared.



# Array for Simultaneous Detection of Multiple Citrus Pathogens



**Fig. 1.** An array showing layout of wells used for testing three plant samples, and positive controls.

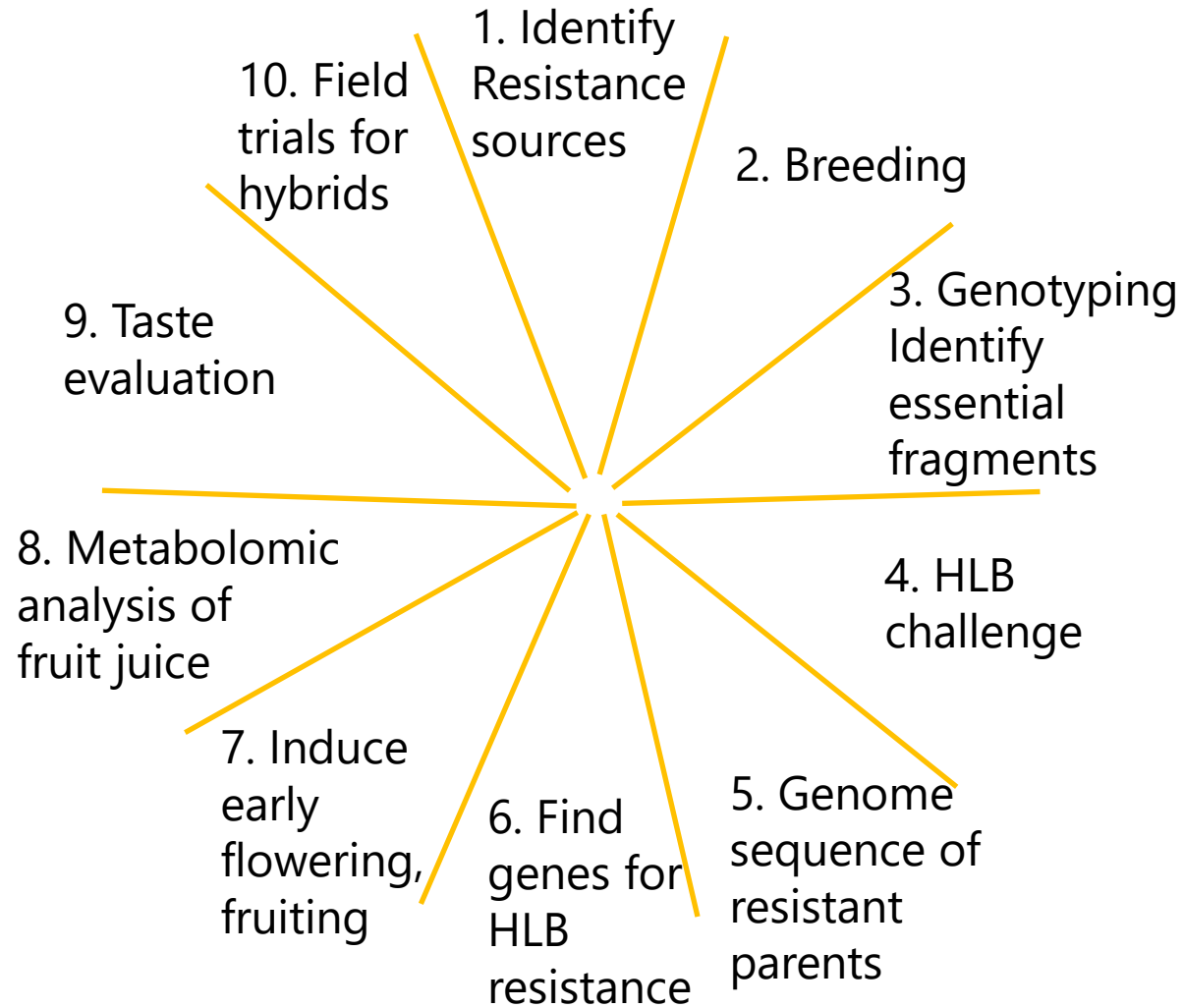


**Fig. 3.** Actual test results of about 50 plants using array. Positives are highlighted. Last column (green) is internal control.

| Number | Pathogen abbreviation | Name of the pathogen                                      |
|--------|-----------------------|-----------------------------------------------------------|
| 1      | CVDI                  | Citrus viroid I                                           |
| 2      | CVDII                 | Citrus viroid II                                          |
| 3      | CVDIII                | Citrus viroid III                                         |
| 4      | CVDIV                 | Citrus viroid IV                                          |
| 5      | CVDV                  | Citrus viroid V                                           |
| 6      | CVDVI                 | Citrus viroid VI                                          |
| 7      | CEVD                  | Citrus Exocortis viroid                                   |
| 8      | CTV                   | Citrus tristeza virus                                     |
| 9      | CTLV                  | Citrus tatterleaf virus                                   |
| 10     | CLRV                  | Citrus leaf rugose virus                                  |
| 11     | CVV                   | Citrus variegation virus                                  |
| 12     | CVEV                  | Citrus vein enation virus                                 |
| 13     | CLBV                  | Citrus leaf blotch virus                                  |
| 14     | CPV                   | Citrus psorosis virus                                     |
| 15     | CCGV                  | Citrus concave gum associated virus                       |
| 16     | CIVA                  | Citrus virus A                                            |
| 18     | CVC                   | Citrus variegated chlorosis ( <i>Xylella fastidiosa</i> ) |
| 19     | XYL                   | <i>Xylella fastidiosa</i> (generic)                       |
| 20     | SC                    | <i>Spiroplasma citri</i>                                  |
| 21     | CLAS                  | <i>Candidatus liberibacter asiaticus</i>                  |
| 22     | LIBU                  | Liberibacters_universal                                   |
| 23     | CLAM                  | <i>Candidatus Liberibacter americanus</i>                 |
| 24     | PP2                   | Phytoplasma                                               |
| 25     | MDH_R                 | Malate dehydrogenase RNA                                  |
| 25     | MDH_D                 | Malate dehydrogenase DNA and RNA                          |
| 26     | BWYV                  | Beet western yellows virus                                |
| 27     | CYVaV                 | Citrus yellow vein associated virus                       |
| 28     | CBaPRV                | Citrus blight-associated pararetrovirus                   |

**Fig. 2.** Names and abbreviations of 26 citrus pathogens included in the array. Note that #17 is deleted, and #25 is a citrus gene used as a control.

# Program overview



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Questions?