Grapefruit Breeding for the now canker/HLB endemic Florida

UF UNIVERSITY of FLORIDA
IFAS
Citrus Research and Education Center

Jude Grosser
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UF-CREC Citrus Genetic Improvement Team
2020

SWEET CITRUS FRUIT HYBRIDS

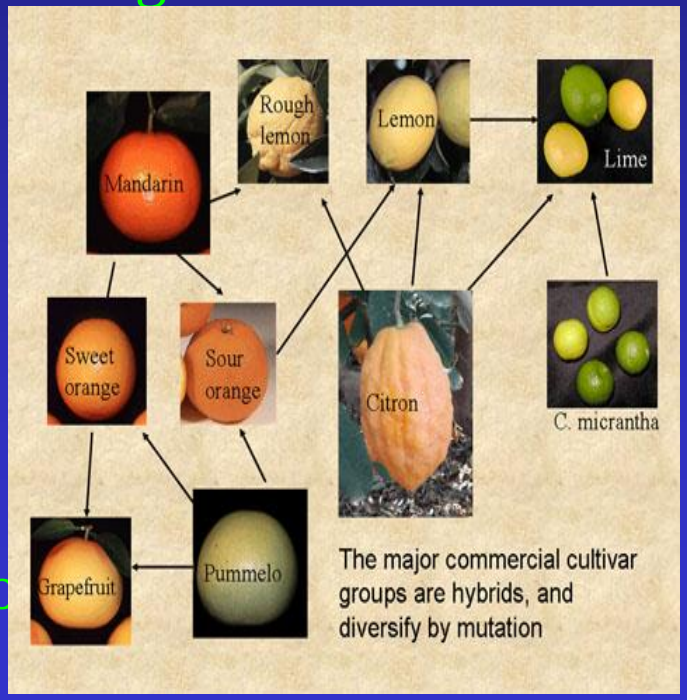
Sweet orange (*C. sinensis*): pummelo (*C. Grandis*) x mandarin (*C. reticulata*)/*C. unshiu*/*C. deliciosa*

Grapefruit (*C. paradisi*): pummelo x sweet orange

Tangelo: mandarin x grapefruit
Minneola, Orlando

Tangor: mandarin x sweet orange
Murcott, Temple

Mandorange: sweet orange x tangor/tangelo
New Triploids



1. **Somaclonal Variation**

2. **Interploid Hybridization to produce seedless triploids**

3. **Somatic Cybridization**

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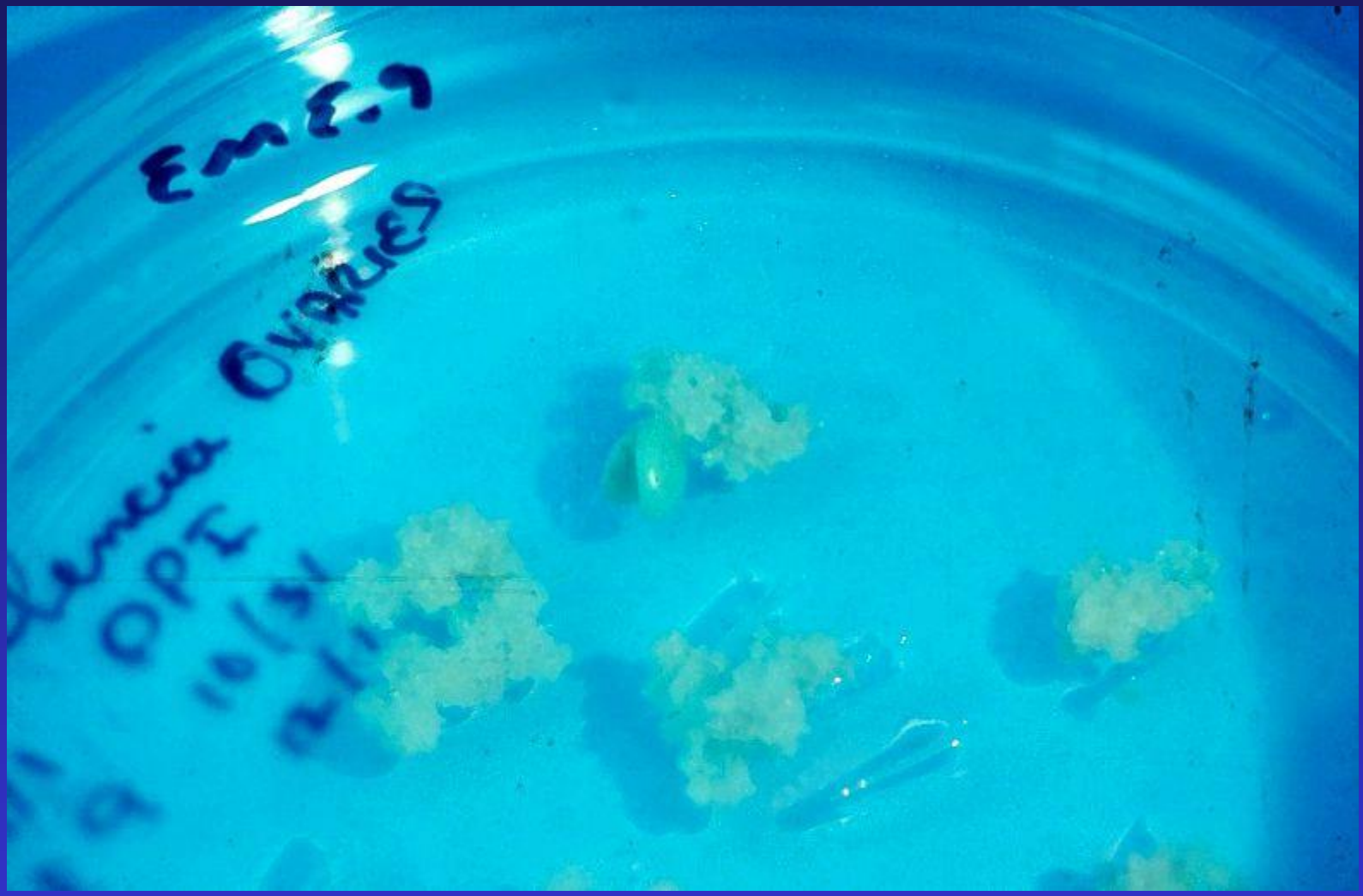
Our Primary Approach for Developing Novel Seedless Fresh Citrus Fruit – Triploidy! (just like banana and new watermelons)

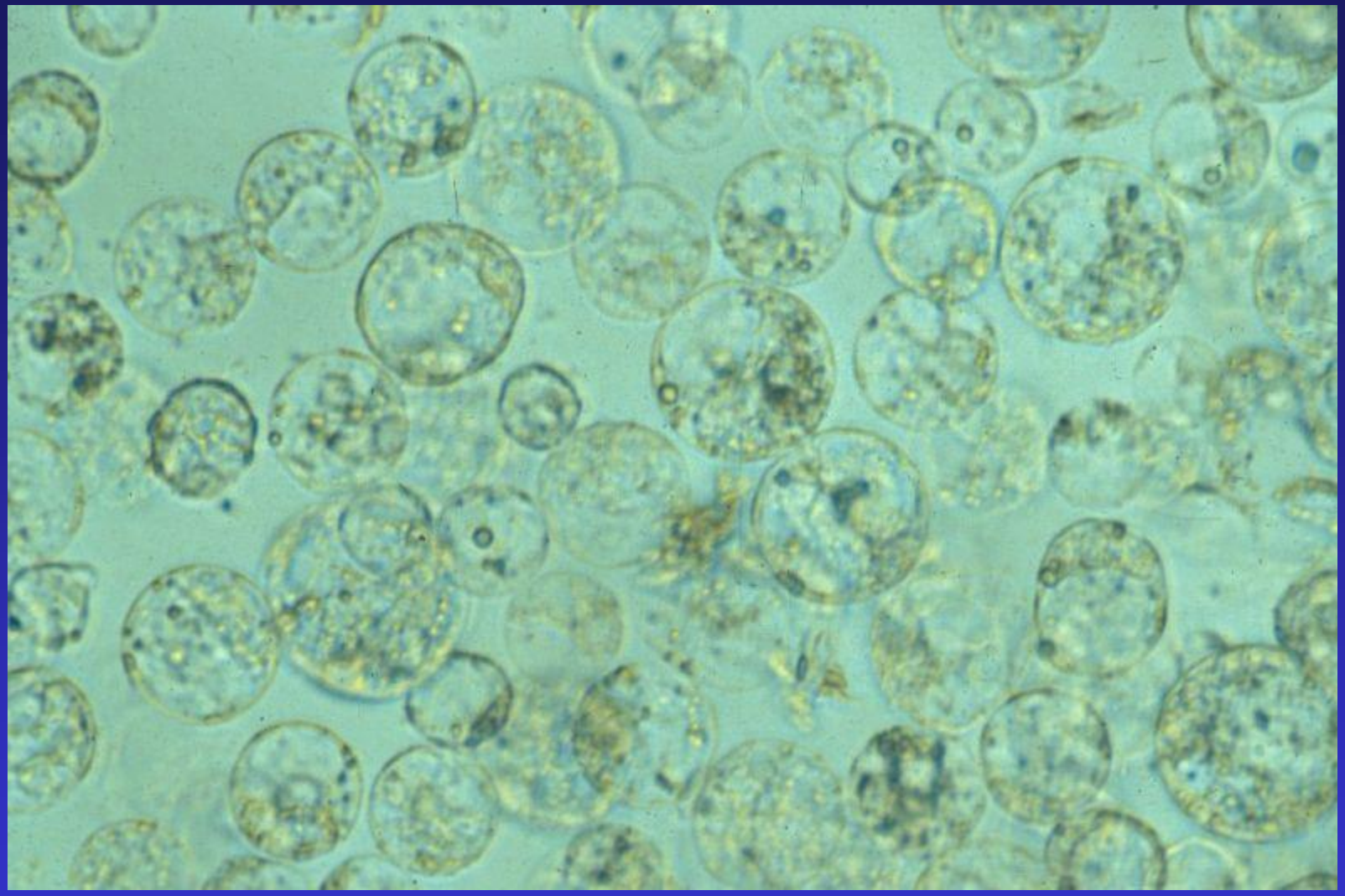
1. Somatic Hybridization Via Protoplast Fusion to Generate Elite Allotetraploid Breeding Parents (Mostly Interspecific Combinations)

2. Interploid Hybridization (Mostly Interspecific)– Crosses of Diploid Monoembryonic Citrus with Selected Tetraploids as Pollen Parents (Reciprocal Crosses when Possible)

3. Embryo Rescue and Micro-grafting to Expedite Seedless Triploid Recovery (Crosses with Tetraploids as the Female do not Require Embryo Rescue)



SWEET ORANGE EMBRYOGENIC CALLUS



SWEET ORANGE SUSPENSION CULTURE PROTOPLASTS

Somaclonal Variation: variability in plants regenerated from tissue culture that is either induced or uncovered by a tissue culture process. Most somaclonal variation is negative, but if enough plants are examined, positive changes can usually be recovered.

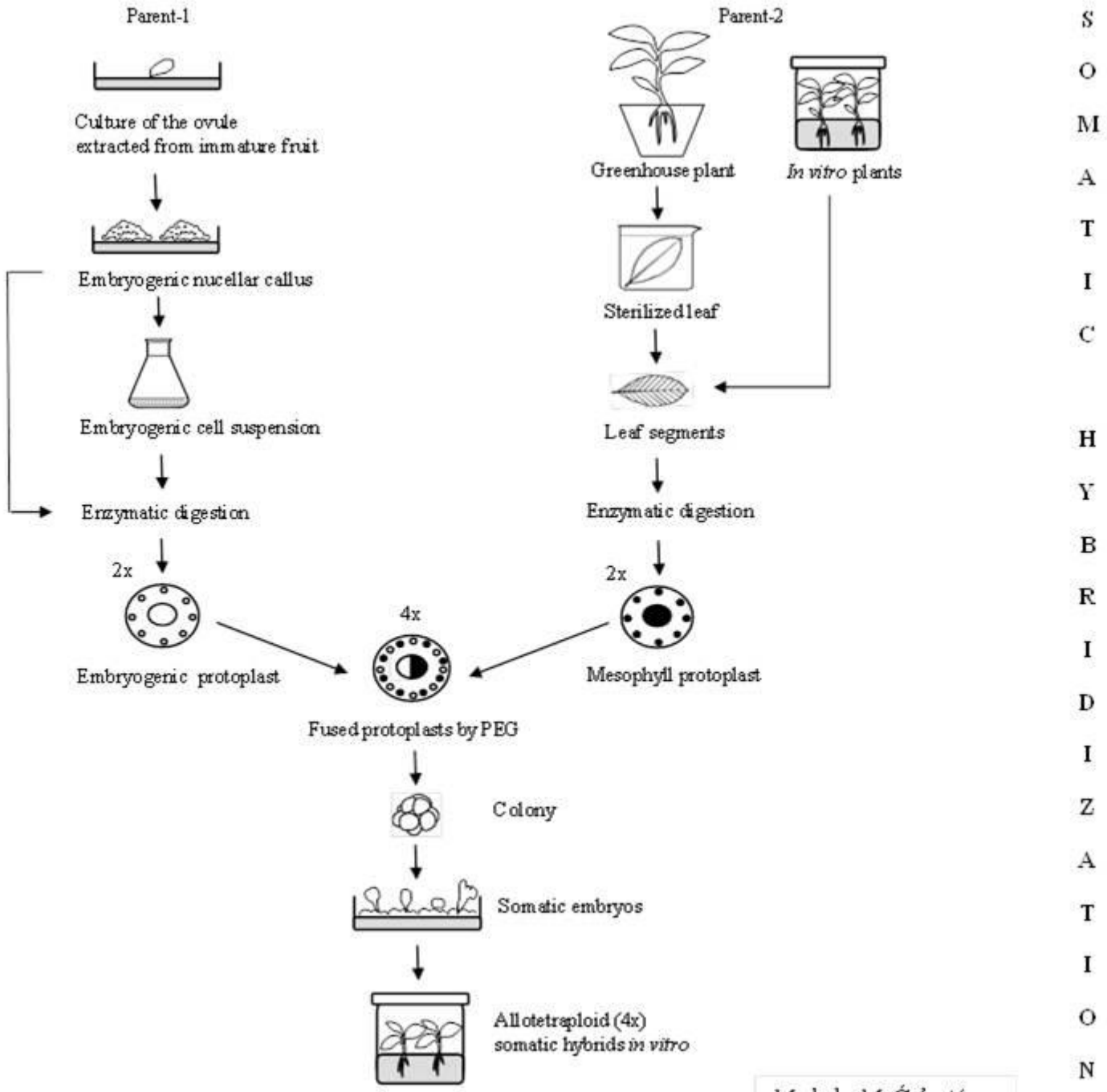
Sources of somaclones in citrus:

organogenesis, somatic embryogenesis, protoplasts

New Red Grapefruit Somaclones – In DPI Parent Tree Program - Clean budwood available for trial under MTA

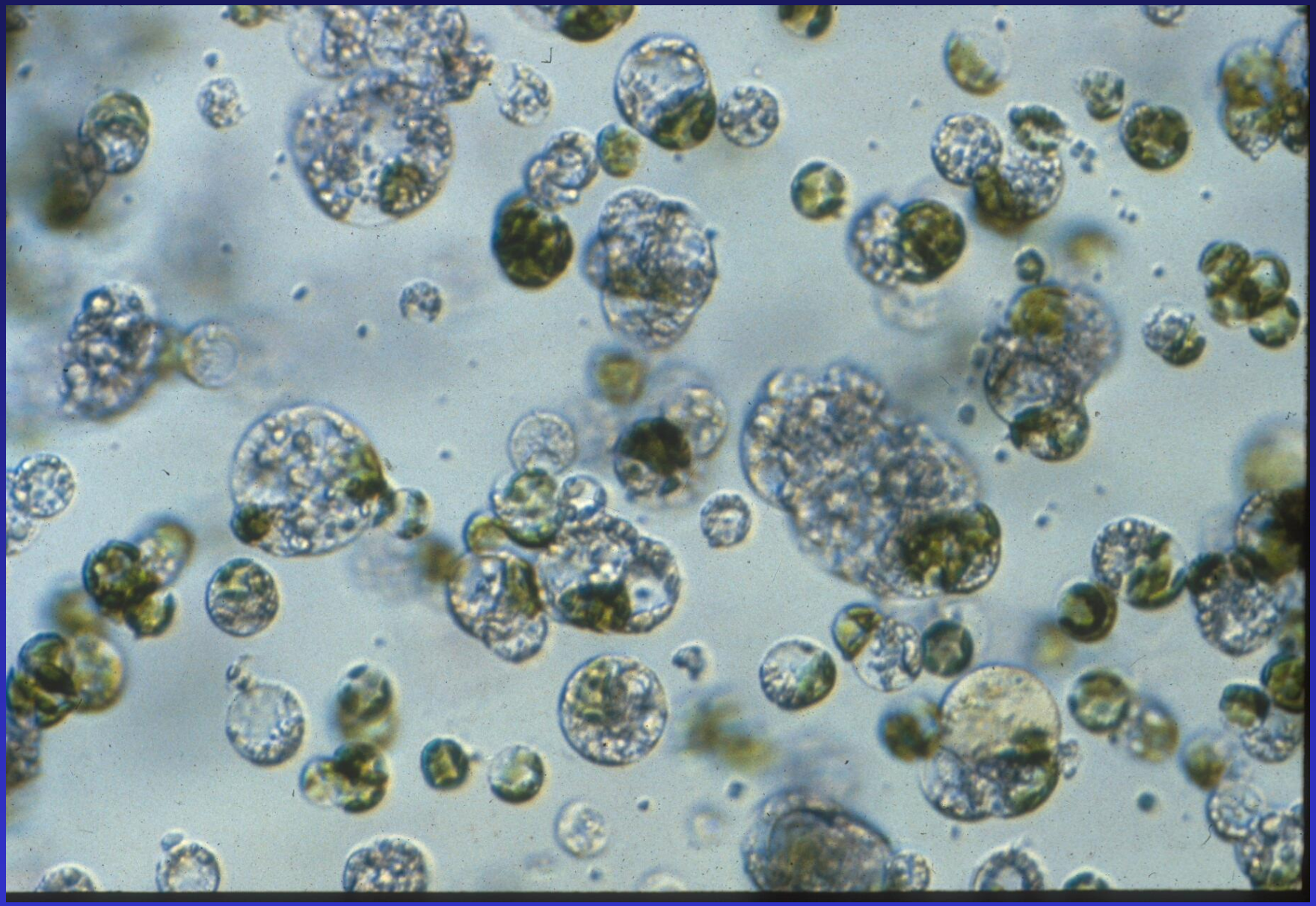
1. Red Grapefruit N40-16-11-11; DPI-435-0003 bright red, high solids
2. Red Grapefruit N-11-17; DPI-435-0040 dark red, good blush
3. Red Grapefruit N11-29; DPI-435-0041 bright red, high yield
4. Red Grapefruit N40-11-7; DPI-435-0102 dark red, high brix, blush
5. Red Grapefruit N40-16-11-15; DPI-435-N40-16-11-15 dark red, good blush
6. Red Grapefruit N40-16-11-3; DPI-N40-16-11-3 dark red, high brix

Reached 12 brix in October

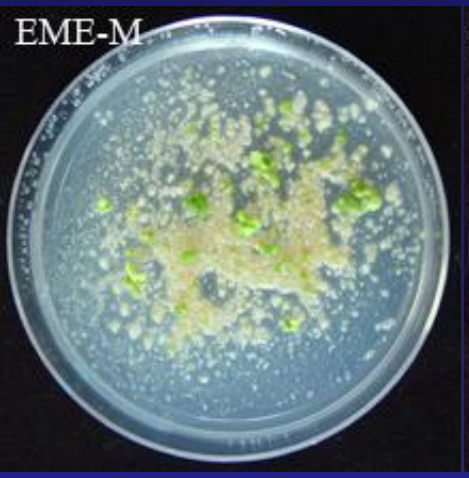


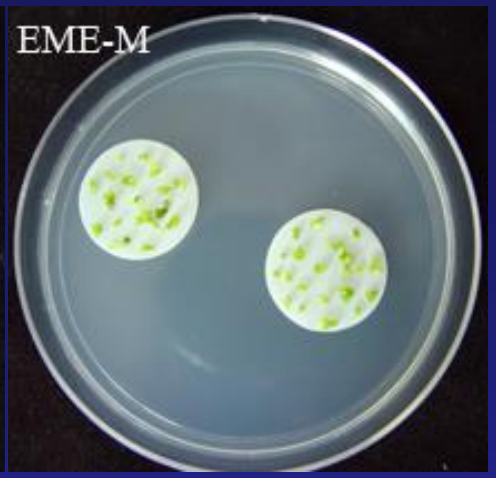
SOMATIC HYBRIDIZATION

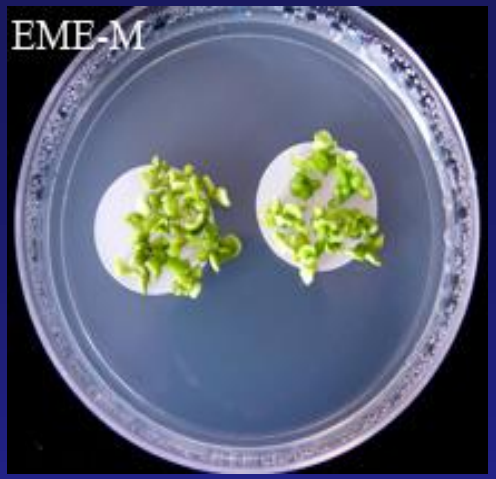
SUCROSE-MANNITOL GRADIENT FOR PROTOPLAST PURIFICATION

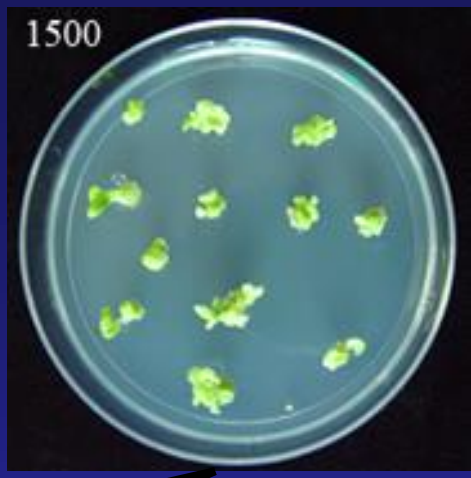


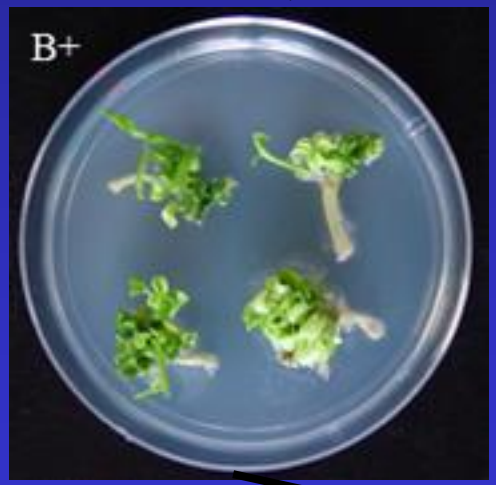
TYPICAL SUSPENSION PROTOPLAST + LEAF PROTOPLAST PEG-INDUCED FUSION

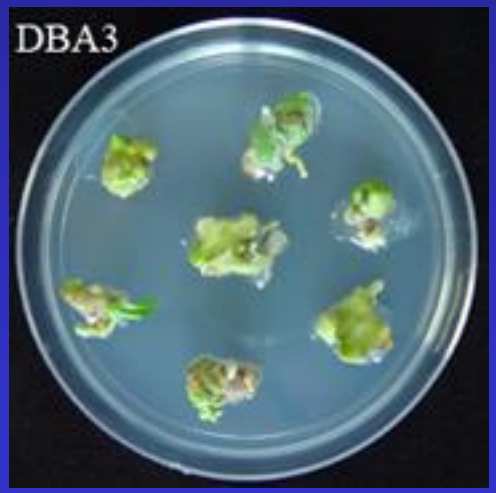


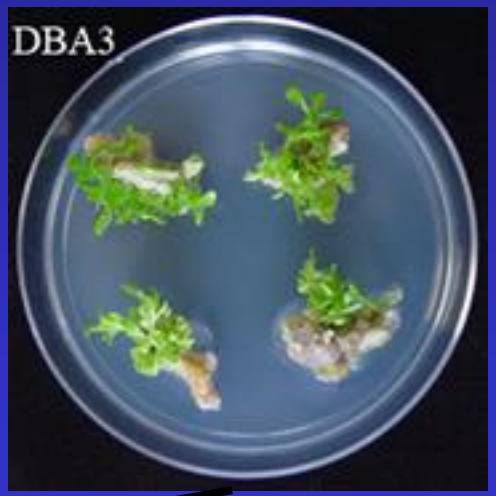




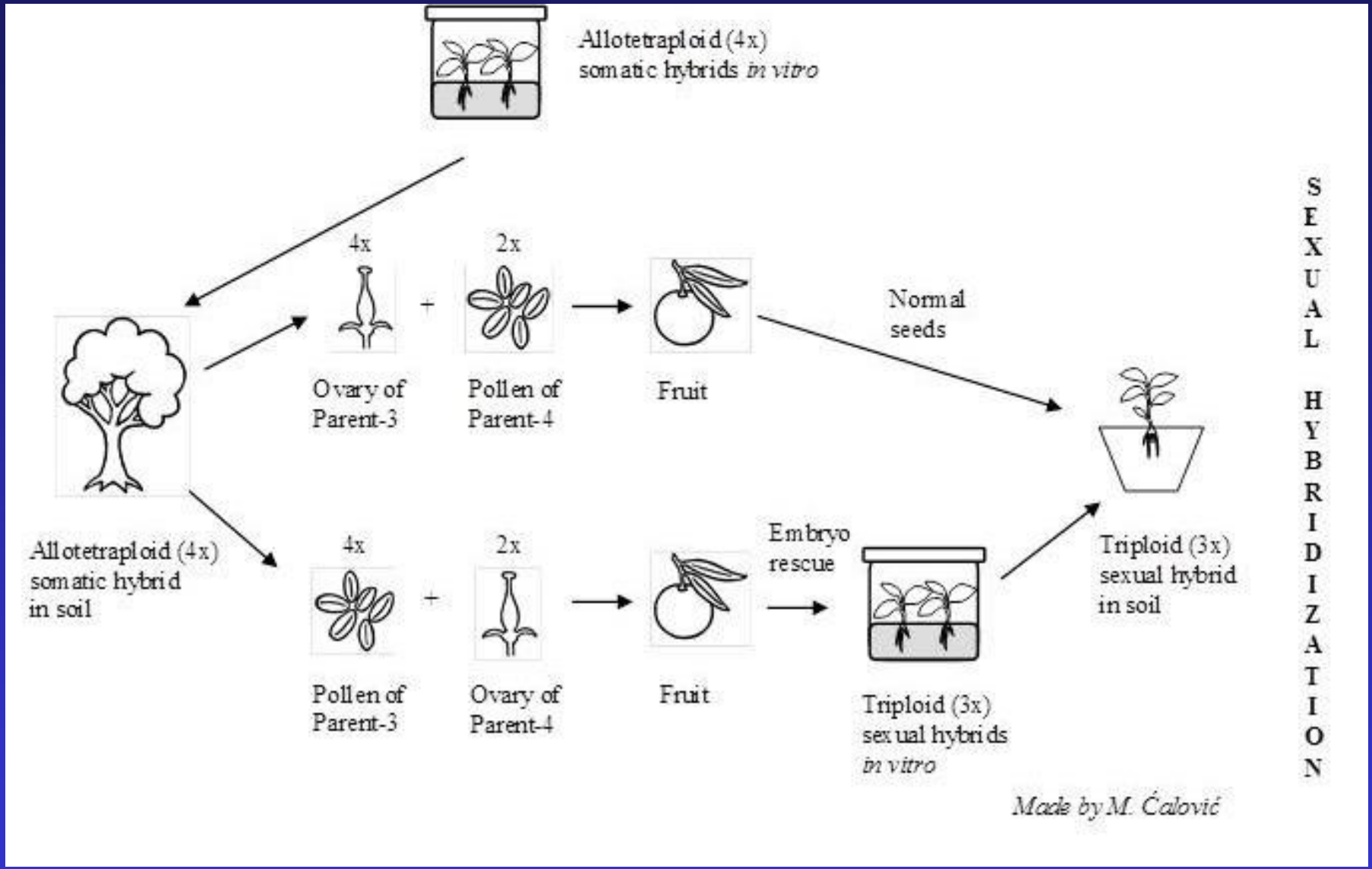


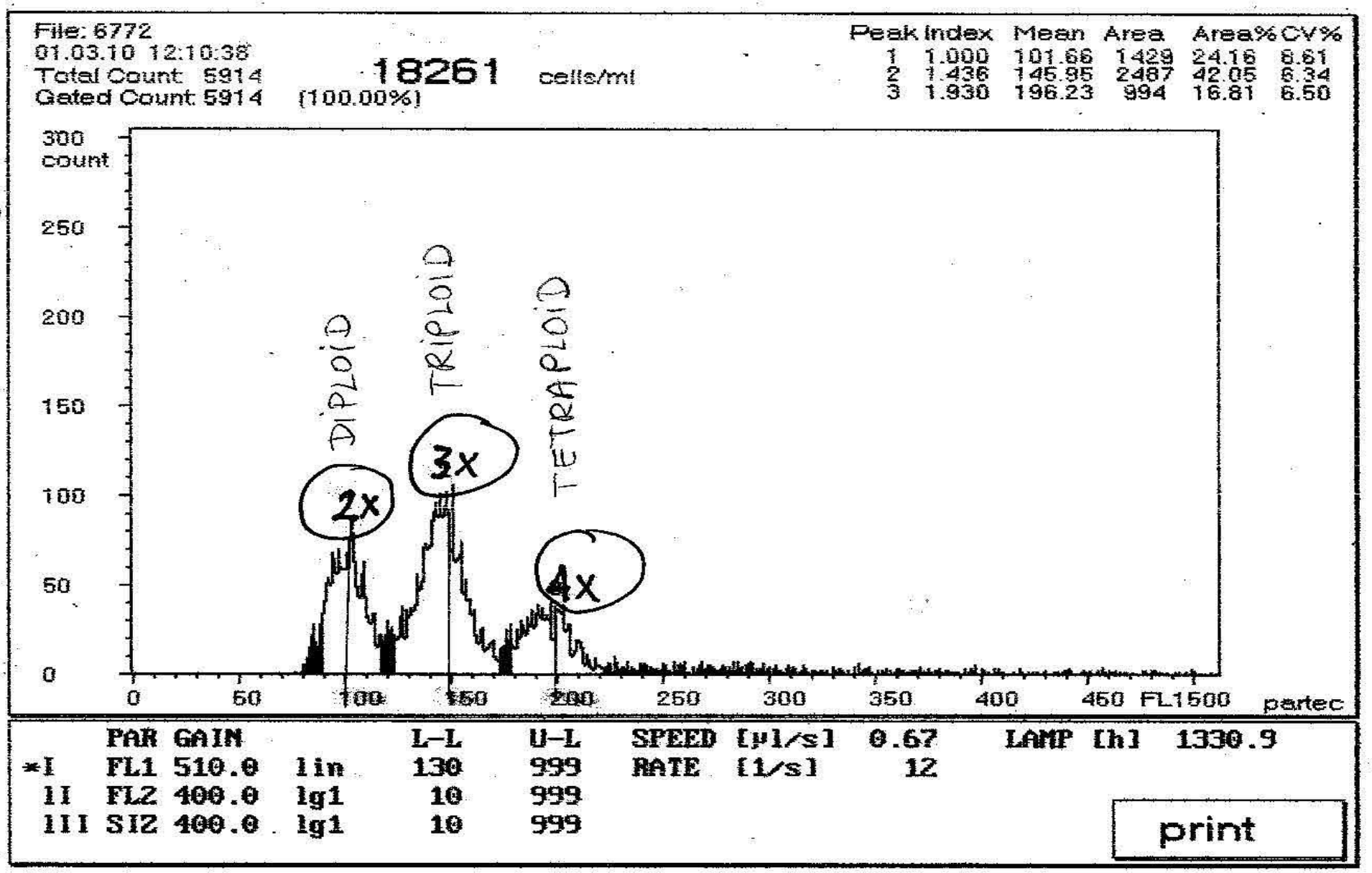






RMAN


Allotetraploid (4x)
somatic hybrids in vitro
4x
2x
+
Ovary of
Parent-3
Pollen of
Parent-4
Fruit
Normal
seeds
Allotetraploid (4x)
somatic hybrid
in soil
4x
2x
+
Pollen of
Parent-3
Ovary of
Parent-4
Fruit
Embryo
rescue
Triploid (3x)
sexual hybrids
in vitro
Triploid (3x)
sexual hybrid
in soil
SEXUAL HYBRIDIZATION
Made by M. Ćalović



Ploidy Analysis Showing Diploid, Triploid and Tetraploid Peaks , Using a Partec Tabletop Flow Cytometer

Tetraploid Breeding Parents Being Used For Grapefruit/Pummelo Improvement Include:

1. Succari sweet orange + Hirado Buntan sdl. Pummelo (yellow flesh, male and female)
2. Chandler #A1-11 + Murcott (canker and HLB tolerant)
3. Chandler #80 + Murcott (canker and HLB tolerant)
4. 4x Hirado Buntan zygotic (pink flesh, canker and HLB tolerant)
5. 4x Hirado Buntan zygotic (red flesh, canker tolerant)
6. 4x 5-1-99-3(2) zygotic (red flesh, canker tolerant)
7. 4x Hudson (dark red grapefruit, pollen parent only)

- more than 2500 triploid plants produced to date

Breeding Canker Tolerant Triploid Seedless Grapefruit-like Hybrids

Tetraploid Somatic Hybrid

X

Canker Tolerant Pummelo

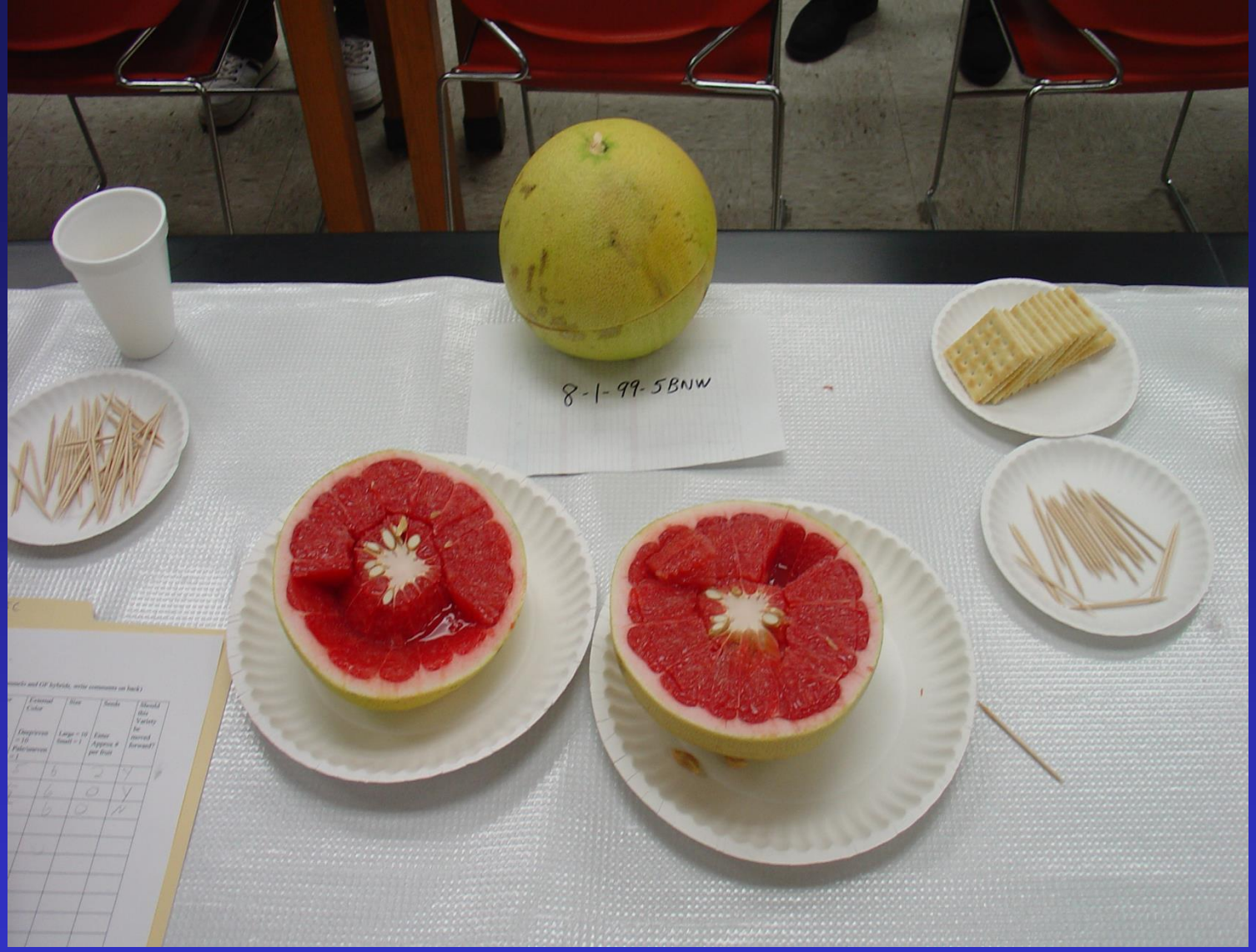
-Several good breeding parents now flowering
-Hundreds of triploid hybrids already produced
-Embryo rescue not required when tetraploid parent is used as the female

Red pummelo 8-1-99-5B

Murcott+Chandler #80
somatic hybrid (4x)

neighbor

Breeding for canker & HLB resistance: canker epidemic causing a naturalscreen of CREC germplasm – leading to the identification of superior canker &HLB tolerant diploid and tetraploid breeding parents for use ininterploid crosses to generate seedless triploids – crosses underway! Hundreds of triploids have been produced.



Another new diploid grapefruit breeding parent – a delicious, early-maturing red pummelo – first fruit 2009.
VERY EARLY MATURITY AND CANKER TOLERANT!

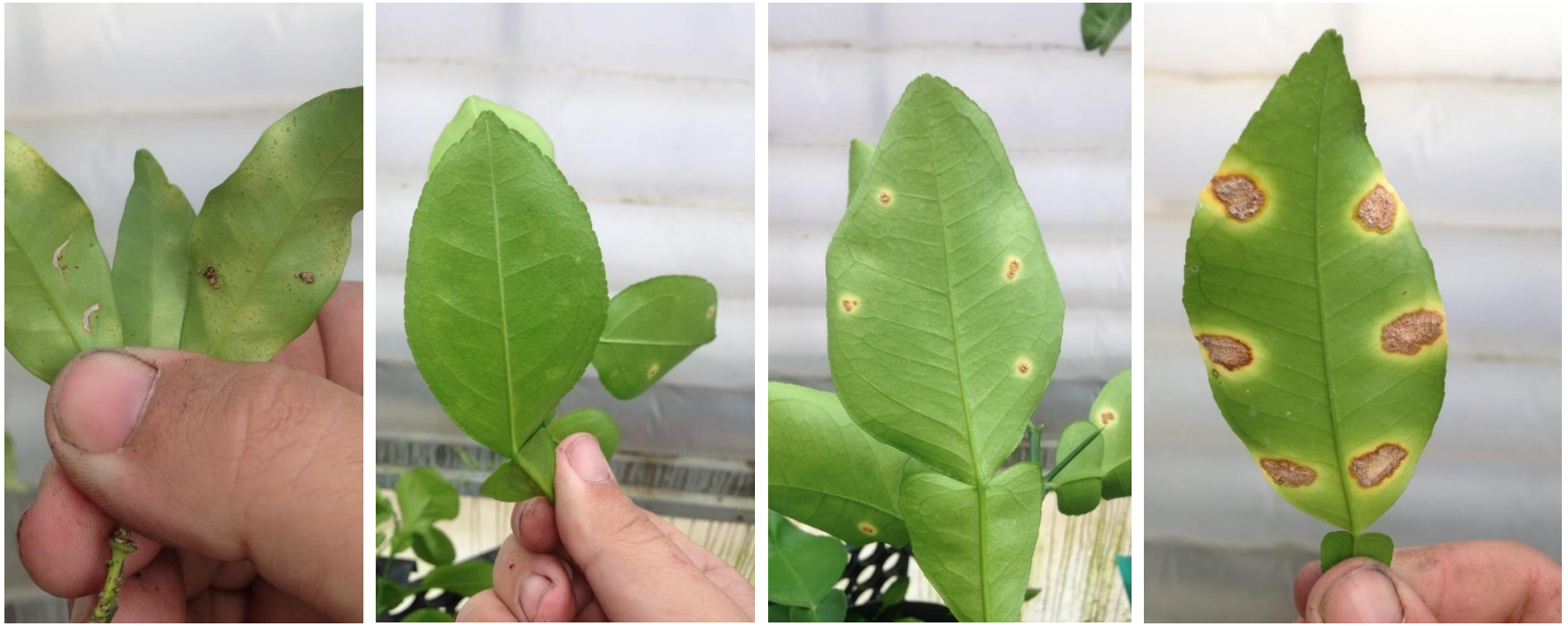
Triploid hybrid C2-5-3
2/3 pummelo, 1/3 sweet orange;
Canker tolerant & 15 ratio October 1st!

HLB-tolerant red grapefruit hybrid 1862

HLB-tolerant red grapefruit hybrid 1903

HLB-tolerant red grapefruit hybrid 1861

Testing of Triploid Pummelo/Grapefruit Hybrids for Citrus Canker Resistance



Citrus leaves 4 weeks post inoculation with *Xanthomonas citri* var. *citri*, Meiwa kumquat on left

CITRUS CANKER RESISTANCE SCREENING (from the Jim Graham Lab)

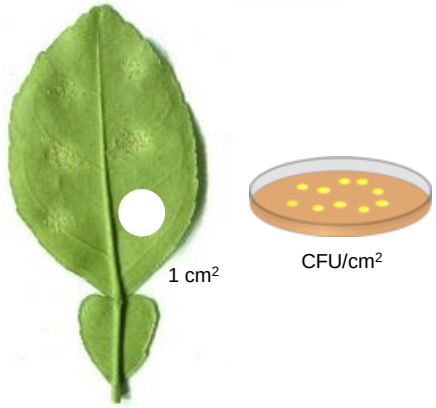
Infiltration inoculation assay (attached leaf assay) – used for new hybrids and cybrids

- 18 cybrids and positive (Marsh grapefruit) and negative controls (Meiwa kumquat)
- Bacterial suspension (Xcc): concentration 10^4 CFU/ml
- 6 inoculation point per leaf: ~ 2 µl (water soaked area of 5-6 mm)

- 6 leaves per plant
- 4 plants per genotype

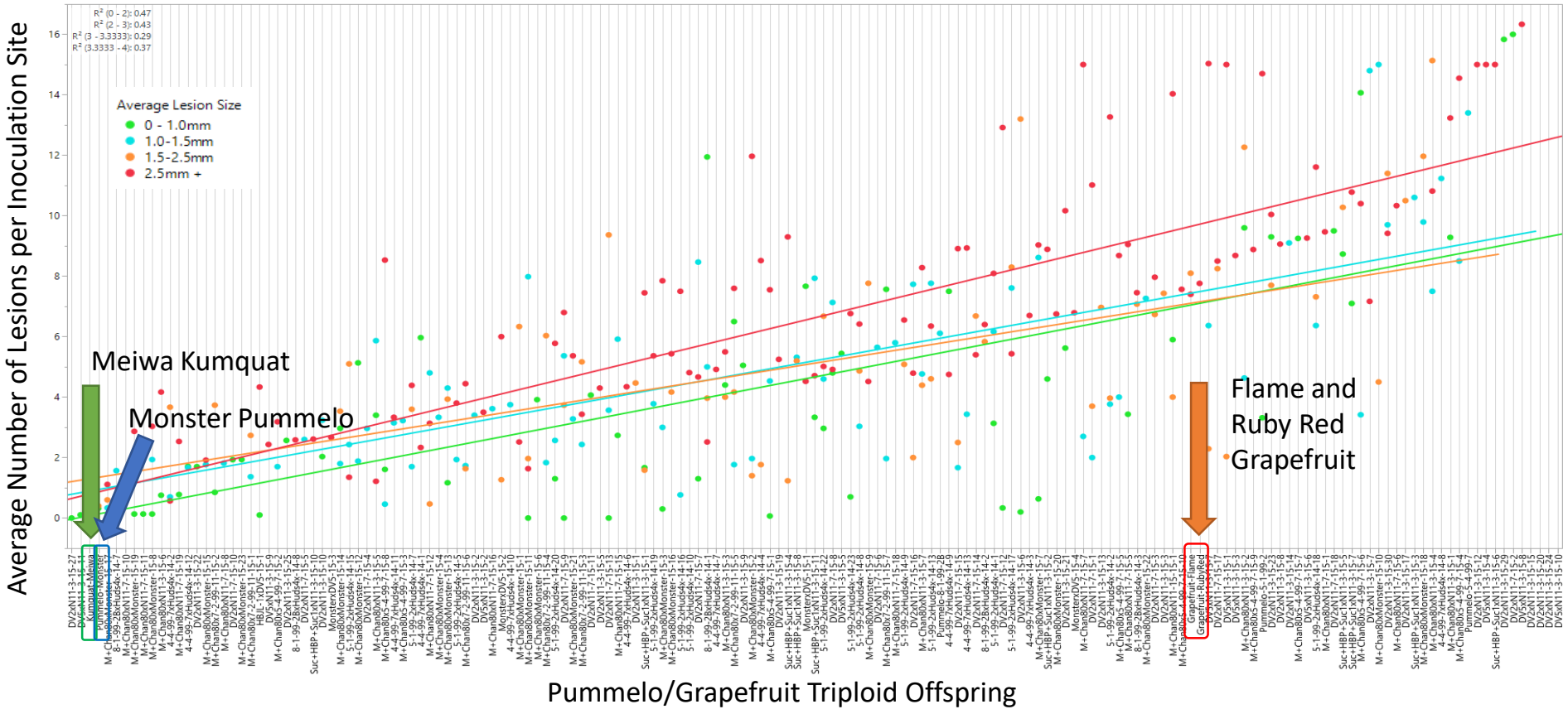
} Number of lesions counted 14 days after inoculation

Growth Curve in plant



- 2 cybrids and positive (Marsh grapefruit) and negative controls (Meiwa kumquat)
- Bacterial suspension (Xcc): concentration 10^4 CFU/ml
- 6 inoculation point per leaf: ~ 2 µl (water soaked area of 5-6 mm)
- 1 disc (1 cm^2) per day
- 3 plants per genotype
- Collection days: 0, 1, 5, 8, 11, 14 days after inoculation

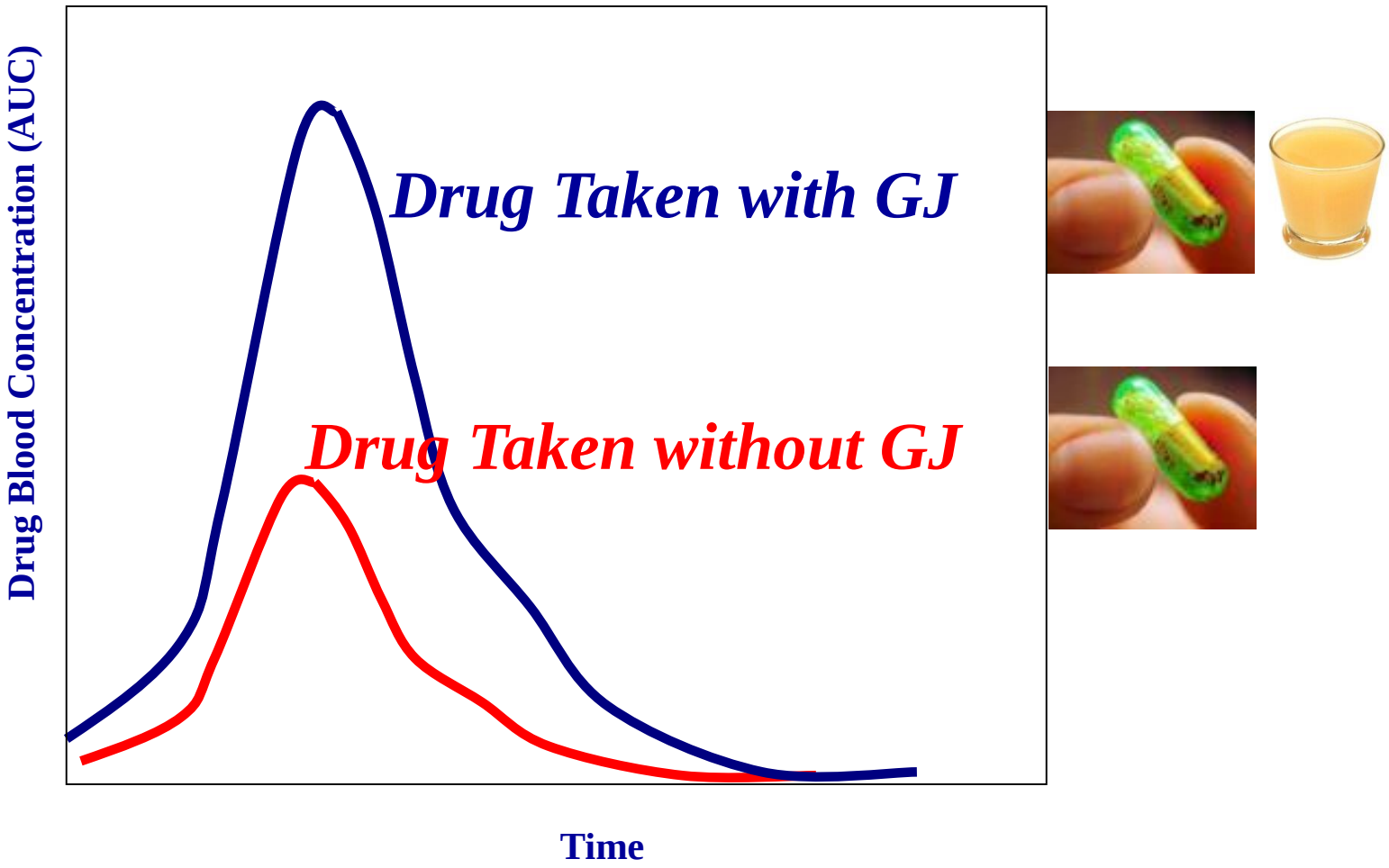
Wide Range of Response of Inoculations with Citrus Canker Among Offspring



Ethan Nielsen PhD dissertation

Love grapefruit but can't eat it because you're taking prescription drugs?

We may have the answer!!!



AUC - Area under the plasma concentration versus time curve

UF 914

- Red flesh color
- Attractive peel blush
- Grapefruit size (+)
- Grapefruit flavor and aroma
- Tender and juicy flesh
- Very low in FC’s (GJE)
- Seedless
- Good brix, lower acid
- Slightly thicker peel than grapefruit

- There is clearly a potential medical claim to make, to recapture the Senior Statin Market
- Focus group showed it is clearly distinguishable from grapefruit to ordinary consumers
- Is there a reason to market UF 914 other than the counter to the drug interaction? Yes – FLAVOR!
- South Korea, what they buy, and how they use it
  - Red grapefruit, to mix with sugar and tea or “shojiu”
  - White fleshed “Sweeties” for fresh, but red is BETTER!

What to think about UF 914?

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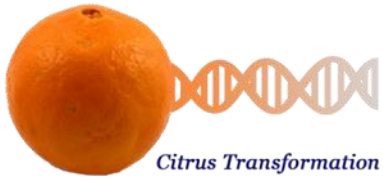
SOMATIC CYBRIDIZATION



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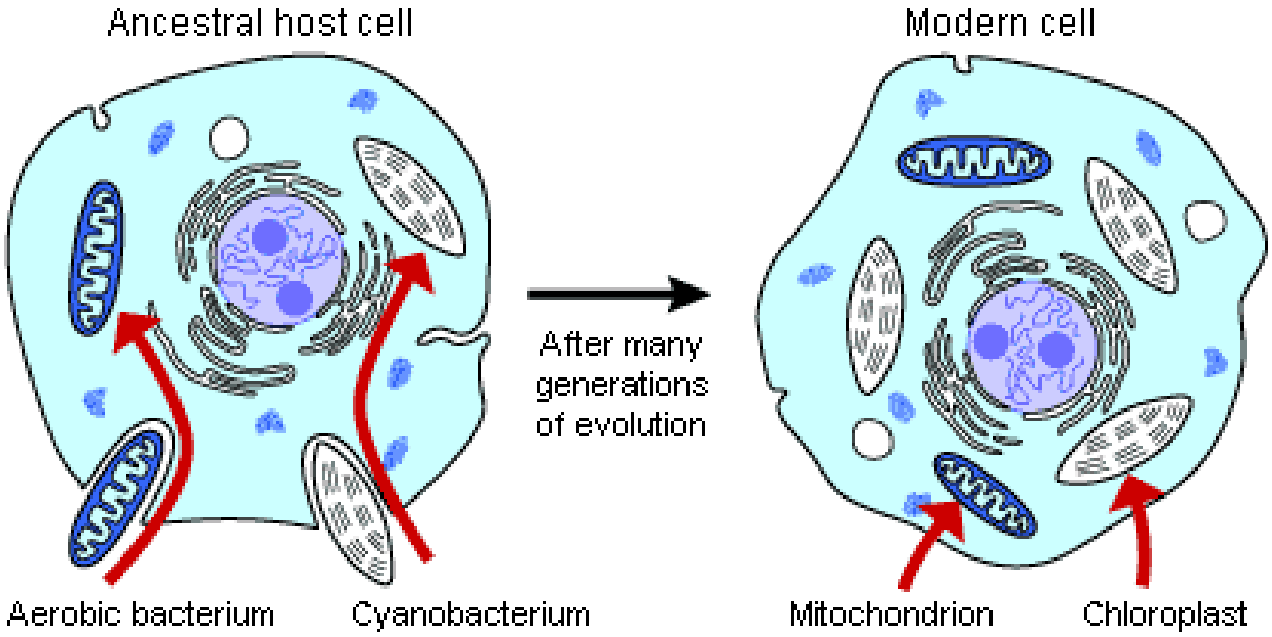
Lynn Margulis



From: http://evolution.berkeley.edu/evolibrary/article/history_24

EndoSymbiosis Theory – Lynn Margulis (1970) published her argument in: *The Origin of Eukaryotic Cells*.

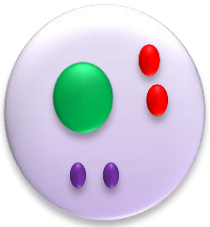
CYBRIDIZATION PROCESS

Ahmad Omar

Highly Susceptible
Fresh fruit market

Ahmad Omar

Highly Resistant



Grapefruit
Citrus paradisi

Kumquat
Fortunella crassifolia

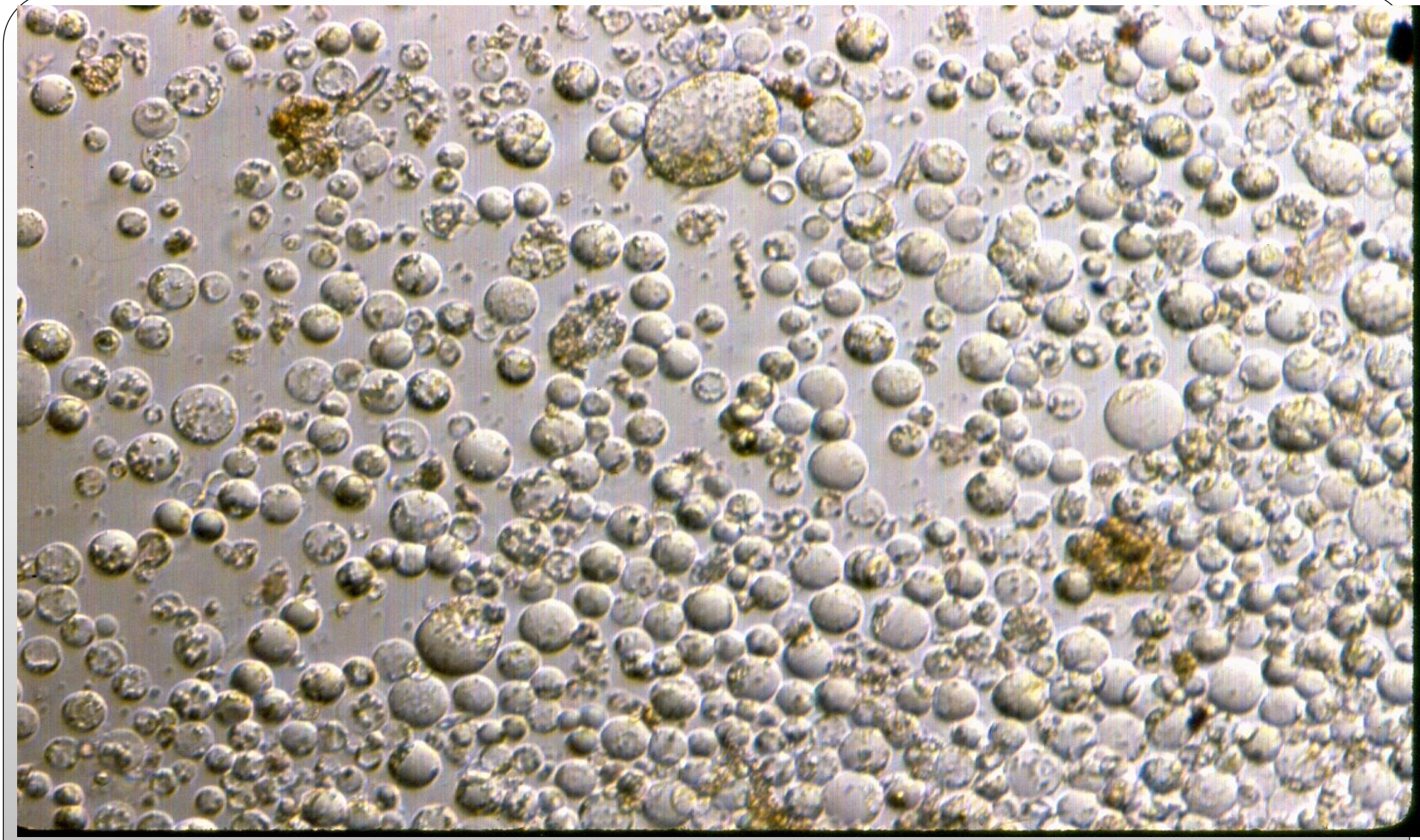
- All genes are not housed in the nucleus. Both mitochondria and chloroplasts have small genomes.
- Citrus cybridization often occurs in citrus somatic hybridization experiments (via protoplast fusion).
- Citrus cybrids from embryogenic culture + leaf cell fusions always have the mitochondrial genome from the culture cell parent, the chloroplast genome is generally randomly inherited. Allows for study of nucleo-cytoplasmic interaction.

Accidental Cybridization – creation of the N2-28 'Summer Gold' grapefruit

Efforts to generate triploids directly by fusing protoplasts isolated from mandarin embryogenic callus with protoplasts isolated from grapefruit pollen tetrads (haploid) resulted in the regeneration of plants with grapefruit morphology. These plants produce delicious grapefruit with an exceptionally long harvest window. We hypothesize that they regenerated from fusions of mandarin suspension cells with contaminate grapefruit pollen wall cells.

UF-CREC Citrus Genetic Improvement Team
2013

Isolated pollen tetrads of Ruby grapefruit



Haploid protoplasts isolated from Ruby grapefruit pollen tetrads, probably contaminated with diploid anther wall protoplasts.

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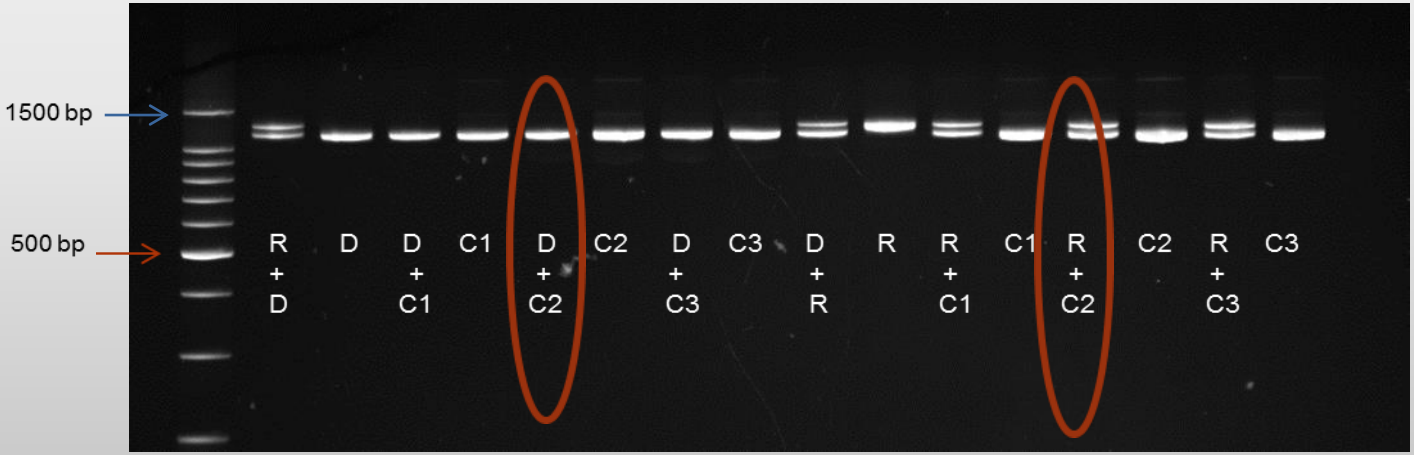
Mitochondrial Genotyping

PCR amplified Cybrid DNA (C1, C2 and C3) in mixture with Dancy (D) and Ruby Red (R) DNA to reveal polymorphism using intron based marker *nad7i1*.



The *nad*7i1 primer set developed by Grosser et al. (in preparation) amplifies a mitochondrial genome target

N2-28 Summer Gold Grapefruit

Table 1. Comparison between summer N2-28 'Summer Gold Grapefruit' and controls 'Ruby Red' and 'Pink Marsh" for the Brix, color and titrable acidity value (average of 20 fruit per selection, test conducted July, 2013).

	Brix°	Color	Titrable Acidity (ml)
Summer Gold N2-28	11.6	34	0.98
'Ruby Red' grapefruit	9.4	34.5	0.85
'Pink Marsh' grapefruit	8.2	34.3	1.2

- Cybrid with 'Dancy' cytoplasm
- Sweeter than Ruby Grapefruit
- Harvest from December to August!
- No granulation or seed germination

TARGETED SOMATIC CYBRIDIZATION

Ahmad Omar and Mayara Murata

Somatic cybrid plants of Grapefruit containing Mewia ‘Kumquat’ cytoplasm (mitochondrial genome).

Somatic cybrid plants of White Marsh, Flame and red somaclone N11-11 grapefruit containing Meiwa Kumquat cytoplasm (mitochondrial genome)

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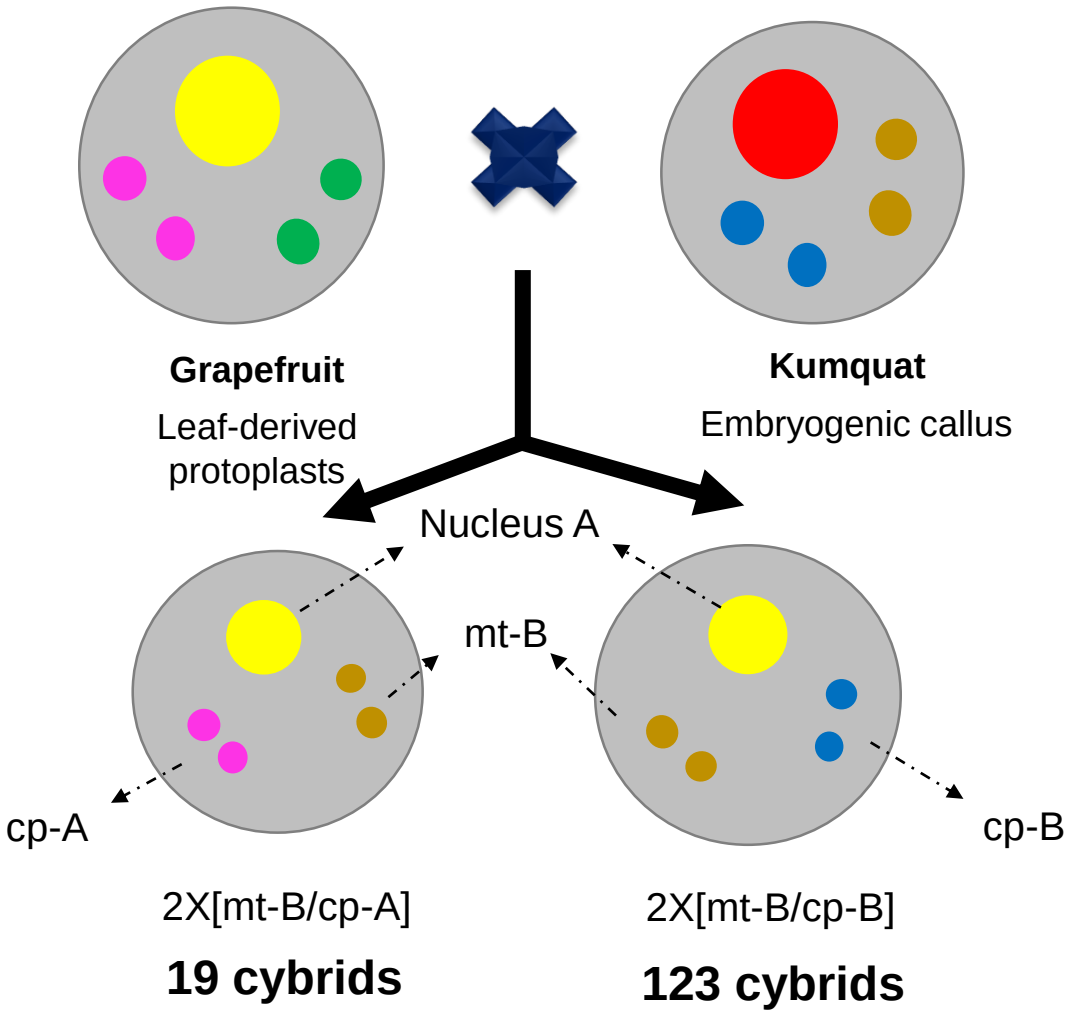
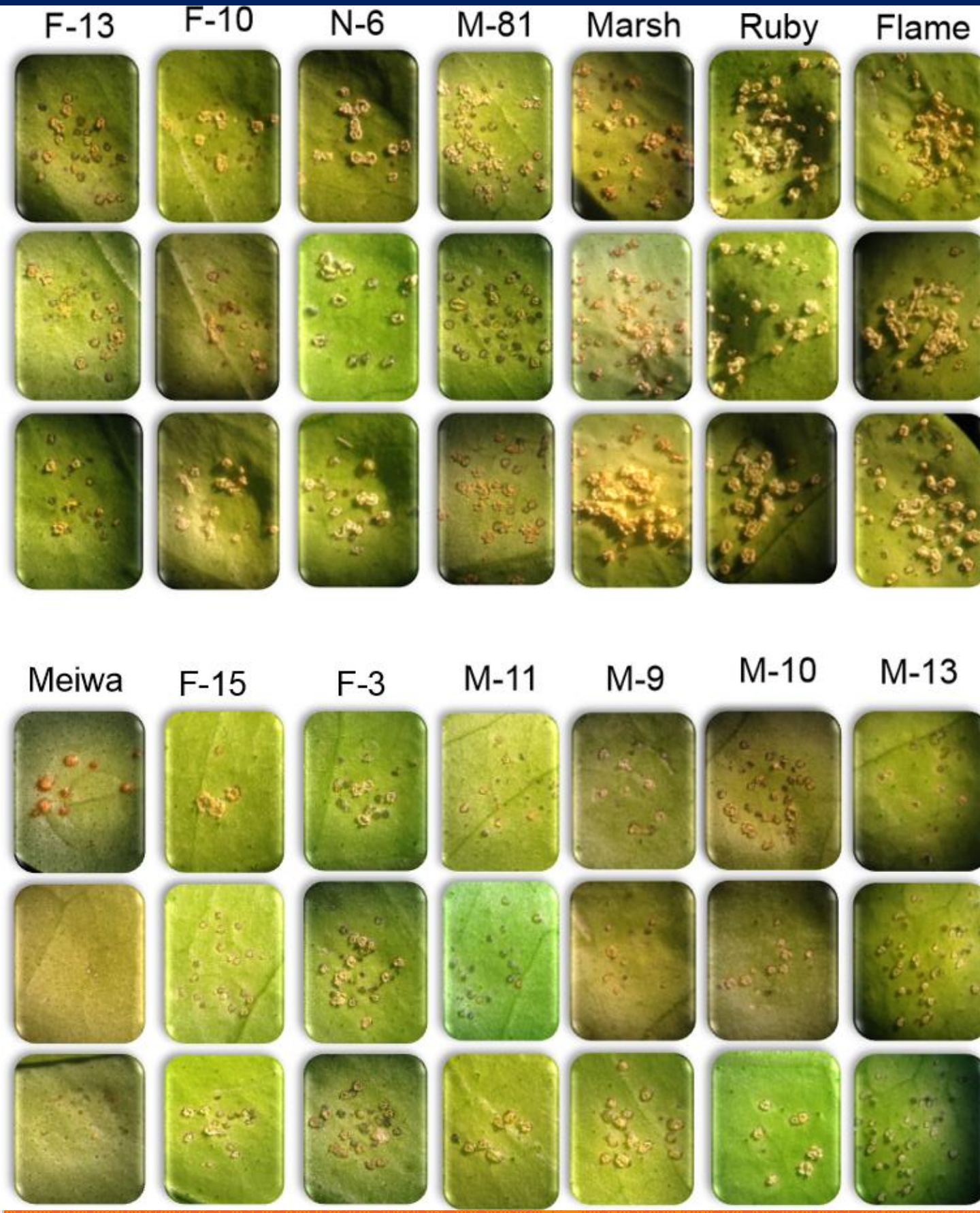
Example of grapefruit cybridization with kumquat cytoplasm

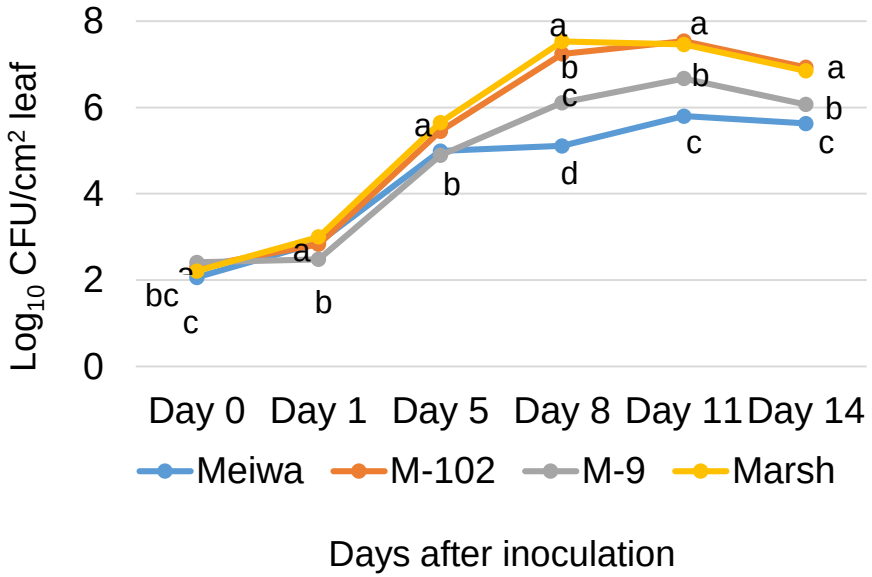




OBJECTIVE 2

CITRUS CANKER RESISTANCE SCREENING
Lesions per leaf comparison

Figure 1. Xcc growth curves. Growth curves of *Xanthomonas citr*i subsp. *citri* in citrus leaves of 6 genotypes [x].



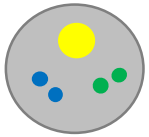
	Genotype	June 2016 # of Lesions	June 2016 Xcc Population
Control	Flame	138.17 a	9.39 abcd
Control	Marsh	104.73 b	9.50 ab
Control	Ruby	101.70 b	8.84 hijk
Grapefruit chloroplast	Cybrid N-4	103.10 b	9.09 defgh
Grapefruit chloroplast	Cybrid M-102	99.37 b	9.02 fghi
Grapefruit chloroplast	Cybrid M-78	98.13 b	8.57 k
Grapefruit chloroplast	Cybrid M-81	98.03 b	9.15 cdefgh
Kumquat chloroplast	Cybrid N-8	76.13 cd	8.56 k
Kumquat chloroplast	Cybrid M-13	85.26 c	9.02 fghi
Kumquat chloroplast	Cybrid N-10	78.36 cd	9.03 efghi
Kumquat chloroplast	Cybrid N-18	72.70 de	8.64 jk
Kumquat chloroplast	Cybrid F-2	70.23 de	9.34 abcdef
Kumquat chloroplast	Cybrid F-5	64.33 ef	9.20 defg
Kumquat chloroplast	Cybrid M-10	63.27 efg	8.51 k
Kumquat chloroplast	Cybrid F-13	61.87 efg	8.72 ijk
Kumquat chloroplast	Cybrid F-3	58.14 fgh	9.46 abc
Kumquat chloroplast	Cybrid M-11	56.73 fgh	8.65 jk
Kumquat chloroplast	Cybrid F-15	55.83 fgh	9.31 abcdef
Kumquat chloroplast	Cybrid M-9	53.90 fgh	8.93 hij
Kumquat chloroplast	Cybrid N-6	50.93 gh	8.71 ijk
Kumquat chloroplast	Cybrid F-10	48.57 h	9.36 abcd
Kumquat chloroplast	Cybrid F-6	46.10 h	9.43 abcd
Kumquat chloroplast	Meiwa	3.87 i	7.57 l

Table 1. Mean of total number of lesions per leaf after infiltration inoculation of *Xanthomonas citri* subsp. *citri* for regenerate cybrids and their progenitors [x]

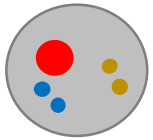
Data indicates that canker tolerance in grapefruit cybrids comes from the kumquat chloroplast, and not the kumquat mitochondria.

Marsh grapefruit

Cybrid M-102



Meiwa kumquat



Cybrid M-9

Conclusions:

- **6 high quality somaclone grapefruit clones (red and dark red) are available for field trials under MTA.**
- **Several new high quality grapefruit-like seedless triploid hybrids are available for trial, including C2-5-3 that matures in September; most are canker tolerant and some are showing better HLB tolerance than grapefruit. Many more in the pipeline!**
- **Fast Track selection UF-914 is commercially available; dark red flesh and superior flavor. Very low in furanocoumarins that interfere with prescription drugs.**
- **Fast Track selection Cybrid N2-28 'Summer Gold' can be harvested through July, commercially available.**
- **Non-GMO cybrid grapefruit clones (white, red and dark red) containing kumquat chloroplasts are showing significantly improved canker tolerance, and may have better HLB tolerance.**

Funding: NVDMC, CRDF, and the Citrus Research and Education Foundation (CREF) for grove support.

Thanks also to: Chris Chase, Mayara Murata, Ahmad Omar, Jim Graham, Diane Bright, Fred Gmitter, Qibin Yu, Mauricio Rubio, JoLisa Thompson, Karen Plant, Johnny Ferrarezi and many others, and especially Troy Gainey and the CREC Grove Crew.

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Thanks!

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2020