U.S. Horticultural
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Breeding Citrus for HLB Resistance

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ARS Citrus Improvement

The oldest citrus breeding program in the world

W. T. Swingle

H.J. Webber

Sub-Tropical Laboratory
Eustis, Fla.
Swingle and Webber
1893-1897

>75% of US citrus industry has rootstock and/or scion from USDA program…. mainly rootstocks!

USDA Citrus Scion Releases

Year	Release	Pedigree
1930	Minneola Orlando	Duncan x Dancy
1959	Robinson Osceola Lee	Clementine x Orlando
1963	Page	Clementine x Minneola
1964	Nova	Clementine x Orlando
1979	Sunburst	Robinson x Osceola
1987	Fallglo	(Clementine x Orlando) x Temple
1987	Flame	Nucellar sport of 'Ruby Red'
1987	Midsweet	Nucellar sport of 'Homosassa'
1989	Ambersweet	(Clementine x Orlando) x midseason orange
2009	US Seedless Pineapple	Irradiated Pineapple
2009	US Early Pride	Irradiated Fallglo

Earlier USDA Released Rootstock Varieties

Variety	Year	Industry %
Carrizo/Troyer citrange	1934	30
Swingle citrumelo	1974	46
Sun Chu Sha mandarin	1988	< 1%

New Rootstocks from USDA

- **US-852 released in 1999**
- **US-812 released in 2001**
- **US-802 released in 2007**
- **US-897 released in 2007**
- **US-942 for release in 2010**

Focus on Developing HLB-and ACP Resistant Citrus

- HLB likely the single greatest threat to citrus
- No strong HLB resistance has been identified in cultivated Citrus scion varieties
- Transgenics appear to be the only medium term solution for strong HLB resistance
- Goal: add genes to reduce survival, growth, and/or virulence of causal pathogen, genes to deter psyllid vector, possibly suppress host disease response
- With little known about host /pathogen interaction, antimicrobial peptides have been a major focus- may also confer resistance to canker and CVC
- Other types of genes also being explored…….

Harnessing nature's genetic engineer: *Agrobacterium tumefaciens*

A. tumefaciens **causes crown gall disease in many plant species**

By removing At genes for growth regulators and replacing with:

1) **promoter**
2) **gene of interest etc.**
3) **gene for selectable marker (antibiotic)**

can express genes when and where you want, without gall formation.

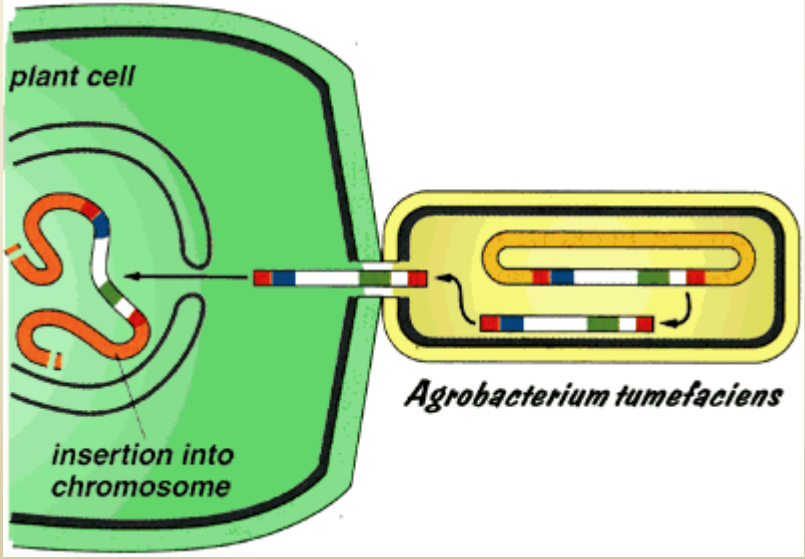
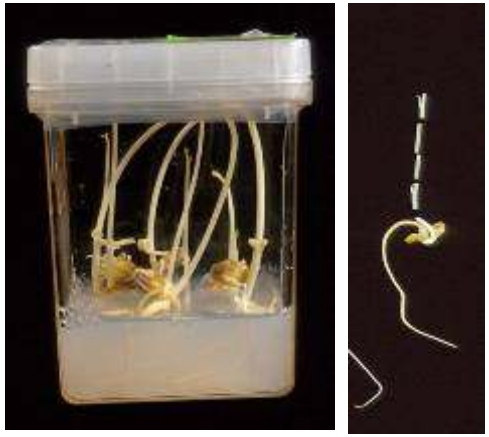


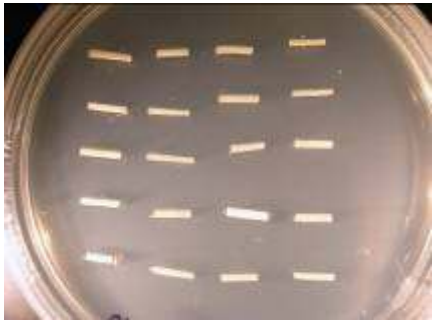
Image: www.plantsci.cam.ac.uk/.../GFP/plantrans.html

Citrus Transformation: Major Focus for HLB and Canker Resistance

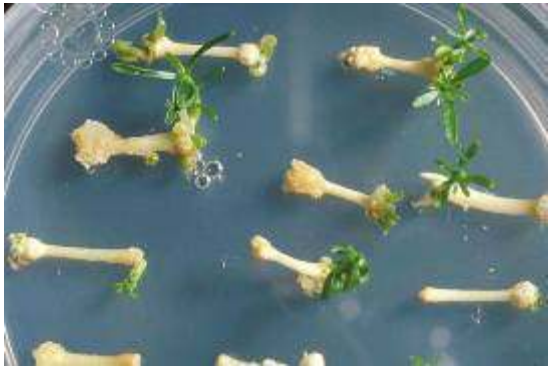
Transformation



Selection



Regeneration

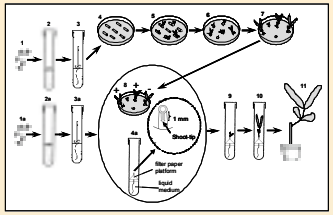


Micro-grafting

Grow out

Evaluation

Transgenic Project: Parallel Tracks



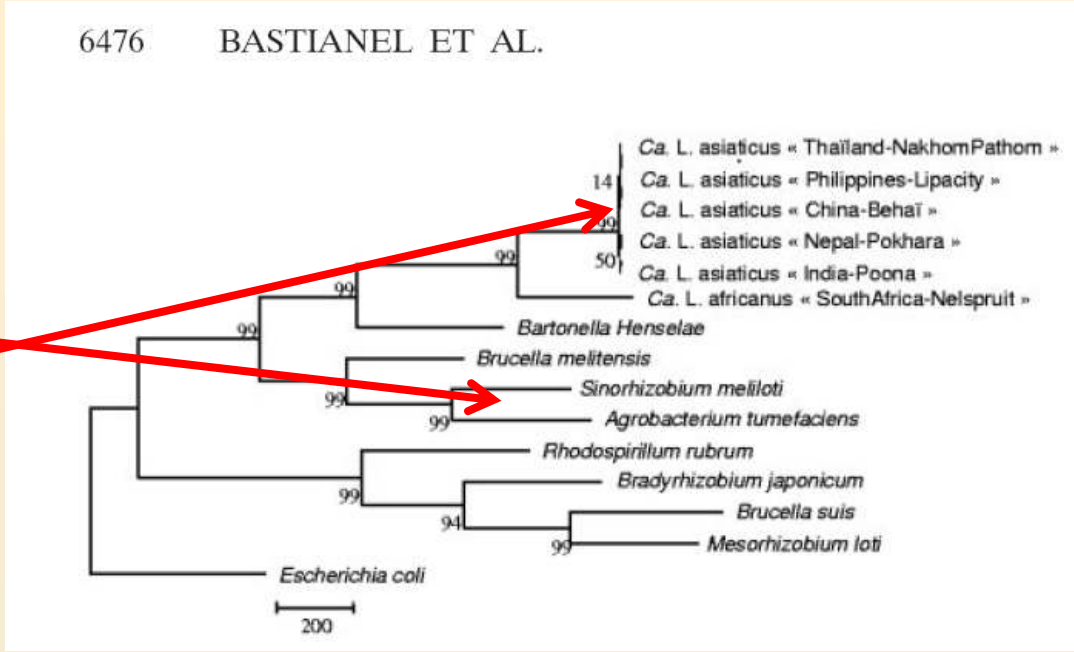
- Fastest track- possible “home run” using best available technology on rootstocks, sweet orange and grapefruit- high throughput.
  - Goal is earliest possible resistant variety in field
  - Emphasizing components which are deregulated in crop plants
- Experiments to overcome transformation bottlenecks
- Identifying new targets for transgenes
- Exploring other promoters, constructs, etc. first with easily transformed rootstock types

Antimicrobial Peptides

- Broadly active against groups of micro-organisms
- First line of active defense to combat infection in multicellular organisms
- Most are very small molecules, MAY move systemically
- Results in microbial death or prevents growth

In –Vitro AMP Screening

- *Agrobacterium* and *Sinorhizobium* are related to *Liberibacter*
- Also using *Xanthomonas citri*
- Best AMPs, including D4E1 are effective in 1 µM range

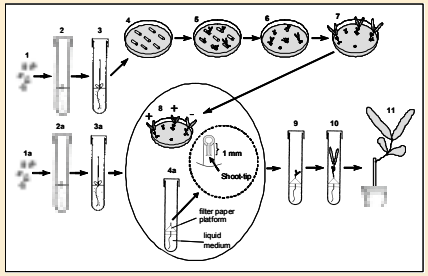


Other Transgenes for HLB Resistance

- Transmembrane protein from Liberibacter is a target identified from the *Liberibacter asiaticus* genome (working with Duan group at USHRL)
- Phloem-specific protein induced during HLB infection (Bowman data USHRL)
- Working with ARS scientists in California to develop constructs so that ALL inserted genes are from Citrus!

➢300 independent scion (Hamlin, Midsweet, Valencia, and Ray Ruby) and >3500 independent rootstock regenerants established as micrografts

Transgenic Project: Challenging with HLB etc.



- Lots of plants of trifoliate hybrids transformed with D35S/D4E1 have been challenged

- No major differences, so far, in HLB symptoms or Liberibacter levels. D4E1-GE plants have better growth.

- Now using more active promoters and phloem specific promoters as well as different transgenes

d35S:GUS Activity in most tissue

SS:GUS activity limited to phloem!

D4E1Hamlin challenged with CBC

Control

D4E1 Transformed
Plant # 88

D4E1 Transformed
Plant # 161

HLB-resistance from distant citrus and relatives

- reports of HLB resistance in different species related to citrus
- broad search may yield greater resistance
- Experiments include more than 50 genotypes- collaboration Riverside Repository
- May identify genes which can then be used to transform commercial citrus
- Citrus gene that should permit flowering within year of seed germination, permitting rapid use of genes from distant relatives

Carrizo transformed with D35S:: Citrus FT

Gloria Moore- UF Horticulture

Evaluating varieties for HLB-tolerance

- Appears that citrus varieties vary widely in their rate of developing HLB
- Identified 8 groves in the Indian River area reporting presence of HLB and multiple specialty cultivars
- Avoided reported edge effects and bias, randomly sampled 20 trees /cultivar /grove
- Ran q-PCR using 16S CLas rDNA “Wenbin” primers (APHIS standard); 760 trees were sampled

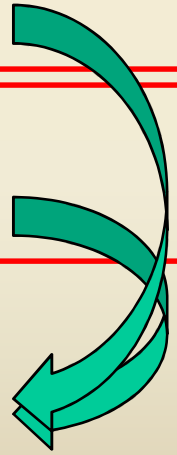
Random sampling of interior trees in commercial groves with multiple specialty cultivars: % of 20 trees with Ct <36 from q-PCR of CLas rDNA (April 2010)

Cultivar	Indian River	Martin 1	Martin 2	St. Lucie 1	St. Lucie 2	St. Lucie 3	St. Lucie 4	Palm Beach
Sweet orange	0	35	50	0	25	30	20	
Grapefruit	0	20	45	0	0		30	5
Sunburst	0	0					25	
Temple	0	5	10	5	0	5		55
Fallglo		15						20
Minneola	5	55	35	0	25	25		80
Murcott			65			5	65	
Ortanique	30							

ANOVA on qPCR Ct data for CLas 16S rDNA for citrus cultivars in 6 Indian River area groves: April 2010. The lower the Ct value, the higher the CLas titer.

(Data excluded for the 2 groves with no CLas detected in sweet orange)

	N	Mean Ct		ΔCt grove normalized		Mean # CLas genome/sample*	% trees Ct<36
Minneola	99	35.2	d	-2.4	c	304	43%
Murcott	61	35.9	cd	-1.3	bc	168	44%
Sweet orange	98	37.1	bc	-0.4	b	236	31%
Grapefruit	100	38.1	ab	1.1	a	40	20%
Temple	101	38.8	a	1.3	a	9	15%
Fallglo	40	38.6	a	1.5	a	13	18%
Sunburst	39	38.7	a	1.6	a	107	13%

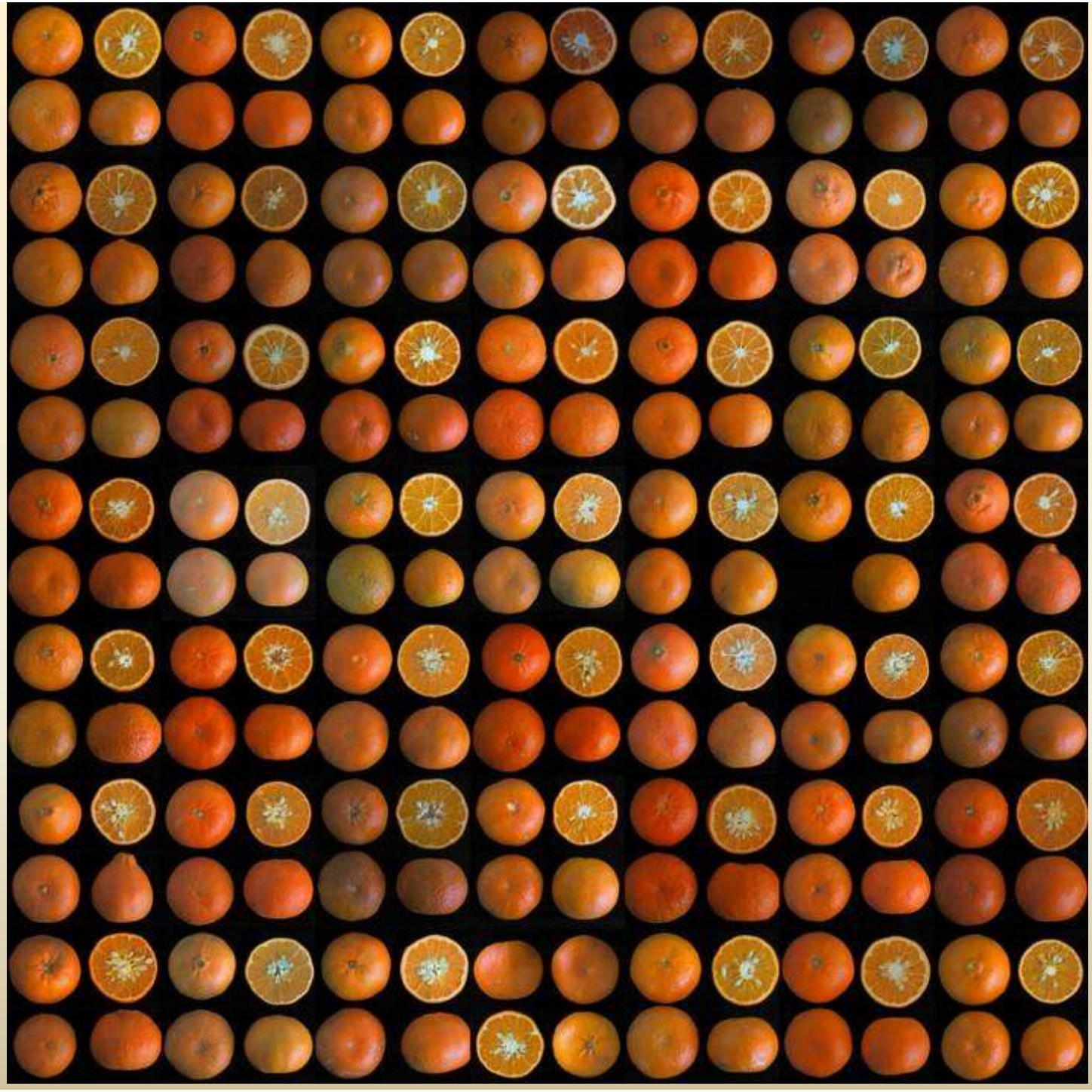


Issues regarding HLB/ACP-tolerance

- If resistance or tolerance is confirmed, how may this benefit citrus industries?
- How much of a delay in symptom development / compromise of cropping is needed to be useful?
- Could resistant/tolerant types be used to protect more susceptible cultivars?
- NOT suggesting we all grow ‘Temple’! If significant U1 resistance/tolerance is found in conventional cultivars, may find in related hybrids that are very sweet-orange or grapefruit like
- As well as possibly identifying genes for intragenic use

Diapositiva 21

U1 REd text: Confusing
USHRL, 02/06/2010



Thanks!

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