Paths to the Future Citrus Varieties in Florida

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Clearwater Beach, Florida
September 2022
Florida’s citrus production has been reduced by ~80%

Businesses have closed, some after >100 years

Groves abandoned and land sold for development

People have lost their employment

Huge social and environmental consequences

No HLB in the title, but...this is all about HLB!
• But we can begin to see the Phoenix rising from the ashes

• ‘What doesn’t kill me, makes me STRONG’

• Research is showing a way forward, with improved horticultural practices, nutrition, soil management, and insect control strategies

• Genetic solutions developed by breeding will underly the future success in Florida

No HLB in the title, but...this is all about HLB!
HLB Tolerant Cultivars Available Now!

FIRST GENERATION
PRE-HLB CREATIONS
Sugar Belle® near Vero Beach, HLB+ >8 years!
• Sugar Belle harvest periods are compressed, 3-4 weeks
• How much fruit can move through a narrow window?
• Can Sugar Belle® contribute to the OJ stream?
• Can clever marketing move more fresh fruit?
• Marketing quality requires quality
• 2020 was a bad year for Sugar Belle® quality; will our changing climate mean more bad years?
Marathon Mandarin
• **Seedless under all circumstances, and easy to peel**

• **Good color, good flavor, and segment structure**

• **Very early maturity, with long on-tree storage capacity**

• **Firm fruit that may be harvested without clipping, saving labor costs at harvest**

• **Responds well to ethylene, and performs very well in long term cold storage**
• Fresh fruit cultivar, not intended for juice

• Early and attractive, easy to peel and always seedless, good quality; competitive with imported southern hemisphere fruit (late season W. Murcott/Tango)?

• Longer harvest season, September to December

• Good HLB tolerance, very productive

• Clipping likely is *not* required

• No long-term experience with growers until now, so risk is higher
• Lemons are among the most HLB tolerant commercial citrus

• Lemon production in Florida ended because of freeze damage

• Freeze protection using microsprinklers makes production feasible

• Florida lemons are not targeting fresh market; grower inputs minimized

• Can enable greater utilization of existing processing capacity

Why lemons for Florida?
• Lemon budwood irradiated and tissue culture-derived plants were planted in in 1999

• >4000 trees, multiple cultivars evaluated for oil yields from 2002-07 and beyond

• Large scale trial underway in South America; combinations are on 5 ha

• Agronomic performance (tons /ha) and industrial yields (kg oil/MT, juice/ha, etc.)

• Demonstration plantings exist in Florida, more trials to be planted
Most fruit are completely seedless, occasionally a few are found

Equivalent to the current commercialized seedless lemons

Fruit and peel oil yields have been in the mid to high ranges, across several seasons

Oil quality analyses demonstrated the oil is commercially acceptable

Fruit shape, size, juice percentage, aroma and flavor are typical of Eureka lemon

Selected in 2017, because of seedlessness and early productivity
Sweet Orange OLL-8 on UFR-4 (4 years)
7–year–old OLL–8 trees in 2021
HLB-tolerant Grapefruit-like Hybrids
1924 (left) and 1862 (right)
Grapefruit Rootstock Trial
HLB Tolerant Rootstock
• All the above came from pre-genome based breeding

• Breeding as a lucky roll of the dice: random segregation of genes, multiple characteristics to select, requires large families grown in the field to be able to find rare individuals that combine all desired characteristics

• Genome-based breeding strategies allow us to “load the dice”: select best parents, early selection of plants with high likelihood of combining all desired characteristics

• Genome sequence information has implications for commercialization of new HLB tolerant citrus cultivars
The International Citrus Genome Consortium

- Compact genome ~ 300 Mb
- Mandarins derived from 2 ancestral species
- Pummelo admixture found in all mandarins
- Mangshanyegan, thought to be a mandarin, is a distinct citrus species

# Citrus Octet & 2 Reference Genomes

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Abbr.</th>
<th>Scientific Name</th>
<th>Seq. Platform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siamese Sweet pummelo</td>
<td>LAP</td>
<td><em>C. maxima</em></td>
<td>Illum.</td>
</tr>
<tr>
<td>Chandler pummelo</td>
<td>CHP</td>
<td><em>C. maxima</em></td>
<td>Illum.</td>
</tr>
<tr>
<td>Ponkan mandarin</td>
<td>PKM</td>
<td><em>C. reticulata</em></td>
<td>454/4x</td>
</tr>
<tr>
<td>Willowleaf mandarin</td>
<td>WLM</td>
<td><em>C. x deliciosa</em></td>
<td>Illum.</td>
</tr>
<tr>
<td><strong>Sweet orange</strong></td>
<td>SWO</td>
<td><em>C. x sinensis</em></td>
<td>454</td>
</tr>
<tr>
<td>Seville sour orange</td>
<td>SSO</td>
<td><em>C. x aurantium</em></td>
<td>Illum.</td>
</tr>
<tr>
<td>Clementine mandarin</td>
<td>CLM</td>
<td><em>C. x clementina</em></td>
<td>Illum.</td>
</tr>
<tr>
<td><strong>Haploid Clementine</strong></td>
<td>HCR</td>
<td><em>C. x clementina</em></td>
<td>Sanger</td>
</tr>
<tr>
<td>W. Murcott mandarin</td>
<td>WMM</td>
<td>Undefined</td>
<td>Illum.</td>
</tr>
</tbody>
</table>
Two Scales in Citrus Sequence Diversity

SWO nucleotide diversity

inter-specific ~ 2%
within-species ~ 0.6%
The two pummelos are pure *C. max*

PKM and WLM are NOT pure *C. ret*

Genome reconstruction of the ancestral species is complicated by the absence of a ‘pure mandarin’ genome.
Admixture Proportion in 9 Citrus Genomes

1) no pure C. ret among 8 genomes
   PKM has max/max segments
2) SWO is mostly max/ret, but also has ret/ret and max/max
3) SSO is an F1 cross = max x ret
Windows into the future
breeding new hybrids with disease tolerance
and nutritional values
• Deep understanding of origins and evolution of *Citrus*, and the nature of contemporary cultivars of commercial significance

• Provide multiple tools for knowledge advancement
  
  • Applications to breeding
  
  • Dissection of fundamental host-pathogen interactions leading to disease or tolerance
  
  • Clear identification of genetic targets for breeding, gene editing

Value of Citrus Genomic Studies
Case Study: An HLB-tolerant Valencia Mutant
(Wu et al. in review)
First reference-level phased sweet orange genome: > 99.999% base accuracy; 99.2% gene annotation BUSCO completeness.
• Based on a new chromosome level phased diploid Valencia assembly
  • Base accuracy= 99.99%
  • BUSCO score= 99.2% (highest BUSCO for any citrus genome assembly thus far)

• Enabled determination of allele-level gene expression profiles of sweet orange and other pummelo-mandarin introgressions

• Enabled structural variant identification associated with mutagenesis, and linked these to changes in allele-specific gene expression associated with host response to CLas

• Multiple HSP genes at a translocated segment terminal were upregulated, perhaps maintaining homeostasis of phloem proteins and thus minimizing CLas-induced stress and increasing tolerance
Miracle Hamlin Trees?
Scion, rootstock, or combination?
UF 1859
Sweet orange-like hybrid
• November-January maturity

• Medium-large fruit, resembling orange in size and shape

• Fruit tend to have a unique blocky shape, because of flattening at stem and blossom ends

• Deep orange-red external color, and deep orange flesh, providing strong fresh market appeal

• Excellent flavor

• Brix = 14; acid = 0.8; ratio = 17.5 (January 2021)
• Orange juice standards require use of Citrus sinensis (sweet orange)

• New HLB tolerant trees will likely NOT only be Citrus sinensis

• Codex Standard 2005 247:
  • C. sinensis and up to 10% C. reticulata

• EU fruit juice Directive 2021:
  • C. sinensis only

• FDA US 21 CFR 146.135:
  • C. sinensis, Ambersweet, up to 10% C. reticulata in other forms such as POJ building block for other juice forms

  Genome sequence analysis has revealed that C. sinensis IS NOT a true species!

The Sweet Orange : Citrus sinensis Dilemma
ADMIJXTURE PROPORTIONS
• A first step to associate specific regions of citrus genomes that contribute to a desired characteristic

• A project supported by The Coca-Cola Company, with genetic contributions from UF and USDA citrus breeders

• Over 200 accessions representing a wide range of genetic diversity have been planted in a trial to collect information on HLB responses and fruit/juice quality

• Genome sequence data have been produced for all accessions

• Multiyear plans in place to collect performance data
• Several hundred hybrid families produced over the past 30 years

• Great genetic diversity

• Nearly 9000 trees have been assessed

• ~4.3% superior in response to CLas

• Next steps: genotyping and association
“Manhattan Plot” showing SNPs responsible for HLB on citrus nine chromosomes
Genomics assisted-breeding
LB8-9 Sugar Belle®

• Amazingly durable vs. HLB
• High Brix & yield during early season
• Included in MAC, topworking, and nursery trials
• Challenges need to be worked out for juice processing

Sugar Belle is already a shining star in the topworking trial!
C4-10-42

• HLB tolerance not yet known
• High Brix & yield during Early season
• Juice has rich color & flavor
• Included in topworking and nursery trials
• Juice processing characteristics TBD

This sample was 18 Brix in January 2022!
T19RBA-22-29
Valencia

- Valencia mutant from irradiation experiment
- Selected as HLB tolerant, >22 years in the field, replicated
- All standard Valencias and other oranges long in decline
- Research underway to determine tolerance mechanism
- Typical Valencia juice quality
- Included in MAC, topworking, and nursery trials
- Among the best in Polk County MAC trial
UF 1859 Hybrid

- Original tree has good HLB tolerance
- High Brix, tropical flavor notes
- Attractive fruit for fresh market
- Juice processing characteristics TBD
- Included in MAC, topworking, and nursery trials

18 year old tree in 2021
Conclusions

• Several tolerant options currently exist for the near term, originating from the pre-genome-based, pre-HLB era

• Genome sequencing technologies have provided great opportunities for further genetic advance with efficiency
  • Targeted breeding based on prediction
  • Understanding the nature of tolerance
  • Support the rationale for commercialization

The Future is Bright!