Progress report on TREC's effort to develop GM PRSV resistant papaya

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5-15-14

Acknowledgments

- Michael J. Davis, Plant Pathologist (retired) Primary investigator for this project
- Thomas L. White, Sr. Plant Biologist (retired) Primary technician for this project
- Zhentu Ying, Biotechnologist (retired) Tissue culture and gene jockey
- Tropical Fruit Advisory Council
  - Initial grant to begin the project
- USDA-ARS subsequent grantees

Background for the project

- PRSV is the limiting disease constraint to papaya production world-wide
  - Intolerance
  - Limited tolerance
- No natural genetic resistance found in *Carica papaya*
- GM PRSV papaya were developed in Hawaii during the 1990s and have been grown commercially since 1998
- Local commercial producers asked UF/IFAS/TREC to develop PRSV resistant papaya for south Florida growers

PRSV resistance

- How is it done
  - Pathogen derived resistance (PDR)
  - PDR resistance results when genes of a disease organism inserted into a host plant (papaya) confers resistance or tolerance to the pathogen (PRSV)
- We selected a strain of the PRSV (HK1) and took its protein coat (CP)
- Inserted the CP into the DNA of the papaya
- PRSV can no longer replicate in the cells of our transformed papaya plants
- So there is very, very low concentrations of CP in GM papaya, much less than in a PRSV infected papaya plant

General selection criteria and goals

- PRSV resistance
- Vigor and growth stature
- Fruit and fruit column uniformity
- Fruit quantity and quality
- General resistance to other diseases and pests
- Resistance to environmental stresses
- Market preferences

PRSV symptoms

Comparisons at TREC

GM vs non-GMO

‘Tainung No. 5’, PRSV tolerant

‘Waimanalo’, PRSV intolerant

Comparisons

Non-GM in Homestead

GM at TREC

Long-time-line

- 1999: Diversity of PRSV in south Florida determined and PRSV Florida isolate H1K selected for transformation
- 2000, R_d: F65 papaya callus transformed with PRSV coat protein constructs; grow out plants in greenhouse

- 2000, R_0: Sexually mature plants frown from transformed callus: Cross female transgenic plants with pollen from elite lines: Tainung No. 5 (T5), Solo 40 (S40), Solo Sunrise (SR); and Puerto Rico 6-65 (PR) to produce hybrids

- 2001, R_1 to 2007, R_7: Field plantings: evaluation, selection, crossing and backcrossing
- 2007: IR-4, UF/IFAS, and EPA meeting on deregulation

Long-time-line

- 2008 to 2011
- Dr. Davis moves to CREC
- Papaya breeding project suspended

- 2011: USDA-IR4 Bio-pesticide grant awarded to complete EPA request for independent GM tranformation verification method
- 2012: Two new GM papaya plantings established at TREC
  - Accession block to re-start program and evaluation
  - T5 planting for yield and cultural trials
  - Seed germination and tissue culture propagation trials

- 2013: GM transformation verification method development completed and submitted to USDA-IR4
- 2013-2014: Regeneration of accessions, evaluations continued, propagation trials continued

Deregulation time-line

- 2008 - deregulated
- Food and Drug Administration

- 2009 - deregulated
- USDA-APHIS

- 2014 new petition submitted (IR-4 assistance)
- Environmental Protection Agency

TREC GM lines

GM line	Parent cross	Hybrid	Purpose
X17-2	Solo Sunrise	X17-2 x SR	Dessert fruit
X17-2	Solo 40	X17-2 x S40	Green market
X17-2	Puerto Rico 6-65	X17-2 x PR	Dessert and green market
X17-2	Tainung No. 5	X17-2 x T5	Dessert, green market, ornamental

X17-2 x T5 (Accession 2562)

Accession collection

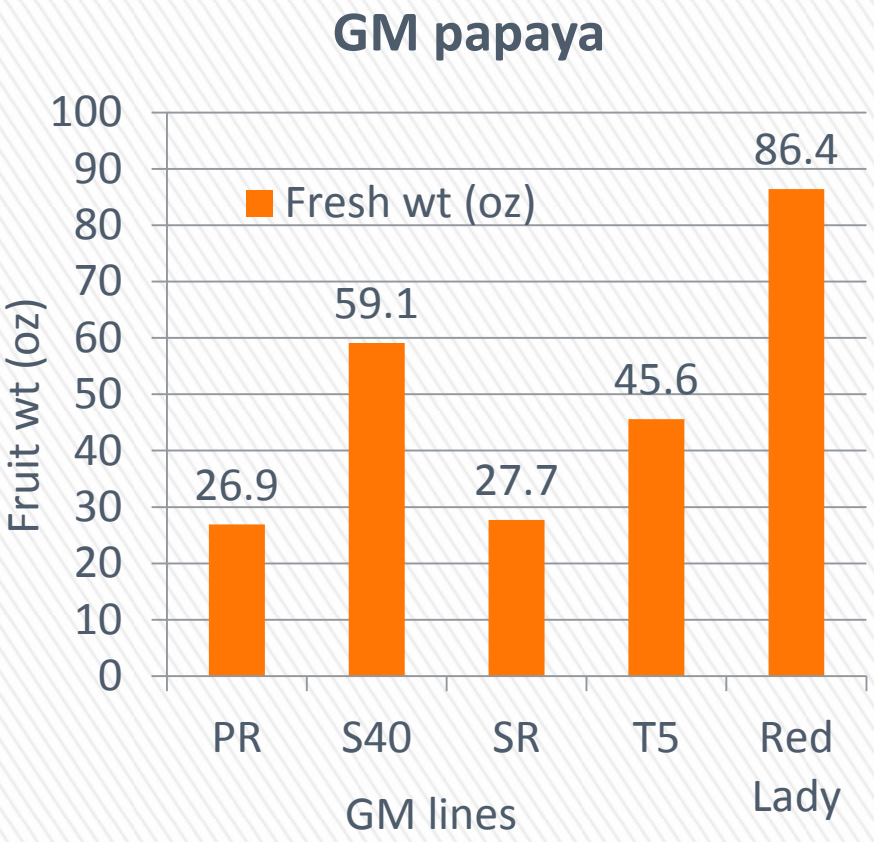
X17-2 x PR (2043)

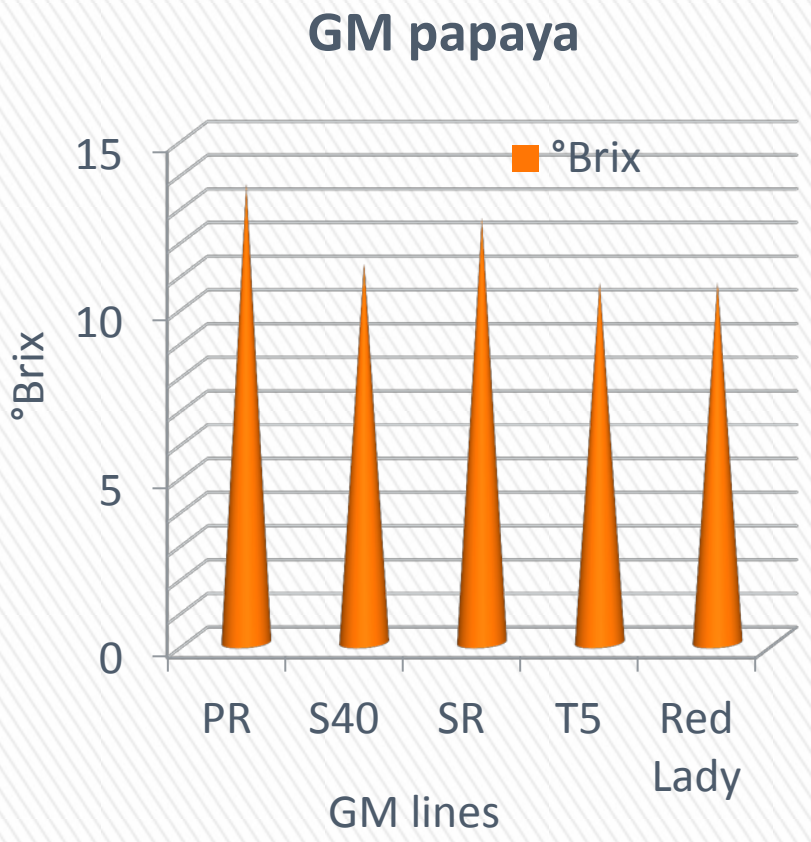
Accession collection

X17-2 x SR (1943)

X17-2 x SR (1971)

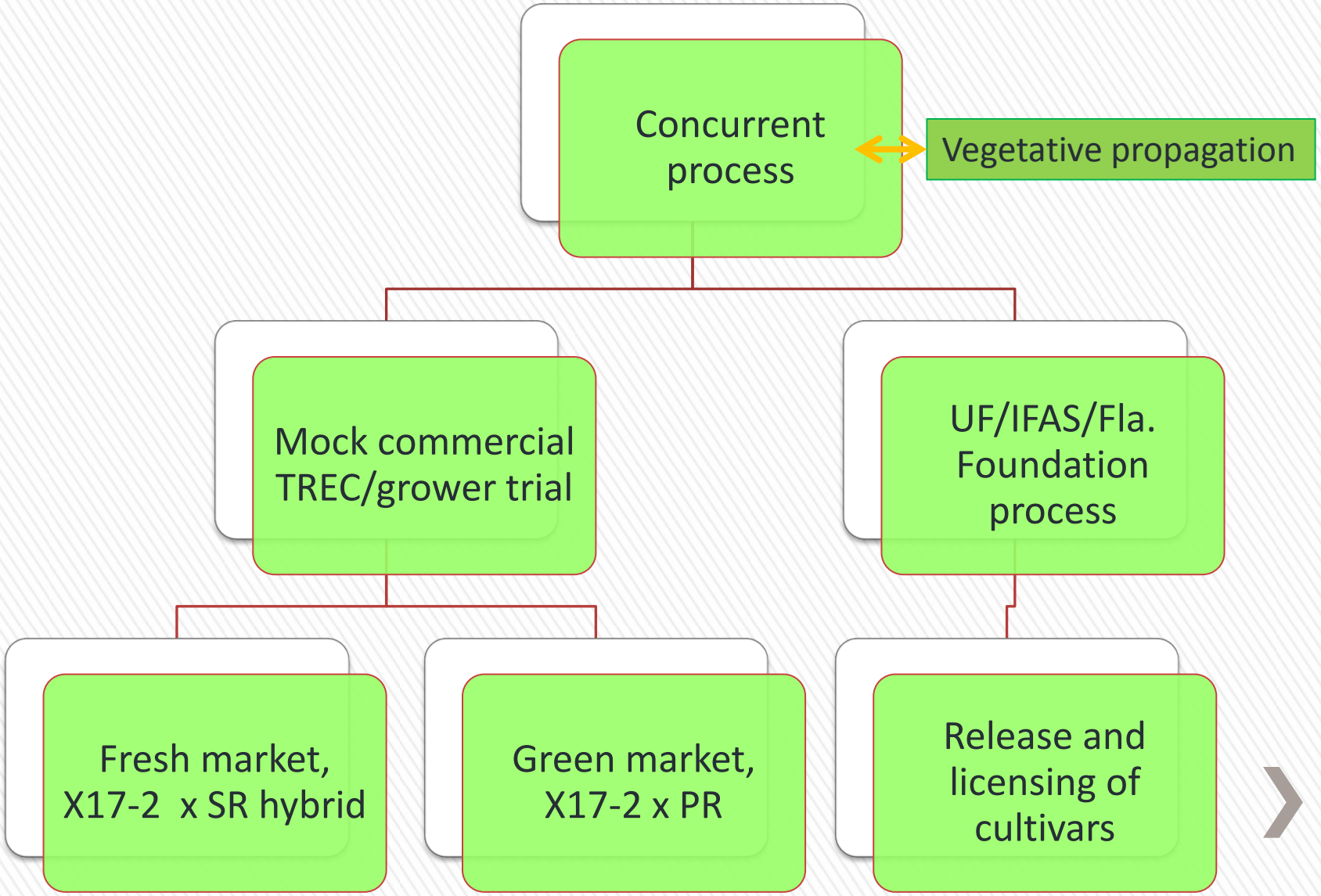
GM Florida X17-2 papaya





Mean fresh wt and quality data 2013

Next steps 2014-?



GM and non-GM comparisons

Nutrient and toxicity comparisons

Seventeen nutrients were compared among four GM X17-2 Florida lines, two GM 'Sunset' lines, and nutrient standards from USDA, New Zealand's Institute for Crop and Food Research (NZ)

- No difference in
  - Protein content
  - Fat content
  - Fiber content
  - Ash content
- GM X17-2 Florida lines were higher in Vitamin A and sodium than the non-GM from NZ
- GM X17-2 Florida lines were higher in beta carotene than non-GM USDA reference
- Potential allergens were investigated
  - No allergens were detected
- No differences among the GM and non-GM hybrid
- Benzyl isothiocyanate (BITC) concentration in papaya
  - Range similar and within what is reported for non-GM and GM papaya from Hawaii and Taiwan

Nutrient and toxicity comparisons

Thirty-six nutrients were compared

among 'Rainbow' GM and non-GM hybrid 'Sunrise' x 'Kaphoo'

- No difference in
  - Protein content
  - Fat content
  - Fiber content
  - Ash content
- GM 'Rainbow' was higher in Vitamin A than the non-GM hybrid
- GM 'Rainbow' was lower in calcium content than the non-GM hybrid
- Potential allergen concentrations were compared
  - Papain
  - Benzyl isothiocyanate (BITC)
- No differences among the GM and non-GM hybrid
- BITC concentration in papaya
  - 1000 times lower than *Brassica* spp.
  - Conc. not altered by GM
  - BITC within range of other papaya

Tripathi et al., 2011. J. of Food Composition and Analysis 24:140-147

Nutrient and toxicity comparisons

Allergen and toxicity results on

GM 'Rainbow' and GM 'SunUp' papaya

- The coat protein (CP) genetic sequence was used in a similarity search of 3 allergic protein databases (>4,201 allergen sequences)
  - No known allergen was found to be similar to the CP used to transform the Hawaiian papaya plants
  - No allergens detected

Cultivar	Level of virus coat protein (ppm)
Rainbow (GM)	6.3
SunUp (GM)	nondetectable
Sunset (non-GM)	nondetectable
Kamiya (non-GM)*	48.5
* Infected with PRSV	

Fermin et al. 2011. J. Agric. Food Chem. 59:10006-10012

Nutrient and toxicity comparisons

Allergen and toxicity results on
GM 'Rainbow' and 'SunUp' papaya

Amount of PRSV coat protein consumption per year (mg/year)			
Fruit consumption rate	'Rainbow' GM	'SunUp' GM	Virus infected fruit
1 per day	1306	52	10,055
1 per week	186	7	1433
1 per month	43	2	331

(1) Each fruit weights 19 oz.; (2) 1 teaspoon of salt weights about 6,000 mg

Take home message: If you want to avoid eating virus protein, eat GM papaya

Thanks and Questions?