USDA ARS National Clonal Germplasm Repository for Citrus and Dates, Riverside, CA

Brief Report for W6 2014

June 2014

Mission

- Access, evaluate, maintain, preserve, and distribute germplasm of *Citrus*, *Citrus* relatives, and date palms (*Phoenix dactylifera*) and *Phoenix spp*.
- Maintain informational files on accessions
- Conduct research that is compatible with primary mission

Collections at Riverside

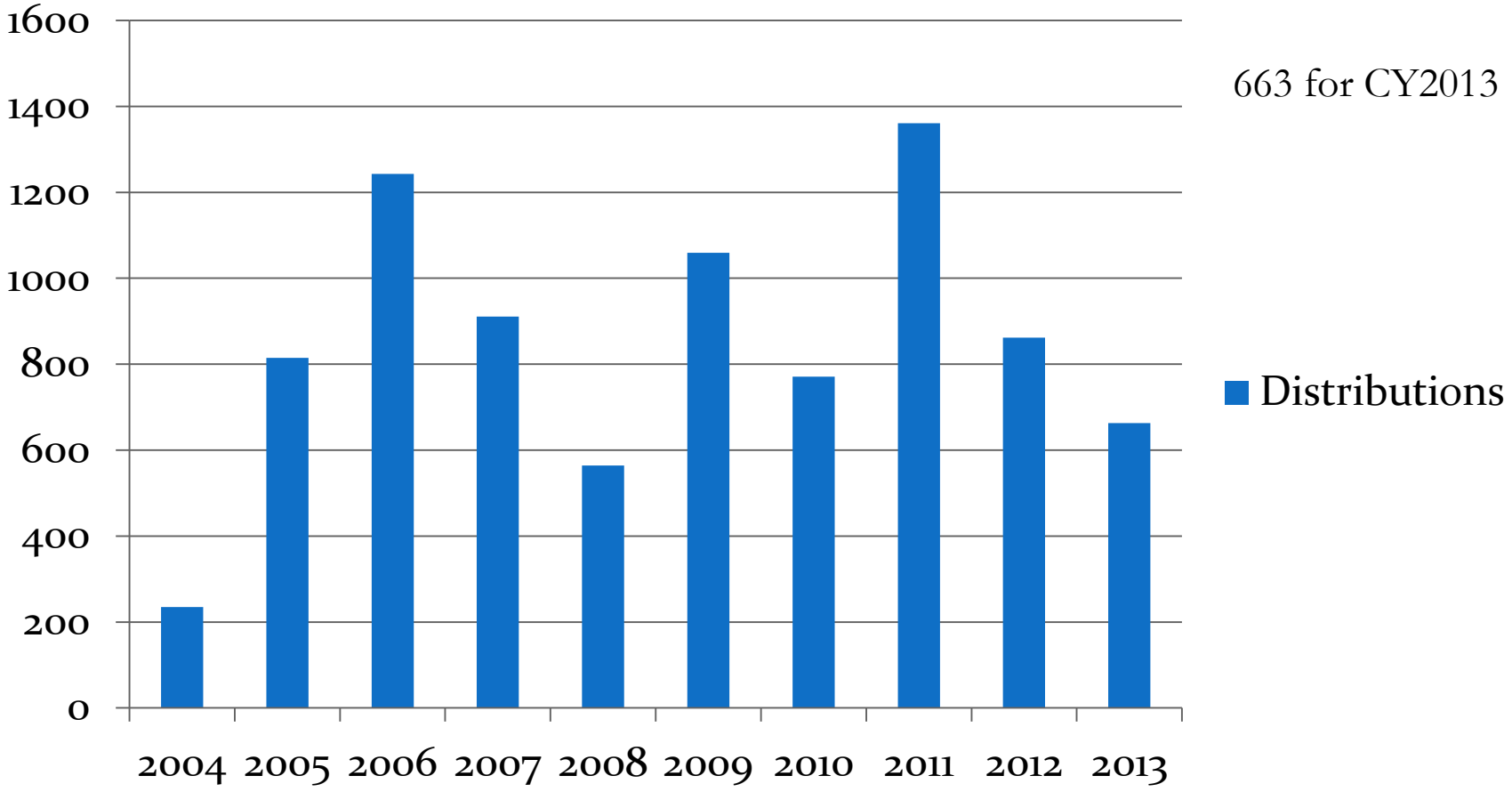
- Citrus Variety Collection in collaboration with Univ. California Riverside – over 1200 accessions in the field

- Citrus Relatives Collection -- 85 accessions of genera other than *Citrus*; 28 of 33 genera of Aurantoideae family; over 1000 inventory items held at Riverside, Coachella (CVARS), and Irvine (SCFS)

- Protected Collection (for distribution of budwood) – over 450 accessions under protected conditions, available for distribution of budwood

- Date Palm Collection, at Coachella (CVARS) – 133 total accessions with 117 *Phoenix dactylifera* accessions, 585 plants

Distributions



663 for CY2013

Repository function:

- 9 new *Citrus* accessions in 2013
- 8 accessions released from quarantine status and placed in the protected collection in May 2014
- 57 additional accessions should be ready for release from quarantine by Oct 2014
- 58 accessions into cryopreservation in cooperation with USDA ARS Plant Germplasm Preservation Unit and California Citrus Research Board; support for 3 years to do the entire protected collection

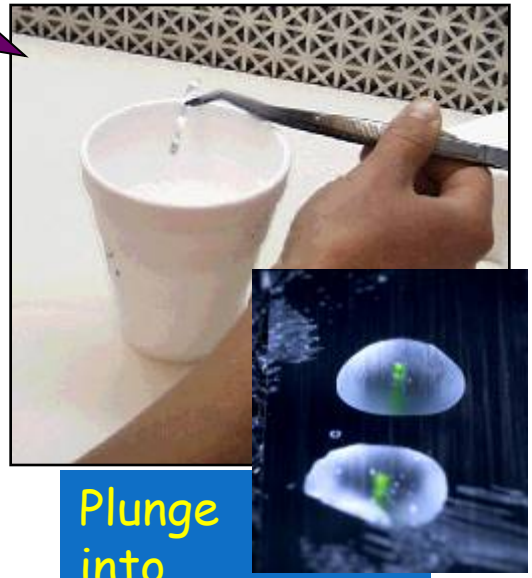
Research Progress:Cryotherapy

- Cryotherapy protocol for elimination of graft transmissible pathogens from citrus has been developed by modification of cryopreservation protocol in collaboration with USDA ARS Plant Germplasm Preservation Unit, Ft. Collins, CO
- Expertise may be developed within days rather than the months required for traditional shoot tip grafting

Diagram of cryotherapy protocol:

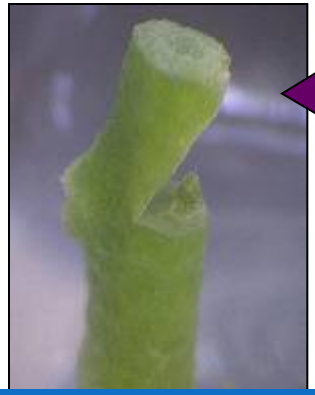
Excise shoot tips

0.3 M sucrose overnight
2M glycerol/0.6 M sucrose
PVS2 30 min 0°C



Plunge
into
Liquid nitrogen
(-196C)

Warm in 1.2 M sucrose



Micrograft onto
Seedling rootstock

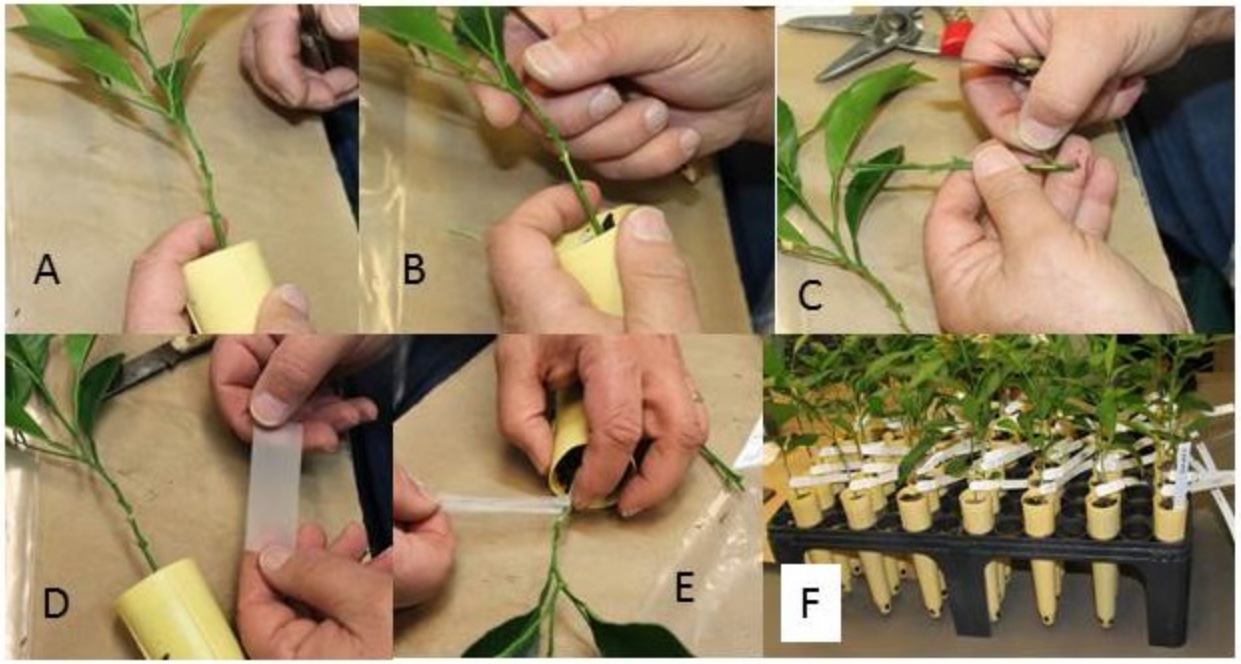
Elimination of graft transmissible pathogens from citrus by use of cryotherapy

Pathogen	Number treated	No. testing negative	Percent Testing clean
CTLV	32	32	100
Viroids	14	11	78
HLB	98	95	97
CLBV	6	6	100
CPsV	25	25	100
Stubborn	10	10	100
Concave gum	25	Not tested	
Vein enation	10	Not tested	

Research Progress: Small Plant Bioindexing Protocol

- Biological indexing is required to release quarantine accessions
- Traditional method takes 10-14 months to grow the indicator plant and perform the test, requires lots of greenhouse space, and cool temperature pathogens can only be indexed for during the winter months
- Protocol developed for using indicator plants 75-90 days post sowing

Research Progress:
Small Plant Bioindex Protocol



A: 90 day old seedling; B: chip is cut from stem of seedling indicator; C: chip of similar size cut from plant requiring indexing; D. chip placed on seedling indicator, wrapped with parafilm-like grafting tape; E: wrapping the chip; F: inoculated indicator plants in a rack and tagged for identification. Symptoms are read as in traditional biological index.

Publications:

- Razi, M.F., M.L. Keremane, C. Ramadugu, M. Roose, I.A. Khan, and R.F. Lee. 2014. Detection of citrus huanglongbing-associated 'Candidatus Liberibacter asiaticus' in citrus and Diaphorina citri in Pakistan, season variability, and implications for disease management. Phytopathology 104:257-268.

- Wang, J., O. Bozan, S.-J. Kwon, T. Dang, T. Rucker, R.K. Yokomi, R.F. Lee, S.Y. Folimonova, R.R. Krueger, J. Bash, G. Greer, J. Diaz, R. Serna, and G. Vidalakis. 2013. Past and future of a centry old Citrus tristeza virus collection: A California citrus germplasm tale. Frontiers in Microbiology 2013; 4: 366. doi: 10.3389/fmicb.2013.00366

- Lee, R.F. and M.L. Kermane. 2013. Mild strain cross protection of tristeza: A review of research to protect against decline on sour orange in Florida. Frontiers in Microbiology 2013 4:259. doi: 10.3389/fmicb.2013.00259

- Ramadugu, C., B.E. Pfeil, M.L. Keremane, R.F. Lee, I.J. Maureira-Butler, M.L. Roose. 2013. A six nuclear gene phylogeny of Citrus (Rutaceae) taking into account hybridization and lineage sorting. PLoS ONE 8(7):e68410. doi:10.1371/journal.pone.0068410

Preserving Citrus Germplasm for Future Generations

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Thank you for your attention!

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