CITRUS RESEARCH BOARD

Citrograph

MAGAZINE

FALL 2022

DWARFING OF COMMERCIAL CITRUS VARIETIES USING TsnRNAs

Figure 1: Karen Westerman, field operations manager, Citrus Pest Detection Program, Central California Tristeza Eradication Agency, stands with a 26-year-old Cara Cara Navel on trifoliate rootstock (1995-2021) inoculated with TsnRNA-IIIb displaying reduced canopy volume.

Irene Lavagi-Craddock, Ashraf El-Kereamy, Subhas Hajeri, Carol Lovatt and Georgios Vidalakis

Project Summary

University of California, Riverside (UCR) researchers utilized 'Transmissible small nuclear ribonucleic acids' (TsnRNAs) to produce dwarfed citrus trees. This project aims to determine whether citrus varieties of high commercial value beyond the originally identified dwarf scion/rootstock combinations can be dwarfed for high-density planting with possible savings in labor.

The commercial use of dwarfed citrus trees can be a highly desirable production strategy in citriculture. Increasing costs of water, land, labor and the threat of huanglongbing (HLB) to California citrus growers have reinvigorated interest in the use of dwarf citrus trees. Additionally, should the California citrus industry be forced to move cultivation to Citrus Undercover Production Systems (CUPS), dwarfed trees would be well suited for this approach. The additive effect of these issues made developing commercially grown dwarfed citrus trees an interesting prospect for citrus nurseries and growers (Lavagi et al. 2018).

Previous Citrus Research Board (CRB)-supported preliminary trials at UCR included the production of Valencia orange and Parent Washington Navel orange trees grown on trifoliate rootstock that displayed a dramatic reduction of tree height (up to 20 percent) and canopy volume (up to 50 percent) following inoculation with *Citrus dwarfing viroid* (CDVd) (Lavagi et al. 2018; Vidalakis et al. 2011). CDVd does not induce any disease symptoms in specific citrus hosts, but rather acts as a regulatory genetic element, which dwarfs trees by modifying tree performance (i.e., vegetative growth and yield per canopy volume) (**Figure 1**). Accordingly, the term 'Transmissible small nuclear ribonucleic acids' (TsnRNAs) was coined to distinguish this dwarfing agent from the term 'viroid,' which typically is used to describe the causal agent of diseases (Semancik et al. 1997).

The use of TsnRNA-IIIb and TsnRNA-IIa to alter citrus canopy size was originally approved for commercial use by the California Department of Food and Agriculture (CDFA) in 2000 under two CDFA permits. However, this technology remained largely underutilized by the California citrus industry. In 2014, after a Citrus Clonal Protection Program (CCPP) walk-through at the Lindcove Research and Extension Center (LREC) in Exeter, California, industry members revisited the possibility of employing TsnRNAs (-Ia, -IIa, and IIIb) to dwarf more citrus scion and rootstock varieties of commercial interest for possible future use.

To address the industry's request, this CRB-funded project includes field trials to determine which commercially valuable rootstock and scion combinations can be dwarfed by TsnRNAs. Selection criteria for the most commercially valuable scion and rootstock varieties for this trial included data from the CCPP budwood distribution database and consultation with the industry (Lavagi et al. 2018). Eighteen rootstock and 16 scion varieties were selected (**Table 1**). To date, the initial stages of this very large-scale screening experiment are well underway; 50 out of 306 rootstock/scion combinations and rootstock seedlings without a scion

Table 1: Scion and rootstock varieties selected for the screening experiment

Scions (16)		Rootstocks (18)	
Tango	mandarins (6)	16-6 Rich	trifoliate (2)
Nules Clementine		Flying Dragon	
Gold Nugget		Carrizo	citranges (trifoliate x sweet orange hybrid) (7)
Daisy LS		Bitters (C22)	
Shiranui		C32	
Okitsu Wase Satsuma		C35	
Parent Washington Navel	sweet oranges (5)	Carpenter (C54)	
Cara Cara navel		Furr (C57)	
Fukumoto navel		Benton	
Moro blood		X639	trifoliate hybrids (other) (4)
Bream Tarocco		US 802	
Allen Eureka	lemons (2)	US 897	
Limoneira 8A Lisbon		US 942	
Persian	lime	Schaub rough lemon	lemon (2)
Rio Red	grapefruit (2)	*C. volkameriana*	
Star Ruby		Sour orange	other (3)
		Alemow (*C. macrophylla*)	
		Sunki	

Total number of treatments was 306. All possible rootstock scion combinations are represented by 16 scions grafted onto 18 rootstocks, for a total of 288 combinations and 18 additional rootstock seedling controls.

Data collection from the 1998 established dwarfed trees, timed harvest and timed inspections.

(included as controls) were inoculated with the mixture of TsnRNAs-Ia+IIa+IIIb in spring 2019 and were planted in the field at the LREC in spring 2021 along with the non-treated controls. From the remaining rootstock/scion combinations, 13 rootstock species are in different production stages (germination, cones, pots). As rootstock seedlings reach appropriate size, the 16 scion varieties will be propagated. These rootstock/scion combinations will be planted in the LREC field as they become available. Trees then will be monitored for three to four years to confirm whether reduced canopy volume is observable in these combinations.

Data from the original 1998 trial with Parent Washington Navel trees grown on trifoliate rootstock indicate a long-term effect of TsnRNA-IIIb on canopy volume reduction (Lavagi-Craddock et al. 2020; Vidalakis et al. 2011). In addition, we recently demonstrated that the overall canopy volume reduction in those trees is due to reduced vegetative growth of the shoots (Lavagi-Craddock et al. 2020). On the other hand, earlier trials with various rootstock scion combinations have indicated that not all citrus species are responding the same way to TsnRNAs treatments. For example, Lisbon lemon (on Citrange and Alemow rootstocks) and Oroblanco and Parent Washington Navel (on Citremon, Citrumelo and Trifeola rootstocks) treated with the TsnRNA mixture –Ia+IIa+IIIb did not reduce in size; and in some cases, the canopy size increased in volume (Vidalakis et al. 2008, Semancik *personal communication*).

Additionally, to gather information on important and commercially relevant horticultural aspects and differences that TsnRNA-IIIb-treated trees may display (e.g., flowering time and time to commercial bearing, as is common in other dwarfed fruit crops), a smaller long-term trial with Tango and Parent Washington Navel trees grown on trifoliate rootstock was included in this project. We will continue our conversations with the industry to review how horticultural traits will be monitored as plants mature over the course of the project.

The increasing interest in CUPS as an HLB management strategy continues to draw attention to the great potential for dwarfed citrus. To evaluate how dwarf trees perform in the CUPS at LREC, some TsnRNA-IIIb-treated trees will be planted indoors in collaboration with the LREC once the structure becomes operational.

In a commercial setting, inoculation with any TsnRNA dwarfing agent ideally should take place prior to shipping or in the field right after planting rather than in the nursery at the time of budding to avoid regulatory issues for production of non-TsnRNA treated trees per the CDFA's Citrus Nursery Stock Pest Cleanliness Program. In general, the timing and method of TsnRNAs inoculation are currently being investigated as a California Citrus Nursery Board (CCNB)-sponsored project.

While our previous data indicate high-density planting advantages for dwarfed navel oranges on trifoliate rootstock treated with TsnRNA-IIIb (Vidalakis et al. 2011), there are no data on the potential of dwarfed trees to generate savings on labor per tree or per acre. To address this question, we are assessing labor time for several horticultural operations such as hedging, pruning, fruit harvesting and pest inspections in collaboration with Ashraf El-kereamy, Ph.D., and Subhas Hajeri, Ph.D., on TsnRNA-treated trees planted in the Central Valley at LREC.

Dwarf trees are key to the successful development of high-density plantings. Such plantings could support horticultural practices, such as efficient pesticide applications, mechanized harvesting, and reduced water usage to meet future citrus production demands and disease challenges. While dwarfing and semi-dwarfing rootstocks have been in use for other tree crops for a very long time (e.g., Webster 2002), evidence for the existence of true citrus dwarfing rootstocks (vs. slow-growing rootstocks such as Flying Dragon) has become available only recently (Bowman et al. 2016). Such rootstocks, however, have not yet been evaluated under California conditions. Therefore, questions about any form of citrus dwarfing require further investigation.

CRB Research Project #5100-154

Glossary

[1]**RNA:** Ribonucleic acid, a nucleic acid present in all living cells. RNAs are transcribed from DNA and link the transcriptional and translation processes that convert DNA to protein. Some viruses store their genetic information in RNA, rather than DNA.

Young plantings of TsnRNA treated plants.

References

Bowman, K.D.; Faulkner, L.; Kesinger, M. 2016. New citrus rootstocks released by USDA 2001–2010: Field Performance and Nursery Characteristics. *HortScience* 51(10):1208-1214.

Lavagi-Craddock, I.; Campos, R.; Pagliaccia, D.; et al. 2020. Citrus dwarfing viroid reduces canopy volume by affecting shoot apical growth of navel orange trees grown on trifoliate orange rootstock. *Journal of Citrus Pathology* 7(1). Retrieved from *https://escholarship.org/uc/item/2497h2fp*

Lavagi, I.; Lovatt, C.; Santiago, L.S.; et al. 2018. Dwarfing of commercial citrus varieties using TsnRNAs. *Citrograph* 9(2):46-50.

Semancik J.S.; Rakowski A.G.; Bash J.A.; et al. 1997. Application of selected viroids for dwarfing and enhancement of production of 'Valencia' orange. *Journal of Horticultural Science* 72(4):563-570.

Vidalakis, G.; Bash J.A.; Semancik J.S.; 2008. Observations on field performance of Tsn-RNA dwarfed *Poncirus trifoliata* hybrids. China Agricultural Press 1:687-691. *Proceedings of the International Society of Citriculture 11th International Citrus Congress*. October 26-30, 2008. Wuhan, China.

Vidalakis, G.; Pagliaccia, D.; Bash, J.A.; et al. 2011. *Citrus dwarfing viroid*: effects on tree size and scion performance specific to *Poncirus trifoliata* rootstock for high density planting. *Annals of Applied Biology* 158(2):204-217.

Webster, T. 2002. Dwarfing rootstocks: past, present and future. *Compact Fruit Tree* 35(3):67-72.

Irene Lavagi-Craddock, Ph.D., is a project scientist at the University of California, Riverside (UCR) and the quality manager for the Citrus Clonal Protection Program (CCPP). Ashraf El-kereamy, Ph.D., is a cooperative extension specialist at UCR and director of the Lindcove Research and Extension Center (LREC). Subhas Hajeri, Ph.D., is a plant pathologist at the Citrus Pest Detection Program, Central California Tristeza Eradication Agency. Carol Lovatt, Ph.D., is an emeritus professor of plant physiology, Department of Botany and Plant Sciences, and a professor of the graduate division at UCR. Georgios Vidalakis, Ph.D., is a professor and extension specialist in plant pathology at UCR and the director of the CCPP. For additional information, contact **georgios.vidalakis@ucr.edu**