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Dwarfing of Clementine Mandarin on Carrizo Citrange Rootstock Associated with Mixtures of Citrus Viroids Performing as Transmissible small nuclear RNAs (TsnRNAs)

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ABSTRACT. Citrus trifoliolate rootstock (*Poncirus trifoliata*) interaction with the Transmissible small nuclear-RNA IIIb (Tsn-RNA IIIb, syn. *Citrus dwarfing viroid*) reduces tree canopy volume and enhances Valencia orange fruit size. Preliminary field trials however, indicated that the trifoliolate hybrid rootstock Carrizo citrange (*Citrus cinensis* x *P. trifoliata*) requires a mixture of the Tsn-RNA Ia (syn. *Citrus bent leaf viroid*), IIa (syn. *Hop stunt viroid*), and IIIb for a tree size reduction. A replicated field trial of Clementine mandarin identified Tsn-RNA Ia as the critical element of the three component mix for the tree size reduction on Carrizo citrange rootstock. However, the presence of a second component, either Tsn-RNA IIIb and/or Tsn-RNA IIa is necessary for the significantly reduced canopy volume of 33-37% (ANOVA, p<0.05).

The control of vegetative growth of tree crops for efficient and economic grove management as well as the achievement of high density plantings for the maximization of production per land surface has been a constant agricultural goal (2, 8). In citrus, the use of some graft transmissible agents, namely viroids, as a mean of reducing the vegetative growth has been under investigation for decades (6). The use of the term transmissible small nuclear ribonucleic acids (Tsn-RNAs) has been introduced to describe well characterized viroids species when disease syndromes are not expressed, but rather act as regulatory genetic elements modifying tree performance (7,8). Even though, the exact growth regulatory mechanism of the Tsn-RNAs is unknown it is evident that the response is specialized and in some cases restricted to specific scion/rootstock/Tsn-RNA combinations (3, 5, 8).

The citrus rootstock trifoliolate (*Poncirus trifoliata*) interaction with the Tsn-RNA IIIb (syn. *Citrus dwarfing viroid*) reduces tree canopy volume of Valencia orange scion (8). These findings prompted the question if the dwarfing of tree growth could be extended to trifoliolate hybrid rootstocks. On a preliminary field screening

of different trifoliolate hybrid rootstocks including among others, citrange (*Citrus sinensis* x *P. trifoliata*), citremon (*P. trifoliata* x *C. limon*), citrumelo (*P. trifoliata* x *C. paradisi*), and trifeola (Minneola tangelo x *P. trifoliata*), the most significant effect was with the trifoliolate hybrid Carrizo citrange treated with a mixture of the Tsn-RNA Ia (syn. *Citrus bent leaf viroid*), IIa (syn. *Hop stunt viroid*), and IIIb where tree size was reduced by approximately 40% (7, 9, unpublished data). Here we report results from a replicated field trial of Clementine (Algerian) mandarin on Carrizo citrange rootstock established to identify the essential components of the mixture of Tsn-RNAs Ia, IIa, and IIIb for reduce tree size on Carrizo citrange rootstock.

Eight trees for each Tsn-RNA treatment of Clementine mandarin (VI 9) scion from the Citrus Clonal Protection Program at the University of California, Riverside (UCR) tested negative for all known graft transmissible pathogens of citrus were budded on Carrizo citrange rootstock seedlings grown from seed produced from disease tested mother plants. At the time of budding, the seedling rootstocks were also grafted with blind buds from reservoir trees of Valencia orange

containing specific Tsn-RNAs. The clementine trees received the three TsnRNAs as four experimental treatments, – Ia-IIa, –Ia-IIIb, –IIa-IIIb, and –Ia-IIa-IIIb. Survival of the Tsn-RNA blind buds was considered evidence of successful treatment and following 1 yr of maintenance under standard greenhouse conditions the trees were field planted at the standard density planting for California orchards (6 x 6.7 m) in an image-mirror arrangement. The Tsn-RNA profile of each tree was evaluated via biological indexing using Etrog citron Arizona 861-S-1 prior to evaluation. Stem sections of the TsnRNA grafted Etrog citron were subsequently assayed by imprint hybridization 16 weeks post inoculation using full length TsnRNA species specific digoxigenin labeled DNA probes as described by Palacio-Bielsa et al. (4). The trees were never pruned and no tools were used during fruit harvesting. All cutting tools were disinfested between tree sampling with 1% sodium hypochlorite solution.

When tree volume of Clementine on Carrizo citrange was estimated as a cylinder, statistical analysis (ANOVA, $p < 0.05$) identified significant reductions (33-37%) in the treatments containing Tsn-RNA Ia (Table 1). Even though the Tsn-RNA Ia is the critical element for the tree volume reduction, the presence of Tsn-RNA IIa and/or IIIb is essential to effect the dwarfing reaction. On the contrary, the mix of Tsn-RNA IIa and IIIb has no significant effect on tree size (Table 1). The dwarfing effect of the Tsn-RNA mixture on the Scion/Carrizo citrange appeared beginning of the fifth year post inoculation while Carrizo seedlings were not reduced in size after 8 yr in the field (average canopy volume of three seedlings; 12.16 and 11.95 m³ for untreated control and Tsn-RNAs treatment respectively). This curious inconsistency for the expression of the dwarfing response might suggest the importance of physiological reactions resulting from the graft union with a different cultivar.

TABLE 1
VEGETATIVE GROWTH EXPRESSED AS CANOPY VOLUME OF CLEMENTINE MANDARIN SCIONS ON CARRIZO CITRANGE ROOTSTOCK ASSOCIATED WITH THE PRESENCE OF MIXTURES OF Tsn-RNA Ia, IIa, AND IIIb (syn. *Citrus dwarfing viroid*, *Citrus bent leaf viroid*, and *Hop stunt viroid*) AFTER EIGHT YEARS IN THE FIELD

Treatment	Scion Volume		
	(m ³)	%	Reduction (%)
Control	18.78 a	100	0
+Ia-IIa-IIIb	11.89 b	63	37
+Ia-IIa	12.59 b	67	33
+Ia-IIIb	12.55 b	67	33
+IIa-IIIb	17.16 a	91	9

Means tested with one way ANOVA, Tukey $p < 0.05$

The question of long term effects of the Tsn-RNAs on trees on Carrizo citrange rootstock requires continuing monitoring. However, previous studies with *P. trifoliata* and the Tsn-RNA IIIb, spanning over two decades, suggest that no deleterious effects will be manifested (7).

Carrizo citrange has been extensively used as citrus rootstock due to desirable traits such as resistance or tolerance to diseases (i.e. tristeza, phytophthora) good yields, and fruit quality in scions (1). The system of tree growth reduction by Tsn-RNA with low cost and the absence of natural transmission vectors of the dwarfing

agents offer a non-genetically engineered dwarfing response. This presents an excellent opportunity for the development and management of more flexible high density citrus groves.

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