Variation in Tree Size and Rootstock Scaling of Grapefruit Trees Inoculated with a Complex of Citrus Viroids

Rivka Hadas and M. Bar-Joseph

ABSTRACT. A marked variation in tree size and degree of scaling was observed among Marsh Seedless grapefruit trees budded on trifoliate orange rootstock and inoculated with graft-transmissible dwarfing (GTD) agent #225 from grapefruit. The GTD #225 inoculated Etrog citrons contained five citrus viroids (CVds) including the 371 base citrus exocortis viroid (CEVd), a 299-base grapefruit strain of hop stunt viroid (HSVd) and three CVds with estimated sizes of 284, 295 and 330 bases. None of the severely dwarfed grapefruit subtypes of GTD #225 lacked the CEVd.

Viroids were originally described as plant pathogens and as such, their elimination from propagative plant material was inevitable (4, 11, 17). Citrus viroids (CVds) cause damage only to a limited number of host plants and most citrus species maintain CVds as latent infections (1, 2, 3, 5, 6). Infection by graft-transmissible dwarfing (GTD) agents now considered to be the result of CVds infection (7, 10, 16), was found to cause reduction of tree size of certain varieties budded to trifoliate orange and Rangpur lime. The GTD sources were empirically selected and used in trials aimed at identifying the stionic combinations and cultural practices which will produce commercially and horticulturally acceptable densely planted (DP) dwarfed citrus groves. This new approach necessitated a critical analysis of the CVds present in the GTD budwood sources. It became clear that the balanced and safe horticultural practice calls not to eliminate nor to conserve all CVds present in these GTDs, but rather to select those CVds which induce a dwarfing effect without causing scaling, gumming and other deleterious effects often associated with CVd infections of sensitive varieties and rootstocks. In addition, the commercial application of CVds induced dwarfing necessitates the identification of budwood sources or other means that will ensure that the treated trees will respond by a horticulturally accepted dwarfing. The present paper presents some recent findings regarding the instability of CVd complexes in grapefruit trees.

MATERIALS AND METHODS

Budwood was collected from a grapefruit GTD source tree (#225-T) and from three of its derivatives which were propagated on trifoliate orange rootstocks at Neve Yaar (NYa). The three NYa trees varied considerably in the degree of their dwarfing, and were designated as very mild (#225 VM) mild (#225 M), or severe (#225 S). Budwood was also collected from 12 grapefruit trees on trifoliate orange rootstock at Kfar Hayarok (KH) for which the GTD #225-T grapefruit tree was used as the source for budwood. Tissue from the grapefruit trees was graft-inoculated to Etrog citrons. The CVds were extracted from Etrog citron leaf samples (18), separated by the sequential polyacryamide gel electrophoresis (PAGE) system (14) and visually detected after silver staining (8).

RESULTS AND DISCUSSION

The reaction of Etrog citron plants inoculated with three subtypes of the GTD source (#225-T) were recently reported (7). In general budwood collected from the less dwarfed subtypes induced a much milder reaction than the severely dwarfed subtype. Symptoms of each of the subtypes were persistent when Etrog citron were kept at 25-30 C, but some of the citrons

inoculated with one of the milder subtypes, which were kept in the greenhouse during the summer months, exhibited a variable reaction with some twigs showing epinasty, whereas other plant parts remained symptomless. Consistent reactions were observed on citron plants following four sub-inoculations with Etrog citron buds carrying these #225 subtypes. Using the sequential PAGE system, distinct patterns of CVds were detected among the different #225 subtypes (7, and Fig. 1). The CVd patterns indicated that viroid segregation was the cause for the differential plant reactions observed. Extracts of #225-S (Fig. 1, lane 2), like #225-T (lane 1), contained at least five viroids, four with estimated RNA lengths of 284

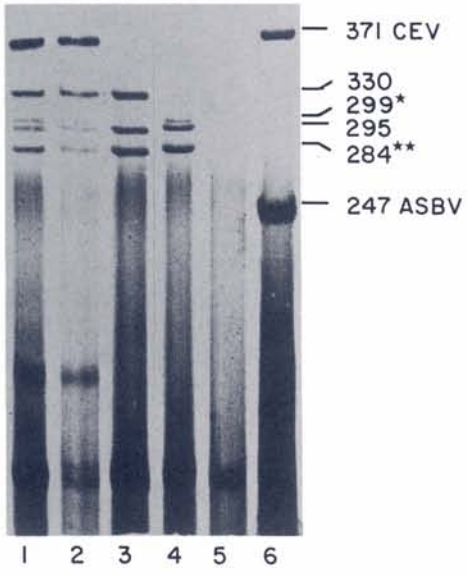

Fig. 1. Polyacrylamide (5%) gel containing 8 M urea stained with silver after processing by sequential PAGE. Nucleic acid samples (2M-LiCl soluble) were prepared from avocado leaves with avocado sunblotch viroid (ASBV) and CEVd infected *Gynura aurantiaca* plants (lane 6); healthy citron leaves (lane 5), and citron infected with GTD #225-T (lane 1) and subtypes #225-S (lane 2), #225-M (lane 3), and #225-VM (lane 4). Note that the CVd complexes presented in this figure are identical to those previously shown in Fig. 1 (7), and the molecular lengths of two viroids identified by one and two asterisks have been changed following recent sequencing data presented in (12) and (13) respectively.

(13), 295, 299 (12) and 330 nucleotides, and one which comigrated and hybridized with a control sample of pure CEVd. Subtypes #225 M (lane 3) and #225 VM (lane 4) lacked the CEVd band, but each contained a total of only three viroids with a size range of about 284, 295, 330 and 284, 295, 299 nucleotides, respectively. Table 1 summarizes the CVd composition from each of twelve grapefruit trees for which the GTD #225-T grapefuit was used as source of budwood. The trees were found to differ considerably in their size and the degree of scaling. Only the 284-base CVd was found to be present in all the tested trees. None of the seven large trees was found to carry the CEVd band, but two of the large trees contained the remaining four CVds present in the GTD #225-T complex. Severe scaling with the exception of tree #4/10, was associated with the presence of CEVd. Trees with and without scaling were found to carry the 284, 295, 299 and 330-base CVds. Trials are in progress with isolated CVds to examine the dwarfing and scaling effects of each CVd.

We have previously presented evidence for the segregation of CVds in citrus budwood source trees and indicated that CVd segregation and exclusion comprises the major cause of the tree size variation observed among the GTD treated grapefruit trees (7). The preservation of all CVds in a receptive host such as Etrog citron appears to be essential in providing a predictable and reliable reservoir for viroid-induced dwarfing.

The potential horticultural advantages of using CVds for moderating tree size in high density plantings include improved growth management, easy fruit picking (2) and tolerance to root-rot (15). However, even though the utilization of CVds is expected to be beneficial, the following safety rules must be considered:

1. Only endemic viroids which are widely spread without causing damage to known crops should be used.

TABLE 1
HORTICULTURAL CHARACTERISTICS AND VIROID CONTENT OF TWELVE GRAPEFRUIT TREES INOCULATED WITH THE GRAFT TRANSMISSIBLE DWARFING AGENT #225-T

Tree no.	Scaling[z]	Tree sizes[y]	Rs/Sc[x] trunk diam (cm)	Viroids observed by PAGE[w]				
				284	295	299	330	371
4/2	IS	L	23/16	+	+	+		
3/2	NS	L	24/17.5	+	+	+	+	+
4/3	SS	S	18/16	+	+	+	+	
4/4	NS	L	20/17	+	+		+	
4/5	VMS	L	29/19	+	+		+	
4/6	SS	S	19/14.3	+				+
4/7	IS	L	29/21	+			+	
4/8	SS	M	21/17	+	+			+
4/10	SS	L	30/23	+	+	+	+	
4/11	VMS	M	25/18	+	+			
5/4	IS	L	–	+		+	+	
3/18	NS	M	–	+		+		

[z]SS = severe scaling of the rootstock; IS = intermediate scaling; VMS = very mild scaling; NS = non scaling
[y]tree sizes: L = Large; M = medium; S = small
[x]Sc = scion; Rs = Rootstock;
[w]Budwood from each tree was grafted on Etrog citron and analysed according to Rivera-Bustamante, *et al.* (14).

2. The selected viroids should have a limited host range and not spread readily to other crops.
3. Only viroids which do not have known synergistic effects with other pathogens should be used.
4. Only genetically stable viroids, which are not prone to variation to severe forms should be selected. Alternatively, a viroid should be used for dwarfing only if a large population of similar viroids already exist in the area.
5. Viroid dwarfing should be practiced only if the potential benefit of dwarfing justifies the inoculation work.
6. Viroids should be used only if the losses incurred by inoculation would be acceptable.
7. Viroid inoculation should be carried out only if methods are available for correcting the negative effects of viroid infection (e.g. the possibility of inarching with a tolerant rootstock).

These rules were adopted following the conditions established for using mild CTV strains for cross protection (9), and should be carefully studied before adopting CVd dwarfing, as a routine practice.

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LITERATURE CITED

1. Albanese, G., R. La Rosa, M. Davino, R. W. Hammond, R. Smith, and T. O. Diener 1988. A viroid different from citrus exocortis viroid found in commercial citrus in Sicily, p. 165-172. *In* Proc. 10th Conf. IOCV. IOVC, Riverside.
2. Broadbent, P., J. B. Forsyth, K. P. Bevington, and R. J. Hutton 1986. Citrus tree size control with dwarfing agents. Citrograph 71: 8-10.

3. Broadbent, P. and S. M. Garnsey
1987. Citrus exocortis, p. 235-245. *In* The Viroids. T. O. Diener (ed.), Plenum Press, New York and London.
4. Diener, T.
1987. The viroids. Plenum Press New York and London.
5. Duran-Vila, N., J. A. Pina, J. F. Ballester, J. Juarez, C. N. Roistacher, R. Rivera-Bustamante, and J. S. Semancik
1988. The citrus exocortis disease; a complex of viroid RNAS, p. 152-164. In Proc. 10th Conf. IOCV. IOVC, Riverside.
6. Gillings, M. R., P. Broadbent, B. I. Gollnow, and C. Lakeland
1988. Viroids in Australian Citrus. Proc. Int. Soc. Citriculture 2: 881-895.
7. Hadas, R., M. Bar-Joseph, and J. S. Semancik
1989. Segregation of a viroid complex from a graft-transmissible dwarfing agent source for grapefruit trees in Israel. Ann. Appl. Biol. 115: 515-520.
8. Igloi, G. L.
1983. A silver stain for the detection of nanogram amounts of tRNA following two-dimensional electrophoresis. Anal. Biochem. 134: 184-188.
9. Lee, R. F., R. H. Brlansky, S. M. Garnsey, and Yokomi R. K.
1987. Traits of citrus tristeza virus important for mild strain cross protection of citrus: the Florida approach. Phytophylactia 19: 215-218.
10. Mendel, K.
1968. Interrelations between tree performance and some virus diseases, p. 310-313. *In* Proc. 4th Conf. IOCV. Univ. Florida press, Gainesville.
11. Navarro, L., C. N. Roistacher, and T. Murashige
1975. Improvement of shoot tip grafting in vitro for virus free citrus. J. Amer. Soc. Hort. Sci. 100: 471-479.
12. Puchta, H., K. Ramm, R. Hadas, M. Bar-Joseph, R. Luckinger, K. Freimuller, and H. Sanger
1989. Nucleotide sequence of a hop stunt viroid (HSVd) isolate from grapefruit in Israel. Nucl. Acid. Res. 17: 1247.
13. Puchta, H. and H. L. Sanger
1990. In vivo RNA recombination between viroids, p. 92 *in*: VIIIth Int. Cong. of Virology, Berlin. (abstr.).
14. Rivera-Bustamante, R. F., R. Gin, and J. S. Semancik
1986. Enhanced resolution of circular and linear molecular forms of viroid and viroid like RNA by electrophoresis in a discontinous pH system. Anal. Biochem. 156: 91-95.
15. Rossetti, V., C. Bove, F. Monier, and Bove, J. M.
1962. Modification biochimiques dans l'ecorce de divers plants d'agrumes atteinte d'exocortis. Fruits 17: 533.
16. Schwinghamer, M. W. and P. Broadbent
1987. Association of viroids with graft-transmissible dwarfing symptoms in Australian orange trees. Phytopathology 77: 205-209.
17. Semancik, J. S.
1979. Small pathogenic RNA in plants - the viroids. Annu. Rev. Phytopath. 17: 461-484.
18. Semancik, J. S., T. J. Morris, L. O. Weathers, B. F. Rodorf, and D. J. Kearns.
1975. Physical properties of a minimal infectious RNA (Viroid). Virology 63: 160-167.