Viroids Associated with Citrus Gummy Bark Disease of Sweet Orange in Turkey

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ABSTRACT. Citrus gummy bark (CGB) is a widespread disorder of sweet orange in the eastern Mediterranean. The etiology of this disease is unknown, but viroids have been suggested as the causal agent. Etrog citron plants were inoculated with buds collected from sweet orange trees asymptomatic or with CGB symptoms and nucleic acid preparations from the inoculated citron leaves were analyzed by sequential polyacrylamide gel electrophoresis. No band associated with a previously unknown viroid was detected in any of the source trees. Gummy bark-affected trees had a viroid profile consisting of citrus exocortis viroid (CEVd), CVd-I, CVd-II group, CVd-III group, and CVd-IV. The asymptomatic trees were only infected with CEVd, except one that also contained CVd-I and CVd-III. The presence of a CVd-II group component in all gummy bark samples and the similarities of CGB symptoms with those of cachexia disease caused by CVd-IIb and CVd-IIc, suggests that a variant of CVd-II may be either the causal agent or a factor involved in CGB.

Citrus gummy bark (CGB) was first reported as a phloem discoloration of sweet orange by Nour-Eldin (6). Besides Egypt, this disease has been reported in many North African and Near East countries including Saudi Arabia, Sudan, Lybia, Iran, Turkey, and Greece (8).

An extensive survey made in the Çukurova region of Turkey indicated a widespread occurrence of CGB on the sweet orange varieties Washington navel, Valencia and Dörtyol, a local cultivar (2). Discoloration and gumming above the budunion are characteristic symptoms on sweet orange scions with variable reddish-brown gum staining under the bark. The similarities of these symptoms with cachexia disease suggested a viroid etiology for CGB (10).

In this study, several gummy bark-infected sweet orange sources were compared with asymptomatic trees from the same location by sequential polyacrylamide gel electrophoresis (sPAGE) analysis after viroid bioamplification in citron.

MATERIAL AND METHODS

Gummy bark disease sources. CGB samples were collected from nine trees (#1 to 9) displaying variable gum staining under the bark. Four asymptomatic trees (#10 to 13) from the same plantings were also selected (Table 1). This source material collected from the Çukurova region in Turkey represented the principle plantings of Washington navel, Valencia, and Dörtyol sweet orange. All source plants were grafted on sour orange rootstock and were 15 to 20 years old. Graft-inoculation on Etrog citron 861-S1 was used to amplify viroid titer from the source trees.

Nucleic acid extraction and viroid purification. After 4 to 12 weeks, young leaves and stems were used for nucleic acid extraction according to Semancik et al. (11). Viroid RNA's were separated by sPAGE (7), detected by silver staining (5), and identified by comparing their electrophoretic mobility with known standards (10).

RESULTS

Field symptoms. Previous surveys of CGB in the Çukurova area indicated that 12 to 48% of the sweet orang e trees were affected (2). We observed CGB symptoms only in trees older than 10 years. When the outer bark of the trunk was scraped above the budunion, reddish-brown gum-stained tissue could be seen

TABLE 1
FIELD CHARACTERISTICS AND VIROID PROFILES OF SELECTED CITRUS GUMMY BARK (CGB) AND ASYMPTOMATIC (NS) SOURCES FROM THE EASTERN MEDITERRANEAN REGION OF TURKEY

	Field characteristics of selected source trees				Viroid profiles of selected source trees[z]				
Sample	Location	Scion	CGB symptoms	Bark lesion[y,x]	CEVd	CVd-I	CVd-II	CVd-III	CVd-IV
CGB sources									
1	Adana	Washington navel	Mild stripe-like gum impregnations	ns[y]	+	+	+	+	+
2	Dörtyol	Local Dörtyol	Severe gum impregnations	2[x]	+	+	+	+	+
3	Dörtyol	Local Dörtyol	Severe gum impregnations	2	+	+	+	+	+
4	Erzin	Washington navel	Moderate gum impregnations	ns	+	+	+	+	+
5	Mersin	Washington navel	Mild stripe-like gum impregnations	ns	+	+	+	+	+
6	Mersin	Washington navel	Mild stripe-like gum impregnations	ns	+	+	+	+	+
7	Dörtyol	Local Dörtyol	Severe gum impregnations	1[x]	+	+	+	+	+
8	Alata	Washington navel	Moderate gum impregnations	1	+	+	+	+	+
9	Alata	Valencia	Moderate gum impregnations	ns	+	-	+	+	+
NS[y] sources									
10	Dörtyol	Local Dörtyol	ns[y]	ns	+	-	-	-	-
11	Mersin	Washington navel	ns	ns	+	+	-	+	-
12	Alata	Valencia	ns	ns	+	-	-	-	-
13	Alata	Washington navel	ns	ns	+	-	-	-	-

[z]Viroid RNAs separated by sPAGE, silver stained, and identified on the basis of their electrophoretic mobilities. + = band detected;
- = no viroid band

[y]ns = non-symptomatic

[x]Bark scaling rating: 1 = moderate bark lesions; 2 = strong bark lesions.

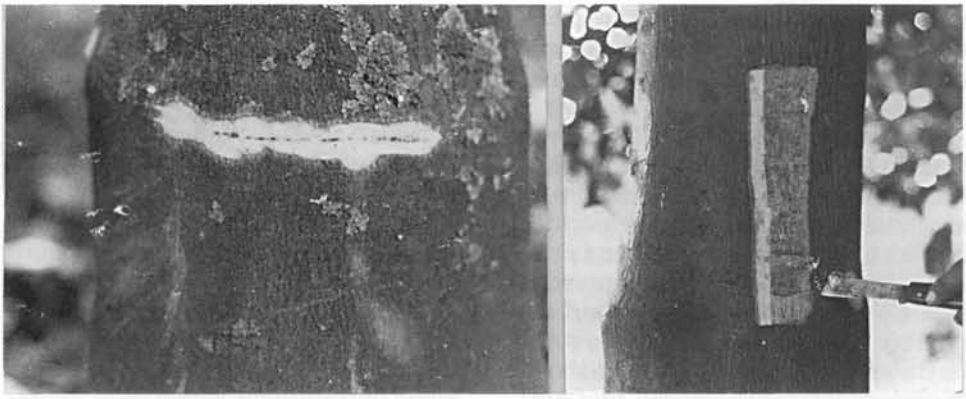

Fig. 1. Symptoms of citrus gummy bark (CGB) diseased trees. Left: mild stripe-like gum impregnations on Washington Navel/sour orange and right: severe gum impregnations on local Dörtyol/ sour orange.

under the bark in variable extents and intensities (Fig. 1). Gum-stained tissue may extend from above the budunion into the main branches. Besides conventional gummy bark symptoms, Dörtyol and Washington navel trees showed bark scaling resembling mild psorosis scaling (Table 1). This symptom was independent of any psorosis infection of the source trees as verified by biological indexing on Madam Vinous sweet orange. In two trees (#2 and 8), we observed stem pitting in the sweet orange scion above the budunion but not in the sour orange rootstock.

sPAGE analysis. The viroid profiles found by sPAGE analysis are presented in Table 1. Independently of symptoms, all samples had the viroid band corresponding to CEVd. With the exception of tree #9, gummy bark sources also had CVd-I. All trees with CGB symptoms contained viroids of the CVd-II group and CVd-III and CVd-IV, contrasting with asymptomatic trees that did not contain these viroids, except for tree #11 that was infected with CVd-III and also with CVd-I (Fig. 2).

DISCUSSION

The symptoms of CGB-affected trees observed in the field were as reported by Nour-Eldin (6). Up to now, only two citrus diseases are known to be caused by viroids: exocortis (CEVd) and cachexia (CVd-IIb), although a viroid electrophoretic profile composed of 12 different bands has been associated with citrus (10).

CGB source trees contained a viroid complex consisting of CEVd, CVd-I, CVd-II group, CVd-III group, and CVd-IV. In contrast, the asymptomatic sources displayed a remarkable lack of viroids considering the

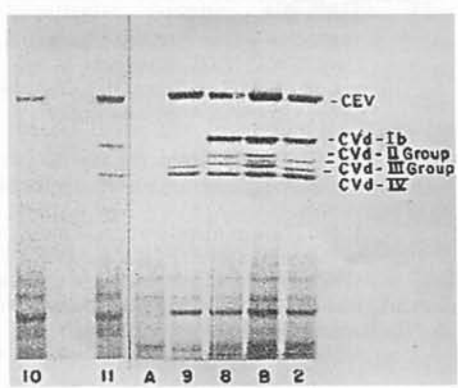

Fig. 2. Polyacrylamide (5%) gel containing 8 M urea and silver stained after processing by sPAGE. Nucleic acid extracts which were from different citrus gummy bark (CGB) diseased trees (lane 2, 8, 9), asymptomatic control trees (lane 10, 11) and two known standards (A: CVd-IIa, CVd-IIb, CVd-IIc; B: CEV, CVd-Ib, CVd-II group, CVd-III group, CVd-IV).

uniform planting. No band that might be attributed to a previously unknown viroid was determined in any of the source trees.

CEVd was the only viroid common to all symptomatic and asymptomatic sources. CEVd is widespread in citrus in the Çukurova region (2, 9). The CVd-II group was found only in CGB- affected trees. The presence of a CVd-II group component in all gummy bark samples and the similarities of CGB symptoms with those of cachexia disease, caused by CVd-IIb and CVd-IIc, suggest that a variant of CVd-II may be either the causal agent or a factor involved in gummy bark expression.

Viroids such as CEVd, CVd-IIa, and CVd-IIIb are widespread both in California and Spain, whereas CVd-Ib, CVd-IIIa, and CVd-IV are restricted to a few sources from Australia, California Israel and Spain (1, 3, 4, 10). From this point of view, it is noteworthy that all CGB sources were infected by CVd-I (except #9), CVd-III and CVd-IV; whereas CVd-I and CVd-III were found only in a single asymptomatic tree. The consistent viroid profile in the CGB source trees may indicate that one or more of these viroids are implicated in symptom expression or are even the causal agents. However, up to now, CGB symptoms have not been successfully reproduced in artificial inoculations which is a necessary step to establish its etiology. Furthermore, the effect of environmental conditions and horticultural practices on CGB expression in the field is not known. Despite that, our studies gave evidence for an implication of viroids in CGB and these field factors may greatly affect occurrence and symptom expression of citrus gummy bark disease.

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