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Differences in Response of Citron Selections to Exocortis Virus Infection

VARIOUS METHODS of detecting exocortis infection have been used (4, 5, 8, 9) since Benton *et al.* (1) proved that exocortis is transmissible by tissue grafts. Salibe (10) in 1961 reported that several varieties of citron (*Citrus medica* L.) are sensitive to exocortis virus. Rapid indexing of exocortis in two citron selections was recorded recently by the authors (2). This paper reports the different reactions obtained by inoculating various clones and seedling selections of citron with exocortis.

Experiments and Results

Four citron varieties (old clones) in the variety collection of the University of California at Los Angeles were used in the first series of tests reported here: Citron of Commerce, CRC (Citrus Research Center, Riverside, California) 649; Diamante, PI (Plant Introduction, U.S. Department of Agriculture) 102895; Italian, PI 701; and Etrog, PI 109620. Rooted cuttings of these varieties were inoculated with different known sources of exocortis and kept for observation in a glasshouse. Etrog citron was the most sensitive indicator and showed the most severe symptoms in the shortest time. Symptoms included dwarfing (Fig. 1), epinasty and curling of the leaves (Fig. 2), cracking of the lower side of the midvein, epinasty of the stem, and cracking and yellow blotching of the bark. Symptoms attributable to exocortis virus infection developed in 5-10 weeks following inoculation. All 4 of these clones carried vein enation virus.

In a second series of tests at Los Angeles and Riverside, cuttings from non-budded citron seedlings were used to obtain material with a good chance of being free of viruses. Cuttings were derived from seedlings

FIGURE 1. *Left: Etrog, PI 109620, grafted with an exocortis-virus-infected grapefruit scion. Right: Etrog, PI 109620, grafted with an exocortis-virus-free grapefruit scion.*

FIGURE 2. *Terminal shoot of Etrog, PI 109620, inoculated with exocortis-virus-infected Eureka lemon.*

of named varieties available at the U.S. Date Field Station, Indio, California (3). The accession numbers of seedlings in this text refer to plants of that collection. Although citron seedlings may resemble the parent variety in general appearance, experience has shown citron to be mostly monoembryonic. It was therefore deemed of interest to determine how the seedlings would react as exocortis indicators.

The following citron seedlings were selected for testing at Los Angeles: 60-16 and 62-118 from Citron of Commerce; 60-10 from Corsican; 60-9 from Diamante; 60-12-2 from *C. medica* var. *dulcia;* 60-7, 60-13, and 62-117 from Etrog; 62-91 from Italian; 62-94 from *C. medica* var. *odorata* Wester; 62-95 from Sicilian; and 60-6-3 from Yemen. Five lots of 3 cuttings from each seedling were used. One lot was rooted as a control, the other 4 lots were grafted with exocortis-infected scions. These scions were free of the viruses of psorosis, tristeza, and yellow vein, but some may have carried other viruses. A splice-grafting method described by Halma (7) was used. The 4 sources of inoculum were: A, an old-clone Eureka lemon [*C. limon* (L.) Burm. F.] tree; B, an old-clone Washington Navel orange [*C. sinensis* (L.) Osbeck] tree; C, an old-clone Citron of Commerce tree carrying a virulent strain of exocortis virus; D, an old-clone Diamante citron tree carrying a virus causing bark mottling without appreciable dwarfing or bark cracking of trifoliate orange [*Poncirus trifoliata* (L.) Raf.]. The graft-inoculated cuttings rooted in 3 weeks after which time they were planted in 1-gallon cans of soil and grown in a glasshouse. Shoots allowed to grow from the citron rootstocks were observed for symptoms.

Similar experiments were made concurrently in a glasshouse at Riverside with cuttings from 6 of the seedlings used at Los Angeles (60-7, 60-10, 60-13, 60-16, 62-91, and 62-95) and from 3 others: 60-8 from Diamante; 62-12-1 from *C. medica* var. *dulcia;* and 60-6-1 from Yemen. Four lots of 4 cuttings from each seedling were used. Each of the cuttings of 1 lot was cut into 2 pieces and the parts grafted together as controls; those of another lot were splice-grafted with scions of an exocortis-free Eureka lemon seedling; those of the other 2 lots were splice-grafted with scions from 2 old-clone Eureka lemon trees, sources E and F. Source E was infected only by exocortis virus and source F by the viruses of exocortis and vein enation. Shoots of the citron rootstocks were observed for symptoms.

Similar results were obtained at both locations. The range of symptoms is illustrated in Figure 3 and summarized in Table 1.

Symptoms did not develop in control plants nor in plants grafted with

FIGURE 3. *Reactions of rooted cuttings of 5 different citron seedlings inoculated with exocortis-virus-infected Eureka lemon. Left to right: 60-16 from Citron of Commerce; 62-91 from Italian; 62-117, 60-7, and 60-13 from Etrog.*

exocortis-free Eureka lemon. Vein enation virus had no obvious influence on exocortis symptoms.

Seedling 60-13 from Etrog and Etrog PI 109620 were similar in their sensitivity to exocortis virus infection and proved to be, by far, the most sensitive selections tested. Plants of these 2 selections inoculated from sources A, B, C, E, and F were severely dwarfed, with tightly-curled leaves, twisted stems, and yellowed cracked bark. The virus from source D caused slight dwarfing, with mild curling of leaves in plants of both selections. Seedling 62-117 from Etrog, from the same parent as 60-13, showed much milder symptoms than 60-13 when grafted with inoculum from sources A, B, and C, and did not react to inoculum from source D. Seedling 62-118 from Citron of Commerce developed moderate symptoms from inoculation with sources A, B, and C, but no symptoms from D. Seedling 60-16, from the same mother tree as 62-118, was a poor indicator, showing only mild epinasty from inoculum A and no symptoms from inocula B, C, or D.

TABLE 1. REACTIONS OF CITRON SEEDLING SELECTIONS TO INOCULATION WITH VARIOUS SOURCES OF EXOCORTIS VIRUS

Reaction	Parent Variety	Seedling Selection	Sources[a] Causing Reaction
Severe[b]	Etrog	60-13	6/6
Moderate	Citron of Commerce	62-118	3/4
	Etrog	62-117	3/4
	Sicilian	62-95	5/6
	Yemen	60-6-3	3/4
Mild to moderate	Corsican	60-10	5/6
	Diamante	60-8	2/2
	Diamante	60-9	3/4
	Etrog	60-7	5/6
	Italian	62-91	5/6
	C. medica var. *odorata*	62-94	3/4
	Yemen	60-6-1	2/2
Mild	*C. medica* var. *dulcia*	60-12-2	1/4
	Citron of Commerce	60-16	3/6
None	*C. medica* var. *dulcia*	60-12-1	0/2

[a]Numerator and denominator are, respectively, number of exocortis-virus-infected sources causing symptoms and total number of sources used.

[b]Except plants inoculated from source D, which caused only a mild reaction on 60-13 and caused no symptoms on any other seedling selection.

Cuttings of Etrog PI 109620 were graft-inoculated singly from 3 stunted citrus trees, 12-40 years of age, having trifoliate orange rootstocks without bark cracking. Rooted cuttings from these rootstocks had been grown for 12 years and were also dwarfed, but showed neither yellow mottling nor cracking of the bark. These sources would seem to be similar to the materials described by Fraser and Levitt (5) and Fraser *et al.* (6). Graft-inoculations from each of the 3 sources resulted in leaf curling and stem epinasty in plants of Etrog PI 109620.

Conclusions

Certain selections of citron appear to be excellent indicator plants for exocortis virus and for an unidentified but possibly related virus causing stunting of trifoliate orange. For use as indicator plants these citron selections must be propagated vegetatively in order to avoid genetic variants having indeterminate reactions to exocortis infection.

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