Elimination of Citrus Ringspot Virus by Shoot-Tip Grafting

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ABSTRACT. The shoot-tip grafting technique has been used to obtain virus-free plants of citrus cultivars, selected from all major citrus growing areas of Argentine. Some years ago a disease was described from citrus plantations of Concordia, province of Entre Rios, with properties similar to those characteristic for citrus ringspot virus (CRSV)-infections. This virus has been described in Misiones province too. The influence of different factors which determine the success of the shoot-tip grafting method in eliminating the Argentine ringspot virus were evaluated. The meristems used in this study were obtained from infected plants, grown under different temperature conditions. Plants obtained from meristem pieces with 4 leaf primordia still contained the virus. Meristem pieces with 3 or 2 leaf primordia produced some virus-free plants, the percentages of which were inversely correlated to the number of leaf primordia on the shoot-tip.

Shoot-tip grafting *in vitro* (STG) has been used in several countries (3, 5) to free citrus plants from viruses and virus-like pathogens. In this work, I will present the results of the application of this technique in an attempt to eliminate citrus ringspot virus (CRSV). This ringspot virus has been isolated from citrus plants in Concordia (Entre Rios, Argentina) (8, 10) and Misiones (Argentina). The similarity of the Argentine virus with CRSV from other countries is striking (11, 13) and although some properties of the causal agent remain unknown, I will use the name CRSV for the Argentine isolate.

MATERIALS AND METHODS

Shoot-tips were isolated from two sources: a) sweet orange originally from Concordia and grown in a warm chamber (30 C), and b) sweet orange field plants with bark scaling and leaf symptoms. The source plants and the shoot-tip grafted plants were indexed for CRSV by grafting two bark chips into Pineapple sweet orange seedlings. If they showed symptoms, mechanical transmissions were performed to *Chenopodium quinoa, Gomphrena globosa,* and *Nicotiana megalosiphon* as described for CRSV in Florida (4). To verify the efficiency of virus elimination, citrus tristeza virus (CTV) was used as a marker. ELISA (enzyme-linked immunosorbent assay) was used as a routine test for CTV detection (1) for all plants.

The shoot-tips were prepared and grafted according to Navarro (6). Shoot-tips with two, three and four leaf primordia were dissected to compare the influence of shoot-tip size on the effectiveness of virus elimination. Shoot-tip grafted plants obtained by STG were removed from the test tubes and grafted on rough lemon seedlings (3) and subsequently tested for CRSV and CTV.

RESULTS AND DISCUSSION

Positive results were obtained when the source plants for shoot-tips were indexed for CRSV on Pineapple sweet orange seedlings. The indicator plants produced shoot necrosis in the first flush after inoculation, whereas leaf flecking, chlorotic spots, blotches and ringspot symptoms appeared in the second flush. Mature leaf symptoms were only rarely observed. Mechanical transmission

TABLE 1
INFLUENCE OF GROWING CONDITIONS OF SHOOT-TIP SOURCE PLANTS AND SHOOT-TIP SIZE ON THE NUMBER OF PLANTS OBTAINED FREE OF CRSV BY SHOOT-TIP GRAFTING *IN VITRO*

Shoot-tip source plants	Shoot tip size (meristem + n. of primordia)		
	mer. + 4 prim.	mer. + 3 prim.	mer. + 2 prim.
Chamber	0/3*	1/6	1/3
Field	0/3	4/7	2/3

* The fractions are: CRSV free/n. tested.

from citrus to herbaceous hosts, produced symptoms similar to those described by Timmer *et al.* (12).

Both source plants indexed positive for CTV by ELISA. All shoot-tip grafted plants tested free from CTV by ELISA.

Table 1 shows the number of CRSV free plants. The number of plants obtained were too small for a proper statistical evaluation, but the results indicate certain tendencies in the relation of shoot-tip size and virus elimination. Most plants derived from shoot-tips with 4 leaf primordia suffered shock, either in the test tubes or after grafting to rough lemon seedlings. In Table 1 only those plants were considered which passed CTV indexing. Shoot-tips with 3 leaf primordia still are extremely big and most contain the CRSV agent. A higher percentage of CRSV-free plants was achieved from pieces which contained only 2 leaf primordia in the apical meristems but success was still not complete.

The symptoms obtained on Pineapple seedlings after transmission from shoot-tip grafted plants were not specific, because in the first flush, all plants were chlorotic in contrast to negative controls, but did not show shock symptoms. The shock reaction occurred in only two cases. However, the leaf symptoms observed on the following flushes were similar to those obtained from the original source plants. Absence of shock reaction in the first flush may have been due to: a) greenhouse conditions because indexing was performed during the summer with temperature peaks of more than 30 C and even positive control plants did not regularly show shock symptoms; b) low virus concentration; c) absence of CTV, which might have a synergistic effect on the symptom expression of CRSV. Such an effect has been reported by Broadbent (2) between CTV and Monak psorosis B, which appears closely related to CRSV (9); and d) that CRSV is composed by a complex of strains, part of which had been eliminated by STG.

The low number of plants used in this study does not permit conclusions to be drawn about shoot-tip source plants nor comparisons with the results obtained by Navarro in his attempts to eliminate psorosis-like leaf symptoms (7). The results prove it is possible to eliminate CRSV by STG, however, the elimination success might be improved by combining STG with other techniques.

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