Effect of Size and Source of Shoot Tips on Psorosis-A and Exocortis Content of Navel Orange Plants Obtained by Shoot-Tip Grafting *In Vitro*

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Navarro, Roistacher, and Murashige (1975) outlined an improved technique for grafting small (0.14 to 0.18 mm) shoot tips of citrus scions to *in-vitro*-grown seedling rootstocks. The effectiveness of this technique for recovering plants free of several citrus viruses, exocortis viroid, and stubborn *Spiroplasma* has been reported by Roistacher, Navarro, and Murashige (1976).

This paper reports the effects of: (a) the size of the shoot tip and (b) the source of the shoot tip on the psorosis virus (PV) and citrus exocortis viroid (CEV) content of Robertson navel orange plants obtained by shoot-tip grafting *in vitro*.

MATERIALS AND METHODS

Plant material. Shoots and buds used were collected from 18 budded plants of Robertson navel orange grown in a glasshouse. These plants were derived from a single source tree which indexed positive for CEV and PV and negative for the viruses of tristeza, vein enation and concave gum. All 18 plants consistently indexed positive for CEV and PV.

Shoot-tip size. Shoot tips used were composed of the apical meristem plus two, four, or six leaf primordia varying in height from 0.1 to 0.7 mm (table 1). They were isolated from young flushes on Robertson navel plants and were grafted on Troyer citrange seedlings grown *in vitro* according to the method of Navarro *et al.* (1975).

Source of shoot tips Shoot tips composed of the apical meristem plus three leaf primordia were isolated from three sources (fig.1): (a) young growing flushes of potted plants; (b) freshly excised inactive lateral buds; and (c) flushes arising from lateral buds cultured *in vitro* according to the method of Navarro *et al.* (1975). In addition, shoot tips were isolated from lateral buds cultured for one month *in vitro* in a medium containing malachite green, 30 mg/l, with the objective of studying the effect of this compound on PV and CEV. All shoot tips were grafted on Troyer citrange seedlings grown *in vitro* by the method of Navarro *et al.* (1975).

In vitro grafted plants were transplanted from the test tube to soil in 10-cm pots and grown in a glasshouse (Navarro *et al.*, 1975). When plants were 10 to 20 cm tall, buds or bark pieces were graft inoculated to sweet orange or Dweet tangor indicator plants to index for the presence of PV, and to Arizona 861 citron budded on Rough lemon to index for CEV. A minimum of two separate index tests were performed for each of the potted plants derived from *in vitro* grafts.

RESULTS AND DISCUSSION

Because of the technical difficulties involved in the *in-vitro* processes used for these experiments, the resulting numbers of plants derived were somewhat small for adequate statistical comparison. Therefore, the results presented are to be considered as trends.

TABLE 1
INFLUENCE OF SHOOT-TIP SIZE ON THE SUCCESS OF GRAFTS AND ON THE PATHOGEN CONTENT OF ROBERTSON NAVEL ORANGE PLANTS OBTAINED BY SHOOT-TIP GRAFTING *IN VITRO*

Shoot-tip size	Height of shoot tip (mm)	% Successful* grafts	No. pathogen-free plants / No. of plants tested	
			Psorosis	Exocortis
Apical meristem and 2 leaf primordia	0.1-0.15	14.6	4/4	4/4
Apical meristem and 4 leaf primordia	0.2-0.3	34.6	9/18	16/18
Apical meristem and 6 leaf primordia	0.4-0.7	47.6	11/24	20/24

*From Navarro, Roistacher, and Murashige (1975).

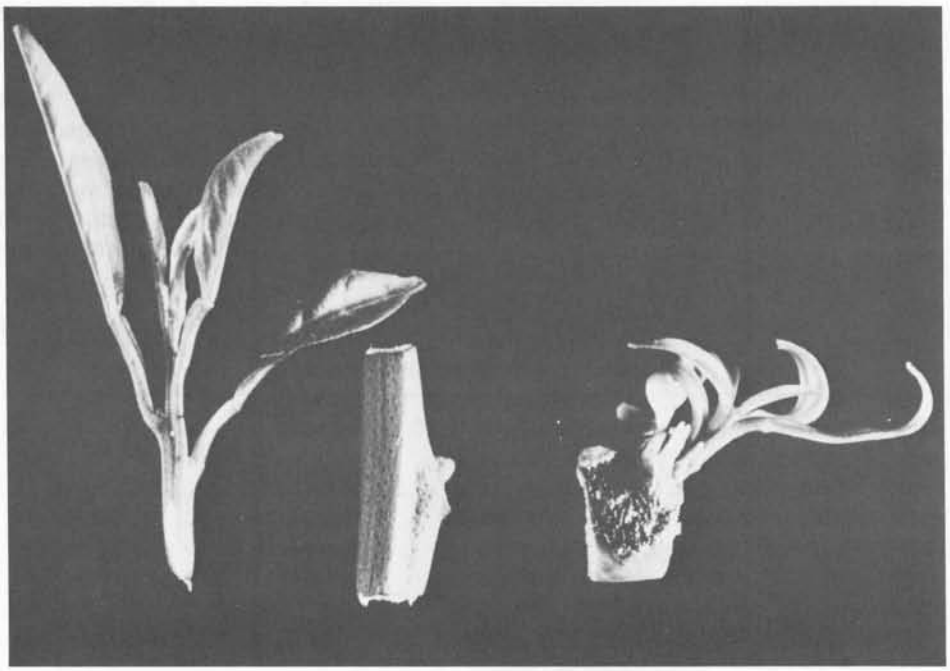

Fig. 1. Sources of shoot tips for shoot-tip grafting. Left, young flush. Center, lateral bud. Right, lateral bud cultured *in vitro* for one month.

Size of shoot tips. Table 1 shows that the number of PV- and CEV-free plants appeared inversely proportional to the size of the shoot tip used for grafting. The two pathogens present (PV and CEV) were differentially separated in relation to size of shoot tips. The number of PV-free plants decreased markedly when the size of the shoot tip was increased from 2 to 4 leaf primordia whereas the number of CEV-free plants decreased only slightly. These results indicate that the pattern of distribution of different citrus pathogens may vary within the same shoot tip. This was confirmed by the data of Roistacher *et al.* (1976) which showed that some viruses (tristeza and concave gum) were easier to eliminate than others (PV and tatter-leaf) and it was possible to separate different viruses and even isolates of the same virus by shoot-tip grafting *in vitro*.

Roistacher *et al.* (1976) reported that one of four isolates of PV and the tatter-leaf virus were not eliminated by *in vitro* grafting of a shoot tip composed of the meristem plus three leaf primordia. It might be possible to eliminate these pathogens by using smaller shoot tips even though this would be more difficult to perform. When the only pathogen present is CEV, a shoot tip composed of an apical meristem and up to six leaf primordia can be used to obtain CEV-free plants with a very good chance of success.

The size of the shoot tip has been considered a very important factor in determining the number of virus-free plants obtained by shoot-tip culture *in vitro* (Murashige and Jones, 1974). Hollings (1965) using shoot-tip culture *in vitro* reported that shoot-tip size (0.25 to (0.75 mm) was not an important factor in obtaining plants free of carnation ring-spot virus, whereas with some other viruses, like carnation mottle, shoot-tip size was very important.

Source of shoot tips. Table 2 shows that the source of shoot tips may have influenced the number of PV- and CEV-free plants obtained by *in vitro* grafting. The best source of shoot tips for obtaining PV-free plants was apparently from young flushes on plants, and the poorest was from freshly excised buds. The best source of shoot tips for obtaining CEV-free plants were the freshly excised lateral buds or shoots obtained from flushes of potted plants. These results suggest that the distribution of viruses within the shoot tips of citrus plants may vary with the stages of flush of the plant. The optimum stage of shoot growth at the time of collection and its relation to virus content remains to be investigated.

The number of CEV-free plants obtained by grafting shoot tips from lateral buds grown *in vitro* was apparently greater when malachite green was added to the culture media, but this chemical had no apparent effect on the number of PV-free plants. Shoot tips from cultured buds in media containing malachite green at 0 and 30 mg/l produced 6 out of 12 and 8 out of 12 PV-free plants and 6 out of 12 and 11 out of 12 CEV-free plants, respectively. Similar results were obtained by Oshima and Livingston (1961) when the percentage of potato virus X-free plants was increased by culturing in a medium with malachite green.

TABLE 2
INFLUENCE OF THE SOURCE OF SHOOT TIPS ON THE SUCCESS OF GRAFTS AND ON THE PATHOGEN CONTENT OF ROBERTSON NAVEL ORANGE PLANTS OBTAINED BY SHOOT-TIP GRAFTING *IN VITRO*

Source of shoot tips	Successful* grafts %	No. pathogen-free plants / No. of plants tested	
		Psorosis	Exocortis
Flushes from potted plants	30-40	17/34	24/26
Flushes from lateral buds cultured *in vitro*	20-30	21/68	44/72
Freshly excised lateral buds	15-25	1/8	8/8

*From Navarro, Roistacher, and Murashige (1975).

The actively growing flush from potted plants appears to be the best source of shoot tips from the standpoint of ease of collection, the higher percentage of successful grafts, and the number of virus-free plants obtained (table 2). Shoot tips derived from inactive axillary buds, though more difficult to isolate, may be useful for separation of viruses (table 2).

The culturing of lateral buds *in vitro* to induce multiple shoots (fig. 1) may have potentially important applications. This may provide a means whereby plants can be transported from one country to another. Budwood could be fumigated and shipped in sealed containers, and the lateral buds excised in the receiving

country and cultured *in vitro* for production of multiple flushes, whose shoot-tips could then be used for grafting. The resulting grafted plants could then be indexed for a broad spectrum of pathogens in a special quarantine facility and destroyed if pathogens were found. An alternative would be to ship grafted plants in a test tube back to the country of origin for transplanting to soil and indexing. This should pose no serious problems with quarantine regulations since all budwood, buds or explants would be under continuous cover. In this manner superior cultivars could be exchanged, pathogens eliminated and pathogen-free, true-to-name selections developed for testing and evaluation. All of this could be accomplished in a relatively short period of time (Navarro, 1976) compared to that required by nucellar embryony (Weathers and Calavan, 1959; Bitters *et al.*, 1972).

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