

Shoot-tip Grafting *in vitro* for Elimination of Viroids and *Citrus psorosis virus* in the Local Arakapas Mandarin in Cyprus

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ABSTRACT. The technique of shoot-tip grafting *in vitro* has been used since 1998 for elimination of *Citrus psorosis virus* (CPsV), Citrus cachexia viroid (CCaVd), *Citrus exocortis viroid* (CEVd) and other related viroids in the local Arakapas mandarin of Cyprus. Twelve isolates of “Arakapas” mandarin were selected on the basis of phenotypic tree characteristics and fruit characteristics. Citrus exocortis and/or related viroids were present in all source plants. Two source plants were infected by CPsV and one by CCaVd. All plants were free of *Citrus tristeza virus* (CTV). About 500 15-20-day-old seedlings of sour orange, Troyer citrange, Valencia, Swingle citrumelo and Carrizo citrange, were micrografted *in vitro* with shoot tips from field trees and from greenhouse plants. About 60 micrografted plants were regrafted on potted sour orange seedlings in the greenhouse with a success of 70%. All plants which survived are being tested for viroids and CPsV. Four of these plants appear to be free of pathogens so far; CCaVd indexing continues. The project will be continued in the next 2 yr for elimination of viroids and CPsV from all 12 selected Arakapas mandarin selections.

Mandarin was introduced in Cyprus in the 19th century (2), possibly as seed, and as a result there are variations among mandarin trees. They are cultivated mainly in the Arakapas area, and for this reason the variety bears the name “Arakapas”. This mandarin is a valuable variety for Cyprus, as its fruit has a characteristic aroma and taste. A study in the early 1990s showed that Arakapas mandarin was totally infected by *Citrus exocortis viroid* (CEVd) and related viroids (4). The technique of shoot-tip grafting *in vitro* which has been used since 1998 for the sanitation of this mandarin and the results obtained so far are described here below.

Selection of mother plants.

Twelve mature Arakapas mandarin trees, grown in commercial groves at Eftagonia, Arakapas and Katydata, were selected as mother plants on the basis of phenotypic tree and fruit characteristics, and their health status (determined as described below). All isolates were mapped and labeled in the field. Young sour orange potted seedlings were grafted with budwood taken

from the selected mother plants in the glasshouse. Five potted plants of each mandarin isolate were established in the glasshouse.

Indexing for viruses and viroids of mother plants. All mother plants were tested for viruses and viroids by biological indexing in the Citrus Virology Glasshouse (with temperatures 15-33°C) and by ELISA for *Citrus tristeza virus* (CTV). Each plant was tested by graft-inoculation on the following indicators: a) Mexican lime for CTV, b) Etrog citron, selection Arizona 861, for CEVd and related viroids, c) Eureka lemon for *Citrus variegation virus* (CVV), d) Madam Vinous or Pineapple sweet orange for *Citrus psorosis virus* (CPsV), congave gum and impietratura, and e) Parson’s special mandarin grafted on rough lemon or Volkamer lemon for Citrus cachexia viroid (CCaVd). Two indicators of each type were used for every isolate. Antisera for ELISA were obtained from Tolkowsky Laboratory, Volcani Center, Israel, and the ELISA method followed was that described by Bar-Joseph and Hadjinicolis (2). All 12 mother trees

were free of CTV and CVV. Two plants were infected by CPsV, one was infected by CCaVd, and all plants were infected by CEVd and/or related viroids.

Shoot-tip grafting (STG) *in vitro*. The standard procedure of shoot-tip grafting technique as described by Navarro et al. (4) was used. Grafting was done on 15-20 day-old seedlings of sour orange, Swingle citrumelo, Troyer citrange, Carrizo citrange and Valencia orange. Shoot tips were obtained directly from the field-grown trees or from potted plants established in the glasshouse. Average micrografting success for 448 attempts was 17%, and of the rootstocks used, sweet orange and Carrizo citrange appeared slightly better than sour orange, Troyer citrange and Swingle citrumelo (Table 1). In general scion material from the field gave higher grafting success than the glasshouse-grown material, in agreement with previous findings by Ioannou et al. (3) during the process of sanitation of the Cyprus local lemon. However, the availability of the field material was season-dependent. In winter there was no suitable grafting material available, while the best scion material was obtained between May and October.

Plant establishment *in vivo* and indexing. Successfully micrografted plants were re-grafted on healthy sour orange seedlings 6-9 mo-old, grown in the glasshouse. The mandarin scion with a small chip from the *in vitro* rootstock was

inserted into a T-incision made on the potted sour orange seedling, wrapped with PVC grafting tape and enclosed in a polyethylene transparent bag which was gradually opened 2-4 weeks after grafting. The whole process was performed in a growth room 20-25°C. Grafted plants were transferred to the glasshouse 3-6 mo after grafting.

All micrografted plants were tested 6-9 mo after their establishment *in vivo* by graft-inoculation on Etrog citron, selection Arizona 861, grafted on sour orange, for CEVd and related viroids. Two test plants were used for every micrografted mandarin. If these plants remained symptomless 6 mo after grafting, the corresponding mandarin plant was retested on Etrog citron, and in addition on Madam Vinous sweet orange or Dweet tangor for CPsV and other diseases producing psorosis-like symptoms, and Parson's Special mandarin grafted on rough lemon or Volkamer lemon for CCaVd. Two indicators of each type were used for every mandarin plant.

Of 60 micrografted plants, re-grafted on sour orange seedlings *in vivo*, 42 were successfully established and transferred to the glasshouse. Results from biological indexing on Etrog citron and sweet orange available for 35 plants so far, of which nine indexed negative for CEVd and related viroids, CPsV and other diseases inducing psorosis-like symptoms on sweet orange. Four of these plants indexed also negative for CCaVd. However, for

TABLE 1
EFFECT OF ROOTSTOCK ON MICROGRAFTING SUCCESS OF ARAKAPAS MANDARIN SHOOT-TIPS

Rootstock	No. of successful grafts/No. grafted (%)
Sour orange	35/248 (14)
Valencia sweet orange	14/70 (20)
Troyer citrange	8/60 (13)
Swingle citrumelo	7/60 (12)
Carrizo citrange	11/60 (18)
Total	75/448 (17)

the remaining five plants, results from Parson's Special mandarin, the indicator of CCaVd, are not yet available. The four virus-tested plants, which have been produced, have originated from four different source mandarin trees.

The process of micro-grafting and indexing will be continued in order to achieve sanitation of all 12 selected isolates of the local Arakapas mandarin. In the meantime efforts are being made to introduce

contemporary indexing techniques for viroids, including sequential polyacrylamide gel electrophoresis and polymerase chain reaction (PCR), which are more rapid and more effective than the conventional biological technique which has been used so far. This is expected to accelerate the process of shoot-tip grafting and indexing and reassure the freedom of the finally selected micrografted plants from all known viroids.

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