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# Results of a Citrus Shoot-Tip Grafting Program at the University of Catania (Italy)

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**ABSTRACT.** In 1980 the Institutes of Fruit Culture and of Plant Pathology, University of Catania, Italy, started a joint program to obtain virus-free trees by shoot-tip grafting (STG). Many orange, lemon, mandarin and grapefruit varieties valuable for commercial plantings and/or for germ-plasm reservoir have been included. The program follows the standard STG procedures with slight modifications. Indexing is carried out by inoculation on indicator plants, enzyme-linked immunosorbent assay (ELISA), and polyacrylamide gel electrophoresis (PAGE). Plants obtained by STG are transferred in the field for horticultural evaluation. Results concerning a first set of candidate trees are presented.

*Index words.* Shoot-tip grafting, indexing.

Micropropagation *in vitro* is a very useful tool to free citrus from virus and viruslike pathogens, and for genetic improvement of citrus trees, as well as a quick increase of healthy budwood to nurseries. Shoot-tip grafting (STG), first reported in 1972 (11), has become an important *in vitro* technique. The STG procedure was improved to obtain virus-free citrus plants quickly, and was standardized (13). In many other countries (7, 8, 9, 12), and also in Italy (15, 16), STG technique has been widely used, sometimes with some modifications in the standard procedure.

Italian citrus trees are almost 100% infected with one or more virus or viruslike diseases (5). Thus, a clean stock program was set up for the most important and/or promising citrus varieties and clones in 1980 by the Institutes of Fruit Culture and of Plant Pathology at the University of Catania.

This paper reports the results of indexing and the horticultural performance of a first set of citrus trees obtained by STG and grown in the field.

## MATERIALS AND METHODS

Over 60 cultivars of sweet orange, mandarin and mandarin like, lemon and grapefruit were originally included in the program. All commercial Italian varieties and some interesting citrus cultivars imported from abroad were included at the be-

ginning, but now new promising clones are being added.

The first set of plants included 24 cultivars. The new trees were obtained by the standard STG procedure (13) modified by changes in the agar medium, seed sterilization, growing of donor plants in a thermotherapy chamber and handling of micrografted plants (4).

Budsticks of STG plants were grafted on Troyer citrange and sour orange rootstocks and planted in the field in 1983 on a 1 x 1.50 m spacing at the Primosole experimental field of Faculty of Agriculture, Catania. The soil is sandy-loam on a level site.

Periodic checks were made to verify bioagronomic characters of trees and to ascertain symptoms of virus or viruslike diseases. Many of them began to bear during the second year and fruit was harvested in December 1985 and January 1986. Fruit were analyzed to determine average weight, rind thickness, juice content, acidity and total soluble solids of the juice.

Mother trees and those obtained from STG were tested for virus and viruslike diseases by inoculation to indicator plants, enzyme-linked immunosorbent assay (ELISA), and polyacrylamide gel electrophoresis (PAGE). Madam Vinous sweet orange, Parson's Special mandarin and S-1 citron grafted on Volkamer lemon were inoculated according to

standard procedures in warm or cold greenhouses (14). Parson's Special mandarin trees that were symptomless after 24 months were transplanted in the field for long-term observations. Tests for impietratura were performed on bearing Marsh grapefruit trees in the field. The fruits were collected every year and peeled to check for gum in the albedo.

Citrus tristeza (CTV) and citrus variegation virus (CVV) were inde-

xed by ELISA (2, 6) and viroids by PAGE (1, 10).

*Spiroplasma citri* was indexed by culturing in 3 G liquid medium and by ELISA (3) using an antiserum purchased from Sanofi Santè animale (France).

## RESULTS AND DISCUSSION

**Indexing of mother trees.** Mother trees were infected by one or more virus or viruslike diseases (table 1).

TABLE 1  
RESULTS OF INDEXING FOR SOME VIRUS AND VIRUSLIKE DISEASES ON MOTHER AND SHOOT-TIP GRAFTED (STG) TREES OF SWEET ORANGE, MANDARIN AND MANDARIN-LIKE, LEMON AND GRAPEFRUIT CULTIVARS

Cultivar	Indexing of mother trees				No. of STG trees tested	No. of STG trees freed from		
	Viroids		PLP <sup>y</sup>	CVV <sup>x</sup>		viroids	PLP	CVV
	PAGE analysis <sup>z</sup>	Citron	(M. Vinous)	(ELISA)				
<b>Sweet oranges</b>								
Golden Buckeye	1	+	—	—	3	3	—	—
Navelina	2	+	+	—	10	9	9	—
Salustiana	2	+	—	—	3	3	—	—
Skaggs Bonanza	3	+	—	—	14	13	—	—
Tulegold navel	1	+	—	—	16	16	—	—
Moro	1	+	—	—	5	5	—	—
<b>Tarocco</b>								
Barresa	2	+	—	—	11	11	—	—
Caudullo	3	+	—	—	7	7	—	—
Cocuzza	3	+	+	+	21	21	21	21
Finocchiara	1	+	+	+	10	10	9	9
Gabella	2	+	—	—	17	17	—	—
Gallo	3	+	—	—	9	9	—	—
Pietraselluta	3	+	+	—	2	2	2	—
<b>Mandarins</b>								
<b>Tardivo di Ciaculli</b>								
apireno	2	+	—	—	1	0	—	—
<b>Clementine</b>								
Nules	3	+	—	—	7	7	—	—
Oroval	3	+	—	—	3	3	—	—
Comune emc 1	2	+	+	+	11	9	9	10
Comune emc 6	2	+	—	—	1	1	—	—
<b>Lemons</b>								
<b>Femminello</b>								
Carnazza	1	+	—	—	2	2	—	—
Continella	3	+	+	+	8	7	7	7
fior d'arancio	3	+	+	+	20	18	16	16
Germaná	2	+	—	+	1	0	—	0
Verna	2	+	—	—	2	2	—	—
<b>Grapefruit</b>								
Star Ruby	2	+	—	—	7	7	—	—

<sup>z</sup>Number of viroid bands detected by polyacrylamide gel electrophoresis.

<sup>y</sup>Psorosislike pathogens indexed on Madam Vinous sweet orange.

<sup>x</sup>Citrus variegation virus: indexed by enzyme-linked immunosorbent assay.

Indexing on S-1 citron trees showed a wide range of reaction from severe to moderate epinasty and vein browning. PAGE analysis detected one to four RNA bands of different viroids (1). Among them, citrus exocortis viroid (CEV) was detected in 20 mother trees and citrus viroid "B" (CBV) was present in 18 trees. A third unidentified RNA close to CBV (1) was detected in Skaggs Bonanza navel, Caudullo, Cocuzza, Gallo, Pietraselluta and Tarocco sweet oranges, Nules and Oroval clementines, and

Continella and fior d'arancio Femminello lemons.

Citrus variegation virus was found in Carnazza, Continella and fior d'arancio Femminello lemons, in Cocuzza and Finocchiara Tarocco sweet oranges.

Indexing on Parson's Special mandarin is in progress, but preliminary results have already shown cachexia-xyloporosis infection in Continella and fior d'arancio Femminello lemons.

Navelina sweet orange showed symptoms of impietratura, psorosis A

TABLE 2  
NUMBER OF BEARING TREES AND FRUIT QUALITY OF  
SHOOT-TIP GRAFTED CULTIVARS

Cultivar	No. bearing trees/ no. total trees	Fruit wt. (g)	Rind thickness (mm)	Juice (%)	TSS <sup>v</sup> (%)	Acid (%)	TSS/Acid
<u>Sweet oranges</u>							
Golden Buckeye	1/3	250	7.0	42.5	8.9	0.92	9.7
Navelina	9/10	230	5.5	49.9	10.5	0.96	10.9
Salustiana	2/3	200	6.5	50.3	10.2	0.74	13.8
Skaggs Bonanza	14/14	255	4.7	48.9	10.0	1.13	8.8
Tulegold navel	14/16	220	5.5	47.4	9.7	0.92	10.5
Moro	4/5	175	5.9	47.3	10.2	1.50	6.8
<u>Tarocco</u>							
Barresa	6/11	176	5.0	49.1	10.0	1.16	8.6
Caudullo	0/7	—	—	—	—	—	—
Cocuzza	10/21	186	4.8	53.6	9.7	1.14	8.5
Finocchiara	0/10	—	—	—	—	—	—
Gabella	3/17	232	4.2	52.2	10.4	1.20	8.6
Gallo	6/9	202	5.7	51.0	9.5	1.04	9.1
Pietraselluta	0/2	—	—	—	—	—	—
<u>Mandarins</u>							
<u>Tardivo di Ciaculli apireno</u>							
	0/1	—	—	—	—	—	—
<u>Clementine</u>							
Nules	6/7	89	2.7	45.4	10.2	0.73	14.0
Oroval	1/3	125	3.8	42.4	10.6	1.01	10.5
Comune cmc 1	7/11	74	3.6	44.1	10.6	0.75	14.1
Comune cmc 6	1/1	72	3.0	47.5	11.7	0.84	13.9
<u>Lemons</u>							
<u>Femminello</u>							
Carnazza	2/2	103	5.5	26.4	8.1	6.49	1.2
Continella	7/8	84	4.3	40.5	8.1	6.79	1.2
fior d'arancio <sup>z</sup>	19/21	137	6.0	30.5	8.3	6.76	1.2
Germaná	1/1	99	4.2	32.3	8.9	6.93	1.3
Verna	2/2	113	5.5	33.2	9.3	7.25	1.3
<u>Grapefruit</u>							
Star Ruby	4/7	400	8.9	48.8	9.5	1.94	4.9

<sup>z</sup>During the indexing one tree of fior d'arancio Femminello lemon died from mal secco disease.

<sup>v</sup>TSS = total soluble solids.

and concave gum. Oroval clementine had symptoms of ringspot only on leaves.

No mother tree was affected by CTV although some cultivars were imported from Spain, where CTV is widespread. No *S. citri* was detected.

**Indexing of STG trees.** STG trees indexing needs to be completed for cachexia-xyloporosis viroid on Parson's Special mandarin, but PAGE analysis showed that almost all cultivars and clones have been freed from citrus viroids (table 1). Only the trees of Tardivo di Ciaculli apireno mandarin and Germaná Femminello lemon are infected by CEV and CBV viroids, one tree of Navelina sweet orange is infected by CBV; and Skaggs Bonanza sweet orange, Continella femminello lemon (one tree), Comune cmc 1 clementine, fior d'arancio Femminello lemon (two trees each) are infected by the third citrus viroid.

It is important to note that in some cases where many viroid bands were detected in mother trees, only exocortis was eliminated whereas

other viroids were still present in STG plants. A different host invasiveness of smaller viroids is therefore suspected (10).

Two trees of fior d'arancio Femminello and the tree of Germaná Femminello lemon were infected by CVV. Both cases happened when field-grown donor plants were used for shoots for STG. When the same variety was grown in a heat chamber before collecting the shoots, all STG propagations were free from CVV (table 1).

**Bioagronomic evaluation.** First observations about morphological characters of STG trees show a true-to-type behaviour. Many trees began to bear during the second year in the field, particularly navel oranges, clementine mandarins and lemons (table 2). Qualitative characters of fruit were determined in December for navel oranges, clementine mandarins and lemons, and in January for blood oranges and Star Ruby grapefruit, and were generally satisfactory and similar to the mother trees.

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