A New Program for Citrus Budwood Improvement in São Paulo, Brazil

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ABSTRACT. In 1988 the Faculty of Agricultural Sciences, Universidade Estadual Paulista and the Center for Agricultural Biotechnology, ESALQ-USP started a joint Program to select outstanding field trees and from these, obtain virus-free budwood by shoot-tip grafting. The first phase of the Program involved the selection of 110 superior trees that were indexed for tristeza, exocortis, psorosis, cachexia and other diseases. Budwood from bearing branches of the top of the trees were used for propagation and then shoot-tip grafted. Re-indexing was carried out to assure disease-free status of the new plants. Preinoculation with a mild strain of citrus tristeza virus was done to prevent infection by severe strains. These plants were called Superplants, and will be used as sources of improved budwood, after horticultural fruit evaluation, has been completed. Due to the rapid spread of citrus variegated chlorosis disease, the Superplants of all commercial varieties are maintained in insect proof cages.

After an extensive field survey, Rossetti and Salibe (4) pointed out in 1961 that virus and virus-like diseases were a major factor restraining vigor and productivity of citrus orchards in São Paulo State, Brazil. Growers were using Rangpur lime as rootstock to replace their original plantings on sour orange devastated by tristeza virus. Exocortis and cachexia viroids, latent up to then, became rampant in this sensitive rootstock. Psorosis disease was also affecting many trees of most varieties.

The establishment of a Citrus Budwood Registration Program in 1961, (5) and the extensive use of nucellar lines of commercial varieties (6), considerably reduced the incidence of pathogens in the new orchards. The official virus indexing work and the Registration Program were unfortunately discontinued in 1980. Some exocortis-infected old lines and new varieties such as Folha Murcha orange, carrying psorosis were then used as budwood sources. Vein enation virus was probably introduced in infected budwood and then spread rapidly by aphids to many mother trees. Other disorders which also made their appearance in the citrus orchards were blight or declinio of unknown etiology starting in 1970, and citrus variegated chlorosis (CVC) caused by the bacterium *Xylella fastidiosa* in the late 1980's.

During the last three decades, the citrus plantings in the São Paulo Plateau (500 to 700 m altitude) expanded from less than 20 million trees in 1960 to about 180 million trees in 1992. Presently, the following virus and virus-like diseases are known to occur in these commercial citrus orchards: endemic citrus tristeza virus (CTV) causing noticeable stem pitting; psorosis and exocortis present in certain lines or varieties; rare occurrence of cachexia; spreading vein enation/woody gall; citrus declinio or blight affecting 4 to 5 million trees yearly and CVC spreading rapidly with an estimated 2 million infected trees.

THE SUPERPLANT PROGRAMME

The objectives of the new citrus budwood improvement program are to produce superior mother trees to provide citrus growers with virus-free propagation material of the highest horticultural quality; to reintroduce old lines with desirable qualities into the industry, to select mother trees of lines producing fruits with higher solids for processing, and also to select cultivars with desirable characteristics for the new citrus area in the southwest of São Paulo State.

The program was planned and implemented by two university groups, the Faculty of Agricultural Sciences, UNESP, in Botucatu and the Center for Agricultural Biotechnology, Escola Superior de Agricultuta "Luiz de Oueiroz" - USP, in Piracicaba. It follows the citrus improvement program in operation in South Africa (7,8).

The development of the shoot-tip grafting technique for freeing varieties from graft-trasmissible agents (2) provided the only practical method to produce virus-free plants of certain varieties, such as Folha Murcha orange and Thompson grapefruit which are periclinal chimeras.

Clean nucellar lines and lines protected by mild CTV, also entered the improvement program. Thus all important commercial varieties and lines were considered in the new improvement program.

MATERIALS AND METHODS

The improvement program for the production of superior citrus mother trees, named "Super Plant" was implemented in 1988. The following steps or Phases have been carried out (Fig. 1).

SELECTION OF OUTSTANDING MOTHER TREES

The program began with a widespread search for superior trees in commercial orchards and research centers, including all commercially important varieties. This field selection, named phase one, was extended to the entire country and involved finding the oldest, most vigorous and productive trees, true-to-type and apparently free from all known diseases and abnormalities. To enter the program, trees had to be 10 or more years old and to produce six or more fruit boxes annually. They were to be the best trees of the best orchards. Budwood was then taken from bearing branches of the top of each selected outstanding tree. Fruit samples were also collected for analysis.

The selection covered the citrus areas of São Paulo and neighbouring states, reaching Bahia, the site of the National Center for Fruit Research. In total over one million hectares is dedicated to citrus growing.

Emphasis was placed on the selection of superior trees of varieties with commercial value, but also included others with potential interest. Old-line trees of commercial varieties were also considered, with the aim of their re-introduction into the industry. Some old lines, like Hamlin orange were, abandoned long ago due to universal exocortis viroid infection and were found only in research centers, when possible, old and nucellar lines of a same variety were selected.

A total 110 superior adult trees representing the best citrus germplasm existing in Brazil were selected during the period 1989-1992.

PROPAGATION AND INDEXING

Buds collected from the selected superior trees were propagated on Rangpur lime seedlings in a greenhouse, as well as in a field nursery. They were indexed for tristeza, vein enation/woody gall and psorosis viruses, and exocortis and cachexia viroids in greenhouses on appropriate indicators (3).

Many horticulturally superior trees found to be negative for viruses and viroids, except CTV which is endemic in Brazil, were immediately used as sources of improved budwood.

VIRUS ELIMINATION

Phase two comprises virus elimination through shoot-tip grafting which is conducted at the Center for Agricultural Biotechnology, in Piracicaba. Budded trees grown in containers in the greenhouse, were transferred from Botucatu to Piracicaba, about 118 km away. One to ten shoot-tip grafted plants were produced from each selected source, as described by Navarro (2). All the shoot-tip grafted plants were returned to Botucatu,

where they were biologically indexed for viruses and viroids in a greenhouse, following the techniques recommended by Roistacher (3).

NUCLEAR MATERIAL

Phase three comprises the propagation of shoot-tip grafted plants on Rangpur lime seedlings in the greenhouse and inoculation with a mild tristeza strain for cross protection. This protective strain is one selected by the Virus Section of the Agronomic Research Institute of Campinas, largely tested for Pera orange.

The cross-protected plants constitute the nuclear material and they are maintained in large containers under greenhouse conditions. Budwood from these plants is being topworked into old trees in Botucatu, for trueness-to-type.

In the final stage, budwood will be provided for the establishment of foundation blocks. Trees there will constitute Super Mother Plants, to be re-indexed every 5 yr. The first release of superior budwood is planned for 1993.

RESULTS AND DISCUSSION

A total of 110 outstanding trees were selected during the field work. The list of varieties and number of selected trees of each, and the results of the indexing procedure for CTV, psorosis, exocortis, cachexia are shown in Table 1.

Pera orange trees comprised nearly 50% of all selected trees because it is the most widely grown variety in the country, representing over 60% of all 188 million citrus trees in São Paulo State. Moreover, there exist many known lines or clones of Pera orange,

TABLE 1
VIRUS INDEXING OF 110 SUPERIOR FIELD TREES FOR THE BUDWOOD IMPROVEMENT PROGRAM OF SÃO PAULO

VARIETY	Total no. of trees	Trees position for:			
		Tristeza	Psorosis	Exocortis	Cachexia
ORANGES					
Pera	48	48	5	2	0
Valencia	7	7	3	1	0
Natal	4	4	1	0	0
Folha Murcha	2	2	2	1	0
Hamlin	4	4	0	3	0
Pineapple	3	3	0	1	0
Barão	3	3	0	1	3
Salustiana	1	1	1	1	0
Acidless	4	4	4	1	0
Others	19	19	5	4	1
MANDARINS AND TANGORS					
Cravo	1	1	0	0	0
Dancy	1	1	0	0	0
Willow Leaf	1	1	0	0	0
Murcott	3	3	0	0	0
Ponkan	3	3	0	0	0
Ellendale	1	1	0	0	0
OTHER VARIETIES					
Bergamota	1	1	0	1	0
Tahiti lime	1	1	0	1	0
Lisbon lemon	1	1	0	1	0
Pernambuco Shaddock	1	1	0	0	1
Flame grapefruit	1	1	0	0	0
TOTALS	110	110	21	18	5

such as Premunizada, Olimpia, Bianchi, Dibbern, Vimusa, Rosa, Tardia, Santa Irene and others, and all need to be represented in the selected germplasm. Old and nucellar lines of Pera orange were found during the search. Emphasis was also placed on all lines of Hamlin, Valencia and Natal oranges. Two superior trees of Folha Murcha orange, a new mutation of Valencia orange, were selected for the program. However, shoot-tip grafted plants did not maintain the main characteristic of this variety, the wilted-like aspect of the leaves.

The CTV stem pitting ratings in the branches of the young propagations of some of the selected trees is shown in Table 2. The selection process appear to have also selected for mild strains of CTV since the severest rating was only 2; Pera sweet orange commonly rates as 3-4 in the field.

Exocortis and cachexia were found in only a few old line varieties. However, psorosis virus was found in several old line varieties and in a few nucellar trees, suggesting slow field spread. Indexing for vein-enation/woody gall is incomplete at present, but symptoms revealed by test plants of Florida rough lemon indicate that this virus is widespread. The presence of this virus in Brazil has only recently been reported (1).

Healthy plants of each one of commercial and potentially important varieties were recovered in this program. The search was conducted before the appearance of CVC. Subsequent indexing of all 110 selected germplasm gave negative results for CVC.

One superior tree of Pera orange, selection number 03M-2, has produced during several crops. fruits with higher solid contents. Thus, the super plant of this Pera orange clone was named Citrovita clone to honor the private company financially supporting the program.

TABLE 2
TRISTEZA STEM PITTING IN THE SELECTED SUPERIOR FIELD TREES

Selection	Rating[a]
Pera orange (SM)	1.0
Pera orange Prem. PP-1	0.3
Pera orange Prem. PP-2	1.3
Pera orange Prem. PP-3	1.0
Pera orange EEL	0.9
Pera orange Santa Irene	0.7
Pera orange Bianchi	0.6
Pera orange S. Manoel	1.0
Pera Dierberger	1.4
Folha Murcha orange	0.8
Valencia orange	0.9
Serra D'Agua orange	0.9
Lima Mineira orange	0.8
Pineapple orange	0.8
Cadenera orange	0.8
Natal orange S. Manoel	0.5
Natal Sete Lagoas	0.8
Verna	2.0
Verna Peret	0.9
Murcott tangor-1	0.2
Murcott tangor-2	0.2
Seedless Murcott	0.1
Cravo mandarin	0.0

[a]Rating: 0 = None, 1 = few pits, 2 = moderate pitting, 3 = severe pitting and 4 = very severe pitting

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