The Citrus Variety Improvement Program in Spain

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Virus and viruslike diseases are the most serious threats facing the Spanish citrus industry today. Tristeza in 1972 affected 82,000 hectares of citrus (Guardiola, 1974), and is now the most dangerous citrus disease in Spain. Because of tristeza, sour orange can no longer be recommended as a rootstock and such tristeza tolerant stocks as citranges and Cleopatra mandarin are being substituted. However, since nearly all trees in Spain may have exocortis (Planes *et al.*, 1968) the use of citranges may lead to another serious problem because of their susceptibility to exocortis. Xyloporosis (cachexia) is widely distributed in Spain (Planes *et al.*, 1973) and its presence may diminish the effectiveness of Cleopatra mandarin as a rootstock. Psorosis and psorosis-related viruses are present in much of our citrus and presumably add to decline and stunting of trees, thereby increasing economic loss. Similarly, impietratura is present in several varieties and is an important factor in diminishing fruit quality. Stubborn disease may be present in Spain, and if so could pose a serious threat to our industry.

In summary, we now have in Spain many virus and viruslike diseases causing great economic loss and action must be taken rapidly or our citrus industry could suffer serious consequences. Steps are now being taken to solve our problem. Investigations are in progress for stock-scion trials for tristeza tolerance; cross protection studies with mild strains of tristeza have been initiated; and a program is now underway for development of "virus-free" budwood of our most important commercial cultivars. This paper will report on this latter program and discuss the role of the newly developed technique of shoot-tip grafting *in vitro* as a means of producing virus-free true-to-name cultivars in a relatively short period of time. I will also discuss our plans for indexing and project an outline for budwood distribution.

PROGRAM DESIGN

Because of the large number of preferred local citrus selections of high quality being grown in Spain, we cannot depend on importation of "virus-free" selections from other countries as a solution. Therefore, a year ago we began a program for obtaining "virus-free" budwood of all the major citrus cultivars grown in Spain with the objective of releasing selections to the growers as quickly as possible. The viruses we hope to eliminate are: tristeza, psorosis, concave gum, exocortis, xyloporosis, impietratura and perhaps other known and unknown entities which may be present in our citrus.

In designing this program we have considered various alternatives for elimination of viruses from our citrus. These include: (1) selecting and indexing of our existing cultivars; (2) developing a nucellar program; (3) thermotherapy; and (4) shoot-tip grafting *in vitro*.

Selecting and indexing our existing cultivars. Under our conditions, with almost 100 per cent infected material, we do not have a reservoir of nucellar or "virus-free" old-line trees to use as parent selections as was done in the Citrus Variety Improvement Program in California (Roistacher, 1975). Guardiola *et al.* (1974) found only one out of 13,000

Navelina sweet orange trees free of tristeza, psorosis, stubborn, impietratura and exocortis. Thus, by initiating a program of selection and indexing of our current cultivars, many trees of superior horticultural value would be discarded due to infection with one or more viruses; this would be a time-consuming process without much benefit. A similar situation was found in Florida, where less than 1 per cent of the old-line trees were found free of tristeza, psorosis, exocortis and cachexia (Childs and Knorr, 1965; Knorr and Childs, 1968).

Developing a nucellar program. Nucellar embryony as a means of obtaining "virus-free" clones of citrus of both polyembryonic and monoembryonic cultivars (Weathers and Calavan, 1959; Bitters *et al.*, 1972) though effective, has certain limitations and liabilities. Nucellar progeny have juvenile characteristics and require many years for the trees to become commercially acceptable. This long-term basis for obtaining "virus-free" material is not fully practical or acceptable under our present emergency. However, we plan to pursue a side program of developing nucellars, and nucellar selections have already been obtained from monoembryonic cultivars via nucellus culture *in vitro* (unpublished results) and from polyembryonic cultivars (González-Sicilia *et al.*, 1973). These plants will be a standby reservoir of "virus-free" cultivars for research and for other purposes.

Thermotherapy. Thermotherapy has been successful in eliminating the viruses of tristeza, seedling-yellows tristeza, psorosis A, concave gum, infectious variegation, tatter leaf, and vein enation, but has failed to eliminate exocortis viroid, stubborn *Spiroplasma*, cachexia (xyloporosis), yellow vein and Dweet mottle viruses (Calavan *et al.*, 1972). Since most trees in Spain carry exocortis viroid and many are infected with xyloporosis virus, thermotherapy has its limitations for our program. However, it will be incorporated as a standby procedure if shoot-tip grafting *in vitro* should fail to eliminate certain pathogens (Roistacher *et al.*, 1976).

Shoot-tip grafting in vitro. The method of shoot-tip grafting *in vitro* (Navarro *et al.*, 1975) has proven effective in recovering citrus cultivars free of tristeza, psorosis A, concave gum, infectious variegation, exocortis, stubborn, and xyloporosis pathogens (Roistacher *et al.*, 1976; Navarro *et al.*, 1976) and the resulting "virus-free" plants were shown to be nonjuvenile, and available for budwood increase in less than one year after *in vitro* grafting. There is every indication that this method will produce trees with growth and fruit characteristics identical to the parent trees, with the possible exception that these pathogen-free trees may appear and bear differently than their parent trees infected with one or more viruses.

The technique of *in vitro* grafting has not been tested against certain viruses, i.e., vein enation, impietratura, and cristacortis, and studies are now in progress testing these pathogens.

Considering the overall potential advantages of shoot-tip grafting *in vitro*, this method has been selected as the primary means for recovering "virus-free", true-to-name cultivars of the best Spanish selections.

PROGRAM OUTLINE

The Citrus Variety Improvement Program of Spain (CVIPS) consists of the following three stages.

1. **Selection of trees.** Trees include representatives of all the major citrus cultivars grown in Spain plus several cultivars of the variety collection at the Burjasot Experiment Station. Based on economic importance, one to five representative trees are selected from each cultivar to be used as sources of shoot tips for *in vitro* grafting. Criteria for tree selection are based primarily on horticultural performance without regard to pathogen content. For old-line cultivars, selection is based on the extensive survey and classification of Spanish varieties made over the past five years by R. Bono

(unpublished) in cooperation with nurserymen, growers, and Extension Service personnel. A large number of trees of each cultivar were observed in the field and those which were true-to-name and had the best horticultural performances were selected and entered in the CVIPS.

For new cultivars, produced by natural mutation, the mother tree in which the mutation was found and one tree of the first progeny were used as sources of shoot tips for *in vitro* grafting. For cultivars at the Burjasot variety collection, the best trees of each selection were chosen.

Also included in the CVIPS are nucellar selections introduced into Spain since 1965 which were certified as "virus-free" with the exception of stubborn *Spiroplasma* (Mather and McEachern, 1974). Since these trees are still young, only one tree of each selection has been chosen for *in vitro* grafting. Others will be selected, if necessary, after they have adequately fruited.

We have already selected 90 trees of 65 different cultivars of sweet orange, mandarin, lemon, and grapefruit. Indexing of these source trees is now in progress.

2. **Shoot-tip grafting in vitro and indexing.** The young growing flushes on field trees are used as the source of shoot tips, which are excised and grafted *in vitro* to appropriate rootstock seedlings, following the procedure of Navarro *et al.* (1975). Shoot tips are composed of the apical meristem plus two to three leaf primordia and measure 0.1 to 0.2 mm in height. A minimum of ten *in vitro* grafted plants are retained for growth, transplanting to soil, and subsequent indexing. Ten plants appear sufficient to assure at least one pathogen-free tree (Roistacher *et al.*, 1976). For cultivars of the highest priority at least 20 plants are retained.

At the time flushes are collected for *in vitro* grafting, leaves and budwood are also collected for indexing. Generally following the procedures for the Citrus Variety Improvement Program in California (Reuther *et al.*, 1972), a minimum of four seedlings or budlings of indicator plants are used for each virus or group of viruses. These include: Mexican lime for tristeza, vein enation, and psorosis; Pineapple or Hamlin sweet orange for psorosis and concave gum; Dweet tangor for concave gum and other psorosis-like viruses; Arizona 861 citron budlings or seedlings for exocortis; and Parsons Special mandarin on Rough lemon rootstock for xyloporosis (Roistacher *et al.*, 1973). In addition, side-graft inoculum will be used in Madam Vinqus sweet orange for subborn. All index plants will be observed for four to six months with the exception of xyloporosis index plants, which will be held for one to two years depending on positive reactions of known very mild controls. Also fruits on source trees will be carefully observed for symptoms of impietratura.

3. **Indexing and propagation of plants derived by in vitro grafting.** Six to eight months after *in vitro* grafting, budwood will be cut for propagation and indexing. Indexing will be limited only to those pathogens found in the source trees with the exception of xyloporosis. Accordingly a minimum of two plants each of Mexican lime, sweet orange, Dweet tangor, citron, and Parsons Special mandarin will be used. Additional indexing for tatter leaf, impietratura, stubborn and cristacortis may be conducted later on selected plants proposed for foundation trees. By these procedures we feel that "virus-free" plants should be available two years after *in vitro* grafting.

Propagations will be made 6 months after starting the indexing of *in vitro* grafted plants (fig. 1). From each plant two propagations will be made to an appropriate rootstock for a foundation planting; two additional propagations will be made on trifoliate orange or Troyer citrange for a separate block to study fruit quality and tree characteristics. In addition, one propagation will be made as a reserve plant. The original *in vitro* grafted plant will be held under glass or screen, and when freedom from viruses is assured, the plant will be propagated for rapid budwood increase (Calavan *et al.*, 1970). The buds obtained by this method will be released to selected nurserymen for a further budwood increase and production of certified trees.

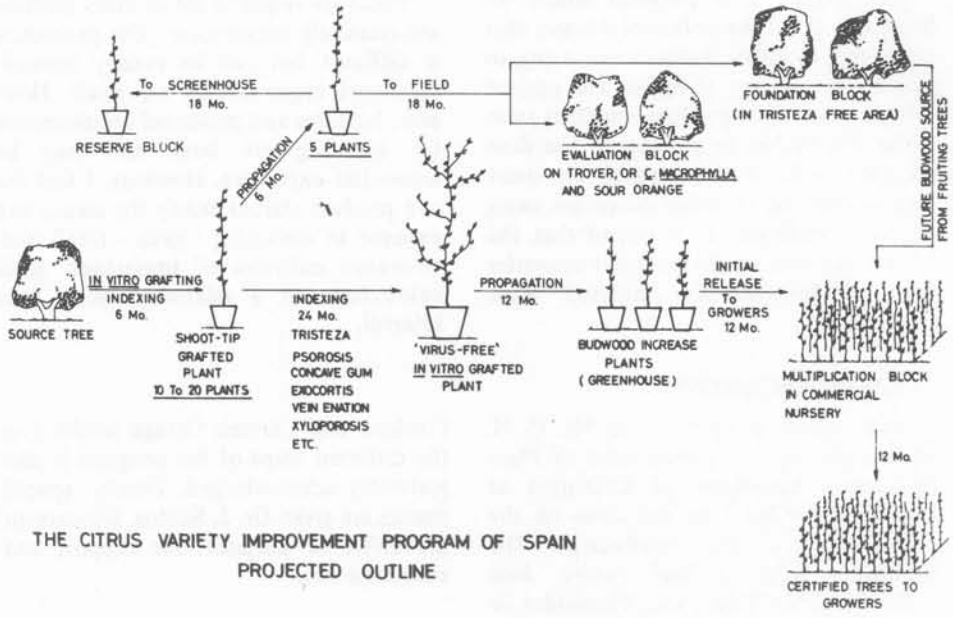

Fig. 1. Diagram of the steps of the Citrus Variety Improvement Program in Spain.

DISCUSSION

In vitro grafting is very similar to standard bud grafting in that progeny should be true-to-name. The difference is the size of the propagating unit. With this in mind, certain risks can be taken, since mutation should not occur with greater frequency than with standard budding. In over 900 plants obtained by *in vitro* grafting there has been no indication of growth or leaf abnormalities, and when plants fruited, fruit appeared typical.

Our program for budwood distribution, as shown in fig. 1, will have three basic blocks. The foundation block, where six trees (two each from three *in vitro* grafted plants) consisting of A, B, and C lines, will provide the major reserve of budwood and trees. This is similar to the California program (Reuther *et al.*, 1972). This foundation block will be located in a tristeza-free zone. A second block, for observation, will be established and devoted to fruit and tree characteristics. Trees can be periodically and critically observed for any indication of off-type or other fruit or tree abnormalities. A third "block" will be composed of plants held in the greenhouse or screenhouse as a reservoir of "virus-free" material.

The first release of "virus-free" plants to the growers will be made by rapid multiplication of buds from the original shoot-tip grafted plant (fig. 1). This should provide budwood to the growers as quickly as possible. Since the number of registered nurseries in Spain is limited, due to very strict quarantine regulations, propagations can be controlled and recalled if off-type or virus-infected plants are discovered during the budwood build-up program. Ultimately, as trees in the foundation block fruit, and prove true-to-name, budwood will be released from these foundation trees for increase and registration. Nursery operations will be supervised by personnel of the Department of Agriculture with periodic indexing of foundation and grower mother block trees to assure freedom from certain pathogens.

CONCLUSION

The CVIPS is a program which we hope will avoid the potential disaster that virus and viruslike diseases can bring to the citrus industry of Spain. The goal of this program is to produce certified trees to be distributed to growers in less than six years. This is a comparatively short time compared to other programs using different methods. It is hoped that the CVIPS can be a model for other countries with similar heavily infected local selections.

Facilities required for *in vitro* grafting are relatively inexpensive. The procedure is difficult but can be readily learned. Personnel requirements are small. However, facilities and personnel requirements for indexing are large and may be somewhat expensive. However, I feel the end product should justify the means and expense in obtaining "virus - free" true-to-name cultivars of important local selections in a relatively short time interval.

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