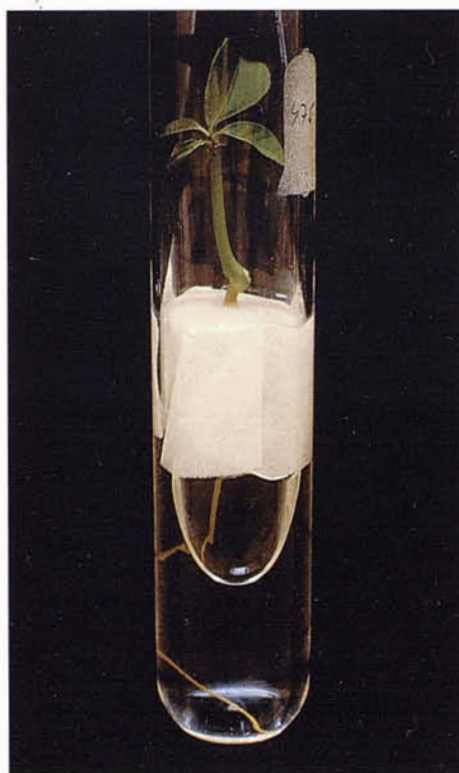


# **VIRUS-FREE CITRUS TREES**

THE AVAILABILITY OF VIRUS-FREE PLANTS IS HAVING AND WILL HAVE A CONSIDERABLE TECHNICAL AND ECONOMIC IMPACT ON CITRUS CULTIVATION IN SPAIN. SPAIN IS THE FIRST COUNTRY IN THE WORLD TO CARRY OUT THIS SORT OF PROGRAMME ON A MASSIVE SCALE, USING WOODY PLANTS AND "IN VITRO" TISSUE CULTIVATION TECHNIQUES.



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**C**itrus cultivation in Spain occupies 580,000 acres, with an average production of some 3.5 million tons, of which 2.1 million are exported. Spain is the fifth biggest citrus producer in the world and the biggest as regards fresh fruit export. Citrus crops are the most important agricultural product our country exports.

The "viroses" of citrus trees, due to viruses, viroids and microplasmas, can lead to a general weakening of the plant, a low yield, and poor quality fruit, restrict the use of many root stocks or even cause the death of the tree. In our country, these diseases are very widespread and represent one of the principal obstacles to citrus cultivation. It is calculated that over 50 % of the trees in commercial plantations are infected with at least three different viroses. This leads to the loss of between 15 and 25 % of the harvest and results in considerable economic losses.

To fight these diseases, the use of virus-free specimens in new plantations is indispensable. However, it has not been possible to find healthy trees of any of the Spanish varieties, and this makes it necessary to resort to methods that allow us to obtain virus-free plants from infected plants. The already existing methods, nuclear embryogeny and heat treatment, present serious limitations to the satisfactory obtention of virus-free plants.

Faced with this situation, it became necessary to find a new method for obtaining virus-free plants and research was started with this aim in mind, using *in vitro* cultivation techniques. These techniques consist basically in the cultivation of cells or vegetable tissues in an artificial nutrient

medium in aseptic conditions and, amongst other advantages, they allow the regeneration of whole plants from cells or tissues.

The virus does not infect all the cells of the affected plant and, in general, the meristematic cells of the caulinary apices of the growing tips remain unaffected. Consequently, plants that have been regenerated from these meristematic cells are free of the virus, even though they come from infected plants.

The difficulty lies precisely in the plant regeneration procedure. In many species, plant regeneration using *in vitro* cultivation of caulinary apices is relatively straightforward, but these techniques cannot be used in the majority of woody species, amongst which we find the citrus.

The perfection of the technique for micro-grafting caulinary apices *in vitro* has solved these problems through the grafting of caulinary apices on to small stocks that have been cultivated *in vitro*. These stocks are obtained from selected seeds which are disinfected and germinated in a test tube containing an artificial nutrient medium. Germination takes place in the dark at 27 °C. After two weeks, the plant has a stem 3-5 centimetres long, which is used as a stock. The caulinary apices are obtained from the growing tips of infected plants. The shoots are disinfected and then, under aseptic conditions and with the help of a microscope and micro-dissection tools, the caulinary apex is isolated. This consists of an apical meristem and three small leaves, about 0.1 mm in size. This apex is inserted into a small incision previously made in the stock. The grafts

are cultivated in test tubes in a liquid nutrient medium (Fig. 1). The cultures are stored at a temperature of 27 °C, with artificial illumination, and after 4-6 weeks the little apex has taken and has developed into 3-4 leaves (Fig. 1). These plants are transferred to a greenhouse so as to carry out virus controls and subsequent reproduction by traditional methods. Using this technique, which has enormous advantages over previously existing techniques, all the viroses that affect the citrus can be eliminated. In Spain, an extensive programme is being carried out, based on the *in vitro* micrograft, to improve the health of citrus varieties. The aim of the programme is to obtain virus-free specimens of Spanish and foreign varieties and distribute them to the growers via the commercial nurseries.

So far, virus-free specimens of more than 150 Spanish and foreign species and varieties of citrus have been obtained and put on the market. Growers started planting virus-free plants from the programme in 1982 and, up until the end of 1986, almost fourteen million had been planted (Fig. 2). This means approximately 14 % of the trees in Spain.

The availability of virus-free plants is having and will have a considerable technical and economic impact on citrus cultivation in Spain. Spain is the first country in the world to carry out this sort of programme on a massive scale, using woody plants and *in vitro* tissue cultivation techniques. The use of the micro-graft technique has later spread to most citrus producing countries and is an example of the enormous economic benefits that biotechnology can bring to agriculture. ●