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Modification of the Shoot-Tip Grafting Method for Citrus

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ABSTRACT. To accelerate growth of shoot-tip grafted (STG) plants, the traditional method used for culturing STG plantlets was slightly modified by omitting the a liquid medium for grafted plantlets and, instead, the plants were transplanted directly into a pasteurized soil medium. Transplant survival rates ranging from 28.5 to 65.1%, depending on citrus cultivar, was achieved. This survival was increased to 88 to 100% by employing the previously developed second grafting to larger seedling technique.

In China, six viral, viroid or bacterial diseases of citrus have been experimentally confirmed (3). Four of the more important pathogens are citrus exocortis viroid, citrus tristeza virus, citrus tatterleaf virus, and citrus huanglongbing. During the period 1990 to 1994, attempts were made to free budwood sources of important citrus cultivars in Sichuan Province of these pathogens by modifying the technique of shoot-tip grafting (STG) as described by Navarro et al. (2). The method used and survival of the test STG plants are presented.

Ten citrus cultivars were used, namely seven sweet oranges (Fuling No. 6 Jingcheng, Fuling No. 12 Jingcheng, Meishan No. 2-2 seedless Jingcheng, Carter No. 2 Valencia, Meishan No 9 Navel, Navelina Navel, and Washington navel) and three mandarins (Nova B7, Satsuma B10 and Cleopatra sour A10).

Trifoliolate orange was used as the rootstock for STG. Seeds were immersed in sterile water for 24 hr after which the outer and inner seed coats were removed under aseptic conditions. The peeled seeds were wrapped in gauze and immersed in 0.5% sodium hypochlorite solution for 10 min and rinsed with sterile water three times. The seeds were then sown on pasteurized Murashige and Skoog culture media (MS) adjusted to pH 5.7. Each culture test tube was sown with 2 to 3 seeds and

incubated at 27°C for 13 to 14 days. This procedure was similar to the methods as described by Navarro et al. (2).

Young shoots were collected from improved mother trees in the orchard or from potted nursery trees grown in the greenhouse. These shoots were treated in sodium hypochlorite and grafted onto the trifoliolate seedlings by the method of Navarro et al. (2)

To accelerate the growth of STG plants, rather than maintaining the STG plantlet on liquid media, the plantlet was planted directly in a small plastic pot (7 × 9 × 10 cm) containing a home-made soil mixture composed of 1/3 rotted sawdust, 1/3 river sand and 1/3 orchard soil which had been autoclaved just prior to use at 15 psi for 20 min and cooled. The soil was moistened with MS medium without sugar and then two or three small grafted plantlets per pot were transplanted. These plants were fertilized with a small amount of sugarless MS solution, and quickly covered with plastic bags. The transplanted plantlets were held under 1,000-1,500 lux fluorescent lamps at 25 to 28°C for 7 to 10 days. Each 3 to 4 days, plants were irrigated and fertilized with nutrient solution. As the tips grew, the plantlets were moved to a shelf by a window for natural light. Plants sprouted 2 to 4 leaves and reached a height of 1 to 1.5 cm in 20 to 60 days.

TABLE 1
THE SURVIVAL OF SHOOT-TIP GRAFTED PLANTS GROWN *IN VITRO* AND TRANSFERRED FROM THE TEST TUBE TO A SOIL MIXTURE

Cultivar	No. survived/ no. grafted	Survival (%)	Avg survival (%)
Jincheng sweet orange			65
Fuling 6	25/32	78	
Fuling 12	6/13	46	
Meishan 2-2	10/18	56	
Valencia sweet orange			64
Carter 2	7/11	64	
Navel sweet orange			29
Navelina	4/14	29	
Washington	6/17	35	
Meishan 9	2/11	18	
Mandarin			57
Satsuma B10	2/4	50	
Cleopatra A10	4/7	35	
Nova B7	2/3	67	

At this time, some of the plantlets were grafted by the method of de Lange (1) to larger seedlings and covered with a plastic bag. Plants were taken to a screenhouse for 15 to 20 days after which the plastic cover was removed and plants were then brought into a screenhouse for further growth.

CONCLUSION

As shown in Table 1, the survival of STG plants ranged from 29 to

65%, depending on the cultivar. The Jincheng orange averaged 65% survival followed closely by the Valencia orange and mandarin cultivars with 64 and 57%, respectively. Navel oranges were the poorest averaging 29% survival. Those plants which were second grafted to large seedlings by the method of de Lange (1) grew well and had 88 to 100% survival.

Using this modification we were able to reduce the time needed to produce STG plants.

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