A Microbudding Technique for Biological Indexing and Ultra-High Density Planting of Citrus

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ABSTRACT. A microbudding technique was developed to produce budded citrus trees faster and cheaper. More than 900 trees were planted in the field, between June and December 1997. The trees grew well in field conditions. Results show that the microbudding technique is useful for commercial production. In addition, preliminary results from virus indexing studies show that microbudding may be useful in biological indexing for citrus tatter leaf virus.

Biological indexing is still the preferred technique used to confirm the presence of most graft-transmissible pathogens in fruit trees including citrus. This indexing process involves budding or grafting a fast growing rootstock or an indicator seedling with the tissue from the test plant (2). If a rootstock is used instead of an indicator plant, a bud from a pathogen-free indicator plant is placed above the tissue from the test plant. Normally, it takes approximately 6 to 9 mo to grow the rootstocks or indicator plants for biological indexing. The foliar symptoms are expressed from wk to a few mo after budding, depending on the pathogen, the indicator plant, and the growing temperature. Biological indexing requires advanced planning to prepare the plants, as well as considerable greenhouse or growth chamber space. A reliable technique that allows the budding process and symptom expression quicker with a lower space requirement would be highly desirable.

Preliminary studies done in my laboratory have shown that a microbudding procedure was useful in developing small budded trees efficiently (3). This procedure was based on budding rootstocks that were approximately 4-mo-old, using small scion buds, but with no taping to keep the bud in place. Instead, the buds were capped with a plastic pipette tip (Fig. 1). Moreover, this technique enabled the production of budded plants faster and at a lower cost compared to conventionally budded trees and required considerably less space. The procedure was successful with several scion-rootstock combinations that were microbudded throughout the year in a greenhouse. Scion buds sliced for this technique were kept refrigerated for a week without significant loss in bud take. This technique is different from previously described microbudding from South Africa (1).

Biological indexing was performed for the identification of citrus tristeza virus, psorosis virus, citrus exocortis viroid (CEVd), and citrus tatterleaf virus (CTLV). Indicator plants of Mexican lime, pineapple sweet orange, Etrog citron, and Troyer citrange were microbudded

Fig. 1. Microbudding steps: microbudding and capping with a pipet tip, followed by cap removal after callusing. The arrow indicates new scion growth.

with buds containing the above mentioned viruses, respectively. After callusing and at least four wk past microbudding, the bud growth was cut off to force new growth of the indicator plant. Early signs of tatterleaf symptoms associated with chlorosis were visible 3 wk after the emergence of new growth in Troyer citrange plants inoculated with CTLV. The initial chlorotic symptoms were clearly visible under a stereoscope. So far, the microbudding system was found more useful for the indexing of CTLV (Fig. 2) compared to other pathogens. Experiments with CEVd showed early symptoms of leaf epinasty in Etrog citron, however, the results were not consistent. Results indicate that the microbudding procedure can be modified to apply for indexing other graft-transmissible pathogens of citrus. Since smaller plants are used, this system is ideal and less cumbersome for growing the indicator plants in small growth chambers at an optimum temperature for symptom expression. Apart from the biological indexing, we were also able to successfully microbud several genetically modified Rio Red grapefruit explants on to rootstocks (5). This enabled genetically engineered explants to grow as plants quicker, and it saved time and effort from a tedious and often less successful attempt to induce roots in these explants.

Production of less expensive trees through microbudding opened up an opportunity to plant an ultra-high density block at a substantially lower cost. In Texas, severe freezes, drought, urbanization and a higher land value, and unpredictable fruit prices in the past several years have been major limiting factors faced by citrus growers. These factors prompted an investigation of the possibilities of a microbudded, ultra-high density planting that would allow a rapid and better economic return for the growers, especially in the early years of an orchard. More than 900 microbudded trees, mainly Marrs orange and Rio Red grapefruit, were planted at a close spacing of 3×6 ft. The objective was to study the field performance of microbudded trees planted at a very young stage. So far, the microbudded trees have grown well in field conditions (Fig. 3) and some orange, grapefruit, and lemon trees produced fruit in less than 2 yr after microbudding (4).

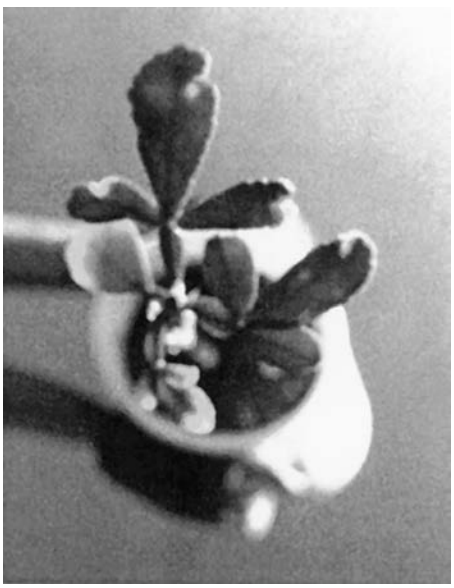

Fig. 2. CTLV induced tattering and chlorosis produced in Troyer citrange, 3 wk after the emergence of new growth.

Fig. 3. Microbudded trees in the field grew very well and out performed comparable trees grown conventionally. The tree in the pot was grown and budded using conventional procedures and rootstock seed were prepared at the same time.

LITERATURE CITED

1, Holtzhausen, L. G., P. J. Muller, and A. P. Vincent
1974. Mikro-okulering: 'n Belowende kwekerypraktyk. Citrus Sub-Trop. Fruit J. 488: 5-8.
2. Roistacher, C. N.
1991. *Graft-Transmissible Diseases of Citrus. Handbook for Detection and Diagnosis*. FAO, Rome. 286 pp.
3. Skaria, M.
1997. Micro-budded plants: A new concept in citrus production and economics. TAMUK Citrus Center Newsletter 15 (4): 1-2.
4. Skaria, M. and Z. Tao
2000. 2-year wonder: Microbudded citrus trees in Texas produce fruit within two years. Citrus Ind. 81(3): 28-19.
5. Yang, Z. N., I. L. Ingelbrecht, E. Louzada, M. Skaria, and T. E. Mirkov
2000. *Agrobacterium*-mediated transformation of the commercially important grapefruit cultivar 'Rio Red' (*Citrus paradisi* Macf.). Plant Cell Repts. 19: 1203-1211.