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MICRO CLONING: A MULTIPLE CLONING TECHNIQUE FOR AVOCADOS USING MICRO CONTAINERS

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SUMMARY

A multiple cloning technique was developed and successfully implemented commercially. This technique is derived from the so-called Frolich technique, which is used as a standard by various nurseries world-wide. This technique implies the positioning of 55 ml micro containers over the multiple etiolated shoots (one per shoot), which developed simultaneously and consecutively from the so called nurse graft. Root initiation occurs within these containers. After successful grafting of these shoots to a commercial scion cultivar, these fully developed avocado plants (clones) are separated from the nurse seedling just above the nurse graft. Usually after hardening off, the wholesale nursery will transplant these micro clones to 7 liter containers (plant bags). However, with this technique it is possible to profitably deliver large numbers of these micro clones to distant growers or satellite nurseries world-wide, to be grown by them to a field transplantable or saleable tree size. Compared to any other, this technique is unique in the sense that it is possible to produce clonal plants small in size, light in weight and in compliance with the highest phytosanitary standards. Consequently massive transport savings over long distances are possible and the export of nursery trees becomes a reality.

Key words: *Persea americana* Mill., clonal propagation, nursery plant, rootstock multiplication.

INTRODUCTION

Avocado rootstocks for many years have primarily been propagated by seed. In fact many leading countries today still give preference to this method of propagation mainly because the available clonal propagation techniques are labour intensive, arduous and to a certain extent uneconomical. The main purpose of cloning avocado rootstocks is to secure genetic uniformity with regard to *Phytophthora cinnamomi* (root rot) tolerance and certain beneficial horticultural characteristics.

Over the years several research workers tried with limited success to propagate avocados clonally by using adult greenwood cuttings. According to Frolich (1951), etiolation was proven very beneficial in rooting avocado cuttings. In an attempt to solve the problem of difficult to root avocado cuttings, Brokaw (1975), Ernst and Holtzhausen (1978), Moll and Wood (1980) and Reuveni and Goren (1982), developed different etiolation techniques, with varied commercial applicability. The techniques developed by Brokaw (1975), Moll & Wood (1980) and Reuveni & Goren (1982), derived from the technique as described by Frolich & Platt (1971-72). This, so-called Frolich technique consisted of etiolating the sprout of a graft (potential rootstock), grafted onto a discardable nurse seedling. After foliation above the etiolated zone, the etiolated sprout was grafted with a fruiting scion and allowed to root in the etiolated zone.

Brokaw (1975) reported on a patented variation of the Frolich method that streamlines the commercial propagation of clonal avocado trees. A planting sleeve (liner bag) configuration, a single etiolated shoot and the addition of a loosely clamped metal ring placed just above the bud union, to constrict and to eventually sever the nurse seedling, forms the basis of the Brokaw technique. A disadvantage of this method is that if the nurse seedling fails to separate or if it is not physically removed, profuse suckering may occur. Accompanied by poor clonal root formation, the resulting effect will be stunted and uneven tree growth in the field.

Ernst (1978) reported on the beneficial effect of the auxin IBA to enhance root formation and to improve root quality of treated etiolated shoots (synergistic reaction). The Frolich technique as modified by Brokaw (1975), still forms the basis of the clonal rootstock propagation techniques used by avocado nurseries internationally.

Due to increased production costs the economical viability of this arduous and time consuming technique is lately under pressure. Unrealistically high world tree prices and the proportion there-of as part of the orchard capital layout costs when considering high-density plantings, highlighted the need for a cost effective technique to be developed. An attempt should be made to lower the transport costs per tree which in turn will have an effect on the above mentioned capital layout costs.

The purpose of this study is therefore to modify the Frolich technique in an attempt to increase efficiency and make it economically viable for both the nurseryman and the intended grower.

MATERIALS AND METHODS

Development of this micro cloning technique, which derived from the Frolich technique, was initiated at Allesbeste Nursery during 1990. This technique is illustrated step by step in Figure 1:

1. A nurse seed (‘Zutano’ or ‘Edranol’) is planted in a long narrow-sleeved polyethylene liner bag (350 x 100 mm; 1 liter content) filled with sterile composted milled pine bark.

2. The seedling is allowed to grow until the stem is thick enough (approximately 6 mm in diameter) to be grafted.

3. As soon as the seedling (nurse seedling) is ready, the desired rootstock (clonal rootstock to be) is grafted onto it, as close to the seed as is practical (approximately 8 weeks after planting).

4. At bud burst (3 to 4 weeks after grafting) the plant is placed in an etiolation room (darkroom) with good ventilation. The temperature in the room should be approximately 25 ºC. The buds will develop in the dark and produce white (etiolated) shoots, sometimes with reddish or red-tinged leaves. Where possible two shoots per graft are allowed to develop.

5. When the etiolated shoots have grown to about 200 to 300 mm in length, the plant is removed from the etiolation room (approximately 4 weeks after bud burst). Indolebutyric acid (IBA) at 0,7% concentration is applied to a small incision (wound) made at the base of the etiolated shoot, approximately 100 mm above the graft union. To support the growth

process 2 g of Plantacote 8M (slow release fertiliser) is applied on the surface of each liner bag. Wire holders are inserted in the liner bags, specially bent to fit 55 ml polyethylene micro containers (30 x 30 mm open end tapered to 10 x 10 mm bottom end; 100 mm in length), which are positioned over the etiolated shoots (one per shoot). After positioning, the micro containers are filled with sterile composted milled pine bark, vermiculite or any other suitable rooting medium.

6. The plant, of which only the upper parts of the etiolated shoots and leaves protrude from the medium filled micro containers, is placed under shade cloth to induce photosynthesis and progressively harden off. Root initiation and shoot elongation progress during this stage.

7. As soon as the shoots have reached the desired length they are grafted at approximately 300 mm above the micro container to a commercial scion cultivar (approximately 8 weeks after IBA treatment). Stage 5 plants suitable for grafting could be grafted while still etiolated (before stage 6). To prevent any interference with the rooting process this could commence approximately 1 week after IBA treatment. After grafting the plants are moved to a greenhouse set at 28 ºC and 75% relative humidity.

8. After bud burst and as soon as the newly developed flushes are approximately 50 mm in length the fully developed avocado plants (micro clones) are separated (severed) from the nurse seedling just above the nurse graft and below the micro container (approximately 6 weeks after second grafting).

9. After separation the original grafted nurse seedling returns to stage 4 to repeat the process for as long as there are sufficient reserves within the seed to support it.

10. The micro clones are then placed into specially designed trays (128 per tray) and kept under high humidity (85% relative humidity) in the micro liner area. Fertigation with a balanced fertiliser mixture commences one week after separation. The conductivity of the leach water should not exceed 200 $mS \cdot m^{-1}$. Hardened off micro clones, with expanded leaves and just before commencement of the second flush (approximately 8 weeks after separation), are sold directly to distant growers or satellite nurseries who then grow these plants to a field transplantable or saleable tree size.

11. If not sold during stage 10, a hardened off micro clone is transplanted to a 7 liter-polyethylene plant bag (420 x 150 x 125 mm), filled with a sterile mixture of 60% composted milled pine bark, 20% river sand and 20% topsoil. Plantacote 8M is added to the mixture at a rate of 15 g per plant bag. Prior to planting the root system is inspected for quantity and quality. Any plant with diseased roots is immediately discarded.

12. The plants are kept under 40% shade until they have reached the desired stage to be sold commercially. To reach a saleable stage takes approximately 3 to 4 months during summer and 6 to 8 months during winter.

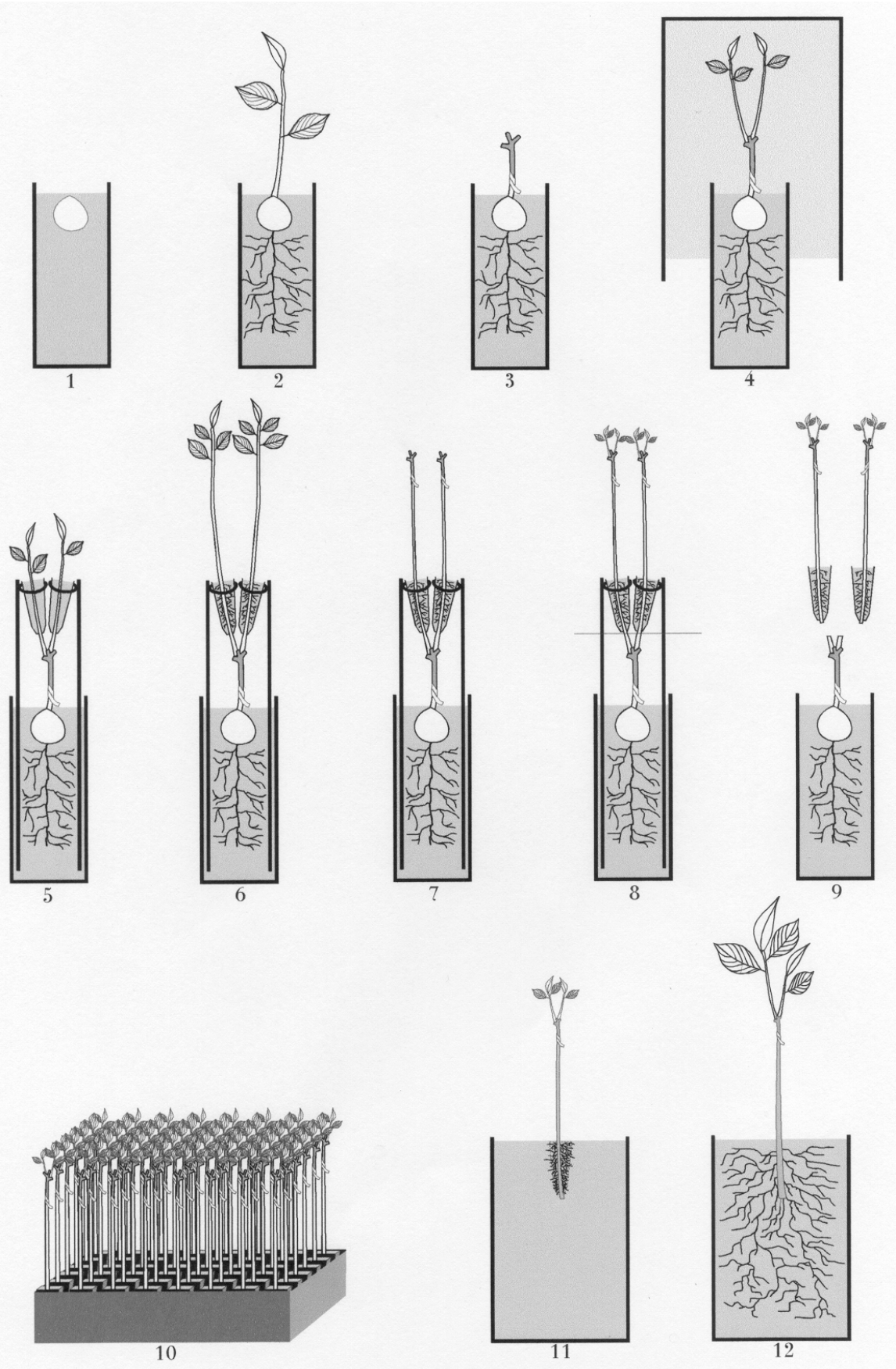

Figure 1. Micro cloning technique for rooting avocado rootstocks

RESULTS AND DISCUSSION

With the micro cloning technique it is possible to produce large numbers of fully developed avocado plants (clones). The propagating process takes approximately 8 to10 months from seed to the hardened off micro clone stage and 10 to 18 months from seed to field transplantable tree size stage (7 liter bag). The latter compares favourable with the Frolich technique as described by Brokaw (1975). The micro cloning technique differs from the Frolich technique in the sense that more than one fully developed clonal plant can be produced per seed. After the first cycle micro clonal plants can successfully be separated above the first graft. The nurse seedling can be allowed to grow and repeat the whole process (second cycle). This process can be repeated until all seed resources have been depleted.

A further advantage of this technique over the Frolich technique as modified by Brokaw (Brokaw, 1975), is that a so-called buffer zone is established between the clonal and seedling root systems. This buffer zone has an important phytosanitary advantage. At transplantation the root system is inspected for quantity and quality. Weak plants can be discarded or be kept in the micro liner area until they are ready to be planted, resulting in a decrease in transplant losses.

The plants kept in the micro liner area are less space consuming. By managing the fertiliser levels in the micro containers the flow of plants (process) can be regulated efficiently.

CONCLUSIONS

A new commercially successful multiple cloning technique, derived from the so-called Frolich technique, has been developed and implemented. This micro cloning technique is rapid, produces large numbers of healthy rooted plants, and does not destroy the nurse seed or graft. Because of the efficiency of this technique a decrease in propagation costs is possible.

With this technique it is possible to profitably deliver large numbers of these micro clones to distant growers or satellite nurseries world-wide, to be grown by them to a field transplantable or saleable tree size. Compared to any other, this technique is unique in the sense that it is possible to produce clonal plants small in size, light in weight and in compliance with the highest phytosanitary standards. Consequently massive transport savings over long distances are possible and the export of nursery trees becomes a reality.

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