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Clone Your Own Avocado at Home

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Editor's Note: *Occasionally, we like to include articles by members of the California subtropical fruit industry. The methods described in this article are not yet commercially implemented in California. We all look forward to its potential development for our industry.*

Researchers and nurserymen have been trying to clone avocado rootstocks for many years. The main purpose for cloning is to propagate rootstocks tolerant to *Phytophthora* root rot. Other reasons for cloning are salinity tolerance, improved productivity, compatibility of the rootstock and scion, and genetic uniformity of the orchard. Avocado wood is difficult to root from cuttings and even harder to grow as a viable plant from the few successfully rooted cuttings. Schroeder, Salazar-Garcia, Ben-Ya'acov, Gustafson, Kadman and many others have reported on vegetative cloning attempts through the years.

E.F. Frolich from UCLA developed an etiolation method, which, although often modified, is widely used internationally for the commercial propagation of clonal avocado rootstocks. The basic procedure is somewhat complicated and time consuming: a large avocado seed is grown in a container to serve as a nurse for the future rootstock. Once the seedling is large enough for grafting, a splice or cleft graft is made, and the graft is left to develop. One bud is selected and allowed to grow. The young plant is placed in a dark room, usually on a table with a dark plastic cover and equipped with an air-circulating fan. The shoot is permitted to grow chlorophyll free until it is between 8 and 16 inches tall, and then it is removed from the dark chamber. Depending on the propagator's preference, a portion of the new stem adjacent to the bud union, which is tender and more readily rooted, is covered with sterile rooting medium, either in a second container or by filling a part of the planting sleeve that previously was folded and not used. If adventitious roots develop successfully, and this is not always the case, the nurse seed is severed from the rooting stem, and the newly rooted shoot is given time to develop under humid conditions. Once the plant hardens off, it is grafted to the preferred variety and is allowed to develop for 2 to 3 months. This double-grafted plant is later transplanted to a larger container and then grown for several months, preferably under shade cloth. Brokaw Nursery patented a variation of the Frolich method that streamlines the commercial propagation of clonal trees. The planting sleeve configuration, the length of the etiolated shoot, and the addition of a loosely clamped metal ring placed just above the bud union are included in Brokaw's technique. The innovation is that the ring is intended to constrict slowly the growing plant, eventually severing the nurse seed. In the case in which the nurse seed fails to separate or if the nurse seed is not physically removed, complications, mainly in the form of profuse suckering and the danger of exposure to root rot and viral infection

through the nurse seed, can occur. The addition of rooting hormones, mainly low concentrations of IBA (indolebutyric acid) and additives such as cytokinins, other auxin derivatives, vitamins, and mycorrhizae to the immediate area of the covered etiolated stem, greatly increases the chance for successful rooting. Some propagators make minor cuts in the base of the stem to encourage the penetration of the auxins and the enhancement of rooting.

To date, most attempts to tissue culture the avocado have not been successful. One promising approach to micropropagating being currently developed by Dr. Litz and Mohamed-Yasseen, in Florida, is the formation of somatic embryos from extracted nucelli. Somatic hybridization techniques are also under study by the same researchers. The cost of buying clonal trees is so high, and justifiably so, that over the past few years, I have been attempting to clone my own rootstocks. Stem rooting was the most difficult in terms of the percent of failures and the fact that even the survivors did not last long. Cloning, using etiolation, was much more promising but extremely slow and tedious. At one level of the learning curve, I began to introduce minimal scoring of the etiolated shoot at the area immediately above the graft. This is the section of the shoot that will be covered by the planting mix and is expected to rot. Additionally, I was applying certain levels of IBA to the wounded area to enhance rooting. Roots grew much more profusely, as compared with the stems that were not scored. My problem was the development of a callus in the area of the cut, which did not appear to interfere with root development but was aesthetically bothersome. Andre Ernst, from South Africa, and Dr. Oded Reuveni, from the Volcani Institute in Israel, came to my rescue during the Third World Avocado Congress that was held in Israel in October 1995. Their advice was that I should not be concerned with the callus phenomenon and that I would find the problem minimized if I were to reduce the level of IBA to 1 %. They were right, and it appears that lower levels of IBA result in almost callus-free rooting.

Since the conversations with Ernst and Reuveni, I have been following a procedure similar to Ernst's propagation method, which he described to me between meetings at the Congress. I have been successfully rooting, with my own modifications, these difficult-to-root avocados (Figures 1 and 2). *This method is rapid, produces a large number of healthy roots, and does not destroy the nurse seed.* The process is as follows: The seedling is grafted and etiolated in the Frolich method as described above. When the etiolated plant is removed from the dark chamber, a long bamboo stake is embedded in the container and placed alongside the shoot. A point about 3 to 4 inches above the graft is selected, the shoot is carefully wounded with a razor blade on two sides, and IBA is applied to the area of the cuts. (Wounding stimulates rooting through the accumulation of auxins and carbohydrates at the injured area. Additionally, etiolation encourages accumulation of auxins that are light sensitive and thus to be unstable in the presence of light.) A 6-ounce clear plastic cup is sliced from top to bottom with a knife. The cut continues to the center of the bottom of the cup, where a pencil-wide hole is made. The cup is placed over the shoot, with the stem fitting through the hole and the wounded part of the stem located about a quarter of the way below the top of the cup. The cup is taped to the stake with masking tape, and then filled with sterile rooting mix. If the shoot is long enough, a second cup is placed in the same manner, about 8 inches above the first cup, following the same procedure. In about a month, depending on the growing environment, there is a full complement of well-developed adventitious roots. When the roots can be seen through the cup, the shoot is

cut at the base of the cup, or cups, dipped in a fungicide, and the cups are placed on a propagating table for further growth. The nurse seed, with its existing original graft, is allowed to grow, and the whole process is repeated again. This system is very efficient: the wounded tissue is juvenile and elongated, and roots proliferate under such conditions. Even if only two plants are produced before the nurse seed is discarded (one clone is generated conventionally and the other using the cup technique), they are still the product of only one nurse seed and one graft, rather than the two seeds and two grafts required by the conventional method. It is likely that if the nurse plant is adequately nourished, it will continue to generate new shoots for further rooting.

Figure 1. Root system of rooted clone.

Figure 2. Cup with rooting mix placed at a point on the etiolated plant and taped to the stake.

The process continues as with any other propagation method: the young plant is transplanted to a larger container, which is placed under shade cloth and later left in the open to harden off.

The fact that fewer seeds are needed to produce a large number of clones can make costly nurse-seed virus indexing less expensive. Indexing is a very meaningful procedure that is not practiced in the United States. In Israel, virus indexing is required once a year. Eventually, our industry may require virus indexing as well. This cloning procedure would minimize the number of tests.

For further reading on avocado tree propagation see:

Propagating Avocados: Principles and Techniques of Nursery and Field Grafting. Publication #21461, $7.50. Available from the: Division of Agriculture and Natural Resources, University of California, 6701 San Pablo Avenue, Oakland, CA 94608-1239, (510) 642-2431, FAX (510) 643-5470, (800) 994-8849 within California.