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EXPERIMENTS WITH CLONING AVOCADO ROOTSTOCKS

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Researchers and nurserymen have been trying to clone avocado rootstocks for many years. The main purpose of cloning is to propagate rootstocks tolerant to Phytophthora root rot. Other objectives of cloning are salinity tolerance, improved productivity, compatibility of the rootstock and scion, dwarfing, 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. Most efforts to tissue culture the avocado have not been successful. One promising approach to micropropagating being currently developed by Dr. Richard Litz, at the University of Florida, is the formation of somatic embryos from extracted nucelli. Somatic hybridization techniques are also under study by the same researcher.

The late E. F. Frolich, at the University of California at Los Angeles, 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 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 two to three months. This double-grafted plant is later transplanted to a larger container and then grown for several additional months, preferably under shade cloth. 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, 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. H. Brokaw 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 can occur, mainly in the form of profuse suckering and the danger of exposure to root rot and viral infection through the nurse seed.

Foreign competition threatens the California avocado industry. Increased productivity is, in my view, the only attainable means by which we will be able to compete economically. Sustained high production of good quality avocados, of over 20,000 pounds per acre (22,400 Kg per hectare), could be achieved with super high density planting and canopy manipulation. With some upright varieties, as many as 730 trees per acre (1,803 trees per hectare) when a 7.5 x 7.5 feet (2.3 x 2.3 meters) spacing, will be required. The current cost of buying clonal trees is high, and for high density plantings it becomes prohibitive.

In the past few years, to reduce the cost of densely replanting our old canopied groves grown on seedling rootstocks, I have been attempting to clone my own. 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 slow and with only 50-60% success rate. 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 root. Additionally, I was applying certain levels of IBA to the wounded area to enhance rooting. Roots grew much more profusely with this practice, as compared with the stems which 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% (currently I use 0.8%). 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 large numbers of healthy roots, and does not destroy the nurse seed or the graft. 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 which are light sensitive and thus are 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 conditions, 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, 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 still viable and adequately nourished, it will continue to generate new shoots for further rooting.

Figure 1.

Figure 2.

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 California. In Israel, virus indexing is required once a year. In South Africa, each tree carries a virus free certificate. Eventually, our industry will have to do the same.

The first year of experimentation with this method brought mixed results. Rooting was highly successful, but growing actual trees from the cuttings was marginal at best. With the help of knowledgeable individuals and better care, we are currently propagating 40,000 seedlings which should give us over 60,000 clones.

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.