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Possible Rejuvenation of Adult Avocado by Graftage onto Juvenile Rootstocks in Vitro

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Abstract. **Avocado *(Persea americana* Mill.) seedlings germinated aseptically were used as rootstocks. Lateral buds excised from greenhouse-grown flowering-age plants and disinfested, were used as scions. About 50% of the shoots that emerged from the scions showed restored rooting competence. The shoots continued to root when the terminals of rooted shoots were severed and retested. Nodal sections from grafted adults also produced rootable shoots. It is suggested that the restored rooting competence reflected a developmental phase reversal from adult to juvenile rather than a simple transfer of rooting cofactors from rootstock to scion.**

Many problems of avocado cultivation could be solved if clonal rootstocks with desirable traits, e.g., salt tolerance and rootrot resistance, were available. A critical factor in rooting avocado cuttings has been the developmental phase of plants from which cuttings are obtained; rooting competence generally diminishes with increasing plant maturation or decreasing juvenility (2, 3, 10). Indeed, ease of rooting is characteristic of plants or organs of the juvenile phase, whereas rooting in the adult phase has been difficult among woody perennials (17, 18).

Frequently, juvenile tissues are obtainable from mature trees. In some genera, they can be observed as unusually vigorous sprouts arising either directly from stem or from special growths, e.g., lignotubers, at the base of trees (e.g., *Eucalyptus* and *Sequoia).* Franclet (5) was able to force sprouts from juvenile reserves in numerous forest genera by severely pruning mature trees.

Grafting is known to cause phase reversion in woody perennials, e.g., *Citrus* (14), *Cupressus* (5), *Eucalyptus* (5), *Hedera* (21), *Passiflora* (11), and *Pseudotsuga* (6). Haas (8) was able to induce rooting in avocado stem sections after graftage onto young seedlings. The rooting was believed to result from stimulants furnished by juvenile tissues. Recent experiments by Shafrir (19) suggest that a phase-reversing factor might

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be transmitted from juvenile rootstocks to adult scions. Repeated grafts of adult avocado onto fresh rootstocks increases the incidence of rootable adult cuttings. Unfortunately, Shafrir (19) did not determine the extent to which the rooting competence could be sustained in tissues of grafted plants.

This investigation considered possible transmission of such juvenility traits as ease of rooting through in vitro grafting of lateral buds from mature avocado trees onto very young aseptically grown rootstock seedlings . Restored rooting competence in sections of shoots that emerged from scions was used as the main indicator of rejuvenation.

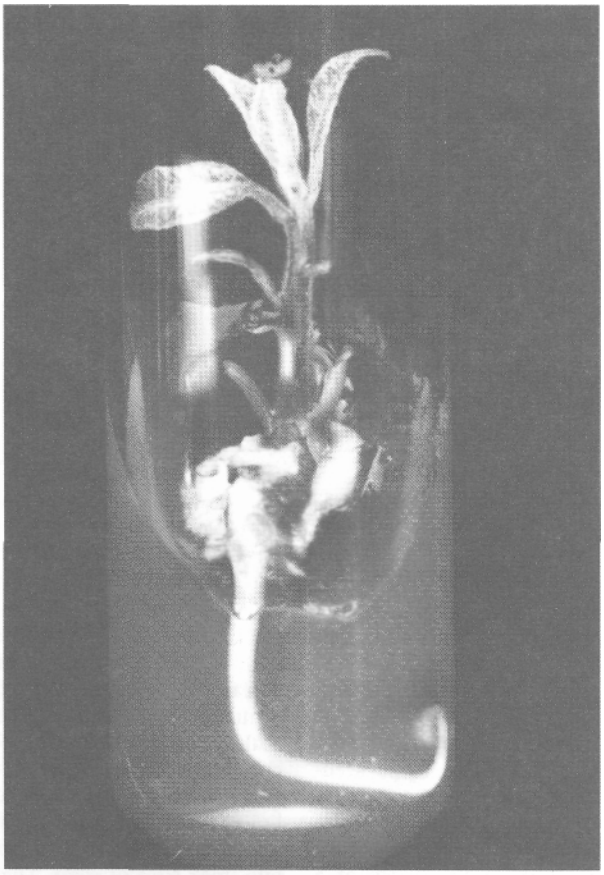

Fig. 1. Aseptically grown avocado seedling prior to use as rootstock in grafting.

Aseptic seedlings were obtained by immersing seeds without seed coats in a solution containing 0.5% sodium hypochlorite and 0.1% Tween 20, dividing them by half into separate cotyledons, excising the plumule-radicle axes together with 1- to 2-cm-thick sections of cotyledon, and transferring them into tubes of nutrient medium. When an avocado seed is halved, the plumule-radicle axis usually is attached to one cotyledon or the other. Explants with the entire cotyledon could not be used because of the risk of contamination, even though vigor of the seedling was dependent on the quantity of attached cotyledonary tissue. The germination medium contained Murashige and Skoog salts (13) and (in milligrams per liter): sucrose, 30,000; *i*-inositol, 100; thiamine -HCl, 0.4; activated charcoal (Sigma, neutralized powder), 1000; and TC agar (KC Biological), 8000. The pH was adjusted to 5.7 before adding agar. Medium was dispensed in 25 ml quantities into 25 x 150 mm glass tubes. The tubes were capped with polypropylene closures and autoclaved for 15 min at 1.05 $kg \cdot cm^{-2}$ and 121°C. The seed germination tubes were maintained upright in 4 x 10 stainless steel racks. One plumule-radicle axis was planted in each tube, which was then incubated at 27° with 16-hr daily exposure to 1000 lux illumination (Sylvania regular spectrum Gro-Lux lamps). Four-week-old seedlings were used as rootstocks (Fig. 1).

The 'Topa-Topa' cultivar, as well as others of the Mexican race, were used as rootstocks. In rooting competence tests, shoots from seedlings of 'Duke 7' were used. Fifteen to 20 shoots were used per treatment.

The shoots of 4-week-old aseptic seedlings were shortened to 0.5 cm and their axillary buds removed. Using a No. 11 blade, an incision to a depth three-fourths of its diameter was made down the length of a shoot. To ensure proper fit of scion in rootstock, the incision was spread open slightly with the dull side of the blade.

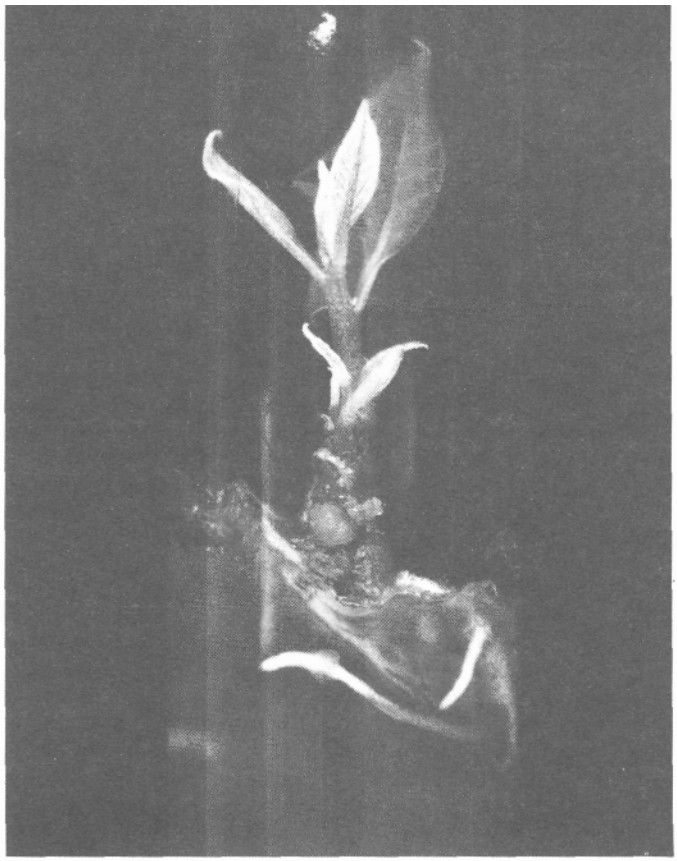

Fig. 2. A successfully grafted avocado in vitro. Elongated shoot is from adult scion bud.

Lateral buds from adult plants were trimmed and disinfested. The outermost bud scales then were removed. Two transverse cuts were made diagonally and downward through the stem, one above and the other below the bud. These cuts isolated the trimmed bud, attached to one end of an ≈*1*mm-thick slice of stem. The stem slice was pared down to a suitable width to fit the incision in the rootstock by removing some tissue along one edge. The scion was finally set into the rootstock, with the stem portion completely enclosed in the incision and the bud portion exposed above the cut surface of the shoot. Dessication, a serious obstacle to grafting, was overcome by applying a thin layer of moist nutrient agar over the graft area, but not on the bud. This layer of agar connected the bud and the graft area with the medium in the culture vessel. The agar was gradually removed from the graft area, starting from the top and progressing to bottom, from 1 week to 3 weeks. Failure to remove the agar interfered with development of good unions. The grafts were incubated in the seed germination medium, supplemented with 222 μM *N*-(phenylmethyl)-1*H*-purin-6-amine (BA) and 616 μM NaH_2PO_4-H_2O. Cultures were maintained erect, rather than as slants. They were incubated at 27°C temperature and 6000 lux illumination. Axillary shoots that emerged from rootstocks were removed. After 6 to 8 weeks, about 70% of the grafts had succeeded and provided shoots for rooting experiments (Fig. 2).

Adult phase shoots used as controls in rooting tests were obtained as nodal segments from greenhouse-grown 'Duke 7' plants. Potted plants were established by grafting scions from fruit-bearing trees onto seedling rootstocks using conventional techniques (9). Source plants were at least 2 years old and had flowered before explants were taken. The nodal segments were obtained from soft, actively growing shoots, 15 to 25 cm long. Leaves were removed and the shoots were disinfested by immersing in dilute

hypochlorite solution for 10 min. The shoots were cut into 1- to 1.5-cm-long sections, which were disinfested further in hypochlorite solution for 10 min, rinsed with autoclaved water, trimmed to remove tissue damaged by disinfestant, and each section placed in a nutrient tube. Nutrient medium was the same as that used for maintenance of the grafts. Shoots were incubated at 27°C temperature and 1000 lux illumination. After 7 weeks, about 50% of the explants produced shoots 0.5 to 1 cm long with one to three small leaves.

Fig. 3. Behavior of juvenile, adult-grafted, and adult avocado shoots in rooting test developed with juvenile explants. The basal medium contained 0.3× Murashige and Skoog salts and, in milligrams per liter: sucrose, 30,000; thiamine·HCl, 0.4; *i*-inositol, 100; and TC agar, 8000. The auxin medium contained 123 μM IBA and the auxin-free step employed no IBA but 1 g·liter^{-1} charcoal. Data were taken after 8 weeks in auxin-free medium.

The rooting characteristics of juvenile, adult, and grafted adult were compared using a two-step rooting procedure. Terminal shoot cuttings from various sources were placed for 3 days in medium containing Murashige and Skoog salts at 0.3 x standard strength and, in milligrams per liter: sucrose, 30,000; thiamine-HCl, 0.4; *i*-inositol, 100; IBA, 25; and TC agar, 8000; and then transferred to another medium containing Murashige and Skoog salts at 0.3 x standard strength and (in milligrams per liter): sucrose, 30,000; thiamine-HCl, 0.4; *i*-inositol, 100; activated charcoal, 1000; and TC agar, 8000.

Juvenile shoots were obtained from seeds germinated in vitro. Adult shoots were obtained as they emerged from nodal segments. Grafted adult shoots were those that arose from adult scion buds following grafts onto young seedlings in vitro. The possibility of phase reversal was assayed by examining effects of repeated grafts of adult shoots onto fresh seedlings rootstocks and sustained rooting among subcultures of grafted shoots.

Shoots from juvenile avocado rooted with virtually a 100% frequency in vitro. In contrast, those that emerged in nodal cultures of ungrafted adult avocado failed to root. The shoots that resulted from grafts of lateral buds of adult sources onto juvenile rootstocks rooted with a frequency of about 30% (Fig. 3). Average number of roots per shoot were 3.5 ± 0.4 and 1.9 ± 0.2 for the juvenile and grafted adult shoots, respectively.

Regrafting of adult shoots onto fresh seedling rootstocks repeatedly (as many as three times) did not improve rooting or general vigor of shoots.

Grafted adult shoots that rooted were recut to remove their rooted bases and retested twice for rooting competence. The cuttings retained their rooting competence.

Perhaps significantly, both grafted and ungrafted adult shoots responded poorly to conditions that favored rooting and growth of juvenile explants. Shoot cuttings of adult origin lost their leaves and many died following culture in IBA-containing medium. Accompanying the loss of leaves was poor rooting of shoots from the grafted source (Fig. 3).

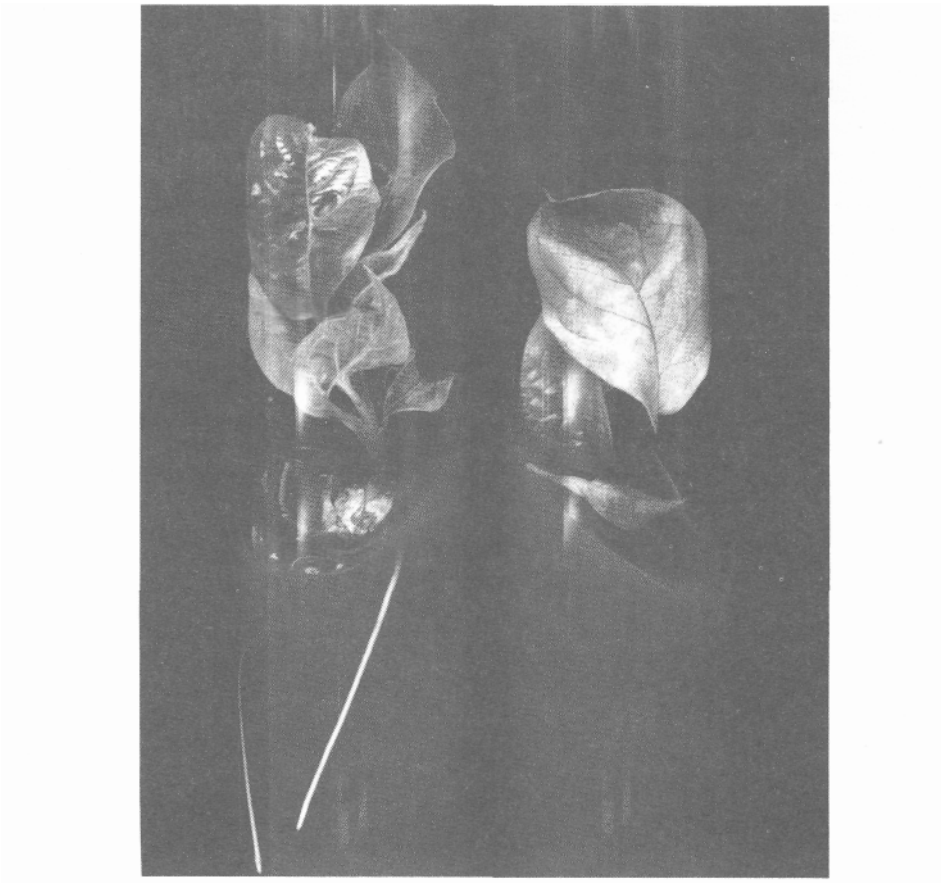

Fig. 4. Prevention of leaf drop in grafted adult avocado shoots in vitro by flooding nutrient surfaces with 1 ml of a 44.4-µM BA solution. **Left**: flooded. **Right**: unflooded.

It appeared that adult shoots, grafted or ungrafted, were sensitive or had a reduced tolerance to 1*H*-indole-3-butyric acid (IBA). A more favorable level of IBA in the rooting test was therefore considered for explants of adult origin. The auxin repressed rooting and caused death of grafted adult shoots in concentrations of 14.7 to 147.6 µM, whereas increased rooting was observed when the first step medium contained 1.4 or 4.9 µM IBA. The 4.9 µM was selected inasmuch as it generated more roots per cutting than other concentrations even though the incidence of rooted cuttings was the same as that of 1.4 µM. Additional tests confirmed that 4.9 µM IBA was optimum and caused 50% of grafted shoots to root with two to three roots per shoot.

Reuveni and Raviv (16) reported the use of cytokinin applications to delay leaf shedding and improve rooting of avocado cuttings in vivo. Flooding of implanted tubes with varying concentrations (0-44.4 µM) of BA during the second step of the rooting procedure did not significantly improve incidence of rooting or number of roots per cutting. Nevertheless, BA treatments delayed leaf injury and abscission and produced vigorous cultures. Representative cultures are shown in Fig. 4.

Juvenile shoots consistently showed rapidly elongating stems with green leaves. In contrast, ungrafted adult shoots showed poor stem elongation, heavy callusing,

retarded enlargement, and a high tendency of leaves to abscise. Following grafts onto young seedlings, the adult shoots retained the tendency to abscise; however, their steins elongated more rapidly and produced less callus and their leaves grew more rapidly than those of ungrafted adults. Nodal sections of grafted adults, after being subjected to the two-step rooting procedure, exhibited axillary shoot elongation that was distinctly more vigorous and luxuriant than that of ungrafted adults.

With a large number of woody perennials, asexual propagation, other than by grafting, requires use of cuttings or explants from sources in the juvenile phase of development. When natural sources of juvenile tissues are absent, the adult plant must be rejuvenated. In tissue cultures, many examples are known where phase-reversal has occurred during the normal course of subculturing or by special culture treatments (1, 4-7, 12, 20, 22). Grafting of adult scions onto juvenile rootstocks has been shown to cause reversion in several species in vivo (5, 6, 11, 14, 21).

With avocado, rootable stem cuttings of adult material have been obtained by grafting the adult onto juvenile rootstock (8, 19). Whether this is rejuvenation or simply a transfer of rooting stimulants from rootstock to scion has remained unresolved. In this investigation, grafts were performed in vitro to explore the question.

Grafting of adult avocado lateral buds onto young seedlings in vitro resulted in scion shoots that rooted with an average 50% frequency. Re-grafts of the emergent scion shoots onto fresh rootstocks three times did not extend the rooting incidence. Additional regrafts might improve rooting further (unpublished data). Subsections of rooted shoots continued to root without further grafting.

The grafted adults produced stems that elongated more rapidly than those of ungrafted adults. The growth characteristics resembled more nearly those of juvenile shoots.

Interestingly, juvenile shoots were more tolerant to high auxin levels than adults. A 123-µM level of IBA was about optimum for juvenile cuttings. This level caused injury to leaves and ultimate defoliation of adult cuttings. It was also lethal to a significant percentage of the cuttings. A 4.9-µM IBA concentration was appropriate for cuttings of adult origin. Porlingis and Therios (15) have also reported a reduced tolerance of adult cuttings to high concentrations of auxin.

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