ROOTSTOCK PRODUCTION AND BUDDING

Brent Holtz, Louise Ferguson, Dan Parfitt, Gerald Allen, and Ron Radoicich

California pistachios are grown primarily on three rootstocks, two species and one interspecific hybrid, all members of the genus *Pistacia*. They include atlantica (*P. atlantica*) Pioneer Gold I (*P. integerrima*), and UCB I (University of California, Berkeley), which is a hybrid between *P. atlantica* ♀ crossed with *P. integerrima* ♂. Two other rootstocks have occasionally been grown in California; they include *P. terebinthus* and Pioneer Gold II (*P. atlantica* ♀ crossed with *P. integerrima* ♂).

Pistachio rootstocks are produced from seeds. All are open pollinated except for UCB I, which is the result of closed pollination. Thus, there is more potential for variability among the non-UCB I hybrids. In the San Joaquin Valley, PG I and UCB I are the most commonly used rootstocks. In colder areas outside the San Joaquin Valley, *P. atlantica* and UCB I are the most commonly planted rootstocks. Only two cultivars, both *P. vera,* are widely used as scions in California. They consist of the female cultivar 'Kerman' and the male pollinizer 'Peters.'

ROOTSTOCK SEED GERMINATION

The requirements for seed germination vary little among the rootstock species. Rootstock seeds are relatively small with a hard unsplit endocarp or shell. Seed coats can be removed by rubbing over screens. Seeds are usually stratified in a refrigerator (36-40 °F / 2-4 °C) for one to three months before they are induced to germinate, usually in February.

After stratification, the seeds usually are soaked in tap water from 2-48 hours at room temperature. Some nurseries dip their rootstock seeds in muriatic or hydrochloric acid (3-5% bleach), followed by a thorough wash. Germination rates of *P. terebinthus* have been doubled and the incubation time reduced by treating seeds with concentrated sulfuric acid (H_2SO_4) to wear away the seed coats (scarification). Thorough rinsing after the acid treatment is essential to prevent complete digestion of the seed coat.

After the acid treatment and wash, seeds are usually soaked for another 1-2 hours before planting. After soaking, water is drained away and the seeds are placed in a single layer in the center of moistened clean (sometimes bleached) white cotton "Turkish Towels." The excess towel is then folded over the seeds and rolled up. Another moistened towel is often placed over the rolled up towel containing the seeds. The seeds are kept moist (sprayed with distilled water) and incubated at temperatures between 70-80 ºF (21-27 ºC) for 3-6 days. Seeds are checked daily (sometimes twice) and removed with sterile tweezers when they have germinated and their hypocotyls have emerged.

PLANTING

After seeds germinate they are planted, usually in Jiffy® liner pots (Jiffy 7 or 757s), flats, fumigated nursery beds, or directly into plastic planting cones. Some nurseries lightly sterilize their germinated seed with low concentrations of bleach (3%) in water. Most commonly, germinated seed is planted into Jiffy liner pots and placed in greenhouses where temperatures are kept between 70-90 ºF (21-32 ºC). The germinated seed should be watered regularly but not over-watered, for over-watering can result in damping off root rot diseases. The benches on which these liner pots or flats are placed should be clean and sterile, for several plant pathogens can survive on dirty benches and infect young pistachio rootstock seedlings.

TRANSPLANTING

The seedlings should be 3-4 inches (7.5 - 10 cm) tall by mid-April; at this point they can be transplanted into pots and placed outside where

they will receive full sun. Seedlings transplanted from flats may initially suffer transplant shock, but will eventually develop a more fibrous root system if broken and damaged roots are pruned and remaining roots uniformly spread out. Seedlings transplanted from the Jiffy peat pots will not suffer transplant shock, but care must be taken not to break down the edge of the peat pot when transplanting. Irregular water distribution may delay seedling root development in the surrounding soil.

Seedlings are usually transplanted into various potting mixtures depending on the nursery. Standard seedling mixes usually contain soil, humus, perlite and peat moss in various concentrations, which are usually amended with sand to improve drainage and structure. Various commercially available Mycorrhizae mixes are often added to the potting mixes to improve nutrient uptake (especially nitrogen and phosphorus) through the symbiotic relationship between the rootstock and fungi. After transplanting, the seedlings are watered and fertilized regularly, and are continuously suckered (every 10 days) in order to keep their terminal buds growing. The rootstocks are tied to three-foot (92-cm) bamboo stakes when they are 10 - 16 inches (25 - 40 cm) tall.

Seedlings may be transplanted into various pots, typically six inches (15 cm) in diameter and 14-16 inches (35 - 40 cm) deep. Some pots, such as tar paper, felt, or paper fiber, can be used and planted directly into the orchard, avoiding transplant shock due to root disruption. Tall plastic pots of similar size also are frequently used, especially by nurseries better suited for the extended maintenance of rootstocks in lath houses and outdoor nurseries. The plastic pots are more durable and reduce evaporation through the pot itself. Plastic pots also provide more protection for the seedlings during shipping and handling, but must be removed at planting. Pistachio rootstocks are highly susceptible to injury, and care should be taken when handling them.

TRANSPLANTING INTO THE ORCHARD

Pistachio rootstocks, whether budded or not, are usually planted from potting containers directly into the orchard. Potted plants are delivered in plastic or fiber pots 14 - 16 inches (35 - 40 cm) tall. It is possible to transplant bare root nursery stock, but because bare rooted trees are very sensitive to drying, this is not a common practice. Proper tree care after planting is more crucial to survival than the type of container the trees came in. Trees should be well watered immediately after planting, and soil moisture should extend well below the three-foot root zone. A second irrigation should follow within two weeks, followed by a regular fertilization and irrigation program. Rootstocks are staked to insure straight tree growth for budding and to protect them from excessive wind.

TRANSPLANTING 3-4 INCH SEEDLINGS DIRECTLY INTO THE ORCHARD

In an effort to reduce the time period seedlings spend in nurseries and consequently the expense of the rootstock; four-month-old UCB I seedlings are sometimes transplanted directly into the orchard. These seedlings are typically germinated in January and planted directly into 2 X 10 inch (5 X 25 cm) plastic planting cones. The germinated seeds are planted approximately ¼ inch deep and hand watered daily with a fine mist. The cones are packed into plastic fresh fruit boxes (15 X 16 X 5 inches or 38 X 40 X 13 cm) and placed in greenhouses where they are bottom heated at temperatures between 70-80 °F (20-28 °C). The seedlings are between 6-10 inches (15-25 cm) high when they are planted in orchards in April and May. At planting time the tip of the planting cone is cut off and the rootstock is easily pushed out of the tube into a small hole created with a 2 inch (5 cm) diameter bulb planter. The planting cones appear to enhance early root growth by providing a wider and deeper space for root growth. Planting germinated seed directly into cones also provides an earlier opportunity to inoculate the planting mix with Mycorrhizae when compared to using Jiffy pots.

Since the seedlings are very small and vulnerable they can be placed in plastic grow tubes which are open at each end and can be attached to planting steaks (Plate 9A-grow tube). The ventilated plastic tubes appear to protect young rootstock seedlings from high winds, rodents, sunburn, and herbicides while providing an ideal micro-climate for growth. The ventilated plastic tubes (Treessentials/Snap N' Grower) can actually snap apart and back together, which makes it ideal to remove and replace when budding. These tubes have been used successfully to start grape vineyards. The bottom end of the tube should be buried at least ½ inch (1-2 cm) under the soil surface in order to avoid creating a "chimney" effect which could cause the seedlings to over heat. Various fertilizers are often added to enhance rootstock growth.

Budding should be done in July as described below. By July the seedlings should be between 20-26 inches (50-66 cm) tall. It may be necessary to snap off some of the rootstock that is above 26 inches to increase girth in order to enhance budding. After budding the grow tubes can again be placed around the seedlings in order to push the Kerman or Peters buds.

UCB I appears to be better suited for early orchard planting since it is able to push more growth in a shorter time period while being more cold tolerant than *P. integerrima*. If the seedlings are not sold the first year the nursery can transplant them into typical potting containers and sell them as 14 month old rootstocks the following year.

BUDDING

Scion cultivars, 'Kerman' and 'Peters,' are propagated via standard budding and grafting techniques. Both budding and grafting are similar in that a portion of one plant is joined to another to form a compound genetic system of one scion and one rootstock cultivar. With budding, a single bud is placed on a stock plant; with grafting, a larger portion of the stem is used as the scion. When planting a new pistachio orchard, rootstocks are planted in February or March and budded in late June, July, or early August. Budding is usually done in early summer when the scion buds are mature and dormant, and the bark on the rootstock is slipping and active. Red leaves on the new rootstock growth is a good indicator that the bark is slipping. If bud take is unsuccessful, rootstocks can again be budded until September. Some rootstocks are budded in the nursery and sold as replants 20-26 months later, either in October/November or the following February/March (Plate 9B-nursery budded). When *P. atlantica* was the rootstock of choice, budding was usually performed the second season, or very late in the first season, since its growth is slower when compared to UCB I or *P. integerrima.*

T-budding is the most efficient and most commonly used method in commercial pistachio production, used 99% of the time. T-budding is performed when the rootstock bark is slipping during the early summer and the scion buds have matured and hardened (Figure 9a). Chip buds and patch buds have also been used successfully to bud pistachio scion onto rootstock (Figure 9b). Patch budding, however, requires a larger caliper rootstock and is therefore more likely to be done in the field in the spring or fall. Chip and patch budding are not common procedures in California. Rootstocks sometimes produce excess sap, called gumming, which can be a problem when budding in early summer.

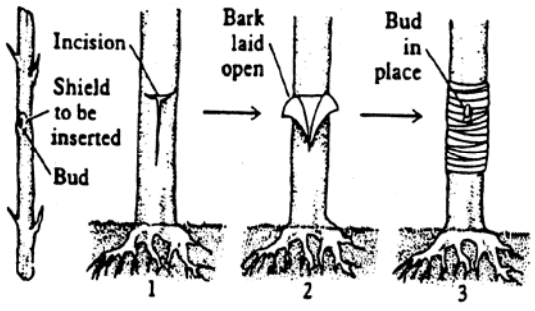

Figure 9a. The T-bud is the most common method used to bud pistachio scion onto rootstock. The shield is cut from the budstick and inserted into a T-cut on the stock.

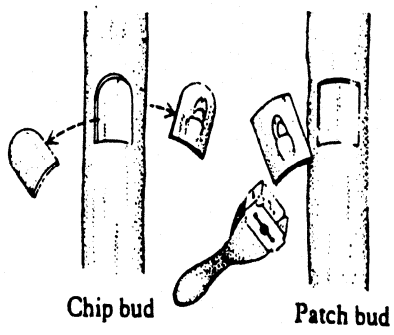

Figure 2b. The chip and patch buds.

Although rarely done, two types of grafts have been used with pistachios. The standard cleft graph (Figure 9c) is the most common and may be done either in a greenhouse or field. The saddle or inverted cleft graft has also been used to graft pistachios. Both are usually more successful if performed in late summer.

Healthy rootstocks are essential for successful grafting or budding of pistachio. Trees should be the correct size for the procedure to be used; scion and rootstock diameters should be similar in size. Generally, trees between ¼ to ½ inches (0.7 - 1.5 cm) are useful for T budding, chip budding and saddle grafts. Larger trees between ½ - ¾ inch (1 - 2 cm) in diameter are needed for patch buds. Trees from ¼ inch (0.7 cm) to more than ¾ inch (2 cm) can be cleft grafted as long as a good cambial match between scion and rootstock is maintained. Accurate matching of cambia is also required for chip buds.

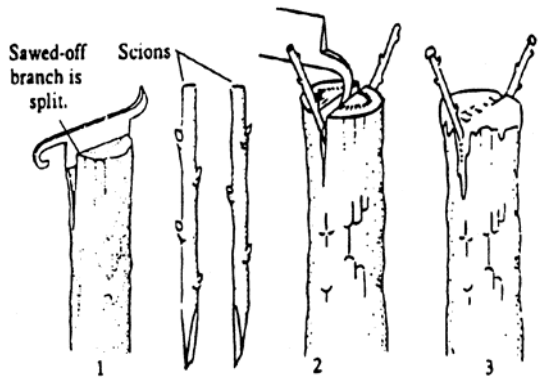

Figure 9c. The cleft graph.

Careful selection of ideal scion buds is essential for successful budding. Selection of scion wood from semi-hardened water sprouts or nonfruiting shoots is generally the best. Since most dormant buds on mature pistachio trees are flower buds, propagators select vegetative buds based on their size and location. Vegetative buds are almost always smaller than flower buds and tend to occur at the base of current-year growth on flowering shoots. Vegetative buds tend to be more pointed than flower or fruit buds that are more rounded. Young, rapidly growing shoots with greater than 20 inches (50 cm) of current growth will tend to have more vegetative buds. Generally, younger trees less than six years old are the best source of budwood.

For successful budding and grafting, a razor- sharp budding or grafting knife should be used to make smooth cuts. The bud or scion should be dormant and the rootstock should be growing in a nursery or planted in an orchard. The cambial zones of the scion and rootstock should make intimate contact in at least one point. With cleft grafting, do not line up the cambia precisely, but slant the scion into the cleft so that the cambia cross at one point. Wrap or secure the union to prevent movement. Budding and grafting wraps can be made of various materials, but the budding rubber is the most common and widely used. It is secured by looping the end under the last wrap. Waxes are available to help prevent drying of the scion and cambial zones, but are not typically used when T-budding.

Good systems for production of clonal scions on seedling rootstock are presently available and are widely used commercially. The exact procedures by which pistachio nurseries produce their products are not given in order to protect their interests.

MICROPROPAGATION OF PISTACHIO ROOTSTOCKS

Clonal micropropagation of pistachio (*Pistacia vera* L.) using tissue culture techniques has been reported in the literature since 1982 (Barazi and Schwabe, 1982). There are now more than 12 papers reporting successful propagation of *P. vera* using various medium modifications and

culture conditions, the most recent being from Onay (2000). Propagation protocols for *P. atlantica*, *P. terebinthus*, and *P. integerrima* have also been reported (Picchioni and Davies, 1990; Pontiks, 1984; Parfitt and Almehdi, 1994). Propagation of *P. vera* is relatively straightforward (Barghchi and Alderson, 1983a,b). However, micropropagation of pistachio rootstock species and hybrids has been more difficult, generally characterized by a lower multiplication rate and greater rooting difficulty.

The general approach involves sterilizing actively growing vegetative shoot tips and placing them in an artificial sterile medium combined with a support matrix. Shoot tips are usually pre-washed in running water for a number of hours. Sterilization is accomplished with a mild Sodium hypochlorite solution (10-15% Chlorox) followed by repeated washes with sterile water. A surfactant or wetting agent such as Tween 20 is often added to the Sodium hypoclorite solution. The sterilization step is critical, since insufficient sterilization results in contaminated shoot tips while excessive sterilization results in dead shoot tips. Exact concentrations of the sterilization solution and length of sterilization vary between investigators and depend in part on where and when the tissue was collected. Generally field collected tissue is more contaminated than greenhouse grown shoot tips. Levels of exogenous and endogenous fungi and bacteria increase later in the season. Internal bacterial contamination is especially problematic (Cassells, 1991) and can be partially avoided by shoot collection from greenhouse grown plants in the spring. Parfitt and Almehdi (1994) avoided many of these problems by using a carbon deficient medium supplemented with CO_2 to propagate the shoot tips.

After sterilization, 1 mm to 1cm shoot tips are placed on a solid medium (Difco agar, Gelrite or other sterile medium into which are incorporated N,P,K, minerals, and combinations of auxin (IAA, IBA) and cytokinin (TDZ, kinitin, BA) growth regulators. The ratio of auxin to cytokinin is critical to successful micropropagation and is usually determined empirically by the investigator. A high auxin to cytokinin ratio will favor rooting while a high cytokinin to auxin ration will favor shoot formation and multiplication. A carbon source (CO_2 or a sugar) is also included. Vitamins are sometimes added to specific media. Most media have been developed from Murashige and Skoog's medium (1962) or the DKW medium (McGranahan et al., 1987). Parfitt and Almehdi (1994) developed a modified medium for superior shoot multiplication of both *P. vera* and *P. integerrima* through manipulation of micronutrient levels and replacement of the sucrose carbon source with CO_2 and high light intensity. After a multiplication phase, explants were separated and moved to a Gelrite based rooting medium without a sugar carbon source, followed by acclimation in a perlite based solid substrate (Plate 9C-clonal UCB-1).

The final and perhaps most critical step in the process involves acclimation of the rooted explants. There are a variety of approaches that can be used, few of which are emphasized or described in the written literature. The general concept is to gradually expose the rooted plantlets to lower levels of relative humidity RH). Because the cultured plantlets are developed in an atmosphere that is maintained at 100% RH, the leaf surfaces do not develop a wax layer to restrict transpiration. Further, in some of the literature, authors suggest that the stomata function at a low level in-vitro and require a period of acclimation to begin to function normally. If cultured plants are taken from medium directly to a pot in the greenhouse or to the field, they will die. A protocol that is often used is to move the plantlets to a perlite solid medium, watered with a balanced N-P-K nutrient solution supplemented with micronutrients, such as Hoagland's solution or a customized solution similar to that used for rooting, but without growth regulators. High humidity conditions can be maintained with clear covers or using a fog system in the greenhouse. Light levels should be increased and humidity reduced over a period of 4+ weeks.

Some general micropropagation references are Bonga and Durzan (1987), Conger (1981), and Debergh and Zimmerman (1991), Ahuja (1993), Hartmann et al. (1997).

References

1. Ahuja, M.R. 1993. Miocrpropagation of Woody Plants. Kluwer Academic Publishers, Dordrecht; Boston. 507 p.
2. Barazi, Z. and W.W. Schwabe. 1982. Rooting softwood cuttings of adult *Pistacia vera. J.* Hortic. Sci. 57(2):247-252.
3. Barghchi, M. and P.G. Alderson. 1983a. In vitro propagation of *Pistacia* species. Acta Hortic. 131:49-60.
4. Barghchi, M. and P.G. Alderson. 1983b. In vitro propagation of *Pistacia vera* L. from seedling tissues. J. Hortic. Sci. 58(3):435-445.
5. Bonga, J.M., and D.J. Durzan (eds.). 1987. Cell and Tissue Culture in Forestry, Vol. 1. Martinus Nijhoff Publishers, Dordrecht, Boston. 422 p.
6. Cassells, A.C. 1991. Problems in tissue culture: culture contamination. In: P.C. Debergh and R.H. Zimmerman (eds.), Micropropagation: Technology and Application. Kluwer Academic Publishers, Dordrecht, Boston. p. 447-469.
7. Conger, B.V. (ed). 1981. Cloning Agricultural Plants. CRC Press, Boca Raton FL. 273 p.
8. Debergh, P.C. and R.H. Zimmerman (eds.) Micropropagation: Technology and Application. Kluwer Academic, Dordrecht, 484 p.
9. Hartmann, H.T., D.E. Kester, F.T. Davies Jr. and R.L. Geneve. 1997. Plan Propagation: Principles and Practices, 6th edition. Prentic Hall, Upper Saddle River NJ. 770 p.
10. McGranahan, G.H., J.A. Driver and W. Tulecke. 1987. Tissue culture of Juglans. In J.B. Bong and D.J. Durzan (eds.), Cell and Tissue Culture in Forestry, Vol. 1. Martinus Nijhoff Publishers, Dordrecht, Boston. P. 261-271.
11. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-479.
12. Onay, A. Micropropagation of pistachio from mature trees. 2000. Plant Cell Tissue & Organ Culture. 60(2). 159-162.
13. Parfitt, D.E. and A.A. Almedhi. 1994. Use of high CO-2 atmosphere and medium modifications for the successful micropropagation of pistachio. Scientia Hort. 56(4): 321-329.
14. Picchioni, G.A. and F.T. Davies, Jr. 1990. Micropropagation of *Pistacia atlantica* shoots from axillary buds. Plant Propagator Newsl., pp. 14-15.
15. Pontikis, E.A., 1984. In vitro propagation of *Pistacia terebinthus* L. Plant Propagator, 30 (3):14-15.