In Vitro Propagation of ‘Canino’ Apricot

Iona Snir
Institute of Horticulture, Agricultural Research Organization, The Volcani Center, Bet Dagan 50-250, Israel

Abstract. Propagation of apricot (Prunus armeniaca L.) has been achieved using several media for establishment, shoot development, and rooting. Most of the rooted plants were established successfully outdoors.

Apricot seedlings are commonly used as rootstocks for apricot cultivars (3); therefore, growing apricot trees on their own roots seems to be a logical possibility. Other fruit trees of the Rosaceae, such as peach (1) and pear (12), were found to be better supplied with mineral nutrients and demonstrated higher productivity when on their own roots than when grafted. Unfortunately, apricot cuttings are difficult to root (3). Therefore, the tissue culture method, which was found successful with other hard-to-root species (5, 9, 10), was tried to solve this propagation problem.

The literature contains only limited information on tissue culture of apricot (8). Thus, the goal of this work was to achieve an easy method for apricot micropropagation.

Budwood of mature ‘Canino’ apricot was grafted on seedlings of the same cultivar. The resulting plants were pruned and placed in a growth chamber in spring, after being left outdoors during winter. Sprouting and growth of the buds took place in a growth chamber at 25°C, with 16 hr light per day at an intensity of 60 μmol s⁻¹ m⁻² at the plant level. Developing shoots were cut into short segments when they reached a length of about 25 cm. After the removal of the leaves, the segments were put through a 2-stage surface disinfection with a solution of 70% ethyl alcohol + 0.05% Tween 20 for 5 min and 2.2% NaOCl + 0.05% Tween 20 for another 5 min, followed by rinsing 5 times with sterilized distilled water. These segments, which contained 1 or 2 buds, were placed on medium A (10 ml) in 25 × 100-mm culture tubes closed with ‘Bellco’ Kaputs.

The following media were examined in this study: medium A, according to Tabachnik and Kester (11), containing macroelements of Knop’s solution (3) with 1 mg/liter 6-benzylaminopurine (BA) and 1 mg/liter indolebutyric acid (IBA); medium B, according to Lloyd and McCown (6), with 2 mg/liter N°-Δ²-isopentyl adenine (2iP); medium C, according to Jones et al. (4), without phloroglucinol but with 1 mg/liter BA and 0.1 mg/liter IBA; medium D, the rooting medium (9), with 0.5 mg/liter 1-naphthaleneacetic acid. Medium C contained Murashige-Skoog (7) macroelements (MS) and medium D contained half strength (MS). The culture tubes were kept in a growth chamber with a light intensity of 40 μmol s⁻¹ m⁻² in a 16-hr light period and 70% relative humidity. A high light intensity of 200 μmol s⁻¹ m⁻² was used also in some.

The buds on the small segments swelled and sprouted and after 40 days they were cut and transferred to media A, B, and C, which are known as media for bud proliferation and development of several plants (4, 6, 9, 11). The apricot buds continued to grow only on medium B (on the other media they became yellow and died), and within a month 70% of the buds on this medium developed into shoots of 3 to 5 cm. These experiments were repeated 7 times in 2 years, containing between 30 to 40 buds per test. Bud development was better at low (40 μmol s⁻¹ m⁻²) than at high (200 μmol s⁻¹ m⁻²) light intensity with regard to percentage of shoots developing from the buds (70% vs. 40%). There was almost no bud proliferation and the propagation process was based mainly on the segments of the shoots which were cut and again planted on medium B to continue the propagation cycle. Between 3 and 5 nodal segments were cut from one shoot after 40 days on medium B. This propagation process has now continued at the same rate for a year.

To induce rooting, shoots were wounded by removing a piece of epidermis (about 5 mm) at one side of their base and planting it on medium D. Rooting initiated within a week and reached a level of 70-90% after 2 weeks. Roots of plantlets left on the rooting medium tended to grow out of the agar (Fig. 1a). The rooted plantlets were transferred to Jiffy-7 pellets and kept at high air humidity (close to 100%) and high light intensity (200 μmol s⁻¹ m⁻²). These conditions were essential at this stage. The plantlet was transferred to a pot with a mixture of 1:1 perlite when the new roots emerged from the Jiffy-7 pellet. For better establishment outside the culture tube, it was best to transfer the rooted plantlet to the Jiffy-7 pellet at an early stage of rooting (root length of 5-10 mm). This process presented other undesirable phenomena such as wilting of upper leaves and some times lower leaves, and the induction of a rest period. The humidity was reduced gradually and the plantlets were fertilized with 1% KNO₃ + 0.1% Sequestrene 138 (Geigy). When the plantlets reached a height of about 15 cm and their leaves expanded to their natural dimensions, they were taken outdoors to a screen house (Fig. 1b). The survival percentage at this stage was close to 100%.

Disinfection problems of the plant material lead us to choose the system cited above. It was almost impossible to take budwood from the orchard because of the heavy bacterial and fungal contamination. The only disadvantage of this system is that most of the apricot stock plants failed to grow again in the same season after the shoot’s removal.

Many woody plants require several subcultures before the shoots reach rooting competence (2). The apricot, in spite of being difficult to root as cuttings, developed a high rooting competence in a very early stage of the propagation process. Two-and-a-half months after the 1st transfer to acetic conditions, the 1st shoots were rooted. The stock plants were not juvenile since they had flowered already and bore fruit.

Literature Cited

Received for publication 1 July 1983. Contribution from Agricultural Research Organization, Institute of Horticulture, No. 678-E. 1983 series. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.
# Propagation of Date Palms by Shoot Tip Cultures

Brent Tisserat  
U.S. Department of Agriculture, Agricultural Research Service, Western Region, Fruit and Vegetable Chemistry Laboratory, Pasadena, CA 91106

## Abstract

Shoot tips, 0.5 mm in length, of date palm (*Phoenix dactylifera L.*) were established on Murashige and Skoog (MS) inorganic salts and (per liter) 30 g sucrose, 0.4 mg thiamine · HCl, 100 mg myo-inositol, 10 mg naphthaleneacetic acid (NAA), 3 g charcoal, and 8 g agar. After 8 weeks, tips were proliferated through axillary bud outgrowths on a liquid medium devoid of charcoal containing 0.1 mg/liter NAA and 10 mg/liter 6-benzylaminopurine (BA). These additional shoots then could be rooted by reculture to an agar medium devoid of BA containing 0.1 mg/liter NAA and successfully transferred to soil.

Date palms are propagated commercially by rooting the limited number of basal offshoots produced by desirable trees. Micropropagation of date palms through tissue culture techniques would offset slow growth rates and limited vegetative propagation potential and would provide large numbers of desirable clones on demand. Palm tissue culture techniques currently have been restricted to the production of aseuxal plantlets via callus (1, 2, 4). This paper describes the production of free-living date palms through lateral bud multiplication *in vitro* using seedling shoot tips as the explant source. Commercial propagation of date palms would be performed using offshoots obtained from choice trees, necessitating modification of the techniques presented.

Shoot tips were dissected from 2-year-old seedlings grown in a 1:1 peat : vermiculite mixture (v/v) in a greenhouse. The foliage and adventitious roots were decapitated, followed by acropetal removal of outward leaves to obtain a shoot tip which was 5 mm in length and about 3 mm in diameter. Tips were surface-sterilized in 2.63% NaOCl for 15 min. Additional leaves were removed and the apical meristem region with 3–5 leaf primordia was planted on nutrient medium. The basal medium consisted of MS salts (3) plus (per liter), 30 g sucrose, 100 mg myo-inositol, 0.4 mg thiamine · HCl, and 8 g Phytagar at pH 5.7. Twenty-five ml were distributed per 25 × 150 mm culture tubes and these were capped withenclosures (Belco Kups). The medium was autoclaved at 11 kg/cm² and 121°C for 15 min. Explants and subsequent cultures were maintained at a constant 28°C under a 16-hr photoperiod at 1 klx. Ten to 20 replicates were employed in each treatment. Cultures were transferred to fresh medium every 8 weeks. Liquid medium was employed in some cases. Cultures were agitated continuously at 70 rpm on a gyrotory shaker in 120-ml polypropylene specimen containers.

### Shoot establishment and proliferation

Additions of BA, indoleacetic acid, NAA, and sulforaphaneacetic acid at 0, 0.1, 1.0, 10, and 100 mg/liter levels to basal medium supplemented with 0.3% charcoal were tested on excised shoot tips in preliminary work. Initial contamination rates varied from 20–40% after the first culture period. High auxin levels stimulated callus growth; low auxin levels aided in shoot differentiation. Addition of cytokinins at any level did not enhance shoot differentiation. Medium containing 10 mg/liter NAA only was found to be satisfactory for shoot tip establishment in culture based on culture survival and length of shoot growth. About 5–10% of the shoot tip cultures grown in this medium produced axillary bud outgrowths. Axillary bud outgrowths from tips cultured in other treatments were not observed. Adventitious root formation from tips was found to occur intermittently when tips were cultured on medium containing NAA; tips cultured in other treatments did not produce roots after 8 weeks in culture.

| Concn (mg/liter) | Axillary budding (%) | No. buds ± SE | culture |
|------------------|----------------------|---------------|
| NAA BA | 16 weeks | 32 weeks |
| 0.1 0 | 12.5 1 ± 0.6 | 1.4 ± 0.8 |
| 0.1 0 | 1.0 0 | 0.0 in agar |
| 0.1 0 | 22.2 1.2 ± 0.8 | 3.5 ± 2.4 |
| 0.1 0 | 22.2 1.2 ± 0.9 | 10.0 in liquid |
| 0.1 0 | 1.0 0 | 27.3 |

10 to 15 replicates originally employed per treatment.

Various combinations and concentrations of NAA (0, 0.1, 1.0, and 10 mg/liter) and BA (0, 0.1, 1.0, and 10 mg/liter) were added to basal medium to test their effect on enhancing shoot proliferation from excised shoot tips explants. Shoot tips exhibited limited axillary bud formation on a wide variety of media (e.g., 0.1 mg/liter NAA with 1.0 or 10 mg/liter BA) (Table 1). Axillary budding was not observed from cultures on media containing 0 or 10 mg/liter NAA regardless of the BA levels. After 2 recultures (4 months), shoots usually grew too large to be recultured in the 25 × 150 mm culture tubes. Adventitious roots commonly developed in several cultures when cultured on media containing 0.1 or 1.0 mg/liter NAA with 0.0–1.0 mg/liter BA. Transfer of 15 established shoots obtained from explants initially cultured on medium containing charcoal and 10 mg/liter NAA to liquid or agar basal medium with 0.1 mg/liter NAA and 10 mg/liter BA in 120-ml specimen containers allowed for further shoot differentiation and proliferation. Motion in the liquid medium often fragmented the cultures; leaves in these cultures became succulent and enlarged considerably compared to those incubated on agar medium.

Axillary shoots grew out from cultured shoots in this environment more frequently than from shoots cultured on agar medium (Table 1; Fig. 1).

### Rooting and transplanting shoots to soil.

Adventitious rooting was obtained readily after reculturing separated shoots to an agar nutrient medium containing 0.1 mg/liter NAA without charcoal, following 8–16