The Use of Fogging and Phototron Units to Acclimatize in Vitro-derived Apple Shoots

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Summary. Acclimatization and growth of in vitro-derived apple shoots of two apple scion apple cultivars were compared under fogged conditions in a greenhouse and in a commercial growth cabinet (Phototron). Plant survival rates of microcuttings of 'Royal Gala' and 'Jonagold' were significantly better when maintained in the Phototron units than when grown in a greenhouse under fog. The number and length of roots on microcuttings was significantly higher in the Phototron than under fog. In the present study, we demonstrated that the Phototron environment was better than a fogged greenhouse for establishing apple shoots ex vitro. However, the Phototron units are so small that they hold no more than 100 to 120 plants at a time. Therefore, the units will be of most value to growers or individuals in laboratories who do not have a constant need for acclimatization facilities. Growers who acclimatize many plants should continue to use fogging or misting facilities.

Tissue culture-derived plantlets must go through a transition phase from the protected environment of the laboratory to the harsh conditions of the greenhouse or field. Survival of microcuttings during this transition depends on the species, previous culture conditions, and hardening-off environment. In general, herbaceous species acclimatize easier than woody species (Marchant, 1982; Mohammed and Vidaver, 1988). In vitro preconditioning treatments have been used to increase survival rate ex vitro (Wardle et al., 1983; Ziv, 1986).

Humidity, temperature, and light are key environmental factors that affect plant survival. High humidity is essential to prevent water loss and desiccation of plant tissue (Donnelly et al., 1986; McCown, 1988). Temperatures around 28C and adequate light favor rapid root development and plant establishment (Moss and Dalgleish, 1985; Shinn, 1985). Normally, these conditions are provided within a greenhouse modified for ex vitro acclimatization. Greenhouse construction and management can be very expensive. In this study we compared ex vitro acclimatization rates of apple shoots in an inexpensive commercial growth cabinet (Phototron) to fogged conditions in a greenhouse.

Materials and methods

Phototron units. Four Phototron units (Pyraponics Industries, San Diego) were used in this study. The units are small (50 cm in diameter × 1 m tall) growth cabinets that were fitted with two shelves. Each unit was connected to a 3.8-liter sonic humidifier via 10-cm-diameter flexible tubing (Fig. 1). The units were operated in a warm room (23 ± 2C); the temperature within the units remained at a constant 27C. The relative humidity within the units remained at a constant 100%. The lights within the chamber [cool-white fluorescent (70 $\mu mol \cdot s^{-1} \cdot m^{-2}$)] were on constantly. The humidifiers were refilled every 12 h.

Fogged greenhouse environment. Fogging was provided by an Agritech 520 fogging unit (Agritech, Broadway, N.C.) that was controlled by a 10-min and a 24-h time clock. The timer was set such that the fogger operated for 6 min and was off for 4 min throughout the daylight hours (7:00 a.m. to 7:00 p.m.). Under these conditions, relative humiditywas maintained at close to 100% throughout the daylight hours. The total output of water was 57 to 76 $liter \cdot h^{-1}$ during the daylight hours. The fog system was turned off at night; the relative humidity fell to about 40% to 60%, and the mean temperature fell to about 20C. Under these night conditions, some leaves began to desiccate. To minimize desiccation, a periodic mist system was operated at night at a frequency of 20 sec of mist per 15-min intervals. The light intensity in the greenhouse was about 1000 $\mu mol \cdot s^{-1} \cdot m^{-2}$ during the day. No supplemental light was provided. Temperature within the greenhouse was maintained at 25 ± 4C by heaters, exhaust fans, and evaporative coolers. All experiments were conducted in Apr. and May 1989.

Rooting medium. Jiffy Mix (Jiffy Mix of America) was used for this study because it was superior to other rooting media for rooting of apple in preliminary experiments (data not presented).

Source of microcuttings. Vigorously growing cultures that had been

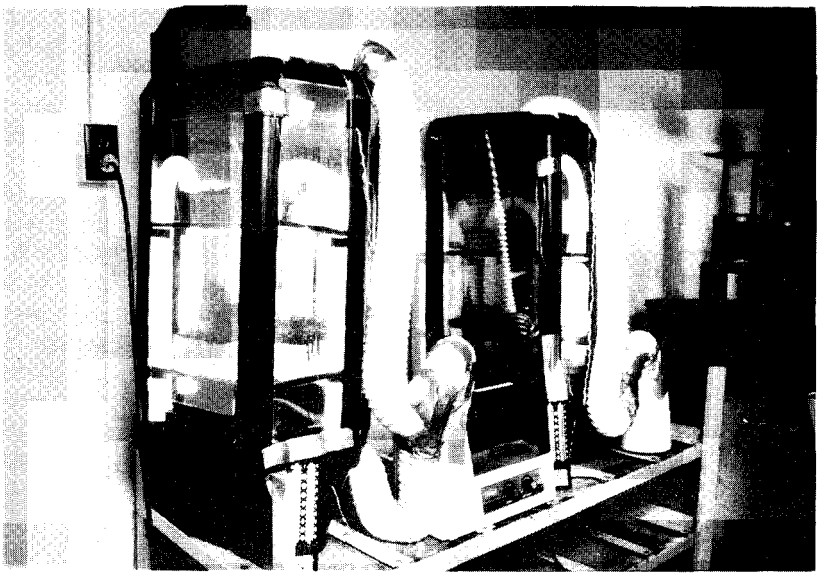

Fig. 1. *A Phototron unit that has been connected to an ultrasonic humidifier via a 10-cm-diameter flexible hose to provide humidity within the chamber of the growth cabinet.*

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established from axillary bud cultures of 'Royal Gala' and 'Jonagold' scion apple *(Malus ×domestica* Borkh.) cultivars were used for these studies. The original stock plant material was virus-free and provided by Stark Brothers Nursery (Louisiana, MO.). The cultures had been established and maintained on shoot proliferation medium that consisted of Murashige and Skoog (MS) (1962) high mineral salts supplemented with Staba vitamins (1969), 6-benzylaminopurine (BAP, 1.0 mg·liter^{-1}), kinetin (3 mg·liter^{-1}), and naphthaleneacetic acid (NAA, 0.1 mg·liter^{-1}), sucrose (30 g-liter-'), and agar (7.0 g·liter^{-1}). pH was adjusted to 5.7 with KOH prior to autoclaving for 15 min at 121C and 1.1 kg·cm^{-2}. The cultures were maintained at 22 to 24C under cool-white fluorescent tubes (60 µmol·s^{-1}·m^{-2}) with a 16-h light photoperiod and subcultured onto fresh medium every 4 weeks.

Root induction. Microcuttings 3 to 5 cm long) of 'Royal Gala' and 'Jonagold' were harvested from proliferating cultures. The basal end was cut at about a 60" angle. These microcuttings were pretreated with indolebutyric acid (IBA) using one of the following methods immediately before planting them in small plastic pots (5 × 5 cm) filled with Jiffy Mix:

1) Dip method. Bases of microcuttings were dipped quickly in a solution consisting of IBA (300 mg·liter^{-1}) dissolved in 50% ethanol (w/v).

2) Liquid medium. Bases of microcuttings were cultured aseptically in 25 × 150-mm culture tubes containing half-strength MS salts and supplemented with full-strength MS vitamins and IBA (2 mg·liter^{-1}). Cuttings were transplanted after incubating for 4 days.

3) Control. Control plants were obtained by transferring some plants directly to Jiffy Mix without IBA treatment.

Plants were divided into two groups and placed either directly in the Phototron units or under fog in a greenhouse Each treatment had six replicates with 12 cuttings each.

After 3 weeks, the number of rooted cuttings, the number of dead cuttings, the number of roots per rooted cutting, and the length of roots of each rooted cutting were recorded.

A general analysis of variance (ANOVA) was conducted for all variables using the SAS program (SAS Institute, 1985). For qualitative factors, the ANOVA was followed by mean separation procedures. Percentage data were transformed to arcsin values prior to analysis. The results are presented in a nontransformed format.

Results

Root initiation and development on 'Royal Gala' and 'Jonagold' was significantly better when microcuttings were maintained in the Photon-on units than when grown in the greenhouse under fog (Tables 1 and 2; Fig. 2).

Table 1. A comparison of root initiation ex vitro of in vitro-derived apple microcuttings under fog in a greenhouse and in a Phototron growth chamber (n = 12 with six replicates).

	Cultivar	
Factor	Royal Gala	Jonagold
	Rooted cuttings (%)[z]	
Environment		
Phototron	70.0 a	46.0 a
Fog	53.0 b	20.7 b
Pretreatment		
Control	31.8 c	10.0 c
IBA dip	62.5 b	37.0 b
Liquid medium	86.8 a	53.0 a

[z]All data were analyzed after transforming them to arcsin values. Mean separation in groups within columns by LSD, 5% level.

Table 2. A comparison of root development ex vitro of in vitro-derived apple microcuttings under fog in a greenhouse and in a Phototron growth chamber (n = 12 with six replicates).

	Cultivar	
Factor	Royal Gala	Jonagold
	Avg. no. roots/ rooted cutting[z]	
Environment		
Phototron	3.5 a	2.7 a
Fog	2.5 b	1.6 b
Pretreatment		
Control	1.8 c	1.1 c
IBA dip	2.6 b	2.4 b
Liquid medium	4.5 a	3.2 a
	Avg length of roots (cm)/ rooted cutting	
Environment		
Phototron	3.5 a	2.2 a
Fog	2.2 b	1.2 b
Pretreatment		
Control	1.6 c	0.8 c
IBA dip	3.1 b	1.8 b
Liquid medium	3.9 a	2.4 a

[z]All data were analyzed after transforming them to arcsin values. Mean separation in groups within columns by LSD 5% level.

Plant survival rates were also greater in the Phototron units than in the greenhouse (Table 3). 'Jonagold' showed signs of bacterial infection, probably of systemic origin, which resulted in the death of a large proportion of cuttings under both environments. Despite this loss, the survival rate of microcuttings was favored by liquid medium pretreatment (Table 3).

The number and length of roots on microcuttings was significantly higher in the Photon-on units than under fog (Table 2). Microcuttings pretreated with liquid medium had the largest number and longest roots; dipped microcuttings were intermediate (Table 3). Under both environments, microcuttings of 'Royal Gala' performed better than those of 'Jonagold'. A higher proportion of untreated microcuttings of 'Royal Gala' produced roots in the Phototron units than in the greenhouse (data not presented).

Discussion

In general, it was observed that the conditions in the Phototron unit were more favorable for both root development and shoot growth than those under fog. The liquid medium pretreatments with IBA survived best, rooted best, and produced the most and longest roots in comparison to control and IBA-dipped shoots. We also noted that the plants grown in the Phototron unit continued to grow more rapidly ex vitro than those from the fogged greenhouse (Figs. 2 and 3). Many of the rooted shoot plants from this experiment were transplanted to a field, where they have continued to grow vigorously for 4 years (data not presented). These plants are now ≈2 m tall; similar in size to 5-year-old grafted controls.

'Jonagold' microcuttings, despite their higher susceptibility to bacterial infection, rooted at rates of 20% under fog and 46% in the Photon-on unit. Successful rooting of difficult-to-root cultivars, such as 'Jonagold', can be achieved under the controlled environmental conditions within a Phototron unit. Further improvement is expected by using better root induction pretreatments and sanitation practices.

The method that we originally used to acclimatize apple shoots to ex vitro conditions relied on the use of fogging a greenhouse (Sriskandarajah et al., 1990). The present study dem-

Fig. 2. In vitro-derived apple shoots that have rooted within a fogged greenhouse (left) and in a Phototron unit (right). Notice the larger leaf size and greater internode length of Phototron-acclimated shoots.

Fig. 3. In vitro-derived apple shoots acclimatized in a Phototron unit have developed new leaves and are growing rapidly.

Table 3. Percentage of in vitro-derived apple shoots that survived ex vitro rooting in a greenhouse under fog and in a Phototron growth chamber (n = 12 with six replicates)

Factor	Cultivar	
	Royal Gala	Jonagold
	Cuttings that survived (%)[z]	
Environment		
Phototron	89.8 a	74.0 a
Fog	77.9 b	66.0 a
Pretreatment		
Control	68.2 c	59.0 b
IBA dip	86.1 b	68.0 b
Liquid medium	96.5 a	82.0 a

[z]All data were analyzed after transforming them to arcsin values. Mean separation in groups within columns by LSD, 5% level.

onstrated that the Phototron unit provided a better environment than the fogged greenhouse for establishing apple shoots ex vitro. The uniform high temperature, relative humidity, and illumination in the Phototron unit provided a more-suitable environment for establishment of microcuttings than the fogged greenhouse. Continuous light and high temperatures (28C) previously have been shown to favor root formation in apples (Lane, 1978; Le, 1985; Sriskandarajah and Mullins, 1981).

Although the Phototron units performed better than fogging for apple acclimatization, the Phototron units are probably too small to be practical for large-scale commercial use. The Phototron units hold no more than 100 to 120 plants per unit at one time. Therefore, large-scale commercial propagators will continue to rely on fogging or misting systems to produce the numbers of plants required to maintain their business demands. However, the Phototron units will have special value for laboratories or companies who do not have constant use for acclimatization facilities.

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Literature Cited

Donnelly, D.J., F.E. Skelton, and H.A. Daubeny. 1986. External leaf features of tissue-cultured 'Silvan' blackberry. HortScience 21:306-308.

Lane, *D.* 1978. Regeneration of apple plants from shoot meristem-tips. Plant. Sci. Lett. 12:281-285.

Le, C.L. 1985. Influence of temperature on in vitro root initiation and development of apple rootstock M26. HortScience 20:451-452.

Marchant, B. 1982. Acclimatizing plants produced by tissue culture. Amer. Nurseryman 15:31-33.

McCown, B.H. 1988. Adventitious rooting of tissue cultured plants, p. 289-302. In. T.D. Davis, B.E. Haissig, and N. Sankhla (eds.). Adventitious root formation in cuttings. Dioscorides, Portland, Ore.

Mohammed, G.H. and W.E. Vidaver. 1988. Root production and plantlet development in tissue-cultured conifers. A review paper. Plant Cell Tissue Org. Cult. 14:137-160.

Moss, G.I. and R. Dalgleisb. 1985. High humidity propagation. Acta Hort. 166:67-73.

Murashige T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 473-497.

SAS Institute. 1985. SAS user's guide: Statistics (1985 ed.). SAS Institute Inc., Gary, N.C.

Shinn, C. W. 1983. Rooting conifer cuttings with a fog system. Intl. Plant Prop. Soc. Combined Proc. 33:110-111.

Sriskandarajah, S. and *M.G. Mullins. 1981.* Micropropagation of 'Granny Smith' apple: Factors affecting root formation in vitro. J. Hort. Sci. 56:71-76.

Sriskandaraiab, S., R.M. Skirvin, H. Abu-Oaoud, and S.S. Korban. 1990. Factors involved in shoot elongation and growth of adventitious and axillary shoots of three apple scion cultivars in vitro. J. Hort. Sci. 65:113-121.

Staba, E.J. 1969. Plant tissue culture as a technique for the phytochemist, p. 75-102. In: M.K. Scikel and V.C. Runeckles eds.). Recent advances in phytochemistry. vol. 2. Appleton-Century Crofts, New York.

Wardle, K., E.B. Dobbs, and K.C. Short. 1983. In vitro acclimatization ofaseptically cultured plantlets to humidity. J. Amer. Soc. Hort. Sci. 108:386-389.

Ziv, M. 1986. In vitro hardening and acclimatization of tissue culture plants, p 187-196. In: L.A. Withers and P.G. Alderson (eds.). Plant tissue cultures and its agricultural applications. Butterworth, London.