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An Efficient Method for Rooting and Acclimation of Micropropagated Apple Cultivars

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Abstract. **To root tissue-cultured apple cultivars, shoots from proliferating cultures were first transferred to root induction medium with IBA for 1 week in the dark. Shoots were later transferred to the same medium without IBA and incubated under light for elongation of the roots. Rooted shoots were then transferred to Jiffy-7s supplemented with biological plant protectant and fertilizer, and incubated in plastic humidity trays. After 2 to 3 weeks, plants were transferred to pots and covered with plastic bags to facilitate acclimation. This technique has resulted in 70% to 100% of shoots selected in vitro producing vigorously growing, healthy plants in the greenhouse. Chemical name used: indolebutyric acid (IBA).**

The efficient and reliable production of vigorously growing plants in soil from in vitro plant material is an important step in the evaluation of transgenic apple (*Malus ×domestica Borkh.*) lines. Research on the factors involved in the development of effective rooting techniques has yielded variable success (Skirvin and Sriskandarajah, 1993; Zimmerman, 1984; Zimmerman and Fordham, 1985). Zimmerman (1984) recommended a combination of treatments for in vitro rooting, which included placing the shoots on rooting medium in the dark for the first week at 30 °C, then moving them in the same medium to a regime of 16 h light/8 h dark at 25 °C. Rooting percentages up to 100% were obtained in vitro but the success rate for acclimation and establishment of the plants in the greenhouse was not reported. Zimmerman and Fordham (1985) reported interaction among light, temperature, type of auxin, salt, and phloroglucinol on root initiation in liquid medium, and later root elongation in preformed peat plugs. Acclimation of these rooted plants and their establishment in the greenhouse are stages where plant loss often occurs, and success rates at this critical step have not been reported. Here we report details of a method that has a high success rate (70% to 100%) for rooting and acclimation of in vitro-derived apple cultivars.

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Materials and Methods

To root tissue-cultured plants, healthy, vigorously growing 4-week-old shoot tips (10–15 mm) were transferred from proliferation medium (Bolar, 1995) into magenta boxes (V8505; Sigma, St. Louis) containing root induction medium. This medium contained Murashige and Skoog inorganic salts (Life Technologies, Grand Island, N.Y.) at half strength, supplemented with the following organic constituents: thiamine-HCl (1 $mg \cdot L^{-1}$), myoinositol (100 $mg \cdot L^{-1}$), and IBA (3 $mg \cdot L^{-1}$). Sucrose (20 $g \cdot L^{-1}$) was the carbon source and the medium was solidified with Difco-Bacto agar (7 $g \cdot L^{-1}$). The pH of the medium was adjusted to 5.6 with 1 N KOH. Nine to 12 shoot tips were placed vertically in each box and cultures were incubated at 25 ± 2 °C in the dark. After 1 week, when the root apices were visible, the shoots were transferred to root elongation medium, which differed from the above root induction medium only by the omission of IBA. These cultures were incubated on racks in a growth culture room at a temperature of 25 ± 2 °C with a 16-h photoperiod provided by white fluorescent tubes at a photon flux of 50 to 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 2 to 3 weeks (Fig. 1A). In the root elongation medium the roots grew ≈10 to 15 mm.

Rooted shoots were rinsed in water to remove any medium, misted with water to prevent wilting, and then transferred to Jiffy-7 plant starter pellets (Jiffy Products, Batavia, Ill.). Prior to transfer, 10 g of biological plant

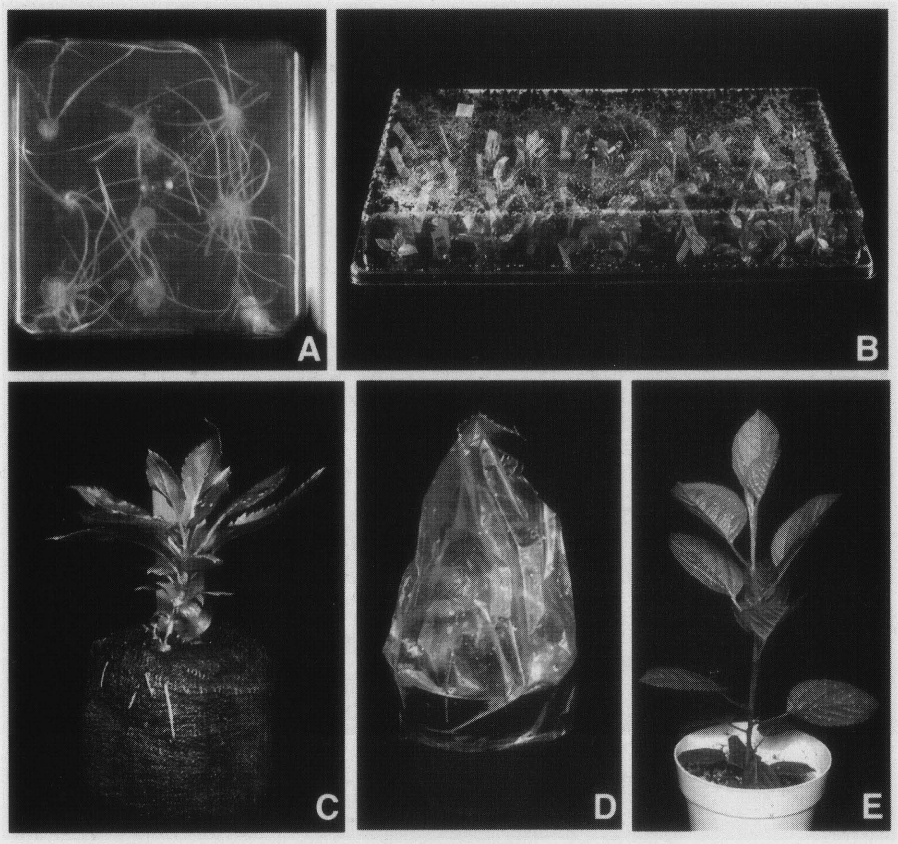

Fig. 1. Stages during rooting and acclimation of in vitro–derived apple shoots: (**A**) production of roots after 2 weeks in root elongation medium; (**B**) Jiffy-7s in humidity tray; (**C**) roots growing out of Jiffys; (**D**) acclimation in pots covered with plastic bags; (**E**) established plants.

protectant (Harman and Lo, 1996) (*Trichoderma harzianum* Rifai strain KRL-AG2, granules T-22G; BioWorks, Geneva, N.Y.) and 10 g of 15N–30P–15K Miracle-Gro fertilizer (Stern's Miracle-Gro Products, Port Washington, N.Y.) were mixed in a liter of water, and the Jiffy-7s were soaked in this mixture. After transfer, the Jiffy-7s were placed in trays under clear plastic humidity domes (Agway, Syracuse, N.Y.) and incubated under light in growth chambers with the same conditions as above (Fig. 1B). After 2 to 3 weeks, when roots began to grow out of the Jiffy-7s (Fig. 1C), the plants were transferred to plastic pots (10.2-mm diameter) containing 4 peat : 1 perlite : 1 vermiculite. The pots were watered, covered with plastic bags, and placed in growth chambers at 70% to 80% relative humidity, at 22 ± 2 °C, under cool-white fluorescent tube lights (16-h photoperiod, 50–60 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Fig. 1D).

To acclimate the plants, after ≈3 d or when growth of a new leaf was observed, the corner of the plastic bag was cut with scissors to make a hole ≈1 cm long, and 4 d later the bag was removed. The pots were watered regularly and fertilized weekly with Miracle-Gro as per the manufacturer's recommendation. The plants were then maintained in the growth chamber or transferred to a greenhouse (Fig. 1E).

Results and Discussion

Zimmerman and Fordham (1985) used nonsterile peat plugs similar to the Jiffy-7s used in this research. Our previous problem of losing 40% to 50% of plants to fungal contamination in the moist Jiffy-7s was greatly reduced by the present protocol after inclusion of the biological plant protectant. Also, fertilizing (the Jiffy-7s) improved the vigor of the rooted plants. Rooting in Jiffy-7s and subsequent transfer of the Jiffy-7s into pots prevented damage to the established roots and was an effective way to handle plants at that stage. Zimmerman and Fordham (1985) acclimated plants by gradually opening the boxes over several days, and also gradually reduced the humidity in the chambers. Skirvin and Sriskandarajah (1993) suggested the use of fogging to acclimate plants. Here the use of plastic bags, and later cutting holes to acclimate the plants, was less cumbersome compared to periodically opening and closing humidity domes, allowed for independent handling of individual plants, and did not require special equipment. This technique has resulted in 70% to 100% of shoots selected in vitro producing vigorously growing, healthy plants in the greenhouse. Thus far, the cultivars Marshall McIntosh, Golden Delicious, Liberty, Royal Gala, and Galaxy, and M26 rootstocks have been rooted and acclimated using this protocol, with similar success rates. For some of the above cultivars, transgenic plants containing foreign genes of interest have been rooted with the same success rate. This report gives a very detailed protocol for shoot proliferation, root initiation, root elongation, acclimation, and establishment of vigorously growing plants in the greenhouse.

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