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In Vitro Propagation of *Monstera deliciosa*[1]

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Abstract. **Shoot tips and lateral buds of *Monstera deliciosa* Liebm were grown on a modified Murashige and Skoog (MS) medium supplemented with combinations of 6 Benzyl-amino-9-(2-tetrahydropyranyl)-9H-purine (PBA) or kinetin and indolacetic acid (IAA). Shoot tips developed much better and faster than lateral buds. More shoot numbers developed with PBA than with kinetin; addition of IAA further increased the shoot number. Highest number of shoots was obtained with 10 mg/liter PBA and 2 mg/liter IAA. Multiplication was higher at 30°C than 21° or 24°. Rooting of shoots was best on MS basal media supplemented with 2 mg/liter IAA. Plants were easily established in soil. The morphology of the *in vitro* propagated plants was similar to the original clones.**

In Denmark, *Monstera deliciosa* is normally propagated by seed imported from Mexico and Brazil. The multiplication of clones vegetatively by cuttings is extremely slow. Our objective in this study was to develop an *in vitro* propagation technique for rapid propagation of *Monstera deliciosa*.

Seedlings between 6 - 8 weeks old and 10 - 15 cm high were used as source material. Leaves and roots were removed and the remaining shoots immersed in 70% ethanol, disinfested for 15 min in 2% sodium hypochlorite with 0.1% Tween 20 and washed twice in sterile tap water. Lateral buds (3 - 5mm) were excised and transferred to the nutrient medium. Apical shoot tips (3 - 5 mm) were divided vertically after the excision, and then transferred to the nutrient media.

The explants were grown in pyrex tubes 25 × 150 mm, each containing 12 ml of medium, and 1 explant. Each treatment consisted of 8 - 16 explants with 2 replicates. The nutrient medium contained inorganic salts according to Murashige and Skoog (1) supplemented with 0.4 mg/liter thiamine, 100 mg/liter meso-inositol, 30 g/liter sucrose and 8 g/liter Bacto agar. The pH of the medium was adjusted to 5.7 with KOH or HCL before autoclaving (15 min at 121°C). The cytokinins (PBA and kinetin) and the auxin (IAA) were dissolved in 0.1N HCL or 0.1N KOH, respectively, and added to the medium before autoclaving.

The experiments were carried out in controlled environment chambers at 30°C (unless otherwise stated) with an alternative cycle 18 hr of light and 6 hr of darkness, using fluorescent tubes (Atlas Cool White). The irradiance was 50 μwcm^{-2} (1500 lux).

Establishment of explants. The influence of PBA and kinetin on establishment was examined in the concentrations 0, 0.2, 2 and 10 mg/liter and IAA in the concentrations 0 and 2 mg/liter. The compounds were examined separately and in combination. Cytokinin was necessary for proliferation and PBA was more effective than kinetin at the concentrations tested. Cytokinin in combination with IAA (2 mg/liter) further increased the development.

Shoot tips were the best explants, lateral buds had only a low survival and

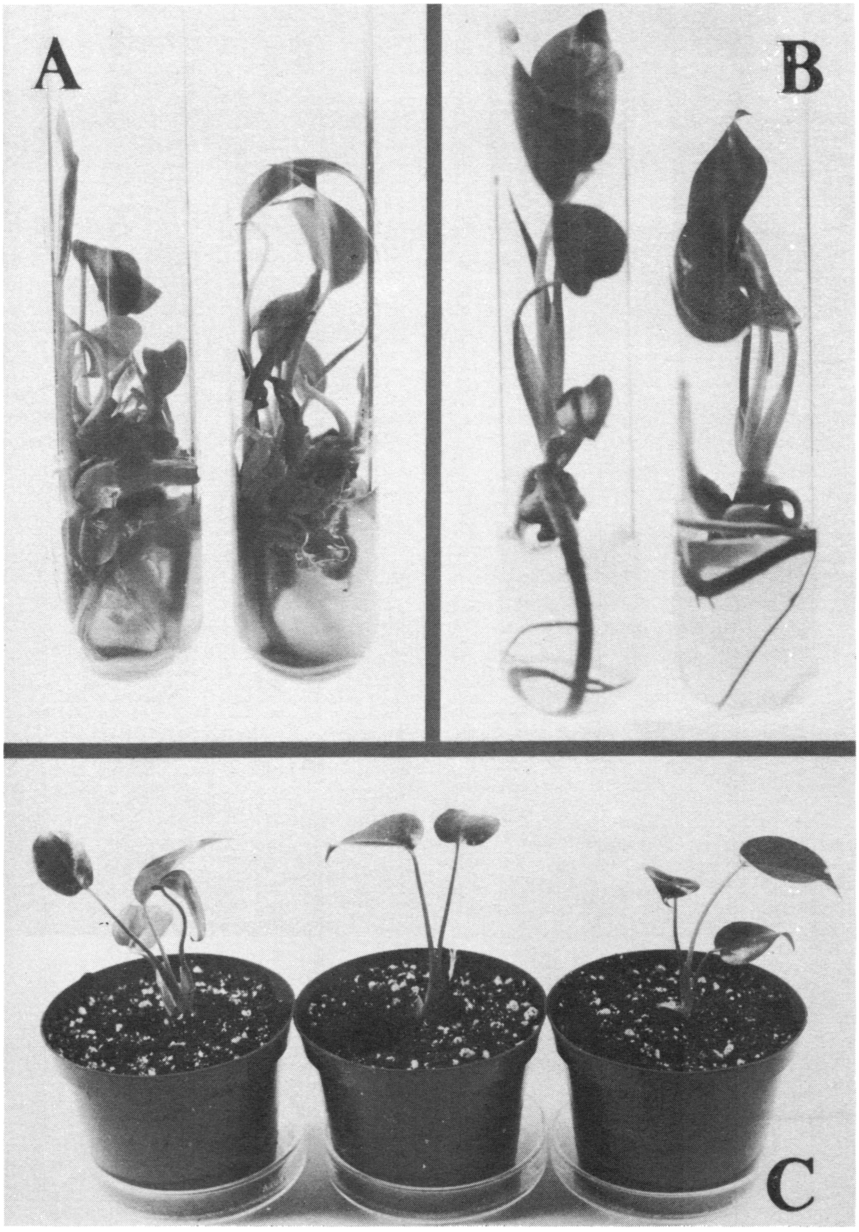

Fig. 1. *In vitro* propagation of *Monstera deliciosa*. A) Multiplication of cultures at 30°C on medium with 10 mg/liter PBA and 2 mg/liter IAA. B) Development of single plants at 30°C on medium with 2 mg/liter IAA. C) Plants established in soil.

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there was very little development.

Temperature. Greater prolification and growth was achieved at 30°C than either 21° or 24°.

Multiplication of cultures. The combination 10 mg/liter PBA and 2 mg/liter IAA was used for the further multiplication of the cultures (Fig. 1A). After 6 weeks 8 shoots developed per culture. The optimum subculture period was 6 weeks.

Development of plants. The development of whole plants from the cultures was examined in experiments with PBA (0, 0.02, 0.2 mg/liter) alone and in combination with 2 mg/liter IAA. These low PBA concentrations were used since the earlier results showed that by using concentrations higher than 0.2 mg/liter more than 1 shoot developed in each culture and the root development was suppressed.

Number and length of shoots, leaves, and roots, was not influenced by low PBA concentration but was increased by 2 mg/liter IAA. This suggests that a medium with 2 mg/liter IAA as the only hormone, will be preferable for development of plants *in vitro* (Fig. 1B).

Establishment in soil. Whole plants with leaves and roots were successfully transplanted to soil (Fig. 1C). Plant morphology of these plants was similar to the original clones.

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In Vitro Micrografting of Apple Shoot Tips[1]

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Abstract. **Micrografting of apple (*Malus domestica* Borkh.) was achieved by placing a 0.1 to 0.2 mm scion explant derived either from field-grown or *in vitro*-grown shoot apices on the hypocotyl of a decapitated 15 day-old seedling. Under asceptic conditons, the scion and hypocotyl unite to form a complete plant which produces 4 to 6 leaves after 6 weeks. These plants were transplanted to sterile vermiculite and conditioned to ambient humidity for 1 week and then transplanted to soil. This technique was used to obtain virus-free plants from virus-infected plants.**

The apple stem grooving virus (SGV) and the symptoms it causes to sensitive herbaceous hosts were described by Lister (8). The virus causes a disease in apple and is not eliminated by thermotherapy (H.W. Guengerich, unpublished data). Most cultivars of apple infected with SGV show no symptoms but the sensitivity of other cultivars has resulted in interstate and international quarantines against the sale of trees that are not certified as free from SGV. Since all clones of the 'Griffith' cultivar[3] were infected with SGV, we initiated a shoot tip culture program as a method of obtaining healthy plants. A satisfactory medium (4) containing the Murashige-Skoog (MS) mineral salts (11) and the vitamins of Gamborg et al. (3) plus 2% sucrose, 0.5 μM 6-benzylamino purine and 0.1 μM gibberellin A_3 was developed but all attempts to promote rooting failed.

The *in vitro* grafting of apple using shoots has been described in a preliminary report (9) and in more detail (7, 13). We have been successful with micrografting of apple (5, 6) as has been described for citrus (10). Here we examined some of the variables which influence successful grafting and determined the efficacy of this method for freeing plants of SGV.

Seedlings of open-pollinated 'Golden Delicious' apple were used as rootstocks. Two media were required, one (MSA) is used for germinating the embryos and a second (MSB) is used to promote growth of the tip meristem sicon. MSA is a solid medium containing the MS inorganics and 0.8% agar. MSB is a liquid medium containing the MS inorganics, B-5 vitamins (3) and 7% sucrose, pH 5.7. Seeds were cold stratified; seed coats removed; and embryos disinfected by a 10 min immersion in 0.5% sodium hypochlorite (10% Clorox) followed by 3 rinses in sterile distilled water. the embryos were placed on MSA and moved to an incubator for 15 days of continuous darkness at 25°C. Seedlings then were removed from the agar and the epicotyls and cotyledons excised. The decapitated seedlings were transferred to flat bottom tubes (2.5 x 11 cm) with a hole in the center in the form of an inverted "U." The radicle of the decapitated seedling was pushed through the hole in the filter paper bridge and a tip meristem scion, either obtained from a 'Griffith' shoot cultured *in vitro* or removed aseptically from shoots of field grown trees, was moistened with MSB then placed in contact with the vascular elements of the exposed hypocotyl. The graft was then exposed to 4 klx of light (10% incandescent, 90% fluorescent) for 16 hr at 27° and 8 hr darkness at 23°. After 1 week, callus was produced on both the hypocotyl and scion and union was achieved. After 6 weeks the scion had developed a shoot with 4 to 6 leaves and the plant was transplanted to a sterilized 11 cm clay pot containing sterile vermiculite. It was then irrigated with MSA and covered with a 400 ml beaker to maintain proper humidity. The plant was irrigated thereafter as needed and the beaker lifted 0.5 cm per day from the vermiculite surface for 6 days. On the 7th day, the beaker was removed and after 2 days the plant was transplanted to a larger sterilized clay pot containing pasteurized soil, then moved to a greenhouse bench.

The probability of successful graft unions increased with the size of the scion piece (Table 1). This was determined from 20 grafts in which the scion consisted of the apical dome only and 20 each of increasingly larger scions (apical dome plus increasing numbers of leaf primordia). When the scion consisted of the dome only, 15% of the graft unions were successful as compared to 65% when the scion included 2 leaf primordia. Since the probability of obtaining the virus-free plants is inversely related to size (10) and since meristems containing the apical dome and 2 leaf primordia will develop into plants 65% of the time, scion explants consisting of the dome and 2 leaf primordia were used in subsequent studies.

The time of year when field grown tips are used as a source of scion explants influenced the success of grafting (Fig.

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[3]Sold commercially as 'Blushing Gold.'