In vitro growth of Magnolia grandiflora L. cv. Bracken's Brown Beauty

by JOHN DAVID TOBE

Introduction

Magnolia grandiflora L. (SOUTHERN MAGNOLIA) displays tremendous morphological variation as well as physiological tolerances. The opportunities for selection of desirable seedlings for use in landscape design have been pursued by horticulturists as is evidenced by the large number of cultivars.

As the average homeowner's property becomes smaller the need for small trees will increase. No doubt the list of *M. grandiflora* cultivars selected by horticulturists for exhibiting smaller size, as well as other traits (e. g. cold tolerance), will increase. Those growers who can supply clonally produced, uniform trees will find a lucrative market in the landscape industry (Dirr and Brinson, 1985). One such grower, Ray Bracken, has done just that with the selection and patenting of a seedling of *M. grandiflora* that is called "Bracken's Brown Beauty" (Figlar, 1988). Thus far his only source of uniform plants has been by rooting cuttings from field grown plants. This has been an unpredictable practice, as well as a destructive one, as the plants will not always give high rooting yields and a yearly supply of young plants must be available for the taking of cuttings.

Tissue culture, or growing plants *in vitro* on a medium supplemented with nutrients and plant hormones, could prove to be a viable alternative to the traditional methods of cutting, grafting and seedling propagation of *M. grandiflora.* Some of the techniques described herein could be used with other species or cultivars of *Magnolia.*

For this particular project two different growth media were used with the addition of two synthetic plant hormones. All cuttings were removed from even aged field grown plants in September of 1989. These were maintained for one year with the assistance of the Clemson University Horticulture Department, during which time the plants were continually monitored for increased growth and axillary bud formation. This study is a preliminary study into the feasibility of micropropagating *M. grandiflora* from shoot tip cuttings.

Materials and Methods

All explant material was selected from a single source of vegetatively propagated plants, which were graciously donated for use by Ray Bracken. The *M. grandiflora* used is a named cultivar from Ray Bracken called, "Bracken's Brown Beauty." All explants were collected on the morning of September 15, 1989 at the Bracken Nursery in Piedmont, South Carolina. The explants were removed with a standard rose shear

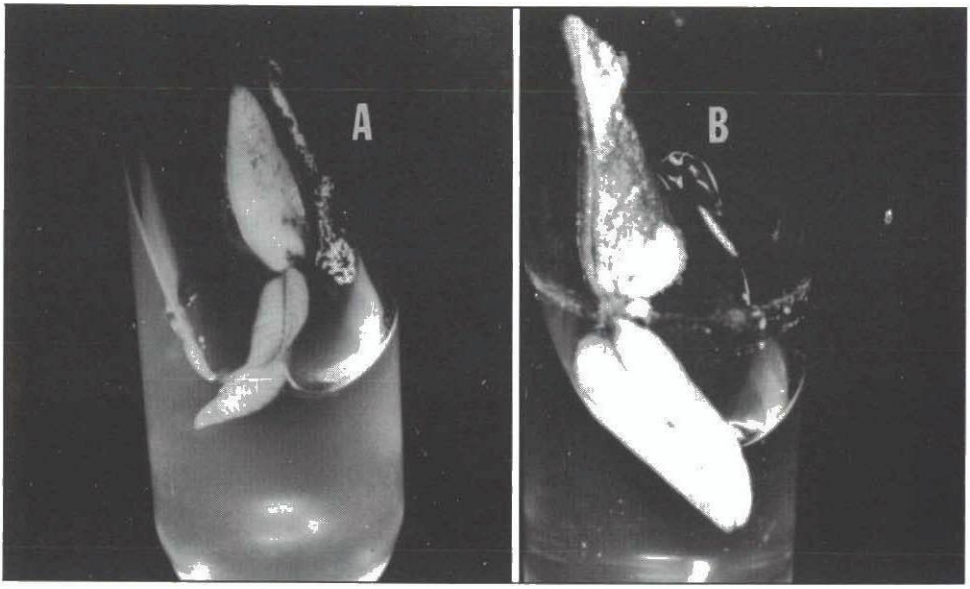

Photo 1. Magnolia grandiflora *'Brackens Brown Beauty' (A) explant after three months growth on MS media with BA at 0.1 mg/l. No callus is produced on these explants. Lifesize. (B) explant after three months growth on WPM with BA at 5.0 mg/l and 0.1 mg/l NAA. The undifferentiated tissue (callus) can be seen toward the apex and base of the shoot.*

and consisted of the shoot tip down to the base of the perules (sheathing stipules). These were immediately placed in plastic ziplock bags (about 70-80 shoots/bag) and placed on ice in the shade. Shoot tip cuttings were taken from young trees (5 years old) as young tissues have been found to root more readily *in vivo* than tissues from older plants (Perry and Vines, 1972). About 400 shoots were collected.

The shoots were placed in a refrigerated room at 10°C until ready for disinfestation (the chemical destruction of fungal spores, insect larvae and bacteria). Disinfestation was carried out by placing 50 shoots in 200.0 mls of 70% ethanol for 1.0 min. and then transferring to 200.0 mls of 20% clorox with 3 drops of TWEEN (a surfacant — used to facilitate the "wetting" of the epidermal hairs) for 20.0 min. The clorox was removed with three rinses in 100.0 mls of sterilized distilled water. The explants or small cuttings consisted of 2.0-4.0 mm of apical bud. Because the shoots began to brown quickly, explant removal was begun immediately after the third rinse of distilled water, and all work was carried out with a dissecting microscope under a laminar flow hood sterilized with 95% ethanol.

Explants were cultured on two types of culture media. Woody Plant Media (WPM) and Murashige and Skoog media (MS) with 0.1, 0.5, 1.0 and 5.0 mg/l of the synthetic cytokinin benzyladenine (BA) added and the synthetic auxin naphthaleneacetic acid (NAA) at 0.1 mg/l added to half of the treatments in a 4 x 2 factorial design for each media type. The WPM pH was adjusted to 5.3 and the MS pH was adjusted to

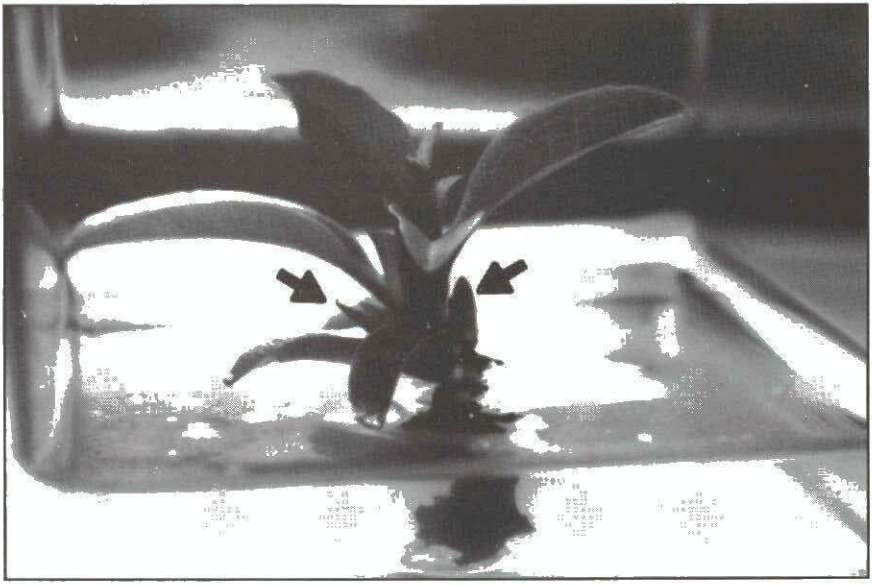

Photo 2. Magnolia grandiflora *'Bracken's Brown Beauty' explant after twelve months growth on MS with BA at 0.1 mg/l and 0.5 mg/l. Multiple shoot growth is indicated at the arrows. X 2.*

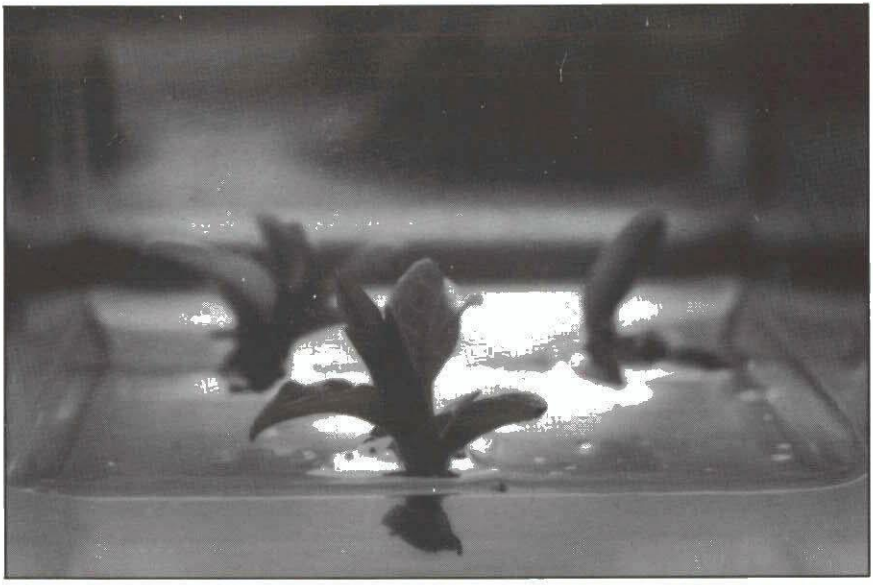

Photo 3. Magnolia grandiflora *'Bracken's Brown Beauty' explants after two months on hormone free MS media. Explants are from the multiple shoot growth in Photo 2. Notice the swelling of tissue at the base of the explant in the front of photo. X 2.*

5.7 prior to autoclaving. The WPM media were solidified with 3 g/l of Difco bacto-agar with the addition of 1.0 g/l of gelrite. Likewise the MS media were solidified with the addition of 6.5 g/l of Difco bacto-agar. The media were poured (aseptically) into disposable sterile Petri dishes. All media were autoclaved for 20.0 minutes on the liquid cycle (121°C). Five replicate samples of each were used for each sample and 5 explants were placed on each treatment with the shoot tip orientated upward. The cultures were grown under fluorescent lamps for 24 hour photoperiod at 25°C.

After four weeks of culture in the plastic Petri dishes, the explants were transferred to glass culture tubes to allow for increased apical growth. After four months the explants were transferred to magenta boxes (larger plastic boxes) and final observations concerning their growth were made from these cultures.

Results and Discussion

Because of the exudates (possibly tannins and phenolics) produced by the explants, transfer of explants onto new media was performed every three weeks. The explants were measured before each transfer and notes on the physical changes were made, e. g. leaf growth, callus growth and necrosis. The concentration of hormones had a significant effect on the growth of the shoot. The largest shoots were produced on the MS medium at the lower concentrations of BA (0.1 mg/l and 0.5 mg/l) with no NAA (A in photo 1). The greatest callus production occurred in the WPM medium at 5.0 mg/l BA with 0.1 mg/l NAA. This was also accompanied by severe deformities of the explants (B in photo 1).

Ultimately all of the explants on MS media died by the fifth month of culture. After six months only 6 explants remained alive out of the original 400, all of which grew on the WPM with BA concentrations of 1.0 mg/l and 5.0 mg/l. These remaining plants were transferred onto WPM with a BA concentration of 2.5 mg/l. After twelve months (August of 1990), all of the remaining explants had produced multiple shoots (the axillary buds had begun to elongate and grow as is shown in photo 2). For root induction half of these were removed from the original explant and placed on WPM with the addition of the synthetic auxin, indolebutyric acid (IBA) at a concentration of 5.0 μM for a period of seven days, then transferred to hormone free media. After two months the plants on the hormone free media are growing vigorously and beginning to swell at their base (photo 3). Thus far root induction has not been achieved.

This study shows that shoot tips of *M. grandiflora* can be cultured and grown *in vitro*. To date there are no published methods for the *in vitro* micropropagation of any species of *Magnolia*. This is no doubt due to the difficulty in finding the right *in vitro* conditions for the growth and development of *Magnolia* explants. Possible extensions to this study might involve the culture of shoots over a longer period of time, with transfers to other kinds of plant hormones to see if this might induce earlier shoot proliferation. Another

important consideration in conjunction with phytohormonal studies might be the age of the plant from which the explants are taken and the source of the explants (lateral versus terminal shoots). It has been established in *in vivo* vegetative propagation that younger trees (2-5 years of age) are a better source for regenerative plant material than older, mature plants (5+ years of age) and that terminal shoots give better percentages of rooted cuttings than lateral shoots (Perry and Vines, 1972).

Although the use of tissue culture techniques will some day be a reasonable alternative for traditional methods of clonal propagation, the time involved in extracting one to three shoots from each bud would either have to be reduced or the individual shoot tips (explants) would need to be manipulated to produce many new shoots in culture. Only then would *in vitro* micropropagation be an economically important alternative to the current use of traditional vegetative propagation for this species.

Acknowledgments

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References

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