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Propagation of Northern Bayberry (*Morella pensylvanica* 'Bobzam') Through Tissue Culture

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Summary

Northern bayberry is difficult to propagate from stem cuttings which limits its availability in the nursery industry. It was shown that cuttings taken in July and treated with IBA rooted at only about 20%. Micropropagation is an alternative to cutting propagation. An efficient micropropagation protocol was developed where microcuttings rooted at 98% and were successfully acclimated to greenhouse conditions.

INTRODUCTION

Northern bayberry (*Morella pensylvanica*) is a valuable native shrub found primarily in coastal areas of the northeastern United States from Virginia to the Canadian Maritimes. It is a suckering, colonizing, semi-evergreen shrub that is useful in landscape situations that present challenging conditions. Bayberries can tolerate salt spray, full sun, reflected light, drought, infertile sandy soils and cold winter temperatures. Its rhizomatous habit allows it to regenerate well following canopy damage resulting from snow clearing activities and people pressure. It is an ideal plant for parking lot islands, seaside plantings and bank stabilization.

Bayberry is a dioecious species, and female plants produce significant amounts of grayish white small fruits that offer seasonal interest, are a source of food for many songbirds and are covered with aromatic wax that can be used for candles. Female plants are more desirable than male plants due to their fruits and their more compact habits. Most bayberry plants are seed propagated because stem cutting propagation is difficult, or at least inconsistent. Plants offered for sale are essentially a 50%:50% mix of males and females, so obtaining a majority of female plants for landscape purposes is frequently not easy to accomplish. Reliable vegetative propagation of northern bayberry has remained elusive for the nursery industry but would provide great advantages over the current seed propagation that is used.

To confirm the general knowledge that northern bayberry is difficult to propagate from stem cuttings, we conducted a rooting study using the female cultivars ‘Bobzam’ and ‘UConn Compact’ and an unnamed male genotype. We collected cuttings at the end of June, end of July and end of August and stuck them individually in 2” pots in one part sphagnum peatmoss, one part horticultural-grade fine perlite, and one part horticultural-grade medium vermiculite under intermittent mist. Cuttings were wounded on one side and dipped in 2000, 4000 or 8000 ppm indole-3-butyric acid (IBA) in 50:50 water and ethanol, along with a 0 ppm IBA control. The male clone could be rooted at 100% using June cuttings treated with 2000 or 4000 ppm IBA and above 70% using 2000 ppm or no IBA. Both female cultivars rooted relatively poorly. ‘Bobzam’ rooted between 55% and 35% using July cuttings treated with between 0 and 4000 ppm IBA. The ‘UConn Compact’ cultivar reached a maximum rooting of only 20% using July cuttings and 2000 ppm and exhibited overall dismal rooting ability across all times and IBA concentrations. Our findings confirm that stem cutting propagation is likely an insufficient method of vegetatively propagating female northern bayberry.

Studies were conducted to determine if micropropagation of northern bayberry in vitro might be a way to efficiently vegetatively propagate a desirable female clone. New shoots (4-6 cm long) were harvested from ‘Bobzam’ plants forced in a greenhouse in late winter and surface sterilized using a 10% bleach solution for 15 min. Murashige and Skoog (MS) and Woody Plant (WP) media were tried in combination with benzyladenine (1 mg/l), or meta-topolin (5 mg/l), or thidiazuron (0.1 mg/l), or zeatin (4 mg/l). All explants on MS media turned black and failed, but many of those on WP with zeatin remained green and showed promise. A second shoot

initiation was conducted using more advanced 10-14 cm shoots with better formed axillary buds (**Fig. 1**) and they were placed on WP medium containing 0, 2, 4, or 6 mg/l zeatin. Explants on 4 mg/l zeatin performed best. They were very slow to acclimate to in vitro culture taking 7 subculture cycles of 3 weeks each (total time of 147 days) to miniaturize and develop a steady 2X shoot multiplication rate (**Fig. 2**). With additional time in culture (beyond 190 days) the multiplication rate increased to 3X every 21 days and eventually reached 4X multiplication.

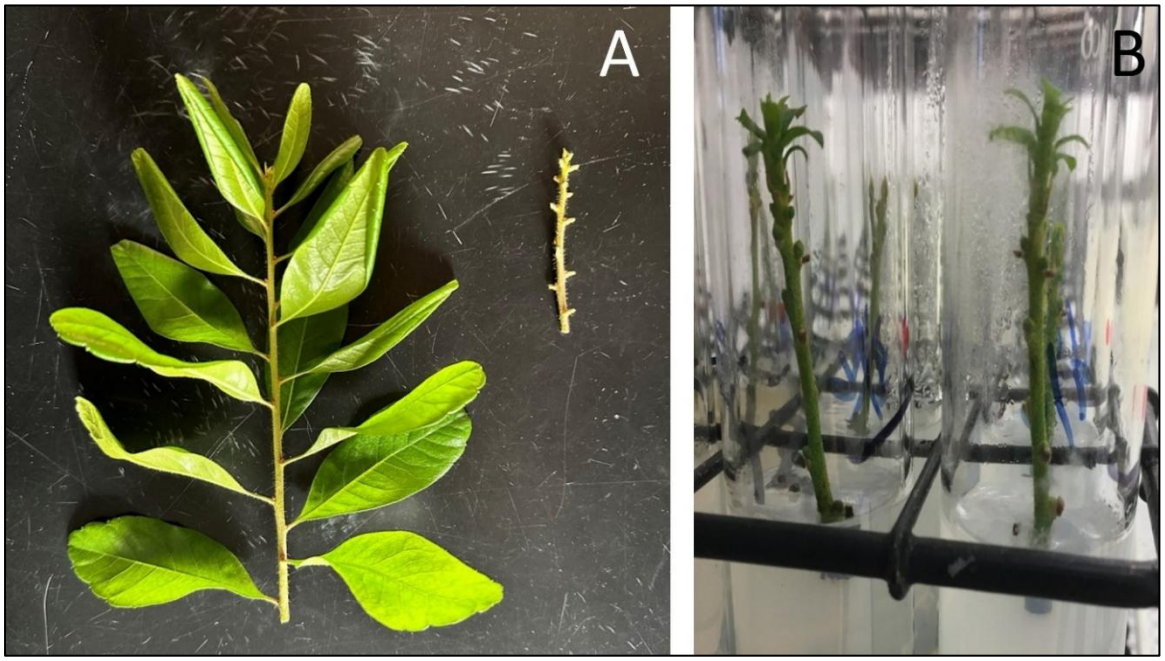

Figure 1. *Morella pensylvanica* 'Bobzam' Bobbee™. A) Well-developed, 10-14 cm shoots that were used to provide starting explants and trimmed shoot tip segment introduced into in vitro culture; B) Initiated shoot tip explants beginning to produce new shoot growth in vitro from apical meristems.

Figure 2. *Morella pensylvanica* 'Bobzam' Bobbee™. A) In vitro shoots developing from lateral buds on nodal explants following subculture; B) In vitro shoot developing from the apical meristem on tip explants following subculture; C) Shoot cultures that have miniaturized and acclimated to in vitro growth at 147 days following culture initiation.

On WP medium with 4 mg/l zeatin, 'Bobzam' cultures produced nicely elongated microshoots with well-expanded leaves. The shoots that were produced were ideal for use as microcuttings for rooting, or they could be cut into 3-5 node basal and apical pieces that could be recultured onto fresh multiplication medium. Apical explant pieces mostly produced shoot expansion only from the apical meristem, developing elongated straight shoots. Basal nodal pieces typically produced 2-5 shoots from axillary buds, but shoots were shorter than those from apical growth (**Fig. 2**).

Microcuttings were easily rooted (98%) in vitro on WP medium containing 1 mg/L indole-3-butyric acid (IBA). Root primordia were visible on the basal portions of microcuttings between day 10 and day 14 following sticking. At this point, pre-rooted microcuttings were transferred to one part sphagnum peatmoss, one part horticultural-grade fine perlite, and one part horticultural-grade medium vermiculite in clear deli tray humidity chambers that were placed under LED lighting providing an 18 hr photoperiod. Microcuttings could also be rooted easily (100%) by directly sticking unrooted shoots from tissue culture into deli tray humidity chambers (**Fig. 3**). Prior to sticking, the basal ends of directly stuck microcuttings were dipped in Hormodin 1 IBA powder (1000 ppm). During the initial 3 weeks of the rooting period, it was important to keep light levels low (below 30 uM/m2/sec) to avoid light stress that tended to turn microcutting leaves red or bronzy-black. Microcuttings rooted after 3-4 weeks and were then potted to 50 plug trays containing screened nursery mix (4:2:1 pine bark:peat moss:sand) and were covered with a clear plastic dome (Fig. 3). Acclimation to greenhouse conditions was accomplished by gradually increasing the number of vent holes in the clear domes to decrease humidity and removing shade cloth to increase light levels.

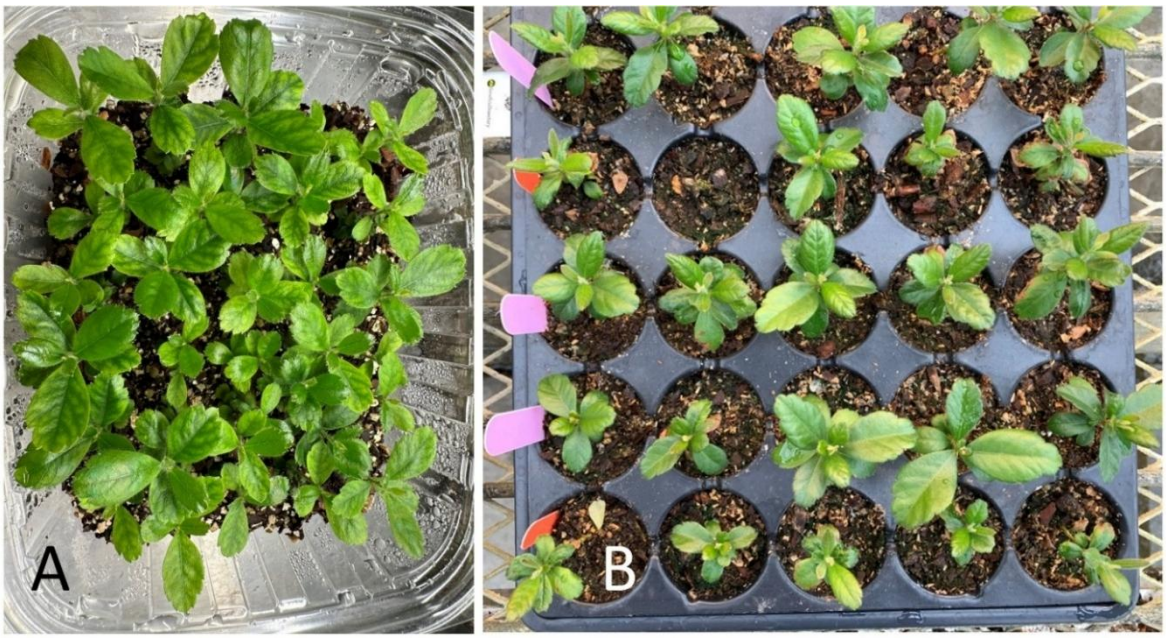

Figure 3. *Morella pensylvanica* 'Bobzam' Bobbee™. A) Well-rooted microcuttings that were directly stuck into peat moss-based rooting medium in humidity chambers; B) Acclimated bayberry plantlets growing in a 50-plug tray in the greenhouse.

Once acclimated to the greenhouse, micropropagated plants were potted into quart containers with nursery mix and then potted up to 1-gallon containers prior to being moved to outdoor, full sun growing conditions. Outdoors plants were provided with controlled-release fertilizer and grew rapidly into normal, well-branched and highly marketable plants (**Fig. 4**). Micropropagated plants were superior in quality (vigor and branching) to stem cutting propagated plants.

Figure 4. *Morella pensylvanica* 'Bobzam' Bobbee™ micropropagated plants after growing for three months in one-gallon containers outdoors.