"Tips" for Increasing Propagation: How Retipping Micropropagated Northern Bayberry [*Morella pensylvanica* (Mirbel) Kartesz.] Multiplies Liner Yield

Victoria M. Burton[1] and Mark H. Brand[1]

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Abstract. **Northern bayberry (*Morella pensylvanica*) is an adaptable, semievergreen, northeastern North American native shrub where fruit-bearing female plants are highly sought after for landscaping purposes. Efficient production of female plants necessitates having a reliable method of clonal propagation because of dioecy. A tissue culture micropropagation method for northern bayberry has been developed using in vitro shoot multiplication and rooting of microshoots. Micropropagation offers propagators a reliable way to clone desirable female bayberry genotypes. Retipping, which is the repeated harvesting of miniaturized shoot tips from recently micropropagated plantlets, has been used to enhance production of some nursery crops such as lilac, mountain laurel, and rhododendron. We explored the use of repeated retipping to amplify the number of plants that can be obtained from an initial micropropagated crop of *M. pensylvanica* 'Bobzam' (Bobbee™) plantlets. Rooted microcuttings direct from tissue culture could be used to provide three harvests of retip cuttings. Following each retip cutting harvest, the bases of the original, rooted microcuttings regenerated shoots that were suitable for a subsequent harvest of retip cuttings in only 4 weeks. The rooting ability of retip cuttings collected during the three harvest cycles did not diminish and remained high, between 90% and 100%. Retip cuttings produce between three to four roots per cutting and initiate new, vigorous shoot growth readily. Rooted retip cuttings can be acclimated to greenhouse conditions with close to a 100% success rate. Furthermore, the original, rooted microcuttings, after supplying three cycles of retip cuttings, can be grown and acclimated alongside retip plantlets to produce additional high-quality plants. Retipping can be used to produce a ~300% increase in salable bayberry plants from just a single crop of rooted microcuttings and three retip cropping cycles.**

Northern bayberry, *Morella pensylvanica* (formerly *Myrica pensylvanica*), is a shrub of considerable ecological and ornamental value from the Myriaceae family. It is native to coastal areas of the northeastern United States, Canadian Maritimes, and parts of the Great Lakes region from hardiness zones 3 to 6 (Dirr 2009). *M. pensylvanica* is valued, in part, for its adaptable nature. Northern bayberry is drought tolerant, has a rhizomatous regenerative habit, and is associated with *Frankia* nitrogen-fixing bacteria (Clawson and Benson 1999). It can grow in a range of poor soil conditions, including high-traffic and high-salt roadsides, with few to no disease or insect problems (Dirr 2009). The female plants of this dioecious shrub boast silvery-gray wax-covered drupes, which are eaten by a variety of songbirds (Bernhardt et al. 2009). These fruit persist through the winter with the semievergreen foliage, adding seasonal interest when used in a landscape setting. The aromatic wax coating on the drupes was used historically to make bayberry candles (Williams 1958).

Primarily female plants are sought for landscaping for their fruit and more compact habit than male plants. Only 20% of male plants is required to achieve optimal fruit set on females (Dirr 2009). Sexual reproduction by seed, which has been the primary propagation method for bayberry, produces a 50:50 mixed male/female population. Those wanting to use primarily female plants and a small number of pollinator males in landscaping must sort through mixed populations to identify female plants. Identification of sexes is not always easy if the plants are not in flower or fruiting. To make this native shrub species more accessible as a landscape plant, efficient asexual reproduction of superior female plants is necessary.

Female cultivars of *M. pensylvanica*, propagated by stem cuttings, have been reported to have poor rooting success, ranging from <35% to 55% (Edgett et al. 2024). For stem cuttings to be economically feasible for nursery production, a rooting percentage of >80% to 95% is required by growers (Brusse 2018; Cartabiano and Lubell 2013). Therefore, in vitro propagation methods may be more effective for clonal propagation of northern bayberry than cutting propagation. An in vitro micropropagation system producing a 3× shoot multiplication rate and ≥ 80% microcutting rooting has been reported previously for *M. pensylvanica* (Edgett et al. 2024).

Our research explored the use of repeated retipping to amplify further the number of plants that can be readily obtained from an initial micropropagated crop of *M. pensylvanica* 'Bobzam' (Bobbee™) plantlets. Retipping is the repeated harvesting and rooting of new shoot tips from recently micropropagated plantlets (Keith and Brand 1995). Often, commercial micropropagation facilities or nursery producers will use retipping to enhance plant propagation yield for crops such as *Syringa*, *Rhododendron*, or *Kalmia* (Lubell-Brand et al. 2021). The objectives of our research were to determine whether retipping could be used to increase successfully the micropropagation yield of northern bayberry at a commercial level and whether multiple cycles of serial retipping could be used.

Materials and methods

In vitro shoot cultures of 'Bobzam' grown and maintained according to Edgett et al. (2024) were used. Typically, subculturing occurs every 4 weeks for 'Bobzam'; however, for our study, cultures were allowed to grow for 8 weeks before use to deplete cytokinin levels to promote microcutting

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[1]Department of Plant Science and Landscape Architecture, Unit 4067, University of Connecticut, 1376 Storrs Rd., Storrs, CT 06269-4067, USA

M.H.B. is the corresponding author. E-mail: mark.brand@uconn.edu.

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rooting. This experiment consisted of rooting microcuttings of 'Bobzam' followed by three retipping events. The experiment was conducted twice. Microcuttings, 3 to 4 cm in length, were harvested from tissue cultures with a razor blade and the bases were dipped in talc-based indole-3-butyric acid (IBA) at 1000 ppm (Hormodin 1; OHP, Mainland, PA, USA) and stuck in clear plastic deli trays (946 mL) with lids (Dart Container Corporation, Mason, MI, USA) filled with ~300 mL of rooting medium consisting of 2:1.7:0.9:0.4 horticultural-grade fine vermiculite (Whittemore Co., Lawrence, MA, USA):pine bark (Fafard Inc., Agawam, MS, USA):sphagnum peatmoss (Fafard Inc.):river-run sand. The medium was screened to exclude particles larger than 5 mm. Five holes were added to the bottom of each tray for drainage. Each tray constituted an experimental unit, and units were arranged as a completely random design with four replications. Trays contained 30 microcuttings, for a total of 120 initial microcuttings, that would become rooted to then serve as the source of retip cuttings over three retipping cycles. Trays were provided a 16-h photoperiod at an initial intensity of 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and irrigation as needed. Full-spectrum white light was provided by Thrive Agritech Infinity LED Linear Fixtures (Los Angeles, CA, USA). After 2 weeks, the light intensity was increased to 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and four holes were added to the deli tray lids to decrease the humidity. After two additional weeks, the light level was increased to 145 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and four more holes were added to the lids. Rooted microcuttings were then grown for 4 weeks at the greater light intensity and lowered relative humidity before the first retipping event was conducted. All microcuttings and retip cuttings were rooted and grown in a growth room with a 25 °C set point.

Retip cuttings were harvested from the tips of the rooted mother microcuttings and were ≥3 cm long and contained approximately three nodes (Figs. 1 and 2). Retip cutting bases were treated with 1000 ppm IBA, and retip cuttings were stuck in trays as described earlier for microcuttings. Each tray contained ~30 retip cuttings, depending on the yield from the microcuttings. Experimental unit, design, and growing conditions are as described for microcuttings. The mother plantlets were allowed to regenerate shoots over a 4-week period and then the second retipping event was conducted as described. All lateral shoots ≥3 cm and with three or more nodes were

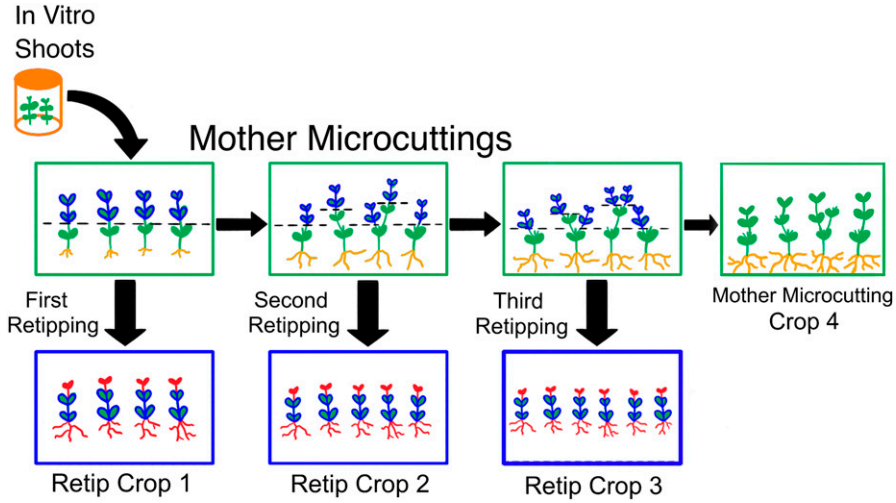

Fig. 1. Flow chart showing the progression of micropropagated mother plantlets of *Morella pensylvanica* 'Bobzam' Bobbee™ from unrooted microcuttings through three sequential retipping events that produce additional retip cuttings for rooting at each harvest. After each retipping event, rooted mother plants produce another growth flush of lateral shoots that can be harvested and rooted to produce more plants. Rooted mother plants regrow shoots at the end of the process to produce a fourth crop of plantlets.

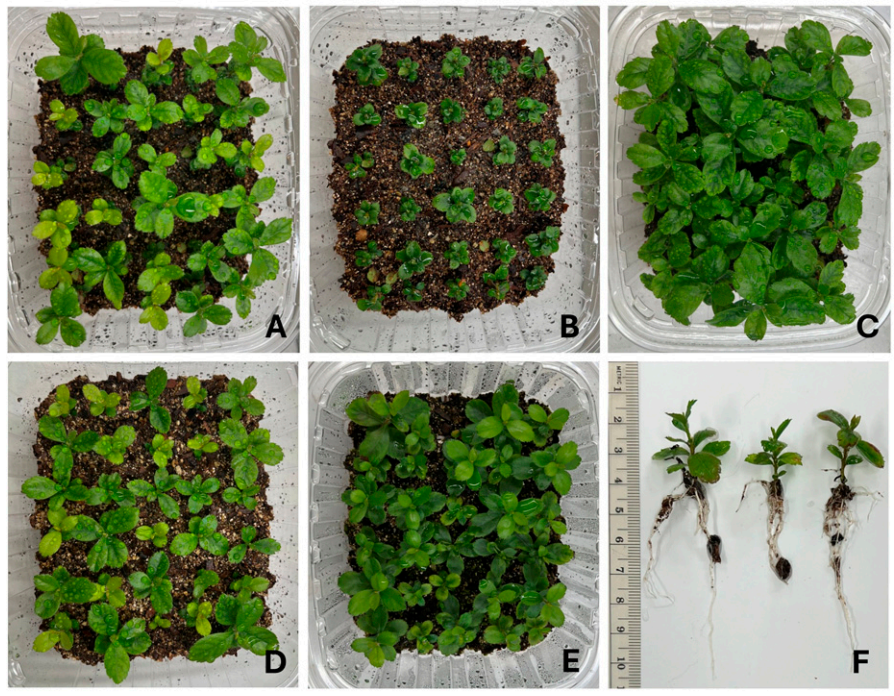

Fig. 2. Bayberry (*Morella pensylvanica* 'Bobzam' Bobbee™) microcuttings, retip cuttings, and plantlets. (A) Rooted microcuttings (mother plantlets) 56 d after they were stuck from tissue culture with new shoot growth. (B) Mother plantlet bases immediately after the harvest of retip cuttings. (C) Mother plantlets with new shoot growth 4 weeks after a retipping event. (D) Retip cuttings stuck for rooting immediately after being harvested in a retipping event. (E) Rooted retip crop 35 d after sticking, showing new shoot growth. (F) Root systems on 35-d-old retip cuttings showing well-developed root systems.

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Table 1. Number of retips per rooted mother microcutting, percent rooting, number of roots per shoot, total length of roots per shoot, and shoot length for two replications and three retipping cycles of *Morella pensylvanica* 'Bobzam' (Bobbee™).

Replication	Retipping event	Retips harvested per mother microcutting (n)[i]	Rooting (%)	Roots per shoot (n)	Total root length per week per shoot (cm)	Shoot length per week per shoot (cm)
Time 1	First retipping	0.99 b[ii]	88.1 b	4.2 a	2.6 b	0.23 c
	Second retipping	1.02 ab	100 a	3.4 b	2.4 b	0.43 b
	Third retipping	1.30 a	100 a	3.8 ab	3.2 a	0.62 a
Time 2	First retipping	1.01 b	99.2 a	4.8 a	3.7 a	0.58 a
	Second retipping	1.53 a	66.3 b	3.1 c	1.8 c	0.26 b
	Third retipping	1.09 b	96.7 a	3.8 b	2.7 b	0.23 c

[i] Microcuttings are those that were rooted and alive at the time of retip harvest.
[ii] Mean separation within columns within time replication (indicated by different letters) according to Wilcoxon's signed-rank test and analysis of covariance $P \leq 0.05$ (n = 4).

removed from the mother plantlets and stuck in trays for retipping event 2. After another 4 weeks, this process was repeated for retipping event 3 (Fig. 1). Trays were fertilized with 20N–4.4P–16.6K water-soluble fertilizer (J.R. Peter's Inc., Allentown, PA, USA) providing 100 mg·L^{-1} N every 2 weeks. Retips were grown for 8 weeks in experiment 1 and for 5 weeks in experiment 2 before root and shoot measurements were taken. Root number and length, number of retips per rooted microcutting, retip rooting percentage, and retip shoot length were recorded. For root number, the number of primary adventitious roots was counted. Root length was the sum of all primary and secondary roots. Shoot length was measured from the media surface to the shoot apex.

A subset of rooted plantlets, including microcutting mother plants and all three retipping cycles, was potted in 50-plug trays and placed in a greenhouse with set points of 21/17 °C day/night temperature thresholds to evaluate growth and performance after propagation. Plug trays were acclimated to greenhouse conditions by gradually increasing light exposure and decreasing relative humidity over a period of 4 weeks. Plants in plug trays were fertilized weekly with the same formulation as described previously at 200 mg·L^{-1}. Beyond the acclimation period, plants were grown for an additional 10 weeks in the greenhouse before being potted in 1.05-L plastic pots and then moved to outdoor growing conditions. Plants were allowed to go dormant and were overwintered in an unheated, white-polyethylene plastic–covered hoop house and then checked for normal budbreak and growth the following spring.

Data were analyzed using Wilcoxon's signed rank test ($P \leq 0.05$) for multiple comparison analysis of nonparametric variables (PROC NPAR1WAY) with SAS v. 9.4 (SAS Institute, Cary, NC, USA). Analysis of the variable root number used analysis of covariance (PROC GLIMMIX) with the number of surviving plants per experimental unit as the covariate.

Results

Bayberry microcuttings directly from in vitro culture rooted at 100% in both replications of the study. This is similar to what Edgett et al. (2024) reported for microcuttings rooted under nonsterile conditions. Rooted microcuttings, when grown out to produce new shoot growth, largely retained their miniaturized condition during the 14-week period of the study and across three retipping cycles (Fig. 2). Rooted, microcutting mother plants produced between 0.99 and 1.53 retip cuttings per mother microcutting at each retip harvest (Table 1). When looking across both time replications of the study, there did not appear to be any decrease in the production of retip cuttings from the first retipping event through the third retipping event. One could expect each mother microcutting to produce an average of at least one retip cutting at each harvest. After harvesting the retip cuttings, the microcutting mother plants required 4 weeks to regenerate new shoots for a subsequent retip harvest (Fig. 2). The rooting percentage for retip cuttings ranged from

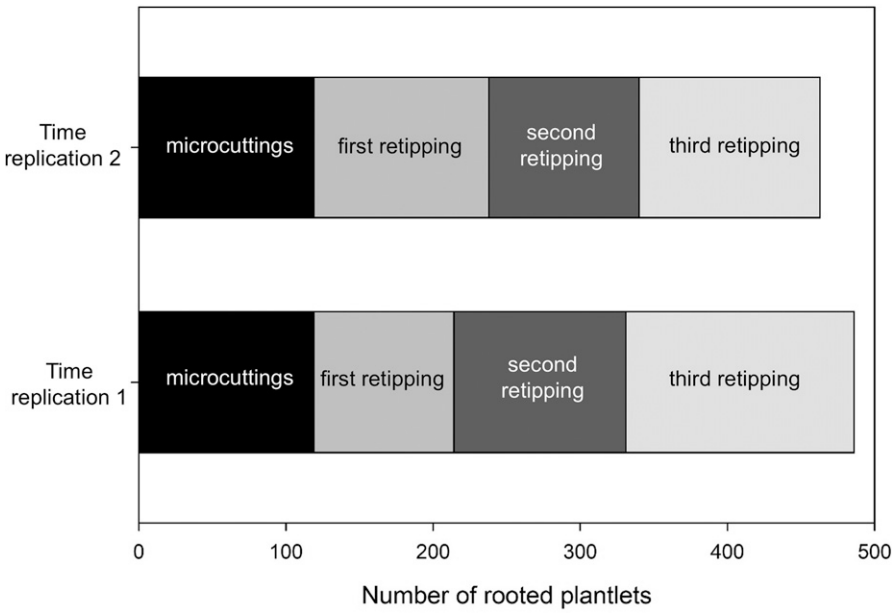

Fig. 3. Number of rooted plantlets produced from microcuttings of *Morella pensylvanica* 'Bobzam' (Bobbee™) over three cycles of retipping and for two replications of the experiment.

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66.3% to 100% across both time replications and was at least at ≥ 90% in most instances (Table 1). Rooting of only 66.3% for the second retipping in time replication 2 was the result of an environmental control malfunction that caused rooting retip cuttings to experience stressful warm conditions for 24 to 48 h and to exhibit a reduced rooting outcome.

Retip cutting rooting ability remained high across all three retipping events, showing no decrease in rooting ability with increasing time since in vitro culture (Table 1). Retip cuttings produced between 3.1 and 4.8 roots per cutting, and the number of roots per cutting was generally consistent across all three retipping events (Table 1). Total root length per week per shoot on rooted retips in time replication 1 ranged from 2.4 to 3.2 cm and in time replication 2 ranged from 1.8 to 3.7 cm (Table 1). Root lengths were longer in time replication 1 than in time replication 2 because data were collected after 8 weeks in replication 1 and after 5 weeks in replications 2, so roots had more growth time to extend their length in replication 1. Data from both time replications show that root growth potential for retips did not diminish with increasing time since in vitro culture. Shoot length per week on rooted retips ranged from 0.23 to 0.62 cm in time replication 1 and from 0.23 to 0.58 cm in time replication 2 (Table 1; Fig. 2). Data from both time replications show that shoot growth potential for retips did not diminish with increasing time since in vitro culture.

A total of 486 plants had been generated over three cycles of retipping at the end of time replication 1, including the original 120 microcuttings rooted directly from in vitro culture (Fig. 3). This represents a 308% increase in plants that were generated from the initial 120 microcuttings. A total of 463 plants were generated through three cycles of retipping at the end of time replication 2, including the original 120 microcuttings. This represents a 289% increase in plants that were generated from the initial 120 microcuttings.

Rooted retip plantlets acclimated easily to greenhouse conditions by gradually decreasing humidity levels and increasing light levels, while also maintaining a 16-h lighted photoperiod. The original rooted, microcutting mother plants, which provided three cycles of retip cuttings, also acclimated easily to greenhouse conditions using the same process as that used for retip plantlets. Plants from retips or microcutting mother plants, when grown for 3 months in 1.05-L pots in the greenhouse, grew rapidly, branched well, and exhibited no abnormalities (Fig. 4). All plants reached a size of ~50 to 65 cm tall and wide. Plants grown from the original rooted microcuttings, retip cycle 1, retip cycle 2, and retip cycle 3 were undistinguishable from each other at the same age after acclimation and were high-quality, marketable plants. The subset of plants that were grown outdoors to evaluate overwintering success of retip-propagated plants went dormant normally in the fall. Plants overwintered successfully in an unheated overwintering hoop house and broke dormancy normally the following spring.

Discussion

Recently, Edgett et al. (2024) developed a method for micropropagating bayberry successfully through tissue culture. Our micropropagation enhancement method, using retipping of rooted microcuttings, represents an effective way to increase plant production by between 289% and 308%. Retipping of micropropagated hemp plantlets was similarly found to increase plant production many-fold when starting with even modest numbers of micropropagated mother plants. There can be other benefits to the use of retipping besides just the increase in plant numbers. Keith and Brand (1995) found that retipping is an effective way to reduce the risk of epigenetic and genetic variation that can be associated with shoots and plantlets derived directly from in vitro culture. Retipping can also be done by less-skilled propagation laborers than

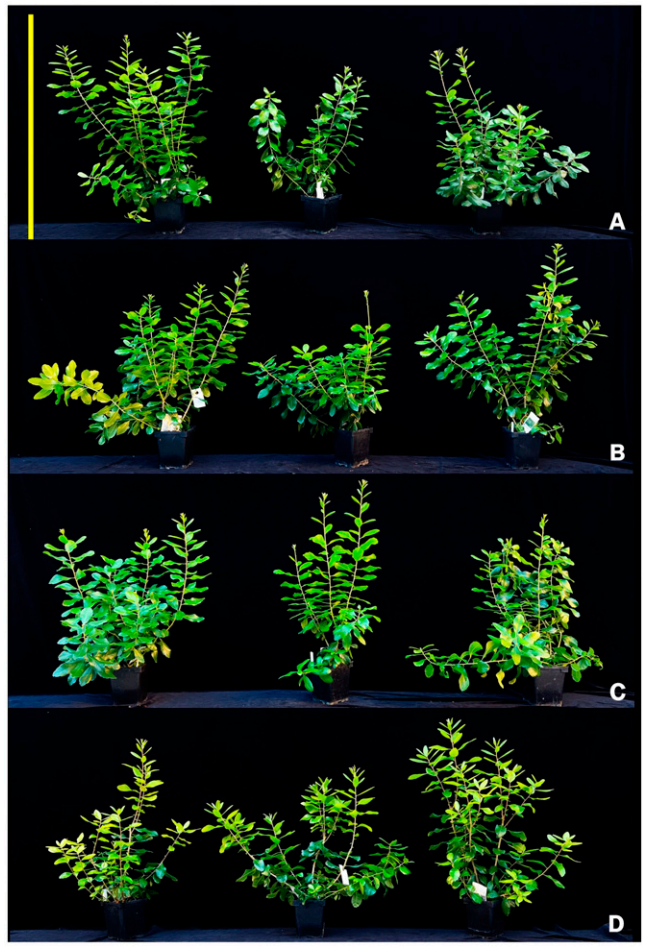

Fig. 4. (A) *Morella pensylvanica* 'Bobzam' (Bobbee™) plants produced from microcutting mother plants. (B) Retip crop from harvest 1. (C) Retip crop from harvest 2. (D) Retip crop from harvest 3 when grown in the greenhouse for 15 weeks after acclimation from rooting. Yellow scale bar = 80 cm.

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micropropagation and requires less-sophisticated facilities. Retip cuttings have a greater rooting capacity than stem cuttings (Edgett et al. 2024), even up to 14 weeks ex vitro. In most ways, retip cuttings offer the propagator the benefits afforded by both micropropagation and conventional stem cutting methods.

Increased rooting ability is a well-documented phenomenon in micropropagated woody plants (Debnath et al. 2012; Edgett et al. 2024). Induced rejuvenation, or reinvigoration, of the in vitro propagules likely plays a role in enhancing rooting ability (Brand and Lineberger 1992; Geneve 2018; Isah 2023). A decline in the rooting ability of retips is likely to occur at some future point, possibly coinciding with the morphological transition out of the miniaturized state. The process of harvesting retip cuttings from microcutting mother plants leads to shoot development from meristems nearer the base of the plant, which retain the miniaturized condition and elevated rooting ability. Because of the high density of axillary buds on the microcutting mother plants, they can regenerate shoots easily after at least three rounds of retipping, and likely even beyond this point. We chose to stop at three cycles to ensure that the mother plants could also be grown out successfully into high-quality plants. Retipping was shown to be an effective way to increase the micropropagation yield of northern bayberry at a commercial level using multiple cycles of serial retipping. It is likely that retipping can be applied to a wide range of plants as a reliable way to enhance clonal propagation output easily from a modest number of rooted microcuttings. More research to confirm application of retipping across a broad range of germplasm could prove fruitful.

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