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Micropropagation of *Mahonia* 'Soft Caress'

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***Abstract. Mahonia* 'Soft Caress' is a unique new cultivar exhibiting a compact form and delicate evergreen leaves. Protocols for micropropagation of *M.* 'Soft Caress' were developed to expedite multiplication and serve as a foundation for future work with other taxa of *Mahonia* Nutt. Combinations of sucrose at 30 or 45 g·L^{-1} in conjunction with Gamborg B5 (B5), Quoirin and Lepoivre (QL), and Murashige and Skoog (MS) basal media as well as other selected growth regulator treatments were evaluated as multiplication media. Rooting of microcuttings was conducted in vitro using combinations of indole-3-butyric acid (IBA) at 0, 2, 4, 8, or 16 μM under either light or dark. Quick dip treatments with aqueous solutions of the potassium (K) salt (K-salt) of IBA at 0, 5.2, 10.4, 20.7, or 41.4 μM were tested in a second experiment for ex vitro rooting. Media containing B5 basal salts and vitamins supplemented with sucrose at 30 g·L^{-1}, 5 μM 6-benzylaminopurine, 5 μM kinetin, 0.5 μM indole-3-acetic acid, and 2.5 μM gibberellic acid yielded 2.80 ± 0.14 microshoots with a mean length of 14.76 ± 0.63 mm over a 6-week culture period and was an optimal multiplication media. Light treatment and IBA concentration had a significant effect on rooting percentages. Microcuttings treated with 8 μM IBA and maintained in the dark resulted in the best rooting (70%) and ex vitro establishment.**

Many years ago, Hibberd (1862) noted "the time is fast coming when gardens of any pretensions to beauty will be judged by their collections of *Berberis*, for there is not any other class of evergreen shrub which affords so many points for interesting observation." Now 150 years later, the genus *Mahonia* Nutt. (syn. *Berberis* L.; for review, see Rounsaville and Ranney, 2010) is finally making a transition from the gardens of collectors into mainstream horticulture. *Mahonia* have tremendous ornamental potential, owing to their durability, showy displays of brilliant-colored flowers, and evergreen foliage. Nevertheless, few selections of the genus have been introduced commercially. *Mahonia* ×*media* cultivars dominate retail sales, although these selections are less than desirable to many consumers as a result of their spindly growth and spine-covered leaflets.

Mahonia 'Soft Caress' (PP20183) is a new cultivar that has shown great promise as a landscape plant. This cultivar arose as a seedling from an open-pollinated *M. eurybracteata* Fedde (Ozzie Johnson, personal communication). Although *M. eurybracteata* is notable for its relative compactness and narrow evergreen foliage, 'Soft Caress' grows considerably denser and was aptly named for its delicate linear leaflets. Hardy to USDA Zone 7, 'Soft Caress' is adorned with terminal yellow racemes during fall and early winter.

Although *Mahonia* sp. can be successfully propagated by stem cuttings, plants tend to have few lateral branches and thus a limited number of shoots available for propagation. Development of in vitro propagation methods would provide a desirable option for more rapid multiplication. In addition to propagation, establishing tissue culture protocols provides an ideal platform for manipulating ploidy level, harvesting chemical compounds, and initiating embryogenesis (Alvarez et al., 2009; Herbert et al., 2010; Pierik, 1997).

Studies on micropropagation of ornamental varieties of *Berberis* and *Mahonia* have been limited to *Berberis thunbergii* DC. 'Crimson Pygmy' (Uno and Preece, 1987); *M. aquifolium* 'Apollo' and 'Undulata' and *M.* ×*media* 'Winter Sun' (Daguin et al., 1992b); and *M. trifoliata* (Mackay et al., 1996). These studies have demonstrated that protocols necessary for shoot growth and development can vary considerably among genotypes and different basal salts and plant growth regulator combinations may be necessary. Woody Plant Medium (WPM) (Lloyd and McCown, 1980) and MS media (Murashige and Skoog, 1962) were used for *Berberis* and *Mahonia* cultures by Uno and Preece (1987) and Daguinet al. (1992b), respectively. Additionally, Mackay et al. (1996) used a combination of WPM salts and MS vitamins for *M. trifoliata*.

In most micropropagation studies, 6-benzylaminopurine (BAP) has been an effective cytokinin for shoot growth for *Mahonia* and *Berberis* species when provided at 5 to 10 μM (Daguin et al., 1992b; Mackay et al., 1996; Uno and Preece, 1987). Mackay et al. (1996) found BAP alone was sufficient for shoot proliferation of *M. trifoliata*; however, for several other *Mahonia* sp., low concentrations of kinetin (Kin) and the auxin, indole-3-acetic acid (IAA), were necessary to stimulate shoot initiation and multiplication (Daguin et al., 1992b). Daguin et al. (1992b) also found it necessary to use higher concentrations of sucrose (45 versus 30 g·L^{-1}), which are sometimes beneficial for younger tissues (Pierik, 1997). Furthermore, Daguin et al. (1992b) incorporated activated charcoal, which is often used to bind organic compounds secreted by plants in vitro, despite the undesirable binding of crucial growth regulators, particularly BAP (Thomas, 2008).

The ability of gibberellic acid (GA_3) to break dormancy, induce bud growth, and promote internodal elongation (Pierik, 1997) makes it a valuable compound for in vitro culture of small bud explants. Daguin et al. (1992b) and Uno and Preece (1987) used GA_3 at 0.6 or 10 μM for shoot multiplication of *Mahonia* and *Berberis* cultures, respectively. GA_3 is also known to promote ethylene-induced necrosis and leaf abscission (Morgan, 1976), which can be a particular problem for cultures in sealed vessels. However, use of ethylene inhibitors such as cobalt(II) chloride hexahydrate ($CoCl_2$), or silver nitrate ($AgNO_3$) can be effective for mediating the negative impacts of GA_3-promoted ethylene (Ma et al., 1998; Misra and Chakrabarty, 2009).

Rooting microcuttings of *Berberis* and *Mahonia* have been successfully achieved both in vitro and ex vitro using a variety of auxins. Mackay et al. (1996) achieved nearly 100% rooting of *M. trifoliata* in vitro using 1.0 μM naphthaleneacetic acid on cultures younger than 6 months. However, as culture age increased, microshoots required a cytokinin-free subculture followed by increased levels of auxin, which ultimately yielded lower (68%) rooting percentages. Conversely, Daguin et al. (1992b) transferred microcuttings of *Mahonia* directly to glasshouse flats after treatment of the microcuttings with a low concentration of IBA and achieved 80% rooting within 6 weeks. Karhu and Hakala (1990) treated microcuttings of *Berberis thunbergii* for 7 d in the dark using liquid media with low levels (2 to 6 μM) of either IAA or IBA (Karhu and Hakala, 1990). Interestingly, a subsequent transfer of the microcuttings to an auxin-free media gave

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significantly better rooting (85%) than those transferred to media with low auxin levels (35%) (Karhu and Hakala, 1990).

With development and introduction of new selections of *Mahonia*, it would be advantageous to have micropropagation protocols to facilitate rapid production and commercialization of these plants. Therefore, the objective of this research was to develop a rapid micropropagation protocol for *Mahonia* 'Soft Caress'.

Materials and Methods

Initiation. During late summer, actively elongating shoots, ≈9 cm long, were collected at 0900 HR from containerized plants maintained in a glasshouse. Leaves were removed without damaging the petiole base and shoots were rinsed under tap water for 4 h. Shoots were surface-sterilized in a 20% (v/v) Ultra Clorox (6.15% NaOCl) solution and two to three drops of Tween 20, periodically agitated for 15 min, and subjected to three rinses in sterile distilled water for 5 min each. Explants consisted of axillary buds excised from the base of leaf petioles.

Excised buds were placed in culture tubes containing 10 mL of initiation media. Based on preliminary trials (data not presented), the initiation media consisted of Gamborg's B5 basal salts and vitamins (Gamborg et al., 1968) supplemented with sucrose at 45 g·L^{-1}, 5 μM BAP, 5 μM Kin, 0.5 μM IAA, 2.5 μM GA_3, 25 μM $CoCl_2$, myoinositol at 0.1 g·L^{-1} 2-(N-Morpholino) ethanesulfonic acid (MES) monohydrate at 0.1 g·L^{-1}, solidified with agar at 7.5 g·L^{-1}, and pH adjusted to 5.75 ± 0.03. Filter-sterilized GA_3 was added to cooled autoclaved media. Excised buds were placed on media and incubated in the dark at 23 ± 2 °C for 2 weeks before being placed under standard culture conditions [23 ± 2 °C and a 16-h photoperiod of 30 μmol·m^{-2}·s^{-1} (400 to 700 nm) provided by cool-white fluorescent lamps].

Shoot proliferation. In the first shoot proliferation experiment, the influence of basal media and sucrose concentration on shoot growth was investigated. Treatments included a factorial combination of three media types, B5 salts and vitamins, QL salts (Quoirin and Lepoivre, 1977) with MS vitamins, and MS salts and vitamins, each in combination with sucrose at 30 or 45 g·L^{-1}, and the same growth regulators as listed for initiation (Table 1). Media were prepared in 180-mL glass jars. Five shoots, ≈10 to 15 mm long, were placed in each jar and incubated under standard culture conditions. After 6 weeks of culture, explants were scored for shoot number and shoot length (longest shoot). The experiment was then repeated by subculturing single shoots (10 to 15 mm long) on fresh identical media to minimize any residual effects from initiation media. Each treatment consisted of six replications (jars) containing five subsamples (explants) each for a total of 30 explants per treatment. All treatments were arranged in a completely randomized design. Data were subjected to analysis of variance (ANOVA) and mean separation using Fisher's least significant difference (LSD) with SAS Version 9.1 (SAS Inst. Inc., Cary, NC).

To further improve multiplication rates and shoot growth, a second shoot proliferation study was conducted using B5 basal media with sucrose at 30 g·L^{-1} (one of the best media from Expt. 1) with selected media components (see Table 2). Media were prepared as described for Expt. 1 and shoots were incubated under the standard culture conditions. Data were collected on shoot number and shoot length (longest shoot) after 6 weeks of culture. Each treatment consisted of six replications arranged in a completely randomized design. Each replicate contained five subsamples each for a total of 30 explants per treatment. Data were subjected to ANOVA, and mean separation using Fisher's LSD with SAS Version 9.1.

Rooting. Experiments were conducted to study both in vitro and ex vitro rooting of *M.* 'Soft Caress'. Media used for in vitro rooting consisted of B5 salts and vitamins and sucrose at 30 g·L^{-1} supplemented with 0, 2, 4, 8, or 16 μM IBA. Microshoots ≈10 mm long were subcultured into 180-mL jars, half of which were subjected to a "dark" treatment achieved by fully enclosing individual jars in aluminum foil. Each of the 10 treatments consisted of six replications with five subsamples each. All jars were arranged in a complete randomized design under standard culture conditions. Foil was removed 4 weeks after the cultures were placed in the dark. After an additional 2 weeks of culture, microcuttings were scored for rooting percentage and root length (longest root) (Table 3). Microcuttings (rooted and unrooted) were then transferred directly to ex vitro cell flats containing a substrate of 1 peat:1 vermiculite (by vol.) with 1.19 kg·m^{-3} micronutrients (Micromax®; Scotts Company LLC, Marysville, OH). Microcuttings were arranged in a completely randomized design and grown in a glasshouse under intermittent mist for 6 weeks and scored for rooting percentage, root number, root length, and shoot length. Data were subjected to regression analyses using SAS Version 9.1.

An additional experiment was designed to study ex vitro rooting of microcuttings. Microshoots growing on multiplication media were trimmed from the bases to ≈10 mm in length and the basal portions dipped for 5 to10 s in 0, 5.2, 10.4, 20.7, or 41.4 μM aqueous solutions of K-IBA. Microcuttings were arranged in a completely randomized design with six replications and five subsamples per replication. Cell trays and rooting substrate were identical to the aforementioned experiment. Microcuttings were set under intermittent mist in a glasshouse and scored for rooting percentage, root number, root length (longest root), and shoot length (longest shoot) after 6 weeks.

Table 1. Effect of nutrient media and sucrose concentration on shoot proliferation of *M.* 'Soft Caress'.[z]

	Subculture 1		Subculture 2	
Treatment	Number of shoots	Shoot length (mm)	Number of shoots	Shoot length (mm)
B5 + 30 g·L^{-1}	2.83 ± 0.27 a[x]	17.93 ± 1.26 ab	2.57 ± 0.26 a	12.00 ± 1.06 a
B5 + 45 g·L^{-1}	2.13 ± 0.23 ac	16.40 ± 1.95 bd	1.83 ± 0.32 ab	11.03 ± 1.95 a
QL + 30 g·L^{-1}	2.57 ± 0.17 a	22.17 ± 2.13 a	2.40 ± 0.36 a	12.87 ± 2.13 a
QL + 45 g·L^{-1}	2.07 ± 0.44 ac	14.23 ± 1.04 bc	1.47 ± 0.18 bc	11.20 ± 1.05 a
MS + 30 g·L^{-1}	1.50 ± 0.18 bc	10.27 ± 1.52 c	1.55 ± 0.13 bc	10.70 ± 1.52 a
MS + 45 g·L^{-1}	2.20 ± 0.35 a	12.10 ± 2.09 cd	2.03 ± 0.30 ac	12.60 ± 2.09 a
Analysis of variance[w]				
Media	NS	**	NS	NS
Sucrose	NS	*	NS	NS
Media × sucrose	*	**	*	NS

[z]All treatments included 5 μM BAP,[y] 5 μM Kin, 0.5 μM IAA, 2.5 μM GA_3, and 25 μM $CoCl_2$. Data were recorded after 6 weeks for each subculture.
[y]Key to media and growth regulator abbreviations: BAP (6-benzylaminopurine), Kin (kinetin), IAA (indole-3-acetic acid), GA_3 (gibberellic acid), $CoCl_2$ (cobalt(II) chloride hexahydrate), B5 (Gamborg's B5 basal salts and vitamins), QL (Quoirin and Lepoivre basal salts and Murashige and Skoog vitamins), MS (Murashige and Skoog basal salts and vitamins).
[x]Mean separation within columns by Fisher's least significant difference at $P < 0.05$. The mean values represent six replications with five subsamples each.
[w]NS,*, **Non-significant or significant at $P < 0.05$ or < 0.01, respectively.

Table 2. Effect of selected growth regulator treatments on microshoot proliferation of *M.* 'Soft Caress'.[z]

Treatment (μM)[y]	Number of shoots	Shoot length (mm)
5 BAP, 5 Kin, 0.5 IAA, 2.5 GA_3, 25 $CoCl_2$	2.92 ± 0.30 a[x]	10.80 ± 0.72 bc
5 BAP, 5 Kin, 0.5 IAA, 2.5 GA_3	2.80 ± 0.14 a	14.76 ± 0.63 a
5 TDZ, 0.5 IAA, 2.5 GA_3	2.76 ± 0.26 a	8.60 ± 0.99 c
5 TDZ, 2.5 GA_3	2.88 ± 0.27 a	8.64 ± 1.00 c
10 Kin	2.28 ± 0.29 a	9.40 ± 0.51 c

[z]Data were recordered after 6 weeks on treatment media.
[y]Key to media and growth regulator abbreviations: BAP (6-benzylaminopurine), Kin (kinetin), IAA (indole-3-acetic acid), GA_3 (gibberellic acid), $CoCl_2$ (cobalt(II) chloride hexahydrate), TDZ (thidiazuron). All growth regulator concentrations are listed in μM.
[x]Mean separation within columns by Fisher's least significant difference at $P < 0.05$. Mean values represent six replications with five subsamples each.

Table 3. Effect of IBA concentration and light treatment on in vitro rooting percentage and root length of *M.* 'Soft Caress'.[z]

Light treatement	IBA[y] (μM)	Rooting (%)	Mean root length (mm)
Light	0	0 ± 0.0	—[x]
Light	2	0 ± 0.0	—
Light	4	0 ± 0.0	—
Light	8	10 ± 4.47	11.7 ± 4.6
Light	16	3 ± 3.33	11 ± 0
Dark	0	0 ± 0.0	—
Dark	2	13 ± 6.67	19.3 ± 10.8
Dark	4	13 ± 4.22	10.0 ± 5.0
Dark	8	37 ± 12.02	36.5 ± 3.5
Dark	16	27 ± 9.54	20.4 ± 4.2
Analysis of variance[w]			
IBA–linear		**	**
IBA–quadratic		*	*
Light × IBA–linear		NS	*
Light × IBA–quadratic		*	*

[z]Data were recordered after 6 weeks on treatment media.
[y]Indolebutyric acid.
[x]Microcuttings failed to produce roots.
[w]NS,*, **Non-significant or significant at $P < 0.05$ or < 0.01, respectively.
The mean values represent six replications with five subsamples each ± SEM.

Results and Discussion

Shoot proliferation. For both subculture periods during the first shoot proliferation experiment, there were significant interactions between basal media and sucrose that affected the number of shoots (Table 1). During both subcultures, cultures produced a higher number of shoots on B5 and QL media containing sucrose at 30 g·L^{-1}, whereas cultures on MS media tended to produce more shoots with sucrose at 45 g·L^{-1}. These interactive effects on shoot number were especially pronounced during the second subculture period, during which cultures on B5 with sucrose at 30 g·L^{-1} and QL with sucrose at 30 g·L^{-1} produced a greater number of shoots (2.6 ± 0.3 and 2.4 ± 0.4, respectively) than cultures on MS with sucrose at 30 g·L^{-1} and QL with sucrose at 45 g·L^{-1} (1.6 ± 0.1 and 1.5 ± 0.2, respectively).

Media, sucrose, and a media × sucrose interaction all significantly impacted shoot length during the first subculture period ($P \leq$ 0.01, 0.05, and 0.01, respectively). Mean shoot lengths recorded during the first subculture again favored B5 with sucrose at 30 g·L^{-1} (17.9 ± 1.3 mm) and QL with sucrose at 30 g·L^{-1} (22.2 ± 2.1 mm), which were significantly greater than MS at either sucrose concentration. Shoot length after the second subculture was not different among treatments.

Overall, microcutting acclimatization after 12 weeks of culture indicated the rates of shoot multiplication among treatments were consistent and significantly different, whereas shoot lengths had stabilized and were not significantly different. The B5 basal media with sucrose at 30 g·L^{-1} was selected for future experiments because it resulted in the highest shoot multiplication rates during both subcultures as well as good shoot elongation after the first subculture. Furthermore, the treatment consisting of the B5 media with sucrose at 30 g·L^{-1} was observed to produce higher quality plantlets with light green expanded leaves compared with other treatments, which tended to produced yellow shoots and narrow folded leaflets. In previous studies on *Mahonia*, total nitrogen (N) and the ratio of NH_4^+/NO_3^- were suggested as factors that influenced in vitro growth (Daguin et al., 1992a). Total N and NH_4^+/NO_3^- ratios in B5, QL, and MS basal salts used in our study were 27.1 mM, 36.2 mM, and 60.3 mM and 8.2%, 16.1%, and 52.3%, respectively. The high total N and NH_4^+/NO_3^- ratios in MS basal salts may have inhibited growth of microcuttings of *M.* 'Soft Caress'. Similarly, Daguin et al. (1992a) reported uptake of critical NO_3^- ions was limited when the NH_4^+/NO_3^- ratio was greater than 50% resulting in poor growth of *Mahonia* sp.

In the second shoot proliferation experiment, shoot multiplication remained high for all media with no significant improvement compared with the media selected in Expt. 1 (Table 2). Conversely, shoot elongation was significantly influenced by media composition. The treatment containing 5 μM BAP, 5 μM Kin, 0.5 μM IAA, and 2.5 μM GA_3 yielded the greatest mean shoot length at 14.8 ± 0.6 mm. By contrast, addition of the ethylene inhibitor $CoCl_2$ to the aforementioned treatment significantly reduced shoot length (10.8 ± 0.7 mm). In beberine-producing plants such as *Mahonia* sp., ethylene has been shown to promote berberine production (Kobayashi et al., 1991; Sato et al., 1990). Inhibition of ethylene led to a decrease in endogenous berberine synthesis and an increase in growth in cell cultures of *Thalictrum minus* L. (meadow rue) (Kobayashi et al., 1991). Surprisingly, in the present investigation, use of $CoCl_2$ caused a decrease in shoot length and an increase in chlorosis, suggesting that in *Mahonia*, ethylene may play a role regulating a desirable level of berberine biosynthesis. Alternatively, the concentration of $CoCl_2$ used may have had a phytotoxic effect, because excess cobalt is reported to inhibit photosynthesis (Palit et al., 1994). Results here also showed a significant decrease in shoot length in those treatments supplemented with thidiazuron (TDZ) (Table 2). TDZ is often an effective cytokinin for in vitro woody plant culture because of its ability to induce adventitious shoots; however, it also tends to reduce apical dominance, thereby leading to poor shoot elongation (Murthy et al., 1998).

Rooting of microcuttings. In vitro root initiation was observed within 3 weeks of culture. Regression analysis indicated that microcuttings subjected to dark treatments had a quadratic response to IBA concentration for both rooting percentage and average root length (Table 3). Treatment of microcuttings with 8 μM IBA/dark yielded the highest rooting percentage (37%) and the longest mean root length (36.5 ± 3.5 mm). Nontreated microcuttings (0 μM IBA) in light or dark as well as 2 or 4 μM IBA under light failed to produce any roots.

Microcuttings (rooted and unrooted) on in vitro rooting media were transferred ex vitro. After 6 weeks, root formation was observed in every treatment. Analysis of variance indicated light and IBA treatment and their interaction had a significant effect on rooting percentage ($P < 0.05$) (Fig. 1). Regression analysis showed the percentage of microcuttings forming roots from in vitro dark treatments increased linearly with IBA concentration, whereas those plantlets from in vitro light treatments had a quadratic response with IBA concentration. All plantlets initiated in the dark gave better rooting percentages than those under lights at corresponding IBA concentrations, whereas the IBA control yielded identical results under both light regimes. Based on regression analysis, the dark treatment at 16 μM gave the highest estimated rooting percentages with 78% of microcuttings forming roots.

The effect of light on root initiation of *M.* 'Soft Caress' is similar to that of *B. thunbergii*, which benefited from an induction period in darkness during rooting (Karhu and Hakala, 1990). Conversely, *M. trifoliolata* was found to root quite readily in vitro with light (Mackay et al., 1996). Although it is known that many woody plant species benefit from darkness during the early stages of in vitro rooting (Rugini et al., 1993), the complexities of the effects are still not fully understood (Nor Aini et al., 2009). Additionally, auxin is only required during the early stages of root growth and development in vitro, and continued exposure to auxin may actually inhibit further growth (De Klerk et al., 1999). Although this was not a factor with *M. trifoliolata* for Mackay et al. (1996), Karhu and Hakala (1990) observed rooting percentages increased from 35% to 85% in *B. thunbergii* after microcuttings were transferred to an auxin-free media after 1 week compared with continual exposure on 4 μM IAA.

By comparison, in vitro rooting percentages in the current investigation were similar to those observed in 21-month-old cultures of *M. trifoliolata* (Mackay et al., 1996). A cytokinin-free subculture followed by transfer

onto media with high concentrations of IBA (4.9 or 12.3 μM) was sufficient to increase rooting percentages from 2% to 79% and 67%, respectively, in *M. trifoliolata* (Mackay et al., 1996). Mackay et al. (1996) interpreted this as residual BAP accumulation in older cultures, because microcuttings less than 6 months old displayed 100% rooting. Although overall rooting percentages of *M.* 'Soft Caress' were comparable to that of *M. trifoliata* over the same duration, improved rooting may be achieved by a cytokinin-free subculture or reduced exposure to plant growth regulators.

IBA had a significant effect on number of roots produced depending on the light treatment ($P < 0.05$) (Fig. 2). For microcuttings with in vitro dark treatments, the number of roots increased linearly with IBA concentration. There was no significant effect of IBA on number of roots for plantlets in the light treatment. Based on regression analysis, dark treatment with 16 μM IBA gave the estimated maximum of 1.6 roots. IBA concentration and light did not significantly affect root or shoot lengths.

Regression analysis performed on ex vitro rooting data revealed no significant linear or quadratic trends. The highest concentration of K-IBA (41.4 μM) yielded the highest rooting percentage at 37 ± 9.2% (data not presented). By comparison, the highest rooting percentage observed during the first 6 weeks of the in vitro study was also 37% (8 μM IBA dark treatment). Interestingly, root morphology appeared to differ after 6 weeks of growth between experiments. Roots formed in vitro under the influence of IBA (and those formed after transfer ex vitro without additional treatment) were considerably thicker, ≈1 to 2 mm in diameter, and in nearly all cases appeared to arise from the basal callus of the microshoots. By comparison, those treated with the K-IBA dip were finer, 0.5 mm or less in diameter, and did not appear to initiate from callus. Proliferation of nonorganized callus can be affected by many variables, including BAP, type of explant, and basal media (Pierik, 1997; Swamy et al., 2004). Although callus formation on microcuttings is known to promote rooting among some species (Mukherjee et al., 2010), it can drastically decrease rooting capacity in others, including *B. thunbergii* (Karhu and Hakala, 1990; Puddephat et al., 1999; Thakur and Kanwar, 2008). Adventitious root initiation on *M.* 'Soft Caress' does not appear to require callus formation as a result of lack of callus on ex vitro plantlets forming roots as well as those in vitro at low IBA concentrations.

In conclusion, a successful micropropagation protocol was developed for *M.* 'Soft Caress'. Shoot culture media provided good multiplication rates and proved effective for maintaining cultures over extended periods of time. Continued studies will focus on improving rooting response of microcuttings, specifically to decrease overall rooting time. These protocols provide a basis for rapid propagation of *M.* 'Soft Caress' and may provide a foundation for other species and hybrids within the genus.

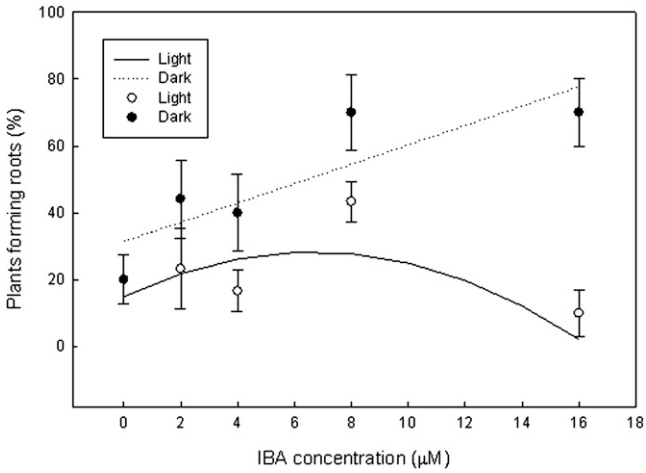

Fig. 1. Effect of IBA and light on percentage of microcuttings of *M.* 'Soft Caress' forming roots. Dotted and solid lines represent trends fitted using linear and quadratic regression analyses: dark (●) = 0.315 + 0.029 (IBA), $r^2 = 0.29$, $P < 0.05$; light (○) = 0.15 + 0.049 (IBA) – 0.003 (IBA^2), $r^2 = 0.15$, $P < 0.05$. Vertical lines = ± 1 SE. IBA = indole-3-butyric acid.

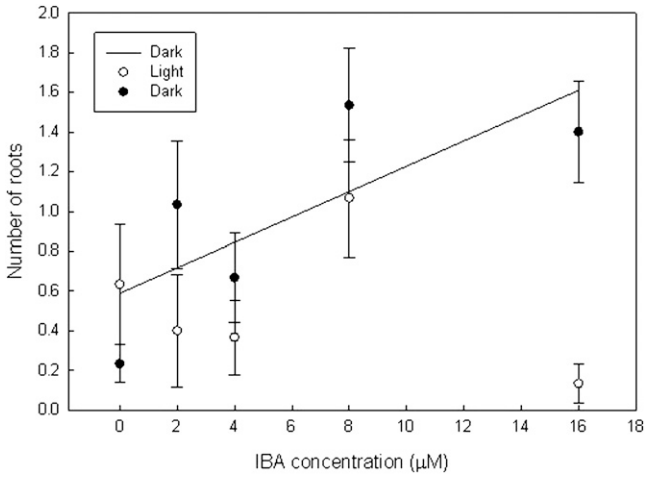

Fig. 2. Effect of IBA and light on number of roots produced on microcuttings of *M.* 'Soft Caress'. Solid line represents a linear trend fitted using linear regression analysis: Dark (●) = 0.59 + 0.064 (IBA), $r^2 = 0.25$, $P < 0.05$. Vertical lines = ± 1 SE. IBA = indole-3-butyric acid.

Literature Cited

Alvarez, M.A., N.F. Eraso, S.I. Pitta-Alvarez, and P.L. Marconi. 2009. Two-stage culture for producing berberine by cell suspension and shoot cultures of *Berberis buxifolia* Lam. Biotechnol. Lett. 31:457–463.

Daguin, F., E. Guyot, A.-C. Cavan, L. Eschbach, and R. Letouze. 1992a. Nitric and ammoniacal nutrition in tissue culture: Behavior of three *Mahonia* clones. Acta Hort. 320:231–239.

Daguin, F., E. Guyot, A.-C. Cavan, and R. Letouze. 1992b. Micropropagation of three *Mahonia* clones. Acta Hort. 320:193–197.

De Klerk, G.J., W. Van Der Krieken, and J.C. De Jong. 1999. The formation of adventitious roots: New concepts, new possibility. In Vitro Cell. Dev. Biol. 35:189–199.

Gamborg, O.L., R.A. Miller, and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50:151–158.

Herbert, C.J., D.H. Touchell, T.G. Ranney, and A.V. LeBude. 2010. In vitro shoot regeneration and polyploid induction of *Rhododendron* 'Fragrantissimum Improved'. HortScience 45:801–804.

Hibberd, S. 1862. The floral world and the garden guide. Volume 5:163. Groombridge and Sons, London.

Karhu, S. and K. Hakala. 1990. Rooting in vitro of micropropagated barberry (*Berberis thunbergii*) shoots. Ann. Agr. Fenn. 29:179–185.

Kobayashi, Y., M. Hara, H. Fukui, and M. Tabata. 1991. The role of ethylene in berberine production by *Thalictrum minus* cell suspension cultures. Phytochemistry 30:3605–3609.

Lloyd, G. and B. McCown. 1980. Commercially feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot tip culture. Proc. Intl. Plant Prop. Soc. 30:421–427.

Ma, J.-H., J.-L. Yao, D. Cohen, and B. Morris. 1998. Ethylene inhibitors enhance in vitro root formation from apple shoot cultures. Plant Cell Rep. 17:211–214.

Mackay, W.A., F. Molinar, Jr., M.M. Wall, and M. Cardenas. 1996. Micropropagation of agarita, *Berberis trifoliata* Moric. HortScience 31:1030–1032.

Misra, P. and D. Chakrabarty. 2009. Clonal propagation of *Rosa clinophylla* Thory. through axillary bud culture. Sci. Hort. 119:212–216.
Morgan, P.W. 1976. Gibberellic acid and indole acetic acid compete in ethylene promoted abscission. Planta 129:275–276.
Mukherjee, P., N. Husain, S.C. Misra, and V.S. Rao. 2010. In vitro propagation of a grape rootstock, deGrasset (*Vitis champinii* Planch.): Effects of medium compositions and plant growth regulators. Sci. Hort. 126:13–19.
Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–497.
Murthy, B.N.S., S.J. Murch, and P.K. Saxena. 1998. Thidiazuron: A potent regulator of *in vitro* plant morphogenesis. In Vitro Cell. Dev. Biol. 34:267–275.
Nor Aini, A.S., B.L. Goh, and R. Ridzuan. 2009. The effects of different indole-3-butyric acid (IBA) concentrations, two light regimes on in vitro rooting and acclimatization of in vitro teak (*Tectona grandis* L.f) plantlets. Afr. J. Biotechnol. 8:6158–6161.
Palit, S., A. Sharma, and G. Talukder. 1994. Effects of cobalt on plants. Bot. Rev. 60:149–181.
Pierik, R.L.M. 1997. In vitro culture of higher plants. Kluwer Academic Publishers, Norwell, MA.
Puddephat, I.J., P.G. Alderson, and N.A. Wright. 1999. In vitro root induction in axillary microshoots of *Quercus robur* L. Ann. Appl. Biol. 134:233–239.
Quoirin, M. and P. Lepoivre. 1977. Improved media for *in vitro* culture of *Prunus* sp. Acta Hort. 78:437–442.
Rounsaville, T.J. and T.G. Ranney. 2010. Ploidy levels and genome sizes of *Berberis* L. and *Mahonia* Nutt. species, hybrids, and cultivars. HortScience 45:1029–1033.
Rugini, E., A. Jacoboni, and M. Luppino. 1993. Role of basal shoot darkening and exogenous putrescine treatments on in vitro rooting and on endogenous polyamine changes in difficult-to-root woody species. Sci. Hort. 53:63–72.
Sato, H., Y. Kobayashi, H. Fukui, and M. Tabata. 1990. Specific differences in tolerances of exogenous berberine among plant cell cultures. Plant Cell Rep. 9:133–136.
Swamy, S.L., J.L. Ganguli, and S. Puri. 2004. Regeneration and multiplication of *Albizia procera* Benth. through organogenesis. Agrofor. Syst. 60:113–121.
Thakur, A. and J.S. Kanwar. 2008. Micropropagation of 'wild pear' *Prunus pyrifolia* (Burm F.) Nakai II. Induction of rooting. Not. Bot. Hort. Agrobot. Cluj 36:104–111.
Thomas, T.D. 2008. The role of activated charcoal in plant tissue culture. Biotechnol. Adv. 26:618–631.
Uno, S. and J.E. Preece. 1987. Micro- and cutting propagation of 'Crimson Pygmy' barberry. HortScience 22:488–491.