In Vitro Propagation of Alexandrian Laurel (*Danae racemosa* L. Moench), a Valuable Ornamental Plant

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Abstract. To overcome the limitations of traditional propagation, this research was initiated to develop an alternative means for efficient production of Alexandrian laurel (*Danae racemosa* L. Moench). An in vitro propagation protocol has been developed for *Danae racemosa* L. Moench using seeds as a source of material for culture initiation. Seedlings were produced after seeds were cultured for 3 months on MS (Murashige and Skoog, 1962) medium. Shoot multiplication occurred on MS medium with or without 6-benzylaminopurine (BAP) with 100% multiplication percentage. However, shoot number was significantly increased from an average of 2.8 to more than six with the addition of 5 or 25 μ M BAP. Among two indole-3-butyric acid (IBA) treatments tested for rooting of seedlings, incorporation of 5 μ M IBA in MS medium significantly increased rooting percentage to 86.4% compared with 71.2% without IBA. The greatest number of roots (three) was produced by 5-minute IBA pulse. However, both IBA treatments significantly reduced root length. The longest root (12.8 mm) was observed on MS medium without any IBA treatment and the shortest (6.1 mm) was produced by IBA pulse. In vitro-propagated plantlets grew well after transfer to a substrate of peat and pine bark (1:1) in the greenhouse. No morphological variation was observed.

Micropropagation can be used as a more efficient way for propagation of plant species that have difficulty in production by traditional means (Debnath et al., 2006; Murashige, 1974). However, this approach has not been successful in *Danae racemosa* L. Moench (Liliaceae/Ruscaceae/Asparagaceae family), commonly called Alexandrian laurel or poet's laurel, the single species in the genus *Danae*. Alexandrian laurel is a perennial evergreen shrub. Grown as ornamental plants, this species has a graceful arching stem with handsome elliptical, glossy, thick, waxy, bright green, evergreen foliage (Fig. 1A), which makes for a permanent attraction in any garden and is also very popular in the flower arrangement industry (Naghi and Dahkaei, 2009). However, known for its notoriously slow growth and challenge to propagate, Alexandrian laurel is not readily available and rarely seen commercialized (Matt, 2005). As an initiative part of a research program toward efficient in vitro propagation and manipulation of this species, this study was conducted with the aims to: 1) develop the in vitro propagation

protocol; and 2) investigate effects of plant growth regulators on shoot multiplication and root formation. Compared with other ornamental plants species, Alexandrian laurel has not been scientifically researched. Information on conventional propagation and

in vitro micropropagation of Alexandrian laurel is scarce. This study will provide helpful information for efficient production of this species.

Materials and Methods

Seed sterilization and culture. Seeds were obtained directly from dried berries (Fig. 1B) collected from a local source in the fall of 2010. The berries were soaked in tap water for a day to rehydrate and the seeds were then manually extracted. The seeds were sterilized by immersing them in bleach solution (0.94% sodium hypochlorite, Bleach-Rite; Current Technologies, Inc.) with a couple of added drops of Tween-20 (Sigma-Aldrich Corp., St. Louis, MO) for 20 min and then rinsed several times with sterile distilled water. The seeds were then soaked in sterile distilled water for 72 h before germination (Fig. 1C). MS (Murashige and Skoog, 1962) medium was used for seed germination. Seeds were placed into GA-7 vessels (77 mm $\times$ 77 mm $\times$ 97 mm; Magenta Corporation, Chicago, IL) containing 50 mL medium. There were four seeds per GA-7 vessel. After 3 months, in vitro-germinated seedlings were used for the subsequent experiments.

Shoot multiplication. The effect of BAP on shoot multiplication was investigated. Shoots were severed from seedlings and cultured on MS medium supplemented with BAP at 0, 1, 5, and 25 μ M. Shoots were cultured in GA-7 vessels containing 50 mL medium. There were four explants per GA-7 vessel and 15 replicates for each treatment. The number of explants with shoot multiplication and the number of shoots per explant

Fig. 1. (A) Leaves of Alexandrian laurel. (B) Red dried fruits of Alexandrian laurel. (C) Seeds of Alexandrian laurel. (D) In vitro-germinated seedlings after 3 months culture on Murashige and Skoog (MS) medium. (E–F) Shoot multiplication on MS medium supplemented with 5 μ M 6-benzylaminopurine (BAP). (G) Single root formation on MS medium supplemented with 5 μ M indole-3-butyric acid (IBA). (H) Double root formation on the same medium. (I) Multiple root formation on the same medium. (J) Acclimatized plants in the greenhouse. Bars = 1 cm.

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were counted at the end of 8 weeks culture. The shoot multiplication percentage was calculated as the number of explants with multiple shoots out of the total number of cultured shoots.

Root induction. The effect of IBA on rooting was examined. Shoots of ≈ 1.5 cm in length were excised individually and two IBA treatments were applied: 1) shoots were cultured on MS basal medium supplemented with 5 μM IBA; and 2) shoots were first dipped in the 5 μM solution of IBA for 5 min (IBA pulse) and then cultured on MS medium. Shoots cultured on MS medium without any IBA treatment served as the control. Shoots were cultured in baby food jars containing 40 mL medium with two shoots per vessel and 24 replicates per treatment. The rooting experiment was repeated once. The number of shoots forming roots, root number, and the length of the longest root of each shoot were recorded after 6 weeks' induction. The root formation percentage was calculated as the number of shoots forming roots out of the total number of cultured shoots.

General culture medium and culture condition. All media were supplemented with 3% sucrose. Media were adjusted to pH 5.8 with 0.1 N NaOH before the addition of 0.4% TC agar (Fisher Scientific, Fair Lawn, NJ) and autoclaved at $1.2 \text{ kg}\cdot\text{cm}^{-2}$ for 20 min. All cultures were kept in a growth chamber at a temperature of $23 \pm 2^\circ\text{C}$ under a 16-h light photoperiod with the light intensity of $97 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided by cool white fluorescent lamps (General Electric F20WT12CW).

Acclimatization. Plantlets longer than 2 cm with expanded leaves were removed from baby food jars. Medium was carefully rinsed off. Plantlets were transplanted individually in plastic pots (20 cm diameter $\times$ 15 cm deep) containing a mixture of peat and pine bark (1:1). All plantlets were maintained in a greenhouse and hand-watered as needed.

Statistical analysis. Experiments were conducted using a completely randomized design. Data were subject to analysis of variance using SAS (SAS Institute Inc., 2008). Mean separation was achieved by the least significant difference test at 95% level.

Results and Discussion

The use of seeds to initiate in vitro culture is common because contamination can be largely or completely prevented. In the current study, disinfecting the seeds in bleach solution (0.94% sodium hypochlorite) for 20 min was effective. No any contamination was observed in the cultures. However, culture medium browning was observed during the culture establishment, and the browning was minimized by frequent transfers. This study showed that seed germination of Alexandrian laurel is a very slow process, and it took up to 3 months for seeds to germinate in vitro (Fig. 1D), although much faster than the conventional method of up to 12 months.

All shoots multiplied on multiplication media. Alexandrian laurel has basal meristems. New shoot buds became visible at the base of shoots within 3 weeks. Leafy shoot clusters were produced by 8 weeks (Fig. 1E–F). Although 100% shoot multiplication was observed on all media tested, the BAP concentrations significantly affected shoot number (Table 1). On medium without BAP, the shoot number was low, only 2.8 shoots per explant. The incorporation of BAP into the medium at increasing concentrations significantly elevated shoot number, and the greatest shoot number of more than six was obtained with BAP at 5 and 25 μM . Although there is no report of BAP promoting shoot proliferation in Alexandrian laurel, a similar stimulatory effect of BAP on shoot multiplication has been extensively reviewed in other species (Afolayan and Adebola, 2004). For example, a maximum number (35) of shoot production was achieved with 2 mg BAP in clonal propagation of *Chlorophytum borivianum* Sant. Et Fernand. (Liliaceae) from immature floral buds (Sharma and Mohan, 2006). In their study on large-scale micropropagation of *Aloe vera*, Oliveir et al. (2009) reported that green apical shoots bearing axillary buds were multiplied four times at 30-d intervals in the MS medium supplemented with 2 $\text{mg}\cdot\text{L}^{-1}$ BAP. The shoot proliferation was also found best (80%) in the MS medium containing BAP at 2.0 $\text{mg}\cdot\text{L}^{-1}$ in micropropagation of *Aloe barbadensis* Miller (Jayakrishna et al.,

2011). These results suggest that the optimal BAP concentration for shoot multiplication is species-specific. Optimization of shoot production for each species is critical for efficient mass production or commercialization.

Root formation readily occurred as noted for single (Fig. 1G), double (Fig. 1H), or multiple root formation (Fig. 1I). After 6 weeks, 71.2% rooting was observed on the control medium without any IBA treatment with a low root number (1.4). The root number was significantly increased to 3.0 by IBA pulse treatment and to 2.2 by the presence of IBA in the medium (Table 2). However, there was no significant change in rooting percentage for IBA pulse treatment compared with the control. Also, no significant difference in rooting percentage was observed between the two IBA treatments. A significant reduction in root length was observed from IBA treatments. The greatest root length (12.8 mm) was observed from the control, whereas the shortest root length of 6.1 mm was observed from the IBA pulse treatment. The beneficial effect of IBA on rooting was noted previously in the other species of the Liliaceae family such as *Lilium longiflorum* Thunb (easter lily) (Hashem et al., 2006); *Lilium davidii* (Liu, 2011); *Asparagus racemosus* (L) (Sharan et al., 2011); and *Chlorophytum arundinaceum* (Samantaray and Maiti, 2011). Our results are consistent with these findings. However, in contrast to general belief that continuous exposure to IBA produces the shortest roots, IBA pulse treatment in this study produced the shortest roots. The reason for this may be attributable to the timeframe for root evaluation. Alexandrian laurel is noted for its slow growth. Rooting performance was evaluated 6 weeks after IBA treatment in this study. Further study on rooting performance over a longer time will help explain this phenomenon.

All proliferated plantlets were acclimatized and grew well under normal greenhouse conditions (Fig. 1J). In vitro-produced plantlets easily adapted to the natural condition and grew normally without any morphological variations.

Conclusions

This study demonstrates that in vitro propagation of Alexandrian laurel could be achieved from seeds, after seed germination, shoot multiplication, root induction, and acclimatization. This protocol provides an alternatively promising means for propagation of this species. However, maximizing shoot multiplication remains a challenge to produce a more efficient mass propagation, although our research significantly improved propagation of Alexandrian laurel. More research is needed on optimization of shoot multiplication and exploration of propagation through organogenesis and somatic embryogenesis.

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Table 1. Effect of 6-benzylaminopurine (BAP) concentrations on shoot multiplication of Alexandrian laurel.

BAP (μM)	Shoot multiplication (%) ^z	Mean number of shoots/explant ^y
0	100	2.8 ± 0.1 c
1	100	5.2 ± 0.1 b
5	100	6.7 ± 0.1 a
25	100	6.3 ± 0.1 a

^{z,y}Means followed by the same letters in each column are not significantly different at the 5% level. Data represent means of 15 replicates, four samples per replicate.

Table 2. Effect of indole-3-butyric acid (IBA) treatments on rooting of shoots of Alexandrian laurel.

IBA (μM)	Root formation (%) ^z	Mean number of roots/explant ^y	Mean length of roots/explant ^y
0	71.2 ± 0.1 b	1.4 ± 0.2 c	12.8 ± 1.0 a
5 (D)	81.6 ± 0.1 ab	3.0 ± 0.2 a	6.1 ± 0.9 c
5 (M)	86.4 ± 0.1 a	2.2 ± 0.2 b	9.9 ± 0.9 b

^{z,y}Means followed by the same letters in each column are not significantly different at the 5% level. Data represent means of two separate experiments, each with 24 replicates and two samples per replicate. (D) refers to shoots dipped in 5 μM IBA solution for 5 min (IBA pulse) and then cultured on Murashige and Skoog (MS) medium. (M) refers to 5 μM IBA included in MS medium.

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