BALANCED SEEDLING DEVELOPMENT OF ALEXANDRIAN LAUREL
UNDER DIFFERENT IN VITRO PGR TREATMENTS

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ABSTRACT

Alexandrian laurel (*Danae racemosa* L.) is a popular evergreen shrub. However, seed germination often takes up to 12 to 18 months with a germination rate of only about 20%. Also, due to its slow-growth rate, it typically takes up to six years to produce a sellable one-gallon size plant. Our research enabled us to develop an efficient *in vitro* germination protocol using a GA3 treatment that took only 10 days to 2-3 months for germination at a rate of 80 to 100%. The micro-propagated plantlets also exhibited more vigorous growth, and had stronger and more abundant roots than conventionally seed propagated plants. However, the seedling development is not balanced, exhibiting too much root growth and too little shoot development. In this research, we tested the cytokinins BA and TDZ as a means of increasing *in vitro* shoot multiplication and seedling quality enhancement. We found that BA balanced seedling development by simultaneously accelerating shoot growth and slowing down root growth, whereas TDZ significantly promoted shoot multiplication and proliferation by producing 5-20 shoots per seed.

INTRODUCTION

Alexandrian laurel (*Danae racemosa* L.) is a highly desirable but difficult to germinate and propagate species. It is one of the finest of the ornamental broadleaf, dark evergreen shrubs, displaying arching branches to three feet in height and four feet wide. The shrub is an excellent choice for the woodland and shade gardens, and is in high demand by landscape architects and designers (Sacco et al 2000, Schmid 2002). The cut foliage is favored for winter arrangements. Currently, the United States is importing almost all cut foliage of Alexandrian laurel for flower arrangement from Italy and other countries (Bellardi et al 2007).

Once established, Alexandrian laurel requires minimal or no care, such as disease and insect control, watering, and deer and other wild animals resistance (Nicholson, personal communication). Normal propagation for this species is by seeds and division. Conventional seed germination takes up to a year and half with a low success rate of only about 20% and typically takes up to six years to produce a saleable one-gallon size plant. The long period of germination and slow growth affect its commercial availability. To improve its commercial value, a shorter germination period and an efficient propagation protocol are desirable. It is also important to produce plants with high quality features.

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Our research objectives were (1). To improve the efficiency of our aseptic seed germination of Alexandrian laurel; (2). To enhance seedling quality with a balanced shoot and root growth; and (3). To develop a multiple seedlings production protocol per seed.

MATERIALS AND METHODS

Alexandrian laurel seeds were disinfected in a bleach solution containing 0.78% NaOCl plus 20 drops Tween-20 for 15 minutes, and then rinsed immediately with sterile purified water 4 times. The disinfected seeds will then be ready for transfer into culture medium.

Woody plant medium (WPM, Lloyd and McCown 1981) was used as our basic culture medium with supplements of 3% sucrose, 0.7% agar, and a pH of 5.8. Plant growth regulators including 6-benzyladenine (BA), gibberellic acid (GA₃), and thidiazuron (TDZ) at different concentrations was added to the basic WPM as treatments to evaluate the growth regulator effects on shoot initiation and multiplication.

A completely randomized design (CRD) was used for this study. All cultures were transferred onto fresh media every 4 weeks and incubated in a plant tissue culture grade growth chamber under a 16-h photoperiod and a photo flux density of 37.6 ± 4.8 μmol s⁻¹ m⁻² light per day provided by cool white fluorescent tubes at 23 ± 0.1°C.

RESULTS

An efficient aseptic germination protocol was developed for this popular ornamental shrub. The germination rate increased by as much as 4-5 times, and the germination time was shortened significantly. Germination for this species, through conventional methods, takes as long as 18 months, and the germination rate is only about 20%. Germination with our micropropagation protocol took as little as 10 days to 2-3 months with a germination rate as high as 80-100%. This is only 2-11% of the time needed by the conventional method.

Also, micro-propagated plantlets demonstrated much more vigorous growth with healthier looking and more abundant roots. One-year-old micro-propagated plantlets are bigger than two-year old conventionally propagated plantlets. In addition to fast growth of plantlets, the abundant root system of the plantlets typically is an excellent indicator of health and vigor of the plantlets.

We were able to balance the seedling growth and produced multiple shoots per seed by adding plant growth regulators such as BA or TDZ in the culture medium. BA balanced seedling development by simultaneously promoting shoot growth and slowing down root growth. TDZ enabled multiple seedling production by producing 5-20 shoots per seed.

REFERENCES