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# In Vitro Propagation of Dwarf Poinciana Using Temporary Immersion Bioreactors

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**The dwarf Poinciana (*Caesalpinia pulcherrima* L.) is a tropical tree in the Fabaceae family. It is fast-growing and produces a stunning arrangement of red, orange, and yellow flowers, and is highly desirable for home and roadside landscaping. The species also has medicinal properties, producing antibacterial and antiviral compounds. Seed germination and rooting of cuttings are the usual methods for propagation of this species. However, seed material usually lacks uniformity and cuttings do not root properly. Furthermore, traditional propagation methods are prone to disease infection. Therefore, the development of a large-scale in vitro propagation system would be of benefit to the nursery industry to allow the production of a large number of uniform and disease-free plant material. The objective of this study is to develop a system for in vitro propagation of dwarf poinciana using advanced temporary immersion bioreactor technology. Different plant growth regulators were evaluated at different concentrations for induction of pro-embryogenic masses (PEMs) and consequently, somatic embryos. Preliminary results indicate that 2,4-D resulted in higher induction of PEMs and conversion of PEMS into somatic embryos. However, somatic embryo formation was low and new studies are necessary to fine tune the concentrations of both 2,4-D and 6-BA in combination to optimize somatic embryo formation. Subsequently, studies will focus on somatic embryo conversion into plantlets, which will be placed in the greenhouse for acclimatization and eventually transferred to the field for performance evaluation.**

The dwarf poinciana (*Caesalpinia pulcherrima* L.) is a tropical tree that belongs in the Fabaceae family with a height at maturity between 8–12 feet and a spread of about 10–12 feet. The tree produces a stunning arrangement of red, orange, and yellow flowers and is highly attractive for landscape use. Therefore, it is often found as an ornamental addition planted on roadsides and as a focal point in landscaping plots for homes and businesses (Rahman et al., 1993). The tree can also be used as a biocontrol for air pollution (Manjunath and Reddy, 2017). The species has important medicinal properties and benefits, such as antibacterial compounds that assist with gastrointestinal problems (Alanis et al., 2005), antiulcer properties (Sharma and Rajani, 2011), antifungal properties (Sudhakar et al., 2006), and antimicrobial properties for combating *Escherichia* coli *E. coli*. Additional medicinal uses include antiviral properties against various forms of the herpesviruses and adenoviruses (Chiang et al., 2003). Furthermore, extracts of this plant can be formed into a dye to make graphene ink used in Inkjet-printed graphene electrodes used in sensors and other electronics (Dodoo-Arhin et al., 2016).

Dwarf poinciana is traditionally propagated either by seed or cuttings. However, seed-derived material lacks uniformity in size and quality; in addition, rooting of cuttings has been reported to be poor (Rahman et al., 1993). Micropropagation is an important tool for the clonal propagation of trees, especially those difficult to propagate by traditional propagation methods, and could be a feasible alternative for dwarf poinciana. The development of a large-scale in vitro propagation system would be of benefit to the nursery industry by allowing the production of a large number of uniform and disease-free plant material. The objective of this study is to develop a system for in vitro propagation of dwarf poinciana using advanced temporary immersion bioreactor technology.

## Materials and Methods

**Plant material and explant disinfection.** Immature flower buds of *Caesalpinia pulcherrima* were collected in Sept. 2017 from young trees growing at the Tropical Research and Education Center, Homestead, FL. (Fig. 1A). The immature flower buds were surface disinfected using the following protocol: 1 min. in 70% ethanol, 3 min. in 3% sodium hypochlorite, 3 min. in 0.01N hydrochloric acid, and three rinses of 3 min. each in sterile distilled water. After surface disinfection, explants were placed on a sterile petri dish with filter paper to dry before cut in half and placed onto initiation culture media.

**In vitro culture establishment and multiplication.** Explants were placed on 60 × 15mm disposable plastic petri dishes containing a culture medium composed of WPM salts (Woody Plant Media, Lloyd and McCown, 1980), supplemented with 20 $g \cdot L^{-1}$ sucrose and solidified with 3 $g \cdot L^{-1}$ Phytagel (Sigma, St. Louis, MO). The medium pH was adjusted to 5.7 before autoclaving at 121° C for 25 minutes at 1.2 $kg \cdot cm^{-2}$. Two to three explants were placed per petri dish.

For induction of callus, the WPM medium was supplemented with 2.27 $\mu$M 2,4-dichlorophenoxyacetic acid (2,4-D) and 2.78 $\mu$M 6-benzylaminopurine (6-BA) (Table 1). Cultures were maintained in a Percival E30B incubator (Percival Scientific, Perry, IA) at 27

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Table 1. Different treatments used on dwarf poinciana (*Caesalpinia pulcherrima* L.) explants for inducing callus formation, multiplication in semisolid medium and multiplication in liquid medium (temporary immersion bioreactor system).

| Medium[z,y] | 2,4-D ($\mu$M) | 6-BA ($\mu$M) |
|---|---|---|
| Induction WPM | 2.27 | 2.78 |
| Multiplication WPM I with charcoal | 4.53 | |
| | 1.11 | |
| Multiplication WPM II with charcoal | 9.06 | 1.11 |
| Multiplication WPM II (liquid) | 9.06 | 1.11 |
| Basal MS | 0 | 0 |
| Basal WPM | 0 | 0 |

[z]Woody Plant Media (WPM).
[y]Murashige and Skoog.

± 2° C in the dark for 65 days. Cultures were monitored weekly for callus formation. Afterward, cultures were transferred to a multiplication medium, consisting of the same WPM medium used for callus induction, but supplemented with 3 g·L$^{-1}$ activated charcoal to reduce oxidation, plus 4.53 $\mu$M 2,4-D and 1.11 $\mu$M 6-BA (Table 1). Cultures were maintained under the same environmental conditions as described above and evaluated weekly for callus multiplication and induction of pro-embryogenic masses. After 55 d in multiplication medium, cultures were subdivided and half of the cultures were transferred to fresh semi-solid multiplication medium in petri dishes supplemented with 9.06 $\mu$M 2,4-D. The other half of the cultures were transferred to a SETIS temporary immersion bioreactor system (Vervit, Belgium) containing liquid multiplication medium, consisting of the same WPM multiplication medium without Phytagel (Table 1). Cultures were maintained at 27 ± 2° C in the dark. The bioreactor parameters consisted of immersion of explants every 240 min with a duration of 1 min per immersion. Cultures in both semi-solid and liquid media were evaluated for callus growth and PEM production.

**Somatic embryo formation.** Following induction and multiplication of callus, cultures were subdivided again and transferred to a fresh semi-solid WPM medium and a MS medium (Murashige and Skoog, 1962) without plant growth regulators for somatic embryo induction (Table 1). Cultures were maintained at 27 ± 2 °C in the dark. Cultures in both media were evaluated for PEM formation and somatic embryo induction.

## Results and Discussion

**Callus formation.** After six days in the induction media, callus formed on most of the explants (Table 2). A small percentage of fungal contamination was observed (Table 2; Fig. 1B), and the contaminated petri dishes were discarded. Continuous callus growth and development was observed on the explant, ranging from a dark brown to a white color with elongated translucent cells. After increasing the levels of 2,4-D from 2.26 $\mu$M to 4.25 $\mu$M and decreasing the levels of 6-BA from 2.77 $\mu$M to 1.11$\mu$M, then once again increasing the levels of 2,4-D from 4.25 $\mu$M to 9.06 $\mu$M, the cultures began to develop roots (Table 2). This could be the result of an increase in the auxin (2,4-D) and a decrease in the cytokinin (6-BA). As a consequence, cultures were subdivided and transferred to basal WPM and MS media to allow the callus to rest and to compare both media regarding their potential for inducing somatic embryos (Table 1).

**Proembryogenic Masses (PEMs) and Embryo Formation.** One hundred and seventy days after initiation of the explants in the induction media, the first somatic embryos began to form. Proembryogenic mass (PEM) formation was observed in both the WPM and MS basal media (Fig. 1C, Table 2). PEMs are well defined structures formed from the callus, usually with a globular shape and smooth structure. However, somatic embryos were only observed on the WPM basal media (Fig. 1D, Table 2). Somatic embryos derive from the PEMs further changing from a globular shape into a cotyledonary stage. Cultures in the bioreactor showed signs of contamination and were quickly discarded (Table 2).

The successful formation of somatic embryos provided valuable information for the implementation of additional studies, which will be subsequently initiated with focus on cultures in WPM medium and with adjustments in the levels of different plant growth regulators. These studies will focus on somatic embryo induction and multiplication, as well as the transition from the

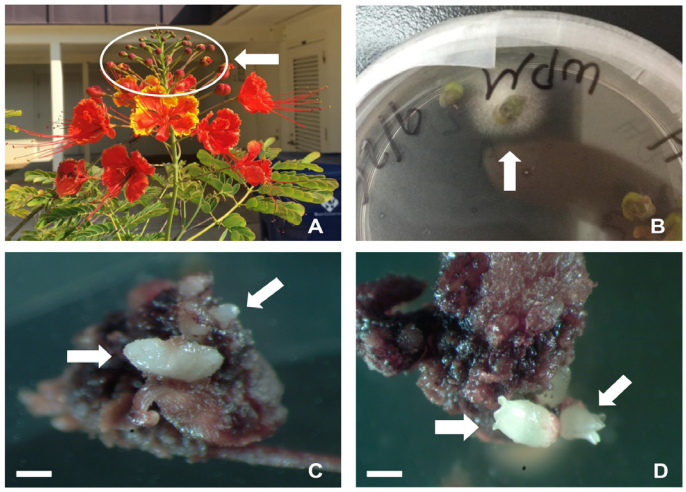


Fig. 1. *Caesalpinia pulcherrima* L. explant source. (**A**) Notice immature flower buds. (**B**) Explant showing fungal contamination. (**C**) Flower bud explants showing proembryogenic masses (PEMs) formation in woody plant culture medium (WPM) with no plant growth regulators. Bar = 2mm. (**D**) Cotyledonary stage somatic embryos (arrows) formed on callus cultures. Bar = 2 mm.

Table 2. Number of dwarf poinciana (*Caesalpinia pulcherrima* L.) explants showing contamination, callus, roots, proembryogenic masses (PEMs), and somatic embryo induction after 28 weeks in Woody Plant Medium (WPM) and Murashige and Skoog (MS) media with or without Plant Growth Regulators (PGRs).

| Culture medium | Explants contaminated | Callus formation | Callus with roots | PEMs | Somatic embryos |
|---|---|---|---|---|---|
| Induction WPM | 14 | 235 | 0 | 0 | 0 |
| WPM with PGRs and Charcoal I | 20 | 78 | 2 | 1 | 0 |
| WPM with PGRs and Charcoal II | 1 | 65 | 6 | 1 | 0 |
| Basal MS | 2 | 46 | 5 | 1 | 0 |
| Basal WPM | 2 | 45 | 5 | 2 | 2 |
| **TOTAL** | **25** | **235** | **18** | **5** | **2** |

semi-solid medium to the liquid medium in bioreactors. Additionally, a protocol should be developed to reduce contamination in liquid medium under temporary immersion bioreactors.

These preliminary results indicate that the induction of somatic embryos in dwarf poinciana is feasible. This is the first report of somatic embryogenesis in *Caesalpinia pulcherrima* L.

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