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PROPAGATION OF YELLOW KAMPONG ROYAL POINCIANA VIA SOMATIC EMBRYOGENESIS

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Abstract. ***Delonix regia*** **(Boger.) Raf. (Royal Poinciana) 'Yellow Kampong' is a very attractive ornamental flowering tree with showy yellow flowers. In South Florida, Royal Poinciana has commercial value and interest for nursery production and potential for use in the landscape. Although Royal Poincianas grow best from seed, seed propagation has several limitations and rooting of cuttings is reportedly inefficient. Furthermore, the 'Yellow Kampong' has very low seed production. Hence, we successfully induced somatic embryogenesis in Royal Poinciana. Cultures were initiated from immature zygotic embryo, comparing two levels of plant growth regulators for the rate of embryogenic induction: 0.5 mg L^{-1} of 2,4-D + 0.1 mg L^{-1} of BA (D1) and 1.0 mg L^{-1} of 2,4-D + 0.125 mg/L of BA (D2). Some contamination was observed at culture establishment and contaminated cultures were discarded. Somatic embryos developed from both treatments. However, treatment D2 had a somatic embryo formation rate of 26.2% compared to 2.4% for D1. Future perspectives include additional studies with plant growth regulators to obtain higher embryogenic rates. Current studies include the establishment of regenerated plantlets for subsequent transplanting to the greenhouse. This technique provides a means for the propagation of Royal Poinciana and a model for other woody species that are difficult to propagate by conventional methods. This is the first report of somatic embryogenesis in Royal Poinciana 'Yellow Kampong'.**

*Delonix regia (*Boger.) Raf. is an ornamental flowering tree related to the mimosa tree family (*Leguminosae*, subfamily *Caesalpinioideae*) and is native and endemic to Madagascar. This species is known in Florida as Royal Poinciana. The tree can reach up to 40 feet tall, usually with a canopy wider than its height (Turner, 1999). Considered one of the five most beautiful flowering trees in the world, it blooms between spring and early summer. The flower is very showy and its color varies from orange to orange-red, including yellow and a rare white (Stebbins, 1999). In the United States, specifically in South Florida, Royal Poinciana has great ornamental potential and is frequently used in the landscape. Stebbins (1999) reports that today there are more Royal Poincianas growing in Miami than in Madagascar. The Yellow Kampong Royal Poinciana stands for its unusual yellow flower color. The flower is comprised of five petals, four yellow and one white with yellow markings.

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Although Poinciana trees grow best from seed and flowering can be achieved within 5 to 7 years (Stebbins, 1999), propagation by seeds has several limitations and rooting of cuttings is very inefficient. Germination percentage is reduced due to presence of a hard seed coat (Duarte, 1974) and position of seeds in the pod influences germination and quality of the seed produced (Srimathi et al., 1992). Furthermore, although Royal Poinciana trees are drought-resistant and grow well in climates with both dry and wet seasons (Stebbins, 1999), climate changes during flowering and pollination periods can result in low seed production (Cutteridge and Stur, 1994). The 'Yellow Kampong', in particular, has a very low seed production, although it has not been determined if the reasons for that are related to climate changes (Schokman, 2003, personal communication).

Alternatively, somatic embryogenesis is a tissue culture technique that has been applied to woody species with success, allowing the in vitro propagation of species that are difficult to propagate by conventional methods. In Royal Poinciana, shoot proliferation has been observed from shoot tips excised from seedlings germinated in vitro (Rahman and Hossain, 1992). Somatic embryogenesis has been reported for a number of leguminous plants. However, no reports exist to date on somatic embryogenesis in Royal Poinciana (Lakshmanan and Taji, 2000).

The main objective of this work is to develop an efficient somatic embryogenesis protocol for the in vitro propagation of Royal Poinciana var. 'Yellow Kampong'.

Materials and Methods

Plant material selection and sterilization. Immature seedpods of Royal Poinciana var. 'Yellow Kampong' were collected during September 2003 from a landscape tree located in Miami, Florida. Seedpods were cut in sections and pre-washed with soap (Alconox 1% w/v, Alconox, Inc., New York, N.Y.) and water to remove external dirt and contaminants. Under a sterile laminar flow hood, pre-washed seedpods were placed into a sterile container with a solution of 3% (v/v) sodium hypochlorite. To allow better surface sterilization, 16 drops of polysorbate 20 surfactant (polyoxyethylene sorbitan monolaurate, Tween® 20, ICI Ltd., New Castle, Del.) were added to the hypochlorite solution under agitation for 20 minutes, followed by three15-minute rinses using autoclaved distilled water.

Culture medium preparation. The culture medium used for somatic embryogenesis induction was the Woody Plant Medium (Lloyd and McCown, 1980) modified with 1 g L^{-1} casein hydrolysate, 40 g L^{-1} sucrose and 8 g L^{-1} agar (Fisher Scientific, Fair Lawn, N.J.). Additional modifications in the medium included the addition of 2,4-D at 0.5 or 1.0 mg L^{-1} and BA at 0.1 or 0.125 mg L^{-1}. The culture medium was autoclaved at 121 °C for 20 min at 15 psi, and dispensed into plastic disposable 60 × 15 mm plastic Petri plates.

Plant Growth Regulator Treatments. Preliminary studies indicated that the use of an auxin (2,4-D) and a cytokinin (6 BA)

provided higher induction rates in royal poinciana. The combination of 1.0 mg L^{-1} of 2,4-D + 0.125 of BA provided best rates of embryogenic induction. Higher levels were detrimental to the cultures, while lower levels were not evaluated (Myers, 2004). Therefore, in this study the treatments were: 0.5 mg/l 2,4-D + 0.100 mg L^{-1} BA (D1) and 1.0 mg L^{-1} 2,4-D + 0.125 mg L^{-1} BA (D2).

Culture initiation and somatic embryogenesis induction. After sterilization, seedpods were dissected and the seeds removed under aseptic conditions. In preliminary studies with Royal Poinciana, we evaluated the potential of different seed explants for inducing somatic embryogenesis. Intact seeds and immature zygotic embryos were tested. We found that the use of entire immature zygotic embryos was more efficient in inducing somatic embryogenesis (Myers, 2004). Therefore in this study we used immature zygotic embryos as explants. Before immature zygotic embryos were placed in culture medium, they were nicked with a scalpel by performing an incomplete cross-sectional cut in the embryo surface. Each plate received between 4 explants. Cultures were kept in the dark at 25 ± 2 °C. A total of 80 immature zygotic embryos were established in culture.

Experimental Design and Statistical Analysis. From 20 seedpods collected from the source tree, 80 immature zygotic embryos were extracted and used as explants. For each treatment a total of 10 plates were used. Explants were randomly distributed among the different treatments. The Analysis of Variance (ANOVA) procedure (SAS Institute, 1989) was used to evaluate the effects of each treatment on the induction of embryogenic cultures. The LSD method was used for the separation of means where significant differences existed ($P \leq 0.05$).

Results and Discussion

Embryogenic Callus Formation. After two weeks in culture, callus formed in both treatments. Contamination was observed in 7.2% of the cultures in initial establishment and contaminated cultures were promptly discarded. Callus consisted of disorganized cell growth around the explant, showing round to elongated translucent cells. Callus formation is likely to have resulted from a combined effect of auxin and cytokinin in the culture medium. Auxins can induce the formation of embryogenic cells and promote repetitive cell division, while cytokinins are required to induce embryogenesis in several dicotyledonous species (Gray, 2000). However, response to auxins and cytokinins is variable and depends on the species and type of auxin and cytokinin used (Lakshmanan and Taji, 2000), and relative amounts applied. In *Medicado truncatula* and *M. sativa*, 2,4-D supplemented with BA promoted enhanced embryogenic calli and subsequent embryo differentiation (Trinh et al., 1998). In previous experiments entire seeds produced limited amount of embryogenic callus. Nicking the immature zygotic embryo with a scalpel improved embryogenic callus formation. However, embryogenic callus formation was still poor for both treatments. Additional studies are required to evaluate alternative sources of auxins and cytokinins in different concentrations, as well other inductive factors, such as aminoacids and carbon source. These have been found to increase the frequency of somatic embryogenesis (Lakshmanan and Taji, 2000).

Proembryogenic Masses (PEMs) Formation. Four weeks after cultures were initiated and callus growth was observed, PEMs started to form. PEM formation was observed for all treatments and explants used. However, immature zygotic embryos showed prolific PEM formation.

PEMs distinguished themselves from callus by showing particular characteristics for embryogenic cells. PEMs are groups of cells showing organized growth, with small, densely cytoplasmic (embryogenic) cells of isodiametric shape (Gray, 2000). Overall, PEM formation for all explants indicates an excellent potential for inducing somatic embryogenesis. For treatment D1, 59.5% of the explants formed callus, while 33.3% of the explants showed PEMs. For treatment D2, 47.6% of the explants formed callus and 52.4% resulted in PEMS. These rates are relatively high compared to other species. However, the removal of plant growth regulators could be critical for somatic embryo differentiation and maturation (Lakshmanan and Taji, 2000), and therefore deserves further studies.

Somatic Embryo Formation. Three weeks after PEMS were observed, somatic embryos started forming in both media. The process of histodifferentiation was observed, with three distinct stages of somatic embryos:

a) globular stage, where embryos appear as well-formed round structures (Fig. 1).

b) torpedo stage, showing an elongated axis (Fig. 2).

c) cotyledonary stage, where cotyledons are clearly visible (Fig. 2).

However, somatic embryo development was asynchronous, where different stages of development could be observed within the same treatment and replication unit.

On treatment D1 only 1 fully developed somatic embryo formed, representing a 2.4% embryogenic rate, while for treatment D2, 11 somatic embryos formed, with a embryogen-

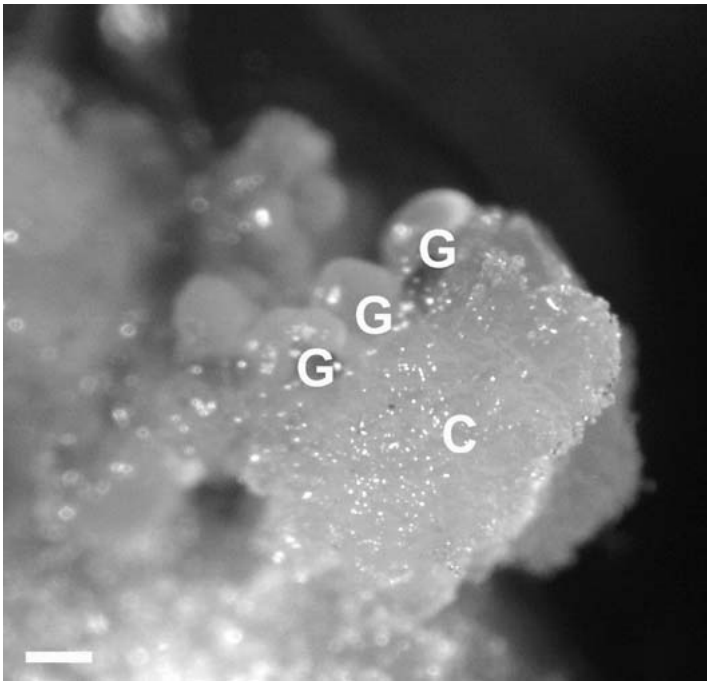

Fig. 1. Embryogenic callus and globular stage somatic embryo induction in *Delonix regia* explants. **C** = callus; **G** = globular stage somatic embryos. *Bar* = 10 mm.

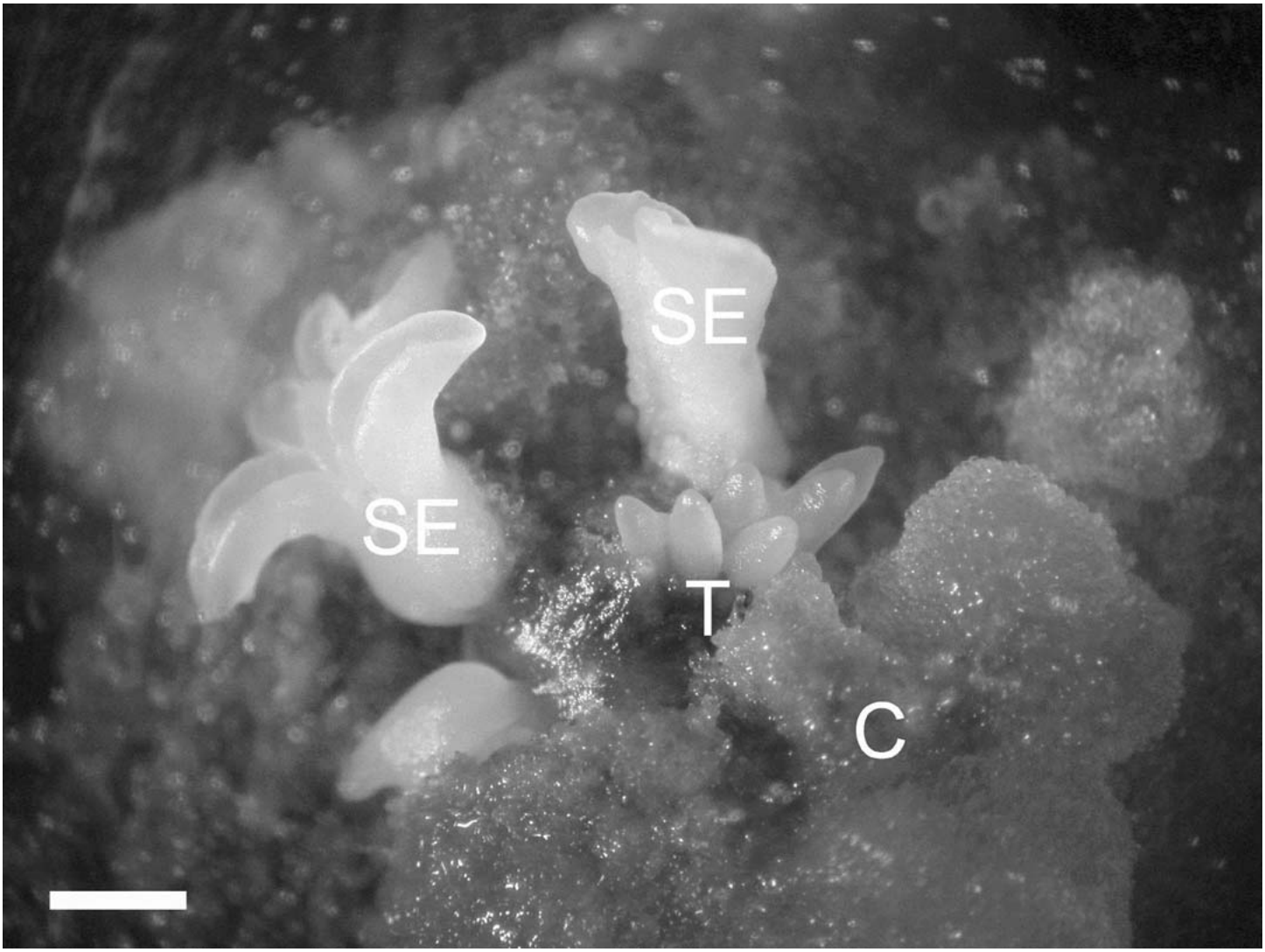

Fig. 2. Somatic embryo induction in *Delonix regia* var. 'Yellow Kampong' explants. **C** = callus; **T** = torpedo stage somatic embryos; **SE** = cotyledonary stage somatic embryos. *Bar* = 150 mm.

ic rate of 26.2%. There were significant differences for the means (P = 0.0094) for somatic embryo formation and the treatments tested (Table 1). Treatment D2 consisting of 1.0 mg L^{-1} of 2,4-D + 0.125 mg L^{-1} of BA provided better somatic embryo induction.

Despite the ten-fold difference in embryo induction between both treatments, the rate is still low and requires additional experiments to improve somatic embryo differentiation. Proposed future experiments include optimization of culture conditions with adjustments of plant growth regulator levels and/or their complete removal. Maturation treatments may be necessary to allow proper accumulation of reserves for histodifferentiation of somatic embryos. This could be a critical stage in development to determine successful germination and regeneration of somatic embryos. A desiccation regime may contribute to the physiological maturity of somatic embryos for proper germination and conversion (Lakshmanan and Taji, 2000).

This study demonstrated the first report of induction of somatic embryogenesis in Royal Poinciana var. 'Yellow Kampong' from immature zygotic embryos. A relative high level of plant growth regulators (2,4-D and BA) resulted in the greatest number of somatic embryos formed, although the rate of formation was still low. Additional studies are necessary to improve induction rates, including alternative plant growth regulator sources at different levels. Cultures have been routinely maintained and additional (repetitive) somatic embryo formation has been observed after the end of this study. Future perspectives include the optimization of the culture system aiming synchronous and increased production of somatic embryos, as well as studies on improved maturation, germination and conversion. Currently, plantlets of Royal

Table 1. Number of explants (mean ± SD) showing callus, proembryogenic masses (PEMs), and somatic embryo induction after five weeks in culture.

Treatment	No. Callus[z]	No. PEMs	No. Somatic Embryos
0.5 mg/l 2,4-D + 0.1 mg/l BA (D1)	2.5 ± 0.7 a	1.4 ± 1.0 a	0.1 ± 0.3 a
1.0 mg/l 2,4-D + 0.125 mg/l BA (D2)	2.0 ± 0.7 a	2.2 ± 0.6 a	1.1 ± 0.7 b

[z]Means followed by the same letter within columns are not significantly different by the LSD test (P ≤ 0.05).

Poinciana 'Yellow Kampong' are being acclimatized to be transplanted and grown under greenhouse conditions.

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