Kinetin and 6-benzyladenine induce different morphogenetic responses in cotyledonary segments of royal poinciana

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Abstract

Understanding the in vitro performance of royal poinciana explants cultured in media supplemented with different types and concentrations of cytokinins may aid in the optimization of regeneration systems established for this woody species. In the present study we evaluate the in vitro performance of royal poinciana cotyledonary explants cultured in the presence of high concentrations of 6-benzyl adenine (BA) and kinetin (KIN). Cotyledonary segments obtained from in vitro germinated seedlings were inoculated in Murashige and Skoog (MS) medium, supplemented with different concentrations (1, 2, 4, 8 mg L⁻¹) of BA or KIN. In the control treatment, no plant growth regulators (PGRs) were added. After 40 days of culture, regardless of the concentration used, the treatments supplemented with BA presented higher calli percentage and fresh mass compared to treatments supplemented with KIN. Adventitious shoots were mainly observed in BA-treatments. Histological analysis showed that adventitious shoots formed at the periphery of callus formed from mesophyll cells in the regions of the explant sectioning. The results obtained provide new information for the establishment of a micropropagation system for royal poinciana, an important ornamental tree species.

Keywords: Delonix regia, flamboyant, cytokinins, micropropagation, shoot regeneration

Introduction

Delonix regia (Bojer ex Hook) Raf., popularly known as royal poinciana or flamboyant, is a Fabaceae tree species widely distributed in tropical and subtropical regions. It is fast-growing and develops an umbrella-shaped cover with recognized agroforestry and ornamental relevance, being used mainly in urban landscapes (Neto and Souza, 2011).

D. regia has also been used as a medicinal agent due its potential for production of antioxidant, antibacterial, anti-inflammatory, antidiarheal, antidiabetic, antimicrobial and gastroprotective compounds (Shahir et al., 2011; Singh et al., 2002; Wang et al., 2016; Fatmawaty et al., 2017). In addition, the gum obtained from D. regia seeds is used as binder in the manufacture of tablets (Adetogun and Alebiowu, 2009; Rodriguez-Canto et al., 2019).
Due to the ornamental and medicinal importance of this species, *in vitro* regeneration systems have been established to contribute to genotype multiplication and especially to assist bioprospection programs. Previous studies have reported the potential of embryonic *D. regia* explants to induce morphogenetic responses (Myers and Vendrame, 2004; Abdi and Hedayat, 2011).

Costa et al. (2019) observed adventitious shoot bud formation from the culture of cotyledony segments in medium supplemented with cytokinin. According to the authors, the morphogenetic responses of the species increased linearly with the increase of the 6-benzyladenine (BA) concentrations (0.125 to 2.0 mg L⁻¹). The highest average number of adventitious shoots per explant was observed in the treatment supplemented with 2.0 mg L⁻¹ BA although, a low rate of shoots has been reported. However, higher concentrations of this plant growth regulator (PGR), or even another type of cytokinin, were not tested.

Kinetin (KIN), for example, is capable of inducing cell proliferation and new adventitious shoot bud formation when added to the culture medium (Frágua et al., 2004; Castilho et al., 2019), and is also recommended for micropropagation of some woody species, for example, *Salix humboldtiana* (Pereira et al., 2000), *Acacia auriculiformis* (Yadav et al., 2016) and *A. leucophloea* (Sharma et al., 2017).

Understanding of the *in vitro* performance of *D. regia* when cultured in medium supplemented with other types and concentrations of PGRs can help to optimize regeneration systems established for this plant species. The present study aims to evaluate the performance of *D. regia* cotyledony explants when cultured in the presence of high BA or KIN concentrations to induce *in vitro* organogenesis to *D. regia*.

### Materials and methods

*D. regia* seeds collected in Jataí (17°52’51"S, 51°42’0"W), in the state of Goiás, Brazil, were subjected to mechanical scarification, on the side opposite the hilum, or even another type of cytokinin, were not tested.

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#### Results and discussion

The presence, concentration and type of cytokinin influenced the induction of morphogenetic responses in *D. regia* (Figure 1A). Organogenic calli were observed only in the presence of cytokinins. In the absence of PGRs, cotyledony explants did not present any morphogenetic response (Figure 1B, C). Supplementation with cytokinin is essential for the induction of morphogenetic responses in *D. regia* cotyledony explants (Costa et al., 2019). During *in vitro* organogenesis, molecular cytokinin signaling is necessary to induce cell proliferation and differentiate adventitious shoot buds promoting the differential gene expression essential for the formation of these organs (Müller and Leyser, 2011; Su et al., 2011).
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In the present study, the percentage of callus fit a quadratic model (Figure 1B). Regardless of the concentration used, BA-treatments presented higher calli percentage compared to treatments supplemented with KIN (Figure 1B). The higher BA efficacy in inducing morphogenetic responses may be related to its lower susceptibility to enzymatic degradation (Magyar-Tabori et al., 2010). BA is a stable cytokinin that persists in the culture medium (Rahman, 2006). It is possible that the BA conjugated amount in the medium was lower than KIN, thus presenting a higher free form quantity readily available for the cotyledonary explants (Buah et al., 2010). This observation is in agreement with Klem et al. (2004) who reported that BA is chemically more stable than other purine-derived cytokinins.

The highest percentage of callus formation for BA and KIN was 88.29% and 72.13% in the concentrations of 4.46 and 4.15 mg L⁻¹, respectively (Figure 1B). On the other hand, the highest concentration of BA and KIN tested (8 mg L⁻¹) inhibited callus induction presenting the lowest calli percentages, 45.77% and 9.5%, respectively. The correct concentration of PGRs is essential for the specification and differentiation of morphogenetic responses and this is a species-dependent feature. For Mimosa caesalpinifolia, another Fabaceae species, was also reported that the greatest number of morphogenetic responses was obtained in the presence of 4 mg L⁻¹ BA and reduced responses in higher concentrations (> 6 mg L⁻¹) of this same PGR (Bezerra et al., 2014). Similarly, García-Angulo et al. (2018) showed that the increase over 5 mg L⁻¹ BA significantly reduced the number of morphogenetic responses of Populus hybrids. For D. regia, previously studies recommended the use of low concentrations (0.125 - 2.0 mg L⁻¹) of PGRs (Myers and Vendrame, 2004; Abdi and Hedayat, 2011; Costa et al., 2019). However, the effect of higher concentrations (> 2 mg L⁻¹) of cytokinins to induce morphogenetic responses had not been tested until this present study.

The treatments supplemented with BA showed higher fresh weight values and fit a quadratic model (Figure 1C). According to the fitted model, the highest value was observed in the treatment supplemented with 4.95 mg L⁻¹ BA. For KIN-treatments the highest fresh weight value was observed at 4.74 mg L⁻¹ although it has been lower than BA-treatments (Figure 1C). This variable seems to be related to the callus formation once the same overall pattern has been observed for both parameters (Figure 1B, C).

Adventitious shoot formation (Figure 1D) was observed in almost all BA-treatments (2, 4, and 8 mg L⁻¹) and only in the 4.0 mg L⁻¹ KIN-treatment. The highest average of shoots was obtained in the treatment supplemented with
2.0 mg L⁻¹ BA, although, no differences were observed among the treatments that induced shoots (Figure 1D). The efficiency of BA to induce shoots in comparison to other cytokinins was reported by Zarinjoei et al. (2014), using calli derived from cotyledon segments of *Gleditsia caspica*, another woody Fabaceae species. According to these authors, the highest bud formation was observed in MS medium supplemented with 1 mg L⁻¹ BA. At this concentration, 94.3% of the cultivated calli produced shoot buds with an average number of 4.3 buds/explants. Similar results were also reported for *Citrus macrophylla* and *C. aurantium*, in which the number of shoots was superior in culture medium supplemented with 2 and 3 mg L⁻¹ BA, respectively, compared to treatments supplemented in conjunction with KIN (Tallón et al., 2013). In *Punica granatum*, the greatest formation of adventitious shoots from cotyledonary explants was also obtained in a medium supplemented with 2 mg L⁻¹ BA (Parmar, 2012). The number of adventitious shoots observed for *D. regia* in the present study was low. Previous studies with this species also reported the low morphogenetic potential of *D. regia* that presented few morphogenetic responses (Myers and Vendrame, 2004; Abdi and Hedayat, 2011). Further studies are still needed to optimize the induction and elongation stages of *D. regia* adventitious shoots.

The histological analysis corroborated the macroscopic observations showing that no morphogenetic response was observed in the explants cultured in the absence of cytokinin (Figure 2A). In treatments supplemented with KIN and BA, morphogenetic responses started from mesophyll cellular divisions, in the explant section region (Figure 2B, C). The cotyledonal explant mesophyll was dorsiventral consisting of 3-4 layers of palisade parenchyma and 10-15 layers of spongy parenchyma (Figure 2A).

**Figure 2.** Histological characterization of *D. regia* cotyledonal explants after 40 days of culture. (A) Cotyledonal explant cultured in MS medium without plant growth regulator. (B, C) Cotyledonal explants cultured in media supplemented with 2.0 mg L⁻¹ of KIN (B) and BA (C). Abbreviations: arrowhead – promeristem; asterisk – callus; fp foliar primordium; me mesophyll. Bars = 500 µm.

The mesophyll cells of cotyledonal explants cultured in media supplemented with cytokinins presented an intense process of cell division (Figure 2B, C). However, in the treatment supplemented with KIN, a progressive effect of cell proliferation was observed (Figure 2B). Most peripheral cells divided and hypertrophied, appearing voluminous and vacuolized, the typical appearance of callus cells (Figure 2B). No other morphogenetic response, except for the callus, was observed. Calli were also observed in the treatment supplemented with BA. However, in the callus periphery, adventitious shoots were histologically structured consisting of leaf beginnings and a promeristem, consistent with the organogenic regeneration pathway (Figure 2C). The results obtained suggest there are differences in the recognition or action mechanism of BA and KIN, since adventitious shoot differentiation was observed mainly in the presence of BA, even if both constitute purine-derived cytokinins.
Conclusions

Cytokinin supplementation was essential to induce in vitro morphogenetic responses from D. regia cotyledonary explants. All treatments supplemented with BA showed higher percentage of calli and fresh weight gain in comparison to KIN. Adventitious shoots were observed mainly in BA-treatments too. Although the conversion of shoots into plants was not observed, we believed that the results obtained may contribute to the establishment and optimization of in vitro regeneration systems of this important ornamental woody species.

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Author Contribution


References


