Phytomorphology Модерн

Influence of different concentrations of jasmonic acid on *in vitro* development of *Catasetum fimbriatum* Lindl. (Orchidaceae)

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Abstract

In vitro seeding constitutes an indispensable tool for propagation of the main commercial species of orchids. This study aimed to analyze the *in vitro* development of *Catasetum fimbriatum* under different concentrations of jasmonic acid in Murashige & Skoog culture medium compound of ½ macronutrients. After 180 days of cultivation, concentration of 0.25 and 0.50 µL • L⁻¹ caused a significant increase in number of roots and leaves. The concentration of 1.00 µL • L⁻¹ showed the best result for the length of longest root and largest leaf, the total length of seedling, and the total fresh and dry masses. Nevertheless, the use of this plant regulator on *in vitro* culture media still requires further investigations to determine the optimal concentration in order to obtain desirable phytotechnical characteristics in different orchid species.

Keywords: *Catasetum fimbriatum*, jasmonic acid, plant regulator, *in vitro* propagation

Introduction

Including 7 genera that inhabit tropical regions of the Americas, Catasetinae Lindl. (Orchidaceae) spreads from sea level to locations with more than 1,000 m of altitude (Hoehne 1949; Romero 1990; Pridgeon *et al.* 2009; Pedroso-de-Moraes *et al.* 2012). These deciduous, sympodially growing orchids have well developed pseudo-bulbs, related to the storage of water during periods of drought (Dodson 1975; Moraes & Almeida 2004). The representatives of the subtribe occur in treetops or on the litter, from where their fleshy roots absorb nutrients (Hoehne 1938).

The main Brazilian genus, under intense extractivism, for having species of high commercial value, is *Catasetum* Rich. ex Kunth (Joly 1998; Pedroso-de-Moraes *et al.* 2007). It presents plants with extensive eco-morpho-phisiological specializations related to cross fertilization (Zimmerman 1991; Moraes & Almeida 2004). In particular, sexual trimorphism described for flowers of subgenus *Orthocatasetum* Mansf. (Hoehne 1938) drew attention of many orchidologists (Moraes & Almeida 2004).

The genus *Catasetum* is composed of about 300 species, which can present diclinic masculine, feminine and monoclinic flowers; such floral typologies can coexist even on the same floral rachis (Hoehne 1938; Zimmerman 1991; Bicalho & Barros 1998; Pridgeon *et al.* 2009). This fact gave rise to controversies regarding its adaptive derivational status (Zimmerman 1991; Moraes & Almeida 2004).

Catasetum fimbriatum Lindl. is a monoic species that show male and female diclinic flowers quite different morphologically from each other, as well as incomplete monoclines (Pedroso-de-Moraes 2002) are frequently visited by bumble bees (*Euglossa* sp.), which pollinate them (Cardoso 2014). The male inflorescences are more frequent, longer, with 10–15 flowers of 7–9 cm diameter, with durability between 15–30 days after anthesis, colored from green to yellow (Cardoso 2014). It has fleshy roots related to the fixation and absorption of decaying organic matter, as well as thin secondary roots related to aeration of the root system (Pedroso-de-Moraes 2002). Pseudobulbs have an average length of 12–27 cm and a diameter of 6–13 cm. Leaves usually light green and pleated, 30–36 cm of length in average (Pedroso-de-Moraes 2002; Cardoso 2014).

Catasetum orchids are considered ornamental plants of great prominence in orchid culture, mainly due to the exoticism of their flowers, being used as potted plants. In addition, the high valuation on the market is due to slow metabolism of the majority of orchids, resulting the lower rates of conventional vegetative propagation and higher expenses for cultural treatment (Pedroso-de-Moraes 2000). Therefore *in vitro* propagation, whether by sowing or regenerating tissues, is actively applied to increase the production of seedlings of high genetic quality and reduction of production costs (Stancato *et al.* 2001).

There are no specific culture media suitable for particular orchid's taxa. It is difficult to explain why in certain combinations of components of environment and culture conditions one results are successful, whereas others – not (Ventura *et al.* 2002). This question is even more ambiguous when analyzing data on the use of different concentrations of recently discovered phytohormones and plant regulators, such as jasmonic acid (JA).

For jasmonates, in relation to morphophysiology, both promoter and inhibitory effects are reported on representatives of different plant groups. A number of studies were carried out to investigate its role on the regulation of several physiological processes, such as: senescence (Parthier 1990), accumulation of storage proteins (Staswick 1992), development of embryos (Wilen *et al.* 1991) and biosynthesis of secondary metabolites (Facchini *et al.* 1996). Such mechanisms of action are result of alterations in gene expression (Reinboth *et al.* 1994).

Exogenous application of JA promotes senescence, petiole abscission, root formation, coiled tendrils, ethylene and β-carotene synthesis (Staswick 1992). In addition to the promoter effects, this plant growth regulator can inhibit seed germination, and inhibit or stimulate callus growth, root growth, chlorophyll production and pollen grain germination relating from applied concentrations (Parthier 1990; Vick & Zimmerman 1986).

In relation to the *in vitro* culture, the influence of different concentrations of JA on the development of post-germination plantlets of *C. fimbriatum* is unknown. Since, the aim of this work was to evaluate the seedling development of *C. fimbriatum* in MS medium (Murashige & Skoog 1962) influenced by different concentrations of JA after 180 days of *in vitro* culture.

Material and methods

Seeds were obtained from mature plants (9 months of development) after artificial cross fertilization and were provided by the Pedroso-de-Moraes Orchidarium (Santa Cruz das Palmeiras, SP, Brazil).

For *in vitro* seeding, MS media (Murashige & Skoog 1962) were composed of half of the macronutrient concentration, supplemented with 0, 0.25, 0.5 and 1.0 µL • L⁻¹of JA, 1 gL⁻¹ of activated carbon and 30 gL⁻¹ of sucrose, with 5.8 pH adjusted before the addition of 7 gL⁻¹ of agar. Then, 50 ml of each culture medium containing the different JA concentrations were poured into four 250 ml sterilized flasks and autoclaved at 121 °C and 1 atm pressure for 20 minutes (Arditti & Ernst 1992).

For disinfestation, seeds were agitated in the solution of 5 % sodium hypochlorite for five minutes in centrifuge tubes. Tubes later were immersed in 70 % alcohol and taken to the laminar flow chamber, where the seeds were washed four times with distilled water and deposited in the flasks containing the culture media (Pedroso-de-Moraes *et al.* 2009).

Four flasks were seeded by treatments with 1 g of inoculated seeds per container. The seeded flasks were sealed with a transparent or metallic plastic cap and maintained for 180 days in a climatic chamber (BOD MA 403) at constant temperature of 25 °C, under a photoperiod of 12 hours and light intensity of *cca.* 116 µmol • m⁻² • s⁻¹ (Dezan *et al.* 2012).

The following biometric phytotechnical characters were evaluated: number of roots (NR), number of leaves (NL), total length of seedling (LS), length of the largest root (LR), length of the largest leaf (LL), total fresh mass (FM) and total dry mass (DM). Total dry mass was calculated after drying of the material at 65 °C until reaching the constant dry mass (Dezan *et al.* 2012).

The results were processed through polynomial regression analysis using BioEstat 5.3 (Ayres *et al.* 2007). In order to select the regression model that best fits the obtained data, we considered the non-significance of regression deviation, the degree of significance present for the highest order model, and finally the value of the coefficient of determination (R^2) (Fernandes *et al.* 2012).

Results and discussion

Number of roots under all applied concentrations was higher than in control group of *C. fimbriatum* (Fig. 1 A). In general, it confirms assertion that the exogenous application of JA increases the rhizogenesis (Staswick 1992). In case of *C. fimbriatum*,the addition of 0.25 µL • L⁻¹ of JA to the culture medium showed the best result for NR in comparison to other concentrations. However, it also was shown that increasing concentration of JA determines the decrease of the number of roots for *Cattlianthe* Jewel Box (Borin *et al.* 2015).

Application of 0.25 and 0.50 µL • L⁻¹ concentrations of JA resulted in increasing number of leaves (Fig. 1 A). Application of 0.50 µL • L⁻¹ of JA showed the highest influence on foliar genesis. However, decrease in leaf formation occurred with the use of 1.00 µL • L⁻¹ of the plant regulator, indicating a phytotoxic effect. For *Cattlianthe* Jewel Box, the addition of 0.25 µL • L⁻¹ of JA to the culture medium had no statistically significant influence on the number of leaves. For this hybrid, the increase in concentrations in the culture media (from 0.50 µL • L⁻¹ to 1.00 µL • L⁻¹) resulted in decrease of NL (Borin *et al.* 2015) and foliar senescence. In case of *Phalaenopsis* Blume, addition of 0.25 µL • L⁻¹ of JA promoted the number of leaves, while further increase of concentrations decreased it (Hsu 2003).

Our findings regarding NR and NL stimulation by JA are in agreement with those obtained for *Zea mays* L. (Vick & Zimmerman 1986). However in other plant groups, the application of JA caused inhibition of rhizogenesis and decreased number of leaves (Staswick 1992).

Application of 0.25 µL • L⁻¹ concentration of JA showed the weakest result in LR and LS in comparison to control (Fig. 1 B). However, there was an increase in LR and, consequently,
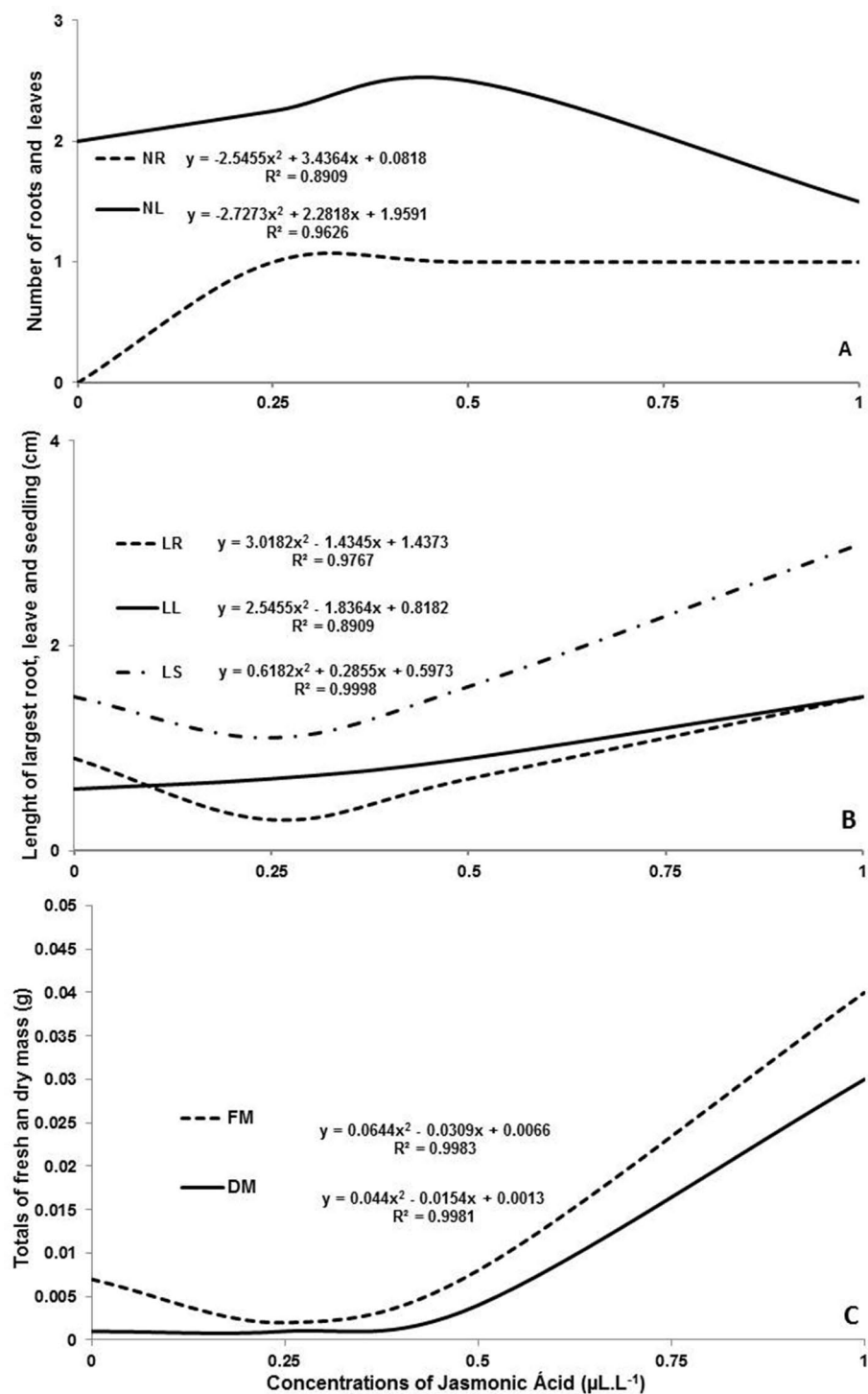
Fig. 1. Polynomial correlation of biometric variables of *Catasetum fimbriatum* seedlings: **NR** – number of roots; **NL** – number of leaves, **LR** – length of largest root; **LL** – length of largest leaf; **LS** – total length of seedlings; **FM** – fresh mass; **DM** – dry mass.

of LS with the increasing concentrations of JA; application of 1.00 µL • L⁻¹ of JA demonstrated the highest results of LR and LS. In case of LL, all applied concentrations were effective, with the concentration of 1.00 µL • L⁻¹ presenting the best result (Fig. 1 B). Such outcomes contradict to findings reported for *Cattlianthe* Jewel Box, for which the concentration of 0.25 µL • L⁻¹ was the most effective for LR, LL and LS, with increasing concentration generating the worse results (Borin *et al.* 2015). Regarding LL, our results corroborate the observations recorded for *Allium cepa* L., *Phaseolus coccineus* L. (50 µM) and *Zea mays* (0.1, 10 and 100 µM), with highest concentrations increasing the length of leaves (Parthier 1990; Maksymiec & Krupa 2007). From other side, for the *Phalaenopsis* the lowest concentration of JA (25 µM) promoted the highest increase in leaf length (Hsu 2003).

Regarding the FM variable, application of 0.25 µL • L⁻¹ concentration of JA added demonstrated the inhibitory effect. However, increasing concentration of the plant regulator promoted FM, where application of 1.00 µL • L⁻¹ of JA was the most effective (Fig. 1 C). This confirms results obtained for soybean, when the increase of JA concentrations resulted in increased fresh mass of seedlings (Koda 1992). These findings also confirms that JA has a positive effect on the cellular water balance, promoting smaller reductions in contained water contents, and promoting greater tissue turgidity (Koda 1992; Maksymiec & Krupa 2007; Kerbauy 2008).

Similarly, our studies showed that the increase in JA concentration (mainly 1.00 µL • L⁻¹) in the culture media promoted an increase in the dry mass of seedlings, (Fig. 1 C). It was also shown that application of JA, pure or fermented by *Botryosphaeria rhodina* (Berk. & M.A. Curtis) Arx, promotes significant increases in the dry mass of floral buds and fruits of *Capsicum frutescens* L. and *Physalis angulata* L., up to the highest (1.00 µM) treated concentration (Linares *et al.* 2010). The increase in dry mass induced by JA can be explained by increasing gene expression related to photosynthetic processes and carbohydrate assimilation (Parthier 1990; Koda 1992).

Conclusions

Based on the results of this study it is evident that: a) the concentration of JA of 0.25 µL • L⁻¹ and 0.50 µL • L⁻¹ showed a significant increase for number of roots and number of leaves respectively; b) application of 1.00 µL • L⁻¹ of JA was the most effective for increasing in length of the largest root, length of the largest leaf, length of seedlings, as well as for fresh and dry mass; c) the use of JA as a plant growth regulator for *in vitro* cultivation still requires further investigation to determine the concentration to be applied by producers, with the aim of obtaining a higher number of desired phytotechnical characteristics in different species of orchids.

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