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Ethylene and gas exchange: A study on the impact on the *in vitro* development of *Dipteryx alata* seedlings

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ABSTRACT

Barueiro (*Dipteryx alata* Vog.) is a native species found in the Brazilian Cerrado, playing a significant role in the biodiversity of this biome, and holds significant economic value due to its versatile applications, including its seeds, which are widely used in the food and cosmetics industries. This study aimed to evaluate the effects of ethylene on the *in vitro* development of barueiro seedlings. Nine treatments were used to modify the *in vitro* environment: Control without gas exchange; Control with gas exchange; Ethylene without gas exchange; Ethylene with gas exchange; Potassium permanganate without gas exchange; Potassium permanganate with gas exchange; Control with atmosphere renewal on the 7th day; Ethylene added on the 7th day; and Potassium permanganate added on the 7th day. Phytotechnical parameters, such as shoot length, root length, stem diameter, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight, were evaluated. Among the parameters analyzed, only Leaf Area, Fresh Root Mass, Root Dry Mass, and Shoot Dry Mass showed statistically significant differences. The reduction promoted on these parameters by ethylene was not superior to having an environment with greater gas exchange restriction, and for some parameters, the simple fact of reduced gas exchange promoted effective inhibition. Therefore, it is recommended to use *in vitro* conditions that increase gas exchange, resulting in more vigorous barueiro seedlings with a greater capacity for morphogenic response.

Index terms: Ambience; Cerrado; volatile.

INTRODUCTION

The Cerrado is one of the most important biomes in Brazil, characterized by its vast biodiversity of fauna and flora, and is recognized as the richest savanna in the world (Lambers et al. 2020). Among the native species of the Cerrado is the barueiro (*Dipteryx alata* Vog.), also known as cambaru and coumaru. The barueiro is widely used by local populations as a source of family income (Lima et al. 2022). According to Ribeiro et al. (2019), this species is at risk of extinction due to factors such as high exploitation, agricultural expansion, deforestation, fires, and projected climate changes. This is a major concern, particularly given the barueiro's potential and economic importance (Santos et al. 2024).

The *in vitro* culture of barueiro offers a promising strategy for large-scale propagation and conservation, ensuring seedlings with genetic and phytosanitary quality. However, research in this area is still limited, with studies focusing mainly on *in vitro* germination, morphogenic responses of juvenile tissues, and asepsis protocols (Araruna et al., 2017; Barbosa et al., 2021; Rezende et al., 2019; Targa et al. 2021).

Given the difficulties in establishing *in vitro* culture from adult material, either due to challenges in asepsis or lack of morphogenetic response, most publications involving this species use material from seeds germinated *in vitro* (Silva et al., 2016a; Silva et al., 2016b; Pinhal et al., 2017; Silveira et al., 2022). It is worth noting that the barueiro seed does not present any difficulties

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in terms of germination, presenting a high rate of germination and the absence of dormancy (Correa et al., 2000).

The classic tissue culture environment is typically characterized by reduced atmospheric exchange between the *in vitro* and ex vitro environments, generating a completely different environment from that which the plant would find in nature (Chen et al., 2016; Matuszkiewcz et al., 2018; Araújo et al, 2023). For most plants, the condition of reduced atmospheric exchange has a negative impact on their development, especially when related to the higher *in vitro* concentration of ethylene.

Ethylene is one of the compounds present in the *in vitro* environment, and its effects can vary, influencing both the inhibition and promotion of morphogenesis in some species. It can also lead to early leaf abscission, the appearance of hyperhydricity, and inhibition of adventitious root formation, making the regulation of ethylene a crucial factor in micropropagation (Carrari-Santos et al. 2023; Castro-Camba et al. 2024). However, few studies evaluate whether ethylene is really the main inhibitory factor in an environment with restricted gas exchange.

In recent years, several studies have reported differences in physiological and morphogenic responses due to increased gas exchange in the *in vitro* environment. These responses may be related to the reduction in humidity, oxygen, carbon dioxide concentrations, as well as ethylene levels (Lazzarini et al., 2019; Silveira et al., 2020; Barba-Espin et al., 2020; Ševcikova et al., 2021; Fortini et al., 2021; Carrari-Santos, et al. 2023; Carrari-Santos et al., 2024).

Thus, the present study aimed to evaluate the influence of gas exchange, and the inhibition of ethylene action, in the initial development of barueiro seedlings.

MATERIAL AND METHODS

The barueiro seeds used in the experiment were obtained from the Municipal Market of Pirapora, Minas Gerais (MG), Brazil. The experiment was conducted in Laboratory of Plant Physiology and Applied Genetics, in Federal University of São João del Rei, campus Sete Lagoas. Seed asepsis was performed by washing them in 70% ethyl alcohol for 1 minute, followed by immersion in 50% commercial sodium hypochlorite for 15 minutes, with subsequent rinsing three times in autoclaved distilled water. This final procedure was conducted in a laminar flow hood.

The experimental design was completely randomized, consisting of nine treatments, each with five replications. Each replicate consisted of a 350 mL flask with two inoculated seeds. The treatments were as follows: Control without gas exchange (CN); Control with gas exchange (CY); Ethylene without gas exchange (EtN); Ethylene with gas exchange (EtY); Potassium permanganate without gas exchange (PPN); Potassium permanganate with gas exchange (PPY); Control with atmosphere renewal on the 7th day (C7); Ethylene added on the 7th day (Et7); and Potassium permanganate added on the 7th day (PP7). Gas exchange was facilitated by using flask lids with two small openings containing the homemade microporous membrane (Saldanha et al., 2012), allowing for the entry and exit of gases. For the last three treatments (C7, Et7, and PP7), caps that prevented gas exchange with the external environment were used.

The culture medium consisted of a mixture of 20 g/L sucrose and 1/4 strength MS salts with vitamins (Murashige and Skoog, 1962). The salts of MS medium were added to the culture medium as powdered pre-mix (Sigma Chemical Company, St. Louis, MO, USA). The pH of the medium was adjusted to 5.7 using a pH meter. The medium was then gelled with agar (6 g/L) (Dinâmica Química®) and autoclaved for 20 minutes for 121 ºC and 1,5 atm. .During the experiment setup, an autoclaved 1.5 mL microtubes was placed in each flask, except for the CN, CY, and C7 treatments. These tubes contained either 400 µL of ethylene or 20 mg of potassium permanganate in granular form (Labsynth). Ethylene was generated using Ethrel®☐ (Bayer S.A. South Carolina, USA), with the addition of 50 µL of 1 mol/L hydrochloric acid to the microtubes to facilitate ethylene release. In the Et7 and PP7 treatments, ethylene and potassium permanganate were added, respectively, seven days after inoculation. Treatment C7 served as a control, where the flask was simply opened and closed in the laminar flow hood on the 7th day. All treatments were placed in a growth room for 30 days, under a light intensity of 50 µmol m^2/s, a photoperiod of 16 hours, and a temperature of 25 °C ± 2 °C.

Data relating to the germination percentage of the different treatments were evaluated.

After 30 days of inoculation, the following parameters of barueiro seedlings were evaluated: root length - cm (RL), shoot length - cm (SL), stem diameter - cm (SD), number of leaflets (NL), leaf area (LA, calculated as the mean of the length and width of three leaflets), fresh root mass- g (FRM), shoot fresh matter mass - g (SFMM), dry root mass - g (DRM), and shoot dry mass - g (SDM).

The data for the measured parameters were subjected to the Bartlett test to verify the homogeneity of variance and to the Shapiro-Wilk test to verify the normal distribution, and were subjected to analysis of variance (ANOVA) and Tukey's means test at a 5% significance level. The analyses were conducted using the Sisvar software, Version 5.7 (Build 91) (Ferreira, 2011).

RESULTS AND DISCUSSION

No differences in germination percentage were observed in the different treatments.

When comparing treatments that differ exclusively by the presence or absence of membranes, enabling greater gas exchange, it is clear how much the limitation of gas exchange inhibits seedling development (Figure 1). These results are in agreement with what was previously observed by in studies with Brazilian ginseng (*Pfaffia glomerata* Spreng.) (Saldanha et al. 2012), candeia (*Eremanthus incanus*) (Miranda et al. 2016), ornamental pepper (*Capsicum annuum*) (Batista et al. 2017), torch ginger (*Etlingera elatior*) (Pinheiro et al., 2021), *Solanum lycopersicum* L. cv. 'MicroTom'(Pepe et al. 2022), *Bauhinia forficata* (Pinheiro et al. 2023) among others.

Despite being visually different, only Leaf Area (LA), Fresh Root Mass (FRM), Root Dry Mass (RDM), and Shoot Dry Mass (SDM) showed statistically significant differences among the parameters analyzed in the different treatments (Table 1). Leaf area parameters and variables related to the root system are more sensitive to the treatments.

Leaf area was largest in the condition with gas exchange and in the combined condition of gas exchange and ethylene sequestration. In general, ethylene has a negative correlation with leaf growth. (Dubois et al., 2018). Exogenous ethylene promotes changes in central metabolism, reducing growth (Nascimento et al., 2021).

Figure 1: Overview of flasks with barueiro seedlings after 30 days in treatments Control with gas exchange (CY); Control without gas exchange (CN); Ethylene without gas exchange (EtN), and Potassium permanganate without gas exchange (PPN).

Table 1: Average Leaf Area (LA), Root Fresh Mass(RFM), Root Dry Mass (RDM) and Shoot Dry Mass (SDM) of barueiro seedlings *in vitro* by the different treatments, Control without gas exchange (CN); Control with gas exchange (CY); Ethylene without gas exchange (EtN); Ethylene with gas exchange (EtY); Potassium permanganate without gas exchange (PPN); Potassium permanganate with gas exchange (PPY); Control with atmosphere renewal on the 7th day (C7); Ethylene added on the 7th day (Et7); and Potassium permanganate added on the 7th day (PP7) .

Treatments	LA	RFM	RDM	SDM
CN	0,660 c	0,345 b	0,085 b	0,135 a b
CY	3,500 a b	0,970 a	0,204 a	0,368 a
EtN	0,840 a b c	0,375 b	0,057 b	0,107 b
EtY	2,072 a b c	0,754 a b	0,092 a b	0,164 a b
PPN	1,9525 a b c	0,385 b	0,072 b	0,175 a b
PPY	3,5925 a	0,697 a b	0,142 a b	0,330 a b
C7	1,708 a b c	0,538 a b	0,098 a b	0,202 a b
Et7	1,102 a b c	0,606 a b	0,084 b	0,138 a b
PP7	1,865 a b c	0,795 a b	0,152 a b	0,250 a b

Tukey test with α at 5% significance. Means followed by the same letters do not differ from each other in the same column. CV = 55.99%. CV = 33.76%. CV = 42,29%. CV = 45,35%.

Although it has not been evaluated, it is clear that the concentrations of photosynthetic pigments present in treatments with the addition of ethylene are lower than in other treatments. The presence of ethylene induces lower chlorophyll production, in addition to inducing chlorophyll degradation in mature leaves (Ceusters et al., 2018).

It is interesting to note that the root system development is extremely sensitive to ethylene (Qin et al., 2019), which corroborates what was observed in our work. The gas exchange condition also resulted in a greater mass of the root system, both in fresh root mass and dry root mass (Table 1). However, this was statistically similar to the EtY, PPY, C7, Et7, and PP7 treatments. The CN, EtN and PPN treatments differed statistically from the gas exchange condition.

Potassium permanganate ($KMnO4$) is a substance that sequesters ethylene from the atmosphere. Its action occurs through the oxidation of the phytohormone, resulting in water, carbon dioxide, and manganese and potassium dioxide (Gutierrez-Aguirre et al., 2023). Comparing the data from the PPN and EtY treatments, a similar behavior is observed, although they do not differ statistically from other treatments. However, it can be inferred that potassium permanganate played an effective role in enabling the reduction of the ethylene concentration rate through its oxidation (Guha and Rao, 2020), regarding gas exchange, which together allowed the plants in this treatment to have better development.

The same trend was observed in the evaluation of Shoot Dry Mass (SDM), with the gas exchange treatment showing higher values than the others, though it did not differ statistically from the CN, EtN, PPN, PPY, C7, Et7, and PP7 treatments (Table 1). The EtN treatment showed a lower result, differing significantly from the gas exchange condition.

It is noteworthy that all treatments involving flask opening and atmosphere renewal (C7, Et7 and PP7) showed higher values compared to those without gas exchange. Even the flask without membranes, when its atmosphere was renewed on the seventh day, resulted in more robust plants. This improvement can be attributed to the enhanced gas exchange dynamics, which affects multiple physiological processes. Specifically, the renewal of the flask atmosphere promotes better growth and higher metabolic rates through the regulated supply of oxygen, carbon dioxide, and water vapor. Additionally, it reduces the relative humidity within the flasks, increasing transpiration and enhancing water and nutrient absorption (Kozai, 2010; Xiao et al., 2011).

Ethylene is the best known of the organic volatiles that accumulate in the atmosphere *in vitro*. When analyzing the treatment with permanganate, which directly impacts the action of ethylene, it is observed that in the environment with restricted gas exchange, despite the lower values than in the environment with gas exchange, there is no statistical difference between the treatments. The relationship between ethylene and plant growth *in vitro* is complex and multifaceted (Neves et al. 2021; Shi and Zhu, 2022). While ethylene is often considered an inhibitory volatile compound, its effects vary significantly based on concentration, timing, and species-specific responses (Ahammed et al., 2020).

Recent research has highlighted complex interactions between ethylene and other phytohormones in regulating plant growth and development (Dias et al., 2010; Dubois et al., 2018; Qin et al., 2019; Ahammed et al., 2020; Nascimento et al. 2020; Zhou et al. 2023; Mai et al. 2025). Ethylene interaction with cytokinin during root development (Street et al., 2015), interaction with abscisic acid (ABA) and gibberellin during seed germination and abiotic stress (Corbineau, 2024), and interaction with auxin (Hu et al. 2017), are examples of the dynamics of ethylene crosstalk. These interactions involve multiple signaling pathways and metabolic processes that collectively should be influence plant responses to *in vitro* conditions.

The production of various organic volatiles under increased gas exchange conditions suggests a broader network of chemical signals affecting plant development (Lazarini et al., 2019; Araújo et al. 2023).

Barbosa et al. (2021), when working with zygotic baru embryos, did not observe any difference in the use of different seals. This was possibly due to some relationship with cotyledon reserves and the speed of initial development, with a consequent change in sensitivity to ethylene.

An important observation to be made is that according to work carried out by Jiang and collaborators (2024), an environment with high humidity concentration induces an increase in ethylene production. At the same time, ethylene plays a key role as a modulator of responses in high humidity (Jiang et al., 2024). Thus, it is observed that classical *in vitro* conditions are conducive to the induction of ethylene production, and not merely an accumulation of ethylene and its autocatalytic effect.

In our study, it was demonstrated that treatments with ethylene addition (EtY, EtN and, Et7) or with its suppression (PPN, PPY, and PP7) were not the most inhibitory for development. At the same time, the simple restriction of gas exchange is already inhibitory. This suggests that successful *in vitro* culture requires conditions that promote lower environmental humidity will consequently allow for less ethylene synthesis and, a lower inhibitory effect of ethylene on development.

CONCLUSION

Potassium permanganate positively influenced the *in vitro* development of barueiro, showing that seedlings are sensitive to ethylene in the *in vitro* environment. However, it is important to highlight that the increase in gas exchange promotes more significant growth and development of barueiro seedlings *in vitro*, with reduced humidity and consequently lower ethylene production.

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