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Research Article

Influence of nitric oxide donor on the physiological quality of seeds of *Myrcia retorta* Cambess

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Abstract

Brazil, with its vast biodiversity, is home to several species of the Myrtaceae family with significant ornamental potential that is still underutilized. *Myrcia retorta* Cambess is a endemic Brazilian plant wich stands out as a promising species for ornamental cultivation. The objective of this study was to evaluate the effect of the nitric oxide donor S-nitrosoglutathione (GSNO) on the germination of *M. retorta* seeds, with the aim of optimizing germination conditions for the domestication of this species. The seeds were divided into two batches based on their morphology: Batch 1 (spherical seeds) and Batch 2 (flattened seeds). The seeds were treated with five concentrations of GSNO (0, 2.5, 5, 10, and 15 mM) and the parameters evaluated included germination percentage, shoot and root length, and time to stabilization of germination. In batch 1, doses of 2.5 mM and 5 mM GSNO resulted in faster germination stabilization (6 and 7 days, respectively) compared to the control (9 days). In addition, these doses increased germination percentage by 15% and 12%, respectively. However, higher concentrations (10 mM and 15 mM) were found to be phytotoxic, reducing shoot length by 30% and 45% and root length by 28% and 42%, respectively. In batch 2, although no statistically significant differences were observed, a trend towards reduced germination and growth with increasing GSNO doses was observed. It is concluded that GSNO positively affects the germination and initial development of *M. retorta* at low concentrations, especially in spherical seeds, suggesting its potential in the domestication process of the species for ornamental purposes. However, doses higher than 5 mM should be avoided due to toxicity risks, highlighting the need to optimize concentrations for practical application.

Keywords: Brasilian flora; Myrtaceae; seed morphology; seed vigor; angiosperms; germination capacity; plant growth regulator.

Introduction

Brazil has a great diversity of myrtaceae, with approximately 1030 species distributed in 145 genera, making it the eighth largest family of angiosperms (Saber et al., 2024).They have applications ranging from the production of pharmaceuticals, fruit growing, urban tree planting and landscaping, with the *Myrcia* genus being one of those with the largest number of species in the family in the country (Gressler, Pizo e Morellato, 2006; Lorenzi, 2016). This genus has species with the most diverse applications, with studies reporting their use as anti-inflammatory, anti-haemorrhagic, antioxidant and aiding in antimicrobial activities (Cascaes, Guilhon, Andrade, Zoghbi, & Santos, 2015).

Myrcia retorta Cambess, a species endemic to Brazil, can be found in fields, highland fields, rupestrian fields, savannahs and restingas and is a species endemic to Brazil, occurring in the states of Paraná, Santa Catarina, Rio Grande do Sul, São Paulo and Minas Gerais, with a distribution center in Santa Catarina and Paraná (Flora e Funga do Brasil [REFLORA], 2024). It is a shrub or tree, 1.5 to 6 m high with twisted cylindrical non-nodal branches, characteristics that make it attractive for use as an ornamental plant.

Seed germination, the processes involved and the substances capable of altering it are extremely important. Understanding aspects that can affect the germination capacity of a species helps to improve its reproduction and management, and can be manipulated according to the desired results (Boucelha & Djebbar, 2015).

The usual form of reproduction for species of the *Myrcia* genus is by seed germination, but there is a lack of protocols for the genus, since no species is described in the Seed Analysis Rules (RAS) (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2009; Ferreira & Maria, 2013; Lorenzi, 2016).

Nitric oxide (NO), a plant growth regulator, is a free radical found naturally in the gaseous state, acting as one of the main forms of nitrogen used in plant signaling. NO has been highlighted in the scientific community due to its involvement in various plant processes, such as flowering, germination, growth, and acting directly in response to abiotic stresses (Corpas & Palma, 2018; Nabi et al., 2019).

Due to its gaseous nature and short half-life, the direct application of NO to plants is unfeasible, so donor molecules are used as NO reservoirs. Among the donors, S-nitrosoglutathione (GSNO) is considered a natural reservoir of NO in cells, releasing it spontaneously (Mata-Pérez et al., 2020).

Given the need for studies involving the germination of potentially ornamental native species and the interaction of NO with plants, the aim of this work was to evaluate the effect of doses of the nitric oxide donor S-nitrosoglutathione (GSNO) on the germination of *Myrcia retorta* Cambess seeds.

Materials and Methods

The experiment was carried out at the seed analysis laboratory of the State University of Londrina (UEL). Fruits with visible characteristics of maturity, identified by their vinaceous color and the texture of the epicarp, were collected in Moro da Pedra Branca, Serra do Cadeado, Ortigueira, Paraná (23°58'02"S; 51°04'33"O) in May 2022, using five mother plants.

After collection, the fruit was stored in a refrigerator at 10º C in airtight glass jars for 15 days to carry out the seed viability test and then the experiment. The fruit was stored instead of the already processed seeds due to the high number of myrtaceae with recalcitrant seeds.

To assess the viability of the batches, a tetrazolium test was carried out with 4 replicates of 50 seeds, in which the seeds were soaked for 3 hours at 25ºC and then placed in 0.1% tetrazolium salt for 3 hours at 35ºC (Lamarca & Barbedo, 2014).

To pulp the fruit, we followed the methodology proposed by Lorenzi (2016), who recommends conditioning the fruit in closed plastic bags for a week at room temperature to soften the pericarp. Subsequently, the pericarp was removed using a 1mm sieve under running water, leaving only the seeds.

Following the processing process, the seeds were separated into two batches, taking into account the number of seeds per fruit, with batch I - completely spherical seeds from fruit with one seed, and batch II - seeds with one of the sides flattened from fruit with two seeds, fruit found with three seeds were insufficient for the composition of a batch, all the mother plants analyzed had fruit containing one, two and three seeds.

The seeds were disinfected in a 1% sodium hypochlorite solution for 2 minutes, then immersed in 70% alcohol for 2 minutes, washed in running water for a further 2 minutes and placed in distilled water. They were then placed in an Erlenmeyer flask wrapped in aluminum foil to avoid light, containing 300 mL of the solution in the different doses of GSNO (2.5 mM, 5 mM, 10 mM and 15 mM) and kept stirring for 5 minutes at room temperature. For the control treatment, distilled water was used to soak the seeds.

The following parameters were assessed: germination (GERM), Days to Stabilization (DTS), aerial part length (APL), root part length (RPL) and total length (TL). For germination, a Mangelsdorf germinator was used at a temperature of 30ºC. The seeds were placed on Germtest paper rolls soaked in distilled water containing 2.5x the mass of the dry paper (MAPA, 2009).

The design chosen for the experiment was completely randomized. The factorial scheme adopted was 2 x 4 + 1, given that there were 2 batches of seeds, 4 treatments and 1 control. For the germination test, 4 replicates of 50 seeds per treatment were used, and 1800 seeds for the experiment as a whole, the result expressed as a percentage obtained from the average. The length test was conducted with 20 seeds per repetition, with 2 repetitions per treatment, and the result expressed as the average of the two repetitions (MAPA, 2009).

The assumptions of normality of errors and homogeneity of variances were tested by Shapiro-Wilk and Bartlett ($p \geq 0.05$). Subsequently, the data was subjected to analysis of variance (ANOVA) at 5% significance and the F-test for the means. When a significant effect of the GSNO doses was observed, linear or non-linear regression analysis was carried out (quadratic, 3- or 4-parameter logistic, segmented, Brain-Cousens model), statistical calculations were performed using AgroR software (RStudio, 2020; Shimizu, Marubayashi and Gonçaves, 2022).

Results and Discussion

In batch 1, no statistical difference was observed in the germination percentage, while for the parameters of days to stabilization, aerial part length, root length and total length, the means in the treatments differed statistically and were then subjected to regression analysis (Table 1 and Figure 1).

In relation to DTS, the application of low doses of GSNO caused the germination process to stabilize earlier, i.e. the seeds germinated faster. In the control treatment the average was 30.5 days, at the lowest dose stabilization occurred at 18.5 days, and rose as the doses increased, with the highest concentration (15 mM) even being higher than the control treatment, making it possible to see a toxic action at high doses of GSNO for this parameter (Figure 1).

In relation to length, both total, aerial part and root part, the application of GSNO led to a reduction in these parameters. The 15mM dose of the compound was the one with the lowest average for the three variables (Table 1).

Table 1 - Parameters assessed in the germination test of batch 1 as a function of GSNO doses: germination (GERM), Days to Stabilization (DTS), aerial part length (APL), root part length (RPL) and total length (TL).

GSNO	GERM(%)ND	DTS*	APL(cm)*	RPL(cm)*	TL(cm)*
0mM	52.5	30.5	5.58	7.94	13.52
2.5mM	49.5	18.5	5.15	7.11	12.27
5mM	42.5	26.75	3.79	5.68	9.47
10mM	44	30.75	4.15	6	10.15
15mM	45	32	3.88	5.6	9.47
CV(%)	20.31	22.28	12.87	11.34	10.46

ND= did not differ statistically, *= means differed in the F test at 5%.

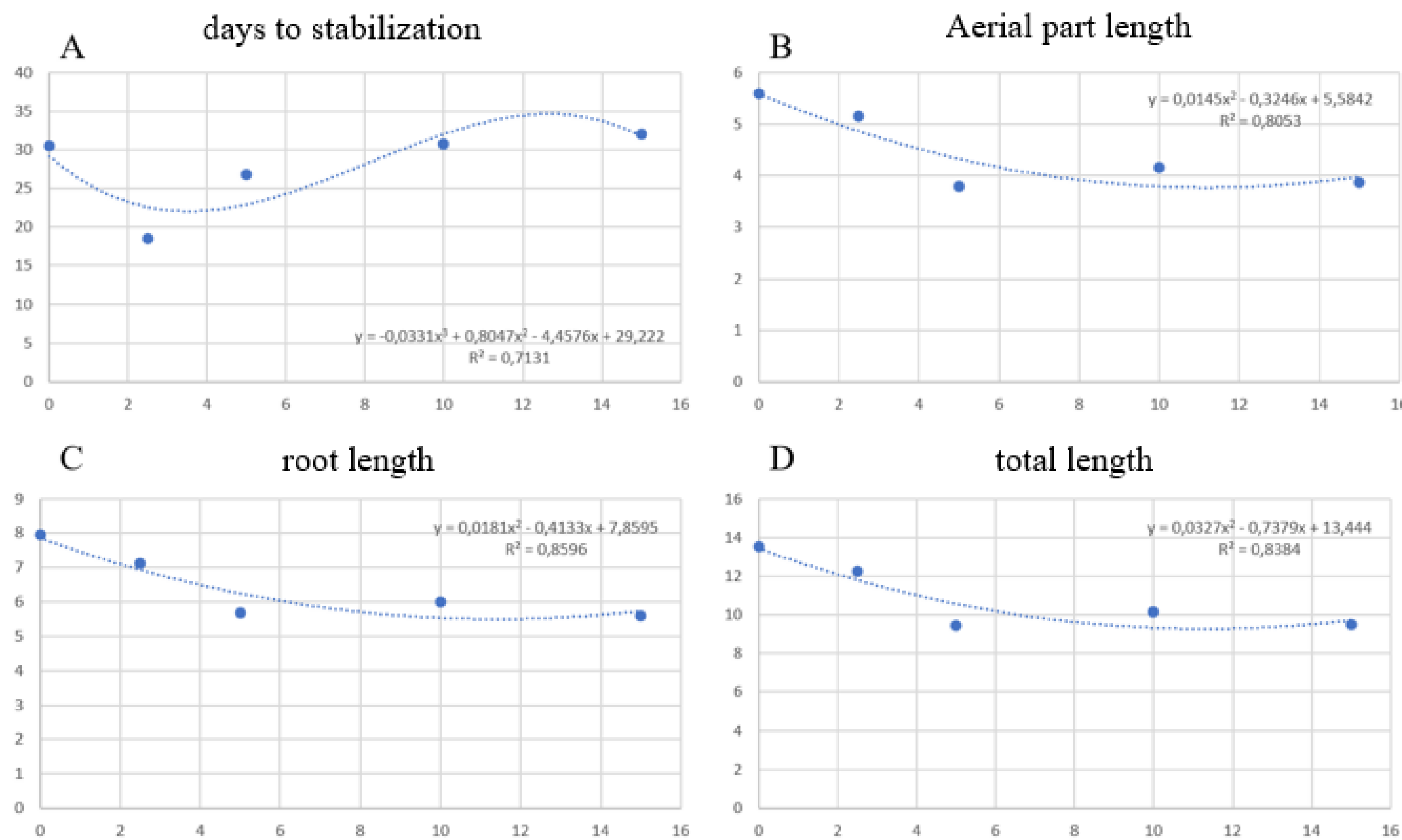

Figure 1 - Polynomial regression for the variables that showed a statistical difference as a function of GSNO doses for batch 1. (A) days to germination stabilization, with the x-axis being the GSNO doses and the y-axis being the days to stabilization. (B) Aerial part length, with the x-axis being the doses and the y-axis being the size in cm. (C) Root length, with the x-axis being the doses and the y-axis being the size in cm and (D) total length, with the x-axis being the doses and the y-axis being the size in cm.

In batch 2, none of the parameters analyzed showed any statistical difference, although there was a tendency for all the parameters to decrease when the highest dose of GSNO (15 mM) and the lowest dose (2.5 mM) were observed, meaning that the compound had an effect on the species (Table 2).

For cumulative germination, the total number of seeds germinated each day was observed and the graph with the values is shown in Figure 2. It is possible to observe a greater speed of germination for the seeds in batch 1, where by day 40 most of the repetitions in all the treatments had already stabilized. When we look at batch 2, we can see that a greater number of days are needed for the experiment to stabilize, with an average of 76 days needed for all treatments and repetitions to stabilize.

Table 2 - Parameters assessed in the germination test of batch 2 as a function of GSNO doses: germination (Germ), days to stabilization (DTS), aerial part length (APL), root part length (RPL) and total length (TL).

GSNO	Germ(%)^{NF}	DTS^{NF}	APL(cm)^{NF}	RPL(cm)^{NF}	TL(cm)^{NF}
0mM	52.5	30.5	5.58	7.94	13.52
2.5mM	52	48.25	5.14	8.52	13.66
5mM	51	32.75	5.22	8.22	13.45
10mM	58	52	4.87	8.23	13.1
15mM	49	32	4.65	7.61	12.26
CV(%)	18.84	40.94	11.47	15.28	13.03

NF= did not differ statistically, **= means differed in the F test at 5%.

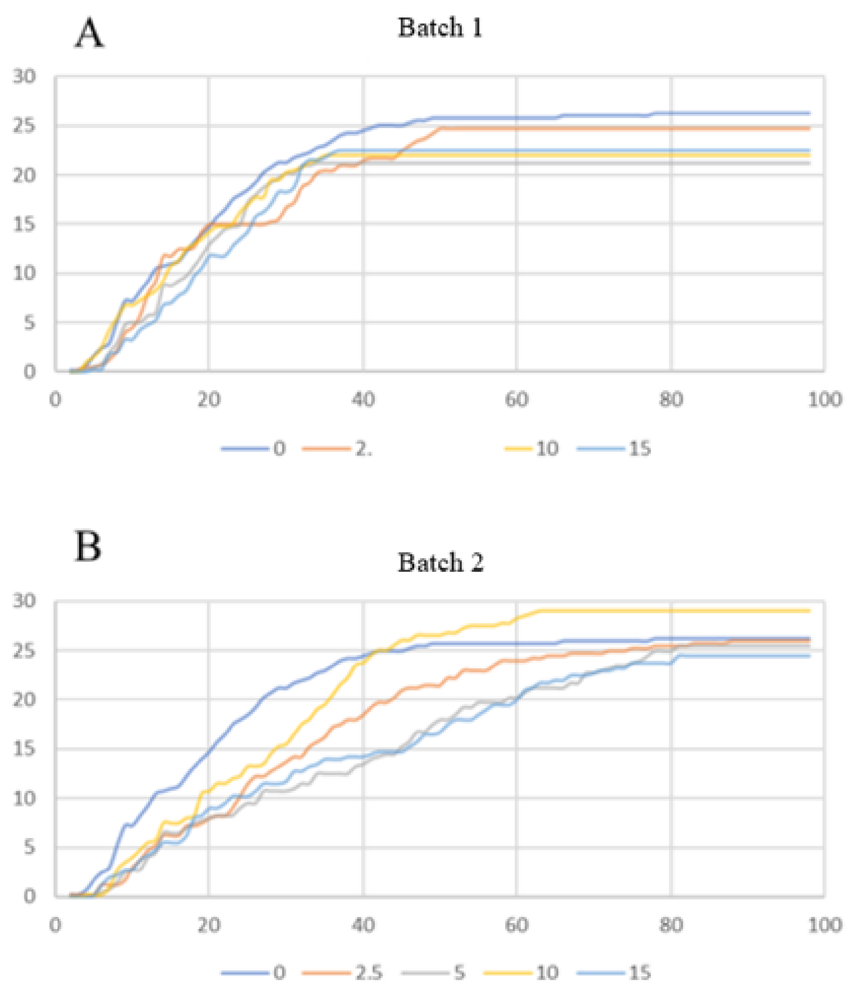

Figure 2 - Graphs of accumulated germination of *M. retorta*. (A) Germination of batch 1. (B) Germination of batch 2. x-axis is represented by the days to stabilization and the y-axis by the number of seeds germinated out of 50 seeds.

Nitric oxide is known to induce the breaking of dormancy and produce stimuli for seed germination, as observed in several species (Signorelli & Considine, 2018; Monteiro et al., 2023). In *Arabdopsis* seeds, Liu et al. (2009) observed an accumulation of NO possibly in the endosperm of the seeds, which enabled the catalysis of ABA (abscisic acid), breaking dormancy, which may explain the lack of interaction between NO and *Myrcia retorta* in the germination percentage, given the tiny amount of endosperm due to the presence of leafy cotyledons.

Some other studies have also shown differences with seeds treated with NO, which would enable the degradation of the endosperm, thus acting to increase germination (Signorelli & Considine, 2018), with results opposite to those found for the Myrtaceae species studied, making it necessary to carry out more studies on the interaction of nitric oxide with seeds from this family, in view of the low availability of literature and being justified by the wide morphological variety of seeds found in the family.

In relation to the doses of GSNO studied for the germination of *Myrcia retorta* seeds, no differences were observed in the same way as those obtained by Monteiro et al. (2023), where the compound increased the germination rate of *Dyckia excelsa* seeds, and no interaction was observed with the length of the plants obtained, which was statistically different in this study.

Conclusions

It is concluded that GSNO positively affects the germination and initial development of *M. retorta* at low concentrations, especially in spherical seeds, suggesting its potential in the domestication process of the species for ornamental purposes. However, doses higher than 5 mM should be avoided due to toxicity risks, highlighting the need to optimize concentrations for practical application.

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