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Seeds

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Dormancy overcoming in seeds of *Myrciaria glomerata* O. Berg.

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Abstract - *Myrciaria glomerata* O. Berg. (Myrtaceae) is a fruit tree native to the Brazilian Atlantic Forest with potential for use as food and medicine, besides having good ecological potential. However, this species is limited by low germination rates. Thus, this study aimed to evaluate different methods to overcome dormancy in seeds of *M. glomerata* and to improve seedling vigor. For this, two experiments were conducted. In Experiment 1, seven treatments were tested: a control treatment, immersion in water at 100 ºC for 30 seconds, immersion in concentrated sulfuric acid (H_2SO_4) for 1 and 5 minutes, immersion in an aqueous solution of gibberellic acid (GA_3) at the concentrations of 250 and 500 mg L^{-1} for 24 h, and seeds homogenized with an aqueous solution of Stimulate® at a concentration of 5 mL kg^{-1} seed. In Experiment 2, six doses of Stimulate® were tested: 0, 5, 10, 15, 20, 25, and 30 mL kg^{-1} seed. The immersion of seeds in 250 and 500 mg L^{-1} GA_3 contributes to increase germination rates, seed length, fresh mass, and dry mass compared to the control and the other methods tested. Immersions in water at 100 ºC and in H_2SO_4 are not recommended for *M. glomerata*. The treatment with Stimulate® at 15–25 mL kg^{-1} seed increased all characteristics analyzed. Immersion in 250 mg L^{-1} GA_3 and Stimulate® at 20–25 mL kg^{-1} seed efficiently stimulated seeds to overcome dormancy in *M. glomerata*, as well as improved seedling vigor.

Keywords: gibberellic acid; Stimulate®; physiological quality; germination.

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Superação de dormência de sementes de *Myrciaria glomerata* O. Berg

Resumo - *Myrciaria glomerata* O. Berg. (Myrtaceae) é uma frutífera nativa da Mata Atlântica com potencial alimentício, medicinal e ecológico, mas com baixa taxa de germinação. Objetivou-se com este trabalho avaliar diferentes métodos na superação da dormência de sementes e no vigor de plântulas de *M. glomerata.* Foram realizados dois experimentos. No experimento 1, foram testados sete tratamentos: controle; imersão em água a 100 ºC por 30 segundos; imersão em ácido sulfúrico concentrado (H_2SO_4) por 1 e 5 minutos; imersão em solução aquosa de ácido giberélico (AG_3) 250 e 500 mg L^{-1} e imersão em solução aquosa com 5 mL de Stimulate® kg^{-1} de sementes. No experimento 2, foram testadas sete doses de Stimulate®: 0; 5; 10; 15; 20; 25 e 30 mL kg^{-1} de sementes. A imersão das sementes em 250 e 500 mg L^{-1} AG_3 contribui na velocidade de germinação e maior comprimento, massas frescas e secas, em comparação às do controle e aos outros métodos testados. A imersão em água a 100 ºC e em H_2SO_4 não é recomendada para *M. glomerata*. As doses de 20 a 25 mL de Stimulate® kg^{-1} de sementes proporcionam incremento para todas as características analisadas. Imersão em AG_3, na concentração de 250 mg L^{-1}, e doses de 15 a 25 mL de Stimulate® kg^{-1} de sementes são eficientes para a superação da dormência de sementes e o vigor de plântulas de *M. glomerata*.

Termos de indexação: ácido giberélico; Stimulate®; qualidade fisiológica; germinação.

Introduction

Myrciaria glomerata O. Berg. (commonly known in Brazil as "cabeludinha", Myrtaceae) is a fruit tree species classified as early secondary in the ecological succession (CAMARGOS et al., 2013). It is native to the Brazilian Atlantic Forest, with natural occurrence in different phytophysiognomies in the phytogeographic domains of the Cerrado, Amazon rainforest, and Caatinga (SOBRAL et al., 2015).

The fruits of *Myrciaria glomerata* can be consumed in natura, consisting of globose berries with a thick peel in "canary yellow" color that contain one or two large seeds with astringent taste (LORENZI, 2002) and anti-inflammatory property (PACHECO-SILVA; DONATO, 2016). In addition, its seedlings can be inserted into agroforestry systems and/or recovery programs for degraded areas. Considering the importance of the species, its ex situ cultivation aimed at conservation and sustainable exploitation is necessary.

Although *M. glomerata* is propagated by its seeds, they have low germination potential (PINTO et al., 2017), depending on the dormancy mechanism or seed quality. The occurrence of seeds that do not germinate or present low germination even under apparently favorable environmental conditions is common in native species, as there are few technical descriptions in the literature. These seeds are considered dormant and may need additional treatment to germinate according to their physiological quality. We emphasize that studies aimed at establishing techniques for the physiological management of seeds are important for the subsequent production of seedlings. According to Taiz et al.

(2017), germination is affected by different hormones, being promoted by some and inhibited by others.

Biostimulants/bioregulators such gibberellins in the form of gibberellic acid (GA_3) and Stimulate®, which consists of cytokinin, gibberellic acid, and butyric indole acid, for example, are used as germination promoters for triggering embryo growth, weakening the endosperm layer surrounding the embryo, and mobilizing energy reserves (SKUBACZ; DASZKOWSKA, 2017; TAIZ et al., 2017; SILVA et al., 2021), as well as assisting in the development of the root system and other processes of plant metabolism and development (SANTIAGO et al., 2019; HOSSEINI et al., 2020). Other methods, such as immersion in sulfuric acid and high temperature water to overcome tegumentary dormancy should also be studied, mainly due to the lack of knowledge on the type of dormancy of the seeds of *M. glomerata*.

Information on seed placement and seed efficiency, such as choosing the optimal doses of hormones and methods of overcoming physiological or tegumentary dormancy for native species is scarce. We hypothesized that the treatment used in seeds can contribute to overcome dormancy and improve physiological quality. Thus, the aim of the present study was to test different methods for overcoming dormancy in seeds of *M. glomerata* and improving seedling vigor.

Material and methods

Two experiments were conducted independently in the Laboratory of Nutrition and Plant Metabolism/Federal University of Grande Dourados (UFGD) between October 2018 and January 2019 (Experiment 1) and October 2019 (Experiment 2). Fruits of *M. glomerata* were collected from twenty plants belonging to a six-year orchard located in the area of fruit farming of the UFGD, university city, municipality of Dourados, Mato Grosso do Sul State (MS), Brazil. The fruits were taken to the laboratory and manually depulped under running water. The mucilage surrounding the seeds was removed using a sieve, with seeds being subsequently dried for 40 min on a paper towel under ambient conditions (25 ± 1 °C and 60% humidity relative).

The following procedures were performed for the two experiments:

Seeds were sanitized with 2.5% sodium hypochlorite for 5 min, washed under running water, and distributed on three Germitest® paper towels previously moistened with distilled water at a ratio of 2.5 times the mass of the dry paper (BRASIL, 2009), being subsequently packed inside transparent plastic boxes (11.5 x 11.5 x 3.5 cm) placed in biochemical Oxygen demand (BOD) incubators at 25 °C under constant white light. The seed moisture content was 47% at the beginning of the experiment. In both experiments, three replicates of 25 seeds were used per treatment.

Taking into consideration the scarcity of information and the limitation of the seeds of *M. glomerata*, the criteria used by, Dresch et al. (2012), who worked with a species belonging to the Myrtaceae family, were adopted for installing the experiment.

Experiment 1

The experiment consisted of the following treatments: 1) control (no treatment), 2) immersion in water at 100 ºC for 30 seconds, 3) immersion in concentrated sulfuric acid (H_2SO_4) for 1 minute, 4) immersion in concentrated (98%) sulfuric acid (H_2SO_4) for 5

minutes, 5) immersion in aqueous solution of GA_3 (250 mg L^{-1}) for 24h, 6) immersion in aqueous solution of GA_3 (500 mg L^{-1}) for 24h, and 7) homogenization using a solution with 5 mL kg^{-1} of Stimulate® (seeds were packed in a plastic bag, the solution was poured in the seeds, and the sample was maintained at rest for 24h).

Stimulate® presents in its composition 0.009%, 0.005%, 0.005%, and 99.81% of cytokinin, gibberellic acid, butyric indole acid, and other ingredients, respectively, according to manufacturer's specification. Exp. 1 was determined based on the evaluation of methods for overcoming dormancy in native species.

Experiment 2

The following doses of Stimulate® were tested: 0 (control), 5, 10, 15, 20, 25, and 30 mL kg^{-1} seeds (according to the description in Exp. 1), with seeds being sown after applying the solution.

Evaluated characteristics

The percentage of normal seedlings and germination speed index were evaluated at 30 days after sowing (DAS), while seed vigor and length were evaluated at 35 DAS for both experiments as follows:

Percentage of normal seedlings: calculated using the number of developed shoots and roots as criterion (BRASIL, 2009).

Germination speed index (GSI): calculated by the sum of the number of seeds sprouted each day divided by the number of days elapsed between sowing and germination, according to Maguire (1962):

$$GSI = (G_1/N_1) + (G_2/N_2) + (G_3/N_3) + (G_n/N_n)$$

Where: GSI = germination speed index, G_1, G_2, G_3, ..., G_n = number of seedlings computed in the first, second, third and last count; and N_1, N_2, N_3, ..., N_n = number of days after sowing in relation to the first, second, third, and last count.

Seedling length: the length of the roots and shoot of the seedlings was measured using a graduated ruler (centimeters) and the results were expressed in cm.

Fresh mass of seedlings: calculated from the weight of roots and shoots after the end of the experiment. The masses were determined in a precision scale (0.0001 g) and the results were expressed in g per seedlings.

Dry mass of seedlings: roots and shoots were placed in paper bags and maintained in an oven regulated at 60 °C for 48 hours. When they reached constant mass, the dry weight was determined in an analytical scale (0.0001 g) and the results were expressed in g per seedlings.

Statistical analysis

A completely randomized experimental designed was used for the two experiments. Germination data were subjected to homogeneity of variance and normal distribution by the Bartlett and Shapiro-Wilk tests, respectively. In Exp. 1, data were submitted to analysis of variance (test F, $p \leq 0.05$) and averages were compared using the Scott-Knott test at $p \leq 0.05$ for all treatments. In Exp. 2, data were submitted to analysis of variance and regression analysis, testing the significance of the linear and quadratic model ($p \leq 0.05$) with a coefficient of determination ≥ 0.60. Statistical analysis was performed using the SISVAR software (FERREIRA, 2014).

Results

Experiment 1

We observed significant effects of treatments regarding the analyzed characteristics, as shown in Tables 1 and 2. Higher values of normal seedlings, GSI, length, fresh mass, and dry mass were obtained by pre-imbibition of seeds in GA_3 at 250 and 500 mg L^{-1}, while root dry mass was higher when using GA_3 at 250 mg L^{-1}. On the other hand, seeds that were immersed in water at 100 ºC for 30 seconds and in sulfuric acid (H_2SO_4) for 1 and 5 minutes did not germinate (Table 1 and Figure 1), while controls presented low germination rates.

Experiment 2

The percentage of normal seedlings of *M. glomerata* responded linearly to increasing doses of Stimulate®, reaching 100% germination with 30 mL kg^{-1} seeds (Figure 2a). The different doses of Stimulate® had a quadratic effect on GSI, with the maximum value (1.14) being obtained at the concentration of 20.95 mL kg^{-1} seeds of Stimulate® (Figure 2b).

Table 1. Percentage of normal seedlings (NS) and germination speed index (GSI), shoot length (SL), and root length (RL) of treatments for overcoming the dormancy of seeds of *Myrciaria glomerata* O. Berg.

Treatments	NS (%)	GSI	SL (cm)	RL (cm)
Control	12.67 c	0.72 c	2.11 c	5.31 c
Water 100 ºC (30 s)	0.00 d	0.00 d	0.00 d	0.00 d
H_2SO_4 (1 min)	0.00 d	0.00 d	0.00 d	0.00 d
H_2SO_4 (5 min)	0.00 d	0.00 d	0.00 d	0.00 d
250 mg L^{-1} GA_3	100.00 a	2.22 a	8.22 a	10.88 a
500 mg L^{-1} GA_3	96.00 a	2.23 a	7.97 a	10.10 a
Stimulate® (5 mL)	46.00 b	2.00 b	3.87 b	8.75 b
C.V. (%)	8.40	10.02	13.46	22.04

Means followed by equal letters in the columns do not differ by the Scott-Knott test ($p \leq 0.05$).

Table 2. Fresh mass of shoots (SFM) and roots (RFM) and dry mass of shoots (SDM) and roots (RDM) of seedlings of *Myrciaria glomerata* O. Berg. subjected to treatments for overcoming seed dormancy at 35 days after sowing.

Treatments	SFM (g)	RFM (g)	SDM (g)	RDM (g)
Control	0.036 c	0.058 c	0.007 c	0.014 d
Water 100 ºC (30 s)	0.000 d	0.000 c	0.000 c	0.000 e
H_2SO_4 (1 min)	0.000 d	0.000 c	0.000 c	0.000 e
H_2SO_4 (5 min)	0.000 d	0.000 c	0.000 c	0.000 e
250 mg L^{-1} GA_3	0.338 a	0.539 a	0.127 a	0.162 a
500 mg L^{-1} GA_3	0.326 a	0.502 a	0.116 a	0.142 b
Stimulate® (5 mL)	0.134 b	0.276 b	0.036 b	0.069 c
C.V. (%)	19.27	25.87	40.45	10.86

Means followed by equal letters in the columns do not differ by Scott-Knott test ($p \leq 0.05$).

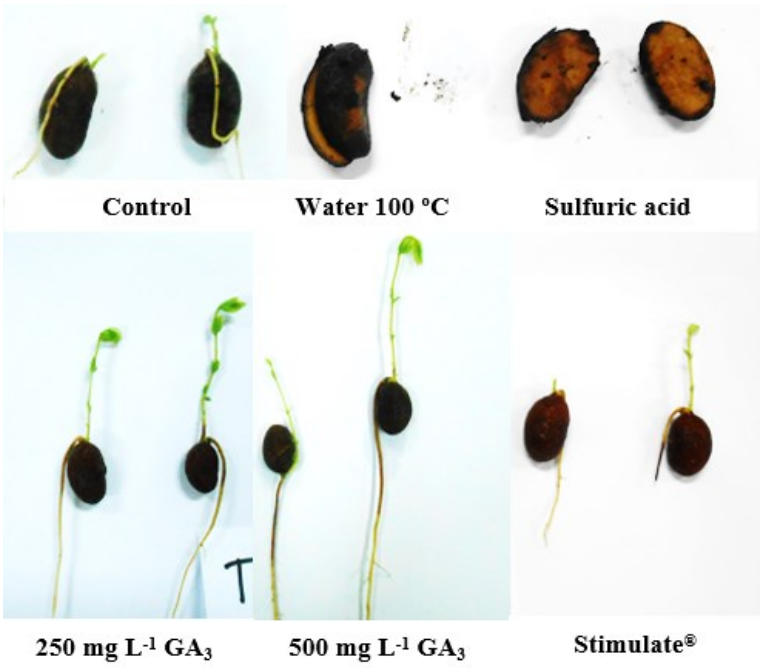

Figure 1. Visual aspect of seeds of *M. glomerata* in the control treatment (not treated), immersion in water at 100 °C for 30 seconds, immersion in concentrated sulfuric acid (H_2SO_4) for 1 and 5 minutes, immersion in aqueous solution of GA_3 (250 e 500 mg L^{-1}) for 24h, and using a solution with 5 mL kg^{-1} of Stimulate®. Source: the authors.

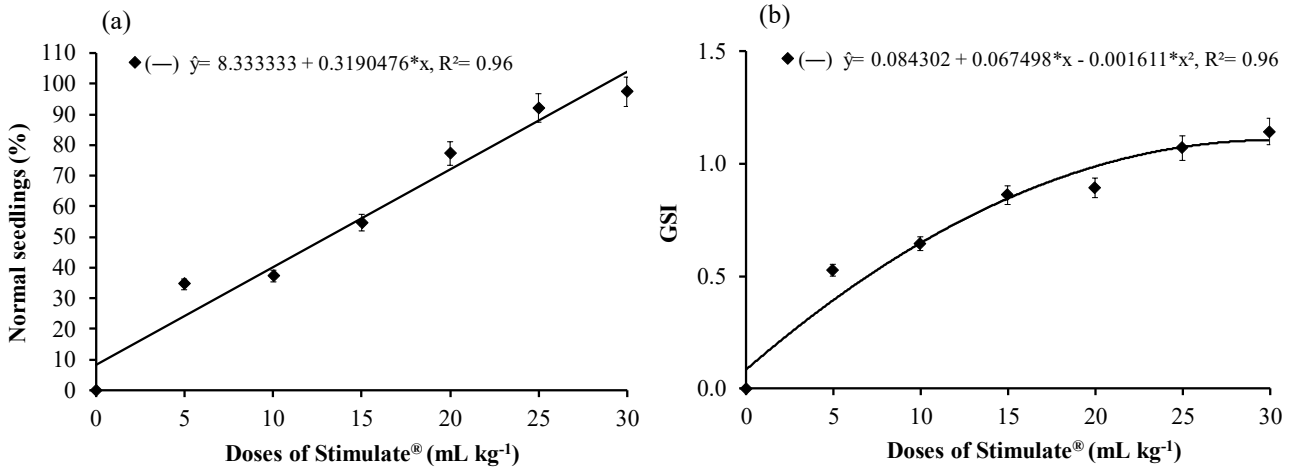

Figure 2. Percentage of normal seedlings – (a) and germination speed index – (b) (GSI) of seeds of *Myrciaria glomerata* O. Berg treated with different concentrations of Stimulate® at 30 days after sowing.

Doses of Stimulate® promoted a quadratic effect on shoot length (Figure 3a), root length (Figure 3b), and total length (Figure 3c). Maximum shoot growth (6.08 cm) was observed with Stimulate® at 20.19 mL kg^{-1} seeds, while maximum root and total length of seedlings were observed at 24.42 and 26.03 mL kg^{-1} seeds, respectively.

The fresh and dry mass of shoots and roots and total mass showed a quadratic effect as a function of doses of Stimulate® (Figures 4 and 5). The highest values of shoot, root, and total seedling fresh mass were 0.059, 0.186 and 1.553 g per seedlings at 20.15, 24.22, and 21.37 mL of Stimulate® kg^{-1} seed, respectively (Figure 4a, b, c). The highest values of dry mass of shoots and roots and total dry mass of seedlings were obtained (0.0205, 0.0833, and 0.6681 g per seedling) at 19.93, 30, and 21.42 mL kg^{-1} seed, respectively (Figure 5).

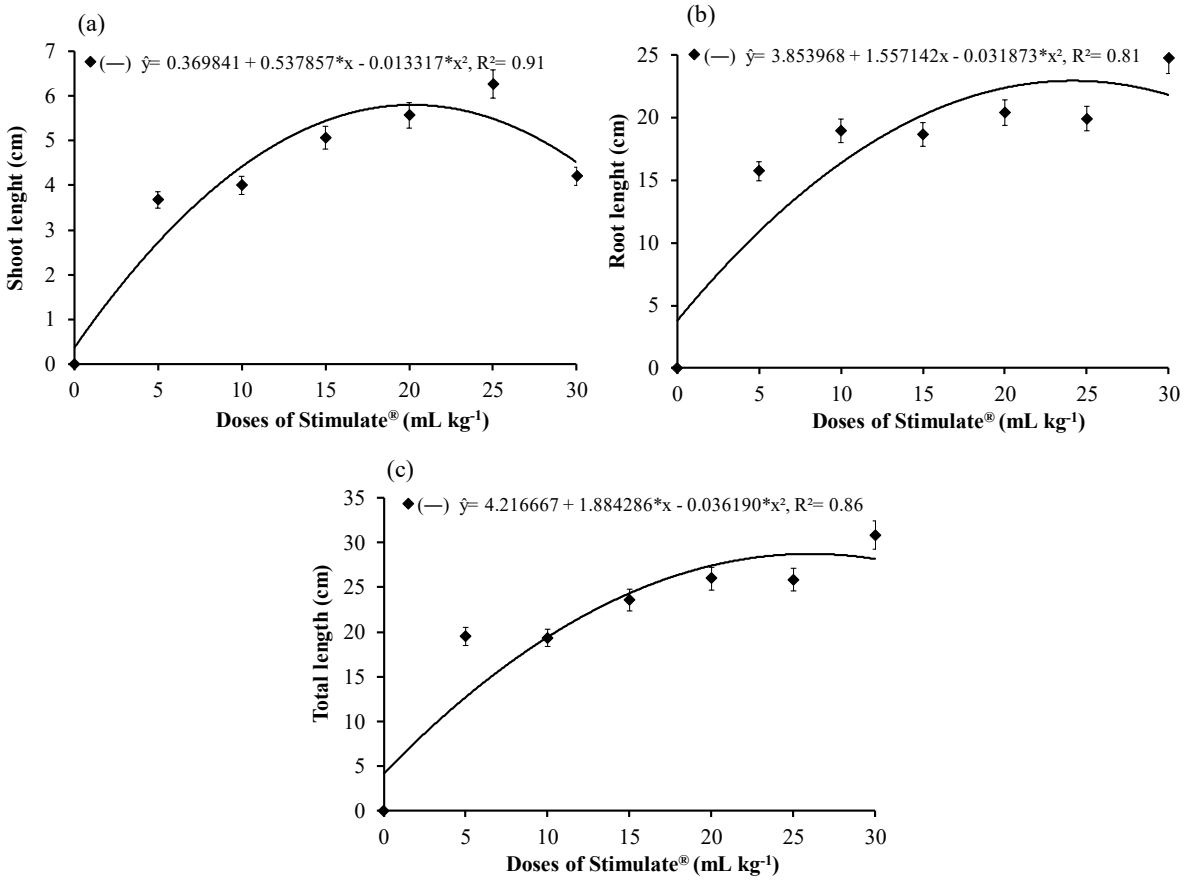

Figure 3. Shoot length – (a), root length – (b), and total length (c) of seedlings of *Myrciaria glomerata* O. Berg treated with different concentrations of Stimulate® at 35 days after sowing.

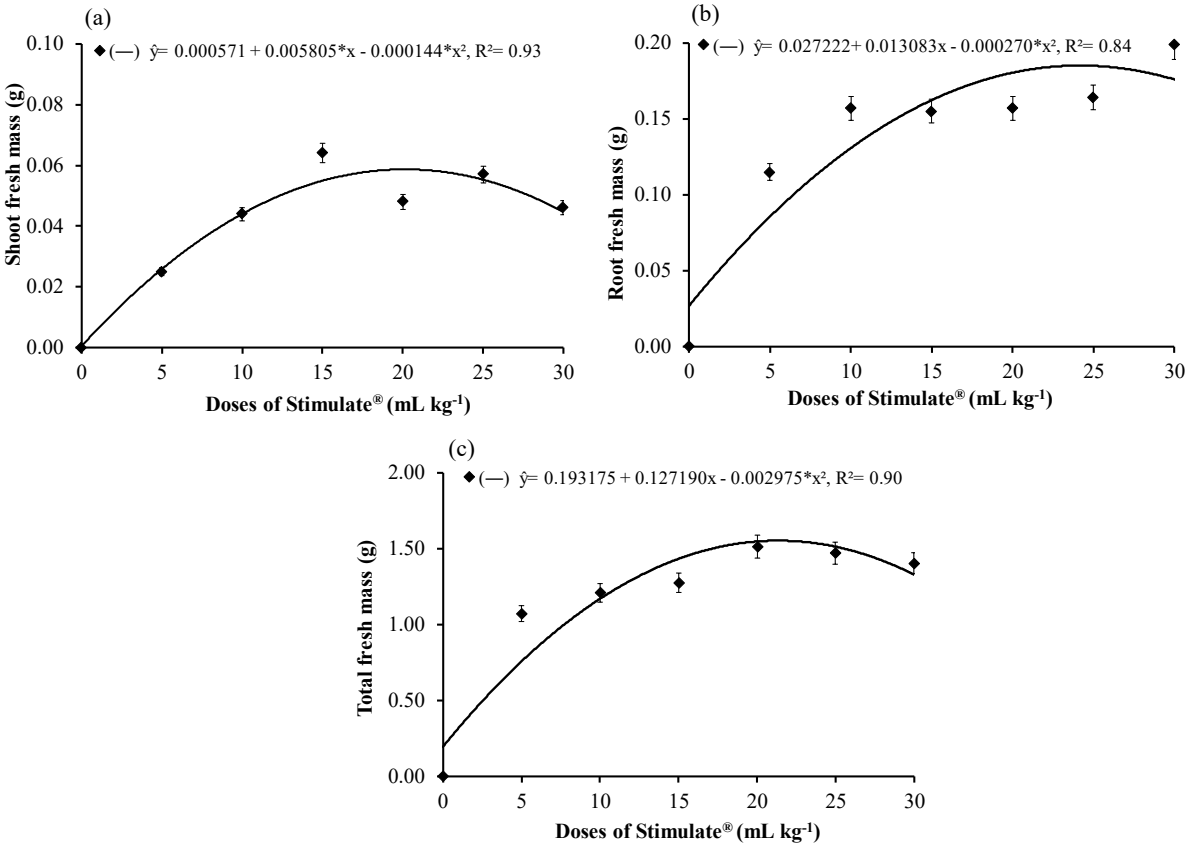

Figure 4. Shoot fresh mass – (a), root fresh mass – (b), and total fresh mass (c) of seedlings of *Myrciaria glomerata* O. Berg treated with different concentrations of Stimulate® at 35 days after sowing.

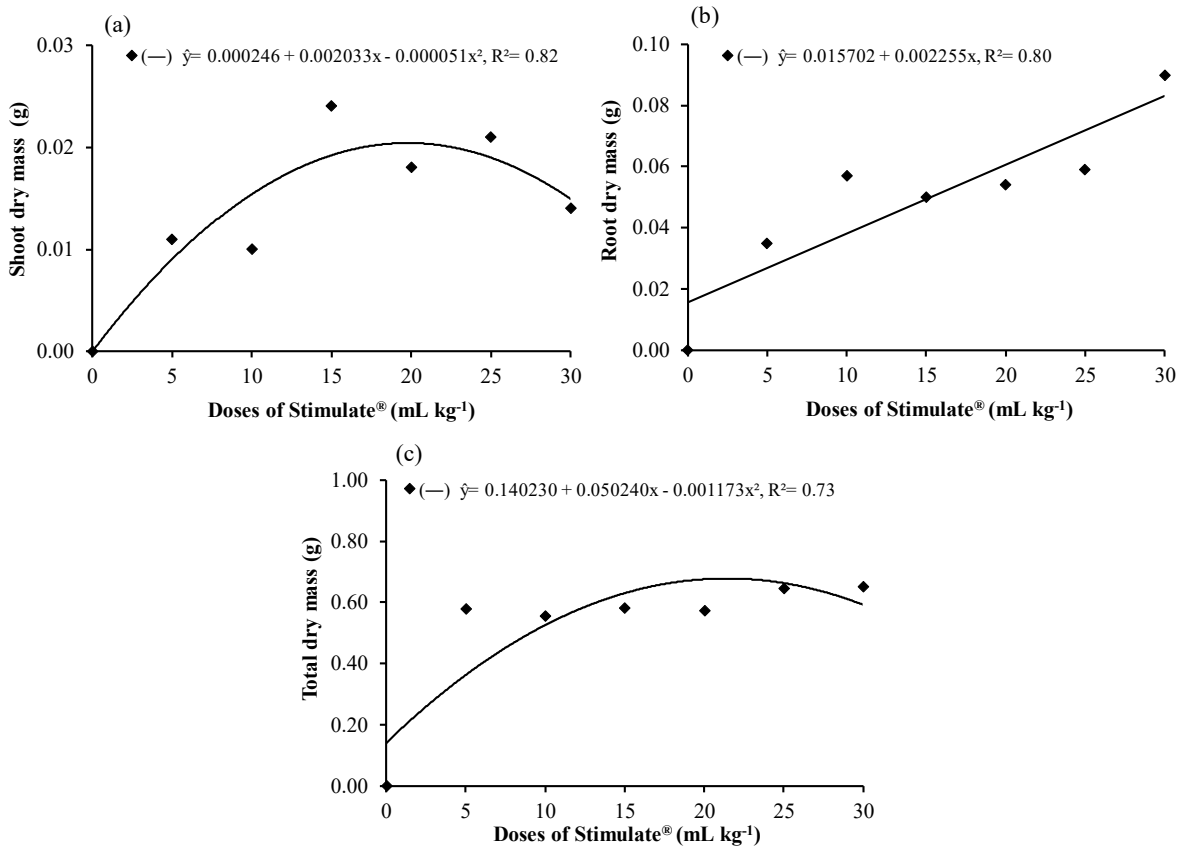

Figure 5. Shoot dry mass (a), root dry mass (b), and total dry mass (c) of seedlings of *Myrciaria glomerata* O. Berg treated with different concentrations of Stimulate® at 35 days after sowing.

Discussion

Experiment 1

The low germination speed index and seedling heterogeneity of *M. glomerata* observed for the control treatment may have resulted from an unfavorable balance between endogenous growth promoters and inhibitors. However, the treatment with GA_3 resulted in a higher number of normal seedlings for promoting cell elongation, which contributes for the seed germination of this species. Treatment with GA contributes to the biosynthesis of the amylase that acts in the starch hydrolysis, being converted into soluble sugars and favoring seedlings formation (BEWLEY et al., 2013; GARCIA et al., 2021).

Thus, the treatment with adequate concentrations of GA_3 may result in a more homogenous germination and a higher germination rate in seeds with relatively low endogenous GA_3 contents (CORNEA-CIPCIGAN et al., 2020; SILVA et al., 2021). In our study with seeds of *M. glomerata* treated with low doses of gibberellic acid (250 e 500 mg L^{-1} GA_3) higher values of GSI, length, and biomass were obtained, which is important as it reduces production costs in the acquisition of this hormone, contributing to overcome dormancy.

Responses regarding GA_3 vary according to the species. According to Silva et al. (2019), seeds of *Annona sylvatica* A.St.-Hil pre-imbibed for 24 h with 1,200 mg L^{-1} of GA_3 showed higher GSI. For *Psidium guajava* L., the use 0.1% GA_3 contributes to germination and normal seedlings (HOSSEINI et al., 2020). The physiological effects of plant growth regulators and biostimulants depend on factors such as dose and formulation, plant species, and environmental conditions, including nutrient availability.

In addition, we reinforce that the immersion in water at 100 ºC and in H_2SO_4, besides not contributing to overcome dormancy, also hampered germination as 12.67% of normal seedlings were formed in the control treatment. From these results, it is possible to infer that the dormancy of *M. glomerata* is physiological and not tegumentary. Generally, H_2SO_4 and boiling water are mostly used to reduce the hardness of the seed integument and facilitate the subsequent process of imbibition. For *M. glomerata*, the use of these methods compromised the physical integrity and reserves of seeds, being an unfeasible practice (Figure 1).

Similarly, Pirola et al. (2021) reported that it is not recommended to use water at 80 ºC and sulfuric acid to overcome the dormancy of seeds of *Eugenia uniflora* L., *Plinia peruviana* (Poir.) Govaerts, *Plinia cauliflora* (Mart.) Kausel, *Eugenia involucrata* DC., *Myrcianthes pungens* (O. Berg) D. Legrand, and *Campomanesia guazumifolia* (Cambess.) O. Berg, species belonging to the Myrtaceae family. These authors described that these two methods may have damaged the seed embryo, reducing the germination potential.

The performance of seeds treated with Stimulate® was lower than expected. Treatment with products containing plant growth regulators such as gibberellin, cytokinin, and auxin during germination may improve seed performance in several plant species, but the dose may have been low, which prompted us to carry out the second experiment.

Gibberellin causes primary roots to break the tissues that restrain their growth, such as the endosperm and seed and/or fruit tegument, whereas cytokinin and auxins supplement the action of gibberellins, inducing cell division and promoting radicle and shoot growth (FERREIRA et al., 2016; TUAN et al., 2018; ATROCH et al., 2020).

Experiment 2

The results of our study indicate that higher concentrations of Stimulate® should be used to achieve the maximum germination (normal seedlings) of *M. glomerata*, indicating its physiological effect as a growth regulator in this species. Stimulate® had a synergistic effect due to its balanced composition of plant growth regulators (0.005% indole butyric acid, 0.009% kinetin, and 0.005% GA_3). Physiologically, the balance of these hormones act on starch hydrolysis (BEWLEY et al., 2013), which favors the availability of reserves for formation of roots and hypocotyl (SKUBACZ; DASZKOWSKA, 2017; TAIZ et al., 2017).

This was likely responsible for the higher values of shoot length and shoot and root dry weights, as Stimulate® increases plant growth and development by stimulating cell division, differentiation, and elongation (SANTOS et al., 2013; SMIDERLI et al., 2022), making it a tool for the improvement of seed and plant physiology. This product can usually be used in the treatment of seeds, but there are also studies on the management of application using other products. In this case, attention should be paid to other factors.

Stimulate® presents a cytokinin concentration of 50 mg L^{-1} kinetin, while the dose of 25 mL kg^{-1} seeds contained 125 mg L^{-1} kinetin (DANTAS et al., 2012), indicating a possible phytotoxic effect at higher doses of Stimulate®. On the other hand, the use of this biostimulant at appropriate doses was promising for the physiology of germination of seeds of *M. glomerata*. There are few studies in the literature related to the use of Stimulate® specifically in Myrtaceae. Thus, we also describe the responses of other fruit species.

Stimulate® has been used in several crops with satisfactory results regarding shoot growth, as observed for *Hymenaea courbaril* L. (SMIDERLI et al., 2022). Hormonal treatment in seeds positively contributed to germination and seedling formation of Myrtaceae species (HOSSEINI et al., 2020; PIROLA et al., 2021), which is similar to what was observed for *M. glomerata*. For seeds of *Physalis angulata* L., an increase in the first germination count, germination speed index, and seedling formation was observed when seeds were pre-imbibed in Stimulate® solution 1.50% (SANTIAGO et al., 2019).

These properties are consistent with the higher values obtained for root length and total length of seedlings, as well as with the considerably higher fresh and dry weight observed for *M. glomerata* in the present study. Stimulate® is an efficient alternative for obtaining high germination rates and seedling growth in *M. glomerata*, and the results of this study are important for the establishment of protocols based on physiological management aimed at the propagation and ex situ cultivation of this species.

Conclusion

Treatment with GA_3 (250 mg L^{-1}) and Stimulate® (20-25 mL kg^{-1} seeds) efficiently overcome dormancy in seeds of *Myrciaria glomerata* O. Berg., resulting in higher germination rates and seedling vigor.

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