Effect of Pre-treatments on Seed Germination and Seedling Growth in *Psidium guineense* Swartz

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Summary

This work aimed to evaluate the effect of pre-treatments with gibberellin, the initial substrate humidity, and incubation temperature on the germination of *Psidium guineense* Swartz seeds. The seeds were submitted to four pre-treatments with gibberellin (GA_3) (50 and 100 mg L^{-1}) and distilled water during 24 hours, and control. Sowing was carried out on Germitest® paper that was moistened with distilled water at humidity levels corresponding to 1.5 and 2.5 times the weight of dry paper. The seeds were then incubated at a constant temperature (25 or 30 °C) and alternate temperatures (20-30 °C). Experiments were randomized, with a factorial scheme 4 × 2 × 3 (pre-treatment × initial substrate humidity × temperature) with four replicates of 25 seeds each. Germination rate and seedling growth of *P. guineense* were not affected by pre-germination treatments. For an optimum germination rate and seedling growth, seeds of *P. guineense* should be sown at alternate temperatures of 20-30 °C or 25 °C and in substrate moisture of 1.5 or 2.5 times the dry paper mass.

Keywords: Myrtaceae, Brazilian Savanna, temperature, gibberellins

Resumen

Efecto de pre-tratamientos sobre la germinación de semillas y crecimiento de plántulas *Psidium guineense* Swartz

El objetivo de este trabajo consistió en evaluar el efecto de los pre-tratamientos con giberelinas, la humedad inicial del sustrato y la temperatura de incubación sobre la germinación de *Psidium guineense* Swartz. Las semillas se sometieron a cuatro tratamientos previos con giberelina (GA_3) (50 y 100 mg L^{-1}) y agua destilada durante 24 horas y un control. La siembra se hizo en papel Germitest® humedecido con agua destilada a niveles de humedad correspondientes a 1,5 y 2,5 veces el peso de papel seco. Luego las semillas se incubaron a temperaturas constantes (25 y 30 °C) y a temperaturas variables (20-30 °C). El procedimiento estadístico utilizado fue un diseño factorial 4 x 2 x 3 (pre-tratamiento x humedad inicial del sustrato x temperatura) completamente al azar con cuatro repeticiones de 25 semillas cada una. La germinación de las semillas y el crecimiento de las plántulas de *P. guineense* no se vieron afectados por los tratamientos pre-germinación. Para una germinación y crecimiento óptimos las semillas de *P. guineense* deberían sembrarse a temperaturas de 20-30 o 25 °C y a una humedad del sustrato de 1,5 o 2,5 veces la masa del papel seco.

Palabras clave: Myrtaceae, sabana brasilera, temperatura, giberelinas

Introduction

The Brazilian savanna is considered one of the richest vegetation environments of the planet, with high diversity of tree and shrub species with potentially great food and medicinal importance. However, these species have been little known and studied and have suffered great human pressure, mainly due to agricultural activity and livestock breeding (Klink and Machado, 2005; Bernardes *et al*., 2008; Scalon *et al*., 2009). There is currently a significant market for native savanna fruits, although most of them have been harvested only from wild plants using aggressive extractivist and predatory techniques that increase the risk of extinction of various species. Therefore, studies on propagation and production of seedlings of these species are important and urgent.

Among these species is the genus *Psidium*, one of the fruit tree species from the family Myrtaceae of great economical potential. The fruits of this genus are popular for *in natura* consumption for their high content of vitamin C, which is up to four times higher than in citrus fruits. Besides, their high capacity for fruition and dispersion and resistance to diseases and weeds (except to fruit flies) indicates an adaptation to different environments (Cisneiros *et al*., 2003). *Psidium guineense* Swartz is found in Tropical America, from southern Mexico to northern Argentina and Brazil (González *et al*., 2005).

The propagation of *P. guineense* is mainly by seeds since vegetative propagation has not proportionated satisfactory and conclusive results (Caldeira *et al*., 2004; Bezerra *et al*., 2010). Moreover, Cisneiros *et al*. (2003) observed that *P. guineense* seeds germinated more rapidly when stored in normal laboratory environment than in a freezer.

Germination is an intricate process that depends on internal and external factors, of which temperature, light, water, and oxygen are the most important. Studies on the effect of these factors on species from the savanna are only incipient. The influence of the temperature on the germination process is essential to understanding the ecophysiological and biochemical aspects of the process. The temperature range between 20 °C and 30 °C has been considered as adequate for seed germination of many subtropical and tropical species, since these are the temperatures commonly found in their native regions at the time of natural germination (Sugahara and Takaki, 2004; Scalon *et al.* 2009; Rego *et al*. 2009, Zucareli *et al*., 2009; Kissmann and Scalon, 2011; Dresch *et al*., 2012).

In addition to temperature, phytohormones such as gibberellin (GA) have a stimulant effect on both dormant and non-dormant seeds (Bernardes *et al*., 2008). According to the review by Baskin and Baskin (2004), the amount of GA required for germination of ripe seeds is controlled by ABA concentrations during seed development. Thus, seeds with low levels of ABA produced during their development ('lightly dormant') require low amount of GA to germinate and vice versa, seeds with high concentration of ABA produced during seed development ('deeply dormant') require high amount of GA to germinate. The gibberellins such as GA_1, GA_3, GA_4, and GA_7, which are biologically active compounds, can reduce the time from sowing to germination of dormant seeds or the time required for seedling production (Baskin and Baskin, 1998).

Little is known about the effect of exogenous hormones on seed germination in the Brazilian savanna species, which could improve seed propagation and seedling production. Thus, the aim of this work is to evaluate the effect of pretreatments, initial substrate humidity, and incubation temperatures on seed germination in *P. guineense*.

Materials and Methods

This work was carried out at the Seeds Laboratory at the Department of Agrarian Sciences, University of Grande Dourados (UFGD), Dourados, Mato Grosso do Sul, Brazil.

The fruits were collected from the plants in Dourados-MS and stored at room temperature (22 ± 1 °C) for 90 days (the time until the pre-test) at the Laboratory of Vegetative Physiology. The fruits were manually pulped; seeds were washed with running water and placed on a towel paper to dry at room temperature and then visually selected to ensure their uniformity in size, color, and level of conservation. At that point, the fruits contained 9 % of moisture (fresh weight basis).

The seeds were then submitted to four pre-germination treatments, which consisted of immersion in 50 mg L^{-1} or 100 mg L^{-1} gibberellin solutions (GA_3) and distilled water over a 24-hour period, whereas the seeds with no treatment served as control. The sowing was carried out on three sheets of Germitest® paper roll with a moisture level corresponding to 1.5 and 2.5 times the dry paper mass, hereafter referred to as 1.5 and 2.5, respectively. The rolls were moistened with distilled water to the desired humidity level and kept into transparent polystyrene bags. These humidity levels were kept for 10 days and then standardized. The seeds were incubated in three type germinators vertical Biochemical Oxygen Demand (BOD) at constant temperatures of 25 and 30 °C and temperatures alternating between 20 and 30 °C.

Physiological potential of the seeds was evaluated with germination test as described here. Water content: 3 g of

seeds in four replicates were dried at 105 ± 3 °C for 24 h; the results were expressed in percentages of the wet mass. First germination count: the number of germinated normal seedlings was counted 20 days after sowing and expressed as percentage. Germination: it was evaluated 42 days after sowing and expressed as a percentage of total sawn seeds. Germination Speed Index (GSI): normal seedlings were evaluated daily for 40 days as the minimum parameter established (2 cm), and the GSI was calculated according to Maguire (1962). Seedling length: the length of the roots and aerial parts were measured 42 days after sowing, and the results were given in cm seedling^{-1}, with a precision of two decimals. Total seedling dry mass: it was calculated from the roots and aerial parts of seedlings. The roots and aerial parts of the seedlings were placed inside paper bags and dried in an oven at 60 °C for 48 h, until they reached constant dry mass. Their mass was measured using an analytical balance (0.0001g) and expressed in mg seedling^{-1}.

The experiments were randomized with a factorial scheme 4 × 2 × 3 (pre-treatments × initial substrate humidity × temperatures) and four replications of 25 seeds each. The data were subjected to the analysis of variance (ANOVA), and the means were compared by Tukey test, at 5 % probability level using SANEST.

Results

According to the first count, the germination percentage did not vary significantly between the pre-germination treatments at 20-30 °C temperatures. The seeds incubated at 30 °C had the lowest means in all treatments (Figure 1A, 1C).

The alternate temperature (20-30 °C) associated with substrate humidity of 1.5 resulted in the highest means in all variables. Pre-treatment with 100 mg L^{-1} of gibberellin gave the lowest means in all variables, indicating that this dose of gibberellin did not stimulate initial germination, especially in substrate humidity of 2.5 (Figure 1B).

There was no significant difference in seed germination between the pre-germination treatments with 20-30 °C incubation temperature, but lower means were observed at 30 °C. Seed germination declined at 30 °C for all pre-treatments, except for the pre-treatment with distilled water, which did not differ from the germination rate at 25 °C (Figure 1D).

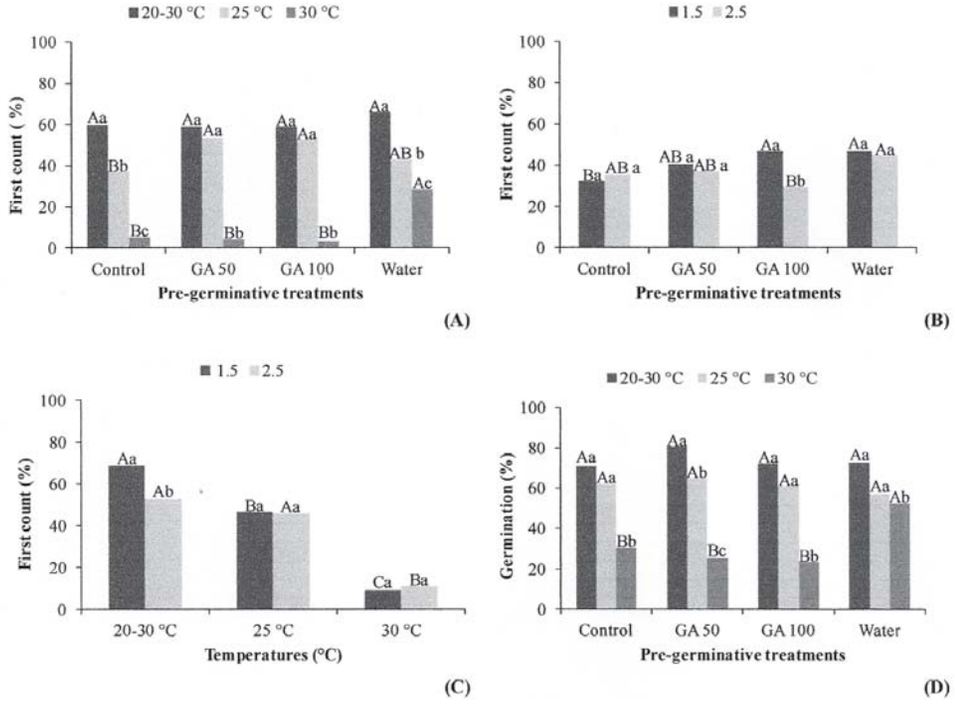

Figure 1. First count germination (A, B, C) and percentage germination (D) of the *Psidium guineense* seeds. Capital letters compare the same temperature (A, D) and substrate humidity (B, C) in different pre-germinative treatments (A, D, B) and temperatures (C). Small letters compare different temperatures (A, D) and substrate humidity (B, C) in the same pre-germinative treatment (A, B, D) and at the same temperature (C).

There was no significant difference in GSI between the pre-germination treatments at the incubation temperatures of 20-30 °C and 25 °C, whereas the lowest means GSI were observed at 30 °C, except for the germination treatment with distilled water, which did not differ from the GSI at 25 °C (Figure 2A).

The GSI was not influenced by the interaction pre-germination treatments × substrate humidity, except for the pre-germination treatment with 100 mg L^{-1} of gibberellin in substrate and humidity of 2.5, which had the lowest mean GSI (Figure 2B). There was no significant difference in GSI among the substrate moisture at 20 °C and 30 °C temperatures. The lowest means of GSI were observed at 30 °C incubation temperature, independent of the substrate humidity (Figure 2C).

There was no significant correlation between the seedling root length and the treatments. The highest values were observed for seeds immersed in water for 24 h (mean 1.82 cm $seedling^{-1}$) at a temperature of 25 °C (mean 1.88 cm $seedling^{-1}$). Similarly, there was no significant difference in the length of the aerial parts among the pre-germination treatments at the incubation temperatures of 20-30 °C and 25 °C (Figure 3A); the lowest mean length of the aerial parts was observed in treatments at 30 °C.

These results are in accordance with the results of the seed germination test. Thus, at 20-30 °C and 25 °C temperatures and no pre-germination treatment seedlings were higher. The incubation at 20-30 °C resulted in the highest mean seedling length in both substrate humidity levels, and did not differ significantly from that at 25 °C and substrate humidity of 2.5 (Figure 3B). The seedling growth was the lowest at 30 °C.

The seedling total dry mass did not significantly differ among the treatments and different substrate humidity, except for the pre-treatment of seeds with 50 $mg.L^{-1}$ of GA (Figure 4A) at 25 °C and substrate humidity of 1.5 (Figure 3B), which resulted in the lowest mean seedling total dry mass. The seedling total dry mass was the highest in treatments with substrate moisture of 2.5 (Figure 4B, 4C) and incubation temperature of 30 °C (Figure 4C). Thus, the lower substrate moisture highlighted the requirement for mild temperature in order to attain higher translocation of the re-

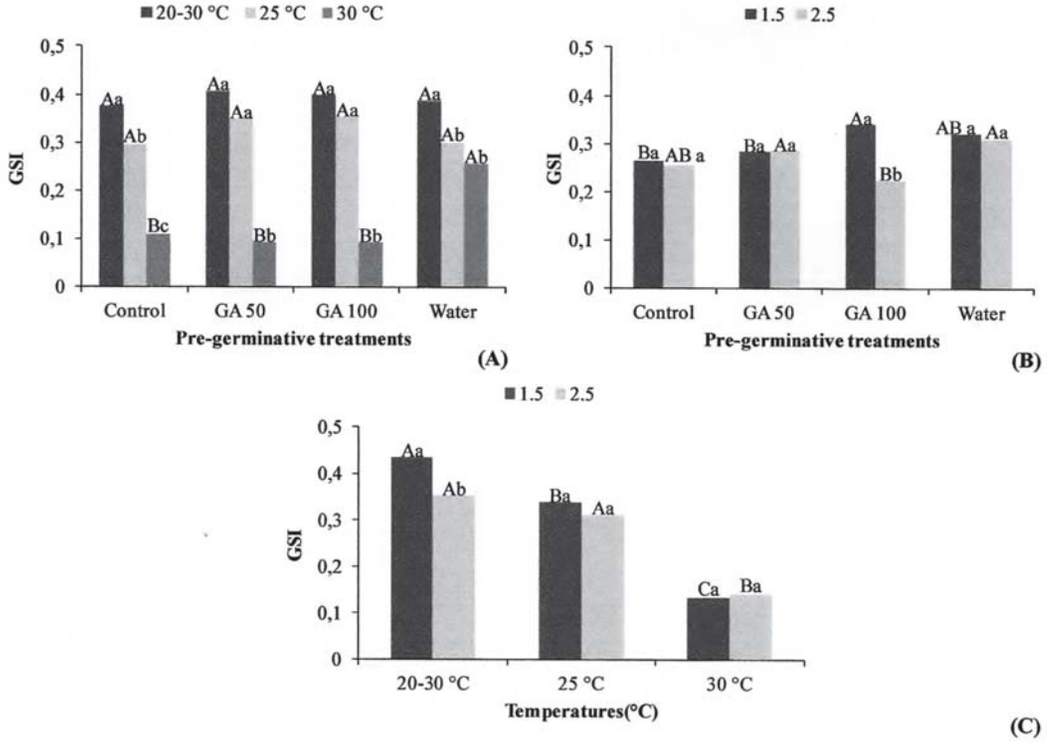

Figure 2. Germination speed index (GSI) of the *Psidium guineense* seeds (A, B, C). Capital letters compare the same temperature (A) and substrate humidity (B, C) in different pre-germinative treatments (A, B) and temperatures (C). Small letters compare different temperatures (A) and substrate humidity (B, C) in the same pre-germinative treatment (A, B) and at the same temperature (C).

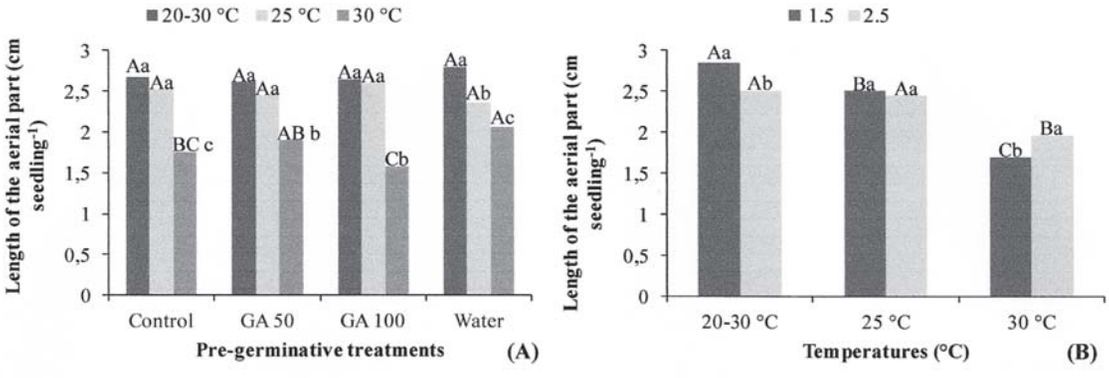

Figure 3. Length of the aerial part (cm seedling^{-1}) (A, B) of the *Psidium guineense* seedlings. Capital letters compare the same temperature (A) and humidity (B) of the substrate in different pre-germinative treatments (A) and temperatures (B). Small letters compare different temperatures (A) and substrate humidity (B) in the same pre-germinative treatment (A) and at the same temperature (B).

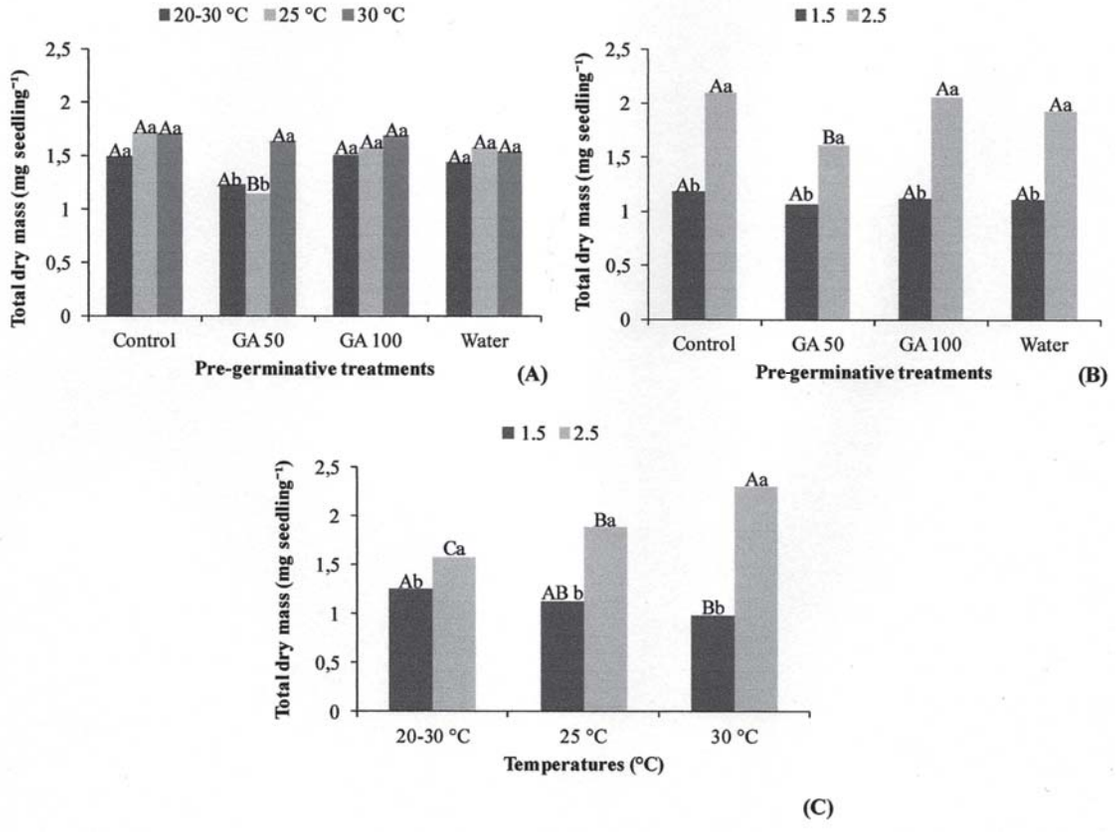

Figure 4. Total dry mass (mg seedling^{-1}) (A, B, C) of the *Psidium guineense* seedlings. Capital letters compare the same temperature (A) and same substrate humidity (B, C) in different pre-germinative treatments (A, B) and temperatures (C). Small letters compare different temperatures (A) and humidity of water (B, C) in the same pre-germinative treatment (A, B) and at the same temperature (C).

serve material to the embryo during the seedling's growth. Additionally, translocation of the reserve material to the seedlings occurred even at higher substrate moisture (2.5) and the highest temperature (30 °C) (Figure 4C).

Discussion

The germination process of *Psidium guineense* seeds appears to depend more on the temperature ranges between 20 °C and 30 °C and 25 °C than on the pre-germination treatments with GA and substrate moisture. The lower values for measured variables were observed at 30 °C (constant temperature) regardless of the pre-germination treatments, suggesting that the germination of this species is sensitive to high temperatures. The reduction in germination rate at 30 °C, according to Zucareli *et al*. (2009), may be related to oxygen availability, because at temperatures above 25 °C oxygen is progressively less soluble in water and reaches the embryo in lower quantities. Additionally, high temperatures may trigger responses to thermal stress in the embryo, thus favoring seed deterioration. Although primary root and normal seedlings are formed, the speed of germination and seedling growth are hampered, thereby limiting the area of species establishment and regeneration.

In general, substrate moisture did not affect the germination and seedling growth at temperatures of 20-30 °C and 25 °C. These data suggest that the humidity levels used provided adequate hydration during imbibition, allowing the reactivation of metabolic processes and growth of embryo (Marcos Filho, 2005). Similarly, Rego *et al*. (2009) did not observe a significant difference between different amounts of water (1.5; 2.5; 3.5; and 4.5 times the weight of the substrate) in the substrate (vermiculite) at three studied temperatures, 20, 25, and 30 °C on the germination of *Blepharocalyx salicifolius* (Myrtaceae) seeds.

In some species, including *Psidium guajava*, small changes in temperature are sufficient for a complete germination of seeds (Sugahara and Takaki, 2004). However, Scalon *et al*. (2009) did not observe a significant difference between the temperatures (18, 20-30, and 30 °C) on the germination of «guavira» seeds *(Campomanesia adamanthium* Camb. Myrtaceae), a native species from the savanna, although the GSI was higher at 30 °C compared to other temperatures. Likewise Santos *et al*. (2004) observed that germination of seeds of *Acca sellowiana* (Berg.) Burnet, *Campomanesia xanthocarpa* Berg, *Myrcianthes pungens* (Berg.) Legr., and *Psidium cattleyanum* Sabine, all from Myrtaceae, increased for 75 % 90 days after sowing at constant temperatures of 15, 20, 25, and 30 °C or alternating temperatures of 15-30 °C.

The seed germination rate and GSI values observed in the present study were higher than those observed by Cisneros *et al*. (2003). In their study of *P. guineense* seeds stored for 180 days in laboratory conditions and in the freezer, the initial germination rate was 60 % after scarification with sulfuric acid for 5 min. Their results showed that the germination capability of the seeds stored in the laboratory environment had decreased gradually, reaching a minimum level of 47 % on the 180th day since the treatment. Possibly, in addition to the seed storage conditions, the low results presented by Cisneiros *et al*. (2003) might be associated with the type of pre-treatment applied on *P. guineense* seeds (sulfuric acid), which could have caused damage to the seeds. On the other side, our results show that *P. guineense* seeds do not require any treatment to reach high germination rate.

The seedling root length in *Campomanesia adamantium* (Camb.) O. Berg (Myrtaceae) also did not show significant correlation to the temperature and substrate humidity; the roots were longer at 25 °C compared to other temperatures (Dresch *et al*., 2012).

The application of gibberellin and water immersion have not optimized the germination rate and initial seedling growth, probably because the concentration of GA and immersion time in GA and water were not sufficient to induce endogenous synthesis of GA nor the translocation of reserves to the embryo. Responses to GA varied and contrasting the application of GA on seed germination have been studied in several species including *Caryocar brasiliense* Camb. (Caryocaceae) (Bernardes *et al*. 2008), *Bertholletia excelsa* Bonpl. (Lecythidaceae) (Silva *et al*., 2009), *Thlaspi caerulescens* J & C Presl (Brassicaceae) (Guimarães *et al*., 2010), *Butia capitata* (Martius) Beccari (Arecaceae) (Lopes *et al*., 2011), *Stryphnodendron* spp. (Fabaceae) (Kissmann and Scalon, 2011), and *Myracrodruom urundeuva* Allemão (Anacardiaceae) (Scalon *et al*., 2012), although the literature argues that the active endogenous GA levels regulate their own synthesis by activating or inhibiting the transcription of genes coding for enzymes that participate in the biosynthesis or degradation of GA (Taiz and Zeiger, 2012).

Results similar to those found for *P. guineense* were observed in a study of *B. capitata* by Lopes *et al.* (2011); they showed no effect of GA and immersion in water on both final emergence and growth. However, Bernardes *et al.* (2008) observed that the treatment of *C. brasiliense* with up to 350 mg L^{-1} of GA increased the emergence rate and that the

height, diameter, and dry matter of the aerial parts of the seedlings were linearly correlated with the concentration of GA. Responses to GA vary among species of the same genus; the rate of seedling emergence of *Stryphnodendron adstringens* (Mart.) Coville is higher when the seeds have been scarified and treated with GA or soaked in water compared with the control, whereas in *S. obovatum* Benth. and *S. polyphyllum* Mart., the rate does not change after immersion in water or treatment with GA (Kissmann and Scalon, 2011).

Despite the increase in seedling dry mass at raised temperatures, the rate of seed germination and vigor suggest that the temperature of 30 °C is not an efficient treatment for the optimum germination rate in this species. Thus, high seed germination rate and seedling growth of *P. guineense* can be reached with temperatures of 20-30 and 25 °C either in 1.5 and 2.5 moisture substrate without any pre-germinative treatment.

In conclusion, the germination and seedlings growth of *Psidium guineense* were not affected by pre-germinative treatments. The seeds should be sown at the temperatures of 20-30 or 25 °C, and in moisture substrate of 1.5 or 2.5 times the dried paper mass.

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