CIÊNCIA e NATURA

ISSN 2179-460X
Acesso aberto

Ci. e Nat., Santa Maria, v. 43, e27, 2021 • https://doi.org/10.5902/2179460X39563
Submissão: 15/08/2019 • Aprovação: 11/11/2020 • Publicação: 18/06/2021

Biology-Botany

Seed germination and seedling formation of *Bromelia antiacantha* Bertol. at different temperatures and substrates

Germinação de sementes e formação de plântulas de *Bromelia antiacantha* Bertol. em diferentes temperaturas e substratos

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ABSTRACT

Bromelia antiacantha Bertol. is a native species of Brazil with food, medicinal, industrial and forest restoration potential. The objective of this study was to test different substrates and temperatures in the germination and seedling formation of *B. antiacantha*. Seeds obtained from fruits collected in situ from three localities, one in Paverama/RS and two in Taquari/RS, were washed in running water and homogenized in a single batch. After drying, the seeds were disinfested and seeded in medium sand and germibox paper, and exposed to constant temperatures of 25 and 30ºC. Firstly, the water content of the seeds were determined and, after installation of the experiment, the percentage of germination (G) and seedlings formed (SF), germination mean time (MTG) and seedling formation mean time (MTS), germination rate index (GRI) and seedling formation rate index (SRI) were calculated. The experimental design was completely randomized, in a 2 x 2 factorial arrangement with 4 replicates of 50 seeds. The water content and the weight of one thousand seeds corresponded to 17.02% and 27.09 g, respectively. The paper was superior to sand, with an average of 87% of germinated seeds, as well as a percentage of 86.25% of normal seedlings formed. Regarding the temperature, at 25 ºC, the seeds showed lower MTG and MTS and higher GRI and SRI. *B. antiacantha* seeds showed superior germination and initial seedling formation when sown on paper substrates and at 25 °C.

Keywords: Native species; Thermal requirement; Vigor

RESUMO

Bromelia antiacantha Bertol. é uma espécie nativa do Brasil com potencial alimentício, medicinal, industrial e para restauração florestal. O objetivo deste estudo foi testar diferentes substratos e temperaturas na germinação e formação de plântula *B. antiacantha*. Sementes obtidas de frutos coletados *in situ* de três localidades, sendo uma em Paverama/RS e duas em Taquari/RS, foram lavadas em água corrente e homogeneizadas em um único lote. Após a secagem, as sementes foram desinfestadas e semeadas em areia média e papel germibox e expostas às temperaturas constantes de 25 e 30 ºC. Inicialmente, determinou-se o teor de água das sementes e, após instalação do experimento, calculados o percentual de germinação (G) e de plântulas formadas (SF), o tempo médio de germinação (MTG) e de formação de plântulas (MTS), o índice de velocidade de germinação (GRI) e de plântulas formadas (SRI). O delineamento experimental foi inteiramente casualizado, em arranjo fatorial 2 x 2 e 4 repetições de 50 sementes. O teor de água das sementes correspondeu a 17.02%. O papel demonstrou superioridade em relação à areia, com média de 87% de sementes germinadas, bem como, um percentual de 86.25% de plântulas formadas. Em relação à temperatura, a 25 ºC, as sementes apresentaram menor MTG e MTS e maior GRI e SRI. As sementes de *B. antiacantha* demonstraram superioridade na taxa de germinação e de formação inicial de plântulas quando semeadas em substrato papel e sob temperatura de 25 °C.

Palavras-chave: Espécie nativa; Requerimento térmico; Vigor

1 INTRODUCTION

Bromelia antiacantha Bertol., commonly known as “caraguatá” or “bananinha-do-mato”, is a native species from Brazil and occurs from Bahia to Rio Grande do Sul (RS) in the Atlantic Forest and the Pampa biome (FLORA DO BRASIL 2020 in construction). It is a large-sized terrestrial plant that belongs to Bromeliaceae family and reaches 2 to 3 m in height, inhabiting areas of restingas, forests of humid soil and environments of secondary vegetation in dense clusters (REITZ 1983; FILLIPON *et al.* 2012). In addition, the species has thick stem and rhizomes and spiny leaves in rosette format, which center becomes reddish when approaching the emission of the inflorescence, composed of actinomorphic flowers of violet-blue coloration (REITZ 1983; SANTOS 2001). The fruits are of the berry type, yellowish, oval and composed of several seeds (REITZ 1983).

It consists of a species of multiple potentials: fruits are widely used in the manufacture of sweets and in folk medicine in the treatment of respiratory diseases, kidney stones and worm infestation (FILLIPON *et al.* 2012; KRUMREICH *et al*. 2015); and the leaves are used in the production of fibers for fabric and cordage

(FILLIPON *et al.* 2012). In addition to food, medical and industrial use, recently GERBER *et al.* (2017) demonstrated the potential of *B. antiacantha* for use in forest restoration projects, where the species presented high survival and regeneration power.

According to TAIZ *et al.* (2017), the germination starts with the absorption of water by the seed and ends with the emission of the embryonic axis. In this process, temperature, oxygen, water, and light are essential. Among these factors, temperature and substrate can be managed to increase the percentage, speed and uniformity of germination, which reflects on seedlings of greater vigor and thereby, reducing production costs (NASSIF *et al.* 2004).

The temperature exerts a great effect on the percentage and speed of germination, since it is strictly related to the biochemical reactions that initiate and regulate the resumption of embryo growth and water absorption (CARVALHO & NAKAGAWA 2012; BEWLEY & BLACK 1994). Seed behavior in relation to temperature varies according to species (CARVALHO & NAKAGAWA 2012), and therefore each should be considered individually.

Besides temperature, the type of substrate influences the germination process and the subsequent development of seedlings, considering that each one presents different characteristics of structure, aeration and retention of water (CARVALHO & NAKAGAWA 2012). Therefore, one of its main functions is to provide an adequate proportion of moisture and aeration for seeds, in order to avoid the formation of a film that prevents the entry of oxygen and promotes the development of pathogens (FIGLIOLIA *et al.* 1993; POPINIGIS 1985).

However, despite multiple uses and economic potential, there are few studies about the physiology of germination of *B. antiacantha*. This study aimed to test different substrates and temperatures in the germination of *B. antiacantha*.

2 MATERIAL AND METHODS

The experiment started in June 2017, when ripe fruits were collected from adult plants of *B. antiacantha* (Fig 1A-E) *in situ* in four sites, of Rio Grande do Sul: São Francisco de Assis (29‹‹06.33‹28°S 55‹‹18.87‹07°W), Guaporé‹‹54.36‹51°28) S 51‹‹57.36‹53°W ,(Paverama (29«24.91‹33°S 51«30.65‹44°W) and Taquari (29‹‹35.57‹45°S 51«23.06‹52°W). In each location, an infructescence (bunch(was collected, each with about 80 fruits. After collection, the fruits were transferred to the Laboratório de Botânica of the Universidade do Vale Taquari (UNIVATES), and with a blade, cut in half for manual seed removal. On average, 30 seeds were removed from each fruit, which were washed for pulp removal and homogenized in a single batch. The seeds were dried on paper towel and kept on laboratory bench for seven days. After this period, the seeds were taken to the Laboratório de Biotecnologia of the Departamento de Horticultura e Silvicultura of the Faculdade de Agronomia of the Universidade Federal do Rio Grande do Sul, Porto Alegre/RS, and maintained in a refrigerator at 7 ± 2 °C until the studies were carried out in September 2017.

After fruit processing, the seeds were systematized in one batch approximately 6.300 seeds, and randomly, mini lots with about 60 seeds, were removed for the tests. Before the installation of the experiment, the test to determine the water content of the seeds was carried out, using the oven-drying method at 105ºC, described by BRASIL (2009). These rules state that under controlled heat conditions, the method will extract the water contained in the seeds through the steam, resulting in the weight loss of the sample, which will express the weight of the original sample in percentage. For this, three containers with samples of 1.0 g of seeds were weighed on an analytical balance and then placed in an oven at 105 ºC ± 3 ºC until acquiring constant weight. After, the containers were removed and weighed again (BRASIL 2009).

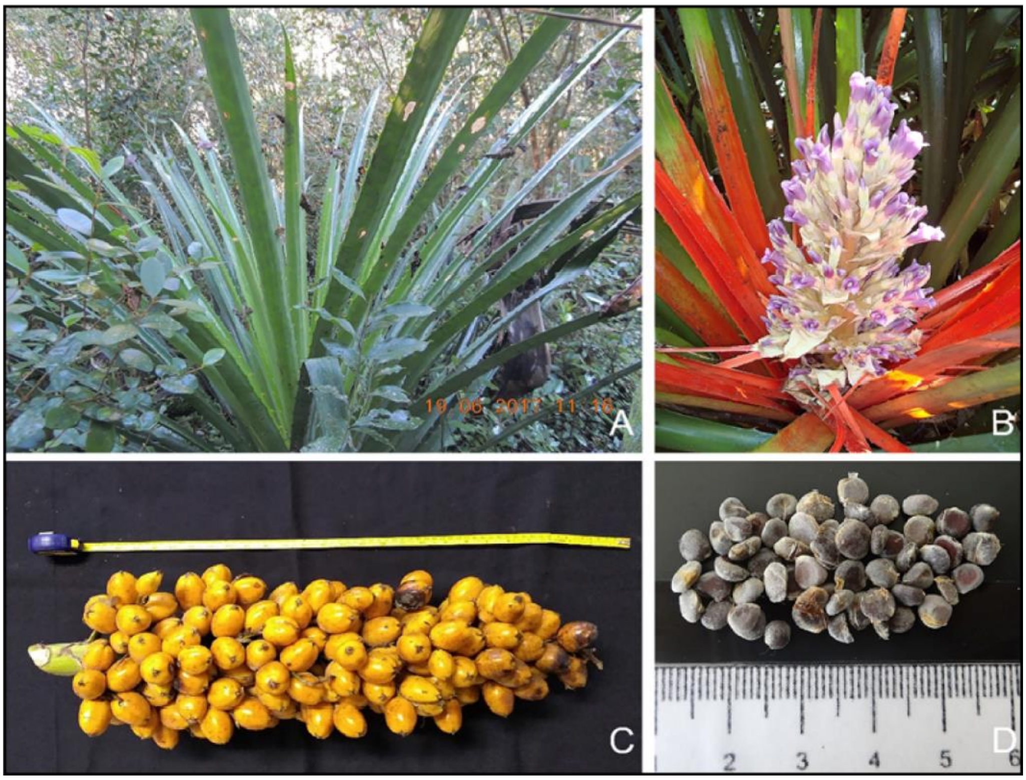
Germination tests started with the disinfestation of the seeds by immersion in 70% ethanol for one minute, followed by 10 minutes in 1.5% sodium hypochlorite plus Tween 20® in the proportion of 0.10%, both under agitation. Then, the seeds were washed three times in autoclaved deionized water, each wash taking one minute to remove residues of disinfestation agents. After this process, the seeds were placed in transparent gerbox-type boxes on the autoclaved substrates: sheet of germibox paper and medium sand. The paper was moistened with a volume of deionized water 2.5 times the mass of the dry paper, and the sand with a volume of water equivalent to 60% of the water holding capacity (BRASIL 2009). The boxes were kept in Biochemical Oxygen Demand (BOD) incubator adjusted with photoperiod of 14 hours, luminous intensity of 27 to 33.75 μmol m^{-2} s^{-1} and constant temperature of 25 and 30 °C.

To evaluate the effect of substrates and temperatures on seed germination and seedling formation, the following variables were evaluated: percentage of germination (G) and seedlings formed (SF), germination mean time (MTG) and seedling formation mean time (MTS), germination rate index (GRI) and seedling formation rate index (SRI), according to the sum of the number of germinated seeds or seedlings formed in each evaluation and divided by the respective time, in addition to the percentage of seedling formation formed on the number of germinated seeds (SF / G).

The first count of germinated seeds was carried out 16 days after the installation of the experiment, and repeated every 2 days. The seeds that presented protrusion of the radicle with 2 mm in length were considered germinated and fully formed seedlings those that had a fully formed aerial part and root system. The experiment was completed in 65 days when no new germination or seedling formation was observed after three consecutive evaluations, considering that the seedlings were studied until the stage when the first two leaves were completely expanded.

The treatments were distributed in a completely randomized design with 4 treatments and 4 replicates of 50 seeds for each treatment. Data were submitted to analysis of variance (ANOVA) and followed by a comparison of means with LSD test (Least Significant Difference) at 5% of probability of error using the CoStat 6.4 *software*. The MTG data were transformed to x, and the MTS and SRI were analyzed by Generalized Linear Model using the SPSS 23.0 *software*.

Figure 1 – *Bromelia antiacantha* Bertol. A. Habit. B. Inflorescence. C. Representative infructescence. D-E. Seeds



3 RESULTS AND DISCUSSION

The water content of *B. antiacantha* seeds was 17.02%. According to COIMBRA *et al.* (2009), the initial water content of seeds is an important factor to standardize seed vigor and viability tests, as well to obtain consistent results. The statistical analysis showed that there was no interaction between substrate and temperature for the analyzed variables. About the substrates, a significant difference was verified only for the percentage of germination and seedling formation. For the temperatures, there was significance for germination mean time, seedling formation mean time, germination rate index and seedling formation rate index (Table 1).

Table 1 – Analysis of variance of germination percentage (G), percentage of seedlings formed (SF), percentage of seedling formation on the number of germinated seeds (SF/G), germination mean time (MTG), seedling formation mean time (MTS), germination rate index (GRI) and seedling formation rate index (SRI) of *Bromelia antiacantha* submitted to different types of substrates and temperatures

Variables	**Substrates**	**Temperature**	**Interaction**	**Overall Average**	**CV (%)**
G (%)	p= 0.016	p= 0.267^{ns}	p= 0.267^{ns}	84	5.12
SF (%)	p= 0.021	p= 0.159^{ns}	p= 0.228^{ns}	83.38	5.19
SF/G (%)	p= 0.745^{ns}	p= 0.239^{ns}	p= 0.670^{ns}	99.26	1.49
MTG (days)	p= 0.414^{ns}	<0.001	p= 0.885^{ns}	22.52	5.8
MTS (days)	p= 0.805^{ns}	<0.001	p= 0.991^{ns}	29.78	8.12
GRI	p= 0.979^{ns}	<0.001	p= 0.654^{ns}	1.98	10.19
SRI	p= 0.882^{ns}	<0.001	p= 0.714^{ns}	0.034	6.49

^{ns} not significant at 5% of probability of error. CV (%) = coefficient of variation

Regarding the substrate, the paper showed superiority compared to sand with an average of 87% of germinated seeds and 86.25% of seedlings formed (Table 2). According to Seed Analysis Rules, paper is one of the substrates most used during the implantation of germination tests, since it provides to seeds the amount of water required to the process, keeping it adequately moist (BRASIL, 2009).

The substrate favors seed germination, as it influences essential factors such as seedling structure, percentage and speed of germination, aeration, water retention capacity, low degree of infestation by pathogens, biochemical reactions, among others (SORANA *et al.* 2019). Besides, to choose a substrate it is necessary to consider seed size, light sensitivity, water availability requirements as well as facility during seedling formation analyzes (BRASIL 2009).

The number of seedling formation on the number of germinated seeds, germination mean time, seedling formation mean time, germination rate index and seedling formation rate index, showed no statistical differences between the substrates tested (Table 2). Both sand and paper presented advantageous characteristics for time and speed of germination and seedling formation of *B. antiacantha*, indicating that this native species is possibly adaptable to different conditions. In a study with another bromeliad, *Aechmea angustifolia* Poepp. & Endl., satisfactory germination results were also observed with the use of paper substrate, with an average of 83% of germinated seeds (VIZENTIN *et al.* 2016). According to SANTOS (2011), the paper provides favorable conditions due to its greater contact with seeds and seedlings, which allows a greater capacity of retention of water and, thus, a decrease in respiratory rate processes.

Another important factor during germination and seedling formation processes is temperature. As reported by DINIZ *et al*. (2020), temperature controls all biological processes, interfering with the dynamics of water absorption, the number and speed of germination and chemical reactions and physiological processes. The percentage of germination, seedling formation and seedling formation on the number of germinated seeds did not show significant differences between the temperatures tested (Table 2). The tests demonstrated that the species develops rapidly until the seedling phase (aerial part and completely formed root system) in places with temperature of 25 to 30 ºC. From this result, it is possible to infer that *B. antiacantha* has initial acclimatization plasticity, in other words, an indicative of its adaptation to different environmental conditions.

At 25 ºC, the seeds of *B. antiacantha* showed a lower germination mean time and seedling formation mean time, with averages of 19.28 and 28.14 days, respectively (Table 2). As stated by FERREIRA & BORGHETTI (2004), seeds that are more vigorous will germinate faster, indicating that the mean germination and seedling formation time is a simple parameter to verify seed vigor. Thus, seeds of rapid germination and seedling formation indicate that the species can establish itself in the environment as soon as possible, taking advantage of favorable conditions (MELO *et al.* 2018). Such fact also indicates that the species studied can be easily propagated in degraded soil sites due to its vigor and rapid seed germination in the environment.

Table 2 – Percentage of germination (G), percentage of seedlings formed (SF), percentage of seedling formation on the number of germinated seeds (SF/G), germination mean time (MTG), seedling formation mean time (MTS), germination rate index (GRI) and seedling formation rate index (SRI) of *Bromelia antiacantha* submitted to different types of substrates and temperatures

Treatments		**G (%)**	**SF (%)**	**SF/G (%)**	**MTG (days)**	**MTS (days)**	**GRI**	**SRI**
Subst.	Paper	87.00 a	86.25 a	99.13^{ns}	23.09^{ns}	29.67^{ns}	1.98^{ns}	0.0340^{ns}
	Sand	81.00 b	80.50 b	99.38	21.95	29.88	1.99	0.0342
Temp.	25 ºC	85.25^{ns}	85.00^{ns}	99.72^{ns}	19.28 a	28.14 a	2.25 a	0.0356 a
	30 ºC	82.75	81.75	98.80	25.76 b	31.41 b	1.71 b	0.0326 b

^{ns} not significant at 5% of probability of error. Means followed by different letters in the column differ from each other by the LSD test a at 5% of probability of error. Subst. = substrates; Temp.= temperatures

In addition to shorter germination and seedling formation time, the seeds kept at 25 ºC presented superiority in the rate of germination and seedling formation (2.25 and 0.0356, respectively) (Table 2). Therefore, the temperature has an important influence on the germination of seeds, especially in the aspects of speed and uniformity. BASKIN & BASKIN (2014) reported that maximum or minimum temperatures, in relation to the optimum temperature, presupposes a reduction in the speed of the germination processes, leaving the seedlings exposed to adverse factors for long periods. Teixeira *et al*. (2018) claim that the germination process occurs within certain temperature limits. For this reason, obtaining an optimum temperature range favors seed germination, since the germinative processes happen quickly and efficiently. However, this is a dependent factor of each species (OLIVEIRA & BARBOSA 2014) and the region of origin, that is, its respective geographic distribution area (CARVALHO & NAKAGAWA 2012).

Most bromeliad species are characterized as tropical, where they develop in locations at moderate temperatures and suitable moisture indices (ESPEJO-SERNA & LÓPEZ-FERRARI 2018). Plants, such as Bromeliaceae, likely to occur in tropical and subtropical latitudes of the Americas, are likely to have germinative success among the most varied conditions of altitude, temperature and humidity. Rios *et al.* (2016) mention that temperatures between 25 °C and 35 °C were promising for *Aechmea constantinii* (Mez) L.B.Sm. during germination processes. Thus, based on the results obtained in this study, 25 °C is the temperature that benefits seed germination and seedling formation of *B. antiacantha*.

Brazil is a country with great diversity of species of the Bromeliaceae. Only in the Brazilian Atlantic rainforest are more than half of the species recorded (RIOS *et al.* 2016), becoming one of the centers of family diversity (ZANELLA *et al*. 2012). *Bromelia antiacantha* is an important species due to its medicinal (expectorant syrup production), food, ornamental and industrial characteristics (FILIPPON *et al.* 2012; KRUMREICH *et al.* 2015), as well its potential for use in the recovery and conservation of areas with degraded soils.

Also, the knowledge about the temperature and substrate for the initial culture of the species is extremely important since it aims to obtain information for its establishment in disturbed places and subsequent economic exploitation. The understanding of optimum conditions during the period of germination is relevant, mainly the temperature and substrate, because they are elements that vary between the seeds of different species (PILAU *et al*. 2012; BOVOLINI *et al*. 2015; FIGUEIREDO *et al*. 2019).

Therefore, the management of native plants becomes an efficient alternative to increase the income of rural producers at the same time that it's an option for recovery of natural environments, to rescue and spread traditional knowledge, and to promote the conservation of the entire ecosystem through its use (FILIPPON *et al.* 2012; RIVAS & BARBIERI 2014).

4 CONCLUSION

The seeds of *B. antiacantha* showed superior germination and initial seedling formation when sown on paper substrates and maintained at 25 °C.

ACKNOWLEDGMENTS

The authors thank the Ministry of Science, Technology and Innovation of Brazil (MCTI) and the National Council for Scientific and Technological Development (CNPq) for funding the research (CHAMADA UNIVERSAL, MCTI / CNPq Nº 01/2016).

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How to quote this article

PAIM, L.P; *et al*. Seed germination and seedling formation of *Bromelia antiacantha* Bertol. At different tempera-tures and substrates. **Ciência e Natura**, Santa Maria, v. 43, e27, p. 1-15, 2021. DOI 10.5902/2179460X39563. Available from: https://doi.org/10.5902/2179460X39563. Accessed: Month Abbreviated. day, year.