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Article

Germination of *Stigmaphyllon blanchetii* Seeds in Different Temperatures and Luminosity

*Germinação de Sementes de **Stigmaphyllon blanchetii** em Diferentes Temperaturas e Luminosidade*

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ABSTRACT - The objective of the present work was to study the influence of temperature and light on germination of seeds of *Stigmaphyllon blanchetii*, popularly known as rat tail. The first stage of the research evaluated the effect of three constant temperatures (20 °C, 25 °C and 30 °C) and an alternating temperature (20-30 °C). In the second stage, for simulation of different types of light, four conditions of luminosity (white, red, far red and absence of light) were used. The temperatures that provided the best germination and development for *S. blanchetii* are the constant (30 °C) and the alternating (20-30 °C) ones. The seeds germinated both in the presence and absence of light, but there was greater germination and seedling development in the absence of light; thus they were classified as preferential negative photoblastic seeds.

Keywords: weed, *Stigmaphyllon blanchetii*, photoblastism, phytochrome.

RESUMO - O presente trabalho teve por objetivo estudar a influência da temperatura e da luz sobre a germinação das sementes de Stigmaphyllon blanchetii, conhecida popularmente como rabo-de-rato. Na primeira etapa da pesquisa, foi avaliado o efeito de três temperaturas constantes (20 °C, 25 °C e 30 °C) e uma alternada de 20-30 °C. Na segunda etapa, para simulação da luz sob diferentes qualidades espectrais, foram utilizadas quatro condições de luminosidade (luz branca, luz vermelha, vermelho distante e ausência de luz). As temperaturas que proporcionaram melhor germinação e desenvolvimento para S. blanchetii foram a constante de 30 °C e alternada de 20-30 °C. As sementes germinaram tanto na presença como na ausência de luz, porém houve maior germinação e desenvolvimento das plântulas na ausência de luz, podendo ser classificadas em fotoblásticas negativas preferenciais.

Palavras-chave: planta daninha, rabo-de-rato, fotoblastismo, fitocromo.

INTRODUCTION

Stigmaphyllon blanchetii C.E. Anderson, popularly known as rat tail, is a climber plant belonging to the family Malpighiaceae, native to Brazil, typical of the Northeast region. It is found in areas of tablelands, mainly in sugar cane crop areas (Lorenzi, 2008). Its distribution and adaptation for the Northeast region is concentrated in the states of Alagoas, Bahia, Paraíba, Pernambuco, Rio Grande do Norte and Sergipe. This species also occurs in the Southeastern region, in the states of Espírito Santo and Minas Gerais (REFLORA, 2018).

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Weeds alter the structure of the ecosystem in which they are inserted (Minchinton et al., 2006). They compete with crops of economic interest for environmental resources, such as water, light, nutrients and space (Gorchov and Trisel, 2003), thus decreasing plant growth and profitability. Therefore, more detailed knowledge is needed about the biology of the study species.

The germination test of weed seeds is an essential tool that provides further knowledge about the biology of species, their ecological function in the field, adaptation capacity, and potential of infestation, for the purpose of further development of management strategies (Bastiani et al., 2015). Germination is regulated by many factors, such as seed viability, seed dormancy and environmental conditions. Temperature can affect it directly or indirectly, through dormancy breaking, embryonic growth potential, deterioration, water absorption rate and biochemical reactions of the germination process (Marcos Filho, 2015). Light is also a significant factor which controls the beginning of germination of photosensitive seeds, and phytochromes are responsible for perception and transduction of the light signal. This chromoprotein has two interconvertible forms: an inactive form, which is activated while absorbing the red light, inducing the production of Ga3 and triggering the beginning of germination, and an active state, which is inactivated when illuminated with far-red light, with consequent production of abscisic acid (ABA), inducing the seeds to a photodormant state (Takaki, 2001; Seo et al., 2009).

Plants can be classified according to their need of light for occurrence of germination: some need light to germinate (positive photoblastic), some require absence of light (negative photoblastic) and some are considered to be neutral, i.e., light does not interfere in the germination process (neutral photoblastic) (Galindo et al., 2012; Marcos Filho, 2015). There are situations in which seeds are classified as preferentially positive photoblastic (quantitative character), because they also germinated in the absence of light (Klein & Felippe, 1991; Melo et al., 2014). As regards phytochrome forms, there are positive photoblastic seeds, whose PhyB controls germination through low fluency response; negative photoblastic seeds, in which PhyA controls germination through high irradiance response; and light insensitive seeds, whose PhyA controls germination through very low fluence response (Takaki, 2001).

Light requirements vary across weed species: *Conyza bonariensis* and *Conyza canadensis* are positive photoblastic (Vidal et al., 2007); the species *I. grandifolia*, *I. nil* and *Merremia aegyptia*, are negative photoblastic (Orzari et al., 2013); and *Sorghum halepense* and *Sorghum arundinaceum* are neutral photoblastic (Krenchinski et al., 2015).

In natural environments, there is variation of light and temperature, which can be changed by canopy structure. When reaching the soil, solar radiation is changed because of selective absorption of leaves, especially chlorophylls (Smith, 2000), resulting in a low red:far-red ratio in the filtered light (Fenner, 1995); thus, there is inhibition of seed germination of pioneer species (Válio and Joly, 1979). The seeds of positive photoblastic species, which are present at deeper layers in the seed bank, do not receive light; thus, they are not able to respond to fluctuations in temperature. However, the same seeds, when placed on the soil surface, but under shade, also did not respond to changes in temperature because they were in the absence of light (Ghersa et al., 1992).

In addition, few studies to date have reported information on the biology of rat tail seeds. Thus, the objective of this work was to study the effects of temperature and light on germination of *Stigmaphyllon blanchetii* seeds.

MATERIAL AND METHODS

Fruit collection

The fruits of *S. blanchetii* were collected at various points in sugarcane crop areas, in the town of Atalaia, in the state of Alagoas (Brazil). Table 1 shows the geographical coordinates of the collection sites. The fruits were collected when they were in the senescence stage. After fruit collection, in all areas, through a simple sample, the composite sample was prepared for analysis. In the laboratory, the seeds were sterilized with sodium hypochlorite solution at 1% for two minutes, and afterwards they were cleansed with distilled water (Ferreira, 2001). The seeds were stored for 15 days after collection in ambient temperature until use.

Table 1 - Geographical coordinates in UTM (Universal Transverse Mercator) of collection sites of *Stigmaphyllon blanchetii* fruits

Collection point	Latitude	Longitude	Collection point	Latitude	Longitude
1	9.455814	36.0264171	12	9.4574258	36.0275644
2	9.4539362	36.0273238	13	9.4603352	36.0241357
3	9.4522076	36.0292902	14	9.4628195	36.0163415
4	9.4521817	36.0292923	15	9.4683390	36.0066888
5	9.4518349	36.0334302	16	9.5103738	35.9836321
6	9.4518375	36.0334298	17	9.5130414	35.9841175
7	9.4574202	36.0330462	18	9.5128321	35.9878726
8	9.4574084	36.0330634	19	9.5101034	35.9876457
9	9.4575841	36.0328670	20	9.5124934	35.9939366
10	9.4575804	36.0328586	21	9.5148070	35.9945108
11	9.4567474	36.0307308	22	9.5174425	35.9930990

Temperature test

The first test evaluated germination of *Stigmaphyllon blanchetii* seeds on the basis of temperature. The test used a completely randomized design, with five replicates of 20 seeds. Constant temperatures of 20 °C, 25 °C, 30 °C and alternate temperature of 20-30 °C were used under a 12-hour photoperiod. The seeds were placed in transparent plastic boxes (13 x 11 x 5 cm) containing sterile germitest paper as substrate, previously moistened with distilled water equivalent to 2.5 times the weight of the dry paper, which was remoistened when necessary (Brasil, 2009).

Light test

In the second test, the seeds of the study species were incubated in a germinator set at the alternating temperature of 20-30 °C, under different types of light (white, red, far red) or in the absence of light. The different types of light were obtained by combining red and blue cellophane paper. For the red light, the transparent plastic boxes were covered with two sheets of red cellophane paper, and for the far-red light, they were covered with overlapped sheets of red and blue cellophane paper. For the white light, transparent plastic boxes were used. They were kept inside closed plastic bags to prevent dehydration (Coimbra et al., 2007). Absence of light was obtained by using black plastic boxes, and the counts were made under green light.

Germinated seeds were counted daily for a period of 25 days. Seeds were considered as germinated when they had primary root protrusion (Carvalho and Nakagawa, 2012). At the end of the evaluations, counts were made for number of normal seedlings (NS), shoot length (SL) and root length (RL), germination speed index (GSI), mean germination time (MGT) and dry matter (DM), for both experiments (Nakagawa, 1999; BRASIL, 2009).

The statistical design was completely randomized with five replications of 20 seeds.

Statistical analysis

Analysis of variance was made with the original data, comparing the means by Tukey's test at 5% probability, using the software SISVAR (Ferreira, 2011). For completion of analysis of variance, data on germination percentage were subjected to arcsine transformation $\sqrt{x/100}$, and after that, all data were transformed into $\sqrt{x}$ (Costa et al., 2010).

RESULTS AND DISCUSSION

Stigmaphyllon blanchetii seeds showed distinct behaviors in their germination when subjected to different temperatures. The highest germination percentages occurred between 20 and 30 °C, and they were not different at the temperature of 30 °C. The same behavior also occurred in all study variables (Table 2). Thus, these temperatures can be considered as the most favorable to germination of this species.

Table 2 - Germination percentage (G), germination speed index (GSI), mean germination time (MGT), normal seedling (PN), dry matter (DM), shoot length (SL) and root length (RL) of *Stigmaphyllon blanchetii* on the basis of different temperatures

Temp. (ºC)	G (%)	SGI	MGT (days)	NS (%)	SL (mm)	RL (mm)	DM (g)
20	37.95 c	0.71 b	15.05 a	1.80 c	0.99 b	4.04 b	0.28 b
25	42.24 bc	0.72 b	14.48 a	4.80 b	1.90 b	6.94 ab	0.30 b
30	57.48 ab	1.94 a	10.19 b	9.60 a	5.66 a	8.78 a	0.54 a
20-30	66.37 a	1.85 a	9.94 b	13.60 a	6.95 a	7.53 a	0.52 a
“F”	8.18**	18.60**	15.23**	26.99**	32.88**	6.76**	8.34**
CV (%)	10.39	14.94	6.32	18.73	18.04	13.80	14.02

Means followed by the same letter in the column do not differ statistically by Tukey’s test at 5% probability of error. ** Significant at 1% of probability by the F-test.

An optimum temperature results from the combination of percentage and speed of germination, which have the most satisfactory results (Marcos Filho, 2015). In this case, the constant temperature of 30 °C and the alternating temperature (20-30 °C) yielded higher results for percentage and speed of germination. There are species that have higher rates of germination when subjected to alternating temperatures, which is indicative of their ability to adapt to temperature variations of the environment (Martins et al., 2010). Similarly to these results, Silva et al. (2017) found that *Parkia platycephala* seeds showed higher germination and vigor at alternating temperatures, which shows that this species tolerates adverse conditions and, thus, it has a higher percentage of success in the field.

There are several conditions that affect seed germination, and temperature is a factor that can interfere in water absorption speed, in germination speed and uniformity, and in chemical reactions that occur during the process (Marcos Filho, 2005; Carvalho and Nakagawa, 2012). The preference of *S. blanchetii* for alternating temperatures may be due to the conditions achieved during its process of formation and seed development, and the optimum temperature for germination is associated with ecological characteristics of the species (Probert, 1992).

Temperatures higher or lower than the optimum temperature decrease germination speed, which can cause total reduction of germination (Carvalho and Nakagawa, 2012). When the seeds of *S. blanchetii* were subjected to temperatures of 20 °C and 25 °C, they showed a significant reduction in germination percentage, GSI, MGT, NS, DM, SL and RL. Thus, they were not indicated for the seed germination test of this species. A similar behavior was found by Zucarelli et al. (2015) in *Passiflora incarnata* seeds, whose germinability was higher as germination increased, especially at 35 °C and at the alternating temperature of 30-20 °C.

Similar results were found by Martins et al. (2010): the alternating temperature of 20-30 °C promoted the highest germination percentage in seeds of *Borreria densiflora* var. latifolia, and speed was inhibited when they were incubated at a temperature of 20 °C. Unlike such results, the temperatures of 20 °C and 25 °C provided higher percentage and speed of germination in seeds of *Ipomea grandifolia*, *Ipomea nil* and *Merremia aegyptia* (Orzari et al., 2013), corroborating the findings of Borges and Rena (1993), i.e., seeds have a variable behavior depending on temperature, and there is no optimum temperature and uniform germination for all species.

Mean germination time shows the time required for maximum germination to occur; the shorter the time, the higher the SGI. There was a reduction in MGT according to the increase of temperature; there was no statistical difference between the temperatures of 30 °C and 20-30 °C, which showed the lowest values (Table 1). This fact is indicative that these temperatures not only increased germination percentage and germination speed index, but also made germination faster. These results corroborate those of Alves et al. (2015) in seeds of *Psidium guajava*. They found that the highest GMT values occurred at 20 °C and the lowest, at temperatures between 20 and 30 °C.

For percentage of normal seedlings, at a temperature of 20 °C and 25 °C, there was a reduction in the number of normal seedlings and in root and shoot length. This was possibly due to the fact that low temperatures cause changes in cell structure, including the plasma membrane and the cell wall. Moreover, they reduce relative plant growth and increase the amount of flavonoids (Matsuura and Obata, 1993; Lozovaya et al., 2005).

Also, the analysis of seedling length (shoot + root) showed that the temperature of 30 °C and the alternate temperature of 20-30 °C led to balanced shoot and root growth, while at the other temperatures, there was increased root production but decreased shoot production. Mean shoot length and mean root length are key variables to identify the most vigorous plants in the germination test. Differences between the analyzed treatments were relative to more vigorous plants as a result of differential growth and, therefore, there was maximum dry matter production (Nakagawa, 1999).

The results of plant dry matter showed that the seeds subjected to temperatures of 30 °C and 20-30 °C had the best results. According to Freitas et al. (2009), seedling dry matter values enable an evaluation of growth because plants have greater or lesser competitive capacity. At these temperatures, *S. blachetii* seedlings showed higher dry matter values, and their competitive capacity was greater. There is greater production at short intervals and there is an advantage in competition for available resources, which may retard or suppress growth of other plants. Thus, it can be inferred that *S. blanchetii* seeds have better conditions of development when exposed to a temperature of 30 °C or alternate temperature between 20 and 30 °C.

Light is another factor that is connected with germination; it can be influenced by temperature in some cases, and germination may not occur (Santos and Pereira, 1987). In this sense, by evaluating the influence of light and temperature on the development of *Amburana cearenses*, Almeida et al. (2017) found that the species has better growth when under a temperature of 25 °C and 30 °C and in the presence of light.

As regards the influence of light, the largest percentages and speed of germination occurred when the seeds were under red light and absence of light, without differences among themselves, while white light caused lower germination percentage and germination speed index. However, with far-red light, there was no difference in the remaining luminosities for germination purposes (Table 3). These results are different from those of Thomas (1974) and Alves et al. (2016), who reported that red light provides a similar effect to that of white light in spectral composition and absorption characteristics of phytochromes.

Alves et al. (2012) found different results: in *Clitoria fairchildiana* seeds under alternating temperatures of 20-30 °C, there was no difference in germination percentage when they were

Table 3 - Germination percentage (G), germination speed index (GSI), mean germination time (MGT), normal seedling (PN), dry matter (DM), shoot length (SL) and root length (RL) of *Stigmaphyllon blanchetii*, on the basis of different types of light, at a temperature of 20-30 °C

Light	G (%)	GSI	MGT (days)	NS (%)	SL (mm)	RL (mm)	DM (g)
W	61.80 b	2.00 c	7.40 a	8.00 bc	5.80 c	8.80 a	0.54 b
R	71.40 a	3.00 ab	5.40 b	9.00 ab	9.20 b	4.00 b	0.62 a
F.R.	66.60 ab	2.80 b	5.60 b	7.00 c	9.60 b	5.20 b	0.46 c
D	72.60 a	3.60 a	4.20 c	10.60 a	12.60 a	5.80 ab	0.65 a
“F”	8.17**	17.47**	16.60**	8.13**	30.98**	10.30**	68.53**
CV (%)	2.88	6.09	6.13	6.76	6.55	13.69	1.99

W = white light; R = red, F.R. = far-red, D = dark light; *Means followed by the same letter in the column do not differ statistically by Tukey’s test at 5% probability of error. ** Significant at 1% of probability by the F-test.

subjected to different spectral qualities, while germination speed was lower when the seeds were exposed to absence of light.

This germination behavior under different types of light can be influenced by the active form of the phytochrome present in seeds in sufficient quantity to start the germination process. In principle, the phytochrome is inactive (710 nm); when absorbing red light, it becomes active (660 nm), thereby initiating germination, together with other factors, such as hormone synthesis and activation of genetic transcription (Bewley et al., 2013; Marcos Filho, 2015).

Importantly, there was a reduction in mean germination time as the germination speed index increased (Table 3). For the seeds kept in the dark, MGT was shorter when compared to that of seeds kept in the light (Table 3). *Sida rhombifolia* seeds also have lower MGT values in the absence of light, which makes germination slower (Carvalho and Carvalho, 2009). According to Labouriau and Agudo (1987), this delay in germination may increase the likelihood that seedlings will find favorable conditions for development.

It was found that the seeds kept in the dark-showed higher values for seedling shoot length, when compared with those incubated in the light. In the absence of light, seeds need to use more of their reserves for shoot elongation in the search for light in order to start photosynthesis. This fact was observed in this study (Taiz and Zeiger, 2013). There was no difference for root length among seeds subjected to the presence and/or absence of light. Different results were found by Paiva et al. (2016): *Salvia hispanica* seeds showed greater seedling growth and dry matter accumulation in the presence of light.

For dry matter, the best results were found in seeds incubated in red light and in the dark. The low dry matter values are related to the treatment with white light and far-red light (Table 3). This fact was possibly due to prolonged exposure of seedlings; high irradiance cause greater absorption of light photons than allowed, and as a result, there may occur photoinhibition and even plant death (Kitao et al., 2000). Carvalho et al. (2006) found that, for *Syagrus coronata*, there was a reduction of seedling shoot dry matter as brightness increased. By contrast, Alves et al. (2012) found that the dry matter of *Clitoria fairchildiana* roots, at alternating temperatures of 20-30 °C, was lower when subjected to the absence of light. Also, they reported higher dry matter values in the presence of far-red, red and white light. This may be explained by the fact that the most vigorous seeds have greater capacity to transfer their reservations to the embryonic axis, resulting in seedlings with a higher growth rate (Nakagawa, 1999).

Seeds can be classified, according to need of light for germination to occur, into positive photoblastic, when they need light for germination; negative photoblastic, when germination occurs in the dark, and non-photoblastic or indifferent, when light does not interfere in the germination process (Marcos Filho, 2015). For Klein and Felippe (1991) and Melo et al. (2014), the photoblastic character may have a preferential element (quantitative character), when there is a small amount of seeds germinated in the dark, or an absolute character, when germination only occurs in the presence of light. Thus, *S. blanchetti* seeds germinated in the presence and in the absence of light, but there was higher germination and seedling development in the absence of light. Thus, they may be possibly classified as negative photoblastic. The same behavior was found in *Myracrodruon urundeuva* seeds (Silva et al., 2002). *Echium plantagineum* seeds are considered to be preferentially positive photoblastic (Roso et al., 2017).

Seeds can also be classified according to light in relation to phytochrome forms. There was higher percentage and speed of germination when seeds were exposed to red light and absence of light; thus, this species probably has the phytochrome form phyA controlling germination through high irradiance (Takaki, 2001). Different results were found by Stefanello et al. (2006), who found that seeds of *Foeniculum vulgare* Miller were indifferent to light, and phytochrome phyA was responsible for germination through very low fluence response. The same behavior was found by Alves et al. (2012) in light-insensitive *Clitoria fairchildiana* seeds, since they have phytochromes controlling germination through very low fluence response. In *Muntingia calabura* seeds, phytochromes control germination through low fluence response (Leite and Takaki, 2001).

Thus, *Stigmaphyllon blanchetii* seeds germinate and grow best at temperatures of 30 °C and 20-30 °C, preferably in the absence of light.

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