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Germination Behavior of Psidium Rufum Dc. Seeds at Different Temperatures, Substrates and Conditions of Light

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ABSTRACT

Background: The performance of the germination of each species varies at different temperatures, substrates and sometimes light conditions. Therefore, in the conduct of germination tests in laboratories, the knowledge the influence of these components on the germination of each species is of fundamental importance. *Psidium rufum* DC. is a species native to Brazil, belonging to the family Myrtaceae, characteristic of Biomes Cerrado and Atlantic Forest. It is a initial secondary species, including used for the restoration of degraded areas, with high survival rates. Knowledge of *P. rufum* are incipient and literature are rare information about the species and the performance of your germination process**. Objective:** Therefore, this study aimed to verify the germination behavior of *P. rufum* seeds at different temperatures, substrates and conditions of presence and absence of light. **Results:** Analysis of variance revealed a significant interaction ($P < 0.05$) between the substrate and temperature variables, indicating that the seed germination of *P. rufum* laboratory depends on the temperature and the type of substrate, except for germination percentage in which the effects of the factors studied proved to be independent. When placed in the substrate blotter paper 25 °C, the seeds had higher germination percentage, higher speed of germination, smaller average time and higher average speed of germination. The maximum synchronization of germination ($U = 0$) in *P. rufum* was observed of temperature 30 °C, the roll paper substrate, however, was the treatment had the lowest percentage of germination. Considering the distributions of the frequency in the germination of *P. rufum*, such isothermal patterns used (20, 25 and 30 °C) differ in the number, location and frequency of fashions, where all distributions showed character multimodal. The germination of *P. rufum* was lower in the dark, revealing preferred positive photoblastic. **Conclusion:** The best results for the germination test were obtained with blotter paper substrate at 25 °C. The light influence the germination of *P. rufum*, with the species considered as the preferred positive photoblastic**.**

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INTRODUCTION

The success in the formation of seedlings of a forest species depends, among other things, knowledge about their germination process. The analyzes of seeds of forest species have gained the attention of the scientific community, in order to acquire information about the ideal conditions for the germination of seeds of many species. These analyzes are carried out by means of a set of procedures approved by the Rules for Seed Analysis (Brazil, 2009).

The performance of the germination of each species varies at different temperatures, substrates and sometimes light conditions. Therefore, in the conduct of germination tests in laboratories, the knowledge the influence of these components on the germination of each species is of fundamental importance.

According to Carvalho and Nakagawa (1983), temperature performance affects the germination process in three ways: on the total, the rate and uniformity of germination. In general, we can identify three critical points of temperatures for germination, which are the calls cardinal temperatures: the minimum temperature, below which there is no visible sprouting in reasonable period of time; the maximum temperature, that above this there is no germination; and the best temperature, at which the maximum number of seed germination, a minimum period of time (Popinigis, 1985; Carvalho and Nakagawa, 1983). According to

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Borges and Rena (1993), the range 20-30 °C proved to be adequate for germination of many subtropical and tropical species.

Another important factor for germination is the substrate as it influences germination due to its structure, aeration, water holding capacity, willingness to infestation by pathogens, among others, could be favorable or detrimental (Martins *et al*., 2011) . In laboratory tests, the choice of the substrate must be taken into account the size of the seed, its requirement with respect to the amount of water, their sensitivity to light, the facility it offers for carrying out and evaluating counts of seedlings (Brazil, 2009).

There are species whose seed germination is influenced, positively or negatively, by the light and seed indifferent to it (Borges and Rena, 1993). This variation of behavior allows us to gather and sort the species into three groups according to their responses to the intensity of light on the germination: the positive photoblastic who have greater ability to germinate in light condition; negative photoblastic, which germinate better in the dark; and neutral photoblastic that germinate well in the absence or presence of light (Oliveira, 2012).

Psidium rufum DC. is a species native to Brazil, belonging to the family Myrtaceae, characteristic of Biomes Cerrado and Atlantic Forest (Sobral *et al*., 2014). It is a initial secondary species, including used for the restoration of degraded areas, with high survival rates (Santos, 2011). As for the utility, its wood is suitable for light carpentry, packaging, tools cable and tools, as well as firewood and charcoal. It is also a recommended species for afforestation of narrow streets and under power lines, for being small. Its fruits are edible, but laxatives, and are well consumed by birds (Lorenzi, 2009). Knowledge of *P. rufum* are incipient and literature are rare information about the species, reasons that this study aimed verifying the germination behavior of the species at different temperatures, substrates and conditions of presence and absence of light.

MATERIAL AND METHODS

The fruits of *P. rufum* were collected by Society Chauá and obtained from matrices located in the cities of Curitiba and Ponta Grossa, Paraná State, Brazil. Extraction of the seeds was carried out manually by means of maceration and washing the fruit in water. The physical analysis of seed were determined the number of seeds per kilogram and the thousand seed weight were used eight repetitions of 100 seeds. The initial moisture content was determined with 50 seeds replicated three times at 105 ± 3 °C/24 hours.

Germination tests were conducted at the Forest Seed Laboratory (Department of Forest Sciences, Federal University of Parana, Brazil) and used four different types of substrates: Roll paper, blotter paper, sand and vermiculite, and three different temperatures 20, 25 and 30 °C, with five replicates of 40 seeds each per treatment. The substrates were sterilized in accordance with the Rules for Seed Analysis. Subsequently, the seeds were placed on their respective substrates and placed in chamber germination type Biomatic regulated at the temperatures 20, 25 and 30 °C with 10 hours photoperiod in the light and 14 hours dark.

After determining the best substrate and temperature, tested the light effect (absence and presence of light) to the seeds of *P. rufum* using gerbox acrylic black and transparent gerbox. Was used blotter paper substrate and a temperature of 25 °C with five replications of 40 seeds each per treatment. The corresponding measurements to gerbox acrylic black were performed in the dark under a lamp covered with green filter with a wavelength between 490-560 nm.

For the statistical analysis of the data of germination was used a completely randomized design in a 3x4 factorial design (3 temperatures and 4 substrates), and the data of germination percentage were converted to arc sen. $\sqrt{x/100}$. To evaluate the results we used the sanest program - Statistical Analysis System (Zonta *et al*., 1985), and the treatment means were compared by Duncan test, considering a level of 95% probability.

Assessments were made daily until the seeds do not germinate more, or get in a state of deterioration. Were considered the ones germinated seeds that had radicle emission with at least two millimeters. The variables analyzed were: (1) index of velocity germination (Maguire, 1962), (2) germination percentage, (3) average germination time (Labouriau, 1983), (4) relative frequency, (5) mean rate and (6) synchronization index (Labouriau and Valadares, 1976), presented below expressions:

$$IVG = \frac{G_1}{N_1} + \frac{G_2}{N_2} + ... + \frac{G_n}{N_n} \quad (1)$$

Being: IVG - index of velocity germination; G_1, G_2 e G_n - number of normal seeds computed in the first, second and last count, respectively; N_1, N_2 e N_n- number of days after the test deployment.

$$\%G = \left(\sum n_i . N^{-1}\right) 100 \quad (2)$$

Being: %G - germination percentage, N- number of seeds placed to germinate; $\sum n_i$ - total number of germinated seeds.

$$\bar{t} = \sum_{i=1}^{k} n_i t_i \Bigg/ \sum_{i=1}^{k} n_i \quad (3)$$

Being: $\bar{t}$ - average germination time; n_i - number of seeds that germinate in time t_i (not the cumulative number, but the number reported for the i-th observation); t_i - time between the start of the experiment and the i-th (day or hour) observation; k– last day of observation.

$$f_i = n_i \Big/ \sum_{i=1}^{k} n_i \quad (4)$$

Being: f_i - relative frequency; n_i - number of seeds that germinate in time t_I; k- last day of observation.

$$V_m = \frac{1}{\bar{t}} \quad (5)$$

Being: V_m – mean rate of germination; $\bar{t}$ – average germination time.

$$U = -\sum_{i=1}^{K} f_i \log_2 f_i \quad (6)$$

Being: U- synchronization index or uncertainty; f_i - relative frequency of germination; k– last day of observation.

RESULTS AND DISCUSSIONS

The thousand seed weight was equal to 77.31 grams (CV = 2.18%), with 12,935 seeds per pound with 16% water content. Do not have information in the literature about data thousand seed weight, number of seeds per pound and water content of the seeds of *P. rufum.*

Analysis of variance revealed a significant interaction (P <0.05) between the substrate and temperature variables, indicating that the seed germination of *P. rufum* laboratory depends on the temperature they are subjected and the type of substrate, except for germination percentage (Table 1) in which the effects of the factors studied proved to be independent.

Table 1: Summary of the analysis of variance for germination percentage (%G), index of velocity germination (IVG), average time in days (AT), mean rate of germination (MR) and synchronization index of germination (U) of seeds of *P. rufum* submitted the four substrates and three temperatures.

MEAN SQUARES						
Source of variation	Degrees of freedom	%G	IVG	AT	MR	U
Temperature	2	26377,0***	9,56***	340,9***	0,000694***	27,76***
Substrate	3	225,7*	0,19***	13,8**	0,000052***	1,03*
Sub. x Temp.	6	144,6^{ns}	0,05*	11,6***	0,000028***	2,05***
Residue	48	67,9	0,02	2,4	0,000004	0,33
C.V (%)		14,9	14,3	6,0	5,4	21,1
MEAN		54,96	0,95	25,99	0,039	2,72

ns: not significant; *, ** and ***: significant at 0.05, 0.01 and 0.001, the F test, respectively

The data on the percentage of germination are shown in Table 2, in which the only variable was analyzed, which revealed no significant interaction (P <0.05) between the substrate and temperature. It was found that the temperature of 25 °C provided the seeds higher germination rates, as well as on paper substrate blotter. The same was observed by Rickli (2012) in which to *Vochysia bifalcata* seed germination tests recommended the use of paper substrate blotter at 25 °C. The vermiculite and paper roll resulted in the lowest germination percentage with 50.9 and 48.3%, respectively, with no significant statistical differences, as well as the temperature of 30 °C, which notably had the lowest average germination in all substrates.

For the index of velocity germination (Table 3), the ideal combination was blotter paper at 25 °C, confirming the superior performance of both, as already observed in the percentage of germination, and that provided the seeds highest germination rates . The substrate blotter paper is revealed as the most suitable for use in *P. rufum* of germination, and also stands out for its use of practicality and be more economical than other substrates.

It was also found that the 30 °C, regardless of the type of substrate, no satisfactory results of germination for the variables analyzed, indicating a species sensitivity to high temperatures. Unlike what was found by Flores *et al*. (2013), in which the germinating seeds *Melanoxylon brauna* was favored in the 30 °C temperature. The behavior of *P. rufum* in relation to temperature fits in observation of Brancalion *et al*. (2010) to check the best temperature for germination of seeds of tree species Brazilian, in which, it has been observed that, through the constant temperature of 25 °C, the species of biomes Cerrado and Atlantic Forest had the highest percentage of germination and for species of the Amazon biome, the temperature of 30 °C is recommended for the tests of germination.

Table 2: Percentage of seed germination *P. rufum* submitted the four substrates and three temperatures.

Substrates	Temperature (°C)			
	20	25	30	MEAN
Sand	72,6 Aa	80,4 aA	13,6 bA	55,1 AB
Blotter paper	71,0 Ba	86,6 aA	22,7 cA	61,2 A
Vermiculite	63,5 bA	79,5 aA	12,3 cA	50,9 B
Paper roll	71,6 Aa	82,5 aA	2,5 bB	48,3 B
MEAN	69,7 b	82,3 a	11,6 c	

Means followed by the same letter, lowercase letters in lines and uppercase letters in columns, do not differ by Duncan test at the level of p<0.05

Table 3: Index of velocity germination of seed *P. rufum* submitted the four substrates and three temperatures

Substrates	Temperature (°C)			
	20	25	30	MEAN
Sand	1,21 bA	1,5 aBC	0,2 cAB	0,9 B
Blotter paper	1,3 bA	1,7 aA	0,3 cA	1,1 A
Vermiculite	1,0 bB	1,3 aC	0,2 cAB	0,8 C
Paper roll	1,2 bA	1,5 aB	0,03 cB	0,9 B
MEAN	1,2 b	1,5 a	0,2 c	

Means followed by the same letter, lowercase letters in lines and uppercase letters in columns, do not differ by Duncan test at the level of $p<0.05$

Table 4: Average germination time of *P. rufum* submitted the four substrates and three temperatures.

Substrates	Temperature (°C)			
	20	25	30	MEAN
Sand	24,7 bAB	22,6 cB	30,4 aA	25,9 B
Blotter paper	22,8 bB	20,7 cB	31,2 aA	24,9 B
Vermiculite	26,5 bA	25,6 bA	29,6 aA	27,2 A
Paper roll	24,1 bB	22,2 bB	31,4 aA	25,9 B
MEAN	24,5 b	22,8 c	30,6 a	

Means followed by the same letter, lowercase letters in lines and uppercase letters in columns, do not differ by Duncan test at the level of $p<0.05$

Table 5: Mean rate of seed germination *P. rufum* submitted the four substrates and three temperatures.

Substrates	Temperature (°C)			
	20	25	30	MEAN
Sand	0,041 bB	0,045 aB	0,033 cA	0,039 B
Blotter paper	0,044 bA	0,048 aA	0,032 cA	0,041 A
Vermiculite	0,038 aC	0,039 aC	0,034 bA	0,037 C
Paper roll	0,042 bAB	0,045 aB	0,032 cA	0,040 B
MEAN	0,041 b	0,044 a	0,033 c	

Means followed by the same letter, lowercase letters in lines and uppercase letters in columns, do not differ by Duncan test at the level of $p<0.05$

Table 6: Synchronization index of germination of seeds *P. rufum* submitted the four substrates and three temperatures.

Substrates	Temperature (°C)			
	20	25	30	MEAN
Sand	3,52 aA	3,34 Aa	1,41 bB	2,89 A
Blotter paper	3,15 aA	3,20 aA	2,33 bA	2,89 A
Vermiculite	3,45 aA	3,53 aA	1,69 bAB	2,76 AB
Paper roll	3,52 aA	3,49 aA	0,00 bC	2,33 B
MEAN	3,41 a	3,39 a	1,36 b	

Means followed by the same letter, lowercase letters in lines and uppercase letters in columns, do not differ by Duncan test at the level of $p<0.05$

Table 7: Percentage, mean time and germination rate index of seeds of *P. rufum* submitted the presence and light no.

	Germination percentage	Average time	Index of velocity germination
With light	81,4 A	19,7 A	1,67 A
Without light	19,4 B	21,8 A	0,27 B

Means followed by the same letter, lowercase letters in lines and uppercase letters in columns, do not differ by Duncan test at the level of $p<0.05$

For the seeds of *P. rufum*, in optimal temperature of 25 °C the smaller and better values for the average time of germination were found in sandy substrates, blotter paper and paper roll, where no significant differences were found, with mean values of approximately 22 days. The vermiculite was not beneficial and provided the highest average durations of germination in temperatures of 20 and 25 °C, with a mean of 26 days. The temperature of 30 °C there were no significant differences for the variable in question, being that for all substrates, the results were not satisfactory compared to other temperatures (Table 4).

The mean rate of germination, being an inverse function to the average time showed similar behavior and unlike the latter, but with even lower coefficient of variation. One can infer that, as for the other variables, the best combination for this variable occurred in the substrate blotter paper at 25 °C and less than satisfactory results was held at the temperature of 30 °C regardless of the substrate analyzed (Table 5). The average time and the mean rate of germination *P. rufum* seed, reinforce what was observed here that, when placed in the substrate blotter paper 25 °C, had higher germination, higher rate of germination, smaller average time and higher mean rate of germination.

The maximum germination synchronization (U = 0) in *P. rufum* was observed of temperature 30 °C, the roll paper substrate (Table 6), however, was the treatment had the lowest percentage of germination. The other temperatures did not differ, as well as the substrates sand and blotter paper, featuring the biggest U-values (less synchronization). Laboriau

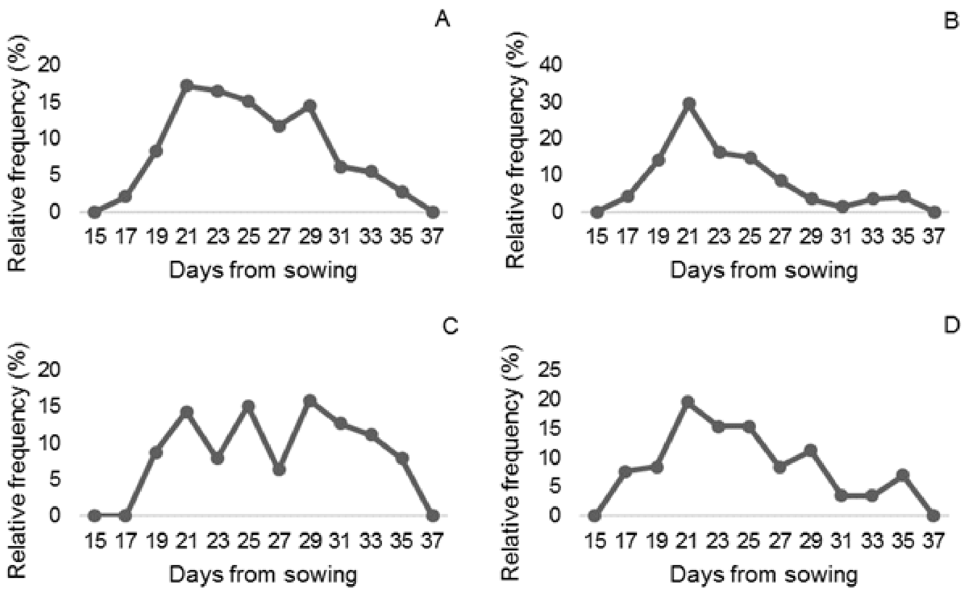
Australian Journal of Basic and Applied Sciences, 9(7) April 2015, Pages: 564-570

and Valadares (1976) who studied the germination of seeds *Calotropis procera* observed that germination percentage and synchronization germination discriminate different temperatures "great". Similar results were also found by Nakao (2012) when working with seed *Urochloa brizantha* where found different optimal temperature ranges for the variables germination rate, germination and synchronization, also implying that the timing of germination was higher at elevated temperatures.

The study of synchronization of seed germination leads to consider the distribution of germination frequencies. Considering the frequency distributions in the germination of *P. rufum* seed (Figure 1), such isothermal patterns used (20, 25 and 30 °C) differ in the number, location and frequency of fashions, where all distributions showed character multimodal.

It is found that at temperatures of 20 to 25 °C, the substrates sand and blotter (Figures 1 A, B, E, F) is the presence of a leading fashion at 21 days after sowing, over germination time of a few seeds appear more distributed. The interaction between the substrate temperatures and vermiculite paper roll (Figures 1-C, D, G, H, K, L) provided with a distribution greater number of modes or peaks, characterizing further multimodal character. The delay in germination at 30 °C proves to be a possible adaptation to external conditions, may increase the likelihood of seedlings find favorable conditions in the environment.

The heterogeneity distributions may be indicative of the effect of environment (temperature x substrate) in embryo growth in germinating seed (Labouriau and Valadares, 1976). The trend towards increased multimodality in the frequency distribution for the isothermal germination, was also found to *Salvia hispanica* L. seed (Labouriau and Agudo, 1987) and *Pterogyne nitens* Tul. (Nassif and Perez, 2000).



Australian Journal of Basic and Applied Sciences, 9(7) April 2015, Pages: 564-570

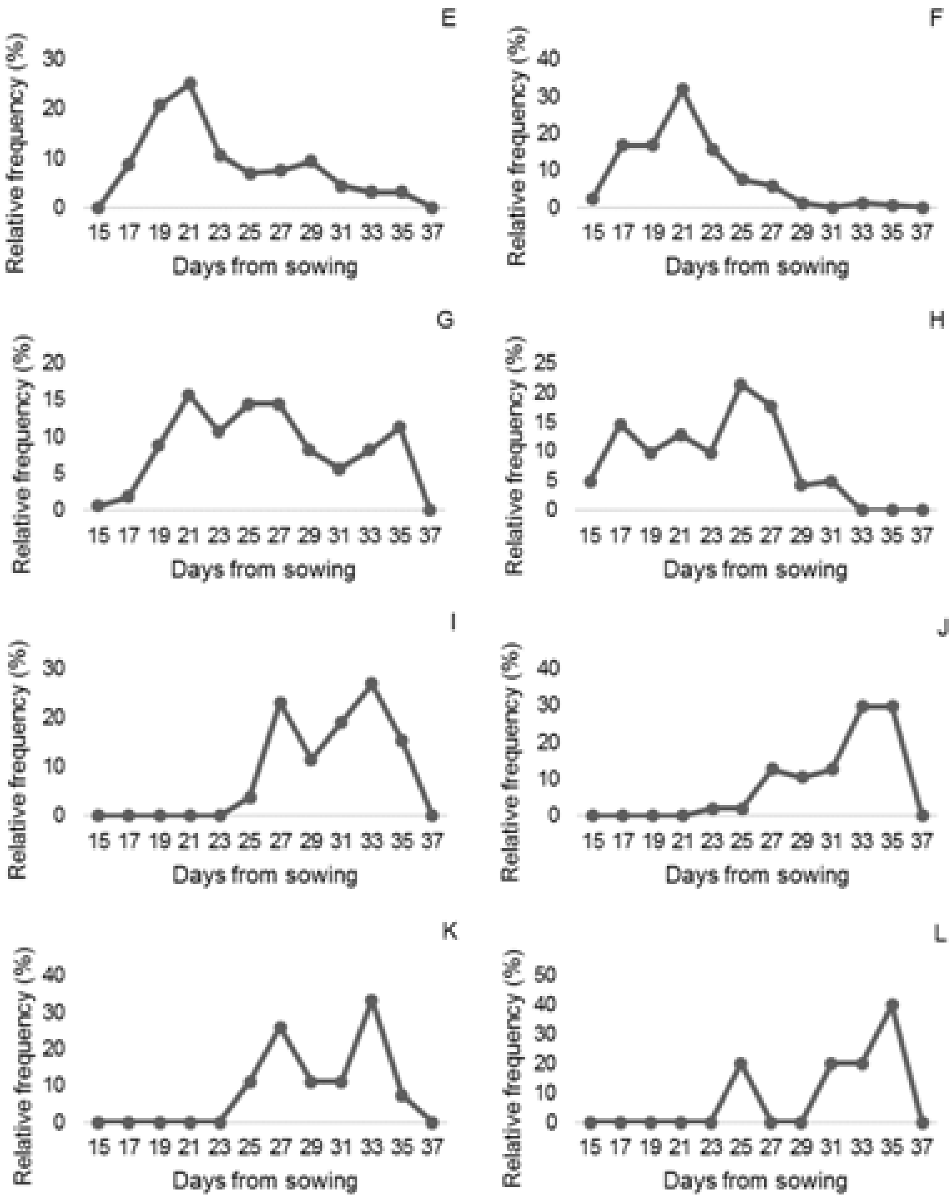


Fig. 1: Polygons frequency on the seed germination *P. rufum* in different substrates and temperatures. A - 20 °C / sand substrate; B - 20 °C / blotter paper substrate; C - 20 °C / vermiculite substrate; D - 20 °C / roll paper substrate; E - 25 °C / sand substrate; F - 25 °C / blotter paper substrate; G - 25 °C / vermiculite substrate; M - 25 °C / roll paper substrate; I - 30 °C / sand substrate; J - 30 °C / blotter paper substrate; K - 30 °C / vermiculite substrate; L - 30 °C / roll paper substrate.

Table 7 shows the Duncan test results to verify the effects of light on germination and seed vigor of *P. rufum.* The results, the germination of *P. rufum* was lower in the dark, revealing positive fotoblastia. The germination average time was around 20 days for both treatments, with no significant differences.

It's worth noting that, according to Klein and Felippe (1991), the positive photoblastism is not always absolute, for many species that behave as positive photoblastic have at least some germination in the dark. In other cases, although statistically species may be considered positive photoblastic, this character is only quantitative, since both in the presence and absence of light is considerable seed germination, these being classified as preferred species photoblastic. Considering the affirmative, it is emphasized that, due to the observed outcome, *P. rufum* seeds can then be identified as preferred positive photoblastic. When working with species *Ocimum gratissimum*, Martins (2006) found that seed can be considered preferred positive photoblastic since under alternating temperature in the absence of light, there was a slight germination. Lucho (2014) also found evidence that *Symplocos uniflora* seeds fall into the same category.

Conclusions:

- ✓ For tests of germination in the laboratory it is recommended to use the substrate blotter paper at a temperature of 25 °C.
- ✓ The species can be classified as being preferentially positive photoblastic.

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