

Diaspores biometry, temperatures and light regime on seed germination of *Ptychosperma macarthurii* (Arecaceae)

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Abstract

This work aimed to analyze diaspores biometric characteristics, the effect of different temperatures and light regimes on seed germination, and the initial seedlings' growth of *Ptychosperma macarthurii*. The experiment was carried out at the Laboratory of Horticultural Seeds at São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal. The individual diaspores biometric analysis was performed by measuring the length, diameter, mass, and weight of a thousand diaspores, the data were subjected to descriptive statistical analysis. It was adopted a completely randomized design in a 5 × 2 factorial with four replications of 25 seeds per treatment. Five temperatures (25; 30; 35; 20-30 and 25-35 °C) and two light regimes (presence and total absence of light) were evaluated. The variables analyzed were germination, germination speed index, the average length of the primary root and shoot, diameter of the stem, and seedlings total dry mass. The length, diameter, and average weight of the diaspores were 12.54 mm, 6.05 mm, and 0.1610 g, respectively. The seeds of *Ptychosperma macarthurii* germinated in a wide temperature range, reaching a higher germination percentage at 25 °C and a higher germination speed index at 30 °C, being classified as neutral photoblastics. The constant temperature of 30 °C provided higher mean values for all seedlings' biometric characteristics.

Keywords: Heat, growth, photoblastia, palm tree

Introduction

Ptychosperma macarthurii [(H. Wendl. ex H.J. Veitch) H. Wendl. ex Hook.f.] is a very elegant cespitose or solitary palm, native to New Guinea and northeastern Australia. It has usually multiple stems, very similar to bamboo, pinnate leaves with short petiole, inflorescences affixed below the palm heart and small globose fruits, with an intense red color and a single wrinkled seed. It bears abundant fruit during the winter months. In Brazil, it is widely cultivated, mainly in the Southeast, in the composition of vases, parks and gardens as an isolated plant or in groups (Lorenzi et al., 2004).

The propagation of palm trees is mainly by seeds and, in general, it shows uneven and slow germination, caused by several factors (Meerow & Broschat, 2015) while temperature, humidity, light and oxygen are considered fundamental (Carvalho & Nakagawa, 2012).

Germination is dependent on well-defined

temperature limits, characteristic for each species (Bewley et al., 2013), considering that, at optimal temperature, there is a higher percentage of germination in a shorter period of time, and at maximum and minimum temperatures there is a lower percentage of germination or embryo death (Carvalho & Nakagawa, 2012).

Regarding the light stimulus in the germination process, those seeds that need light are called positive photoblastic, while those that germinate better in the absence of light are negative photoblastic and when there is no light interference in germination the seeds are considered neutral photoblastic (Nogueira et al. al., 2014). Light can have a stimulating or inhibiting effect on germination depending on the light wavelength to which it was submitted (Carvalho & Nakagawa, 2012).

Both temperature and luminosity can be controlled and, in this way, can contribute to improve percentage, speed and synchronization of germination.

Thus, it is important to evaluate alternating and constant temperatures and the need for luminosity in the germination test to determine the best conditions for seed germination (Lone et al., 2014).

In addition to these factors, the classification of seed weight or size is a strategy that can be adopted to standardize the emergence and initial growth of seedlings (Carvalho & Nakagawa, 2012). In the case of species of the Arecaceae family, biometric studies are important to explore the landscape potential of these species, especially to collaborate with research aimed at sexual propagation (Domingos Neto & Ferreira, 2014).

In this context, the present work aimed to analyze the biometric characteristics of diaspores, the effect of different temperature conditions and light regimes on seed germination, as well as on the initial growth of *Ptychosperma macarthurii* [(H. Wendl. ex. H.J. Veitch) H. Wendl. ex Hook.f].

Material and Methods

The experiment was carried out at the Laboratory of Horticultural Seeds, São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal.

The fruits of *Ptychosperma macarthurii* were harvested on March 6, 2020 at their optimum point of maturation, identified by the red color of the epicarp, from mother plants grown in the Nursery of Ornamental and Forestry Plants of São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, and then they were transported to the Laboratory of Horticultural Seeds. In the laboratory, the fruits were pulped (removal of the epicarp and mesocarp) by manual friction with a steel mesh sieve (6 mm). The diaspores were subsequently aseptically soaked in sodium hypochlorite solution (2%) for 10 minutes, then rinsed with running water.

Weight of 1000 and 100 diaspores - the weight of 1000 diaspores was obtained using eight repetitions of 100 diaspores, which were weighed on an electronic scale (TOLEDO® Prix 3) for a capacity of 20 kg (Brasil, 2009). Subsequently, homogenization and separation of a sample containing 100 diaspores was carried out, and they were individually weighed on a digital bench scale with a precision of 0.001 g (SHIMADZU®, model AY220), meanwhile the results for both estimates expressed in grams.

Length and diameter of diaspores - with the same sample of 100 diaspores used previously, biometric characterizations were performed, and the measured data were: length (considering the distance between

the base and the apex) and diameter (considering the median portion). Measurements were performed using a digital caliper with a precision of 0.01 mm (Western® PRO DC-6).

The data of each biometric characteristic were submitted to descriptive statistical analysis, and the calculated data were: lowest value, highest value, mean, variance, standard deviation, standard error of the mean and coefficient of variation, performed by the AgroEstat® statistical software. Relative frequency was performed through frequency distribution and plotted on frequency histograms using Microsoft Excel® version 2016 software.

Evaluation of temperatures and light regimes - completely randomized experimental design, 5 × 2 factorial arrangement, with five temperature conditions (constant: 25, 30 and 35 °C; alternating: 20-30 °C and 25-35 °C) and two light or photosensitivity regimes (photoperiod: 08 h day, 16 h night and no light). Four replications of 25 seeds per treatment were used, totaling 1000 seeds.

For the treatments of presence and absence of light (photosensitivity), transparent plastic boxes of the "gerbox" type and black, respectively, sized 11 × 11 × 3 cm were used. Expanded vermiculite® of medium granulometry was used as substrate, maintained at 100% of its water retention capacity. The boxes were wrapped in transparent plastic bags and placed in a Biochemical Oxygen Demand (BOD) germination chamber (ELETROLAB®, model EL202/4), with their respective temperature and photoperiod conditions for each treatment.

The germination evaluation was performed daily, and the seeds that emitted the germinative bud were considered germinated, until there was stabilization of germination for all treatments. For the treatment with total absence of light, the evaluations were carried out in a darkroom, using a flashlight covered with two sheets of green cellophane paper (Coelho et al., 2012).

To determine the water content of the seeds, two subsamples with 10 seeds each were separated, using the oven method at 105 ± 3 °C, for 24 hours (Brasil, 2009). The germination percentage was determined using the formula proposed in the Rules for Seed Analysis (Brasil, 2009). To evaluate the Germination Speed Index (GSI), the formula established by Maguire (1962) was used, based on the daily germinated seed count values.

At 52 days after sowing, the experiment was concluded, and the average length of the primary root (ALPR) and average length of the shoot (ALS) were

measured with the aid of a graduated ruler (cm), collar diameter (CD) measured using a digital caliper with precision of 0.01 mm and total dry mass of the seedlings (TDMS), which were placed in a previously identified paper bag and taken to the forced ventilation oven, regulated at 65 °C until reaching constant weight (48 h), being subsequently weighed on a precision analytical balance of 0.001 g and the results expressed in g seedlings⁻¹.

Twenty seedlings were used per treatment, five seedlings were randomly chosen per repetition, and it was measured only those considered normal, according to Brasil (2009). The seedlings of the treatment referring to the temperature of 35 °C for both light regimes were not measured, because they did not meet the pre-established criterion.

The data were submitted to the Shapiro-Wilk normality ($p > 0.05$) and Levene's homogeneity of variances tests, with the germination percentage previously transformed into arc sine $(x/100)^{1/2}$, in view of failure to meet assumptions. Then, analysis of variance was performed using the F test, and the comparison of means was performed using the Tukey test, adopting a significance level of 5% ($p \leq 0.05$), using the software statistician AgroEstat® version 1.1.0.711 (Barbosa & Maldonado Júnior, 2015).

Results and Discussion

Biometric characterization of diaspores

The weight of a thousand *P. macarthurii* diaspores was 357.58 grams (g), and one kilogram (kg) contained approximately 2,828 diaspores. Lorenzi et al. (2004) reported that the same species has approximately 6,969 diaspores in one kilogram, a value higher than that found in this research. Close value for the weight of a thousand diaspores was obtained by Rodrigues et al. (2015) with the species *Bactris marajá* Mart., 572.08 g, however the number of diaspores per kilogram was lower, 1,748. Batista et al. (2011) show that genetic and climatic factors, fruit maturation stage and water content of diaspores can interfere in the amount of seeds kg⁻¹.

The diaspores of *P. macarthurii* presented average weight, length and diameter ranging from 0.07 to

0.25 g, 10.70 to 14.40 mm and 5.0 to 6.9 mm, respectively. It is also noted that the lowest values for the coefficient of variation were obtained by the variables length in cm (6.12) and diameter in cm (7.64), thus demonstrating that there is low variability between the matrices from which the fruits were harvested. Therefore, the weight variable obtained medium variability, with a coefficient of variation of 18.83%. It was also verified that the sampling used was accurate, in view of the low standard error presented by all analyzed variables (Table 1).

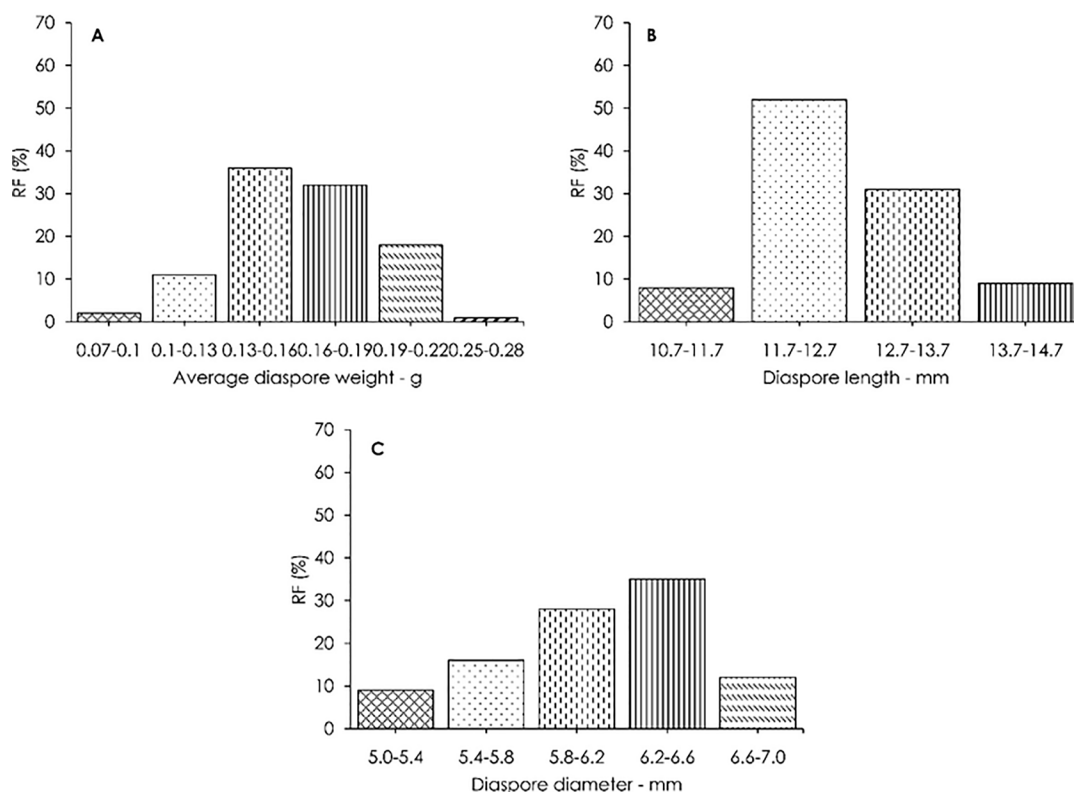
Higher values than those found in this research for diaspore weight were obtained by Rodrigues et al. (2015) evaluating *Bactris marajá* Mart., with diaspore weight ranging between 0.44 and 0.94 grams, while Felizardo et al. (2015) with the species *Oenocarpus bataua* Mart. obtained variation between 4.89 and 7.93 grams, characterizing divergence, directly influencing the evaluated parameters.

It can be seen in Figure 1A that the weight of diaspores obtained six classes, with the highest frequencies in the intervals of 0.13-0.16 g and 0.16-0.19 g, where 36% and 32% of diaspores were classified, respectively. For diaspore length (Figure 1B), the classes with the highest frequencies were between 11.7-12.7 mm (52%) and 12.7-13.7 mm (31%). Regarding the diameter of the diaspores (Figure 1C), higher frequencies were observed in the classes 5.8-6.2 mm (28%) and 6.2-6.6 mm (35%).

There is a great variability among the species of the Areacaceae family regarding the biometric characteristics of the fruits and diaspores, a fact that was observed for the species *Oenocarpus bataua* Mart. studied by Felizardo et al. (2015) and by Moura et al. (2019), who found different behavior of the length and diameter of seeds of the species *Syagrus coronata* (Mart.) Becc. For Silva et al. (2014), the variations found in the variables weight, length and diameter may occur due to environmental factors during flowering and development of fruits and seeds, as well as may serve to indicate the high genetic variability existing among the population of individuals of the species.

Table 1. Descriptive statistics of the variables weight, length and diameter of *Ptychosperma macarthurii* diaspores [(H. Wendl. ex. H.J. Veitch) H. Wendl. ex Hook.f.]. Jaboicabal, SP, 2020.

Statistical parameters	Weight (g)	Length (mm)	Diameter (mm)
Lower value	0.0703	10.70	5.00
Highest value	0.2544	14.40	6.90
Average	0.1610	12.54	6.05
Variance	0.0009	0.59	0.21
Standard deviation	0.0303	0.76	0.46
Mean standard error	0.0030	0.07	0.04
Coefficient of variation (%)	18.83	6.12	7.64



RF: relative frequency in percentage.

Figure 1. Frequency distribution for the classes of variables mean weight (A), length (B) and diameter (C) of the diaspore of *Ptychosperma macarthurii* [(H. Wendl. ex. H.J. Veitch) H. Wendl. ex Hook.f.]. Jaboticabal, SP, 2020.

Temperatures, light regimes and seedling biometry

The water content in the seeds was 41.01% and they began to germinate 21 days after installation of the experiment at constant temperatures of 30 °C and alternating 25-35 °C, both in dark conditions.

For germination percentage, there was an effect for the variables temperatures ($p < 0.01$; $F = 20.29$) and interaction between the factors (temperature x light regimes) ($p < 0.05$; $F = 3.69$), there was no effect for the light regimes alone ($p > 0.05$; $F = 0.45$). The germination process occurred for all imposed conditions and there was no significant difference between the two light regimes for germination percentage (Table 2). Regarding the evaluated temperatures, it is noted that only the temperature of 35 °C differed from the others, with the temperature of 25 °C being the one that reached the highest percentage of germination.

Regarding the germination speed index (GSI), there was a significant effect for all sources of variation analyzed, obtaining ANOVA estimates of ($p < 0.01$; $F = 67.59$) for the variable light regimes, ($p < 0.01$; $F = 68.66$) for the different temperatures and ($p < 0.01$; $F = 19.90$) for the interaction between the factors temperature x light regimes). There was a statistical difference for the

light regimes, and the condition in the absence of light had the highest GSI value. The temperatures of 30 and 35 °C were statistically different from the others, which had higher and lower values for GSI, 11.400 and 1.321, respectively.

Table 2. Comparison of means for germination percentage and germination speed index of seeds of *Ptychosperma macarthurii* [(H. Wendl. ex. H.J. Veitch) H. Wendl. ex Hook.f.], under two light regimes and five temperatures. Jaboticabal, SP, 2020.

Averages	Germination (%) ¹	Germination speed index (GSI) ²
Light	72.80 a	3.804 b
Dark	71.26 a	7.147 a
MSD (5%)	4.69	0.83
25 °C	80.95 a	3.802 c
30 °C	77.17 a	11.400 a
35 °C	51.77 b	1.321 d
20-30 °C	75.40 a	4.530 bc
25-35 °C	74.86 a	6.324 b
MSD (5%)	10.54	1.86
CV (%)	10.09	23.48

¹ Data transformed into arc sine $(x/100)^{1/2}$; ² Untransformed data;

Means followed by the same letter do not differ from each other in the column, by Tukey's, at 1% probability. MSD (%): minimum significant difference and CV (%): coefficient of variation, expressed as a percentage.

Higher temperatures increase membrane fluidity and metabolite concentration up to a threshold of protein denaturation (Zinn et al., 2010). Thus, it is assumed that

such factors may have interfered with the germinability of seeds at a temperature of 35 °C.

It is observed that even the condition in the presence of light reached the highest value for germination (%), it reached the lowest value for the GSI (Table 2). Similarly, the temperature of 25 °C reached the highest value for germination (%), however the temperature of 30 °C was the one with the highest value for GSI. It is noteworthy that for germination (%), these temperatures would not differ statistically, with differences between them only for the variable GSI. The optimal temperature for total germination is different from the optimal temperature for the speed of seed germination, because at higher temperatures the speed of water absorption and chemical reactions is greater, which justifies the seeds germinating faster (Carvalho & Nakagawa, 2012).

It is also noted that the alternating temperatures of 20-30 and 25-30 °C reached values above 70% for germination and values close to GSI, 4.530 and 6.324, respectively (Table 2). This is because a large number of species have a favorable germinal reaction to changes in temperature, due to the similarity of what happens in the environment where they develop, in which daytime temperatures are higher than nighttime temperatures (Carvalho & Nakagawa, 2012).

Melo et al. (2018) report that germination speed is a good index to measure the occupation of a species in a given environment, since rapid germination is characteristic of species whose strategy is to establish themselves in the environment as quickly as possible because it takes advantage of favorable conditions. Thus, the condition at a temperature of 30 °C provides a quick establishment for the species *P. macarthurii*, as seen in table 2.

Regarding the germination process of the seeds for the imposed light regimes, a similar behavior was observed (Table 2), in this way they can be classified as neutral photoblastic. Maekawa et al. (2010) show that the different responses to light, in the germination process, would be imposed by differences in the ability to filter sunlight, presented by the tissues that protect the seed.

Satisfactory results on the germination of palm seeds submitted to conditions of alternating temperatures were found by Almeida et al. (2018) in *Mauritia flexuosa*, 20-30 °C; Beckmann-Cavalcante et al. (2012) in *Euterpe edulis* and *Euterpe oleracea*, 20-30 and 25-35 °C and 30, 35, 20-30, 25-35 °C, respectively; Ferreira & Gentil (2017) on *Phytelephas macrocarpa*, 26-40 °C; Rodrigues et al. (2014) in *Bactris maharaja*, 26-40 °C; Viana et al. (2016b)

on *Livistona rotundifolia*, 25-35 °C.

For constant temperatures, better germination results were obtained by Aguiar et al. (2017) in *Euterpe edulis*, 25, 28 and 30 °C; Costa et al. (2018) in *Euterpe precatory*, 20 °C; José et al. (2012) In *Oenocarpus bacaba*, 30 °C; Luz et al. (2014) in *Sabal mauritiiformis*, 30°C; Masetto et al. (2012) in *Copernicia alba*, 30°C.

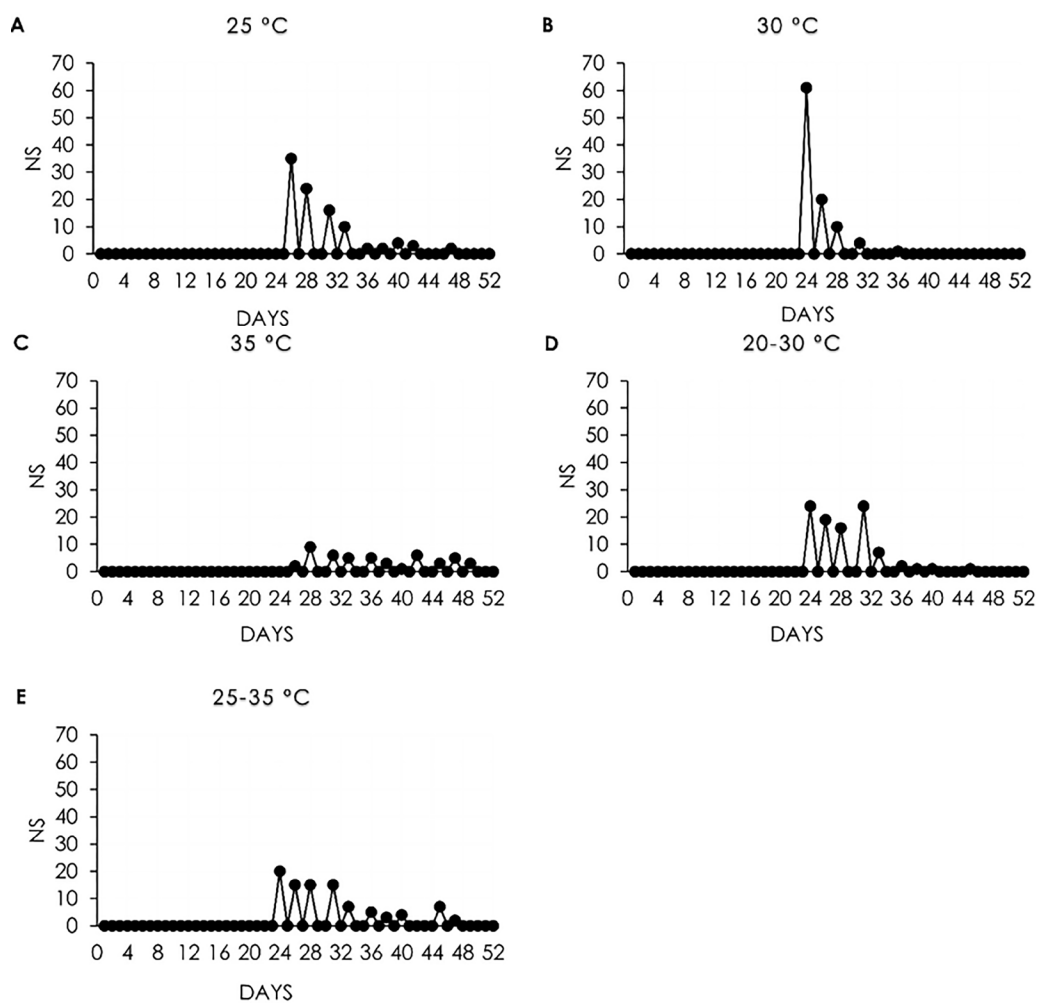
According to figures 2 and 3, it is observed that the germination of *P. macarthurii* seeds behaved heterogeneously for all temperatures in the presence and absence of light, reinforcing what was reported by Meerow & Broschat (2015), when describing that palm seeds in general have uneven and slow germination, being caused by several factors such as seed maturation stage and presence of mechanical dormancy caused by fruit structures, such as the rigid endocarp, which confer resistance to embryo expansion.

The seeds submitted to the presence of light at a constant temperature of 30 °C and alternating between 20-30 and 25-35 °C began their germination process on the 24th day after the experiment was installed and, later, on the 26th day, those submitted to constant temperatures of 25 and 35 °C. (Figure 2). The highest peak for 25 °C occurred between the 26th and 31st day, 24th and 26th day for 30 °C, 31st and 42nd day for 35 °C, 24th and 31st day for alternating temperatures of 20-30 °C and 25-35 °C.

Regarding the germination process at different temperatures and absence of light (Figure 3), germination of seeds submitted to temperatures of 30 °C and 25-35 °C started on the 22nd day, and for constant temperatures of 25 °C and 35 °C and alternating 20-30 °C occurred on the 24th day. Higher germination peaks were observed on the 31st day for 25 °C, on the 22nd day for 30 °C, on the 40th day for 35 °C, on the 24th day for alternating temperatures of 20-30 °C and 25-35 °C.

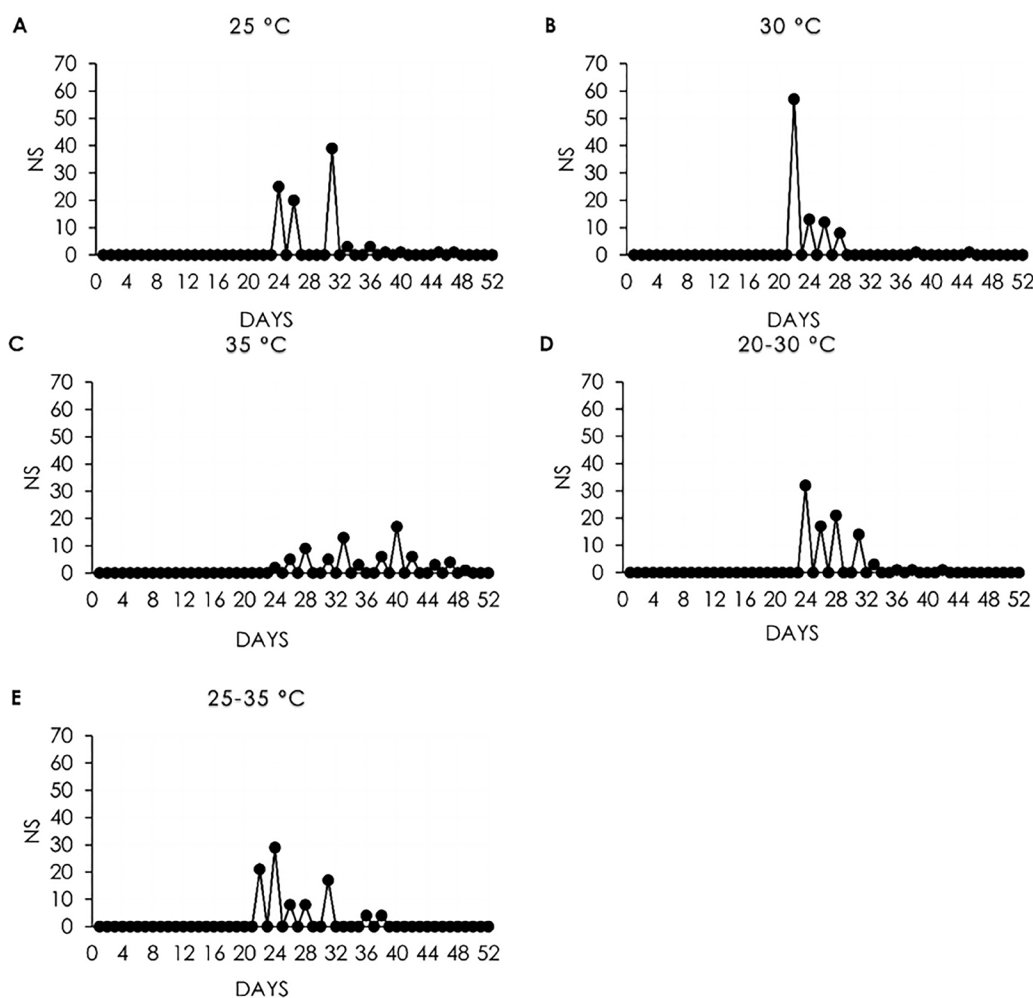
Heterogeneous germination was also observed by Aguiar et al. (2017) for *Euterpe edulis*; José et al. (2012) for *Oenocarpus bacaba*; Luz et al. (2008) for the species *Dypsis decaryi*; Luz et al. (2014) for *Sabal mauritiiformis*; Masetto et al. (2012) for *Copernicia alba*; Viana et al. (2016a) for *Livistona rotundifolia*.

According to Carvalho & Nakagawa (2012), the heterogeneity of germination within the same seed lot distributes the establishment of individuals over time and, therefore, the population survives the stages that are inappropriate for its development in the field. However, in large-scale seedling production, this characteristic becomes a barrier for the producer who seeks rapid and homogeneous germination (Shimizu et al., 2011).



NS: number of seeds germinated day⁻¹, over 52 days.

Figure 2. Distribution of seed germination of *Ptychosperma macarthurii* [(H. Wendl. ex H.J. Veitch) H. Wendl. ex Hook.f.], subjected to five temperatures and the presence of light. Jaboticabal, SP, 2020.



NS: number of seeds germinated day⁻¹, over 52 days.

Figure 3. Distribution of seed germination of *Ptychosperma macarthurii* [(H. Wendl. ex. H.J. Veitch) H. Wendl. ex Hook.f.], subjected to five temperatures and absence of light. Jaboticabal, SP, 2020.

Regarding the biometric parameters of the seedlings, there was a significant effect between the temperature factors ($p < 0.01$; $F = 214.74$) and light regimes ($p < 0.01$; $F = 85.14$) in isolation, not with significance for interaction ($p > 0.05$; $F = 2.29$) between them for average primary root length (APRL). For collar diameter (CD) there was an effect for different temperatures ($p < 0.01$; $F = 12.52$) and light regimes ($p < 0.01$; $F = 10.90$), with no significant interaction between the same ($p > 0.05$; $F = 2.20$). For average shoot length (ASL) there was an effect ($p < 0.01$; $F = 37.53$) only for the different temperatures. As for the total dry mass (TDM) there was a significant effect for the different temperatures ($p < 0.01$; $F = 73.43$) and interaction between the factors (temperature x light regimes) ($p < 0.05$; $F = 3.18$), with no effect for the light regimes in isolation ($p > 0.05$; $F = 1.40$).

It is possible to notice that there was no statistical difference for APRL and TDM in relation to the light regimes (Table 3). A difference is observed for ASL, where the condition in the absence of light (continuous dark)

provided greater root length, which is explained by its ability to explore the deeper layers in search of water and nutrients. For CD, there was also a significant difference, however, the presence of light was the condition that provided the highest mean value.

Still in table 3, it is possible to observe that the constant temperature of 30 °C provided higher mean values for all the characteristics evaluated. It is important to emphasize that the constant temperature of 30 °C provided a higher GSI, thus having a direct relationship between GSI and the evaluated biometric characteristics.

For the PRL characteristic, there is a statistical difference for both temperature conditions under study, with the alternating temperature of 25-35 °C providing the lowest average length for the roots. For TDM, lower biomass accumulation was obtained by temperatures, constant at 25 °C and alternating at 25-35 °C (Table 3). Regarding the PRL and CD characteristics, the lowest average was obtained by the constant temperature of 25 °C.

Table 3. Comparison of means for mean primary root length (PRL), mean shoot length (MSL), collar diameter (CD) and total dry mass (TDM) of *Ptychosperma macarthurii* seedlings [(H. Wendl. ex HJ Veitch) H. Wendl. ex Hook.f.], subjected to different light and temperature regimes. Jaboticabal, SP, 2020.

Averages	PRL (cm)	MSL (cm)	CD (mm)	TDM (g seedlings ⁻¹)
Light	3.77 b	1.23 a	1.32 a	0.0149 a
Dark	488 a	1.23 a	1.21 b	0.0143 a
MSD (5%)	0.23	0.13	0.06	0.00
25 °C	3.95 c	0.92 c	1.16 c	0.0116 c
30 °C	6.50 a	1.81 a	1.41 a	0.0198 a
20-30 °C	4.61 b	1.19 b	1.30 ab	0.0164 b
25-35 °C	2.24 d	0.99 bc	1.19 bc	0.0105 c
MSD (5%)	0.44	0.24	0.11	0.00
CV (%)	17.56	34.05	16.23	21.86

Means followed by the same letter do not differ from each other in the column, by Tukey's, at 1% probability. MSD (%): minimum significant difference and CV (%): coefficient of variation, expressed as a percentage.

Temperature is also critical for the development of the seedling's initial structures, such as the primary root, and plays a crucial role in the seedling establishment process (Oliveira et al., 2014), with inadequate temperatures directly affecting the root, which is in a rapid process of cell division and any adverse environmental factor can lead to a decrease in development capacity (Larcher, 2003). Thus, the alternating temperature of 25-35 °C negatively affected the development of the root system, as well as the accumulation of biomass, as observed in the present study.

The highest value for shoot growth achieved by the constant temperature of 30 °C can be explained by the fact that it is considered optimal for cell division for most species (Ferreira & Borghetti, 2004). Regarding the diameter of the collar, a higher value was observed in the presence of light and this may indicate that there is a greater availability of photoassimilates mobilized by the shoot, that is, greater gain in carbon with positive effects on growth (Chiamolera et al., 2011).), however, under conditions of reduced light availability, the production of adenosine triphosphate necessary for biosynthesis and carbohydrate incorporation may be incipient (Shao et al., 2014), causing reduced plant growth (Taiz & Zeiger, 2017), noticed by the statistical difference between the light regimes for the CD characteristic.

Based on the characteristics evaluated, it is noteworthy that the species under study has the ability to settle in regions with different temperature ranges and light conditions, however, such attributes directly affect its germination process and initial development of seedlings.

Conclusions

There was no single representative class for the biometric variables of diaspores, with the average length, diameter and weight of 12.54 mm, 6.05 mm and 0.1610 g, respectively.

The seeds germinated in a wide temperature

range, and it is possible to obtain a higher percentage of germination at 25 °C and a higher germination speed index at 30 °C, so it can be classified according to the light stimulus as neutral photoblastic.

The constant temperature of 30 °C provided higher mean values for the biometric characteristics of the seedlings.

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