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Optimal conditions for germination of seeds of *Epiphyllum oxypetalum*

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Queen of the night (*Epiphyllum oxypetalum*), an ornamental cactus, is widespread in many countries. The demand for this species is increasing because esthetic quality of its flowers is appreciated. This study aimed to analyze the influence of temperature, substrate and luminosity on the germination of *E. oxypetalum* seeds. A completely randomized design with four replications was used in a 3 x 2 x 2 factorial scheme, corresponding to three temperatures (20, 25 and 30°C), two substrates (blotting paper and sand of average particle size) and two luminosity conditions (light and dark). The percentage germination, germination speed index (GSI) and mean germination time (MGT) were evaluated. The 20°C treatment combined with the sand substrate and the presence of light provided favorable conditions for the germination of the seeds of *E. oxypetalum*, yielding a higher GSI and a shorter MGT; however, the species may be considered as preferentially photoblastic because of its ability to germinate in darkness.

Key words: Cactaceae, luminosity, ornamental plant, substrate, temperature, vigor.

INTRODUCTION

The family Cactaceae (eudicotyledon) comprises between 120 and 200 genera and between 1,500 and 2,000 species, of which approximately 40 genera and 200 species occur in Brazil; many of these species are used as ornamental plants (Souza and Lorenzi, 2012). Queen of the night [*Epiphyllum oxypetalum* (A.P. de Candolle) Haworth] is an ornamental plant that is native to Mexico, widely cultivated in the tropics and distributed in Mexico, Guatemala, Honduras, Nicaragua, El Salvador, Costa Rica, Venezuela and Brazil (Anderson, 2001).

The genus *Epiphyllum* comprises epiphytic cacti that thrive in shaded environments and acid substrates (Mace and Mace, 2009). The species *E. oxypetalum* is a robust and branched plant with a cylindrical primary branch (2 to 3 m long). The secondary branches, which are approximately 30 cm in length and between 10 and 12 cm in width, are flattened, elliptical, thin, marginally cut, wavy and leafy in appearance. The flowers are aromatic with nocturnal anthesis, 25 to 30 cm long and 12 to 17 cm in diameter, and have a perianth that is externally reddish and white on the inside. The buds begin to form

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in early spring and bloom in the fall (Anderson, 2001).

Because of its ease of cultivation and attractiveness stemming from the abundance and size of its flowers, the demand for this species is increasing. Research indicates that the stem of *E. oxypetalum* has a broad spectrum of activity against pathogenic bacteria and a high nutritional value and may thus be employed for medical and nutritional purposes (Upendra and Khandelwal, 2012).

The temperature, substrate and lighting conditions are of significant importance for germination and can be manipulated to optimize the percentage, speed and uniformity of germination, resulting in vigorous seedlings and reduced production costs (Pacheco et al. 2006).

Temperature acts as an inducer of germination and plant development and may change the speed of water absorption and chemical reactions that trigger the unfolding, reserve transportation and synthesis of substances for the seedling (Coelho et al., 2008). The optimum temperature interval for most tropical species is between 15 and 30°C, wherein the seed expresses its maximum potential for germination in the shortest time, whereas higher or lower temperatures tend to inhibit this process. The best germination temperatures are not sharply defined, but critical points can be identified, such as the maximum and minimum values that hinder the process (Stefanello et al., 2006; Renner et al., 2007; Carvalho and Nakagawa, 2012).

The cardinal temperatures for seed germination help explain the biogeographic peculiarities of Neotropical species (Borghetti, 2005). The study of these temperatures is important to provide useful information on seed technology and for understanding the physiological ecology of plant species (Ferreira and Borghetti, 2004).

The substrate also affects germination because the structure, aeration, water retention capacity, degree of infestation by pathogens and other factors may vary according to the type of material used (Popinigis, 1985). According to Abreu et al. (2005), the substrate is a factor that affects both the speed and percentage of germination; thus, in choosing the substrate, the size of the seed, the moisture and light requirements and the effort required to install and evaluate the seedlings must be considered.

Species have different luminosity demands because light is required for the germination of seeds (positively photoblastic seeds) in some species, inhibits the germination process in other (negatively photoblastic seeds) and, in some species, germination occurs either with or without light because the seeds are insensitive to this factor (neutrally photoblastic) (Ferreira and Borghetti, 2004; Yamashita, 2008).

Temperature and substrate are basic components for conducting germination tests (Stockman et al., 2007). As the seeds present variable physiological responses upon exposure to different temperatures, substrates and luminosities, it is recommended that the influence of these components on the germination of each species of interest be studied, thus providing support for the analysis of these influences (Guedes et al., 2009). Therefore, this study aimed to analyze the influence of temperature, substrate and luminosity on the germination of *E. oxypetalum* seeds.

MATERIALS AND METHODS

The experiment was conducted at the Seed Laboratory of the Londrina State University (Universidade Estadual de Londrina - UEL), which is located in the northern part of the State of Paraná, Brazil (23°23'S and 51°11'W). Queen of night (*E. oxypetalum*) seeds from mature fruits produced in the experimental area of the Department of Agronomy of UEL were used. The fruits were manually pulped with a spoon, and the pulp with the seeds was transferred to a 2 L beaker containing an aqueous sugar (25 g L^{-1}) solution. This mixture was left for 48 h at room temperature to encourage fermentation. After 2 days of fermentation, the pulp-seed mixture was placed in a fine-mesh sieve and washed in running water to remove the mucilage from the seeds, which were placed on filter paper to dry for 48 h in the shade at room temperature.

For the germination study, three temperatures (20, 25 and 30°C), two substrates (blotting paper and sand of average grain size) and two luminosity conditions (light and dark) were used in a completely randomized 3 x 2 x 2 factorial design with four replications. The substrates were placed in covered crystal polystyrene boxes with lids (L11 x l11 x h3 cm) that each contained 50 seeds and constituted one replicate. For the treatment with the paper substrate, a sheet of white blotting paper (10.5 x 10.5 cm, 250 mg) moistened with distilled water (2.5 times the dry weight of the paper) was used in each box (Brazil, 2009); for the treatment with the sand substrate, each box contained 200 g of sand (particles size of 0.25 to 0.50 mm) and 50 ml of distilled water. The chemical properties of the sand are pH($CaCl_2$) = 6.7; Al^{3+} = 0.0 $cmol_c\ dm^{-3}$; (H+Al) = 1.51 $cmol_c\ dm^{-3}$; Ca^{2+} = 0.34 $cmol_c\ dm^{-3}$; Mg^{2+} = 0.16 $cmol_c\ dm^{-3}$; K^+ = 0.005 $cmol_c\ dm^{-3}$; $CTC_{pH=7.0}$ = 2.015 $cmol_c\ dm^{-3}$.

The treatments subject to darkness were packed in plastic boxes, which were wrapped in foil and black plastic; whereas the other were maintained in constant light, using a fluorescent lamps of the photon flux density of 9.84 µmol $m^{-2}\ s^{-1}$ (±0.69 of standard deviation), according to the average of 10 measurements. The treatments subject to darkness were evaluated in a dark room with the aid of green lighting, using a lamps of the intensity of quantum irradiation of 1.07 µmol $m^{-2}\ s^{-1}$ (±0.13 of standard deviation), according to the average of 10 measurements, at a distance of 47 cm from the sensor and light source. Each replicate was always maintained in germinators at the 3 chosen temperatures.

The treatments were evaluated daily up to stabilization of germination, 26 days in this experiment. The seeds with a root length equal to or greater than 2 mm were considered to have germinated. The evaluated variables were the percentage of the seeds that germinated (G), the germination speed index (GSI), calculated according to Maguire's formula (1962), and the mean germination time (MGT) in days, calculated according to Lima et al. (2006). The data were subjected to analysis of variance, and the means were compared by Tukey's test.

RESULTS AND DISCUSSION

The three-way interaction among the temperature, substrate and luminosity factors was statistically significant for each of the analyzed variables (Table 1). In

Table 1. Germination (G), germination speed index (GSI) and mean germination time (MGT) of *E. oxypetalum* seeds subjected to different temperature, substrate and luminosity conditions.

Source of variation	Variable		
	G (%)	GSI	MGT (d)
Temperature (T)			
20°C	99.25[a]	16.65[a]	7.01[c]
25°C	91.37[b]	13.72[b]	8.52[b]
30°C	79.75[c]	11.87[c]	12.29[a]
Substrate (S)			
Sand	92.83[a]	15.47[a]	8.68[b]
Paper	87.42[b]	12.69[b]	9.87[a]
Luminosity conditions (L)			
Light	98.50[a]	20.72[a]	5.05[b]
Dark	81.75[b]	7.44[b]	13.50[a]
F Value			
Temperature (T)	35.37*	73.06*	522.31*
Substrate (S)	8.09*	73.35*	75.79*
Luminosity conditions (L)	77.35*	1668.27*	3788.15*
T*S	2.44[ns]	0.70[ns]	3.47*
T*L	28.30*	31.21*	420.23*
S*L	15.16*	3.23[ns]	1.90[ns]
T*S*L	2.74**	7.12*	21.60*
CV (%)	7.32	8.00	5.13

[ns] Not significant, * Significant at $p < 0.05$, ** Significant at $p < 0.07$.

Tables 2, 3 and 4, the interaction of the various factors (temperature, substrate and luminosity) is shown for G. The effect of the temperature factor on G is shown in Table 2 at each level of the substrate and luminosity factors. For the sand substrate, the 20 and 25°C treatments produced better G than the 30°C treatment in the dark, but no significant difference occurred among the temperature treatments in the light. For the paper substrate, the best and worst G occurred in darkness at 20 and 30°C, respectively, but no differences occurred in the light. For Rojas-Aréchiga and Vázquez-Yanes (2000), the optimum temperature interval to get over 75% germination for some cactus species is between 15 and 35°C, but 20°C provide appropriate conditions for germination rate in a wide range of genera. However, the optimum temperature for most cactus seed germination is 25°C (Rojas-Aréchiga and Vázquez-Yanes, 2000; Nobel, 2003). Other studies show that both 20 and 25°C are favorable for certain species, since the better performance was observed at 25°C in two cactaceous species, *Melocactus bahiensis* (Britton and Rose) and *Schlumbergera truncate* (Haw.) Moran (Lone et al., 2007, 2010). By contrast, Almeida (2008) evaluated the germination of *Cereus fernambucensis* Lem., other cactaceous species, and found that 20°C provided the best germination, result that agrees with those obtained in the present study (Table 2).

The substrate factor effect at each level of the luminosity and temperature factors is shown in Table 3. The paper substrate showed higher germination in the light at 30°C, with no significant difference between substrates at the other temperatures. In the dark, the sand substrate showed higher germination when subjected to 25 and 30°C, but there was no difference at 20°C. Lone et al. (2007), who examined the seeds of *M. bahiensis*, observed greater germination using sand as the substrate. According to Cavalcanti and Resende (2007), sand is the standard substrate for the germination of several species of cacti. However, Andrade et al. (2008), who worked with the seed germination of the cactus *Hylocereus undatus* (Haworth) Britton and Rose on different substrates, obtained better results with paper than with sand.

Varela et al. (2005), who studied the influence of temperature (20, 25, 30 and 35°C) and substrate (sand, paper and vermiculite) on the seeds of *Acosmium nitens* (Vog) Yakovlev, found greater germination for the sand substrate at temperatures of 20, 25 and 30°C.

In Table 4, the luminosity factor effects on G are shown at each level of the substrate and temperature factors. In

Table 2. Temperature factor effect on the percentage of *E. oxypetalum* seeds germinating (G) at each level of the substrate and luminosity factors.

Temperature	Sand		Paper	
	Light	Dark	Light	Dark
20°C	98.5[a]	100.0[a]	100.0[a]	98.5[a]
25°C	98.0[a]	95.0[a]	100.0[a]	72.5[b]
30°C	96.0[a]	69.5[b]	98.5[a]	55.0[c]

Means within columns followed by the same letter do not differ by Tukey's test at $p < 0.07$.

Table 3. Substrate factor effect on the percentage of *E. oxypetalum* seeds germinating (G) at each level of the luminosity and temperature factors.

Substrate	Light			Dark		
	20°C	25°C	30°C	20°C	25°C	30°C
Sand	98.5[a]	98.0[a]	96.0[b]	100.0[a]	95.0[a]	69.5[a]
Paper	100.0[a]	100.0[a]	98.5[a]	98.5[a]	72.5[b]	55.0[b]

Means within columns followed by the same letter do not differ by Tukey's test at $p < 0.07$.

Table 4. Luminosity factor effects on the percentage of *E. oxypetalum* seeds germinating (G) at each level of the substrate and temperature factors.

Luminosity	Sand			Paper		
	20°C	25°C	30°C	20°C	25°C	30°C
Light	98.5[a]	98.0[a]	96.0[a]	100.0[a]	100.0[a]	98.5[a]
Dark	100.0[a]	95.0[a]	69.5[b]	98.5[a]	72.5[b]	55.0[b]

Means within columns followed by the same letter do not differ by Tukey's test at $p < 0.07$.

the sand, G was greater in the light than in the dark for seeds held at 30°C, but there were no luminosity effects at the lower temperatures. However, light improved G on the paper at both 25 and 30°C compared with darkness, with no significant luminosity effect on the paper at 20°C. Previous studies with *E. phyllanthus* (L.) Haworth seeds also demonstrated their sensitivity to light, which promotes their germination (Simão et al., 2010).

A total germination was observed both in the presence and absence of light. Similar results were obtained by Cotá-Sanchez and Abreu (2007), who obtained values greater than 90% in studies on the germination of *E. phyllanthus*. Thus, it is possible to achieve high germination percentages by adjusting the temperature, substrate and luminosity according to the needs of each species. Light has been recognized as necessary for the germination of many species and is considered by some authors to be a factor overcoming seed dormancy (Bewley and Black, 1994; Baskin and Baskin, 2001). Positive photoblastism is a mechanism for the preservation of species that prevents germination at great depths, where seedling emergence would not succeed because of limited nutritional reserves (Bewley and Black, 1994). The seeds of *E. oxypetalum* were able to reach the peak of germination both in the dark and the light; however, because of its lower percentage of germination in darkness, the species displays a preferential photoblastism (Table 4).

In Tables 5, 6 and 7, the interaction of the various factors (temperature, substrate and luminosity) is shown for the GSI. The analysis of the effect of temperature at each level of the luminosity and substrate factors is presented in Table 5. The highest GSI occurred at 20°C for the seeds germinated on the paper substrate, both in the light and in darkness, but only in darkness in the sand substrate. However, in the sand substrate, both 20 and 30°C in the presence of light provided higher GSIs.

According to Bewley and Black (1994); Carvalho and Nakagawa (2012), the higher the temperature (up to a certain limit), the faster and more efficient the germination process. Lone et al. (2007) obtained a higher GSI at 25°C for *M. bahiensis*, and Almeida (2008), evaluating the influence of temperature and luminosity on the germination of *Himatanthus drasticus* (Mart.) Plumel

Table 5. Temperature factor effect on the germination speed index (GSI) of *E. oxypetalum* seeds at each level of the substrate and luminosity factors.

Temperature	Sand		Paper	
	Light	Dark	Light	Dark
20°C	23.49[a]	12.05[a]	21.16[a]	9.88[a]
25°C	20.69[b]	9.73[b]	18.31[b]	6.14[b]
30°C	23.02[a]	3.84[c]	17.63[b]	3.00[c]

Means within columns followed by the same letter do not differ by Tukey's test at $p < 0.05$.

Table 6. Substrate factor effects on the germination speed index (GSI) of *E. oxypetalum* seeds at each level of the luminosity and temperature factors.

Substrate	Light			Dark		
	20°C	25°C	30°C	20°C	25°C	30°C
Sand	23.49[a]	20.69[a]	23.02[a]	12.05[a]	9.73[a]	3.84[a]
Paper	21.16[b]	18.31[b]	17.63[b]	9.88[b]	6.14[b]	3.00[a]

Means within columns followed by the same letter do not differ by Tukey's test at $p < 0.05$.

Table 7. Luminosity factor effects on the germination speed index (GSI) of *E. oxypetalum* seeds at each level of the substrate and temperature factors.

Luminosity	Sand			Paper		
	20°C	25°C	30°C	20°C	25°C	30°C
Light	23.49[a]	20.69[a]	23.02[a]	21.16[a]	16.31[a]	17.63[a]
Dark	12.05[b]	9.73[b]	3.84[b]	9.88[b]	6.14[b]	3.00[b]

Means followed within columns by the same letter do not differ by Tukey's test at $p < 0.05$.

seeds, observed that 25 and 30°C in darkness promoted a higher GSI, which differed from the results obtained for the species under study here (Table 5). Temperature variations affect the percentage, speed and uniformity of germination. Therefore, the optimum temperature is that which allows the most efficient combination of the percentage and speed of germination (Marcos Filho, 2005), which was 20°C in the present study.

The analysis of the substrate factor at each level of luminosity and temperature is shown in Table 6. The sand substrate showed a higher GSI than the paper at the evaluated temperatures both in the presence and absence of light, but the difference was not statistically significant at 30°C in darkness. Iossi et al (2003), evaluating the effect of substrate and temperatures in the germination of *Phoenix roebelenii* O'Brien seeds, had a higher GSI at 30°C using sphagnum or sand.

In Table 7, the luminosity factor effects on the GSI are shown at each level of the substrate and temperature factors. Light produced a higher GSI than darkness regardless of the evaluated temperature and substrate. For *Pimpinella anisum* L. seeds, both in the light and in darkness, the highest GSIs occurred at temperatures of 20 and 25°C (Stefanello, 2005).

In Tables 8, 9 and 10, the interaction of the various factors (temperature, substrate and luminosity) is shown for the MGT. Table 8 presents the analysis of the temperature factor effects at each level of the substrate and luminosity factors. The 20°C treatment produced a shorter MGT under the evaluated luminosity and substrate conditions, although the difference was not statistically significant in the sand substrate in light.

While evaluating the influence of temperature, luminosity and substrate on the germination of *Caesalpinia echinata* Lam. seeds, Mello and Barbedo (2007) observed that the MGT tended to decrease in both light and darkness from 15 to 35°C; thus, the seeds proved indifferent to luminosity for germination. According to Almeida (2008) and Lone et al. (2007), 25°C for *C. fernambucensis* and 30°C for *M. bahiensis* caused the fastest germination.

The analysis of the substrate factor effects at each level of the luminosity and temperature factors is shown in Table 9. The sand substrate provided the shortest

Table 8. Temperature factor effects on the mean germination time (MGT) of *E. oxypetalum* seeds at each level of the substrate and luminosity factors.

Temperature	Sand		Paper	
	Light	Dark	Light	Dark
20°C	4.33[a]	8.51[c]	4.80[b]	10.40[c]
25°C	4.93[a]	10.46[b]	5.64[a]	13.05[b]
30°C	4.36[a]	19.46[a]	6.20[a]	19.14[a]

Means within columns followed by the same letter do not differ by Tukey's test at $p < 0.05$.

Table 9. Substrate factor effects on the mean germination time (MGT) of *E. oxypetalum* seeds at each level of the luminosity and temperature factors.

Substrate	Light			Dark		
	20°C	25°C	30°C	20°C	25°C	30°C
Sand	4.33[a]	4.93[b]	4.36[b]	8.51[b]	10.46[b]	19.46[a]
Paper	4.80[a]	5.64[a]	6.20[a]	10.40[a]	13.05[a]	19.14[a]

Means within columns followed by the same letter do not differ by Tukey's test at $p < 0.05$.

Table 10. Luminosity factor effects on the mean germination time (MGT) of *E. oxypetalum* seeds at each level of the substrate and temperature factors.

Luminosity	Sand			Paper		
	20°C	25°C	30°C	20°C	25°C	30°C
Light	4.33[b]	4.93[b]	4.36[b]	4.80[b]	5.64[b]	6.20[b]
Dark	8.51[a]	10.46[a]	19.46[a]	10.40[a]	13.05[a]	19.14[a]

Means within columns followed by the same letter do not differ by Tukey's test at $p < 0.05$.

MGT in the presence of light at temperatures of 25 and 30°C, but did not differ from the paper substrate at 20°C. In darkness at 20 and 25°C, the sand substrate provided better performance than the paper, without differing at 30°C.

In Table 10, the luminosity factor effects are shown at each level of the substrate and temperature factors. Germination in light provided the shortest MGT for the evaluated temperatures and substrates. However, Amaro et al. (2006) observed that 25 and 30°C in darkness reduced the MGT in *H. drasticus*.

Studies such as this verify the importance of determine the temperature, substrate and luminosity factors and provide an account of the influence of these factors on the percentage, speed and uniformity of seed germination. So, the results indicated that the germination of *E. oxypetalum* seeds is favored by 20°C, a sand substrate and the presence of light; however, the species may be considered as preferentially photoblastic because of its ability to germinate in darkness.

Conclusion

The temperature of 20°C combined with the sand substrate and the presence of light provided optimal conditions for the germination of *E. oxypetalum* seeds measured by a higher GSI and a shorter MGT.

Conflict of Interest

The authors have not declared any conflict of interest.

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