

The effects of light wavelength and intensity on the germination of pitaya seed genotypes

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Abstract

Pitaya is the name given to the fruits of several climbing cactus plants (Cactaceae), some of which are potentially suitable for commercialization. Studies that focus on various factors, such as light, that affect germination are necessary to understand the germination process of pitaya genotypes. The aim of this study was to evaluate the germination of the seeds of five pitaya genotypes under light of different wavelengths and intensities. The genotypes studied were *Hylocereus undatus* (HU), *H. Polyrrhizus* (HP), *Selenicereus megalanthus* (SM), *H. undatus* x *H. costaricensis* (PH1) and *H. costaricensis* x *H. undatus* (PH2). The wavelengths tested were red (R), far-red and white light, at intensities of 3.4, 10.1 and 17.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The absence of light was also tested. Each treatment consisted of four replications of 50 seeds of each genotype. The variables evaluated were germination percentage, germination speed index and average germination time. All genotypes germinated in the dark, but light produced favorable results in the germination percentages of SM, PH1 and PH2. Light wavelength did not affect the germination percentage of the *Hylocereus* genotypes. Light intensity was favorable for the germination speed index and average germination time. Intensities of 17.9 and 10.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were the most suitable for germinating HU, HP and PH1, while 17.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was most suitable for SM and 17.9, 10.1 and 3.4 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were suitable for PH2. All light wavelengths were favorable for germinating HU and PH2.

Keywords: Cactaceae; Dragon fruit; Germination test; *Hylocereus*; *Selenicereus*.

Abbreviations: AGT_average germination time; GSI_germination speed index; HU_*Hylocereus undatus*; HP_*Hylocereus polyrrhizus*; Pfr_far-red phytochrome; PH1_*Hylocereus undatus* x *Hylocereus costaricensis*; PH2_*Hylocereus costaricensis* x *Hylocereus undatus*; SM_*Selenicereus megalanthus*; HC_*Hylocereus costaricensis*.

Introduction

Pitaya is the name given to the fruits of various climbing cactus plants that contain small digestible seeds and can have either thorny or non-thorny skins, which can easily be removed during maturation (Nerd 2002). Some species, including HU (oblong fruit with red skin and white flesh), HP (oblong fruit with red skin and red flesh), HC (globular fruit with red skin and red flesh) and SM (oblong fruit with yellow skin and white flesh), are suitable for commercialization (Mizrahi et al. 1997; Nerd 2002). Pitayas can be propagated either vegetatively or from seed. Growth from seed is the preferred method for breeding programs because materials with different genetic information can be obtained, allowing their various characteristics to be exploited. Therefore, plants produced by sexual propagation exhibit wide variability, allowing the selection of offspring with desirable characteristics, such as productivity, external appearance, flesh color and suitability for different climatic conditions (Andrade et al. 2008). The environmental factors most commonly known to regulate germination are the absence or presence of light, light wavelength, temperature and temperature changes, and the concentration of gases and water around the diaspore (Ferreira et al. 2001). Light is necessary for seed germination in some species, which are identified as positively photoblastic. Negatively photoblastic species germinate better in limited light; the germination of

light-insensitive species is not affected by light. The seeds of most cultivated plants germinate in both the presence and absence of light, although non-photoblastic seeds may require light under unfavorable environmental conditions. The classification of seeds in terms of light sensitivity is an important factor in germination testing (Villiers 1972; Mayer and Poljakoff-Mayber 1989). A seed's sensitivity to light varies according to the properties of the light, its intensity and the irradiation time, as well as the soaking time and temperature (Toole 1973; Labouriau 1983). Plants detect the properties of light with phytochromes, a class of pigments that consists of five distinct forms. Phytochrome B is responsible for detecting the R: FR ratio (Red:Far-Red, ratio between the 655-665 nm and 725-735 wavelengths) and rendering seed germination (Takaki 2001). According to the Brazilian Seed Analysis Rules, the intensity of light that is necessary to satisfy the requirements of all light-sensitive seeds must be between 10.1 and 17.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and for seeds not sensitive to light, the intensity can be 3.4 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Brazilian Ministry of Agriculture and Agrarian Reform 1992, 2009). The aim of this study was to evaluate the germination of the seeds of five genotypes of pitaya at different light wavelengths and intensities, to elucidate the effect of light on pitaya seed germination.

Table 1. The germination percentages for pitaya genotypes at different light intensities ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and wavelengths (white, red – R - and far-red - FR).

Genotype ^x	Intensity	White light	R	FR	Averages
HU	17.9	90 ^{ns}	87 ^{ns}	87 ^{ns}	88 ^{ns}
	10.1	86	89	88	88
	3.4	87	86	88	87
	Darkness	85	85	85	85
	Averages	87	86	87	
	CV (%)	5.11			
HP		White light	R	FR	Averages
	17.9	91 ^{ns}	88 ^{ns}	85 ^{ns}	88 ^{ns}
	10.1	93	88	86	89
	3.4	94	86	89	90
	Darkness	90	90	90	90
	Averages	92 ^{ns}	88	88	
	CV (%)	8.3			
SM		White light	R	FR	Averages
	17.9	89 ^{Aay}	81 ^{Aab}	76 ^{Bb}	82 ^A
	10.1	89 ^{Aa}	82 ^{Aa}	90 ^{Aa}	87 ^A
	3.4	88 ^{Aa}	77 ^{Ab}	81 ^{ABab}	82 ^A
	Darkness	30 ^B	30 ^B	30 ^C	30 ^B
	Averages	74 ^a	67 ^b	69 ^{ab}	
	CV (%)	8.49			
PH1		White light	R	FR	Averages
	17.9	67 ^{Aa2}	71 ^{Aa}	71 ^{Aa}	70 ^{AB}
	10.1	77 ^{Aa}	73 ^{Aa}	75 ^{Aa}	75 ^A
	3.4	68 ^{Aa}	64 ^{Aa}	73 ^{Aa}	69 ^B
	Darkness	67 ^A	67 ^A	67 ^A	67 ^B
	Averages	69 ^a	69 ^a	72 ^a	
	CV (%)	7.53			
PH2		White light	R	FR	Averages
	17.9	98 ^{Aa2}	95 ^{Aa}	97 ^{Aa}	97 ^A
	10.1	96 ^{ABa}	96 ^{Aa}	97 ^{Aa}	97 ^A
	3.4	96 ^{ABa}	96 ^{Aa}	95 ^{ABa}	96 ^A
	Darkness	93 ^B	93 ^A	93 ^B	93 ^B
	Averages	96 ^a	95 ^a	96 ^a	
	CV (%) ^z	2.46			

^xHU: *H. undatus*; HP: *H. polyrhizus*; SM: *S. megalanthus*; PH1: *H. undatus* X *H. costaricensis*; PH2: *H. costaricensis* X *H. undatus*. ^yAverages followed by the same uppercase letter in the column and lowercase letter in the row did not differ in the Tukey test ($p \leq 0.05$). ^{ns}Not significant. ^zCoefficient of variation.

Results

Germination percentage

The seeds of all genotypes germinated in the dark, but the presence of light, even at low intensity, boosted the germination percentage for SM, PH1 and PH2. The wavelength of light did not affect the germination percentage of *Hylocereus* (HU, HP, PH1 and PH2). One illumination effect was observed to affect the germination of SM only: white light produced better results than red, and for far-red, an intensity of $10.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ was better than $17.9 \mu\text{mol m}^{-2} \text{s}^{-1}$. Additionally, the separate analysis of the effects of intensity at the different wavelengths showed that light intensity did not affect the germination of the PH1 genotype (Table 1).

Germination speed index

In terms of the GSI, the three intensities of light tested exhibited higher averages by comparison with a dark environment for HP and PH2. For the other genotypes, intensities of 17.9 and $10.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ were better than $3.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ and darkness. Only SM showed any significant

difference with respect to wavelength, exhibiting an average higher than that obtained for white light (Table 2). Upon analyzing the GSI values for each intensity, the highest values were found for 17.9 and $10.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ for all genotypes, with the exception of PH2 under white light. Under red light, all intensities were better than darkness for all genotypes, and a similar result applies for far-red light with the HP, PH1 and PH2 genotypes (Table 2).

Average germination time

For the AGT, light intensities of 17.9 and $10.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ were favorable for HU, HP and PH1. For SM, $17.9 \mu\text{mol m}^{-2} \text{s}^{-1}$ was favorable, while for PH2, all light intensities were better than darkness. No significant difference was observed for the HU and PH2 genotypes in terms of wavelength. For HP and PH1, far-red produced better results than white light, and for SM, white light was better than far-red (Table 3). Under white light, intensities of 17.9 and $10.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ were favorable for all genotypes, but $3.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ was also favorable for SM and PH2. Under red light, all intensities were better than darkness for all genotypes. A similar result was obtained under far-red light for HP, PH1 and PH2 (Table 3).

Table 2. The germination speed indexes (GSIs) for pitaya genotypes at different light intensities ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and wavelengths (white, red - R - and far-red – FR - light).

Genotype ^x	Intensity	White light	R	FR	Averages
HU	17.9	12.25 ^{Aay}	10.73 ^{ABb}	12.3 ^{Aa}	11.76 ^A
	10.1	11.81 ^{Aa}	11.65 ^{Aa}	11.88 ^{Aa}	11.78 ^A
	3.4	10.47 ^{Ba}	10.78 ^{ABa}	10.19 ^{Ba}	10.48 ^B
	Darkness	9.98 ^B	9.98 ^B	9.98 ^B	9.98 ^B
	Averages	11.13 ^a	10.79 ^a	11.09 ^a	
CV (%)	6.34				
HP		White light	R	FR	Averages
	17.9	12.94 ^{ABa2}	12.44 ^{Aa}	12.08 ^{Aa}	12.49 ^A
	10.1	13.37 ^{Aa}	11.94 ^{Aa}	12.52 ^{Aa}	12.61 ^A
	3.4	11.19 ^{BCa}	11.36 ^{ABa}	12.52 ^{Aa}	11.69 ^A
	Darkness	9.74 ^C	9.74 ^B	9.74 ^B	9.74 ^B
Averages	11.81 ^a	11.37 ^a	11.72 ^a		
CV (%)	9.15				
SM		White light	R	FR	Averages
	17.9	7.59 ^{ABa2}	6.73 ^{Aa}	6.94 ^{Aa}	7.09 ^A
	10.1	8.18 ^{Aa}	6.19 ^{Ab}	6.14 ^{Ab}	6.84 ^A
	3.4	6.61 ^{Ba}	6.19 ^{Aa}	4.73 ^{Bb}	5.84 ^B
	Darkness	1.54 ^C	1.54 ^B	1.54 ^C	1.54 ^C
Averages	5.98 ^a	5.16 ^b	4.84 ^b		
CV (%)	11.10				
PH1		White light	R	FR	Averages
	17.9	8.14 ^{Aa2}	8.29 ^{Aa}	8.74 ^{Aa}	8.39 ^A
	10.1	9.00 ^{Aa}	8.51 ^{Aa}	9.11 ^{Aa}	8.88 ^A
	3.4	6.45 ^{Bb}	7.14 ^{ABab}	7.90 ^{Aa}	7.17 ^B
	Darkness	6.24 ^B	6.24 ^B	6.24 ^B	6.24 ^C
Averages	7.46 ^a	7.55 ^a	8.00 ^a		
CV (%)	10.28				
PH2		White light	R	FR	Averages
	17.9	13.42 ^{Aa2}	12.92 ^{Aa}	13.54 ^{Aa}	13.30 ^A
	10.1	12.62 ^{Aa}	13.44 ^{Aa}	12.96 ^{Aa}	13.01 ^A
	3.4	12.54 ^{Aab}	13.48 ^{Aa}	12.31 ^{Ab}	12.78 ^A
	Darkness	9.69 ^B	9.69 ^B	9.69 ^B	9.69 ^B
Averages	12.07 ^a	12.38 ^a	12.13 ^a		
CV (%) ^z	5.51				

^xHU: *H. undatus*; HP: *H. polyrhizus*; SM: *S. megalanthus*; PH1: *H. undatus* X *H. costaricensis*; PH2: *H. costaricensis* X *H. undatus*.^yAverages followed by the same uppercase letter in the column and lowercase letter in the row did not differ in the Tukey test ($p \leq 0.05$). ^zCoefficient of variation.

Discussion

The presence of light, regardless of wavelength, was positively correlated with germination for most of the genotypes studied. Consistent with our study, Yamashita et al. (2008), working on the germination of yerba porosa (*Porophyllum ruderale*), and Lopes et al. (2005), working on climbing spinach (*Basella rubra*), also observed no difference between white, red and far-red wavelengths, all of which produced better results than the absence of light. Simão et al. (2010), working on the germination of seeds of *Epiphyllum phyllanthus*, an epiphyte cactus, obtained better germination percentages under white light in comparison with a dark environment, which is also consistent with our observations.

Various germination behaviors related to the effect of light on cactus seed germination are reported in the literature. Ortega-Baes and Rojas-Aréchiga (2007), working on the germination of *Trichocereus terscheckii*, and Meiado et al. (2010), working on *Cereus jamacaru*, both found that the seeds of these cacti did not germinate in the dark, i.e., the seeds of these species are positively photoblastic. However, Pedroni and Sánchez (1997) found no sensitivity to light over

a wide range of temperatures for *Pereskia aculeata* (Cactaceae). The wavelengths of light did not affect the values of GSI for *Hylocereus* genotypes. Rebouças and Santos (2007), working on the germination of seeds of *Melocactus conoideus* (Cactaceae), also found no significant differences in germination speed between various wavelengths of light (red, far-red and white light). Light intensity proved favorable to both the measurements of GSI and AGT, and intensities greater than $3.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ gave the best results for all pitaya genotypes except PH2, which showed no sensitivity to light intensity but was adversely affected by darkness (Table 2 and 4). Simão et al. (2007) also detected no differences in the germination percentages of seeds of *Selenicereus setaceus* (Cactaceae) germinated under differing light intensities, finding that only germination speed increased in direct proportion to light intensity. Considering germination percentage on its own, the HU and HP genotypes can be classified as neutral because there was no difference in germination based on the presence or absence of light (Villiers 1972; Mayer and Poljakoff-Mayber 1989). The SM, PH1 and PH2 genotypes should preferably be classified as

Table 3. The average germination time (AGT - days) for pitaya genotypes at different light intensities ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and wavelengths (white, red - R - and far-red - FR).

Genotype ^x	Intensity	White light	R	FR	Average				
HU	17.9	4.04	Aaby	4.31	Ab	3.77	Aa	4.04	A
	10.1	4.03	Aa	4.12	Aa	3.82	Aa	3.99	A
	3.4	4.48	Ba	4.23	Aa	4.58	Ba	4.43	B
	Darkness	4.72	B	4.72	B	4.72	B	4.72	C
	Averages	4.32	a	4.35	a	4.22	a		
CV (%)			4.95						
HP		White light	R	FR	Average				
	17.9	3.79	Aa2	3.84	Aa	3.85	Aa	3.83	A
	10.1	3.8	Aa	3.99	Aa	3.71	Aa	3.84	A
	3.4	4.75	Bb	4.11	Aa	3.77	Aa	4.21	B
	Darkness	5.11	B	5.11	B	5.11	B	5.11	C
Averages	4.36	b	4.26	ab	4.11	a			
CV (%)			6.77						
SM		White light	R	FR	Averages				
	17.9	6.44	Aa2	6.64	Aa	6.11	Aa	6.40	A
	10.1	6.40	Aa	7.32	Aab	8.05	Bb	7.26	B
	3.4	7.29	Aa	7.13	Aa	9.42	Bb	7.95	B
	Darkness	11.03	B	11.03	B	11.03	C	11.03	C
Averages	7.79	a	8.03	ab	8.65	b			
CV (%)			9.38						
PH1		White light	R	FR	Averages				
	17.9	4.63	Aa2	4.72	Aa	4.50	Aa	4.62	A
	10.1	4.79	Aa	4.61	Aa	4.41	Aa	4.61	A
	3.4	5.88	Bb	4.99	Aa	4.95	Aa	5.28	B
	Darkness	5.93	B	5.93	B	5.93	B	5.93	C
Averages	5.31	b	5.06	ab	4.95	a			
CV (%)			5.91						
PH2		White light	R	FR	Averages				
	17.9	3.74	Aa2	3.85	Aa	3.69	Aa	3.76	A
	10.1	3.91	Aa	3.72	Aa	3.88	Aa	3.84	A
	3.4	4.01	Aa	3.75	Aa	4.04	Aa	3.93	A
	Darkness	5.09	B	5.09	B	5.09	B	5.09	B
Averages	4.19	a	4.1	a	4.17	a			
CV (%) ^z			5.96						

^xHU: *H. undatus*; HP: *H. polyrhizus*; SM: *S. megalanthus*; PH1: *H. undatus* X *H. costaricensis*; PH2: *H. costaricensis* X *H. undatus*.

^yAverages followed by the same uppercase letter in the column and lowercase letter in the row did not differ in the Tukey test ($p \leq 0.05$).

^zCoefficient of variation.

positively photoblastic because the germination percentage was higher in the presence of light in comparison with darkness (Klein and Felipe 1991). However, taking GSI and AGT together, the best results for all genotypes were obtained in the presence of light. According to Takaki et al. (1983), positively photoblastic seeds can be characterized as needing a high threshold of far-red phytochrome (Pfr) for germination to occur because they contain an insufficient quantity of Pfr to initiate the germination process. This means that a period of exposure to light is necessary to boost Pfr to sufficient levels to initiate germination. According to Takaki et al. (1983) and Takaki (2000), neutral or non-photoblastic seeds have a sufficient Pfr threshold to initiate germination without exposure to light. The fact that some seeds germinated in the dark in our study can be attributed to a pre-existing level of Pfr that was sufficient to induce germination in some of the seeds in the sample (Takaki 2001 and Simão et al. 2010). The results obtained in this study partially elucidate the adaptation and development of pitaya species, both in shady and sunny environments, as reported by Le Bellec et al. (2006). The results also partly explain the vivipary reported by Cota-Sánchez (2007) for some species of the Hylocereae tribe, which includes the *Hylocereus* and *Selenicereus* genera. This study showed that light intensities

of 17.9 or 10.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ are recommended for germinating *H. undatus*, *H. polyrhizus* and *H. undatus* x *H. costaricensis*, 17.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for *S. megalanthus* and 17.9, 10.1 or 3.4 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for *H. costaricensis* x *H. undatus*. This study also showed that all wavelengths are favorable for germinating *H. undatus* and *H. costaricensis* x *H. undatus*, that far-red light was better than white light for *H. polyrhizus* and *H. undatus* x *H. costaricensis* and that white light was better than far-red light for *S. megalanthus*.

Materials and Methods

Plant Materials

The experiment was conducted in the plant science laboratory at the State University of Londrina (Parana State) using seeds of different pitaya genotypes taken from fruits acquired from individual collectors in the Londrina region and producers in São Paulo state and Bogota, Colombia. Three species and two hybrids were tested, identified as HU, HP, SM, PH1 and PH2. The flesh was manually removed from the fruits using a spoon, and then the flesh of each fruit was packed in a plastic container with water (2 L) and sugar (25 g L⁻¹ sucrose) and left to ferment for 48 hours at ambient temperature to facilitate seed extraction. The solution was then sieved under

running water to eliminate the flesh residues and retain the seeds, which were then placed on filter paper to dry in the shade for 48 hours at ambient temperature. The seeds were then packed in polyethylene and stored for 20 days in a cold chamber (6 - 9°C, 75% RH.). The seeds were homogenized before setting up the experiment.

Light treatments

Red, far-red and white light wavelengths were used at intensities of 3.4, 10.1 and 17.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$ ($\pm 0.48, 0.75, 0.46 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively). One treatment consisted of keeping the seeds in the dark. All treatments were subject to four replications of 50 seeds, sown on blotting paper moistened with distilled water (2.5 times the dry packed weight of the paper) and stored in germination boxes (Gerbox). To test for the effect of white light, the germination boxes were placed in opaque plastic boxes (28 x 18 x 10 cm) internally lined with aluminum foil (two germination boxes per lined plastic box). Red light wavelengths were obtained using two sheets of red cellophane, and far-red light was achieved by using two sheets of red and two sheets of blue cellophane (Almeida and Mundstock 2001). The cellophane sheets were fixed to the longer sides of pre-cut paper bags, into which the aluminum-lined plastic boxes were inserted. The seeds were sown in two germination boxes containing blotting paper moistened with distilled water (2.5 times the dry blotting paper weight), which were placed in each plastic box. To test under zero light conditions, the aluminum-lined plastic boxes were wrapped inside with two black polyethylene bags. The experiment was conducted in a growing room with 40 W daylight fluorescent tubes Philips® 2x9, at a temperature of 25°C (± 0.5) and photoperiod of 12 hours. To test all light intensities, the boxes were placed at a distance of 34 cm (± 2) from the light source (fluorescent tube) for direct exposure at 17.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$. To reduce the intensity to 10.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$, half the area of the tubes was covered with 5 cm strips of black polyethylene, blocking 50% of the light (commercial information). To produce an intensity of 3.4 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the tubes were entirely covered with the same type of polyethylene. In the 10.1 and 3.4 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatments, the light source distance was also 34 cm (± 2).

Statistical analysis

The experimental design was fully randomized in a 4 x 3 factorial arrangement of 4 light intensities and 3 wavelengths. Each treatment was replicated four times for each genotype with a sample size of 50 seeds. Evaluations were made daily for 30 days in a room with a green safety light, and seeds exhibiting a hypocotyl were considered to have germinated. The GSI, calculated using the formula proposed by Maguirre (1962), and average germination time (AGT - days), calculated according to Lima et al. (2006), were evaluated as the variables.

For the purpose of statistical analysis, the germination percentage values were transformed into arcsines ($x/100$)^{0.5} for normalization, although the values shown in the tables are not transformed. variance was carried out, and the averages were compared using the Tukey test ($p < 0.05$).

Conclusion

Light intensities of 17.9 and 10.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ are the best indicated values for the germination of PB, PV, and PH1,

while 17.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$ is the best indicated for PA, and 17.9, 10.1 and 3.4 $\mu\text{mol m}^{-2} \text{s}^{-1}$ are indicated for PH2.

All of the light levels were favorable for the germination of PB and PH2; VE was higher than white light for PV and PH1; and white light was higher than VE for PA.

Acknowledgments

We thank the National Council for Scientific and Technological Development (CNPq), Araucária Foundation and CAPES for funding the project and the scholarship provided.

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