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Piptadenia stipulacea (Benth.) Ducke Seed Germination in Response to Temperature, Light and Water Stress

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Abstract

The current study aimed to investigate the effects of the temperature, light, and water stress on *Piptadenia stipulacea* seed germination. It assessed germination percentage, speed and average germination time, root and stem length as well as the dry weight of seedlings subjected to the constant temperatures of 20˚C, 25˚C and 30˚C and alternating temperatures from 20˚C to 30˚C. A 12-hour photoperiod was established in addition to the following light conditions: white, darkness, red and far red. The experimental design was completely randomized and four replicates of 25 seeds were performed for each treatment. Regarding water stress, seeds were subjected to osmotic potentials of 0, −0.2, −0.4, −0.6, −0.8, −1.0, and −1.2 MPa, at 30˚C and 12 h light/12 h darkness photoperiods. After they were mixed, 100 seeds were randomly selected for biometric measurement and they were found to be uneven with respect to size and weight. *P. stipulacea* seeds germinated under all tested temperature and light conditions. Germination under water stress occurred up to −0.8 MPa. The conclusion is that there was no germination from −1.0 MPa. The seeds are light-indifferent and germinate at the constant temperatures of 20˚C, 25˚C and 30˚C and alternating temperatures from 20˚C to 30˚C.

Keywords

Forest-Tree Seeds, Native Species, Semi-Arid Region, Polyethylene Glycol

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1. Introduction

Piptadenia stipulacea (Benth.) Ducke, Fabaceae, is found in "Não me deixes" Farm, a Natural Heritage Private Reserve located in Quixadá County, Ceará State, Brazil, within an area covered by Caatinga vegetation. Its seeds show integumentary dormancy [1], which is a very common phenomenon in seeds from Caatinga area tree species, in the Brazilian Northeast semi-arid region [2]. It is an advantageous evolutionary trait for species that need to ensure their diaspores dispersal in time and space within regions subjected to water restrictions related to drought [3] [4]. The species can also be found in areas disturbed by anthropic activities [5] and its seedlings require light for their initial establishment [6]. These features make *P. stipulacea* widely used in forest restoration and agroforestry systems.

Once dormancy is broken, the morphologically developed seed requires environmental stimuli such as temperature, light (both quantity and quality) and, above all, water availability for the germination and establishment of new seedlings in a safe environment. In addition to such stimuli, seeds size and vigor are features that must be taken into consideration when one studies native species. Seeds biometry provides important subsidies to differentiate species from the same genus and it gives information about seeds' health and conservation status [7]-[9].

Investigating light and temperature influence on the seed germination process is the basis for understanding native species ecological and physiological behavior. According to Bewley *et al*. [10], depending on the species, the seeds can germinate after long or brief exposure to light; germinate in the darkness or with light and darkness periods, whereas others are light-indifferent. In general, the ideal temperature range in which the seed is able to germinate depends on its endogenous limits, thermal characteristics and on the moisture in the place where it has dispersed [11] [12].

In addition to the appropriate temperature and light conditions, water availability is another important abiotic factor responsible for the plant species germination success. Water is responsible for activating different metabolic processes that lead to seed germination, and each species requires a minimum amount of available water in order to germinate [10].

Knowing how abiotic factors such as light, temperature and water affect the *P. stipulacea* seed germination process may contribute to its seeds rational use and to a more efficient seedling production for planting. Knowing seeds biometry allows producers to select those with good size and devoid of physical damage. The current study aimed to investigate the effects of temperature, light and water stress on *P. stipulacea* seed germination.

2. Material and Methods

2.1. Seeds Collection and Storage Place

P. stipulacea fruits were manually collected from five selected trees in September 2013. The harvesting took place in a Caatinga vegetation area in the Natural Heritage Private Reserve "Não me deixes" Farm (4˚49'34"S, 38˚58'9"W and 210 m above current sea level), in Quixadá County, Ceará State, Brazil.

The fruits were dried in an oven (45˚C/3days) and processed for seed extraction. After processing, seeds were separated from impurities (part of fruits, leaves, petioles) and from other seeds which were damaged by insects or that were broken. They were then placed in plastic containers and stored (for two months) in a cold chamber at an average temperature of 12˚C and relative humidity of 55% until the beginning of the experiments.

The experiments were conducted in the Seed Analysis Laboratory (LAS—Laboratório de Análise de Sementes) from the Plant Science Department, at Federal University of Ceará (UFC). Botanical material containing *P. stipulacea* leaves and fruits was collected for specimen preparation and was deposited at Prisco Bezerra Herbarium—EAC, Federal University of Ceará, under protocol number 054121 (EAC).

2.2. Biometry and Thousand Seed Weight

After they were mixed, 100 seeds were randomly selected for individual measurement. Biometric featuring was performed by means of digital caliper (0.01 mm) through which the following variables were measured: length, width, thickness and weight (in precision scale). The length was measured from the base to the apex and width and thickness were measured on seeds midline. Data were subjected to descriptive statistics in Excel application in order to calculate arithmetic mean, standard deviation, standard error, coefficient of variation and confidence interval for each biometric feature.

P. stipulacea thousand seed weight was calculated in accordance with the Rules for Seed Analysis recommendations [13]. The number of seeds per kilogram was determined from the thousand seed weight results.

2.3. Temperature and Light

The dormant seeds [1] were manually scarified with n. 80 sandpaper, opposite to the micropyle, in order to wear the integument. After scarification, they were treated with sodium hypochlorite to avoid fungi attack during germination. The seeds were placed in Becker and carefully and completely immersed in 5% sodium hypochlorite for 5 minutes. They were then transferred to a fine mesh steel sieve, rinsed in running water and dried with paper towel.

The treated seeds were sown on two sheets of germitest filter paper arranged in 9.50 cm diameter Petri dishes. The Petri dishes with the sheets of germitest filter paper were previously autoclaved at 120˚C for 20 minutes. The substrate was moistened with distilled water in the ratio of two and a half times the weight of the paper [13].

Temperature and light effects were checked by using the completely randomized experimental design. Treatments were distributed in 4 × 4 factorial arrangement, and subjected to constant temperatures of 20˚C, 25˚C and 30˚C and alternating temperatures from 20˚C to 30˚C, under a 12-hour photoperiod and the following light conditions: white, darkness, red, and far red, with four replicates of 25 seeds. During alternating temperatures, the light period corresponded to the higher temperature. As for the white light condition, the Petri dishes were placed in transparent plastic bags to prevent water loss. In the absence of light (darkness), seeds were individually wrapped in aluminum foil and placed inside black plastic bags. For the simulation of red light (660 nm) and far red light (730 nm), the method described by Almeida and Mundstock [14] was adopted. The red light was obtained by using two sheets of red cellophane whereas the far red light, was obtained by using two sheets of red cellophane and two sheets of navy blue cellophane as described by Bickford and Dunn [15].

The Petri dishes were placed in a BOD (Biological Oxygen Demand) germination chambers regulated in their constant and alternating temperature regimes. The germinated seeds were daily recorded for 10 days, and the evaluation criterium was the presence of a radicle of at least 2 mm. Security green light was used to count seeds subjected to darkness, red and far red light. During this period, the Petri dishes were carefully remoistened with distilled water when necessary. Finally, the following variables were evaluated:

a) Germination percentage, % G = $(N/A) \times 100$, where: N = number of germinated seeds and A = total number of sown seeds.

b) Germination speed index, according to Maguire [16]: GSI = $G_1/N_1 + G_2/N_2 + \ldots + G_n/N_n$, where: G_1, G_2 and G_n = number of germinated seeds in each day and N_1, N_2 ... N_n = number of days elapsed since the day of sowing.

c) Mean germination time, according to Ranal and Santana [17]: MGT = $(\Sigma G_i * T_i)/\Sigma G$, with the results expressed in days, where: G_i = number of germinated seeds within a given time interval T_i, G = number of germinated seeds, T_i = days of germination.

d) Length: the length of seedlings root and shoot was measured separately, discarding the cotyledons, with the help of a millimeter ruler.

e) Dry weight: obtained from material placed in paper bags duly identified and dried in the greenhouse at 80˚C, with forced air circulation for 24 h. After this period, the seedlings were weighted in precision scale and the results were expressed as g/seedling.

2.4. Water Stress

The water restriction simulation on *P. stipulacea* germination under laboratory conditions was tested by means of polyethylene glycol solutions (PEG 6000), which satisfactorily simulates low water potentials. PEG is chemically inert and does not show toxicity to the seeds [18]-[21].

The seeds were placed in Petri dishes (9.50 cm diameter) and the germitest paper substrate was moistened with distilled water (witness) and PEG 6000 solution in different concentrations. The used polyethylene glycol concentrations (PEG 6000) were −0.2; −0.4; −0.6; −0.8; −1.0 and −1.2 MPa water potential, obtained for the constant temperature of 30˚C, according to Villela *et al.* [18]. The experiment was set up in completely randomized design with four replicates of 25 seeds each.

The Petri dishes were kept in germination chamber (BOD) under constant temperature of 30˚C for a 12-hour photoperiod. The germitest paper substrate and PEG solutions were replaced every 48 hours aiming at main-

taining the experiment initial conditions. The counts of germinated seed were carried out daily for 14 days, and the germination percentage (% G) was determined at the end of the experiment.

2.5. Statistical Analysis

All the dependent variables were analyzed with respect to normality by using Kolgomorov-Smirnov test, and the homogeneity of variances was analyzed by using the Levene test. After the two criteria were met, the data were subjected to ANOVA, and averages were compared by Tukey's test at 5% significance level. When they were not met, the data were subjected to non-parametric statistics by the Kruskal-Wallis test and evaluated by non-parametric multiple comparisons at 5% significance level [22].

3. Results

3.1. Biometric Characteristics

P. stipulacea seeds are uneven regarding size, and show variation in length (6.71 to 8.67 mm), width (4.78 to 6.32 mm) and thickness (1.88 to 3.07 mm). The unit seed weight ranged from 42.9 to 80.28 mg. Seeds descriptive statistics is presented in **Table 1**. The thousand seed weight was of 44.134 g, which allows inferring that a kilogram of *P. stipulacea* seeds can contain 22,658 seeds.

3.2. Effect of Temperature and Light on Germination

Temperature and light quality (white, darkness, red and far red) evaluations for *P. stipulacea* seeds germination percentage (% G) showed similar means ($H_{(3.15)}$ = 32.96, p = 0.0048). The data presented in **Table 2** show that the species germinated regardless the presence and absence of light, both at the constant temperatures of 20˚C, 25˚C and 30˚C and at the alternating temperature from 20˚C to 30˚C.

The ANOVA result for *P. stipulacea* seed germination showed that abiotic factors such as temperature, light and their interactions exerted significant effects for all variables under analysis, with the exception of the light for seedling dry weight (**Table 3**).

The interaction between light and temperature influenced the germination speed index (GSI). It is possible to see that the highest germination speed index (10.52) occurred at 30˚C under far red light condition, and the lowest one (4.3) was observed under darkness condition at 20˚C (**Table 4**).

There was a significant statistical effect of temperature and light treatments on *P. stipulacea* seeds Mean Germination Time (MGT). The lower MGT was obtained at 30˚C under red and far red light conditions. The

Table 1. Mean, standard deviation, standard error, coefficient of variation and confidence intervals (CI) relating to biometric measurements (length, width, thickness and weight) in a sample of 100 *Piptadenia stipulacea* seeds.

Variable	Mean	Standard deviation	Standard error	Coefficient of variation	CI 95%
Length (mm)	7.7	0.51	0.05	6.65	7.69 ± 0.09
Width (mm)	5.44	0.35	0.04	6.4	5.44 ± 0.06
Thickness (mm)	2.27	0.20	0.02	8.95	2.27 ± 0.03
Weight (mg)	63.48	8.34	0.83	13.14	63.48 ± 1.63

Table 2. Germination percentage of *Piptadenia stipulacea* seeds subjected to different temperature and light treatments (mean ± standard deviation, n = 4).

Temperature (˚C)	Germination percentage (G%)			
	Light			
	White	Darkness	Red	Far red
20	93 ± 5.03 aA	86 ± 2.30 aA	87 ± 6.83 aA	87 ± 3.82 aA
25	91 ± 3.82 aA	88 ± 0.00 aA	93 ± 2.00 aA	93 ± 2.00 aA
30	95 ± 3.82 aA	93 ± 3.82 aA	95 ± 2.00 aA	97 ± 2.00 aA
20/30	93 ± 3.82 aA	95 ± 3.82aA	97 ± 3.82 aA	95 ± 5.03 aA

Means followed by the same letter do not significantly differ from each other by nonparametric multiple comparisons at 5% probability.

Table 3. Results concerning analysis of variance (ANOVA), variance factor (VF), coefficient of variation (CV) and degrees of freedom (Df) for Germination Velocity Index (GVI), Mean Germination Time (MGT), shoot (cm) and root (cm) length and dry weight (root + shoot, g/seedling) of *Piptadenia stipulacea* seeds and seedlings treated under different temperature and light conditions.

Variance factor	F values					
	Df	GVI	MGT	Shoot	Root	Dry weight
Temperature (T)	3	63.97**	16.09**	23.79**	0.52**	0.0247**
Light (L)	3	3.71**	0.58**	18.54**	1.17**	0.0002 ns
T × L	9	2.25**	0.21*	0.93**	0.29**	0.0031*
Residual	48	0.58**	0.10**	0.29**	0.09**	0.0014**
CV (%)	-	10.57	8.95	11.26	15.97	17.04

**Significant at 1% probability level by F test; *Significant at 5% probability level by F test and ns: non-significant.

Table 4. Germination Speed Index of *Piptadenia stipulacea* seeds subjected to different temperature and light treatments (mean ± standard deviation, n = 4).

Temperature (˚C)	Germination Speed Index (GSI)			
	Light			
	White	Darkness	Red	Far red
20	4.76 ± 0.41 aA	4.3 ± 0.30 aA	4.74 ± 0.39 aA	4.36 ± 0.31 aA
25	6.89 ± 0.39 aB	7.35 ± 1.37 aB	7.71 ± 0.50 aB	6.72 ± 0.34 aB
30	7.93 ± 1.02 aB	8.35 ± 0.68 aB	10.25 ± 1.20 bC	10.52 ± 0.51 bD
20/30	6.89 ± 0.50 aB	8.68 ± 1.60 bB	8.17 ± 0.24 abB	8.47 ± 0.67 bC

Means followed by the same lowercase (lines) and uppercase (columns) letters do not significantly differ from each other by the Tukey's test at 5% probability.

largest MGT was obtained at 20˚C in all evaluated light conditions (**Table 5**).

The white light was more efficient in stimulating *P. stipulacea* seedlings' root growth. The highest root length mean occurred at 20˚C (2.55 cm) under white light condition, although the other temperatures evaluated under the same light condition were statistically similar (**Table 6**).

The lowest mean shoot length occurred at 20˚C in the four evaluated light conditions. There was the highest mean shoot length in all treatments (**Table 7**).

The dry mass was lower at 20˚C regarding the four evaluated light conditions. There were small variations in the means obtained from the other treatments (**Table 8**).

3.3. Water Stress

P. stipulacea germination percentage was significantly influenced by different treatments with polyethylene glycol (PEG 6000), ($F = 331.29$, $p < 0.001$). Seed germination was not affected by PEG 6000 concentrations up to −0.4 MPa. However, it was reduced at the concentrations of −0.6 and −0.8 MPa. There was no seed germination at −1.0 and −1.2 MPa (**Figure 1**).

4. Discussion

Seeds size and weight depended on the year they were produced and on where they were dispersed. Such features can vary in the same individual and within the same functional group [23]. In general, seeds size may vary between five to six orders of magnitude in most habitats [24] [25]. *P. stipulacea* seeds are classified as small [13]. Water capture is more efficient in small seeds. Large seeds may have difficulty in obtaining water for germination from temporary water supplies because of their low surface to volume ratio [26]. Thus, small seeds germinate first, since they require less water, which is an advantageous feature for Caatinga species, because water availability is restricted to three or four months during the rainy season [27]. *P. stipulacea* seedling producers can decide for early or late seedlings depending on the seeds size.

P. stipulacea seeds germinated under all tested temperature and light conditions. Such fact may reveal the

Table 5. Mean Germination Time of *Piptadenia stipulacea* seeds subjected to different temperature and light treatments (mean ± standard deviation, n = 4).

Temperature (˚C)	Mean Germination Time (MGT)			
	Light			
	White	Darkness	Red	Far red
20	5.05 ± 0.42 aB	5.15 ± 0.20 aB	4.79 ± 0.07 aC	5.13 ± 0.22 aC
25	3.45 ± 0.12 aA	3.27 ± 0.60 aA	3.15 ± 0.20 aB	3.68 ± 0.14 aB
30	3.20 ± 0.58 bA	2.96 ± 0.15 abA	2.45 ± 0.27 aA	2.43 ± 0.15 aA
20/30	3.54 ± 0.33 aA	2.98 ± 0.58 aA	3.00 ± 0.07 aAB	2.98 ± 0.19 aA

Means followed by the same lowercase (lines) and uppercase (columns) letters do not significantly differ from each other by the Tukey test at 5% probability.

Table 6. *Piptadenia stipulacea* root length subjected to different temperature and light treatments (mean ± standard deviation, n = 4).

Temperature (˚C)	Root length (cm)			
	Light			
	White	Darkness	Red	Far red
20	2.55 ± 0.27 bA	1.61 ± 0.34 aAB	2.00 ± 0.38 abAB	1.69 ± 0.19 aAB
25	2.21 ± 0.33 bA	1.58 ± 0.16 aAB	1.91 ± 0.11 abAB	2.26 ± 0.30 bB
30	2.10 ± 0.53 bA	1.61 ± 0.33 aA	1.46 ± 0.35 aA	1.64 ± 0.21 abA
20/30	2.27 ± 0.29 bA	2.08 ± 0.14 abB	2.27± 0.24 bB	1.53 ± 0.35 aA

Means followed by the same lowercase (lines) and uppercase (columns) letters do not significantly differ from each other by the Tukey test at 5% probability.

Table 7. *Piptadenia stipulacea* shoot length subjected to different temperature and light treatments (mean ± standard deviation, n = 4).

Temperature (˚C)	Shoot length (cm)			
	Light			
	White	Darkness	Red	Far red
20	2.17 ± 0.29 bB	3.56 ± 0.41 aB	3.05 ± 0.44 aC	3.36 ± 0.52 aB
25	4.36 ± 0.29 bA	6.37 ± 1.01 aA	4.37 ± 1.10 bB	6.31 ± 0.32 aA
30	4.07 ± 0.13 cA	7.23 ± 0.90 aA	5.48 ± 0.34 bA	6.24 ± 0.38 abA
20/30	3.64 ± 0.16 cA	6.89 ± 0.36 aA	4.66 ± 0.23 bAB	5.50 ± 0.49 bA

Means followed by the same lowercase (lines) and uppercase (columns) letters do not significantly differ from each other by the Tukey test at 5% probability.

Table 8. *Piptadenia stipulacea* dry mass subjected to different temperature and light treatments (mean ± standard deviation, n = 4).

Temperature (˚C)	Dry mass			
	Light			
	White	Darkness	Red	Far red
20	0.16 ± 0.013 aA	0.18 ± 0.02 aA	0.17 ± 0.036 aA	0.17 ± 0.027 aA
25	0.25 ± 0.025 aB	0.21 ± 0.035 aA	0.21 ± 0.072 aAB	0.27± 0.02 aB
30	0.26 ± 0.036 aB	0.24 ± 0.046 aAB	0.27 ± 0.044 aB	0.25 ± 0.027 aB
20/30	0.22 ± 0.042 aAB	0.29 ± 0.022 aB	0.25± 0.043 aB	0.22 ± 0.058 aAB

Means followed by the same lowercase (lines) and uppercase (columns) letters do not significantly differ from each other by the Tukey test at 5% probability.

species ability to adapt itself to thermal fluctuations and natural light levels in the environment in which it is located. According to Guedes *et al.* [28], this characteristic enables the greater ability by seeds to establish themselves in environments with abiotic constraints (temperature, humidity) as those found in the Brazilian northeastern semiarid region. *Myracrodruon urundeuva* Allemão and *Caesalpinia leiostachhya* (Benth.) Ducke seeds,

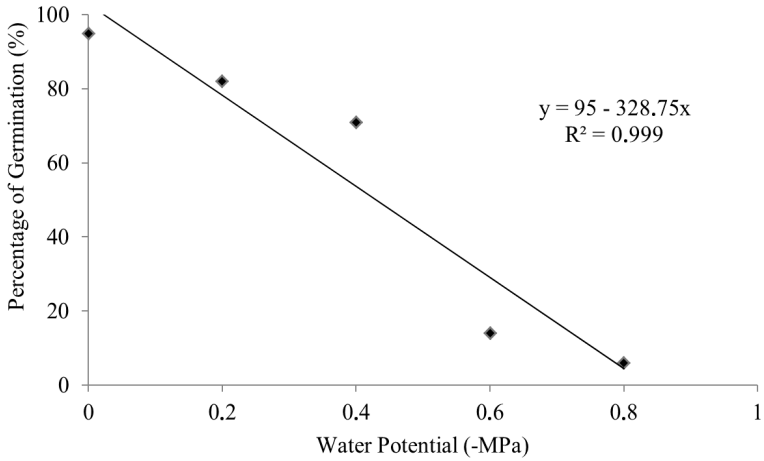

Figure 1. *Piptadenia stipulacea* seeds germination percentage in different osmotic potentials.

which are common tree species in this region, germinate at the optimal temperatures of 20˚C and 30˚C, regardless the presence or absence of light [29] [30].

Given the results shown in **Table 4**, we can observe that the highest germination speed index occurred at 30˚C. There was a trend of increased germination speed index as the temperature increased. Marcos Filho [31] points out that gradual temperature reduction causes a sharp decrease in germination speed index, due to its effects on the absorption and mobilization of the reserves rates. The speed at which seeds germinate is important for the satisfactory seedling establishment in the field. According to Martins *et al.* [32], when seedlings take a long time to emerge from the soil, they become more vulnerable to adverse environmental conditions. As for seedling market, seed germination and uniformity along with the immediate seedling emergence are attributes that should be taken under consideration in the forest seedlings production.

The lowest mean germination time of *P. stipulacea* seeds was observed at 30˚C under red and far red light conditions. As this species occurs in Caatinga areas with average temperature of 30˚C, the seeds mean germination time is important for the satisfactory seedling establishment in the field. Another aspect to be considered is the fact that it is used in forest restoration and agroforestry systems. The light had little effect on germination and seedling development was not assessed. In this case, forest seedlings producers should provide appropriate shading in order to ensure rapid and uniform seed germination and subsequent seedling emergence in greenhouses.

In general, the root and shoot average length and dry weight recorded differences among the treatments in the presence of white light. In the laboratory, such light condition simulates the direct sunlight in the field, in which the root tends to grow since it is stimulated by high light intensity. A deep root system allows the most adapted species to occupy degraded areas or areas undergoing environmental restoration projects.

P. stipulacea seeds germination percentage was affected by PEG 6000 concentrations from 0 (control) to −0.8 MPa. The lack of germination in osmotic potentials −1.0 and −1.2 MPa can be attributed to the very unfavorable water conditions. Under such conditions, seeds avoid germinating as a survival strategy. This way, they can ensure further seedlings development [10].

Seeds from Northeastern semi-arid species show decreased germination when subjected to water stress due to the increased salt concentration up to the critical point, which is typical of each species. Silva *et al.* [33] corroborated this fact in their study on *Cnidoscolus juercifolius* Pax and K. Hoffm. seeds, in which germination was zero at −0.9 MPa.

5. Conclusion

P. stipulacea seeds were found to be uneven with respect to size and weight. *P. stipulacea* seeds germinated under water stress occurred up to −0.8 MPa and seeds are light-indifferent and germinate at the constant temperatures of 20˚C, 25˚C and 30˚C and alternating temperatures from 20˚C to 30˚C.

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