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Dormancy overcoming and germination test in *Piptadenia stipulacea* (Benth.) Ducke seeds[1]

Superação de dormência e teste de germinação em sementes de *Piptadenia stipulacea* (Benth.) Ducke

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ABSTRACT - *Piptadenia stipulacea* (Benth.) Ducke presents slow and uneven germination due to integumentary dormancy. In addition, there are no standardized indications as to the temperature and substrate for conducting the germination test in the laboratory. Thus, the objective was to evaluate methods of dormancy overcoming, temperatures and substrates in the germination and vigor of *P. stipulacea* seeds. For this, two experiments were installed, the first one in a completely randomized design, with 4 replicates of 25 seeds and treatments to overcome dormancy were: immersion in water at 100 °C for 1; 2; 3; 4; 5 and 6 min; immersion in concentrated sulfuric acid (H_2SO_4 - 98% P.A) for 1; 4; 7; 10 and 13 min; scarification on sandpaper nº 80; in the region opposite the micropyle and intact seeds. At 21 days after sowing, emergency, emergency speed index and mean emergency time were evaluated. In experiment II, the experimental design was completely randomized in a 4 x 6 factorial scheme, with four replicates of 25 seeds, using the substrates between sand, paper, paper roll and vermiculite at temperatures of 20; 25; 30; 35; 40 °C and alternated 20-30 °C, the variables analyzed were first count, germination, mean germination time and germination speed index. In order to overcome seed dormancy of *P. stipulacea*, it is recommended that the pre-germinating treatment be set in the region opposite to the micropyle, and to evaluate the germination and vigor of these seeds, the temperature of 30 °C and 20-30 °C can be used with the roller substrate of paper.

Key works: Fabaceae. Jurema-branca. Semi-arid region.

RESUMO - *Piptadenia stipulacea* (Benth.) Ducke apresenta germinação lenta e desuniforme, devido à dormência tegumentar, além disto, não há indicações padronizadas quanto à temperatura e substrato para condução do teste de germinação em laboratório. Objetivou-se avaliar métodos de superação de dormência, temperaturas e substratos na germinação e vigor de sementes de *P. stipulacea*. Para tanto, instalaram-se dois experimentos, sendo o primeiro em delineamento inteiramente casualizado, com 4 repetições de 25 sementes e os tratamentos para superação de dormência foram: imersão em água a 100 °C por 1; 2; 3; 4; 5 e 6 min; imersão em ácido sulfúrico concentrado (H_2SO_4 – 98% P.A) durante 1; 4; 7; 10 e 13 minutos; escarificação em lixa n° 80; desponte na região oposta à micrópila e sementes intactas. Aos 21 dias após a semeadura, avaliou-se emergência, índice de velocidade de emergência e tempo médio de emergência. No experimento II, o delineamento experimental foi o inteiramente casualizado em esquema fatorial 4 x 6, com quatro repetições de 25 sementes, utilizando os substratos entre areia, sobre papel, rolo de papel e entre vermiculita nas temperaturas de 20; 25; 30; 35; 40 °C e alternada 20-30 °C, as variáveis analisadas foram primeira contagem, germinação, tempo médio de germinação e índice de velocidade de germinação. Para superação de dormência de sementes de *P. stipulacea*, recomenda-se o tratamento pré-germinativo desponte na região oposta à micrópila. Pode-se utilizar a temperatura de 30 °C e 20-30 °C com o substrato rolo de papel, na avaliação da germinação e vigor de sementes de *P. stipulacea*.

Palavras-chave: Fabaceae. Jurema-branca. Semiárido.

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INTRODUCTION

Piptadenia stipulacea Benth. Ducke, belonging to the Fabaceae family, popularly known in Brazil as 'jurema-branca', is a small (2 – 4 m) tree species endemic to the semi-arid region of Northeast Brazil. It is a species with high potential for timber, cosmetic and medicinal markets, adding economic and environmental value (FERREIRA *et al*., 2012).

Species of the Fabaceae family usually have seeds with dormancy, which must be overcome before planting. In this context, some studies have been carried out to indicate pre-germination treatments to overcome seed dormancy, and the choice of the method depends on the causes and intensity of dormancy and on species. The available procedures to overcome dormancy include: storage, mechanical and chemical scarification, washing in hot water, stratification, cooling, dry heat, alternated temperatures, gibberellins and light (MARCOS-FILHO, 2015).

Dormancy breaking, besides favoring hydration, synchronizes and intensifies germination and the speed at which it occurs, facilitates the analysis of viability and vigor in laboratory and in the field, as well as the production of early, more uniform seedlings (DAPONT *et al*., 2014). Mechanical scarification has been efficient at overcoming dormancy in seeds of *Caesalpinia ferrea* (COELHO *et al*., 2010), *Acacia caven* (ESCOBAR *et al*., 2010), *Schizolobium amazonicum* (SHIMIZU *et al*., 2011), *Parkia* spp. (MELO *et al*., 2011), *Adenocarpus desertorum* and *Astragalus gines-lopezii* (SCHNADELBACH *et al*., 2016). On the other hand, for *Leucaena leucocephala* (TADROS; SAMARAH; ALQUDAH, 2011), water at 70 ºC for 20 minutes was the most efficient method, and in *Parkia gigantocarpa* seeds (OLIVEIRA *et al*., 2012), acid scarification for 30 and 40 minutes were the best treatments.

In addition to studying the method to overcome dormancy, it is necessary to determine the adequate conditions for the germination test of *P. stipulacea*, which, as for most native forest species, are not yet standardized and have not been included in the Rules for Seed Analysis (BRASIL, 2009) or in the Instructions for Analysis of Forest Species (BRASIL, 2013). In this context, it becomes necessary to determine the adequate substrate and temperature, in order to provide ideal conditions for germination and subsequently for adequate development of seedlings (TONIN; PERES, 2006).

Thus, some characteristics must be considered in the choice of the substrate, such as density, water retention and absorption capacity, aeration and drainage, absence of pests, diseases and toxic substances, seed size and demand for water and light (BRASIL, 2009). Temperature must directly act on the results of final percentage and germination speed, primordial factors to select an ideal temperature, because it will influence particularly water absorption by the seed and, consequently, biochemical reactions which regulate its metabolism (CARVALHO; NAKAGAWA, 2012).

Requirements in terms of temperature and substrate for germination widely differ among forest species. Some species have already been studied with respect to these factors, such as *Adenanthera pavonina* L. (KISSMANN *et al*., 2008), in which the best results were obtained with the substrates paper roll and on paper at temperatures of 18, 25, 30 and 20-30 ºC; for *Caesalpinia pyramidales* Tul. (LIMA *et al*., 2011), temperatures of 20-30 and 20-35 °C were recommended in the substrates sand and vermiculite; in *Myracrodruon urundeuva* (GUEDES *et al*., 2011), the best treatments were at 30 ºC in sand; and in *Parkia platycephala* (SILVA *et al*., 2017), the temperature of 25-35 °C in the substrate vermiculite showed the best results.

Given the above, this study aimed to evaluate methods to overcome dormancy, temperatures and substrates on the germination and vigor of *P. stipulacea* seeds.

MATERIAL AND METHODS

P. stipulacea seeds were obtained from ripe fruits harvested from twenty trees, at least 50 m apart, with good phytosanitary aspect, in September 2011, located in Mossoró-RN, Brazil (5º12'51" S, 37º18'44.7" W) (5º12'58.8" S, 37°18'51.65" W).

After collection, seeds were manually extracted from the fruits, and those that were broken, cracked and attacked by insects were eliminated from the samples. Then, the seeds were placed in glass flasks and remained in a controlled chamber (17 °C ± 50% RH) until the experiments were conducted, in December 2011.

Prior to the experiment, the moisture content was determined by the oven method at 105 ± 3 °C for 24 hours, according to Brasil (2009), with two replicates of 20 seeds. A preliminary test was also conducted to confirm the presence of seed coat dormancy, with two samples of 25 seeds placed between two sheets of blotting paper pre-moistened with a volume of water equivalent to 2.5 times the paper weight. Seeds were weighed at intervals of 2, 4, 6, 8, 24, 48, 72 and 96 hours after soaking, and only 12% of them germinated after 96 hours, while the others remained hard, confirming the presence of seed coat dormancy.

In experiment I, pre-germination treatments to overcome dormancy consisted of intact seeds - control

(T1), immersion in water at 100 °C for 1 (T2); 2 (T3); 3 (T4); 4 (T5); 5 (T6) and 6 minutes (T7); immersion in concentrated sulfuric acid (98%) for 1 (T8); 4 (T9); 7 (T10); 10 (T11) and 13 minutes (T12), scarification with sandpaper n° 80 (T13) and tip removal in the region opposite to the micropyle (T14). The experimental design was completely randomized, with 4 replicates of 25 seeds, totaling 56 experimental plots. After treatment application, seeds were washed in running water and dried in paper towel to remove excess moisture.

Then, sowing was carried out at 1 cm depth on plastic trays (28 x 20 x 7 cm) containing the substrate sand, previously washed and autoclaved, in a greenhouse. Irrigation was performed manually using a watering can by applying a volume of water equivalent to 60% of field capacity (BRASIL, 2009). The following variables were analyzed:

a) Emergence of seedlings - obtained by counting normal seedlings at 21 days after sowing, when emergence stabilized, and the results were expressed in percentage.

b) Emergence speed index (ESI) - carried out along with the test of seedling emergence. ESI was determined for each treatment by summing the number of seedlings emerged every day and dividing it by the respective number of days elapsed since sowing (MAGUIRE, 1962).

c) Mean time of emergence - determined by daily counts, at the same time, of the number of emerged seeds which met the same standards of normality as in the germination test. Calculations of mean time of emergence were carried out using the formula proposed by Labouriau (1983).

Experiment II was conducted at the laboratory, in a completely randomized design, in 4 x 6 factorial scheme, with four substrates (on paper, paper roll, sand and vermiculite) and six temperatures (20; 25; 30; 35; 40 °C and alternated temperatures of 20-30 °C), with four replicates of 25 seeds.

To use the substrate defined as on paper, seeds were arranged in transparent plastic boxes (11.0 x 11.0 x 3.5 cm) containing two sheets of blotting paper, moistened with a water volume equivalent to 2.5 times the dry weight of the substrate. For the substrate paper roll, sowing was carried out beneath three sheets of paper towel, moistened with distilled water at a proportion of 2.5 times the dry weight of the substrate and placed in transparent paper bags to avoid water loss. The substrates sand and vermiculite were arranged in plastic boxes (11.0 x 11.0 x 3.5 cm), moistened to 60% of their capacity, and sowing was carried out at 1 cm depth. After sowing, plastic boxes and paper rolls were transferred to germination chambers, regulated at the respective temperatures and under 8-h photoperiod. The following variables were analyzed:

a) First count of germination - the number of seedlings was counted from the 3rd to the 10th day after sowing, adopting normal seedlings as the criteria (BRASIL, 2009);

b) Germination speed index (GSI) - calculated according to the formula of Maguire (1962);

c) Mean time of germination (MTG) - calculated using the formula established by Labouriau (1983), with results expressed in days after sowing.

Prior to statistical analysis, the data were subjected to normality and homogeneity of variance tests, which indicated that no data transformation was necessary. The data were then subjected to analysis of variance by F test and, in case of significance, Tukey test was applied at 0.05 probability level, using the statistical program SISVAR® (FERREIRA, 2011).

RESULTS AND DISCUSSION

The seeds had 9.4% moisture content when the experiment was conducted. For the pre-germination

Table 1 - Summary of analysis of variance for emergence percentage (E), emergence speed index (ESI), mean time of emergence (MTE) of *Piptadenia stipulacea* Benth. Ducke seeds subjected to different methods to overcome dormancy

Source of variation	Degrees of freedom	Average squares		
		E (%)	ESI	MTE (days)
Treatments	16	1780.68*	7.90	14.11
Error	51	52.47	0.23	1.49
C.V(%)		14.30	18.45	20.61
Overall average		50.64	2.65	5.93

*significant at 5% probability

treatments, the analysis of variance pointed to significant effect of the treatments to overcome dormancy on all characteristics evaluated (emergence, emergence speed index and mean time of emergence) (Table 1).

Seeds subjected to pre-germination treatments with tip removal, sandpaper and hot water for 1 minute showed the highest percentages of emergence, with means of 83, 74 and 72%, respectively, not differing from one another (Table 2). Thus, these treatments caused wear on seed coat without damaging the embryo, allowing water to enter in an amount sufficient for seedling emergence. Additionally, this soaking period was satisfactory for tissue rehydration, with consequent intensification of respiration and all other metabolic activities, which culminate with the supply of energy and nutrients that are necessary to resume embryo axis growth (CARVALHO; NAKAGAWA, 2012).

Results similar to those of the present study were found in *Acacia caven* seeds (ESCOBAR *et al*., 2010), where tip removal and scarification with sandpaper were the most efficient treatments to overcome dormancy. Scarification with sandpaper led to germination above 70% in *Schizolobium amazonicum* seeds, compared to the treatment with immersion in hot water for 2 minutes (SHIMIZU *et al*., 2011). This method also showed efficient results in overcoming dormancy of seeds of *Leucaena leucocephala* (TADROS; SAMARAH; ALQUDAH, 2011) and in *Caesalpinia ferrea* Mart. ex Tul. (COELHO *et al*., 2010).

Tip removal and sandpaper on the end opposite to the micropyle also led to higher means for emergence speed index (Table 2). In general, higher emergence percentages tended to be associated with higher means of emergence speed index, indicating the existence of direct relationship between both processes, demonstrating that these treatments are satisfactory to overcome dormancy and establish uniform emergence of *P. stipulacea* seeds, as also found for seeds of *Adenocarpus desertorum* and *Astragalus gines-lopezii* (SCHNADELBACH *et al*., 2016).

Additionally, with respect to emergence and emergence speed index, chemical treatment with sulfuric acid for 1, 4 and 7 minutes did not differ from the control, being inefficient at overcoming dormancy in this species. This probably occurred because the time of contact between seeds and acid was short, since the immersion for 10 minutes led to higher means compared with the periods of 1, 4 and 7 minutes. The effects observed on seeds subjected to treatments with sulfuric acid in short period of time indicate inefficiency of the method to break the resistance to water entry, allowing seeds to maintain the strategies of dormancy, resulting in uneven germination (CARVALHO; NAKAGAWA, 2012).

Better results for mean time of emergence were found in treatments with immersion in sulfuric acid, sandpaper and tip removal (Table 2), as observed in *Acacia auriculiformis* seeds immersed in sulfuric acid for 2 minutes, which showed shorter mean time of germination

Table 2 - Emergence, emergence speed index (ESI) and mean time of emergence (MTE) of *Piptadenia stipulacea* Benth. Ducke seeds subjected to different pre-germination treatments

Treatments	Emergence (%)	ESI	MTE
Control	5 f*	0.22 f	7 d
Water at 100 °C for 1 minutes	78 ab	6.0 bc	3.75 cd
Water at 100 °C for 2 minutes	79 bcd	5.5 bcd	3.75 abcd
Water at 100 °C for 3 minutes	74 bcd	5.5 bcd	3.75 abcd
Water at 100 °C for 4 minutes	82 bc	5.25 bc	4.0 abcd
Water at 100 °C for 5 minutes	75 cd	5.5 bc	3.75 abc
Water at 100 °C for 6 minutes	78 bcd	5.75 bc	3.75 bcd
Sulfuric acid for 1 minutes	19 f	1.25 ef	4.0 abc
Sulfuric acid for 4 minutes	39 ef	2.75 bcde	4.0 abc
Sulfuric acid for 7 minutes	49 ef	3.5 cdef	3.75 abc
Sulfuric acid for 10 minutes	62 cde	4.5 b	3.5 abc
Sulfuric acid for 13 minutes	76 de	4.75 b	4.0 abc
Sandpaper	80 ab	5.5 a	3.0 ab
Stand out	82 a	6.5 a	3.0 a

*Means followed by the same lowercase letter do not differ by Tukey test at 5% probability

(OLATUNJI; MAKU; ODUMEFUN, 2013). Treatments with mechanical scarification and immersion in sulfuric acid also led to shorter mean time of emergence for *Parkia panurensis* seeds (MELO *et al*., 2011).

Thus, lots of seeds of the same species may exhibit different responses in terms of their utilization, since the speed at which germination occurs is fundamental for survival and development of the species because it reduces the time of exposure of the seeds to adverse conditions and inclement weather (PIVETA *et al*., 2010).

In the second experiment, there was significant interaction between the substrates and temperatures tested for all characteristics evaluated (Table 3).

For first count, it can be observed that the temperature of 30 °C led to values statistically equal to those obtained at 20-30 °C, except in the substrate vermiculite (Table 4). In addition, seeds germinated in paper roll at 30 °C obtained higher germination percentage in shorter time. In study conducted with *M. urundeuva* seeds, evaluating the interaction between temperature and substrate, Guedes *et al.* (2011) also observed that the temperature of 30 ºC combined with the substrates paper towel led to high percentages of germination.

On the other hand, at temperature of 40 °C germination in the first count occurred only in paper roll, probably because this substrate lost less water through evaporation compared to the others (Table 4). At temperatures of 25, 30 and 20-30 °C, with sowing in paper roll, the values were statistically equal to those found in vermiculite. In a survey on the germination of seeds of 272 native tree species, Brancalion, Novembre and Rodrigues (2010) established relationships between optimal temperature and the biome of occurrence, and temperatures of 25 and 30 °C were identified as the most favorable to seed germination.

In *Adenanthera pavonina* seeds, L. Kissmann *et al*. (2008) also obtained higher means at the first count using paper roll. Batista *et al*. (2011), testing different substrates on the germination of *S. oleracea* seeds, observed that vermiculite led to faster germination, compared with other substrates.

Regarding germination percentage, as well as first count, the temperatures of 30 °C and 20-30 °C were statistically equal to one another and superior to the others, regardless of the substrate used (Table 4). The best substrates, regardless of temperature, were paper roll and between vermiculite, corroborating the results of first count. Temperature of 40 °C associated with the substrates between sand, on paper and between vermiculite led to low germination and even zero germination, and germination only occurred in paper roll, with no increment compared with the values observed in the first count, highlighting the superiority of the substrate paper roll. These results corroborate those found by Silva *et al.* (2017), in which *P. platycephala* seeds obtained higher percentage of germination using paper roll as substrate.

The shortest mean times of germination were obtained at temperatures 30 °C and 20-30 °C, regardless of substrate. The substrates sand, paper roll and vermiculite led to the shortest mean times of germination and did not differ from one another. In the substrates sand and paper roll, it was not possible to detect significant difference between the temperatures (Table 4). At all temperatures, the substrate paper roll led to longer mean time of germination for *P. stipulacea* seeds.

Higher germination speed indices were found at temperatures of 30 °C and alternated temperature of 20-30 °C, regardless of substrate. Sowing in paper roll resulted in higher GSI compared with the other substrates, regardless of temperature. At temperatures of 25, 30 and 20-30 °C, the substrates paper roll, on

Table 3 - Summary of analysis of variance for first count (FC), germination (G), germination speed index (GSI) and mean time of germination (MTG) of *Piptadenia stipulacea* Benth. Ducke seeds subjected to different substrates and temperatures

		Average squares			
Source of variation	Degrees of freedom	FC (%)	G (%)	GSI	MTE (days)
S	3	6762,0*	4783.27	34.64	36.58
T	5	10025.2	9019.36	56.26	3.65
SxT	15	2120.4	1327.81	10.77	7.18
Error	72	59.11	76.50	0.38	0.26
C.V(%)		16.45	13.45	13.44	12.78
M.G		47.0	65.0	4.58	4.01

*Significant effect at 5% probability. F.V = source of variation, G.L = degrees of freedom, C.V = coefficient of variation, M.G = general average

Table 4 - Germination first count, germination, germination speed index and germination speed index of *Piptadenia stipulacea* Benth. Ducke seeds subjected to different substrates and temperatures

Temperatures (°C)	Substrates			
	Between sand	Paper roll	About paper	Between vermiculite
Germination first count (%)				
20	68 Aa	21 Db	0 Cc	2 Dc
25	65 Aa	71 BCa	20 Bb	72 Ba
30	57 Ac	90 Aa	75 Ab	89 Aab
35	11 Bc	83 Aba	4 Cc	54 Cb
40	0 Bb	65 Ca	0 Cb	0 Db
20-30	60 Aab	75 ABCa	60 Ab	71 Bab
Germination (%)				
20	83 Aa	79 ABa	59 Cb	81 Aa
25	66 Aa	76 ABa	69 BCa	81 Aa
30	67 Ab	91 Aa	84 ABa	83 Aab
35	14 Bb	87 Aa	12 Db	83 Aa
40	0 Bc	65 Ba	9Dbc	24 Bb
20-30	84 Aa	82 ABa	89 Aa	93 Aa
Germination speed index				
20	6.19 Aa	3.81 Db	2.10 Cc	2.98 Cbc
25	5.44 Ab	6.09 BCa	3.41 Ba	6.31 ABa
30	5.03 Aa	7.53 Aa	6.55 Aa	7.55 Aa
35	1.02 Bc	7.05 ABa	0.97 CDc	5.53 Bb
40	0Bb	5.41 Ca	0.78 Db	0.85 Db
20-30	6.28 Aa	6.49 BCa	6.03 Aa	6.69 ABa
Germination speed index (days)				
20	3.75 Bba	3.17 Aa	7 Cc	4.31 Bb
25	3.05 Ba	3.27 Aa	5.81 Bb	3.43 BAa
30	3.40 Ba	3.04 Aa	3.39 Aa	3.17Aa
35	3.75 Bba	3.17 Aba	6.28 CBc	4.31 Bb
40	0 Aa	3 Ab	7 Cc	7 Cc
20-30	3.71 Ba	3.33 Aa	4.29 Aa	3.73 BAa

*Equal lowercase letters in the row and the same capitals in the column do not differ from each other by the Tukey test at the 5% probability level

paper and between vermiculite led to higher means and did not differ statistically from one another (Table 4). These results corroborate those obtained by Guedes *et al*. (2011), evaluating different temperatures and substrates on the germination of *Myracrodruon urundeuva*. These authors obtained higher germination speed indices at temperature of 30 °C and in the substrate paper roll.

CONCLUSIONS

1. Dormancy of *P. stipulacea* seeds should be overcome by tip removal or scarification with sandpaper, both in the region opposite to the micropyle;
2. Temperatures of 30 °C and 20-30 °C with the substrate paper roll can be used to evaluate the germination and vigor of *P. stipulacea* seeds.

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