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Seed germination and seedling development of *Anadenanthera colubrina* in response to weight and temperature conditions

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Abstract: The aim of the study was investigated the effects of seed weight and temperature on the germination behavior, and development of seedlings of *Anadenanthera colubrina*. Germination was carried out using the five constant temperature regimes (20, 25, 30, 35 and 40ºC) under photoperiod (12h) and two classes of seeds: heavy ($\geq$ 0.095g) and light (<0.095g). The experimental design was totally random, with four replications of 25 seeds. The temperature was significant for germination percentage, germination velocity index and mean germination time. There was no effect of seed weight, and there was no interaction between temperature and seed weight for three response variables. Optimum germination for *A. colubrina* seeds was recorded in four of the five temperatures studied (20, 25, 30 and 35ºC) for both heavy and light seeds. At each temperature, the average root length was similar and, the seedling dry weight was greater for the heavy seed class. There was no effect of temperature on the seed mass to the production of normal seedlings, except at 40ºC. Variation in seed weight, in *A. colubrina*, can be a part of their strategy for increasing the probability of success of seed germination and subsequent survival of the species a regeneration niche.

Keywords: Seed Mass, Semi-Arid, Native Species, Seed Size

1. Introduction

Seed is one of the least plastic organs of a plant (Ruiz de Clavijo 2002), yet variations in size and mass occur due to environmental conditions such as temperature and water availability (Rees and Westoby 1997, Probert and Brenchley 1999) and genetic variations, e.g., number of ovules per flower (Wiens 1984). Despite this variation, all plants should have an ideal seed size that optimizes germination and seedling establishment in natural habitats. Plants with seeds above or below this size will have low or high reproductive fitness (Rees and Westoby 1997, Vaughton and Ramsey 1998).

Seed germination and seedling establishment are stages of the plant life cycle that are related to the regeneration niche (Grubb 1977) and variation in seed mass may have distinct consequences for seed germination and seedling survival (Ruiz de Clavijo 2002, Du and Huang 2008, Parker et al. 2006). Due to larger seed reserves, seedlings from larger and heavier seeds are able to withstand longer periods of drought stress and high temperatures than those of small seeds (Khurana and Singh 2000, Du and Huang 2008). Their seedlings produce longer roots which reaches moisture from the deeper layers of the soil (Baker 1972, Schimpf 1977). As larger-seeded species are able to endure unfavorable conditions for longer (Leishman and Westoby 1994, Liu et al. 2007, Zabala et al. 2011, Harel et al. 2011) they are more often found in dry environments (Wright and Westoby 1999).

Several studies demonstrate the competitive advantage of large seeded plants related to its percentage of germination and seedling establishment (Milberg et al. 1996, Vaughton and Ramsey 1998, Ruiz de Clavijo 2002, Du and Huang 2008). Therefore, some species tend to produce large fruits and seeds to increase their reproductive fitness. Species producing heavier seeds often exhibit seedlings with greater initial height with more chance to reach better light conditions and survivorship (Parker et al. 2006), while light seeded plants usually have lower reproductive performance (Ribeiro et al. 2012).

The semi-arid vegetation of the Brazilian northeast, hereafter named Caatinga, is a tropical forested ecosystem controlled by the timely onset of precipitation and its irregular distribution concentrated from February to May (Ab'Sáber 1974). We believe that in this stressful ecosystem larger seeds are more successful in terms of the time and percentage of germination and seedling traits to endure such unfavorable conditions. To test out hypothesis we studied the relation between seed and seedling size of *Anadenanthera colubrina*, a widely tree species found in the northeast Brazil. The following questions were posed: a) does seed size, in terms of biomass, of *A. colubrina* interfere on the germination rate and time? b) does seedling size related to the seed size? c) Is there any relation interaction between temperature and seed size on the germination and seedling development? To answer these questions the effects of five different temperatures on germination of light and heavy *A. colubrina* seeds were studied.

2. Materials and Methods

Anadenanthera colubrina var. *cebil* (Griseb.) Altschul, Leguminosae, is a tropical tree that reaches up to 10-12 m height and diameter at breast hight of 20 – 30 cm. The fruit is a legume with 17-30 cm length with 8-15 seeds. As a fast growing species it has been used for reforestation of degraded areas (Lorenzi 1992). It is widely distributed throughout Brazil, except in the South and in the southern Bolivia and northern Argentina. It is one of the commonest species in the Caatinga (Prado, 2008). Wood of *A. colubrina* is used in construction, carpentry, firewood and charcoal while seeds and trunk bark are used in tanning industries.

The ripe fruits of *A. colubrina* were collected in November 2010, from three randomly selected trees in an area of Caatinga located in the municipality of Banabuiú, Ceará (38º 56'14,6'' W e 5º 10'03,9'' S). Fruits were taken to the Seed Analysis Laboratory, in the Department of Plant Science, at the Federal University of Ceará. There, seeds were manually removed and stored under an average temperature of 8ºC for approximately twenty months before the experiments.

2.1. Biometrics and Seed Moisture Content

In the initial seed characterization we determined the water content, using an oven at 105 ± 3ºC for 24 hours, according to the method described Brazil (2009). Seeds were mixed and then we took 100 seeds by random for biometric measurement. We measured the weight with a precision scale and length, width and thickness with a digital caliper. Length was measured from the base to the apex and width and thickness measurements were taken from the midline. Seeds were then separated into two size classes: heavy (seeds weighing ≥0.095 g) and light (seeds weighing <0.095 g).

2.2. Germination Tests

Seeds were sown in two sheets of germitest filter paper which was arranged in 14 cm diameter Petri dishes and moistened with distilled water at a ratio of two and a half times the weight of the paper. When necessary, the substrate was re-watered. The Petri dishes were placed in a BOD (Biochemical Oxygen Demand) germination chamber, with a photoperiod of 12 hours of light / 12 hours of dark and constant temperatures (20º, 25º, 30º, 35º and 40ºC) with variation of ± 1ºC. Germination was recorded daily, the evaluation criteria being the presence of a radicle of at least 2mm. Four replicates of 25 seeds each were used for each treatment.

The Percentage of germination was calculated on the basis of 25 seeds per replicate, totaling 100 seeds per treatment. Germination velocity index (GVI) was based on the model of Maguire (1962) as follows: $GVI = G_1/N_1 + G_2/N_2 + ...+ G_n/N_n$, where: $G_1, G_2... G_n$ = number of seeds germinated each day; and $N_1, N_2 ... N_n$ = number of days from sowing to first and last count.

Mean germination time (MGT) was evaluated using the method proposed by Labouriau (1983), with the results expressed in days: $MGT = G_1.T_1 + G_2.T_2 + ... + G_n.T_n/\sum G$, where: G_1, G_2, G_n = number of seeds germinated at the first, second and third counts; and T_1, T_2, T_n = number of days after sowing. Length of seedling root (LSR) was measured from the root tip to the insertion point of the cotyledons and average was calculated following Nakagawa (1999).

To estimate seedling dry weight (SDW) after removed seedlings from the substrate we removed the cotyledons and dried them at 80°C for 24 hours. They were subsequently cooled in a desiccator, and then weighed on a precision balance.

At the end of the germination experiment, which lasted five days, seedlings were classified as normal, abnormal and not germinated, according to Rules for Seed Testing (Brazil 2009). Normal seedlings were considered as those that showed the potential to continue development producing normal plants, plants having all essential structures, when grown under favorable conditions. Abnormal seedlings were those that did not show the potential to continue development producing damaged, deformed or deteriorated seedlings. Seeds were considered dead if they did not germinate and soften with any signs of early germination.

2.3. Statistical Analysis

A multivariate analysis of variance (MANOVA) was used to evaluate the effects of the different assay treatments on seed germination and seedling biometry of *A. colubrina*. Data was analysed using SPSS 20 software for Windows (SPSS Inc., Chicago, Illinois, USA, 2011). Means that showed significant differences were compared using a Bonferroni adjusted alpha level of 0.017 (Germination Percentage, Germination Velocity Index, and Mean

Germination Time) and alpha level of 0.01(average Length of Seedling Root, average Seedling Dry Weight, Normal Seedling, Abnormal Seedling and Ungerminated Seeds). To meet the criteria of normality, germination percentage data was arc sine √x/100 transformed.

3. Results and Discussion

We verified that seeds of *A. colubrina* showed irregularity in size and weight (Table 1), showing variation in length (9.14 to 12.80 mm), width (8.67 to 12.62 mm) and thickness (0.62 to 1.45 mm). Seed weight ranged from 0.042 to 0.130 g. Weight of 1000 seeds was 92.27 grams (sd=1.18) and moisture content of 9.83%, which allows us to infer that a kilogram of *A. colubrina* seeds contain around 10.837 seeds.

Table 1. *Mean, standard deviation (Sd), standard error (Se), and coefficient of variation (CV) of seed traits (length, width, thickness and weight) and for light, and heavy seeds of Anadenanthera colubrina var. cebil (Griseb.) Altschul. Heavy were seeds weighing ≥0.095g and light were seeds weighing <0.095g.*

Variable	Mean	Sd	Se	CV(%)
Length (mm)	11.16	0.71	0.07	6.34
Width (mm)	11.16	0.70	0.07	6.27
Thickness (mm)	0.091	15.82	1.58	17.29
Light (g)	0.081	0.01	0.07	13.58
Heavy	0.104	0.007	0.10	6.73

Anadenanthera colubrina seeds can be classified as medium size (11.16 x 11.16 mm) for woody species that occur in the semiarid Caatinga region of northeastern Brazil. Barbosa (2008) studied the strategies for germination and growth of eleven woody Caatinga species with rapid germination. The species studied presented medium sized seeds, ranging from 12-14.6 x 10-13 mm. In the Caatinga environment, smaller seed sizes are more effective in absorbing water. Thus, seed germination and establishment is in the rainy season from three to four months (Barbosa 2008).

Seeds of *A. colubrina* are not dormant, their sizes, and weights are non-uniform and germinate rapidly with high rates of germination (90%). Germination is immediate, occurring from the second day after sowing, with duration of four days between the beginning and end of germination. *A. colubrina* showed 80-100% germination after 48 hours of observation without requiring treatment to estimulate germination due to the thin integument and presence of a fissure line or pleurogram (Barbosa 2008). According to Melo-Pinna et al. (1999), the pleurogram is a region of increased frailty on the surface integument where the rapid imbibition of water occurs during the short rainy season.

The multivariate analysis of variance (MANOVA) indicated a significant effect of temperature on all three response variables: percentage of germination, germination velocity index, and mean germination time (F= 7.787; p< 0.0001; Wilk's Lambda = 0.061; partial η^2 = 0.503). Through the analysis of dependent variable, using a Bonferroni adjusted alpha level of 0.017, we confirmed such result (Table 2). Contrastingly, we observed no effect of seed size (F= 2.373; p= 0.077; Wilk's Lambda= 0.740; partial η^2 = 0.260) and of the interaction between temperature and seed size for all three response variables (F= 1.397; p= 0.164; Wilk's Lambda= 0.483; partial η^2 = 0.166).

Table 2. *Results of analysis of variance (ANOVA) using a Bonferroni adjusted alpha level of 0.017 for germination percentage (Germination %), Germination Velocity Index (GVI) and Mean Germination Time (MGT) of heavy and light Anadenanthera colubrina var. cebil (Griseb.) Altschul seeds treated at different temperatures. Df=degrees of freedom*

Dependent Variable	Df	Mean Square	F	Sig.	Partial Eta Squared (η^2)
Germination%	4	519.70	5.511	0.002	0.424
GVI	4	43.94	13.033	<0.001	0.635
MGT (days)	4	1.07	15.936	<0.001	0.680

The germination rate of both light (51%) and heavy seeds (71%) were lower at 40ºC. All different temperatures analyzed influenced germination of the *A. colubrina*. Within each temperature analyzed the germination rate was statistically similar between the light and heavy seeds (Table 3). We found no differences on the germination velocity index and mean germination time between light and heavy seeds in all temperatures (Table 3). Also, we didn't observe any difference on the mean germination time between light and heavy seeds which was higher at 20ºC for both seed classes (Table 3).

Table 3. *Averages of germination rate (%), germination velocity index (GVI) and germination time (MGT) of heavy and light seeds of Anadenanthera colubrina var. cebil (Griseb.) Altschul at five different temperature treatments.*

T (°C)	Germination %	
	Seeds light	Seeds heavy
20	(90) 74.18± 11.34aA	(87) 69.64± 7.26aA
25	(79) 64.02± 11.33aA	(80) 63.62± 4.27aA
30	(87) 72.21± 14.17aA	(85) 67.93± 7,16aA
35	(75) 60.73 ± 9.12aA	(93) 77.11± 9.91aA
40	(51) 45.61 ±7.92aB	(71) 58.30± 10.91aB
T (°C)	**Germination velocity index (GVI)**	
	Seeds light	Seeds heavy
20	8.78 ±1.25aA	9.31±1.32aA
25	12.87± 3.15aB	11.12 ±0.75aB
30	11.75± 1.94aAB	11.50 ±1.68aAB
35	12.83± 2.48aB	13.99±2.14 aB
40	6.45 ±1.11aAC	8.83 ±1.18aAC
T (°C)	**Germination time (MGT)**	
	Seeds light	Seeds heavy
20	2.70 ±0.17aA	2.60 ±0.45aA
25	1.70 ±0.10aB	1.88 ±0.08aB
30	1.91 ±0.02aB	1.92 ±0.07aB
35	1.70 ±0.13aB	1.84 ±0.15aB
40	2.06 ±0.31aB	2.21 ±0.53aB

Means followed by the same lowercase (rows, seed weight) and uppercase (columns, temperatures) letters are not statistically different from each other, at Bonferroni adjusted alpha level of 0.01. Values are mean ± standard deviation (n = 4). Values in parentheses represent the non-transformed percentage of germination.

Optimum germination for *A. colubrina* seeds was recorded in four of the five temperatures studied (20, 25, 30 and 35ºC) for both heavy and light seeds. Thus we can assume that there was no effect the seed weight and, seed weight interacted with temperature on germination percentage for *A. colubrina*. We found that seeds of *A. colubrina* differs from other species of semi-arid regions. Generally, environmental factors such as restrictions in resource availability (water) in the growing season induce intra-specific seed size differences (Vaughton and Ramsey 1998). Moreover, in these environments, small seeds are mostly regulated by temperature and only respond to a germination signal when the availability of water and nutrients are sufficient for seedling establishment (Easton and Kleindorfer 2008).

Some species native to regions with seasonal climates use a strategy in which germination is high when environmental conditions are favorable. Perez-Garcia and Gonzalez-Benito (2006) argue that for some Mediterranean plants, the ideal temperature and soil moisture conditions are triggers of germination in early spring which reaches a maximum upon the arrival of the next season (winter), gathering the necessary humidity to ensure seedling establishment. In fact, variation in seed weight of *A. colubrina* can be a part of their strategy to guarantee the germination and subsequent survival of the species (Grubb 1977). The semi-arid climate of north-eastern Brazil é characterized by the unpredictability of the rainy season, in which it may rain or not, and the amount of rainfall is highly variable in time and space (Andrade-Lima 1981). Heavier seeds can germinate during favorable and survival during unfavorable environmental conditions. On the other hand, light seeds would have a better chance to disperse and establish in a safe place.

It is known that, under low temperature, the absorption of water and re-hydration of tissues can occur, but it may be that the recovery of growth of the embryonic axis does not occur or that damage to the embryo precludes the completion of germination. Moreover, at high temperatures imbibition can occur without allowing embryo growth, inducing dormancy (Bewley and Black 1994). In the case of *A. colubrina*, successful germination demonstrated by the percentage of germination in the two seed weight classes showed that seed germination occurs over a wide temperature range. It may be that this is due to the fragile seed integument and presence of a pleurogram which facilitates the absorption of water independent of seed mass. It was found that germination of this species at a minimum temperature of 10ºC, with 42%, at a maximum of 40ºC, with 90%, and optimal within the range 30-35°C, with a percentage of 89-100% (Barbosa 2008).

We observed that seed size influenced all seedlings traits (root length, biomass and morphology-normal x abnormal seedling) (MANOVA, F= 86.551; p< 0.0001; Wilk's Lambda= 0.072; partial η^2= 0.928). Temperature also influenced seedling traits (F= 16.372; p< 0.0001; Wilk's Lambda = 0.013; partial η^2= 0.663) while we observed no interaction between temperature and seed mass for all traits (F= 1.211; p= 0.278; Wilk's Lambda= 0.527; partial η^2 = 0.148). These results were confirmed using a Bonferroni adjusted alpha level of 0.01 for the analysis of each individual dependent variable. Thus, we found no effect of seed size on root length but seedling biomass was positively related to seed size (Table 4). The shortest root length was observed at 40ºC while seedling biomass was significantly different between the five different temperatures (Table 4).

Table 4. *Average root length and seedling biomass from light and heavy seeds of Anadenanthera colubrina var. cebil (Griseb.) Altschul germinated at five different temperatures. Values are mean ± standard deviation (n = 4).*

T (ºC)	Root length (cm/seedling)		Seedling dry weight (g/seedling)	
	Light seeds	Heavy seeds	Light seeds	Heavy seeds
20	2.79 ± 0.15 aC	2.94 ± 0.43 aC	0.067 ± 0.01 bA	0.089 ± 0.0 aA
25	4.71 ± 0.46 aBA	5.07 ± 0.37 aBA	0.062± 0.01 bAB	0.085 ± 0.0 aAB
30	5.03 ± 0.16 aA	5.27 ± 0.19 aA	0.065 ± 0.01 bA	0.088 ± 0.01 aA
35	4.23 ± 0.52 aB	4.51 ± 0.18aB	0.063± 0.01 bAB	0.080±0.01 aAB
40	1.60 ± 0.20 aD	1.51 ±0.30 aD	0.058± 0.01 bBC	0.080 ± 0.0 aBC

Means followed by the same lowercase (rows) and uppercase (columns) letters, are not statistically different, at Bonferroni adjusted alpha level of 0.01.

Variation in seed weight did not influence the speed of germination or the mean germination time of *A. colubrina* seeds. It was expected that heavier seeds would have a faster, shorter germination because of the difference in their content, particularly in starch and protein. Meanwhile, both seed weight classes, heavy and light, showed increased speed and shorter germination times at 25, 30 and 35ºC. This result contradicts other studies in arid regions by predicting that the seed weight affects the timing of seed germination. A study by Easton & Kleindorfer (2008) supports this argument. In their study, heavier seeds of the Frankenia species had the highest level of successful germination (85%) at 17 and 23°C and this decreased at 29ºC (to 59%) when compared with light seeds (83% at 17ºC, 66% at 23ºC and only 40% at 29ºC).

On the other hand, Gross and Kromer (1986) evaluated the effect of different seed weight (0.3282 to 0.6940 mg) on the germination and growth of *Oenothera biennis* and found that it had little effect that did not persist beyond four weeks. But, Paz et al. (1999) found no significant effect between seed mass and emergence rate of seven species of Psychotria. These authors concluded that the effects of seed mass on seedling emergence are driven by external ecological factors more than by intrinsic effects of seed mass. One of the environmental factors that may influence the relationship between seed mass and the emergence rate is the availability of water in the soil. Large seeds may have difficulty in obtaining sufficient water for germination from temporarily available water supplies because of their low ratio of surface to volume (Harper et al. 1970). Consequently, it is expected that small seeds of species that occur in semiarid regions have a competitive advantage to begin the process of germination in dry soils.

As *A. colubrina* survives in soils subject to water stress, increased growth of the main root and greater seedling dry weight were expected in seedlings of heavy rather than light seeds at the five temperature treatments. However, *A. colubrina* root length was similar in both the seed classes, light and heavy. In contrast, seedling dry weight was greater in heavy seeds than in light. In general, in regions with marked climatic seasonality, heavier seeds produce larger seedlings with longer roots to survive periods of low soil moisture (Easton and Kleindorfer 2008). In this case, it is assumed that the greater reserves present in heavy *A. colubrina* seeds allowed the differentiation in seedling dry weight in comparison to the light seeds at the temperatures analyzed. However, the quantity of nutrients present in both weight classes was insufficient to promote differentiation in primary root length.

The percentage of non-germinated seeds and abnormal seedlings were higher at 40ºC, at this temperature heavy seeds produced more normal seedlings than the light ones (Table 5).

Table 5. *Percentage of normal and abnormal seedlings, and ungerminated heavy and light Anadenanthera colubrina var. cebil (Griseb.) Altschul seeds germinated at five different temperatures. Values are mean ± standard deviation (n = 4).*

T (°C)	% Normal seedlings		%Abnormal seedlings		%Ungerminated seeds	
	Light seeds	Heavy seeds	Light seeds	Heavy seeds	Light seeds	Heavy seeds
20	21.50 ±1.29aA	22.50 ±2.36aA	1.75 ±0.96aB	0.50 ±0.58aB	1.75 ±1.50aB	2.25 ±1.89aB
25	19.25 ±2.99aA	20.00 ±1.41aA	4.00 ±1.63aAB	3.75 ±1.26aAB	1.75 ±1.50aB	1.25 ±0.50aB
30	21.75 ±3.40aA	21.25 ±1.89aA	2.00 ±2.71aAB	3.00 ±1.41aAB	1.25 ±0.96aB	0.75 ±0.96aB
35	19.00 ±2.94aA	22.50 ±1.91aA	2.75 2.0±6aAB	1.50 ±1.29aAB	3.25 ±2.22aB	1.00 ±1.41bB
40	12.75 ±3.40bB	17.75 ±3.86aB	5.75 ±3.10aA	3.75 ±2.75aA	6.50 ±1.91aA	3.50 ±1.73bA

Means followed by the same lowercase (rows) and uppercase (columns) letters, are not statistically different from each other, at at Bonferroni adjusted alpha level of 0.01

For the five temperatures studied, the lowest percentage of normal seedlings occurred at 40ºC and at this temperature heavy seeds produced more normal seedlings than the light ones. The production of normal seedlings is a competitive advantage for the successful establishment of the future population.

4. Conclusions

Seed germination of both classes, heavy and light, occurred at temperatures of 20, 25, 30, 35 and 40ºC. There was no effect of seed weight, and there was no interaction between temperature and seed weight for three response variables germination percentage, germination velocity index and mean germination time. At each temperature, the average root length was similar, and the seedling dry weight was greater for the heavy seed class. There was no effect of seed weight on the production of normal seedlings, except at 40ºC. Variation in seed weight, in *A. colubrine* increases the probability of success of seed germination and seedling establishment.

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