

DO SEEDS OF *Anadenanthera macrocarpa* AND *Pityrocarpa moniliformis* FORMED IN DIFFERENT LOCATIONS OF THE FRUIT HAVE SIMILAR PHYSIOLOGICAL QUALITY?

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Received for publication: 16/08/2020 – Accepted for publication: 11/11/2020

Resumo

Sementes de Anadenanthera macrocarpa e Pityrocarpa moniliformis formadas em locos distintos no fruto apresentam qualidade fisiológica semelhante? Árvores da família Fabaceae podem produzir sementes com tamanho e desempenho germinativo contrastantes dependendo da região onde foram formadas no fruto. Essas diferenças causam redução da qualidade fisiológica do lote de sementes. Assim, objetivou-se com este trabalho avaliar a qualidade fisiológica de sementes formadas em locos distintos nos frutos de Anadenanthera macrocarpa e Pityrocarpa moniliformis, e quais suas implicações para a composição final do lote de sementes. A caracterização biométrica dos frutos foi realizada com quatro descritores quantitativos (comprimento, largura, espessura e número de sementes por fruto). Em seguida, sementes formadas em locos da região proximal, intermediária e distal do fruto foram mensuradas (comprimento, largura, espessura) e avaliadas usando testes de germinação e vigor (velocidade de germinação, comprimento e massa seca de plântulas). Sementes destas espécies possuem diferenças biométricas em função da sua origem no fruto, sendo aquelas formadas nos locos da região intermediária do fruto maiores do que as demais. A origem das sementes de A. macrocarpa e P. moniliformis formadas em locos distintos do fruto não apresentam diferenças de qualidade fisiológica, portanto, a triagem de sementes originadas em regiões distintas do fruto não beneficia a qualidade final dos lotes de sementes destas espécies.

Palavras-chave: angico, biometria, catanduva, germinação, sementes florestais.

Abstract

Trees of the Fabaceae family can produce seeds with contrasting size and germinative performance depending on the region where they were formed in the fruit. Such differences reduce the physiological quality of the seed lot. Thus, the aim of the present study was to evaluate the physiological quality of seeds formed in different seminal nucleus of the fruits of *Anadenanthera macrocarpa* and *Pityrocarpa moniliformis* and their implications for the final composition of the seed lot. The biometric characterization of the fruits was performed with four quantitative descriptors (length, width, thickness, and number of seeds per fruit). Then, seeds formed in seminal nucleus of the proximal, intermediate, and distal regions of the fruit were measured (length, width, and thickness) and evaluated using germination and vigor tests (germination speed, length, and dry weight of seedlings). Seeds from these species have biometric differences depending on their origin in the fruit, in which those formed in the seminal nucleus of the intermediate region of the fruit are larger than the other ones. The origin of the seeds of *A. macrocarpa* and *P. moniliformis* formed in different seminal nucleus of the fruit do not show differences in physiological quality. Therefore, the sorting of seeds originated in different regions of the fruit does not benefit the final quality of the seed lots of these species.

Keywords: angico, biometrics, catanduva, germination, forest seeds.

INTRODUCTION

The obtaining quality seeds is essential for commercialization, storage, and sowing (MARCOS-FILHO, 2015) and choosing the seed is an important step for obtaining vigorous seedlings, both in the production and post-planting phases (OLIVEIRA *et al.*, 2013). For this reason, studies that verify the outcome of the seed formation region in the fruit on the germinative performance of forestry species is fundamental for obtaining higher quality lots (FREITAS *et al.*, 2013).

In *Moringa oleifera* Lam., it was verified that seeds formed in the proximal and distal regions of the fruit originated plants more vigorous than the seeds formed in the intermediate region (OLIVEIRA *et al.*, 2013). The effect of the seed formation region in the fruit extends to the seedling production phase of *Bertholletia excelsa* Humb. & Bonpl, in which seeds formed in the proximal region of the fruit provided higher levels of morphological quality (DIONISIO *et al.*, 2019).

Species of the Fabaceae family can produce seeds with contrasting size and germination depending on the region in which they were formed in the fruit (ROCHA *et al.*, 2017; SANTOS *et al.*, 2019). The seeds developed in the distal and proximal regions tend to be smaller than the ones in the intermediate regions (OLIVEIRA; MORAIS, 1997). The size of the seed may or may not affect the final germination rate; however, those of larger size tend to produce more vigorous seedlings, maximizing the uniformity of the seed lot of cultivated plants (CARVALHO; NAKAGAWA, 2012). However, this statement is controversial for forestry species.

Studies evaluating the physiological quality of seeds formed at different locations in the fruit are based on the relationship between the biometric aspects of the seeds and the results of physiological tests. In addition, the biometric characterization of seeds provides relevant information for the seed classification in order to obtain homogeneous lots (DUTRA *et al.*, 2017; SILVA *et al.*, 2017; SANTOS *et al.*, 2018).

The effect of seed formation in different regions of the fruit on germination performance was studied for some forestry species of the Fabaceae Family, including *Leucaena leucocephala* (Lam.) de Wit, *Prosopis juliflora* (Sw.) DC (OLIVEIRA; MORAIS, 1997), *Caesalpinia ferrea* Mart. ex Tul. (NOGUEIRA *et al.*, 2010), *Mimosa caesalpiniiifolia* Benth. (FREITAS *et al.*, 2013), *Enterolobium contortisiliquum* (Vell.) Morong (LESSA *et al.*, 2014) and *Cenostigma pyramidale* (Tul.) Gagnon & G. P. Lewis (MENDONÇA *et al.*, 2016). The results of these studies have not shown a consistent pattern in relation to the physiological quality of seeds formed from distinct seminal nucleus in the fruit.

The sorting of seeds formed in different regions of the fruit could benefit the final quality of the seed lot. Therefore, a hypothesis was raised for two species native to the Caatinga with wide potential for use: do seeds of *Anadenanthera macrocarpa* (Benth.) Brenan (angico) and *Pityrocarpa moniliformis* (Benth.) Luckow & R.W. Jobson (catanduva) formed in different seminal nucleus of the fruit have similar physiological quality?

The *A. macrocarpa* (Fabaceae) has a high tannin composition in its bark and is used as a herbal medicine (ARAÚJO *et al.*, 2018) for treatment of respiratory and inflammatory diseases and healing processes (FIGUEREDO *et al.*, 2013). This species also contributes to the recovery of degraded areas by providing rapid carbon removal and triggering important environmental roles in landscape restoration (MORAIS-JUNIOR *et al.*, 2020). The *P. moniliformis* (Fabaceae) has a high content of antioxidants that fight cancer cells (ALVES *et al.*, 2014) and stands out among the main tree species in the Caatinga for the supply of honey (JESUS *et al.*, 2015).

In view of the hypotheses raised and the potential for the use of these species, the aim of the present study was to evaluate the physiological quality of seeds formed in different seminal nucleus of the fruits of *A. macrocarpa* and *P. moniliformis* and their implications for the final composition of the seed lot.

MATERIAL AND METHODS

Fruits were collected manually in five individuals of *A. macrocarpa* and eight individuals of *P. moniliformis* from populations located in the municipalities of Parelhas (6°42'38" S and 36°36'39" W) and Macaíba (5°53'7" S and 35°21'38" W), Rio Grande do Norte State, Brazil. The fruits were collected in the ripe stage, evidenced by the characteristic color and signs of spontaneous opening.

The biometric characterization of the fruits was performed by measuring length (cm), width (mm), thickness (mm) and number of seeds of 100 samples. Based on the length and number of seeds per fruit, the limits of the proximal, intermediate, and distal regions of the fruits were determined (Figure 1). The seeds from the respective regions of the fruit were separated for biometric characterization and evaluated for physiological quality through germination and vigor tests. The biometric characterization of the seeds was performed by measuring length (mm), width (mm), and thickness (mm) of 100 samples.

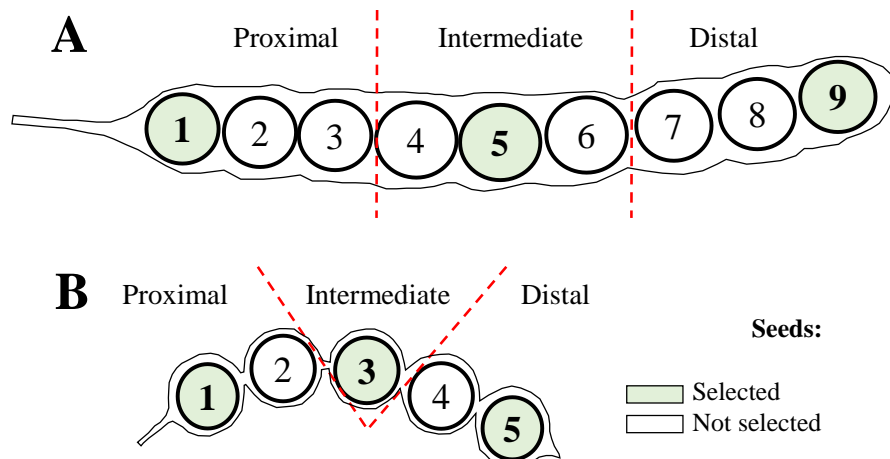


Figure 1. Illustrative representation of the seed formation region in the fruit and selection of *Anadenanthera macrocarpa* (A) and *Pityrocarpa moniliformis* (B) seeds.

Figura 1. Representação esquemática da região de formação da semente no fruto, e seleção das sementes de *Anadenanthera macrocarpa* (A) e *Pityrocarpa moniliformis* (B).

For the germination test, seeds of *P. moniliformis* were previously scarified with metal file in the region opposite the micropyle to overcome physical dormancy. Then, the seeds of *A. macrocarpa* and *P. moniliformis* were disinfected in sodium hypochlorite solution NaClO (2.5% / 5 min) and sown between three sheets of paper towels (Germitest® type) as substrate, which was moistened with distilled water at a rate of 2.5 times the dry weight of the sheet. The paper sheets were arranged in a roll system and incubated in a BOD (Biochemical Oxygen Demand) type germination chamber regulated at a temperature of 25 °C and 12 h photoperiod. The test lasted ten days for *A. macrocarpa* and 21 days for *P. moniliformis*.

The physiological quality of the seeds was evaluated based on the variables: (a) germination - rate of normal seedlings; (b) germination speed index (GSI) - calculated according to Maguire (1962) based on the daily count of the number of seeds germinated in the germination test; (c) length (cm) - measured root and shoot with a graduated scale in mm; and (d) dry weight of seedlings (mg) - root and shoot weight after drying in a convection oven (80 °C / 48 h).

The experiment was performed in a completely randomized design with three treatments (seeds formed in the proximal, intermediate, and distal regions of the fruits) and four replications of 50 seeds for each species. All data were initially subjected to normality tests of Shapiro-Wilk ($n < 50$) and Lilliefors ($n > 50$). Data on the biometric characterization of fruits and seeds were analyzed using descriptive statistics, with the relationship among the variables being revealed by Spearman's rank correlation (r_s). For the size (length, width, and thickness of seeds) and physiological quality of seeds (germination and vigor), the analysis of variance (ANOVA) was applied with comparison of means by the Tukey's test at 5% probability level (parametric data) and Kruskal-Wallis at 5% probability level for non-parametric data. The software used for statistical analysis was BioEstat® (version 5.3).

RESULTS

The fruits of *A. macrocarpa* are a type of legume, long, dry, dehiscent polyspermic, 19.79 ± 0.30 cm long, 17.86 ± 0.19 mm wide and 1.78 ± 0.02 mm thick containing between four and 14 seeds per fruit (average of 9.15 ± 0.2). The fruits of *P. moniliformis* are a type of legume, long, dry, dehiscent polyspermic, 8.56 ± 0.19 cm long, 8.74 ± 0.07 mm wide and 2.80 ± 0.04 mm thick containing between two and nine seeds per fruit (average of 5.33 ± 0.15). Greater and lesser variation (CV) of the data set was attributed to the number of seeds per fruit and fruit width for both species (Table 1), respectively.

Table 1. Descriptive statistics for the biometric characterization of fruits of *Anadenanthera macrocarpa* and *Pityrocarpa moniliformis*.

Tabela 1. Estatística descritiva para a caracterização biométrica de frutos de *Anadenanthera macrocarpa* e *Pityrocarpa moniliformis*.

Descriptors	Min	Max	Average \pm standard error	CV (%)	S	K
<i>Fruits of Anadenanthera macrocarpa</i>						
Length (cm)	10.20	27.50	19.79 \pm 0.30	18.14	-0.01	-0.37
Width (mm)	8.80	22.80	17.86 \pm 0.19	12.53	-0.83	1.83
Thickness (mm)	1.20	2.60	1.78 \pm 0.02	18.87	0.73	-0.13
Number of seeds per fruit	4.00	14.00	9.15 \pm 0.2	26.20	0.05	-0.67
<i>Fruits of Pityrocarpa moniliformis</i>						
Length (cm)	4.10	13.70	8.56 \pm 0.19	22.65	0.33	0.01
Width (mm)	6.90	10.20	8.74 \pm 0.07	8.43	-0.23	-0.82
Thickness (mm)	1.40	3.80	2.80 \pm 0.04	17.43	-0.71	0.65
Number of seeds per fruit	2.00	9.00	5.33 \pm 0.15	28.09	0.43	-0.13

CV: coefficient of variation; S: skewness; K: kurtosis.

CV: coeficiente de variação; S: assimetria; K: curtose.

The normality tests showed no normal distribution of data for the biometric descriptors of fruits in both species. Fruits of greater length ($S = -0.01$) and width ($S = -0.83$), thinner ($S = 0.73$) and higher number of seeds per fruit ($S = 0.05$) were predominant in *A. macrocarpa*, as observed in the displacement distribution curve (skewness) (Table 1). For *P. moniliformis*, fruits of greater width ($S = -0.23$) and thickness ($S = -0.71$), greater length ($S = 0.33$) and lower number of seeds per fruit ($S = 0.43$) were predominant. In all biometric descriptors for both species, platykurtic kurtosis ($K < 3$) was observed, i.e., greater amplitude of the biometric values due to the flatter curve in relation to the normal distribution of the data.

Seeds of *A. macrocarpa* and *P. moniliformis* formed in seminal nucleus of the intermediate region of the fruit show greater length (10.17 and 6.42 mm), width (10.86 and 5.45 mm) and thickness (0.89 and 1.47 mm) in relation to seeds formed in the proximal and distal regions (Table 2). Therefore, seeds from these species formed in the intermediate region of the fruit are larger.

Table 2. Biometric characterization of seeds of *Anadenanthera macrocarpa* and *Pityrocarpa moniliformis* formed at different seminal nucleus in the fruit.

Tabela 2. Caracterização biométrica de sementes de *Anadenanthera macrocarpa* e *Pityrocarpa moniliformis* formadas em locos distintos no fruto.

Seed formation region in the fruit	Length (mm)	Width (mm)	Thickness (mm)
<i>Anadenanthera macrocarpa</i>			
Proximal	9.39 b*	9.84 c**	0.80 b**
Intermediate	10.17 a*	10.86 a**	0.89 a**
Distal	9.53 b*	10.18 b**	0.81 b**
<i>Pityrocarpa moniliformis</i>			
Proximal	6.25 b**	5.04 b*	1.44 b**
Intermediate	6.42 a**	5.45 a*	1.47 a**
Distal	6.10 b**	5.07 b*	1.45 b**

Averages followed by the same letter in the column do not differ by Tukey's test* (parametric data) and Kruskal-Wallis** (non-parametric data) at 5% probability level.

Médias seguidas da mesma letra na coluna não diferem entre si pelo teste de Tukey* (dados paramétricos) e Kruskal-Wallis** (dados não paramétricos) ao nível de 5% de probabilidade.

The biometric characterization of fruits revealed no correlation between length, width, and thickness of fruits for these species, except length and width of fruits of *A. macrocarpa* ($r_s = 0.568^{**}$) (Table 3), showing range of variation in the formation of fruits. A significant correlation for all seed variables formed in seminal nucleus of

the distal and intermediate regions of the fruit is verified, whereas those formed in the proximal region show only a correlation between length and width ($r_s = 0.329^{**}$). For *P. moniliformis*, a significant correlation in all seed variables resulting from the region of formation in the fruit was found for those formed in the proximal and intermediate regions, while there was only a correlation between length and width of the seed formed in the distal region ($r_s = 0.550^{**}$).

Table 3. Spearman's correlation (r_s) for the biometrics of fruits and seeds of *Anadenanthera macrocarpa* and *Pityrocarpa moniliformis* formed in different seminal nucleus in the fruit.

Tabela 3. Correlação de Spearman (r_s) para a biometria de frutos e sementes de *Anadenanthera macrocarpa* e *Pityrocarpa moniliformis* formadas em locos distintos no fruto.

Correlation	Fruits	Seed formation region in the fruit		
		Proximal	Intermediate	Distal
<i>Anadenanthera macrocarpa</i>				
Length x width	0.568 ^{**}	0.329 ^{**}	0.666 ^{**}	0.693 ^{**}
Length x thickness	0.145 ^{ns}	0.058 ^{ns}	0.389 ^{**}	0.408 ^{**}
Width x thickness	0.090 ^{ns}	0.019 ^{ns}	0.384 ^{**}	0.409 ^{**}
<i>Pityrocarpa moniliformis</i>				
Length x width	-0.096 ^{ns}	0.669 ^{**}	0.605 ^{**}	0.550 ^{**}
Length x thickness	0.091 ^{ns}	0.261 ^{**}	0.313 ^{**}	-0.013 ^{ns}
Width x thickness	0.111 ^{ns}	0.327 ^{**}	0.249 ^{**}	0.121 ^{ns}

^{ns}not significant, ^{**}significant at 1% probability level by t test.

^{ns}não significativa, ^{**}significativo ao nível de 1% de probabilidade pelo teste t.

The significant correlations showed marked differences for seed formation in the proximal region of the fruit for *A. macrocarpa* and distal for *P. moniliformis*, suggesting a different pattern of development when compared to those formed in other regions of the fruit in each species.

Seeds formed at different seminal nucleus in the fruits of *A. macrocarpa* and *P. moniliformis* showed similar germination rate and subtle differences in vigor (Table 4). Although the seeds formed in the intermediate (larger) and distal (smaller) regions showed higher germination speed for both species, the seeds of *A. macrocarpa* from the proximal and distal (smaller) regions formed seedlings with greater shoot length and lower dry root weight (Table 4), thus not allowing defining significant differences in physiological quality among seeds formed in different fruit seminal nucleus. Therefore, the compositions of the seed lots for these species may come from different regions of the fruit. Moreover, the seed size is not related to the physiological quality for *A. macrocarpa* and *P. moniliformis*.

Table 4. Germination (G), germination speed index (GSI), shoot length (SL), root length (RL), shoot dry weight (SDW) and root dry weight (RDW) of seedlings of *Anadenanthera macrocarpa* and *Pityrocarpa moniliformis* formed in different seminal nucleus of the fruit.

Tabela 4. Germinação (G), índice de velocidade de germinação (GSI), comprimento da parte aérea (SL) e raiz (RL), e massa seca da parte aérea (SDW) e raiz (RDW) de plântulas de *Anadenanthera macrocarpa* e *Pityrocarpa moniliformis* formadas em locos distintos no fruto.

Seed formation region in the fruit	G	GSI	SL	RL	SDW	RDW
	(%)	-	(cm)	(cm)	(mg)	(mg)
<i>Anadenanthera macrocarpa</i>						
Proximal	97 a [*]	13.1 b [*]	7.34 ab ^{**}	11.18 a [*]	138.86 a [*]	96.88 b [*]
Intermediate	96 a [*]	13.2 ab [*]	6.76 b ^{**}	11.55 a [*]	150.09 a [*]	112.30 a [*]
Distal	97 a [*]	14.1 a [*]	8.01 a ^{**}	11.52 a [*]	151.06 a [*]	99.70 b [*]
<i>Pityrocarpa moniliformis</i>						
Proximal	86 a [*]	16.5 b [*]	4.73 a [*]	4.34 a [*]	6.24 a ^{**}	2.67 a [*]
Intermediate	85 a [*]	21.9 a [*]	4.84 a [*]	5.01 a [*]	6.67 a ^{**}	3.15 a [*]
Distal	91 a [*]	22.0 a [*]	4.96 a [*]	4.75 a [*]	6.43 a ^{**}	2.88 a [*]

Averages followed by the same letter in the column do not differ by Tukey's test^{*} (parametric data) and Kruskal-Wallis^{**} (non-parametric data) at 5% probability level.

Médias seguidas da mesma letra na coluna não diferem entre si pelo teste de Tukey^{*} (dados paramétricos) e Kruskal-Wallis^{**} (dados não paramétricos) ao nível de 5% de probabilidade.

DISCUSSION

Non-domesticated tropical forestry species show wide variability in fruit size, number of seeds per fruit and seed size (PEREIRA *et al.*, 2011; DUTRA *et al.*, 2017). A greater variation in the biometric characters of fruits of *A. macrocarpa* and *P. moniliformis* may occur because the populations are located in anthropized areas since a lower variation is expected for primary vegetation (RODRIGUES *et al.*, 2015). In this sense, the number of seeds per fruit is directly influenced by pollination efficiency, where a certain amount of viable pollen grains is necessary to reach the flower stigma, which will result in a greater or lesser number of seeds formed (NASCIMENTO *et al.*, 2011).

During flowering and fruit maturation, trees are susceptible to changes in conditions (temperature and humidity) and environmental resources (water, solar radiation, and nutrients), thus promoting biometric changes in fruits and seeds (VIEIRA; GUSMÃO, 2008; SILVA *et al.*, 2017). In addition, the nutritional status of trees during flowering, fertilization and maturation and the ability to assimilate/transport reserves also affect the fruit formation (SANTOS *et al.*, 2018).

Seeds of *A. macrocarpa* and *P. moniliformis* formed in seminal nucleus in the intermediate region of the fruit are biometrically larger in all the evaluated aspects (Table 2). This fact is related to the greater translocation of photoassimilates and accumulation of reserves (SANTOS *et al.*, 2018), confirming the tendency for legume species, such as *L. leucocephala* and *P. juliflora* (OLIVEIRA; MORAIS, 1997). Although there are biometric differences in between seeds of *A. macrocarpa* and *P. moniliformis* depending on the region of formation in the fruit, no differences in physiological quality were revealed by the tests of germination and vigor.

For species of the family Fabaceae, the germination and seedling vigor was not influenced by the seed formation region in the fruit of *M. caesalpiniiifolia* (FREITAS *et al.*, 2013), while for *C. pyramidale*, seeds from the distal region showed lower germination in relation to those from other regions in the fruit (MENDONÇA *et al.*, 2016). Higher rate and speed of germination were obtained with seeds of *L. leucocephala* formed in the proximal region. For *P. juliflora*, seeds from the proximal and distal region are of superior quality (OLIVEIRA; MORAIS, 1997). In *E. contortisiliquum*, seeds formed in seminal nucleus from distal region of the fruit germinate quicker compared to the other regions (LESSA *et al.*, 2014).

The seed size also had no effect on the seed germination of *C. pyramidale* (MENDONÇA *et al.*, 2016) and *Caesalpinia ferrea* Mart. ex Tul. var. *leiostachya* Benth.; however, seeds classified as medium and large size produced larger seedlings (ALBUQUERQUE *et al.*, 2018). For *Hymenaea stigonocarpa* var. *stigonocarpa* Mart. ex Hayne, large seeds germinated quicker in relation to intermediate and small seeds (PEREIRA *et al.*, 2011). Lastly, seeds of *L. leucocephala* and *P. juliflora* classified as small provided superior germination (OLIVEIRA; MORAIS, 1997).

In view of the variations in the germination performance and seedling vigor as a function of the seed formation seminal nucleus in the fruit, it is not possible to state that the seed origin in the fruit is related to its physiological quality in all leguminous forestry species neither to say that larger or smaller seeds are related with vigor. Therefore, the composition of the forest seed lot must be studied individually for each species.

CONCLUSIONS

- The origin of the seeds of *A. macrocarpa* and *P. moniliformis* formed in different seminal nucleus of the fruit do not present differences in physiological quality.
- The sorting of seeds formed in different regions of the fruit does not benefit the final quality of seed lots for these species.

ACKNOWLEDGEMENTS

The present study was performed with the support of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001

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