

Physiological Maturity of *Parapiptadenia rigida* Seeds

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Abstract

The establishment of appropriate standards related to the physiological and morphological aspects of the seeds are fundamental procedures to help the nurserymen and seed producers in determining the maturity and the appropriate moment of collection of the fruits. In this sense, the objective of this research is to evaluate the physiological maturation of seeds of *Parapiptadenia rigida* by means of germination and vigor tests, based on the color of the pods. The seeds were collected in June 2017, from three matrices located in the municipality of Marechal Cândido Rondon, Paraná, Brazil. The pods were classified in four stages of maturation, according to the Chart of colors model “Munsell colors chart” for plants tissues, and measured the biometric parameters. The parameters observed to evaluate the germinative potential are: first germination count, germination velocity index, emergency velocity index, and fresh and dry matter masses of seedlings. The experimental design was completely randomized, with five maturation stages and four replicates of 25 seeds each. The averages were compared using the Tukey test at a 5% probability. The germination test showed that the increase in physiological potential of *P. rigida* seeds is associated with the progresses of pod maturation. Therefore, the vigor test demonstrated that the physiological maturation of the species is simultaneous with the change of coloration and maturation of the pods.

Keywords: germination, vigor, maturation stage, native species

1. Introduction

The species *Parapiptadenia rigida* (Benth.) Brenan, belong to the *Fabaceae* family, this species is characteristic and exclusive of the Paraná, Uruguay and its tributaries river basins. *Parapiptadenia rigida* (Benth.) Brenan is recommended for the recovery of degraded areas and forest restoration in areas of permanent preservation. It is an early secondary tree, its wood is heavy, elastic and highly durable, which makes it suitable for rural constructions and for carpentry, besides its use for tanneries, because it is rich in tannin (Lorenzi, 2008).

The need for conservation of tropical forests and the strengthening of environmental policy promoted an increase in the demand for seeds of native species, which constitute a basic input in the ecosystem conservation programs (Carvalho, Silva, & Davide, 2006). The knowledge of the morphological and ecophysiological characteristics of the seeds of native forest species, with a view to the production of seedlings, are important for the biodiversity conservation. Ferreira (2000) observed the lack of basic information about native species makes it difficult to use silvicultural programs, and the germ studies are fundamental for the knowledge of the anatomy, morphology and physiology of the seeds.

The objective of this study is to define the ideal harvest time and the highest quality of seed stage, known as physiological maturity (Popinigis, 1985), which varies according to species and environmental conditions. After this moment, the permanence of the seeds in the field results in their progressive deterioration (Lazarotto, 2011). This process results from morphological, physiological and functional changes, such as increase in size, variations in water content, vigor and accumulation of dry mass, that follow from the fertilization of the ovum until the moment when the seeds are ripe (Carvalho & Nakagawa, 2012).

These modifications include a set of preparation steps for the germination process, essentially characterized by the synthesis and accumulation of reserves (Marcos-Filho, 2015). Figliolia and Kageyama (1994) explained that the knowledge of the physiological maturity point of seeds contributes to understanding the behavior of the species in terms of their reproduction, as well as the obtaining of good physiological quality genetic material,

which is the fundamental basis for the programs silvicultural breeding, genetic conservation and recovery of degraded areas.

The physiological quality of the seed is related to the capacity to perform its vital functions, characterized by its germination, vigor and longevity. Thus, the level of physiological quality of the seed is evaluated by two fundamental parameters: viability and vigor. The viability, measured by the germination test, determines the maximum germination of the seed, offering for this, the most favorable conditions possible. The vigor represents more subtle attributes of physiological quality, being determined in unfavorable conditions or evaluation of the decline of some biochemical or physiological function of the seed (Popinigis, 1985).

Seed vigor can be defined by Baalbaki, Elias, Marcos-Filho and McDonald (2009) as the properties that determine the potential for rapid and homogeneous emergence and the normal development of seedlings under varying environmental conditions. The evaluation of the vigor in the seeds of the forest species is a practice that allows us to estimate and to compare different objectives in many seeds (Valentini & Piña-Rodrigues, 1995). Duarte and Carneiro (2009) claim that the harvesting point depends on the occurrence of the physiological maturity of the seed, which in many cases coincides with the maximum accumulation of dry matter and, when the seeds reach this point stage, generally it is potential for germination and vigor rises.

According to Piña-Rodrigues and Aguiar, (1993) the physiological maturation of the seeds can be detected by physical changes such as changes in color of the fruit, size, odor, dehiscence of fruit, among others. Several studies indicate the change in color of the fruit as a good indicator of physiological seed maturation, *Allophylus edulis* (Kaiser et al., 2016), *Inga laurina* (Schulz, Oro, Volkweis, Malavasi, & Malavasi, 2014), *Jatropha curcas* (Rubio, Meneghel, Gomes, & Malavasi, 2013; Dranski, et al., 2010), *Machaerium brasiliense* (Guimarães & Barbosa, 2007).

It is of fundamental importance to determine adequate patterns related to the physiological and morphological aspect of the seed, as well as to define the maturation point and the appropriate time of fruit harvest, in order to help nurserymen and seed producers. Thus, the objective of the study was to evaluate, through germination and vigor tests, the physiological maturation of *Parapiptadenia rigida* seeds, based on the color of the pod.

2. Materials and Methods





2.1 Collect and Classification Seeds

The experiment was accomplished at the Seed Technology Laboratory of the State University of the West of Paraná (UNIOESTE), Campus Marechal Cândido Rondon, using seeds of *Parapiptadenia rigida*.

The seeds were collected from three matrices located in Marechal Cândido Rondon, Paraná, Brazil. Claim to Köppen, the classification of this region climate is Cfa, mesothermic, humid subtropical (Alvares, et al., 2014), with hot summers (average temperature over 22 °C) and winters with little frost (average temperature below 18 °C) and an average annual rainfall of 1,500 mm.

The collection was carried out directly in the trees, with the aid of pruning shears, composing a lot of pods, which were separated according to the degree of maturation, determined by the color difference. The pods were classified in four maturation stages according to the color chart, Munsell color charts for plant tissues model (Munsell, 1976), in 7.5 GY 5/8, stage I: light green; 2.5 GY 6/10, stage II: dark green; 2.5 GY 5/4, stage III: light brown; 5 YR 3/2, stage IV: dark brown (Table 1). After sorting the pods, the seeds were benefited, being extracted manually, and then mixed and homogenized, constituting representative lots for each treatment.

Table 1. Color of fruits of *P. rigida* in different maturation stages

Maturation stage	Coloro of fruit	Visual Classification	Letter from Munsell
I		Light Green	7.5 GY 5/8
II		Dark Green	2.5 GY 6/10
III		Light Brown	2.5 GY 5/4
IV		Dark Brown	5 YR 3/2

2.2 Biometric Parameters of Seeds

The seeds were manually extracted and the biometric parameters length and diameter using a digital caliper were subsequently measured. The water content was determined by the oven method at 105 ± 3 °C, during 24 hours (MAPA, 2009), with 5 treatments, 4 replicates of 25 seeds each. The mass of one thousand seeds was calculated according to the RAS Seed Analysis Rule (MAPA, 2009).

2.3 Germination Test

The seeds were germinated using germitest paper, moistened with distilled water 2.5 times the value of their weight and packed in a germination chamber type BOD, with constant temperature of 25 °C and photoperiod of 12 hours. Daily tally of normal and abnormal seedlings were performed, with criteria determined by the Rules for Seed Analysis (RSA) (MAPA, 2009). Subsequently, the percentage of germination, the average germination speed (AGS) and the mean germination time (MGT) were calculated. The calculation of the percentage of germination of AGS and MGT were performed according to Labouriau (1983):

$$G (\%) = (N/A) \times 10 \quad (1)$$

Where, G is the percentage of germination; N is the number of germinated seeds (during 40 days); and A is the total number of seeds placed to germinate.

$$MGT (\text{days}) = (\sum n_t \times t_i) / (\sum n_{\text{total}}) \quad (2)$$

$$AGS (\text{days}^{-1}) = 1/t \quad (3)$$

Where, MGT refers to the mean germination time in days; n_t is the number of seeds germinated in a time interval; t_i is the time interval (40 days); n_{total} is the total number of seeds germinated; v is the average speed of germination.

2.3. Vigor Test Based on Seedling Performance

2.3.1 Germination Speed Index

$$GSI = (G1/N1 + G2/N2 + Gn/Nn) \quad (4)$$

Where, GSI = germination velocity index; G = number of seeds germinated; N = number of days of sowing.

2.3.2 Emergency Velocity Index

It was accomplished in a greenhouse, on what the seeds were sown in trays containing commercial substrate, and the number of emerged seedlings with above ground tissues was recorded daily until emergency stabilization, and this was calculated by the formula proposed by Maguire (1962):

$$ESI = (E1/N1 + E2/N2 + En/Nn) \quad (5)$$

Where, ESI = emergency speed index. E1, E2, En = number of normal seedlings computed in the first count, the second count and the last count. N1, N2, Nn = number of days of sowing at the first, second and last count.

2.3.3 First Germination Count

The first germination count was performed in conjunction with the germination test, for which the percentage of normal seedlings was counted on the fifth day after the test installation (MAPA, 2013).

2.3.4 Masses of Fresh and Dry Weight of Seedlings

At the end of germination and emergency test, seedlings were weighed in scales were weighed in an analytical balance with an accuracy of 0.0001 g, to obtain fresh matter mass (FMM). After they were packed in kraft paper bags and placed in a drying oven at 60 ± 2 °C for 72 h, to measure the dry wight mass of seedlings (DWM).

2.4 Statistical Analysis

The experimental design was completely randomized, with 4 maturation stages, 5 replicates of 25 seeds each.

The results were verified for normality and homogeneity and submitted to analysis of variance (F test). When appropriated, means were compared by Tukey test at 5% of probability.

3. Results and Discussion

3.1 Seed Characterization

There was a significant difference to longitudinal diameter seed (LDS), transverse diameter seed (TDS) thousand seed weight (TSW), moisture content (MC), thickness (T) and dry weight mass (DWM) between the treatments (table 2).

Table 2. Biometric characterization of *P. rigida* seeds at different stages of the false fruit maturation

Stage of maturation	LDS (mm)	TDS (mm)	T (mm)	MC (%)	TSW
7.5 GY 5/8	12.73 b	8.94 a	0.97 a	22.73 a	56.36 a
2.5 GY 6/10	13.60 a	9.17 a	0.64 b	18.51 b	43.81 b
2.5 GY 5/4	12.78 b	8.70 a	0.54 c	14.28 b	40.33 b
5 YR 3/2	13.05 ab	9.17 a	0.60 bc	9.87 c	31.52 c
CV%	4.82	5.51	7.43	10.43	7.8

Note. LDS = longitudinal diameter seed, TDS = transversal diameter seed, T = thickness, MC = moisture content, thousand seed weight (TSW). * Means followed by the same letter in the columns do not differ by Tukey test ($p \leq 0.05$).

The seeds of maturation stages II and IV showed higher averages. However, didn't occur significant difference between maturation stages for DTS variable. In terms of the variables T, MC and TSW, verified higher averages for the maturation stage I, 7.5 GY 5/8, as well as progressive reduction for the other stadiums. It can be inferred, through these results, that the seeds of *Paraptadenia rigida*, in the maturation stages evaluated, underwent dehydration concomitant to the advance of the stage of maturation of the pod, culminating in the reduction of the thickness and the water content of the seed. The morphometric data obtained in the study were similar to that measured by Gasparin (2012), and Marangoni Muniz, Binotto, Georjgin, and Maciel (2014) for rigid *Parapiptadenia rigida*.

The results observed for the biometric data of *P. rigida* show that the seeds were harvested when they had reached the point of physiological maturity, because no differences were observed between the stages of maturation for the transverse diameter of the seed. It can be deduced that these had already reached the maximum size, in addition they were verified reductions of the moisture content of the seeds, process that occurs naturally and concomitant to the maturation of the seeds.

Therefore, it is possible to infer that the variations observed for the other morphological characteristics of the seeds of the different stages of maturation are related to the loss of water observed according to the degree of maturation of the same. In this sense, Marcos-Filho (2015) explains that during the development period, the maturation of the seeds go through a series of modifications, among them morphological changes, where the seeds need a high water content for partition of solutes until reaching the maximum accumulation of dry matter, coinciding with the point of physiological maturation, at this moment, the author states that there is the disconnection of these seeds from the mother plant, followed by intense dehydration of the seeds.

3.2 Germination Test

Differences ($p \leq 0.05$) were detected for germination percentage (G%), germination speed index (GSI), mean germination time (MGT) and average germination speed (AGS), as a function of seed maturation stage (Table 3).

Table 3. Germination parameters of *P. rigida* seeds at different maturation stages of false fruit maturation

Maturation stages	G%	MGT (days)	AGS (days ⁻¹)
7.5 GY 5/8	4.00 c	4.60 b	0.080 c
2.5 GY 6/10	55.00 b	7.69 a	0.130 b
2.5 GY 5/4	68.00 a	4.84 b	0.214 a
5 YR 3/2	63.75 a	4.67 b	0.218 a
CV%	5.43	5.97	9.65

Note. G% = Percentage of germination, MGT = mean germination time, AGS = average germination speed. * Means followed by the same letter in the columns do not differ by Tukey test ($p \leq 0.05$).

Seeds of the 2.5 GY 5/4 and 5 YR 3/2 stages showed the highest percentage of germination (68.0% and 63.75%, respectively), as well as lower MGT and higher GSI. There was a drastic reduction in the G% for stage I, however, at this stage a high water content was also observed for the seeds, suggesting that although these seeds have already reached physiological maturation, they are still low potential for harvesting. The lower average MGT observed in stage I is related to the low percentage of germinated seeds, therefore, it is not referred as indicative of high potential.

The results indicate that there is an increase in the physiological potential of *P. rigida* seeds as the pod maturation stage is advanced. In addition, it can be suggested that the maximum physiological potential of *P. rigida* seeds was obtained at the stage of maturation III and remained at the next stage studied (stage IV), according to Marcos-Filho (2015). Since the seed reaches the maximum physiological potential, it can only be maintained or decreased, so this point is considered the physiological peak, and also the initial mark of seed deterioration.

Several studies, carried out with several species, indicate differences in the germination potential of the seeds as a function of the maturation stage of the fruits, for example *Machaerium brasiliense* V. (Guimarães & Barbosa, 2007), *Jatropha curcas* L. (Dranski et al., 2010), *Cedrela fissilis* (Ristau et al., 2017), *Hovenia dulcis* T. (Vera Cruz et al., 2017).

3.3 Vigor Test

Table 4 shows the averages for first germination count (FGC), germination speed index (GSI), emergence speed index (ESI), fresh matter mass (FMM) and dry matter mass (DMM) of *Paraptadenia rigida* seedlings.

Table 4. Averages of germination test of *P. rigida* seeds in different maturation stages

Maturation stages	FGC (%)	GSI	ESI	FMM (g)	DMM (g)
7.5 GY 5/8	1.00 c	0.138 c	0.230 c	1.93 b	0.25 b
2.5 GY 6/10	7.00 b	1.806 b	1.264 b	2.04 b	0.35 b
2.5 GY 5/4	6.67 b	3.238 a	3.624 a	2.53 a	0.57 a
5 YR 3/2	8.75 a	3.432 a	4.046 a	2.25 a	0.63 a
CV%	5.85	10.94	11.5	12.83	4.65

Note. First germination count = FG, germination speed index = GSI, emergency speed index = ESI, fresh matter mass = FMM, dry matter mass = DMM *Means followed by the same letter in the columns do not differ by Tukey test ($p \leq 0.05$).

The first germination count test indicated a progressive increase in the meantime concomitant to the advancement of pod maturation stages, where the highest percentage of germinated seeds at the first count was observed at the maturation stage IV (5 YR 3/2). For the GSI and ESI parameters, the highest indices were obtained in maturation stages III and IV.

The results obtained corroborate Berloff, Graichen, Fernandes, and Silva (2015), where they evaluated the physiological quality of seeds of the same species under different conditions and verified that the highest averages were observed for the dark brown seeds fixed in the plant. Contrary results were observed by Sobral, Brunetto, Belotti, and Batassare (2010), where they verified a reduction in the germination and emergence of *P. rigida* seeds in the last weeks of collection, in which the pods were dark brown.

Seeds belonging to the stages with light brown and dark brown pods presented higher masses of fresh matter and dry matter of seedlings, and it can be inferred that the data obtained are indicative of greater vigor. In this sense, Dan, Mello, Wetzel, Popinigis, and Zonta (1987) affirm that vigorous seeds originate seedlings with higher growth rates due to the greater capacity of transformation and supply of storage reserves and the greater incorporation of these by the embryonic axis. In addition, Marcos Filho (2015) explained that the changes in seed vigor is related to increase dry matter transfer from the adult plant to the seeds, so that the proportion of vigorous seeds increases with maturation.

The results for the vigor tests corroborate with a hypothesis that is a physiological maturation of the seeds of *Paraptadenia rigida* is simultaneous to the change of heart and maturation of the pods, as it is observed an increase in the means, for all tests of seed vigor, according to the increase in pod maturation levels.

4. Conclusion

The maturation stage exerts different results on germination and vigor of seeds. The biometric characteristics, germination and vigor test are parameters that indicate the potentiality and physiological maturity of the seeds of *P. rigida*, for the reason that allowed us to discern that there was an increase in the physiological potential concomitant to the progress of the maturation stage.

The seeds of *P. rigida* belonging to fruits (pod) of light brown to dark brown coloring, still fixed at adult plant, presented better performance as regards viability and vigor when compared to seeds from fruits with greenish coloration.

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