

Dormancy of *Bowdichia virgilioides* Kunth seeds

Dormência de sementes de *Bowdichia virgilioides* Kunth

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Recebido em: 01-06-2020; Aceito em: 29-06-2020

Abstract

The degree of maturity of fruits and seeds can influence the establishment of seed dormancy. Thus, the present study aimed to verify whether dormancy of *B. virgilioides* seeds depends on the degree of fruit maturity. Two experiments were carried out: in the first one, fruits at two maturity stages (green and brown) were collected from seven plants located in the municipality of Conceição da Feira, BA, Brazil. Germination tests were conducted in a completely randomized design in a 2 x 2 factorial scheme, with two levels of fruit maturity (yellowish green with purple edges and brown with blackish spots) and two treatments related to dormancy overcoming (without overcoming and with overcoming). For the second experiment, darkened fruits were collected from three plants located in the municipality of Cruz das Almas, BA, Brazil. Germination tests were conducted according to a completely randomized design with two treatments, seeds with and without dormancy overcoming. Dormancy in *B. virgilioides* seeds depends on the degree of fruit maturity. Dormancy occurs after the seventh week of floral anthesis when the fruits are predominantly black, dry and brittle.

Additional keywords: fruit maturity stage; germination; 'sucupira preta'

Resumo

O grau de maturação de frutos e de sementes pode influenciar no estabelecimento da dormência de sementes. Assim, objetivou-se com este trabalho verificar se a dormência de sementes de *B. virgilioides* depende do grau de maturação do fruto. Realizaram-se dois experimentos, no primeiro foram coletados frutos em sete matrizes situadas no município de Conceição de Feira, em dois estádios de maturação (verdes e marrons). Os testes de germinação foram conduzidos em delineamento inteiramente casualizado em esquema fatorial 2 x 2, com dois estádios de maturação (verdes amarelados com bordas arroxeadas e marrons com regiões enegrecidas) e dois tratamentos relativos à superação de dormência (sem superação e com superação). Para o segundo experimento, coletou-se frutos marrons com regiões enegrecidas em três matrizes situadas no município de Cruz das Almas. Foram realizados testes de germinação, conforme delineamento inteiramente casualizado com dois tratamentos, sementes com e sem superação de dormência. A dormência em sementes de *B. virgilioides* depende do grau de maturação do fruto. O estabelecimento da dormência ocorre após a sétima semana de antese floral quando os frutos estão predominantemente enegrecidos, secos e quebradiços.

Palavras-chave adicionais: estágio de maturação do fruto; germinação; sucupira preta.

Introduction

Bowdichia virgilioides Kunth is a native tree that is distributed throughout the Brazilian territory (Silva et al., 2015; Cardoso, 2019). It has pharmacological potential (Botini et al., 2015; Machado et al., 2018; Silva et al., 2019) and has application in organic production, since its leaves induce the ripening of bananas, replacing calcium carbonate (CaCO₃), a harmful compound to human health (Nascimento et al., 2019). The wood of this species has commercial value and is used for the fabrication of furniture and in civil construction (Lorenzi, 2009).

Studies conducted since the 1990s have shown that *B. virgilioides* seeds have dormancy due to impermeability of the protective cover (seed coat) (Andrade et al., 1997; Smiderle et al., 2003; Albuquerque et al., 2007; Smiderle & Schwengber, 2011; Rosa-Magri & Meneghin, 2014; Coêlho et al., 2019). The Instructions for Analysis of Seeds of Forest Species (Brasil, 2013) record that seeds of this species have seed coat dormancy and indicate pre-germination treatment with chemical scarification using sulfuric acid for 5 to 10 minutes. Another study proves the efficiency of immersing seeds in water at temperature of 100 °C to overcome dormancy (Smiderle & Schwengber, 2011).

Dormancy in seeds is strongly influenced by genetic factors, but it also responds to environmental factors that act in their stage of formation, which explains the existence of varying degrees of dormancy in the same species between different places of origin (Bewley et al., 2013). The impediment of germination may vary depending on the position of the seeds in the pod (Nogueira et al., 2010), on the mother tree (Müller et al., 2016) and on the sites of collection (Botzell et al., 2000).

In addition to genetic and environmental factors, studies confirm that the degree of fruit and seed maturity also influences the establishment of dormancy (Nakagawa et al., 2007; Nogueira et al., 2013; Müller et al., 2016; Leite et al., 2019). The impediment of germination due to seed coat impermeability can be avoided if the seeds are collected at the point of physiological maturity (Cardoso, 2009). Thus, the hypothesis was raised that for *B. virgilioides* dormancy sets in after the point of physiological maturity.

Therefore, given the ecological and economic importance of *B. virgilioides*, added to the fact that it is a species whose dormancy has been confirmed in several studies, the objective was to verify whether the dormancy of its seeds depends on the degree of fruit maturity.

Material and methods

Two experiments were carried out at the Laboratory of Ecology and Forest Restoration of the Universidade Federal do Recôncavo da Bahia (UFRB), on the campus of Cruz das Almas, Bahia, Brazil.

Experiment 1

In the first experiment, the fruits were manually collected from seven parent plants located in the municipality of Conceição de Feira – Bahia, Brazil, at 12°27'05.8 South latitude and 38°59'20.1 West longitude, in December 2018. After harvest, the fruits were sent to the laboratory, processed and classified by color, according to Almeida (2013). Seeds were obtained from fruits at two maturity stages: A - yellowish green fruits with purplish edges, corresponding to the maturity of five weeks after floral anthesis (Almeida, 2013) and B - brown fruits with darkened regions, corresponding to the seventh week after floral anthesis (Almeida, 2013) (Figure 1).

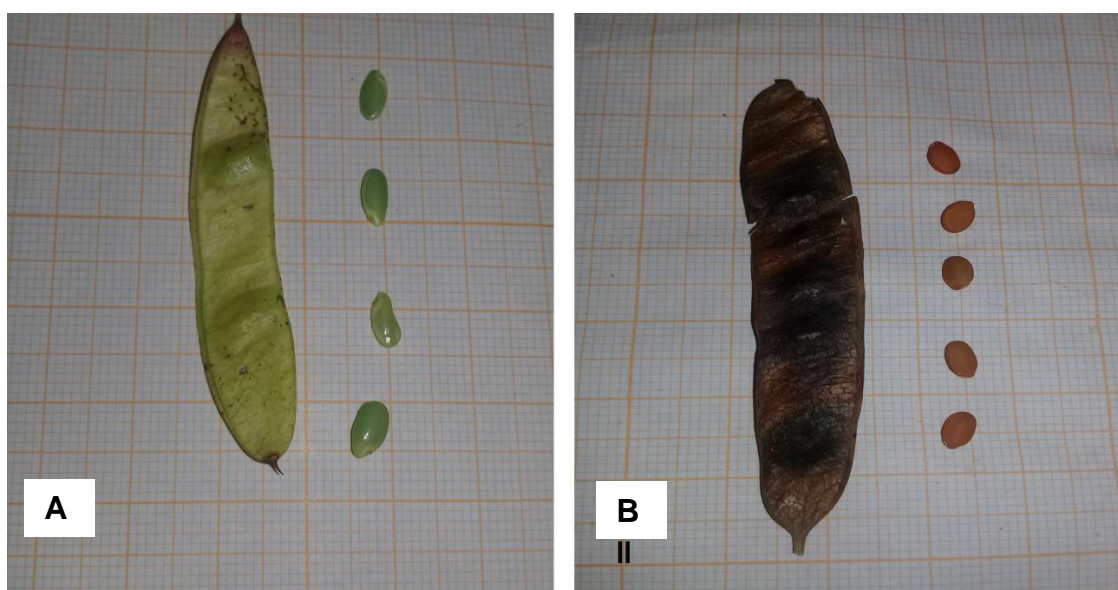


Figure 1 - Fruits and seeds of *Bowdichia virgilioides* Kunth collected from seven parent plants located in the municipality of Conceição da Feira – Bahia, Brazil. A - yellowish green fruits with purplish edges and B - brown fruits with darkened regions.

The moisture content of the recently harvested seeds was determined by the method of drying in an oven at $105 \pm 3^{\circ}\text{C}$ for 24 hours (Brasil, 2009), using four sub-samples of 1 gram of seeds.

The experimental design was completely randomized in a 2 x 2 factorial scheme, with two levels of fruit maturity (yellowish green fruits with purplish edges and brown fruits with darkened regions) and two treatments related to dormancy overcoming (without dor-

mancy overcoming and with dormancy overcoming), with four replicates of 25 seeds each. Therefore, a total of four treatments were used: T1 (seeds from yellowish green fruits with purplish edges with dormancy overcoming), T2 (seeds from yellowish green fruits with purplish edges without dormancy overcoming), T3 (seeds from brown fruits with darkened regions with dormancy overcoming) and T4 (seeds from brown fruits with darkened regions without dormancy overcoming).

For the treatment of dormancy overcoming, the seeds were immersed in distilled water at 100 °C for 10 seconds (Smiderle & Schwengber, 2011).

For the germination tests, four replicates of 25 seeds were arranged in germitest paper rolls, moistened with distilled water in a volume equivalent to 2.5 times the weight of the dry paper (g). Then, the rolls were placed in transparent plastic bags, which were kept in a B.O.D. (Biochemical Oxygen Demand) germination chamber with constant temperature of 30 °C and photoperiod of 12 h (Brasil, 2013). Daily counts were performed until germination stabilized.

The variables evaluated in this experiment were germination percentage, percentage of normal seedlings, percentage of unviable seeds, mean germination time (Bewley et al., 2013), and shoot length and primary root length of normal seedlings, considering the initial number of seeds (Guedes et al., 2009).

Generalized linear models were applied for analysis of the variables (random component): proportion of germinated seeds, proportion of normal seedlings, proportion of unviable seeds and proportion of hard seeds, in a total of 25 seeds, according to Carvalho et al. (2018). The systematic component corresponds to the treatments employed. Inferences of the analysis (deviance) for binomial distribution, with logit link function, were based on the Chi-Square statistic. To verify the amplitudes of differences between treatments, tests of multiple comparisons of means with Tukey fit were used, with correction of confidence interval by the Šidák method, through the packages emmeans (Lenth et al., 2020) and multcompView (Graves et al., 2019), used in the R Core Team 3.5.3 program. (R development core team, 2019).

For the variables mean germination time and mean length of shoot and primary root of normal seedlings, analysis of variance and means comparison tests (Tukey, $\alpha=0.05$) were used. All statistical analyses were performed with the software R version 3.5.3 (R Core Team, 2019).

Experiment 2

For the second experiment, fruits of *B. virgilioides* located at 12°39'43 South latitude and 39°05'51 West longitude were collected. Three flowering trees were marked in October 2019 and visited weekly to observe the fruits. The fruits were collected on February 19, 2020, after the seventh week of floral anthesis (Almeida, 2013), predominantly darkened, very dry and brittle. The moisture content of the recently harvested seeds was determined by the oven method at 105 ± 3 °C for 24 hours (Brasil, 2009), using four 1-ram sub-samples.

The experiment was conducted in a completely randomized design with two treatments, seeds subjected to the procedure to overcome dormancy (immersed in distilled water at 100 °C for 10 seconds) and not subjected to any pre-germination treatment, with 10 replicates and 25 seeds per replicate.

Four replicates of 25 seeds were arranged in germitest paper rolls, moistened with distilled water in a volume equivalent to 2.5 times the weight of the dry paper (g). Then, the rolls were placed in transparent plastic bags, which were kept in a B.O.D. germination chamber with constant temperature of 30 °C and photoperiod of 12 h. Daily counts were performed until 34 days. The end of the counts was defined based on the stabilization of germination, which occurred after one week without germination event.

The variables evaluated in this experiment were germination percentage, percentage of normal seedlings, percentage of unviable seeds and percentage of hard seeds.

For the treatment that showed germination, the confidence interval for binomial probability was obtained for the variables under study through the exact method (Pearson-Klopper), using the binom package (Dorai-Raj, 2014).

Results and discussion

Experiment 1

The average initial moisture content of seeds from green fruits was 54.13%, Confidence Interval- $CI_{95\%}$: $54.13\% \pm 1.04\%$. Seeds from brown fruits showed moisture content of 14.67%, Confidence Interval- $CI_{95\%}$: $14.67\% \pm 0.18\%$. Both the degree of fruit maturity, differentiated by green and brown colors, and the dormancy overcoming procedures influenced germination, formation of normal seedlings and unviable seeds (Table 1).

Seeds harvested from green fruits whose dormancy was overcome with hot water did not germinate and rotted, leading to 100% of unviable seeds (Table 2). Seeds obtained from green fruits without the application of pre-germination treatment showed germination percentage of 99.2% (Table 2). These results allow for two important inferences. The first is that, depending on the degree of maturity of the seeds, certain pre-germination treatments, such as immersion in water at a temperature of 100 °C, can cause damage to the embryo, instead of promoting germination. The other inference is that seeds harvested from yellowish green fruits with purplish edges, which corresponds to the point of maturity, as indicated by Almeida (2013), did not show dormancy.

For seeds collected from brown fruits, the treatment without dormancy overcoming resulted in a higher germination percentage (93.6%), while exposure of seeds to water at 100 °C caused reduction in germination (63.2%) (Table 2). The percentages of normal seedlings and unviable seeds also revealed lower performance of the treatment with exposure of seeds to hot water for seeds obtained from green and brown fruits (Table 2). Thus, seeds collected from brown fruits with darkened spots were not dormant. These results indicate that the green and brown fruits were harvested before the seeds acquired dormancy.

Table 1 - Analysis of deviance for the germination of *Bowdichia virgilioides* Kunth, in response to temperatures, modeled by binomial distribution, with logistic link function.

Source of variation		Germination	
	Deviance	Deviance difference	p-value (Chi-Square)
Null	-	432.99	
Color (C)	45.961	387.03	<0.0001
Overcoming (O)	311.393	75.64	<0.0001
CXO	60.486	15.15	<0.0001
AIC		51.075	
Source of variation		Normal seedlings	
	Deviance	Deviance difference	p-value (Chi-Square)
Null	-	422.76	
Color (C)	39.001	383.76	<0.0001
Overcoming (O)	308.231	75.53	<0.0001
CXO	59.584	15.95	<0.0001
AIC		54.563	
Source of variation		Unviable seeds	
	Deviance	Deviance difference	p-value (Chi-Square)
Null	-	507.17	
Color (C)	138.49	368.68	<0.0001
Overcoming (O)	334.64	34.05	<0.0001
CXO	13.09	20.96	0.0003
AIC		42.325	

AIC – Akaike information criterion

Table 2 - Percentages of germination, normal seedlings and unviable seeds of *Bowdichia virgilioides* Kunth

Treatments	% Germination		% Normal seedlings		% Unviable seeds	
	Brown	Green	Brown	Green	Brown	Green
Water 100 °C	63.2±9.6 b A	0.0±0.0 b B	59.2±9.8 b A	0.0±0.0 b B	10.4±6.2 a B	100.0±0.0 a A
No overcoming	93.6±4.9 a B	99.2±1.8 a A	92.8±5.2 a B	98.4±2.6 a A	0.8±1.8. b A	0.8±1.8. b A

Means followed by confidence intervals, fitted by the Šidák method, and followed by distinct letters, lowercase in columns and uppercase in rows, differ by Tukey test, at 0.05 significance level.

Results reported in studies conducted by Nogueira et al. (2013), Müller et al. (2016) and Leite et al. (2019) demonstrated that other species of the Fabaceae family also do not have dormancy at the point of maturity.

Nogueira et al. (2013) classified the fruits of *Mimosa caesalpiniiifolia* Benth. into five degrees of maturity based on color and defined stage 5 (dry on the ground) as the maturity point, although this stage did not differ statistically from 3 and 4 for germination percentage. In the stage 3 there was no significant difference between treatment with (85%) and without (87%) dormancy overcoming. These authors also concluded that a high percentage of germination was obtained in seeds harvested from pods with light brown color (stage 3) without dormancy overcoming treatments.

Müller et al. (2016) separated the fruits of *Peltophorum dubium* (Spreng) Taub. into five stages of maturity based on color. In stages I and II there was no germination, in stage III germination was equal to 48%, in IV to 75% and in V stage to 18%. Thus, at the stage of maximum vigor and germination (IV), the seeds were not dormant. These authors attributed the reduction of germination in stage V to the possible acquisition of dormancy, but they did not apply dormancy overcoming

treatments or tetrazolium tests to confirm this assumption.

Leite et al. (2019) classified the fruits of *Mimosa ophthalmocentra* Mart. Benth. into five maturity stages, based on color, and performed germination tests for each stage with and without treatment to overcome dormancy. They found that dormancy intensifies from the fourth class (light brown). In class 3 (yellow-brown), the percentage of germination did not differ between treatments with (82%) and without (77%) dormancy overcoming, which indicates that for this species, the seeds are not dormant when at the point of physiological maturity.

When subjected to water at 100 °C, seeds collected from brown fruits with darkened regions showed better germination performance compared to those from green fruits (Table 2). In the absence of dormancy overcoming treatment, seeds obtained from yellowish green fruits with purplish edges (T2) showed a higher percentage of normal seedlings (98.4%) compared to those obtained from brown fruits (92.8%). Therefore, these results corroborate those of Almeida (2013), who identified yellowish green fruits as the point of morphological maturity.

A characteristic of seeds of the Fabaceae family is their thick coat, which makes it waterproof and prevents the entry of water. This occurs because these seeds have some wraps, among them, waxy cuticle, suberin, palisade tissue and the macrosclereid layers (Cardoso, 2004). Lima et al. (2018), performing image analysis technique in *Bowdichia virgilioides* Kunth, observed that this species may show different seed coat

thicknesses.

The mean germination time was not different between treatments (Table 3). It is worth pointing out that the treatment referring to seeds from yellowish green fruits with purplish edges with dormancy overcoming (T1) was not included in the analyses of the variables mean germination time and lengths of shoots and roots since its germination was null.

Table 3 - Mean seed germination time (MGT), shoot length (SL) and primary root length (PRL) of *Bowdichia virgilioides* Kunth. seedlings

Treatments	MGT (days)	SL (cm)	PRL (cm)
T2	13.57 a	1.73 a	2.71 a
T3	13.33 a	1.03 b	1.65 b
T4	16.41 a	1.59 a	2.59 a
MSE	4.18	0.02	0.04
CV%	14.15	9.59	8.84
p-value	0.06	<0.0001	<0.0001

Means in columns followed by equal letters do not differ statistically by Tukey test ($\alpha = 0.05$)

Treatments without dormancy overcoming showed greater vigor of the seeds, considering that the average lengths of shoots and roots, based on the initial number of seeds, are indicators of vigor (Guedes et al., 2009).

average 8.94% with Confidence Interval- $CI_{95\%}$ of 8.94% \pm 0.98%.

In the treatment without dormancy overcoming, in only one replicate, among the 10 used, there was germination of two seeds, and only one formed a normal seedling. In the treatment with immersion of seeds in water at 100 °C for 10 seconds, germination (87.7%) and the formation of normal seedlings (76.8%) were high (Figure 2).

Experiment 2

The moisture content of the seeds from the fruits after the seventh week of floral anthesis was on

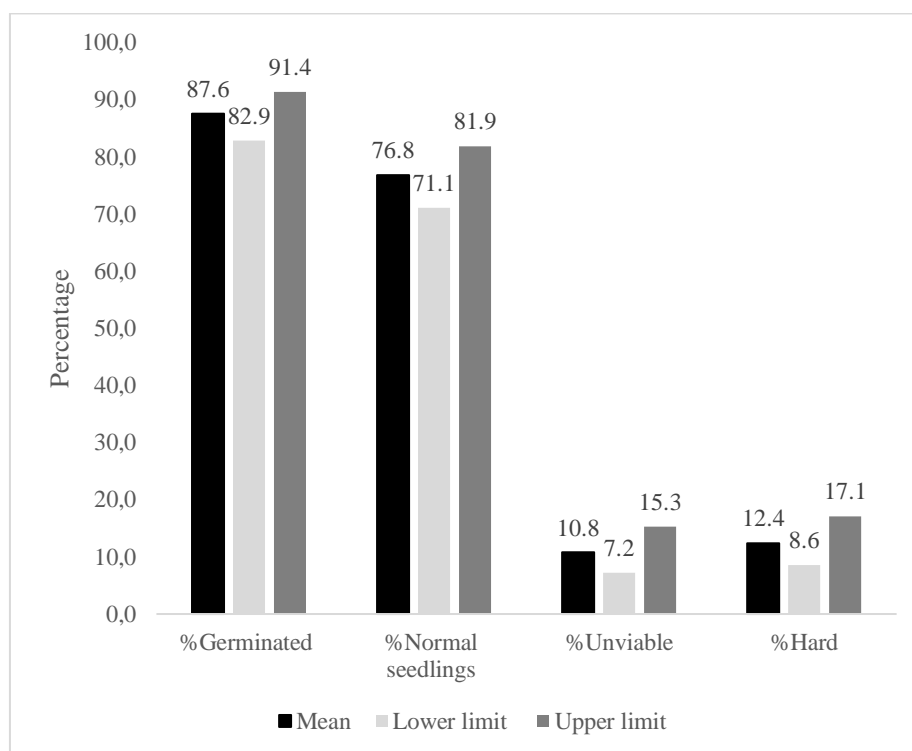


Figure 2 - Confidence intervals ($\alpha = 0.05$) of binomial distribution for germination percentage, percentage of normal seedlings, percentage of unviable seeds and percentage hard seeds of *Bowdichia virgilioides* Kunth, resulting from treatment to overcome dormancy.

Seeds obtained from fruits after the seventh week of floral anthesis showed high degree of dormancy because, without immersion in water at 100 °C for 10 seconds, germination was virtually zero, occurring only in two seeds out of a total of 250. These results confirm that fruit maturity influences the dormancy of *B. virgilioides* seeds.

According to Bewley et al. (2013), seed coat impermeability increases as the moisture content decreases, and the moisture range within which the impermeability is consolidated is between 2 and 20%. For studies that reported dormancy of *B. virgilioides*, the moisture content of the seeds was between 8.6 and 9.0% (Andrade et al., 1997; Albuquerque et al., 2007; Smiderle & Schwengber, 2011), so the moisture range within which the seed of this species becomes impermeable can be between 8.6 and 9.0%. In the present study, the moisture content of dormant seeds was 8.94%.

The results of this study may contribute to the practice of cultivating *B. virgilioides*, since it was found that the application of dormancy overcoming methods will not always be necessary. Reducing the indication of the use of sulfuric acid, the main technique employed to overcome dormancy of *B. virgilioides* seeds, facilitates the use of this species in reforestation programs because, if the seeds are collected at the adequate point of physiological maturity, there will be no need to use sulfuric acid, a product of difficult handling and disposal.

Conclusions

Bowdichia virgilioides seeds, collected from yellowish green fruits and brown fruits with darkened regions, do not show dormancy.

The establishment of dormancy occurs after the seventh week of floral anthesis when the fruits are predominantly darkened, dried and brittle.

Acknowledgments

To CAPES for granting the financial support through the Graduate Scholarship and funding the Institute of Environment and Water Resources - INEMA, through the Reference Center for Forest Restoration of the Atlantic Forest Biome - CRRF/MA.

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