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RESEARCH NOTE

Physiological maturity of *Tabebuia aurea* (Silva Manso) Benth. & Hook. f. ex S. Moore seeds[1]

Brenna Rafaella Veríssimo dos Santos[2], Clarisse Pereira Benedito[2*], Salvador Barros Torres[2], Caio César Pereira Leal[2], Tatianne Raianne Costa Alves[2]

ABSTRACT – The purpose of this research was to verify the efficiency of physical and biochemical indices in determining the physiological maturity of *Tabebuia aurea* seeds, as well as to evaluate the post-harvest storage of fruits with different maturation stages on germination and vigor. For this, 200 fruits were classified as dark green (stage I), light green (stage II), and light brown (stage III) epicarp. Freshly harvested fruits were evaluated for length, width, and weight and their seeds for length, width, thickness, thousand-seed weight, water content, electrical conductivity, and chemical composition. Subsequently, a factorial consisting of three maturation stages and four storage periods of fruits (0, 5, 10 and 15 days) were used, and water content, germination, germination speed index, root length, shoot length, and seedling dry matter were evaluated. Physical and biochemical indices can be used as maturation indicators in *T. aurea* seeds, except fruit length, seed thickness, electrical conductivity, and protein content. The maximum germination and vigor of *T. aurea* seeds were obtained in fruits at stages I (dark green) or II (light green) associated with post-harvest storage of fifteen days.

Index terms: Bignoniaceae, silver trumpet tree, maturation index, forest seeds, post-harvest storage.

Maturidade fisiológica em sementes de *Tabebuia aurea* (Silva Manso) Benth. & Hook. f. ex S. Moore

RESUMO – Objetivou-se verificar a eficiência de índices físicos e bioquímicos na determinação da maturidade fisiológica de sementes de *Tabebuia aurea*, bem como avaliar o repouso pós-colheita dos frutos com diferentes estádios de maturação sobre a germinação e vigor. Para isto, classificou-se 200 frutos com a coloração do epicarpo em verde-escuro (estádio I), verde-claro (estádio II) e castanho-claro (estádio III). Inicialmente, nos frutos recém-colhidos, avaliou-se o comprimento, largura e peso e, para as sementes, o comprimento, largura e espessura, peso de mil sementes, teor de água, condutividade elétrica e composição química. Em seguida, utilizou-se o fatorial de três estádios de maturação e quatro períodos de repouso dos frutos (0, 5, 10 e 15 dias) e avaliou-se o teor de água, germinação, índice de velocidade de germinação, comprimento de raiz, comprimento da parte aérea e massa seca de plântulas. Os índices físicos e bioquímicos podem ser utilizados como indicadores da maturação em sementes de *T. aurea*, com exceção do comprimento dos frutos, espessura das sementes, condutividade elétrica e teor de proteínas. O máximo de germinação e vigor de sementes de *T. aurea* foi obtido em frutos nos estádios I (verde-escuro) ou II (verde-claro), associados ao repouso pós-colheita por quinze dias.

Termos para indexação: Bignoniaceae, craibeira, índices de maturação, sementes florestais, repouso pós-colheita.

Introduction

Tabebuia aurea (Silva Manso) Benth. & Hook. f. ex S. Moore (Bignoniaceae) is known in Brazil as craibeira, para-tudo, caribeira, or ipê-da-caatinga (silver trumpet tree, Caribbean trumpet-tree, or tree of gold), being used for logging, urban afforestation, reforestation, and medicinal purposes. Its multiplication is mainly by seeds, but their fruits

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[2]Departamento de Ciências Agronômicas e Florestais, UFERSA, Caixa Postal 137, 59625-900 – Mossoró, RN, Brasil.
*Corresponding author <clarisse@ufersa.edu.br>

are dehiscent, making harvesting difficult (Lorenzi, 2008).

Quality seeds are essential to obtain healthy and vigorous seedlings. Among the criteria adopted at harvest to obtain quality seeds are their physiological maturity (Barbosa et al., 2015). Usually, seeds present the maximum germination and vigor at this stage, and the study of their maturation aims to determine how and when it is reached for each species. For this, some maturity indices should be verified, such as changes in water content, size, germination, vigor, seedling dry matter, and fruit and seed color (Bewley et al., 2013).

Physiological maturity is reached when seeds are only physically linked to the mother plant, as the transfer of water and nutrients ceases, being exposed to climate conditions, microorganisms, and insects (Marcos-Filho, 2015). In this sense, the postharvest storage technique of fruits can be used to anticipate the harvest. It avoids seed storage in the field and its negative consequences, in addition to eliminating the natural loss of seeds, especially for dehiscent fruits, such as those of *T. aurea*. This technique is widely used for seeds of some fleshy or dry fruit vegetables, but still non-existent for forest seeds.

The studies of Lopes et al. (2014) with cumaru (*Amburana cearensis* (Allem.) A.C. Smith.), Pires Neto et al. (2016) with angico (*Anadenanthera colubrina* (Vellozo) Brenan.), and Kaiser et al. (2016) with chal-chal (*Allophylus edulis* (A. St.-Hil. A. Juss. & Cambess.) Hieron. ex Niederl.) stood out on the physiological maturity of forest seeds. However, no information can be found in the literature on *T. aurea* to evaluate the effect of post-harvest storage on the physiological quality of seeds. Thus, studies with *T. aurea* seed maturation are fundamental since its fruits are dehiscent, and a delay of few days in the harvest may lead to total loss of seed production or may result in immature seeds with high water content and low vigor if it is carried out in advance.

Thus, this study aimed to verify the efficiency of physical and biochemical indices in determining the physiological maturity of *T. aurea* seeds, as well as evaluate the post-harvest storage of fruits with different maturation stages on germination and vigor.

Material and Methods

Fruits of *T. aurea* were harvested in August and September 2016 from approximately 20 native trees spaced at least 50 meters from each other and located on the campus of the Federal University of the Semi-Arid Region (UFERSA) (5°12′15″ S and 37°19′54″ W) in an area close to the School of Nursing and Medicine (FACENE) (5°12′39″ S and 37°19′32″ W), Mossoró, RN. Subsequently, fruits were classified by visual observation of the epicarp color into dark green (stage I), light green (stage II), and light brown (stage III) (Figure 1) using the Munsell (1976) color chart.

Evaluations of freshly harvested fruits and seeds

Initially, 100 freshly harvested fruits were evaluated for the following physical aspects:

Seed water content: determined by the oven method at 105 ± 3 °C for 24 hours, with two replications of 25 seeds (Brasil, 2009).

Fruit and seed weight, length and width: a random sample of 100 fruits and 100 seeds were randomly weighed individually on a semi-analytical scale, and means were expressed in grams. Fruit length was measured from the base to the apex using a ruler graduated in millimeters, and the results were expressed in centimeters. Fruit width was measured from its midline using a digital caliper with a 0.05-mm precision, with results expressed in centimeters. Seed dimensions were measured using a digital caliper (0.05 mm precision).

Thousand-seed weight: determined by randomly counting eight subsamples of 100 seeds, as recommended by Brasil (2009).

Lipid content: determined by the method described by Mizubuti et al. (2009).

Maturation stage	Epicarp color	Visual characterization	Munsell*
I		Dark green	10 Y 5/2
II		Light green	7.5 Y 6/3
III		Light brown	7.5 Y 8/2

Figure 1. Visual characterization of fruits of silver trumpet tree (*Tabebuia aurea* (Silva Manso) Benth. & Hook. f. ex. S. Moore) at different maturation stages.

*Munsell color charts for plants tissues.

Protein content: determined based on the total nitrogen content, dosed by the Kjeldahl method (AOAC, 2016).

Starch content: 0.2 g of dry matter (ground seeds) was added in tubes containing 3 mL of 60% perchloric acid. These tubes were then hermetically sealed and stirred at ambient temperature for five minutes. The supernatant was collected for starch quantification, and the absorbance was measured at 620 nm with the application of the Antrona method (Yemm and Willis, 1954).

Amino acid content: 0.2 g of dry matter (ground seeds) was added into tubes containing 3 mL of alcohol. Subsequently, they were hermetically sealed and heated in a water bath at 60 °C for 20 min. Amino acid contents were quantified by absorbance at 570 nm using the acid ninhydrin method (Yemm and Cocking, 1955).

Evaluations of seeds after post-harvest storage

Approximately 30 fruits from each maturation stage were placed in plastic trays covered with yarn tissue and stored for 0, 5, 10, and 15 days at ambient conditions (30 °C and 45% RH). The following physiological indices were evaluated after each storage period:

Seed water content: determined by the oven method at 105 ± 3 °C for 24 hours, with two replications of 25 seeds (Brasil, 2009).

Germination: four replications of 25 seeds were sown in aluminum trays (41 × 27.5 × 3.5 cm) with washed sand substrate and maintained at 30 °C. Initially, the substrate was moistened with an amount of water equivalent to 60% of field capacity. Seeds that formed normal seedlings were considered germinated. The first and final counts were carried out at 10 and 21 days after sowing, respectively (Brasil, 2013).

Germination speed index (GSI): conducted together with the germination test, with daily counts and values obtained according to Maguire (1962).

Shoot and root length: shoot length was measured from the seedling collar to its apex using a ruler graduated in millimeter. Root length was measured from the collar insertion to the end of the main root. Both results were expressed in cm seedling^{-1}.

Seedling dry matter: normal seedlings were dried in a forced-air ventilation oven at 65 °C for 72 hours, with results expressed in g.seedling^{-1}.

Statistical analysis

A completely randomized design with four replications of 50 seeds was adopted for the first trial, while a completely randomized design in a 3 × 4 factorial scheme (three maturation stages and four post-harvest storage periods), with four replications of 50 seeds, was adopted for the second test. The data were subjected to analysis of variance by the F-test, and the means were compared by Tukey test at 5% probability using the software *Sistema para Análise de Variância* (SISVAR) (Ferreira, 2011). The estimation of regression parameters was evaluated using the software Excel.

Results and Discussion

Seed water content was higher for fruits from stage I (dark green), followed by fruits from stages II (light green) and III (light brown) (Table 1). These differences in water content between maturation stages suggest that seeds obtained from stage I were more immature than the others, followed by stages II and III. In contrast, seeds from stage III probably were already over the point of physiological maturity, as they had lower initial water content, and several fruits were cracked at harvest time, with a mature appearance, initiating the process of natural dehiscence and contributing to greater reduction in seed water content. These observations reinforce reports by Marcos-Filho (2015), who verified that seeds at the initial stage of formation have a high water content, ranging from 70 to 80%, decreasing during the maturation process until reaching equilibrium values with relative air humidity usually between 12 and 15% for orthodox seeds from dry fruits, although it remains relatively high during most of this period since the transfer of dry matter from plant to seeds must occur in liquid medium.

These results agree with those obtained from *Peltophorum dubium* (Spreng) Taub., whose seeds from green fruits also presented initial water content above 70%, and those from light brown fruits (stage III) presented water content of 17%

Table 1. Water content (WC), thousand-seed weight (TSW), length (L), width (W), and thickness (T) of *Tabebuia aurea* (Silva Manso) Benth. & Hook. f. ex. S. Moore) seeds with different stages of fruit maturation.

Maturation stage	WC (%)	TSW (g)	L (mm)	W (mm)	T (mm)
I (dark green)	70.9	26.50 A	16.34 A	12.26 A	1.95 A
II (light green)	59.9	22.82 B	16.10 A	11.94 A	2.02 A
III (light brown)	17.1	7.40 C	15.25 B	10.96 B	1.98 A

Means followed by the same letter in the column do not differ from each other by the Tukey test (p < 0.05).

(Muller et al., 2016). Freshly harvested seeds of stage II had a water content of 59.9%, close to that found for *Tabebuia chrysotricha*, with a value of 61.3% at physiological maturity (Fonseca et al., 2005). However, those of *Amburana cearensis* (Allem.) A. C. Smith. reached physiological maturity with 49.7% of water (Lopes et al., 2014).

A statistical difference was observed between treatments for thousand-seed weight, whose higher values were obtained for stage I seeds, while the more mature fruits (stage III) provided lower weight seeds (Table 1). These results are related to seed water content since those of stage I presented higher water content, while those of stage III had a lower value. According to Bewley et al. (2013), the high water content at the beginning of maturation is necessary for both cell expansion and translocation of plant metabolites to seeds, besides being fundamental for the subsequent accumulation of reserves.

Higher values were observed for seed length, width, and thickness in the stages I and II, showing a significant reduction for seeds from stage III (Table 1). These results are in agreement with those found in the literature when they inform that seeds grow rapidly in size, reaching their maximum in a short period in relation to the total duration of the maturation period. Once the maximum is reached, it is maintained for some time and slightly reduced at the end of the period (Carvalho and Nakagawa, 2012; Bewley et al., 2013; Marcos-Filho, 2015).

No statistical differences were observed between maturation stages for length, width, and weight (Table 2). Seeds of *Moringa oleifera* Lam. (Agustini et al., 2015), *Oreopanax fulvus* Marchal (Pinto et al., 2016), and *Erythrina variegata* L. (Matheus et al., 2011) showed no differences in the length of fruits from different maturation stages. According to Barbosa et al. (2015), fruit and seed sizes are good indicators to assist in the evaluation of seed physiological maturity, which reach the maximum size in the intermediate stages of the maturation process, with a slight reduction at the end due to water loss, a fact also found for *Inga striata* Benth. (Mata et al., 2013), *Allophylus edulis* (A. St.-Hil., A. Juss. & Cambess.) Hieron. ex Niederl. (Kaiser et al., 2016), and *Anadenanthera colubrina* (Vellozo) Brenan (Pires Neto et al., 2016). However, this parameter must be evaluated along with other maturation indices.

Seed biochemical analyses indicated differences between seed categories for protein content means. In turn, the highest lipid, starch, and amino acid accumulation were verified for seeds from stage III, also coinciding with the lower water content (Table 3). These results are in agreement with Marcos-Filho (2015), who reported that protein content is usually constant during seed development, indicating a uniform synthesis rate and an initially low lipid content that increases as physiological maturity approaches. Thus, stage III seeds were possibly closer to the point of physiological maturity. Moreover, in seed maturation studies, the evaluation of the amount of dry matter accumulated during the development process requires frequent sampling at relatively short intervals so that the detection of variations in the amount accumulated by seeds is carried out with precision. This fact did not occur in this study since no collections were carried out at frequent intervals because only differences in fruit color were considered.

The post-harvest storage and fruit maturation stages on seed quality presented linear effects for all maturation stages on seed water content, whose values decreased as the post-harvest storage time increased. However, this reduction was more pronounced for seeds of stages I and II since they had higher initial water content. At 15 days, water content was similar between the three stages, reaching values of 9.0, 8.8, and 7.0% for seeds from stages I, II, and III, respectively (Figure 2). Thus, the post-harvest storage technique was advantageous for *T. aurea* seeds, mainly for fruits harvested greener, as seed water content was gradually reduced,

Table 2. Length, width, and weight of fruits of silver trumpet tree (T*abebuia aurea* (Silva Manso) Benth. & Hook. f. ex. S. Moore) as a function of different stages of fruit maturation.

Maturation stage	Length (cm)	Width (cm)	Weight (g)
I (dark green)	11.1 A	21.98 A	22.53A
II (light green)	11.5 A	21.92 A	21.5 A
III (light brown)	11.8 A	20.58 A	16.1 B

Means followed by the same letter in the column do not differ from each other by the Tukey test ($p < 0.05$).

Table 3. Mean protein, lipid, starch, and amino acid contents of seeds of silver trumpet tree (*Tabebuia aurea* (Silva Manso) Benth. & Hook. f. ex. S. Moore) with different maturation stages.

Maturation stage	Protein (%)	Lipid (%)	Starch (mg/100g)	Amino acid (µmol GLU/g DM)
I (dark green)	19.765 A	9.701 B	0.153 B	2.1135 B
II (light green)	18.743 A	12.017 B	0.116 C	2.020 B
III (light brown)	18.265 A	18.778 A	0.291 A	8.502 A

Means followed by the same letter in the column do not differ from each other by the Tukey test ($p < 0.05$).

indicating their preparation for physiological maturity. It prevented the seeds from remaining in the field, freeing them from unfavorable weather conditions, as well as from the attack of insects and microorganisms.

Germination and germination speed index values are shown in Figures 3A and 3B, respectively. They fit the quadratic polynomial model for stage I, the cubic polynomial model for stage II, and the linear model for stage III. Freshly harvested seeds (time zero) from stages II and III showed maximum germination (61 and 68%, respectively), while those from stage I had lower germination (35%). Five days

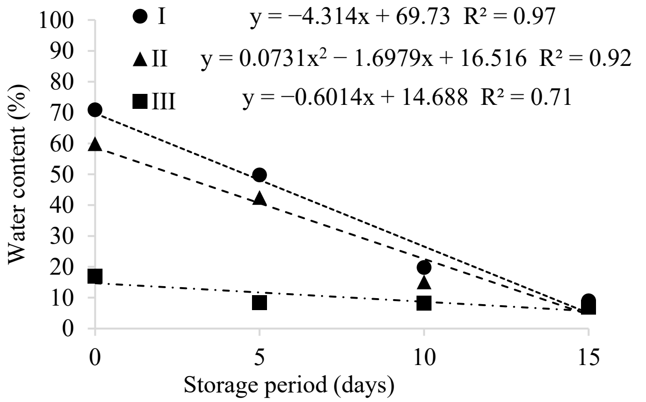

Figure 2. Water content of silver trumpet tree (*Tabebuia aurea* (Silva Manso) Benth. & Hook. f. ex. S. Moore) seeds as a function of different stages of fruit maturation and post-harvest storage of fruits. Stage I (dark green); stage II (light green), and stage III (light brown).

of post-harvest storage led to an increase in the germination of stage I seeds, reaching a higher mean, whereas stage III showed a reduction when compared to stages I and II. It indicates that drying during post-harvest storage of greener fruits resulted in seeds with a higher germination capacity than those dried on the plant itself (stage III). Seeds harvested at stage III possibly had already passed the point of physiological maturity and were theoretically stored in the field, i.e., more deteriorated when compared to those from stages I and II. A reduction in values was verified at ten days of storage when compared to the previous five days. However, germination of stage III seeds was impaired at 15 days, significantly reducing in relation to the others (Figure 3A). Germination results are in agreement with those found by Ricci et al. (2013), who observed that, in general, the germination of seeds that did not reach physiological maturity and are placed to germinate soon after harvest is lower compared to those resulting from germination after a few days of storage. Environmental conditions under which post-harvest storage was conducted may have contributed to accelerating the deterioration progress of mature seeds (Degan et al. 2001), as low germination was observed when seeds were stored at ambient temperature. Chitarra et al. (2008) recommend the harvesting of *Piptadenia gonoacantha* (Mart.) Macbr) fruits of green and brown-green color for immediate sowing, as they result in higher germination values. Similarly, Carvalho et al. (2014) also obtained higher germination in seeds from green fruits of *Physalis angulata*.

The germination speed index (GSI) showed a linear trend for stages II and III and a quadratic trend for stage I (Figure 3B). Seeds from stages II and III presented faster

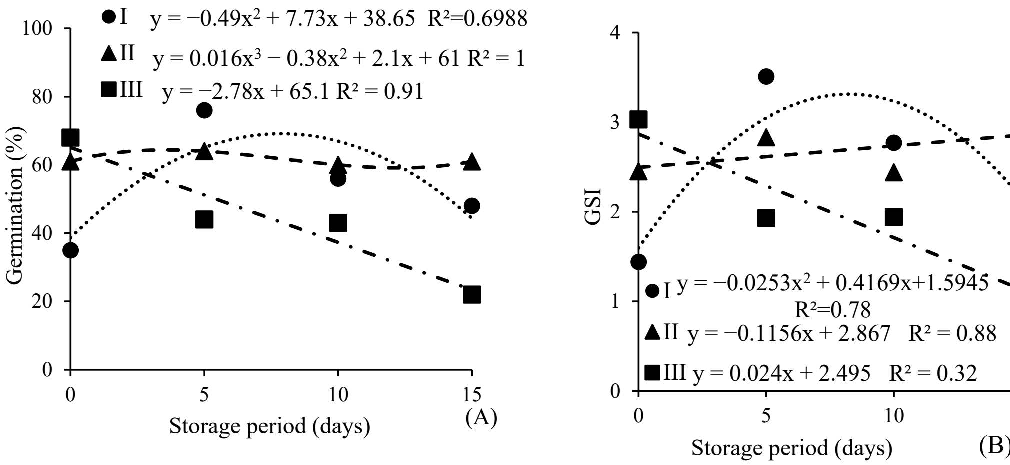

Figure 3. Germination (A) and germination speed index (GSI) (B) of seeds of *Tabebuia aurea* (Silva Manso) Benth. & Hook. f. ex. S. Moore) as a function of different maturation stages before and after post-harvest storage of fruits. Stage I (dark green), stage II (light green), and stage III (light brown).

germination at storage time zero when compared to stage I, showing that seed germination speed is directly related to fruit maturation. This relationship can be explained by a higher vigor and, consequently, higher physiological quality of seeds harvested from older fruits (Nakada et al., 2011). Seeds from stage II had similar behavior in relation to GSI at times zero, five, ten, and 15 days, i.e., the post-harvest storage did not present significant effects on GSI for the seeds of this maturity stage. In contrast, seeds from stage I presented lower GSI at time zero, but it reached maximum values at five, ten, and 15 days of storage, equal to stage II, i.e., postharvest storage provided a positive effect on seed vigor at this maturity stage (Figure 3B). However, stage III seeds had a lower GSI with the increased storage time, showing a lower value at 15 days, evidencing again that the increased post-harvest storage time for these seeds contributed to increasing seed deterioration, resulting in a decrease of vigor. According to Marcos-Filho (2015), seed deterioration process starts progressively from the physiological maturity point, characterized by physical, physiological, and biochemical changes, which determine a decrease in its quality. Thus, the results obtained in this study differ from those found for *Piptadenia gonoacantha* (Mart.) Macbr., whose seeds from freshly harvested green and brown-green fruits presented higher GSI (Chitarra et al., 2008). On the other hand, seeds of *T. impetiginosa* (Mart.) Stand. showed maximum SGI values from seeds harvested mature at the beginning of dispersal (Gemaque et al., 2002).

The highest values for root length at the post-harvest storage zero were obtained from seeds from stages II and III, while those from stage I resulted in a smaller size (Figure 4A). The maturation stages remained stable at five and ten days, but only seeds from stage II presented maximum root length at 15 days. No increase in root length was found for stages I and II as storage time increased, remaining with the same behavior, while seeds from stage III had higher values at times zero and ten days.

Stage II seeds presented the maximum value of total seedling dry matter (Figure 4B) at times zero and five days of storage. However, the values remained similar between seed categories at ten days and reached maximum values at 15 days for seeds from stages I and II.

Dry matter increased at five, ten, and 15 days for seeds from stages I and II between the different storage times. Seeds resulting from stage III had higher dry matter accumulation at ten days (0.065 g.seedling^{-1}) of post-harvest storage. On the contrary, Kaiser et al. (2016) observed that *Allophylus edulis* (A. St.-Hil., A. Juss. & Cambess.) Hieron. ex Niederl. seeds from green fruits resulted in seedlings with a lower dry matter.

Shoot length as a function of post-harvest storage time was longer at five, ten, and fifteen days and shorter for time zero (Figure 5B). Still for this variable, the isolated effect of maturation stages had a higher value for stage II seeds, being statistically higher to the others (Table 4). Negreiros et al. (2006) suggested that the complete physiological seed maturity favors seedling growth. Carvalho and Nakagawa (2012) stated that not fully mature seeds could germinate, but they will not result in vigorous seedlings, such as those from mature seeds.

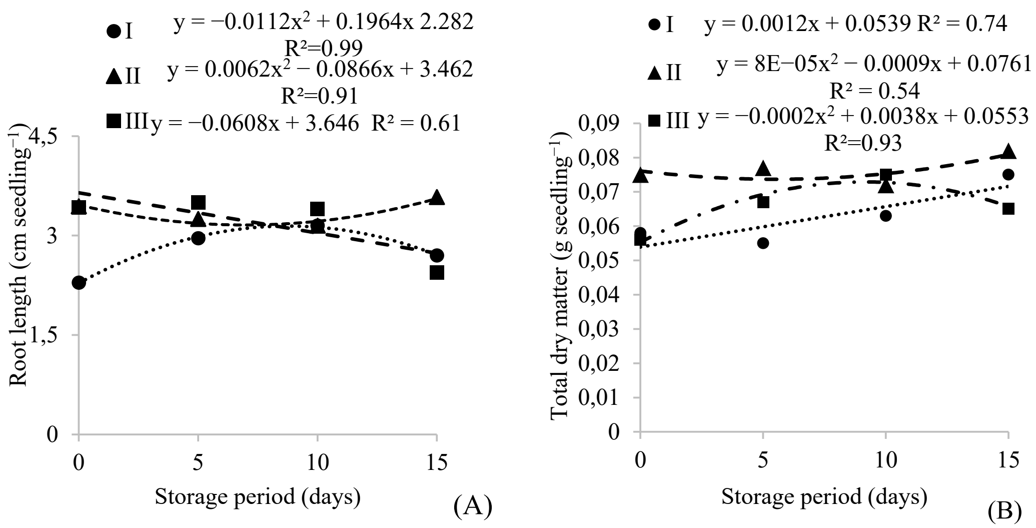

Figure 4. Root length (A) and total seedling dry matter (B) of seeds of *Tabebuia aurea* (Silva Manso) Benth. & Hook. f. ex. S. Moore) as a function of different maturation stages before and after post-harvest storage of fruits. Stage I (dark green), stage II (light green), and stage III (light brown).

The results indicate that fruit epicarp color, percentage of germination, root length, and seedling dry matter can be used as indicators of the physiological maturity of *T. aurea* seeds.

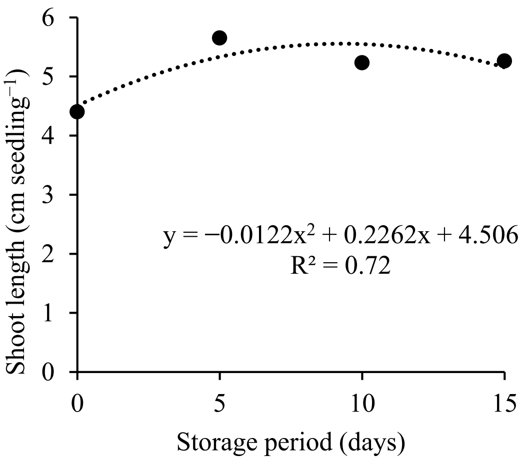

Figure 5. Isolated effect of post-harvest storage for the shoot length of silver trumpet tree (*Tabebuia aurea* (Silva Manso) Benth. & Hook. f. ex. S. Moore) as a function of different maturation stages of fruits before and after post-harvest storage.

Table 4. Isolated effect of maturation stages for shoot length (SL) of silver trumpet tree (*Tabebuia aurea* (Silva Manso) Benth. & Hook. f. ex. S. Moore) as a function of different maturation stages of fruits before and after the post-harvest storage of fruits.

Maturation stage	SL (cm)
I (dark green)	4.51 B
II (light green)	6.28 A
III (light brown)	4.61 B

Means followed by the same letter in the column do not differ from each other by the Tukey test ($p < 0.05$).

Conclusions

Physical and biochemical indices can be used as maturation indicators of *T. aurea* seeds, except for fruit length, width, and thickness, seed thickness, and protein content.

Maximum germination and vigor of *T. aurea* seeds can be obtained from fruits at stages I (dark green) and II (light green), associated with post-harvest storage of 15 days under the environmental conditions of this study.

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