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Physiological maturity of *Cenostigma tocantinum* Ducke (Fabaceae) seeds

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ABSTRACT: Studies on seed maturation directly contribute to obtaining seeds with a higher standard of physical, physiological, and sanitary quality. Thus, the aim of this study was to verify the morphological and physiological changes during the maturation of *Cenostigma tocantinum* Ducke seeds. The maturation stages were analyzed: I (293 days after anthesis – DAA), II (322 DAA), III (350 DAA), and IV (356 DAA) through visual, biometric analyses, and quantification of moisture content, dry mass, viability, and seed vigor. During the development of *C. tocantinum* seeds, changes in fruit coloration and an increase in fruit and seed length, width, and thickness were observed. In the initial stages, the seeds had a high moisture content, which decreased in the later stages. On the other hand, the dry mass of the seeds showed an inversely proportional behavior to the moisture content. Physiological variables performed better in stages III and IV, except for electrical conductivity. It can be concluded that *C. tocantinum* seeds showed superior physiological quality in stages III or IV, recommending the collection of seeds during these maturation periods.

Index terms: *pau-pretinho*, physiological quality, seed development, seed maturation.

RESUMO: Estudos sobre maturação de sementes contribuem diretamente para obtenção de sementes com maior padrão de qualidade física, fisiológica e sanitária. Dessa forma, objetivou-se verificar as alterações morfológicas e fisiológicas durante a maturação de sementes de *Cenostigma tocantinum* Ducke. Analisou-se os estádios de maturação: I (293 dias após antese - DAA), II (322 DAA), III (350 DAA) e IV (356 DAA) através de análises visuais, biométricas e a quantificação do grau de umidade, massa seca, viabilidade e vigor das sementes. Durante o desenvolvimento das sementes de *C. tocantinum* observou-se mudanças na coloração dos frutos e um aumento no comprimento, largura e espessura dos frutos e sementes. Nos estádios iniciais, as sementes apresentaram elevado grau de umidade e redução nos estádios finais. Por outro lado, a massa seca das sementes apresentou comportamento inversamente proporcional ao grau de umidade. As variáveis fisiológicas apresentaram desempenho superior nos estádios III e IV, exceto para condutividade elétrica. Conclui-se que as sementes de *C. tocantinum* apresentaram qualidade fisiológica superior nos estádios III ou IV, recomendando-se a coleta das sementes nesses períodos de maturação.

Termos para indexação: pau-pretinho, qualidade fisiológica, desenvolvimento de sementes, maturação de sementes.

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INTRODUÇÃO

High-quality tree seeds are essential for vigorous seedlings and to meet the forestry sector demands (Piveta et al., 2014; Padilha et al., 2021). Seed quality is linked to genetic, physical, sanitary, and physiological attributes. Of these, physiological quality is of great importance since it is directly associated with plant vital functions, potentially providing longevity and uniformity in production (Popinigis, 1985).

During seed development/maturation, physiological quality increases, with changes in moisture content, accumulation of dry mass, germination potential, and vigor, making it a vital period for development of viable seeds (Carvalho and Nakagawa, 2012; Bing et al., 2023). Consequently, various studies have assessed the changes during seed maturation, aiming to evaluate morphological and physiological modifications and their effects on post-harvest (Muller et al., 2016; Hell et al., 2019; Santos et al., 2019; Guarriz et al., 2022; Mescia et al., 2022).

In each species, these changes may occur in specific periods, associated with fruit coloration, size and weight, presence of predators, fruit dehiscence, and fruit dispersion. These are indicators of the optimal point for the collection of seeds with high physiological potential (Gemaque et al., 2002; Ristau et al., 2020).

However, little is known about the maturation period and hence collection time in many native forest species, such as *Cenostigma tocantinum* Ducke (Fabaceae). This species naturally occurs in the Amazon region and is popularly known as "*pau-preto*," "*pau-pretinho*," and "*cássia-rodoviária*." It can be used in construction, urban tree planting, and recovery of permanent preservation areas (Garcia et al., 2008; Lorenzi, 2016; Rodrigues et al., 2020).

Considering the above, it is of utmost importance to identify the optimal time for collecting seeds with maximum physiological quality to improve their production (Cruz et al., 2021). In this sense, the present study aimed to analyze morphological and physiological changes during the maturation of *C. tocantinum* seeds to determine the optimal time for seed collection.

MATERIAL AND METHODS

The study was conducted on *Cenostigma tocantinum* trees in the city of Ourém - PA, Brazil (01° 30' South latitude and 47° 03' West longitude. Average monthly rainfall was 181 mm during the experiment (INMET, 2022). Twelve *C. tocantinum* mother trees were used to monitor seed development from September 2020 to September 2021. Seed collections were performed at four stages of maturation based on fruit color change: Stage I (293 days after anthesis - DAA), Stage II (322 DAA), Stage III (350 DAA), and Stage IV (356 DAA).

Fruits were collected directly from mother trees using a pruning saw and subjected to visual (coloration) and biometric characterization. They were measured for width, length, thickness, and number of seeds per fruit. Then, seeds were extracted from the fruits, removing dead, fungal, or non-viable seeds, and biometric characterization was conducted to measure length, width, and thickness. In both biometric analyses, eight replications of 25 fruits and seeds were measured using digital calipers.

After each collection, tests for moisture content, dry mass, electrical conductivity, germination, and seedling emergence were conducted to compare the four developmental stages.

Four 10-seed replications were used for moisture and dry mass tests, following the oven-drying method at 105 °C for 24 hours (Brasil, 2009).

For the germination test, four 25-seed replications were disinfested with 2% sodium hypochlorite for 3 minutes, followed by rinsing with deionized water. The seeds were then sown in sterilized vermiculite, autoclaved for 20 minutes at 121 °C, and kept in a Biochemical Oxygen Demand (BOD) germination chamber. A temperature of 30 °C and a photoperiod of 12 hours were used until the establishment of normal seedlings (12 days) (Brasil, 2013), with daily evaluations. At the end of the test, the length of normal seedlings was measured using a millimeter ruler, and then seedlings were kept in an oven at 60°C for 48 hours to determine dry mass. Additionally, percentages of germinated seeds (radicle protrusion ≥ 2 mm), normal seedlings (seedlings with developed root systems and aerial parts without abnormalities), and germination speed index (GSI) (Maguire, 1962) were calculated, considering the speed at which seeds transformed into normal seedlings.

The seedling emergence test was conducted in a nursery with 50% shading, using four 25-seed replications for each maturation stage. The seeds were sown in trays filled with autoclaved vermiculite and manual watering daily. The test was conducted for 12 days, and at the end, variables such as emergence percentage and EVI (Emergence Velocity Index) (Maguire, 1962) were obtained.

Electrical conductivity was tested in four 10-seed replications using 75 mL distilled water. The seeds were placed in a BOD-type chamber at 25 °C, and readings were taken after 10 and 24 hours of soaking using a portable conductivity meter. The results were expressed as $\mu S \cdot cm^{-1}.g^{-1}$.

The experiment was conducted using a completely randomized design (CRD) with four maturation stages (treatments). Data were subjected to the Shapiro-Wilk normality test and Bartlett's test for homogeneity of variances. Due to the non-normality, biometric data were presented in box plots and compared using the Dunn test (α=0.05). Polynomial regression was used to analyze physiological data. Additionally, Pearson's correlation coefficient was calculated to determine the degree of dependence between biometric and physiological traits. Statistical analyses were performed using R software (R Core Team, 2020).

RESULTS AND DISCUSSION

We observed color changes in both *Cenostigma tocantinum* fruits and seeds, along with increases in biometric and physiological variables. Fruits exhibited color variation during development. At 293 days after anthesis (DAA), they were green (Stage I). At 322 DAA, it changed to green-yellow (Stage II). At 350 DAA, the color shifted to brown with brown spots (Stage III). Finally, at 356 DAA, the predominant color was reddish-brown (Stage IV) (Figure 1).

Similar changes were observed in *Paubrasilia echinata* (Lam.) Gagnon, H.C. Lima & G.P. Lewis (*pau-brasil*), which belongs to the same family as the species under study (Fabaceae). *Pau-brasil* exhibited a light green color between 28

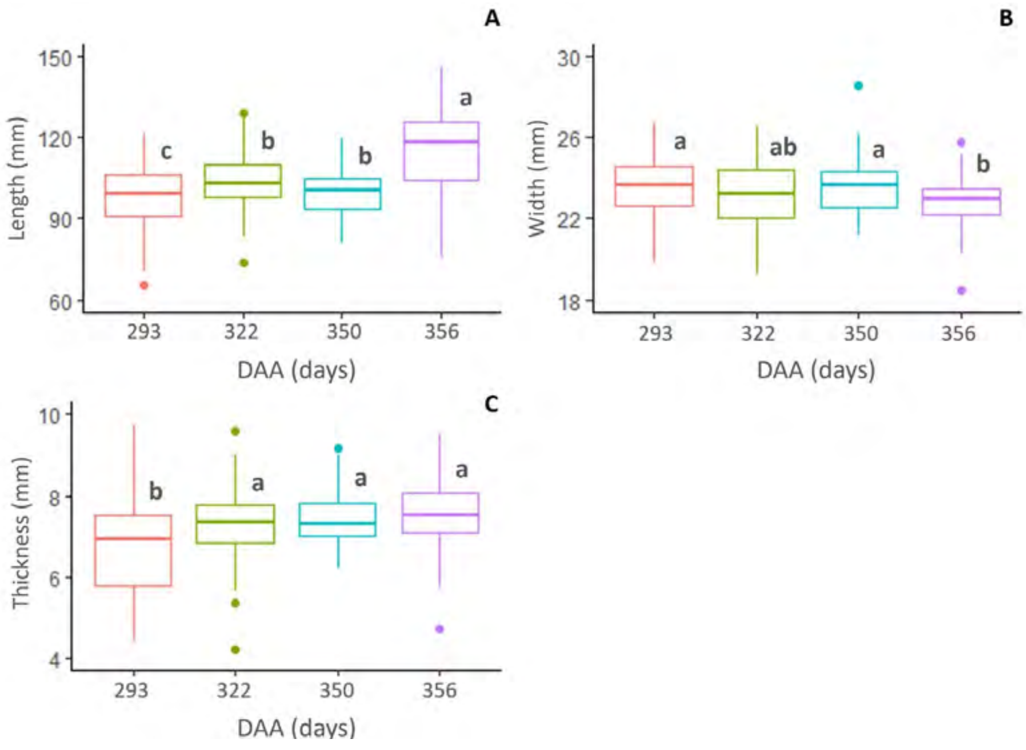
Figure 1. Coloration of fruits and seeds of *Cenostigma tocantinum* at stage I (293 DAA), stage II (322 DAA), stage III (350 DAA), and stage IV (356 DAA).

and 44 DAA, followed by a change to dark green with small light brown spots at 50 DAA and a completely brown color at 60 DAA (Borges et al., 2020). These observed changes are related to the maturation of internal tissues, associated with chlorophyll degradation and synthesis of existing pigments, which determine fruit coloration (Lima et al., 2012).

Similar to fruits, seeds also exhibited changes in coloration during the evaluated periods. In Stage I, the seeds were green; in Stage II, they changed to green-yellow with small red spots; in Stage III, they had a yellow color with red spots; and finally, in Stage IV, the seeds were completely brown with red spots (Figure 1). Likewise, Lopes et al. (2014) observed variations in coloration during the development of seeds of *Amburana cearensis* (Allemão) A.C.Sm, ranging from cream to black during development, which corroborates what was observed in our study. Just as in fruits, this variation in seed coloration may be associated with chlorophyll levels, which are higher at the beginning of seed development and contribute to carbohydrate accumulation (Brazel and Ó'Maoileídigh, 2019; Gupta et al., 2022). In cultivated species like tomatoes, green fruits can contribute up to 15% of photosynthetic activity (Hetherington et al., 1998). Seed and fruit coloration is a practical indicator used in the field to determine the ideal collection time; however, it is necessary to analyze it in conjunction with physiological variables during the same period.

Regarding the length of *C. tocantinum* fruits, differences were observed at all maturation stages, with higher medians in Stage IV (113.31 mm) (Figure 2A). Similar results were found by Duarte et al. (2022) in seeds of *Lecythis pisonis* Cambess., which showed higher values for fruit length in the last stages evaluated (4 and 5 months after anthesis).

For fruit width, no differences were observed between Stages I, II, and III, with a median of 23.40 mm. However, in Stage IV, there was a reduction in this variable (22.85 mm), showing a significant difference compared to Stages I and III (Figure 2B). In contrast, there was an increase in the median thickness of fruits from Stage I (6.72 mm) to Stage II (7.42 mm), remaining stable until Stage IV (7.57 mm) (Figure 2C). In addition, in Stage I, the interquartile range was greater than in the other stages. For the species *A. cearensis*, small variations in fruit width and thickness were observed during the maturation period, with initial values of 12.8 mm and 9.7 mm, respectively, in Stage I (26 DAA), reaching 13.9 mm



*Equal letters do not differ from each other by the Dunn test at a 5% probability level.

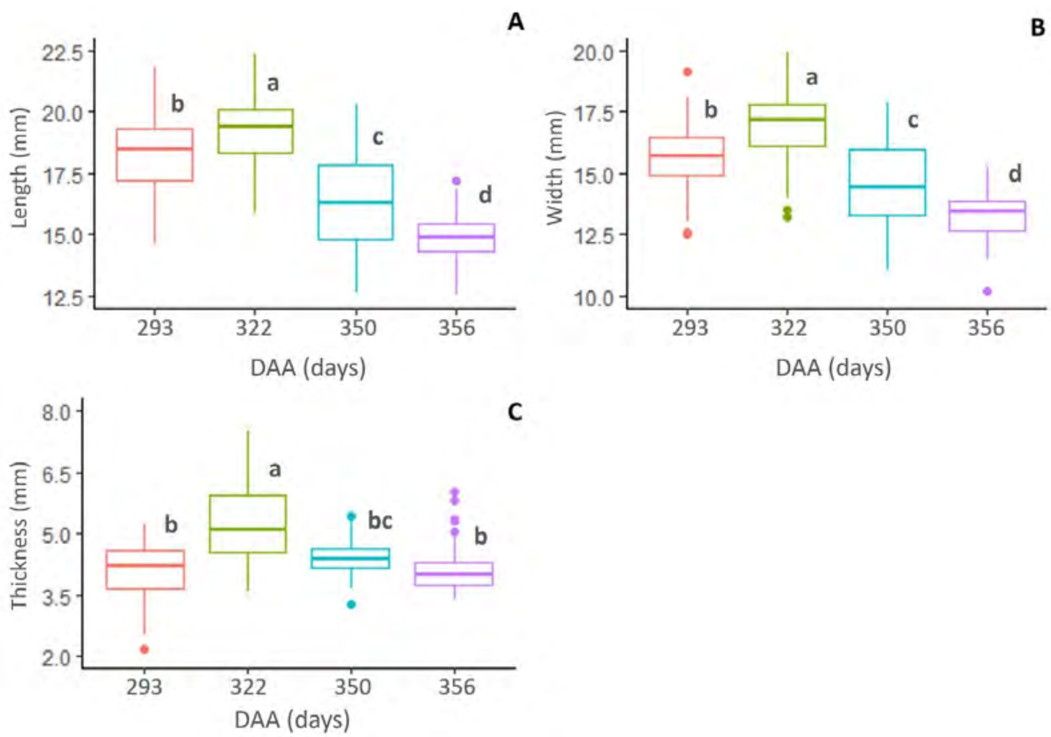
Figure 2. Boxplot of the length (A), width (B), and thickness (C) of *Cenostigma tocantinum* fruits at stage I (293 DAA), stage II (322 DAA), stage III (350 DAA), and stage IV (356 DAA).

in width and 9.6 mm in thickness in Stage V (54 DAA) (Lopes et al., 2014). These biometric variations in fruits and seeds can be related to individual variations that may occur within the same tree or among mother trees due to climatic variations, genetic variability, and physiological changes during maturation (Pereira et al., 2018).

Additionally, these fluctuations in dimensions may be related to climatic factors that affect the synchronization of the fruiting process among individuals during development (Mata et al., 2013) or may also reflect seed quality (Oliveira et al., 2020; Gomes et al., 2023). Due to this variability, for some species like *Mimosa caesalpiniifolia* Benth., fruit biometric parameters were not effective in assisting in maturity point determination (Alves et al., 2005). In the case of *Erythrina crista-galli* L., only fruit length was a complementary parameter for determining physiological maturity because a significant decrease in dimension was observed after maturity (Lazarotto et al., 2011).

For *C. tocantinum* seed biometry, a similar response was observed in all variables analyzed, with maximum values in Stage II, with a length of 19.29 mm, width of 17.02 mm, and thickness of 5.28 mm, followed by a decrease in these variables in Stage IV (Figures 3A, B and C). Similar behavior was observed for the species *Anadenanthera colubrina* (Vell.) Brenan, in which seeds in the early maturation stages had larger dimensions, with reductions at the final stages (Cruz et al., 2021). Likewise, the length, diameter, and thickness of *Albizia niopoides* (Spruce ex Benth.) Burkart showed higher means in Stage I (100% green fruits) and a reduction in Stage IV (100% brown fruits) (Ristau et al., 2020). Variations in dimensions during seed maturation are due to changes in fruit water content. During this period, water loss through transpiration occurs, leading to physiological maturity (Silva et al., 2013).

Moisture content during *C. tocantinum* seed development exhibited a descending quadratic behavior with days after anthesis (DAA), with values of 61.4% in Stage I, 58.9% in Stage II, and a sharp decrease to 35.1% in Stage III and 14.1% in Stage IV (Figure 4A). A similar result was observed for seeds of *Parkia platycephala* Benth. (Fabaceae), which showed high moisture content in the early stages (74.59%), decreasing to 29.65% in the final stage (Santos et al., 2022). The beginning of seed maturation is marked by a period of cell division, expansion, and differentiation,



*Equal letters do not differ from each other by the Dunn test at a 5% probability level.

Figure 3. Length (A), width (B), and thickness (C) of seeds of *Cenostigma tocantinum* at stage I (293 DAA), stage II (322 DAA), stage III (350 DAA), and stage IV (356 DAA).

with a high moisture content during this phase. Subsequently, there is an accumulation of reserves followed by seed desiccation and an increase in dry mass, defining the physiological maturity point (Marcos-Filho, 2015). Such a seed moisture variation is related to the importance of water in seed-filling and maturation processes (Lopes et al., 2014). Additionally, after seeds reach the physiological maturity stage, variations can occur due to environmental conditions in which these seeds are found.

Seed dry mass also exhibited a high coefficient of determination, with results inversely proportional to moisture content. In Stage I, dry mass was 0.30 $g.seed^{-1}$, remaining stable until Stage II (0.28 $g.seed^{-1}$), with greater mass accumulation in Stages III (0.38 $g.seed^{-1}$) and IV (0.46 $g.seed^{-1}$) (Figure 4B). Similarly, Muller et al. (2016) observed an increase in dry mass content until Stage III, reaching 2.87 g for the species *Peltophorum dubium* (Spreng.) Taub. The high or maximum accumulation of dry mass reflects superior seed quality, indicating the ideal period for seed collection (Marcos-Filho, 2015). In the seeds of *C. tocantinum*, we observed that the maximum accumulation of dry mass coincided with the same stages of high or maximum physiological quality.

The parameters germination, normal seedlings, GSI, as well as length and dry mass of normal seedlings, showed a quadratic polynomial response, initially increasing until reaching a maximum point and then decreasing as seed development progressed. The germination process of *C. tocantinum* seeds began in Stage I with 19% germination, followed by a sharp increase to 83% in Stage II, and 98 to 99% in Stages III and IV, respectively (Figure 5A).

Similarly, an increase in the percentage of normal seedlings was observed over the analyzed stages, with higher values in Stages III and IV, at 95% and 99%, respectively (Figure 5B). Studies with *Physalis peruviana* L. seeds found lower germination percentages in the initial stage due to seed immaturity at this physiological state (Barroso et al., 2022). Guariz et al. (2022), in their study on *Bauhinia monandra* Kurz, obtained a higher percentage of normal seedlings for mature seeds, at 96%. The highest germination rates of normal seedlings in the later maturation stages in our study may be associated with the point of physiological maturity of *C. tocantinum* seeds, as higher dry mass accumulation and lower moisture contents were observed in the same stages, allowing for the expression of their maximum physiological potential.

The seed germination rate varies until seeds reach their point of physiological maturity. In the initial stages, germination rates are low due to the embryo immaturity. Only when seeds reach physiological maturity can they express maximum germination and vigor (Popinigis, 1985; Barroso et al., 2017). This response was observed in our study, with seeds having lower germination power in the initial stages (19%) and reaching a maximum in the later

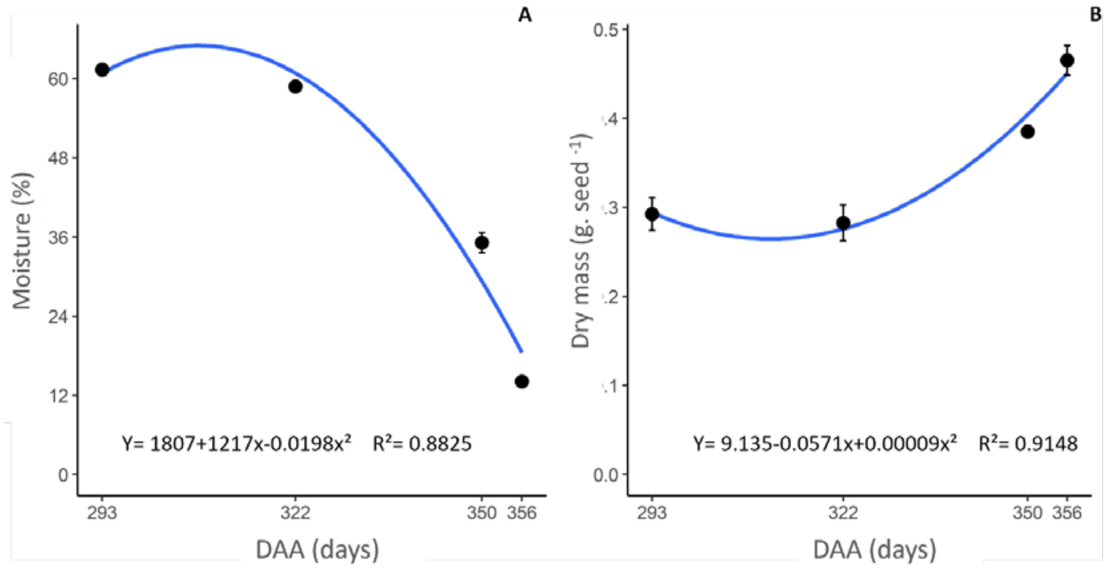


Figure 4. Polynomial regression analysis for the moisture (A) and dry mass (B) of *Cenostigma tocantinum* seeds at stage I (293 DAA), stage II (322 DAA), stage III (350 DAA), and stage IV (356 DAA).

maturation stages (95% in Stage III and 99% in Stage IV). Thus, germination capacity is considered one of the main parameters in a physiological maturity study because it evaluates seed quality and potential for seedling production, directly influencing productivity (Agustini et al., 2015).

For the germination speed index (GSI), no values were observed in Stage I, 0.73 in Stage II, and a maximum in Stage IV with 3.51 (Figure 5C). In the case of *Erythrina variegata* L., a similar behavior was observed, with GSI starting at 0 in the early stages and reaching a maximum (0.78) at 77 DAA (Matheus et al., 2011). It is important to note that there is a progressive increase in seed potential during maturation, with maximum vigor expressed at physiological maturity (Arantes et al., 2018).

For the length of normal seedlings, the highest results were found in Stages III and IV, at 18.42 cm and 16.43 cm, respectively. In terms of seedling dry mass, values obtained were 0.28 g.seedling^{-1} in Stage III and 0.30 g.seedling^{-1} in Stage IV (Figure 5D and E). At the seed maturity point, high vigor coincides with maximum dry mass, resulting in more vigorous seedlings. Although immature seeds can also germinate, they may produce lower-vigor seedlings (Carvalho and Nakagawa, 2012). Similarly, *C. tocantinum* seeds exhibited lower vigor in the early stages compared to the later stages of maturation.

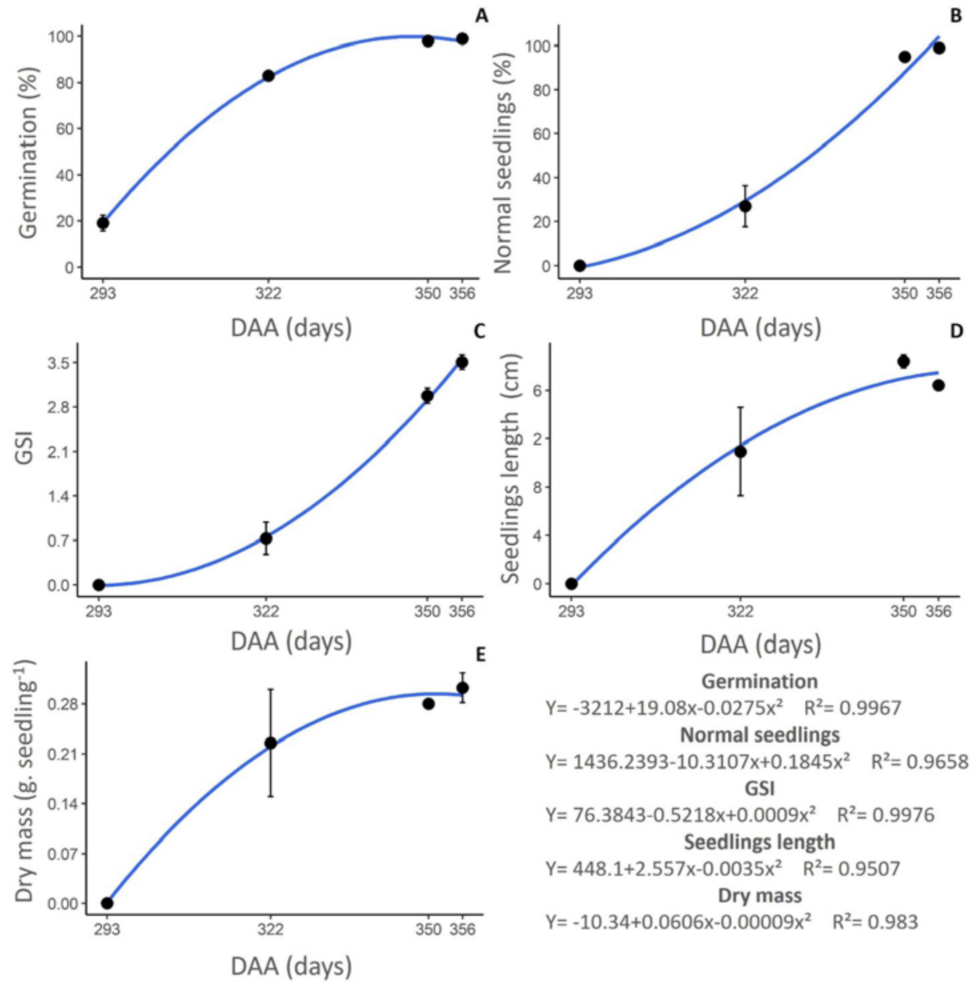


Figure 5. Polynomial regression analysis for the germination rate (A), normal seedlings (B), germination speed index - GSI (C), length (D), and dry mass of seedlings (E) of *Cenostigma tocantinum* seeds at stage I (293 DAA), stage II (322 DAA), stage III (350 DAA), and stage IV (356 DAA).

In the seedling emergence test, higher values were observed in the later stages, at 98% and 97% in Stages III and IV, respectively. Likewise, the emergence speed index (ESI) showed an increase over the stages analyzed, with higher values in Stages III (3.97) and IV (3.33) (Figure 6A and B). Seed maturation studies with *Luffa cylindrica* (L.) M. Roem. seeds found higher percentages of seedling emergence at 50 DAA, with 40% emergence (Medeiros et al., 2019). In seeds of *Hymenaea courbaril* L., a higher value for ESI (0.85) was observed in seeds collected from mature fruits (Guariz et al., 2021). This demonstrates that the seedling emergence test can be a good indicator for differentiating seed vigor. More vigorous seeds produce seedlings with higher potential when compared to less vigorous seeds (Cruz et al., 2021).

In the regression analysis of emergence, along with germination, seedling length, and seedling dry mass, it was possible to establish the moment when they reached their maximum points, resulting in a period of 337 to 369 days. This could be an indication of the periods for collecting *C. tocantinum* seeds.

In terms of electrical conductivity, a similar response was observed after 10 h and 24 h soaking. In Stage I, values were 11.39 $\mu S{\cdot}cm^{-1}.g^{-1}$ and 17.32 $\mu S{\cdot}cm^{-1}.g^{-1}$, respectively, with an increase in exudate loss in the final stage, reaching 33.75 $\mu S{\cdot}cm^{-1}.g^{-1}$ (10 h) and 47.59 $\mu S{\cdot}cm^{-1}.g^{-1}$ (24 h) (Figure 6C and D). Seeds of *Cenostigma pluviosum* var. *peltophoroides* (Benth.) Gagnon & G.P. Lewis also showed higher exudate loss in mature seeds (80.50 $\mu S{\cdot}cm^{-1}.g^{-1}$), along with higher germination potential (Lorenzetti et al., 2018). This change in membrane integrity, as indicated by higher electrical conductivity values, may be indicative of the beginning of structural/biochemical changes in the seeds, although it does not affect physiological quality as determined by the other tests used in the present study.

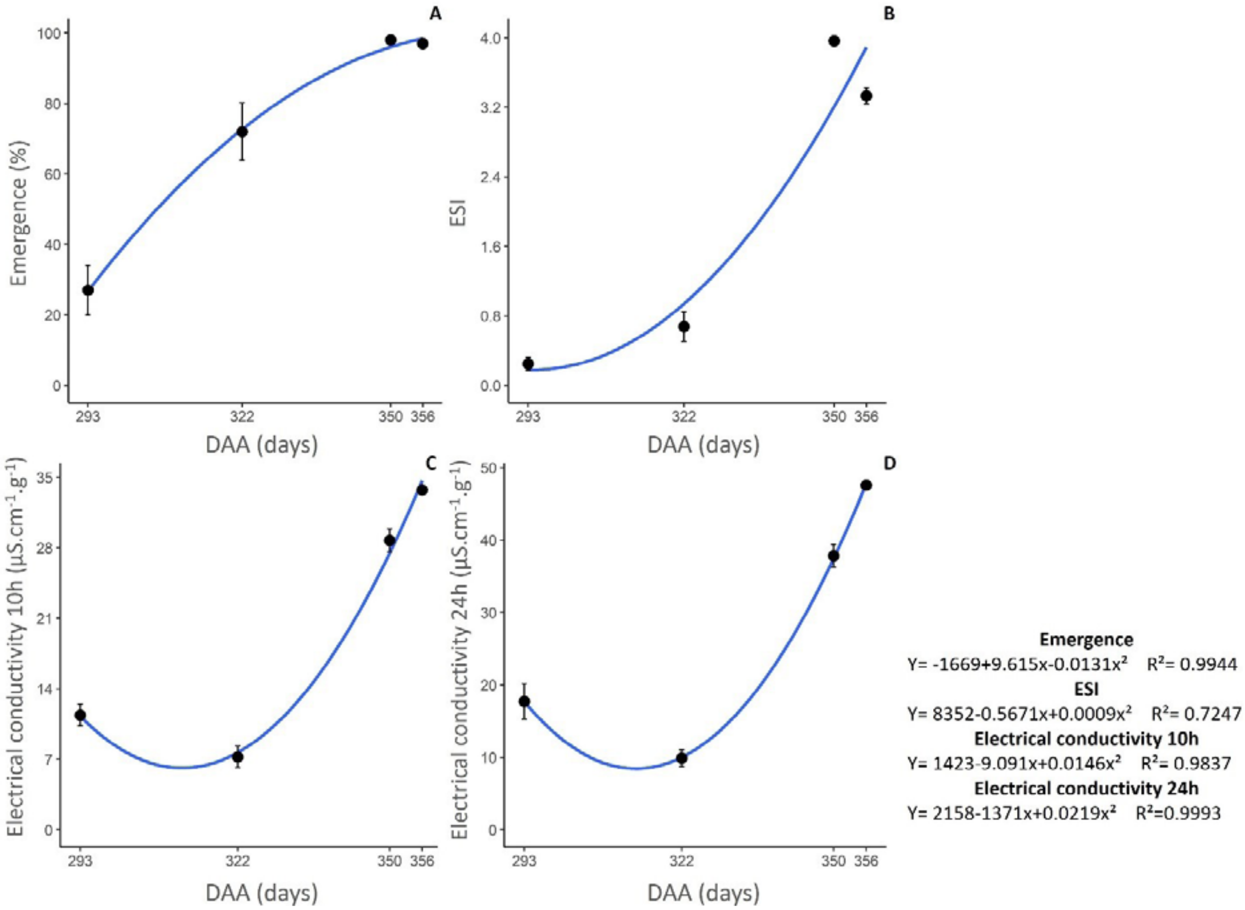


Figure 6. Polynomial regression analysis for the emergence (A), emergence speed index - ESI (B), electrical conductivity at 10h (C), and electrical conductivity at 24h (D) of *Cenostigma tocantinum* seeds at stage I (293 DAA), stage II (322 DAA), stage III (350 DAA), and stage IV (356 DAA).

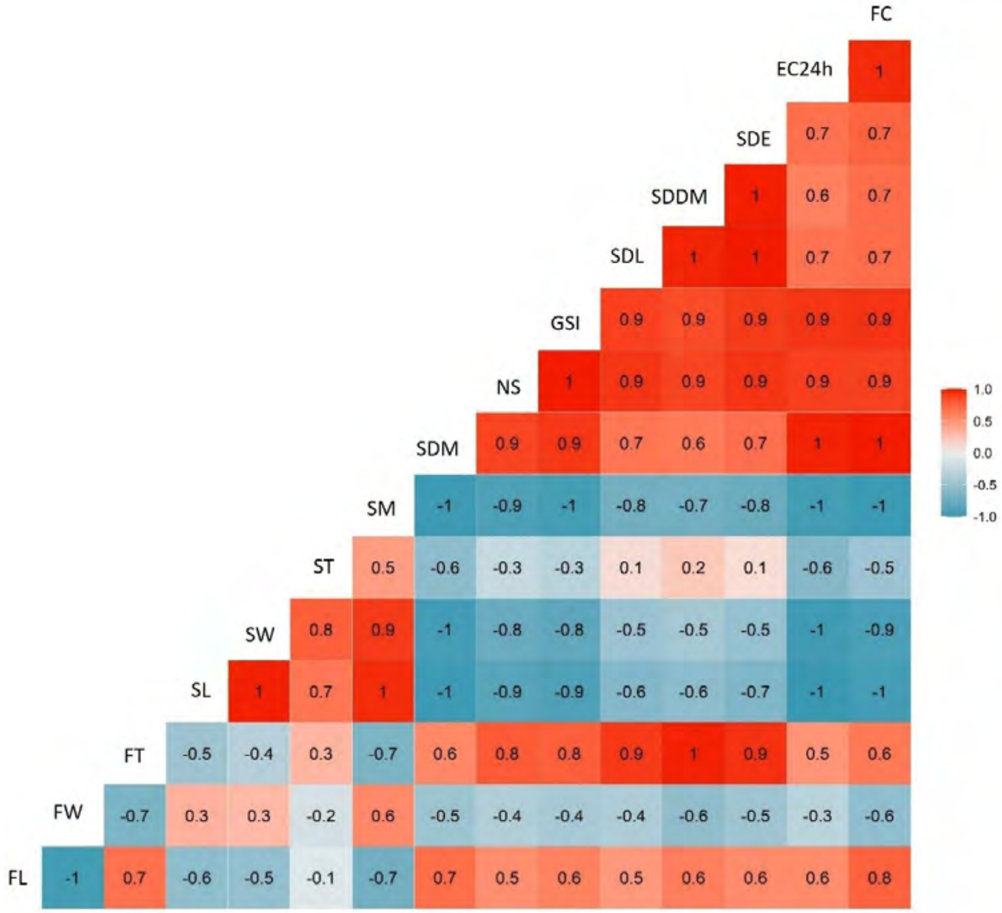


Figure 7. Correlation among the variables: fruit length (FL), fruit width (FW), fruit thickness (FT), seed length (SL), seed width (SW), seed thickness (ST), seed moisture (SM), seed dry mass (SDM), normal seedlings (NS), germination speed index (GSI), seedling length (SdL), seedling dry mass (SdDM), seedling emergence (SdE), electrical conductivity at 24h (EC 24h), and fruit color (FC) of *Cenostigma tocantinum* at stage I (293 DAA), stage II (322 DAA), stage III (350 DAA), and stage IV (356 DAA).

Electrical conductivity is a sensitive biochemical test capable of detecting the first modifications in seeds. Several factors can influence the test response, such as seed coat composition and size of pores (Vasconcelos et al., 2019), which can facilitate or hinder the release of solutes into the solution. Additionally, the deterioration process begins shortly after seed maturity (Marcos-Filho, 2015).

Pearson's correlation analysis showed that fruit color had a positive correlation of 0.9 with normal seedlings and GSI, and 0.7 with emergence, seedling length, and seedling dry mass. Another parameter that correlated positively with vigor indices and normal seedlings was fruit thickness, with values above 0.8 (Figure 7). Therefore, when seeds reached their maximum thickness value, of 7.42 mm, they exhibited high physiological quality and thus can be harvested at this stage.

In summary, considering the set of morphological and physiological evaluations analyzed through correlation for *C. tocantinum* seeds, fruits with brown color and brown spots (Stage III) and reddish-brown color (Stage IV), along with seed dry matter and moisture content, were efficient for determining the physiological maturity of the species. However, the latter two variables are more difficult to quantify in the field; therefore, fruit color and fruit thickness are the most suitable traits for the collection of seeds with high physiological quality.

CONCLUSIONS

The seeds of *Cenostigma tocantinum* exhibit superior physiological quality when collected at stages III (350 DAA) and IV (356 DAA), showing brown coloration with brownish-red patches, respectively. Therefore, for this species, seeds should be collected during these stages of maturation for further use in seedling production and/or direct planting.

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