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Verification of non-deep physiological dormancy and treatment to overcome seed dormancy in *Campomanesia phaea* (O. Berg) Landrum

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ABSTRACT: Cambuci [*Campomanesia phaea* (O.Berg) Landrum] is a native Brazilian fruit tree species from the Atlantic Forest, belonging to the Myrtaceae family, with great potential for commercial exploitation. The propagation of this species is limited to seeds, which exhibit low and uneven germination, posing a challenge for large-scale seedling production due to potential dormancy. The objective of this study was to verify the existence of dormancy in cambuci seeds and determine the most effective dormancy-breaking treatment. First, an X-ray test was conducted on the seeds, and the results were correlated with their germination and tetrazolium test outcomes. Subsequently, different methods were applied to overcome dormancy, including stratification, soaking, and gibberellin treatments. Cambuci seeds exhibit simple non-deep physiological dormancy, which can be overcome by stratification for 28 days at 15 °C.

Index Terms: Atlantic Forest, Brazilian native species, Myrtaceae, stratification, X-ray test.

RESUMO: O cambuci [*Campomanesia phaea* (O.Berg) Landrum] é uma espécie frutífera nativa do Brasil, típica da Mata Atlântica, pertencente à família Myrtaceae, com grande potencial para exploração comercial. A propagação dessa espécie é limitada às sementes, que apresentam germinação baixa e desuniforme, representando um desafio para a produção de mudas em larga escala devido à possível dormência. O objetivo deste estudo foi verificar a existência de dormência nas sementes de cambuci e determinar o tratamento mais eficaz para superá-la. Primeiramente, foi realizado um teste de raio-X nas sementes, e os resultados foram correlacionados com teste de germinação e de tetrazólio. Em seguida, foram aplicados diferentes métodos para a superação da dormência, incluindo estratificação, embebição e tratamentos com giberelina. As sementes de cambuci apresentam dormência fisiológica simples não profunda, que pode ser superada por meio de estratificação durante 28 dias a 15 °C.

Termos para indexação: Mata Atlântica, espécies nativas Brasileiras, Estratificação, Myrtaceae, teste de raios X.

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INTRODUCTION

The genus *Campomanesia* belongs to the Myrtaceae family and comprises 35 species, 25 of which are endemic to Brazil (Oliveira et al., 2024). Cambuci [*Campomanesia phaea* (O.Berg) Landrum] is a native Brazilian fruit tree endemic to the Atlantic Forest. This species plays a crucial ecological role as a food source for insects, birds, and mammals (Nogueira et al., 2016) and holds significant potential for commercial exploitation due to its nutraceutical properties (Tokairin et al., 2018; Stafussa et al., 2021), medicinal value (Donado-Pestana et al., 2021), and suitability for industrial fruit processing (Tokairin et al., 2018).

In general, Brazilian native species are commercially propagated by seeds, and this is also the main method for propagating cambuci (Tokairin et al., 2018). The seeds of this species tolerate desiccation and remain viable until they reach a moisture content of 15%, but they exhibit a low germination speed to form a completely normal seedling (Santoro et al., 2020). This could suggest the presence of dormancy in this species, although no studies have confirmed it. Knowing dormancy to assist in identification of possible dormancy in unexplored species like the cambuci tree, the X-ray test can be performed. It is a rapid, simple, and non-destructive method recommended by the International Seed Testing Association (ISTA, 2004; Gomes-Junior, 2010). The test involves evaluating the internal structure of the seeds and obtaining information about seed integrity and the relationship between seed internal morphology and viability (Xia et al., 2019). Additionally, it can provide insights into abnormalities in seedlings, or the reasons of non-germinated seeds based on the characterization of their internal morphology (Gomes-Junior, 2010; Zacharias et al., 2024). Futhermore, X-ray test can be associated with tetrazolium and germination tests to establish correlations among the obtained results (ISTA, 1995; Gomes-Junior, 2010).

Seed dormancy is a plant strategy that ensures successful germination to maintain its survival and multiplication, even in optimum environmental conditions (Nonogaki, 2014; Cipriani et al., 2019). Seed dormancy can be divided into five different classes, based on the causes: physical, physiological, morphological, morphophysiological, and combinational (Baskin and Baskin, 2021). The phenomenon of seed dormancy is common in Brazilian native species (Santos et al., 2022) and is considered an undesirable characteristic for seedling production, as it results in slow and uneven emergence (Azeredo et al., 2011; Marcos-Filho, 2015), necessitating the use of some treatment to overcome dormancy.

Among the common methods utilized for seed dormancy release, seed stratification, soaking, and gibberellic acid application can be performed, and they demonstrate good results for a wide range of studied species (Baskin and Baskin 2021; Nautiyal et al., 2023).

Information about cambuci, a native fruit species from Brazil, with commercial potential, is scarce in the literature. Regarding seed knowledge, there are few studies, but none with probable dormancy. In this study, we aimed to elucidate and verify the existence of cambuci's seed dormancy by associating X-ray, tetrazolium, and germination tests, in addition to studying methods to overcome seed dormancy.

MATERIAL AND METHODS

The experiments were conduct in the Laboratory of Radiobiology and Environment, Center for Nuclear Energy in Agriculture (CENA/USP) and Seed Analysis Laboratory, in the Department of Crop Science of Luiz de Queiroz College of Agriculture (ESALQ/USP), Piracicaba, São Paulo, Brazil.

Seed material

Mature cambuci fruits were obtained from a commercial orchard located in Natividade da Serra, São Paulo, Brazil (23° 22' 33" S, 45° 26' 31" W, 720 meters altitude). Seeds were extracted from ripe fruits and then rubbed into a sieve with sand to separate the seeds from the remnant pulp. After that, the seeds were washed with tap water and dried at room temperature for 24 hours. Swollen seeds with no apparent damage were selected and treated with fungicide Vitavax® (Thiram 200 SC).

X-ray test

The seeds were affixed to an acetate sheet (210 x 297 mm) using double-sided tape, organized into 16 groups of 25 seeds each, totaling 400 seeds. Thereafter, the acetate sheet was exposed to the digital radiographic system MultiFocus, connected to the Faxitron X-ray equipment, at an intensity of 25 kV for 180 seconds. Due to the lack of information regarding the internal seed morphology of the cambuci, images were obtained and analyzed based on the morphology of species from the same family (Myrtaceae) (Rego et al., 2010). The illustrated glossary of morphology was also utilized (Brasil, 2009b).

Tetrazolium test

The seeds were placed in an individualized cell plastic tray in the same order as they were arranged for X-ray test. For the tetrazolium test, the first 200 seeds were immersed in distilled water for 16 hours in a BOD incubator at 25 °C. Thereafter, the seeds were cut longitudinally and immersed in a tetrazolium solution (2,3,5-triphenyl tetrazolium chloride salt) with a concentration of 0.75% in a water bath at 38°C for 4 hours (Silva et al., 2021). Afterwards, the seeds were washed under tap water and kept immersed in water until evaluation. The classification of the seeds was performed based on the intensity, size, and position of the embryo tissue, as well as its appearance. Viable seeds were identified by their reddish to pinkish and firm tissue, while non-viable seeds were classified based on their milky-white coloration and flaccid soft tissue (Brasil, 2019a; Silva et al., 2021).

Germination test

The remaining 200 seeds from the X-ray test were used to perform the germination test. The seeds were placed in germitest paper rolls, which consisted of three layers of sheets that were moistened to 2.3 times their weight. Subsequently, the seeds were placed in a germination chamber (Mangelsdorf MA 401) at a temperature of 25 °C and a photoperiod of 12 hours of light, with the rolls positioned vertically (Gomes et al., 2016a). Seeds were evaluated weekly over a period of 4 months, recording the number of normal seedlings, abnormal seedlings, and dead seeds. Seedlings were classified as normal when they exhibited fully formed roots and shoots without any visible damage (Brasil, 2009a).

Stratification treatments

Seeds were placed in germination boxes filled with medium-textured sand. The humidity was controlled to reach 60% of the field capacity (Brasil, 2009a), which corresponded to 40 mL of distilled water in 315.80 g of sand. The boxes were covered with plastic bags to maintain humidity and were placed in cold chambers at different temperatures (5, 10, and 15 °C) for different periods (0, 7, 14, 21, and 28 days). After the various cold exposure periods, the seeds were transferred to germination chambers (Mangelsdorf MA 401) at a temperature of 25 °C and a photoperiod of 12 hours of light. The experiment was composed of 15 treatments (temperatures x periods in cold chamber), eight replications of 25 seeds each.

Soaking treatments

For this method, seeds were subjected to different water imbibition times (0, 8, 16, 24, and 32 hours). To prevent seed death, a water pump was used to keep the water oxygenated. After the soaking periods, the seeds were placed in germitest paper rolls in the same way conducted for the germination test. The experiment comprised 5 treatments with eight replications of 25 seeds each.

Gibberellin (GA_3) and stratification treatments

Gibberellic acid (GA_3) at different concentrations (0, 125, 250, and 500 $mg.L^{-1}$) was used in this experiment. The seeds were placed in germination boxes filled with medium-textured sand. The humidity was controlled to reach 60% of the field capacity (Brasil, 2009a), which corresponded to 40 mL of distilled water in 315.80 g of sand. The different concentrations of GA_3 were added to the distilled water used to moisten the sand, and then the boxes were covered with plastic bags to maintain humidity. Afterwards, the germination boxes were placed in a germination chamber (Mangelsdorf MA 401) at a temperature of 25 °C and a photoperiod of 12 hours of light. To confirm the best results obtained in the stratification experiment, two additional treatments with 21 and 28 days of cold exposure (15 °C) were evaluated within the gibberellin experiment. The experiment consisted of 6 treatments, with eight replications of 25 seeds each.

Data collection and statistical analysis

For the X-rayanalysis, the images were individually examined, and the results were compared with the tetrazolium and germination tests conducted for each seed. This comparison aimed to establish a cause-and-effect relationship and assist in determining the occurrence of dormancy.

For the seed dormancy overcome tests, weekly evaluations were conducted over a period of 4 months. The number of normal seedlings, expressed as a percentage of germinated seeds (GS%), was determined (Brasil, 2009a), considering seedlings with fully formed roots and shoots and no visible damage. Additionally, the mean germination time (MGT), expressed in days, was calculated according to Ferreira and Borghetti (2004).

The data were analyzed using Tukey's test, with a significance level of 5%, using SAS software (Version 9.4, SAS Institute Inc., Cary, USA).

RESULTS

Cambuci's embryo occupies the entire seed cavity and is classified as hypocotyledonary, with a well-developed hypocotyl-radicle axis that is curved in a c shape. The cotyledons are vestigial to rudimentary, and there is no endosperm present (Figure 1A).

The X-ray images and longitudinal seed cuts used for the tetrazolium tests enabled the observation of the internal morphology of cambuci seeds. Out of a total of 200 seeds, 184 (92%) were classified as viable (Figures 1B and C), while 16 (8%) were classified as non-viable (Figures 1D and E) by tetrazolium test.

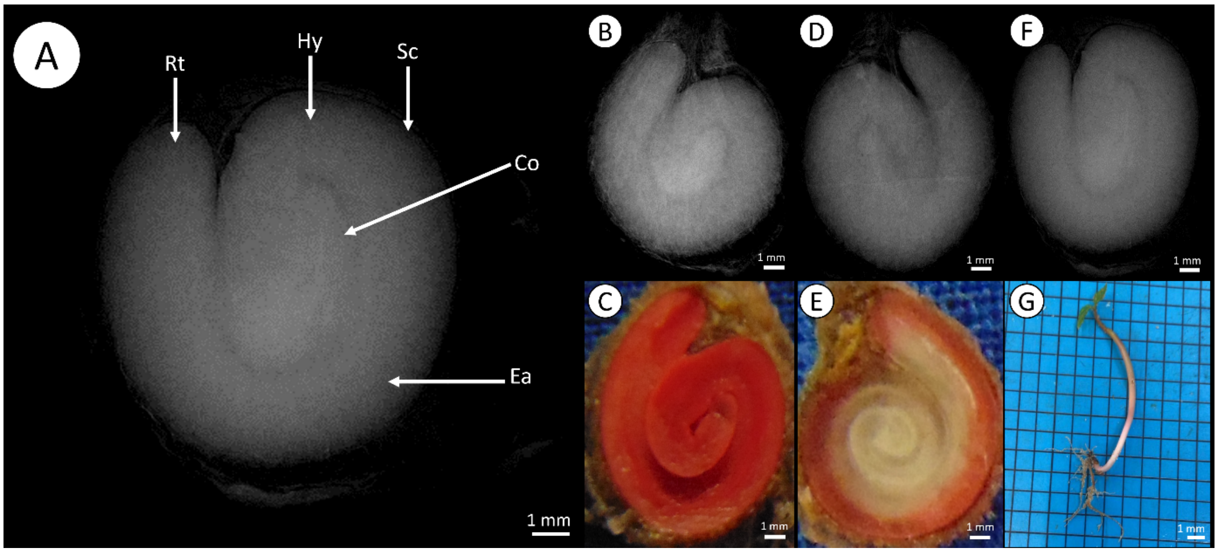

Figure 1. Internal structure, viable and non-viable seeds of cambuci [*Campomanesia phaea* (O.Berg) Landrum]. Seed internal morphology (A), viable seed identified by X-ray test (B), viable seed identified by tetrazolium test (C), non-viable seed identified by X-ray test (D), non-viable seed identified by tetrazolium test (E), viable seed and normal seedling development (F-G). Rt, root tip; Hy, hypocotyl; Sc, seed coat; Co, cotyledons; Ea, embryo axis.

The viable seeds exhibited full and well-formed structures, with no physical damage detected through the X-ray test, and non-viable conditions detected by the tetrazolium test. Within the 16 non-viable seeds classified by the tetrazolium test, 7 exhibited low-density tissue, which appeared as darker regions in the X-ray images. The remaining non-viable seeds were full and well-formed, showing no physical damage, which suggests that physiological characteristics, not detectable by the X-ray test, might have rendered them non-viable.

In the association of the X-ray images and the germination test, it was possible to observe that out of a total of 200 seeds, 164 (82%) seeds were full and well-formed (Figure 1F). Additionally, 124 of the seeds formed normal seedlings (Figure 1G). However, 40 of the seeds, even with no apparent physical damage detected by the X-ray test, formed abnormal seedlings. The remaining 36 (18%) of the seeds from the germination test presented physical damage detected by the X-ray test, causing abnormalities in the seedlings and preventing the seeds from germinating. Factors such as damage in the radicular portion, empty spaces in the seed, malformed embryos, and low-density tissue are responsible for these observations (Figure 2).

X-ray images revealed various seed damages leading to seedling abnormalities. Radicular damage caused rootless seedlings with stunted growth, while embryo axis damage resulted in seedlings with elongated primary roots but no secondary roots and underdeveloped shoots. Low-density tissue and empty spaces in seeds resulted in deformed roots, elongated hypocotyls, and rudimentary or absent shoots. Non-germinated seeds with empty spaces detected by X-ray also died.

In the stratification treatment, there were no significant differences observed in the interaction between the studied factors, temperature and days of exposure. However, a temperature of 15 °C, used in this study, resulted in a higher

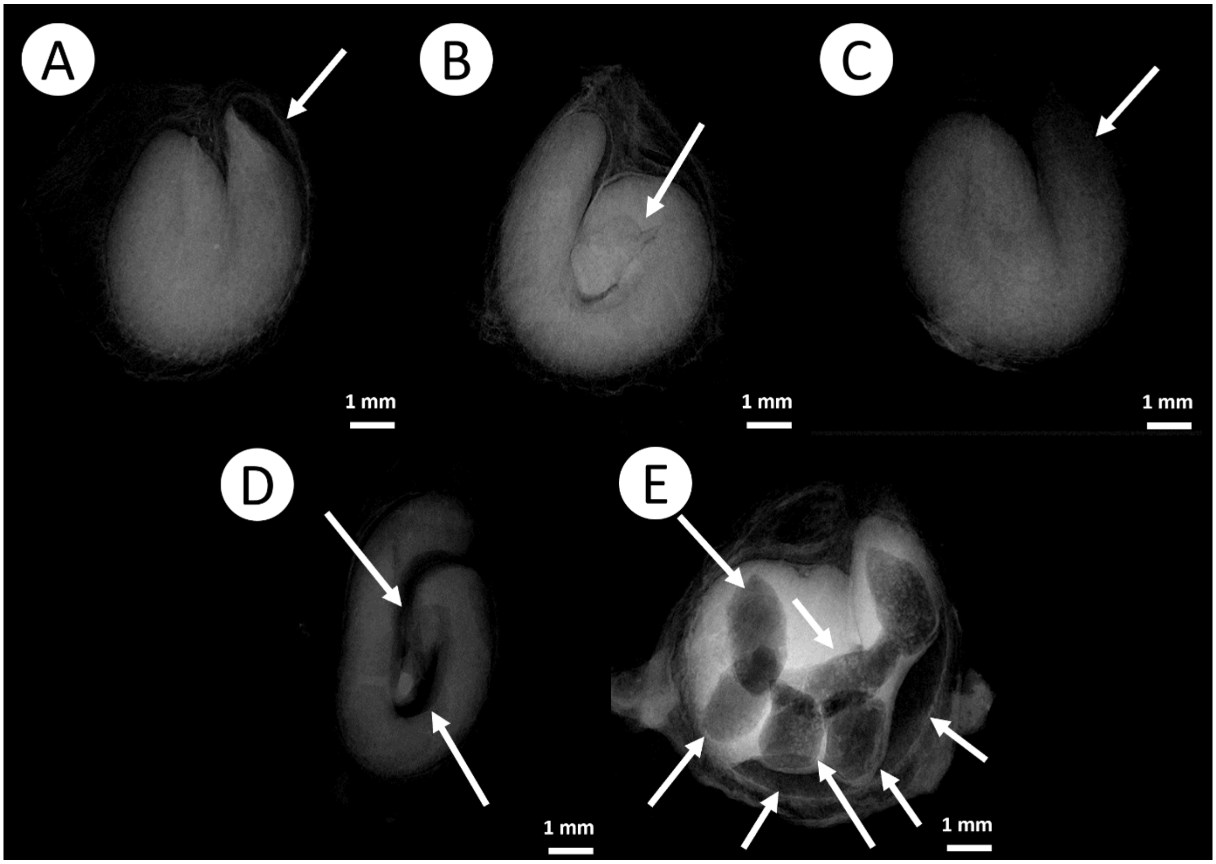

Figure 2. X-ray images of cambuci [*Campomanesia phaea* (O. Berg) Landrum] seeds that developed abnormal seedlings during the germination test. Damage in the radicular portion (A), malformed embryonic axis (B), low-density tissues (C), empty spaces (D), empty spaces and deteriorated tissue (E).

germination percentage of the seeds (Figure 3A). The best results for MGT were observed when seeds were exposed to 15 °C (Figure 3B), and the best result for period of exposure was 28 days, not differing from 21 days (Figure 3C).

The different soaking periods did not influence the germination percentage of cambuci seeds. However, soaking the seeds for a period of 32 hours reduced the MGT (Figure 3D).

The stratification treatments at 15 °C, 21, and 28 days of exposure resulted in higher germination percentages (Figure 3E) compared to control. As for the MGT, exposing the seeds to 15 °C for 28 days provided the best result (Figure 3F).

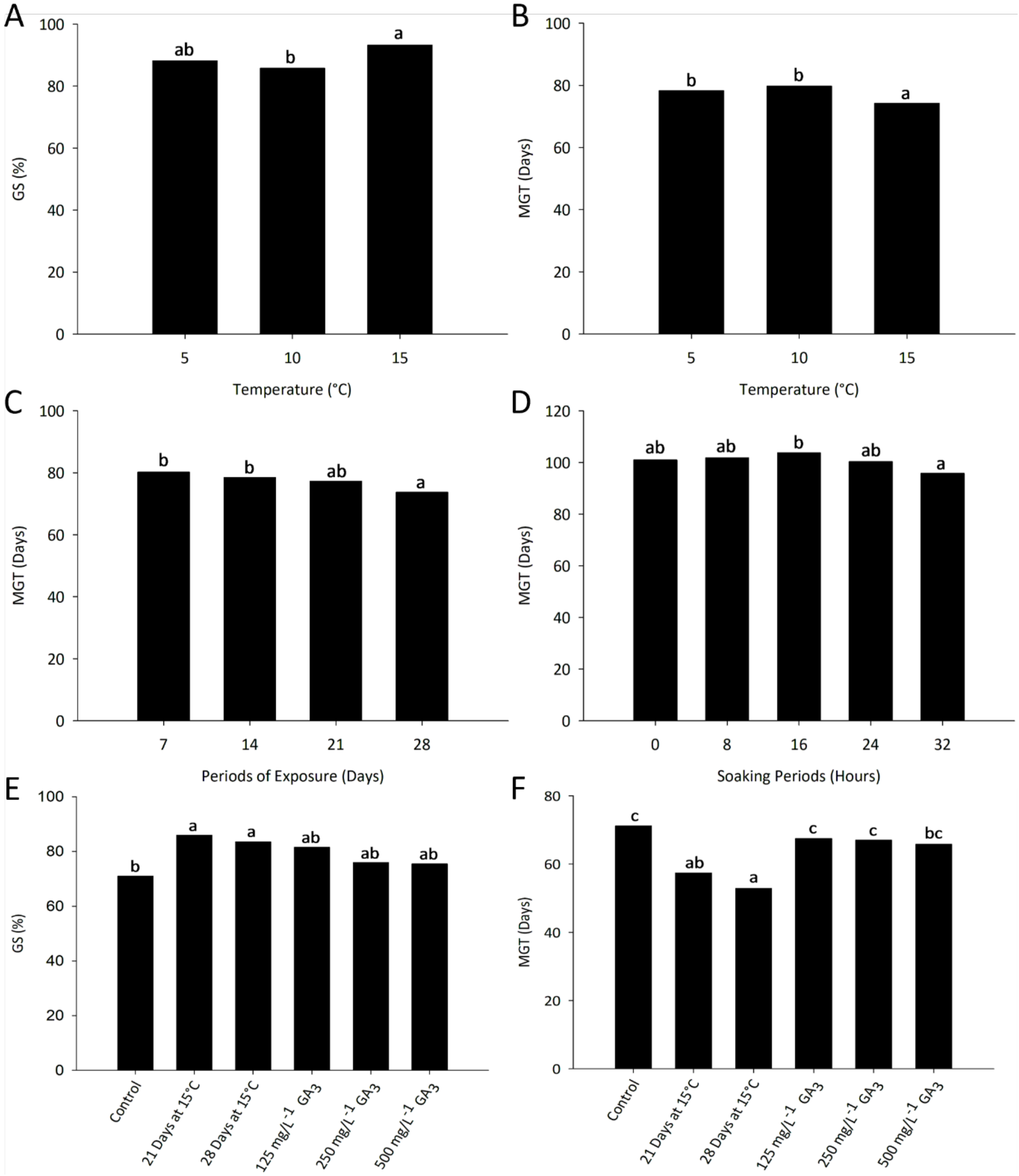

Figure 3. Effects of dormancy-breaking methods on mean germination time and seed germination percentage of cambuci [*Campomanesia phaea* (O. Berg) Landrum] seeds: Germinated seed percentage (GS%) in response to different temperatures (A), mean germination time (MGT) in response to different temperatures (B), MGT in response to periods of exposure to cold temperature (C), MGT in response to different soaking periods (D), GS% in response to GA_3 and stratification treatments (E), MGT in response to GA_3 and stratification treatments (F).

DISCUSSION

The presence of small to rudimentary cotyledons and the absence of endosperm in cambuci seeds are like other species from the same genus, such as guabiroba (*Campomanesia xantocarpa* O. Berg, Myrtaceae) (Vieira et al., 2022). In this study, we classified the internal morphology of cambuci seeds for the first time. Understanding the internal morphology can be useful in identifying factors associated with the physiological potential of the seeds (Gomes et al., 2016b; Amaral et al., 2020) and can serve as a basis for germination and viability tests (Gomes-Junior, 2010).

The observation of the X-ray images combined with the results of the tetrazolium test enabled to identify that most of the seeds were viable with full and well-formed structures. The same results were observed in the X-ray image for another Brazilian native species from the same genera, *Campomanesia pubensces* (Mart. Ex Dc.) O. Berg (Amaral et al., 2020). Within the non-viable seeds, darker regions were observed, which can indicate deterioration or malformed embryo (Silva et al., 2014b), being this the cause of their unviability. For those seeds for which unviability was not detected by the X-ray test and that appeared as full and well-formed seeds, physiological characteristics can be inferred as the cause of unviability. Well-formed seeds classified using X-ray tests can produce abnormal seedlings or be identified as dead seeds since the test can detect damage to the seed tissue that is not related to the physiological process (Silva et al., 2014a).

In the presented study, the primary damages observed in cambuci seeds through the X-ray test were documented (Figure 2). Damaged seeds of *Moringa oleifera* Lam., Moringaceae, classified by the X-ray test, resulted in abnormal seedlings and dead seeds, thereby supporting our findings (Noronha et al., 2018). Furthermore, damaged seeds, whether caused by mechanical factors, deterioration, malformation, or insect injury, and seeds with empty spaces resulting from incomplete embryo development within the seed cavity, have reduced physiological potential, which negatively impacts seed germination (Rego et al., 2023; Silva et al., 2024).

A few fully developed and well-formed seeds, classified through the X-ray test, produced abnormal seedlings during the germination test. Similar observations were documented for cowpea (*Vigna unguiculata* L., Fabaceae) (Rego et al. 2023), and pitanga-do-mato (*Eugenia pleurantha* O. Berg, Myrtaceae) (Masetto et al., 2007). Therefore, all the full and well-formed seeds verified by the X-ray test in conjunction with the germination test can be considered viable, even those that yielded abnormal seedlings in the germination test, as they exhibited no physical damage.

During the experiment, abnormal seedlings predominantly occurred in the later stages of evaluation, in contrast to the normal seedlings, which were observed to develop until the third month of data collection. This observation suggests that the seeds of the cambuci may exhibit dormancy, and once this dormancy is overcome, abnormal seedlings may arise due to tissue deterioration caused by microorganism contamination (Burg et al., 1994; Carvalho et al., 2009).

The stratification procedure is commonly utilized for Brazilian native species (Raseira et al., 2004). Stratification periods promote the activation of hydrolytic enzymes responsible for inhibitors degradation and may increase gibberellin concentrations (Marcos-Filho, 2015), altering the internal balance of phytohormones responsible for dormancy/ germination control (Petri and Herter, 2004; Taiz and Zeiger, 2017). In addition, chilling temperatures activates enzymes responsible to convert the storage substances in more soluble forms (Bewley and Black, 1994).

Stratification at 15 °C for 28 days promoted a higher germination percentage and a shorter mean germination time (MGT) (Figures 3A, B, C, E and F). Reducing the MGT is crucial for seedling production because, apart from shortening the time required to produce seedlings, it results in less variation in emergence, reducing seedling non-uniformity (Monteiro et al., 2019; Regnier, 2020). These results emphasize that sowing the seeds immediately after fruit collection is not the best approach to achieving favorable germination for cambuci seeds. Therefore, stratification is a method capable of improving both the germination percentage and, most importantly, the MGT in this species. It also suggests that cambuci seeds exhibit non-deep simple physiological dormancy. This type of dormancy can be released with a short stratification period (5 to 90 days) and exposure to higher temperatures (15 °C) when compared to the lower temperatures typically used for some species (4 to 6 ^{o}C) (Cardoso, 2009; Baskin and Baskin, 2014; Baskin and Baskin, 2022).

Soaking seeds in water is an efficient method to overcome seed dormancy caused by the hard seed coat that hinders water uptake (Martins et al., 2011). Additionally, the soaking treatment can contribute to the leaching of inhibitory substances present in the seed coat, thereby enhancing the seed germination process (Nautiyal et al., 2023). The soaking treatment did not show significant improvement in seed germination of cambuci seeds. The results can assume that cambuci seeds do not exhibit physical dormancy and inhibitors substances in the tegument, that could be washed away during the soaking treatment, as the result observed for some tropical forest tree seeds (Odoi et al., 2019).

The phytohormones gibberellin (GA) and abscisic acid (ABA) are known as the major regulators for seed dormancy and germination (Shu et al., 2016; Tuan et al., 2018). Changes in their balance and sensitivity within the seed impacts on the dormancy maintenance of some species (Tuan et al., 2018). Therefore, GA enhances the germination process by inducing enzymes which can promote degradation of barriers, as the endosperm, and induce seed storage mobilization, stimulating the embryo growth (Finkelstein et al., 2008). Exogenous GA application can be performed to overcome seed dormancy in some species, increasing seedling production (Silva et al., 2021; Lee et al., 2022). Despite this, exogenous gibberellin treatments did not have any beneficial effects on cambuci seeds.

In our study, the possibility that gibberellin is the only factor of cambuci seed dormancy is discarded. However, other physiological processes may be associated with this dormancy, and there could be an interaction among them.

CONCLUSIONS

Cambuci [*Campomanesia phaea* (O. Berg) Landrum] seeds exhibit non-deep physiological dormancy. Stratification at 15 °C for 28 days is an effective method to overcome this dormancy, enhancing seed germination and reducing the mean germination time for the species.

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AUTHORS' CONTRIBUTION

Mariane Jeronimo Forte Sinhorini contributed with: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Writing - original draft; Marcelo Almeida de Oliveira Junior contributed with: Formal analysis, Data curation, Visualization, Writing—review & editing; Victor Augusto Forti contributed with: Conceptualization, Methodology, Formal analysis, Data curation, Visualization, Writing—review & editing; Simone Rodrigues da Silva contributed with: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Resources, Supervision, Writing - review & editing.

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