

Assessment of *Handroanthus impetiginosus* (Mart. ex DC.) Mattos seed quality through X-ray testing

Thiago Alves da Silva¹, Raimunda Katiane Souza Lopes¹, Deniasse Bernardo Amoda², Letícia de Aguila Moreno³, Heloisa Oliveira dos Santos³, José Marcio Rocha Faria^{1*}

ABSTRACT: Several techniques are used to evaluate seed quality; however, most of them are destructive and require considerable execution time. The X-ray test has proven to be an efficient nondestructive technique for evaluating the quality of seeds. This study aimed to evaluate internal damage in ipê-roxo (*Handroanthus impetiginosus*) seeds through X-ray tests and to investigate how it affects seed germination. Samples from four lots of ipê-roxo seeds were exposed to radiation and, subsequently, the radiographs were analyzed using ImageJ software and the seeds divided into four classes based on their internal morphology: 1) seeds with an embryonic cavity filled with more than 50%; 2) an embryonic cavity filled up to 50%; 3) an embryonic cavity attacked by insects; and 4) an empty embryonic cavity. After that, the seeds were subjected to a germination test and, after 21 days, scored as germinated, nongerminated, dead, and normal and abnormal seedlings. The X-ray test proved to be effective in evaluating the internal morphology of ipê-roxo seeds and their relationship with physiological quality. Radiographic analysis enabled the precise identification of damage, such as embryonic malformation and damage by insects.

Index terms: damage to seeds, germination, physiological quality.

RESUMO: Diversas técnicas são utilizadas para avaliar a qualidade das sementes; entretanto, a maioria deles é destrutiva e requer um tempo de execução considerável. O teste de raios X tem se mostrado uma técnica não destrutiva eficiente para a avaliação da qualidade de sementes. Este estudo teve como objetivo avaliar danos internos em sementes de ipê-roxo (*Handroanthus impetiginosus*) por meio de testes de raios X e investigar como esses danos afetam a germinação das sementes. Amostras de quatro lotes de sementes de ipê-roxo foram expostas à radiação e, posteriormente, as radiografias foram analisadas no software ImageJ, sendo as sementes divididas em quatro classes de acordo com sua morfologia interna: 1) sementes com cavidade embrionária preenchida com mais de 50%; 2) cavidade embrionária preenchida até 50%; 3) cavidade embrionária atacada por insetos; e 4) cavidade embrionária vazia. Em seguida, as sementes foram submetidas ao teste de germinação e, após 21 dias, foram classificadas como germinadas, não germinadas, mortas e plântulas normais e anormais. A análise radiográfica permitiu a identificação precisa de danos, como malformação embrionária e danos por insetos. O teste de raios X mostrou-se eficaz na avaliação da morfologia interna de sementes de ipê-roxo, com boa relação com a sua qualidade fisiológica.

Termos para indexação: danos em sementes, germinação, qualidade fisiológica.

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*Corresponding author
jmfaria@ufla.br

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¹Laboratório de Sementes Florestais,
Departamento de Ciências
Florestais, Universidade Federal de
Lavras, PO Box: 3037, 37200-900,
Lavras, MG, Brasil.

²Laboratório de Nutrição e
Metabolismo de Plantas,
Departamento de Biologia,
Universidade Federal de Lavras,
PO Box: 3037, 37200-900,
Lavras, MG, Brasil.

³Setor de Sementes, Departamento
de Agricultura, Universidade Federal
de Lavras, PO Box: 3037, 37200-900,
Lavras, MG, Brasil.

INTRODUCTION

Over the last few years, the number of studies on forest species has increased, especially due to increasing concerns about environmental issues. The need to recover degraded areas and restore landscapes using native species is fundamental to maintaining the ecosystem (Rodrigues et al., 2020). Among these native species, the genus *Tabebuia* (ipê) stands out and is widely used in reforestation programs, where plant propagation occurs via seeds.

The purple ipê tree, popularly known as *ipê-roxo-de-sete-folhas* and *ipê-roxo*, is a tree species belonging to the Bignoniaceae family and is found throughout the Brazilian territory (Fernandez and Cock, 2020). The ipê trees reach heights ranging from 10 to 20 meters, with abundant flowering, dense and striking flowers, creating an extraordinary landscape effect (Pires et al., 2015). According to the Resolution of the Environment Secretariat, this species is recommended for restoration projects in forest ecosystems and landscaping. The use of high-quality seeds in the production of seedlings is essential since their multiplication essentially occurs through sexual reproduction (Amaral et al., 2011).

One of the techniques used to evaluate seed quality is related to the study of the internal morphology of the seeds through radiograph analyses obtained by X-ray (Noronha et al., 2018). This technique assesses seed quality by X-ray absorption, which varies depending on the thickness, density and composition of seed tissues, as well as the wavelength of radiation (ISTA, 2004). The dark areas of a seed radiograph are the result of an absence of tissue or low tissue density. This occurs because X-rays can easily penetrate these areas. On the other hand, white areas are the result of denser tissues, where X-rays have difficulty penetrating these areas (Silva et al., 2014).

In this way, X-ray is considered a technique that is easy to execute, fast, nondestructive and capable of detecting seeds with empty, damaged, or malformed embryo cavities (Abud et al., 2018; Medeiros et al., 2018). First developed in favor of medicine, this technique is currently used in several areas in the forestry sector, including the inspection of physical seed quality (Rahman and Cho, 2016).

Seed X-ray analysis is a promising tool for evaluating ipê seed quality after harvest and after storage. Considering the need for fast and efficient evaluation methods, this study aimed to assess the internal morphology of ipê-roxo through X-ray images and establish a relationship between radiographic images and the germination process.

MATERIAL AND METHODS

The research was performed at the Central Seed Research Laboratory of the Department of Agriculture of the Federal University of Lavras - Minas Gerais. The ipê seeds were provided by the Forest Seeds Laboratory from the same educational institution.

After harvest, four different seed lots were kept in a dry cold storage room (35-45% RH and 5-8 °C). Each lot was defined by the collection year: Lot 1 – season 2010, Lot 2 – season 2015, Lot 3 – season 2019, and Lot 4 – season 2023. The seeds were subjected to X-ray and germination tests to establish a relationship between the morphological characteristics in the seeds and the possible consequences for germination.

X-ray: The internal morphology of the seeds was evaluated using four replications of 25 seeds in each lot. The seeds were placed on transparent acetate sheets (11 cm × 11 cm). Each seed received a number based on its position on the acetate sheets to facilitate identification during the germination test. Subsequently, the seeds were exposed to 35 kV radiation for 17 seconds at a distance of 45 cm from the emitting source. The distance used was considered the most appropriate for identifying the internal morphology of the seeds using the Faxitron model MX-20 equipment (Faxitron X-ray Corp. Wheeling, IL, USA). The X-ray images were saved on a computer, the seeds were evaluated according to the filling of the embryonic cavity with the aid of ImageJ® software, and grouped into four classes:

- Class 1: Embryonic cavity filled with more than 50%.
- Class 2: Embryonic cavity filled up to 50%.
- Class 3: Embryonic cavity attacked by insects.

- Class 4: Embryonic cavity empty.

After the X-ray process, the previously identified and numbered seeds were set to germinate. Seeds from class 4 (empty embryonic cavity) were excluded from the germination test due to the absence of the structures necessary for the germination process.

Germination: The X-rayed seeds separated according to classes 1 to 3 were subjected to a germination test using the between-paper method with Germitest® paper roll (Brasil, 2009). The germination papers were moistened with water equivalent to 2.5 times their mass. The rolls were kept in a B.O.D. (Biochemical Oxygen Demand) chamber at 25 °C with a photoperiod of 12 hours of light and 12 hours of dark. Counting was performed daily from day one until the end of the test (21 days). The criteria used for the evaluation followed the Instructions for Analysis of Seeds of Forest Species (Brasil, 2013), considering nongerminated (NG) seeds that, at the end of the experiment, had not germinated and did not show visible signs of deterioration. Dead seeds (D) were defined as those that showed signs of deterioration. Only seeds that developed a primary root with a length equal to or greater than 2 mm were considered germinated (G). Normal seedlings (NS) were identified as those whose essential structures for development were intact, while abnormal seedlings (AS) were those that lack one or more of their essential structures. The germination experiment was organized in a completely randomized design, with three treatments (classes 1 - 3) and four replications.

RESULTS AND DISCUSSION

The combination of an exposure time of 17 seconds and a radiation intensity of 35 kV allowed for better visualization of the internal anatomy of the *Handroanthus impetiginosus* seeds. Based on the physical analyses obtained by X-ray, a significant difference was observed among the classes within the same lot. Overall, class 1 (embryo cavity filled by more than 50%) had higher germination rates than did the other classes (Table 1).

Table 1. Percentage of nongerminated seeds (NG), dead seeds (D), germinated seeds (G), seeds that generated normal seedlings (NS), and seeds that generated abnormal seedlings (AS) from four lots of *Handroanthus impetiginosus* seeds.

SEED LOT	CLASS	N° OF SEEDS	NG	D	G	NS	AS
			----- % -----				
2010	1	86	47	37	16	86	14
	2	5	0	80	20	100	0
	3	-	-	-	-	-	-
2015	1	74	24	14	62	85	15
	2	16	6	94	0	0	0
	3	3	0	33	67	100	0
2019	1	81	17	25	58	83	17
	2	2	0	50	50	0	100
	3	8	0	62	38	67	33
2023	1	85	15	26	59	74	26
	2	-	-	-	-	-	-
	3	10	0	20	80	50	50

Classes: embryonic cavity filled with more than 50% (1); embryonic cavity filled with up to 50% (2); and embryonic cavity attacked by insects (3). The percentages of normal seedlings and abnormal seedlings were calculated considering the percentage of germinated seeds as 100%.

The germination test results showed that most of the class 1 seeds (Figure 1) produced normal seedlings, regardless of the lot, as presented in Table 1. Overall, fully formed seeds, characterized by dense tissues, play a crucial role in the development of vigorous and healthy plants (Pinheiro et al., 2022).

According to Medeiros et al. (2020), the filling of the embryo cavity is an indication of the level of integrity of the seed tissues. Fully formed and undamaged tissues offer greater resistance to the passage of X-ray photons, while damaged tissues offer less resistance. Furthermore, a lower tissue density may correlate with fewer reserves, such as proteins, lipids and starch, in addition to embryo malformation. These factors can negatively influence seed germination (Doll et al., 2020).

The absence of development of normal seedlings from seeds that emitted primary roots may indicate an initial stage of loss of viability, according to Socolowski and Cicero (2008). Moreover, it is possible that more than 50% of the seeds with embryonic cavities on the radiographs (Figure 1B), which germinated but did not generate normal seedlings, were attacked by microorganisms that affected the formation of normal seedlings (Sales et al., 2018). Another explanation is that the seedlings did not develop because the reserve tissue lacked energy. Carvalho e Almeida (2016) reported that reserve tissues act as a storage and supplier of organic compounds in a simple form and are used by the embryonic axis from the beginning of germination until the final development of the seedling.

Through image analysis of *Handroanthus impetiginosus* seeds, it was found that most class 2 seeds (with an embryonic cavity filled with up to 50% of the seeds) died at the end of the experiment (Table 1). Dead seeds from class 2 were more frequent in the 2015 lot (94% of the total) (Table 1). Figure 2B shows the radiographic image of a class 2 seed, where it is clear that the embryonic tissue is incompletely developed, indicating a low density of tissue mass inside the seed, resulting in seed death at the end of the germination test, as illustrated in Figure 2C.

A few seeds with embryonic cavities attacked by insects (class 3) exhibited cotyledon damage (Figure 3B), resulting in a negative impact on seedling development (Figure 3C), which can be associated with reserve reduction during the germination phase, according to Pinheiro et al. (2020). Similar results were reported by Medeiros et al. (2019), who studied *Leucaena leucocephala* seeds, and by Pinheiro et al. (2020), who studied *Piptadenia gonoacantha* seeds; both of these studies investigated the relationship between internal morphology and physiological seed quality via X-ray images.

The Rules for Seed Testing (Brasil, 2009) describe that abnormal seedlings have no potential to continue developing and produce normal plants even under favorable conditions, which may be associated with genetic or physical characteristics during embryonic cavity filling. Nevertheless, 100%, 67% and 50% of the class 3 seeds from 2015, 2019, and 2023 lots, respectively, generated normal seedlings (Table 1). This phenomenon can be explained because, depending on the location and intensity of the damage caused by the insect, seed malformation may not interfere with

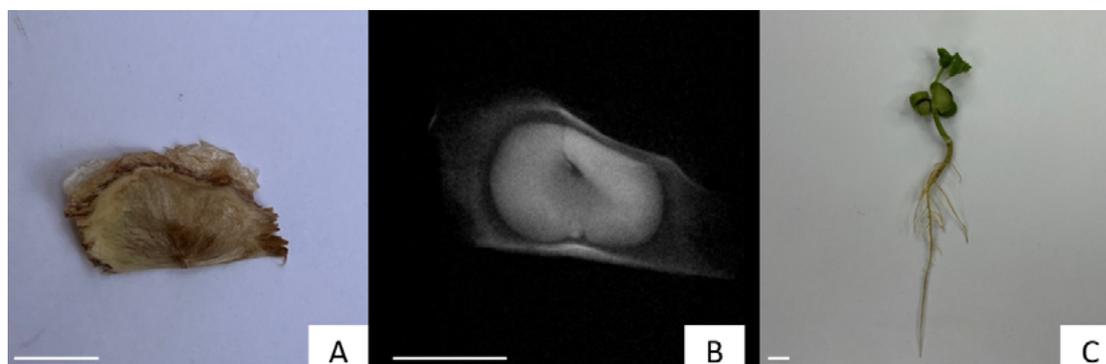


Figure 1. A - *Handroanthus impetiginosus* seed; B – radiograph of the same seed showing the embryonic cavity filled with more than 50%; C – normal seedling originated from the same seed after 21 days of germination test. Bar: 1 cm.

the germination process, allowing the formation of normal seedlings. Torrez et al. (2022) stated that the analysis of radiographic images facilitates the selection of biological material and avoids the total discarding of seeds with cavities, consequently increasing the chances of normal seedling formation.

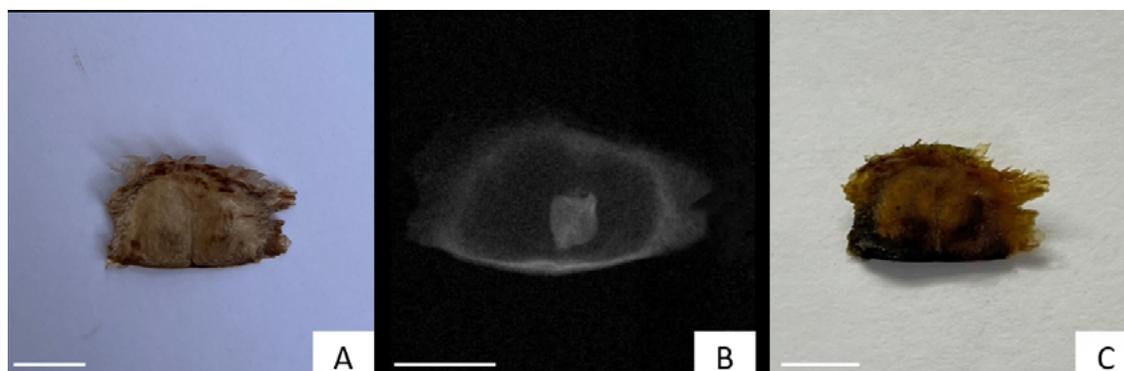


Figure 2. A - *Handroanthus impetiginosus* seed; B - radiograph of the same seed showing the embryonic cavity filled up to 50%; C – the same seed, dead after 21 days of germination test. Bar: 1 cm.

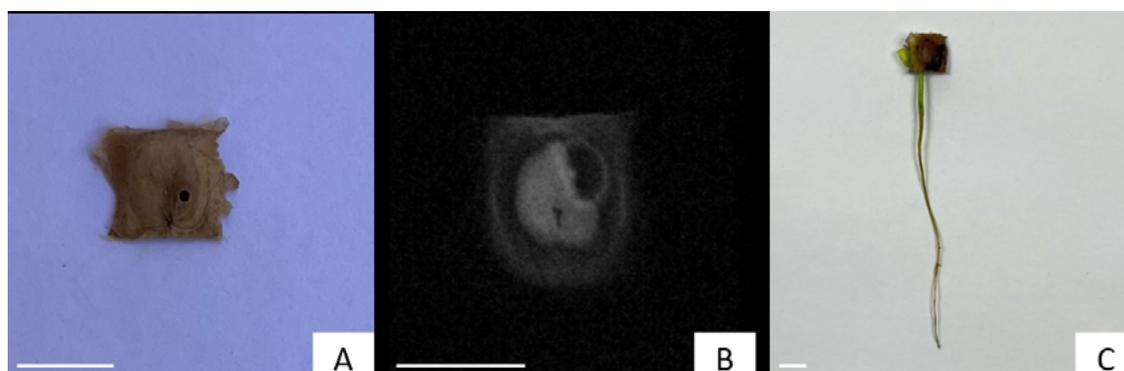


Figure 3. A - *Handroanthus impetiginosus* seed; B - radiograph of the same seed showing the embryonic cavity attacked by insect; C – abnormal seedling originated from the same seed after 21 days of germination test. Bar: 1 cm.

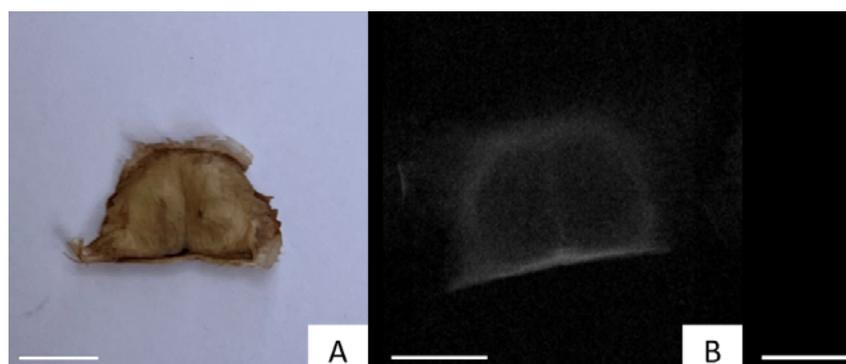


Figure 4. A - *Handroanthus impetiginosus* seed; B – radiograph of the same seed showing an empty embryonic cavity. Bar: 1 cm.

A germination test was not performed for seeds from class 4 (the empty embryonic cavity) (Figure 4B) in any of the lots. The empty cavity from seeds of this class could not be identified externally, which highlights the potential of the X-ray technique in the evaluation of seed physical quality, corroborating the findings of Masetto et al. (2008), who investigated the seed quality of *Cedrella fissilis* by X-ray.

The use of the X-ray technique has proven to be efficient in evaluating the internal cavity of forest seeds. Gomes et al. (2014), when evaluating the morphology of *Terminalia argentea* using the X-ray test, found that this technique allows the detection of embryonic abnormalities in the x-rayed seeds. Similar results were observed in the species evaluated in this study. The authors highlight that the use of X-ray equipment is promising in detecting the quality of seed lots, helping to separate damaged seeds or seeds with abnormalities.

Oliveira et al. (2004), in a study with seeds of yellow ipê (*Tabebuia serratifolia*) and purple ipê (*Tabebuia impetiginosa*), also observed that the X-ray test was efficient in evaluating defects in seeds of both species. Internal damage detected on X-rays affects the germination of these seeds, reducing the quality of the lot.

Marchi and Gomes Júnior (2017), when using image analysis techniques to determine the size of the seed embryo of *Senna multijuga* and its relationship with germination and vigor, observed that the internal mass of the seeds, evaluated by X-rays, is an indicator of its physiological quality. The radiographic images allowed the classification of lots with better and worse quality, highlighting the effectiveness of the technique in evaluating the internal content of the analyzed seeds. Similar results were found by Altizani-Júnior et al. (2023), who highlighted the importance of the non-destructive technique to identify the physical integrity of tissues and relate it to the germination performance of seeds, through the detection of embryonic damage and malformations.

CONCLUSIONS

Radiographic analysis of *Handroanthus impetiginosus* seeds allows the precise identification of damage, such as embryonic malformation and insect attack. Through this analysis, it is possible to establish a direct relationship between the severity of the damage or anomaly and the impact on germination, providing precise information for the selection of seed lots.

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