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X-rays for determination of papaya seeds viability[1]

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ABSTRACT - This study investigated the effectiveness of using the X-ray technique to assess the viability of papaya seeds. The X-ray images were performed in eight replicates of 25 seeds, placed on transparent acrylic plates on double-sided adhesive tape, previously identified, and then submitted to radiation in X-ray equipment "HP Faxitron". After exposure to X-ray radiation, the seeds were submitted to the germination test in rolls of germitest paper and kept in a germination chamber at 20-30 °C for 30 days. Seedlings were photographed and evaluated as normal seedlings, abnormal and non-germinated seeds. Afterward, the X-ray images and the seedlings from the same seeds were visually compared, and the X-ray images clearly showed seeds with and without internal filling. All abnormal seedlings were full seeds; therefore, X-ray images were not able to separate normal and abnormal. A 100% accuracy was verified by comparing the X-ray images and their respective seedlings, full seeds originated normal or abnormal seedlings, and empty seeds corresponded to non-germinated seeds. Therefore, using X-ray images is a promising technique to identify the viability of papaya seeds. The automated analysis of X-ray images is a simple, fast, and efficient technique to provide information on seed viability and generate parameters related to their germination capacity.

Key Words: *Carica papaya* L. Papaya Formosa. Image Analysis. Internal Filling.

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INTRODUCTION

The X-ray image analysis of seeds is recommended by the International Seed Testing Association (ISTA, 2004) and by the Rules for Seed Analysis (BRASIL, 2009) for evaluating the internal morphology of seeds. Compared to other methods, the use of radiographic analysis to determine the seed's physical quality and morphological aspects is advantageous due to the speed and conservation of the viability of the tested seeds, in addition to being a non-invasive method (RAHMAN; CHO, 2016).

This method has proven efficacy to determine the internal morphology and parameters associated with the physiological quality of several plant species, such as *Cucumis sativus* L. (GOMES JUNIOR; CHIQUITO; MARCOS FILHO, 2013), *Cucurbita pepo* L. (ANTONIO *et al*., 2016; SILVA *et al*., 2014), *Solanum aethiopicum* L. (ALVES *et al*., 2018), *Brassica oleracea* L. var. *italica* (ABUD; CICERO; GOMES JUNIOR, 2018), *Helianthus annuus* L. (ROCHA; SILVA; CICERO, 2014), *Crotalaria juncea* L. (ARRUDA; CICERO; GOMES JUNIOR, 2016), *Sesamum indicum* L. (NOGUEIRA FILHO *et al*., 2017), *Acca sellowiana* (SILVA *et al*., 2013), *Swingle citrumelo* (ARRUDA; CICERO; GOMES JUNIOR, 2018), *Anacardium occidentale* L. (SILVA *et al*., 2017), *Brachiaria brizantha*. (JEROMINI *et al*., 2019), *Terminalia argentea* (GOMES *et al*., 2014), among other species. The X-ray image analysis is a method that stands out for its reduction in analysis time and high efficiency.

The principle of the technique consists of the absorption of X-ray waves in different amounts by the seed tissues, which is dependent on their thickness, density, and composition, as well as on the wavelength of the emitted light radiation (ISTA, 2004). The test is considered a fast method, performed in seconds, and non-destructive, which also aims to detect full, empty, and malformed seeds (ISTA, 2004), allowing verification of the evolution of seed development. When associated with computer programs and image interpretation, this technique allows measuring the seed area, calculating the internal space filled by the seed reserve tissues, and relating it to its viability and physiological potential (CARVALHO *et al*., 2010).

In Brazil, most research using X-ray imaging on seeds has been carried out on grain crops and vegetables, with few studies on fruit species. Papaya (*Carica papaya* L.) is a fruit species cultivated and propagated through its seeds. Therefore, studies that seek to study fast and effective methods to identify the viability of papaya seeds are important for Brazilian fruit growing.

Studies on the internal filling of papaya seeds using X-ray images may contribute to advancing research in the fruit seed production sector. Thus, this study aimed to investigate the effectiveness of X-ray images in accurately identifying full and empty papaya seeds and their correlation with seed viability.

MATERIAL AND METHODS

The experiment was carried out at the Seed Technology Laboratory of the Federal University of Mato Grosso do Sul (UFMS), in Chapadão do Sul, State of Mato Grosso do Sul, Brazil, and at the Seed Laboratory of the Instituto Federal Goiano (IF Goiano), in Rio Verde, State of Goiás, Brazil. Papaya seeds were extracted from ripe fruits from the Formosa group (Tainung), collected in Chapadão do Sul-MS, and dried at room temperature in the laboratory for 20 days. Then, the seeds were placed in glass containers and stored for 30 days in a refrigerator at 8 °C. For the analysis of the internal morphology of the seeds, eight replicates of 25 seeds were used to facilitate the capture of images. The samples were distributed in transparent acrylic plates containing double-sided adhesive tape to prevent the seeds' movement and to allow them to be positioned in the same sequence in the evaluation of the germination test.

All the seeds were placed on the plate in a similar position and then placed inside the Faxitron HP digital X-ray equipment, model 43855A, configured with a radiation exposure time of 10 seconds, voltage of 31 kV, focal length of 45.7 cm, and image contrast calibrated at 16383 (width) x 3124 (center). The generated digital images were saved on a computer in TIFF format, processed, and then analyzed at the Seed Laboratory of the IF Goiano.

The images were evaluated using ImageJ software to determine Mode, IntDen, and Solidity parameters. Mode (relative density or average gray) is the sum of the gray values of all pixels in the selected area divided by the number of pixels in the selection, expressed in gray.pixel^{-1}. IntDen (integrated density) is the sum of the pixel values in the image or selection, i.e., equivalent to the product of area and relative density, expressed in grey.mm^2.pixel^{-1}. Solidity (solidity or filling) is the percentage of seed area effectively filled by high-density material.

The second part of the work was developed at the UFMS Seed Laboratory. Water content was determined using the oven method at 105 ± 3 ºC for 24 hours (BRASIL, 2009). Two replicates of 2.0 g were evaluated, and the results were expressed as a percentage. The germination test was installed using the same arrangement and sequence of seeds used in the X-ray images. For this step, the seeds were distributed on the three sheets of germitest paper towels, previously moistened with distilled water in a proportion of 2.5 times the dry mass of the paper. The sheets were then turned into rolls and kept in a germination chamber at an alternating temperature

of 20-30 ºC (night/day). The germination percentage was evaluated on the 30th day after the installation of the germination test, as recommended by the Rules for Seed Analysis (BRASIL, 2009), with normal and abnormal seedlings and non-germinated seeds being photographed and counted.

The results were interpreted using parallel analysis of X-ray images of the seeds and images of their respective normal seedlings, abnormal or non-germinated seeds. Multivariate analysis using principal components was used to analyze the data using the R software, version 3.5.1 (R CORE TEAM, 2018).

RESULTS AND DISCUSSION

The moisture content of papaya seeds was 12%. According to Simak (1991), the seed water content influences the optical density; that is, the lower the seed moisture, the greater the optical density, which enables greater differentiation of the internal structures of the seeds seen on X-ray images. Studies carried out with X-ray analysis in pumpkin seeds (SILVA *et al*., 2014) and passion fruit (SEVERIANO *et al*., 2018) demonstrated the possibility of adequate visualization of the internal morphology of seeds with water content between 6.5% and 12%.

The germination test showed 42% of normal seedlings, 23% of abnormal, and 35% of non-germinated seeds. Analyzing the data obtained from the radiographs, it was verified that the categories of seeds found (full and empty) directly influenced the germination test results.

The X-ray image and the seedling corresponding to the radiographed seed, classified as normal and abnormal seedlings or non-germinated seed are shown in Figure 1. Figure 1 shows that both normal seedlings (Figure 1A) and abnormal seedlings (Figure 1B) originated from full seeds, making it impossible to differentiate them by X-ray images.

Figure 2 shows a radiographic image in which papaya seeds can be visualized with and without internal filling. Severiano *et al*. (2018) reported that the radiographic images made it possible to visualize the internal morphology of the papaya seeds, which is important because the external visualization cannot differentiate between full, empty, malformed, and dead seeds, thus allowing the disposal of seeds damaged or dead. Research on this subject has prioritized the development of methodologies for seed analysis using radiographs for different species, mainly to improve the quality of seed lots about their physical and physiological attributes (GOMES JUNIOR, 2010).

Figure 1 - Normal seedling (A), abnormal seedling (B), and non-germinated seed (C) of papaya

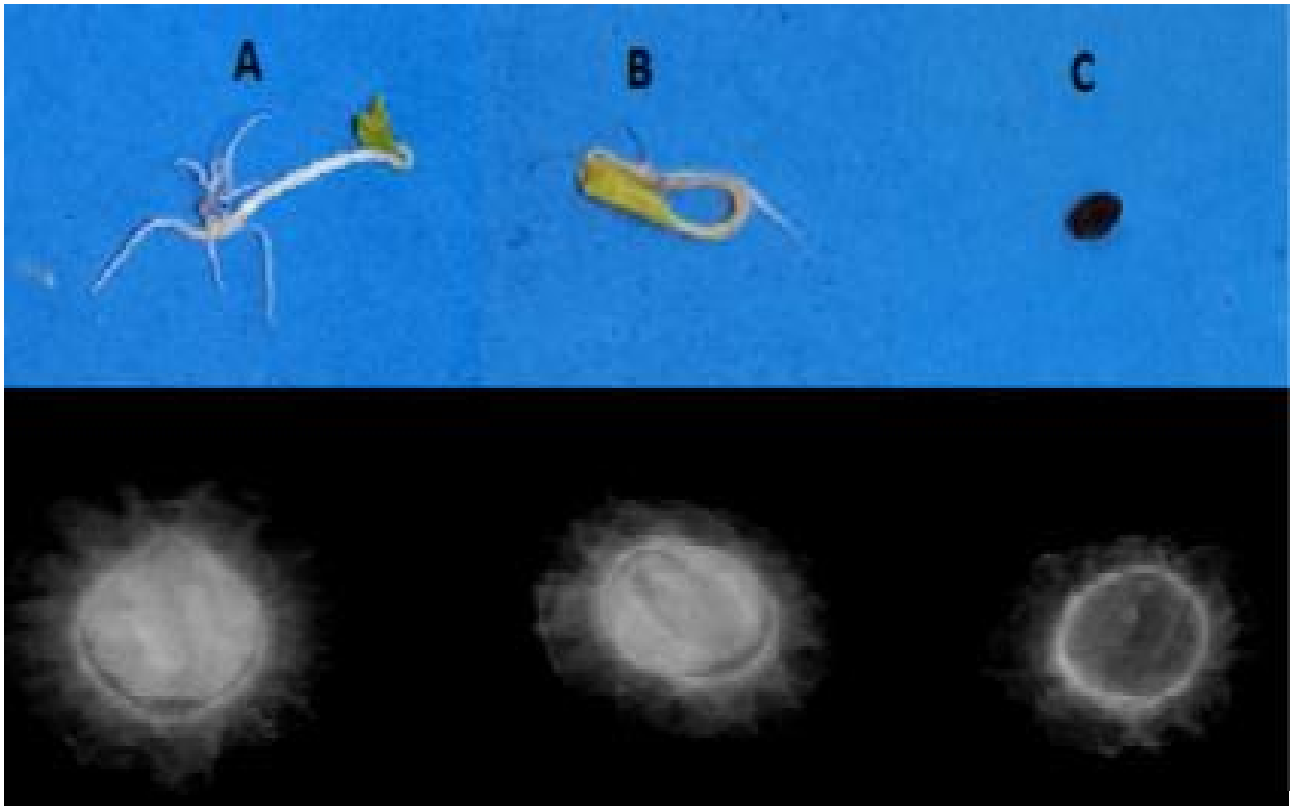
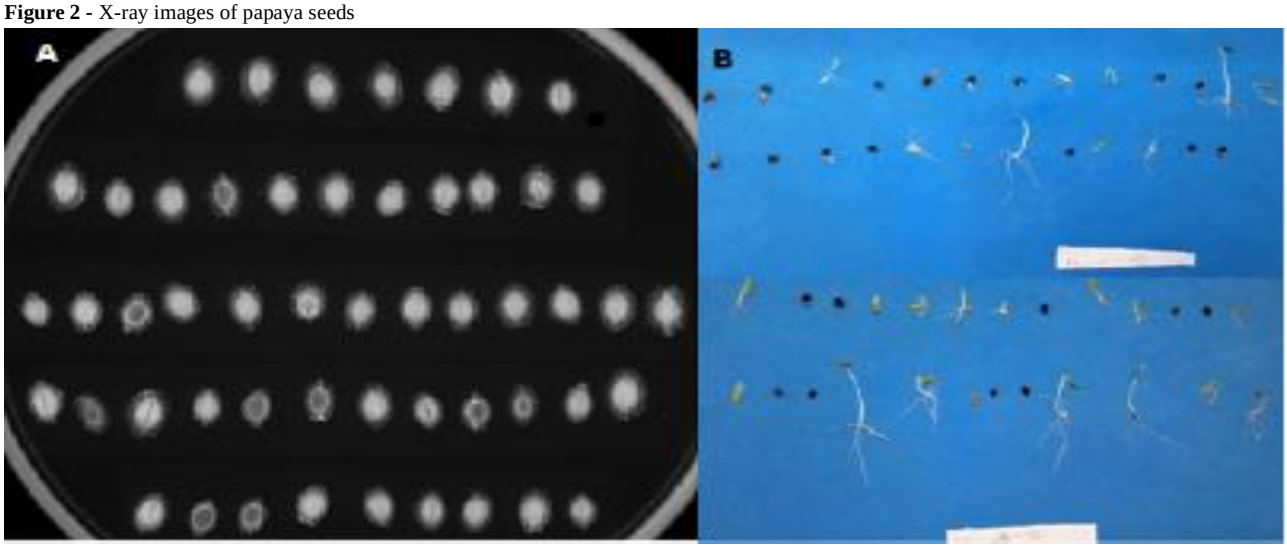


Figure 2 - X-ray images of papaya seeds



The results obtained allow visually relating, with 100% accuracy, the papaya seed germination test with the analysis of X-ray images. Full seeds originate normal or abnormal seedlings, whereas empty seeds correspond to non-germinated seeds. All abnormal seedlings originated from full seeds; therefore, separating the X-ray image between normal and abnormal seedlings is impossible since both correspond to full seeds.

Relative and integrated density are variables used recently in research with seeds, which are still little reported, but with great potential for evaluating seed lots (ABUD; CICERO; GOMES JUNIOR, 2018; MEDEIROS *et al*., 2018). These variables, calculated using the gray values of each pixel in the image, offer an idea of the resistance that a given tissue has to the passage of X-ray waves since the photons in an X-ray beam can be transmitted, scattered (Compton effect) or absorbed (photoelectric collision) when they collide with an object (KOTWALIWALE *et al*., 2014). Thus, higher gray densities indicate denser tissues, with a greater impediment to the passage of X-ray waves.

From the multivariate principal component analysis (PCA), the first two components explained 87.8% of the total variability of the data. In general, the data located far and opposite to the vectors (represented in the circle of correlations centered on the right side of the central ordering diagram) had the lowest values for these characteristics. The higher the value of the Solidity, Mode, and IntDen variables, the more the images are related to full seeds, and the lower the value of the variables corresponds to empty seeds (Figure 3).

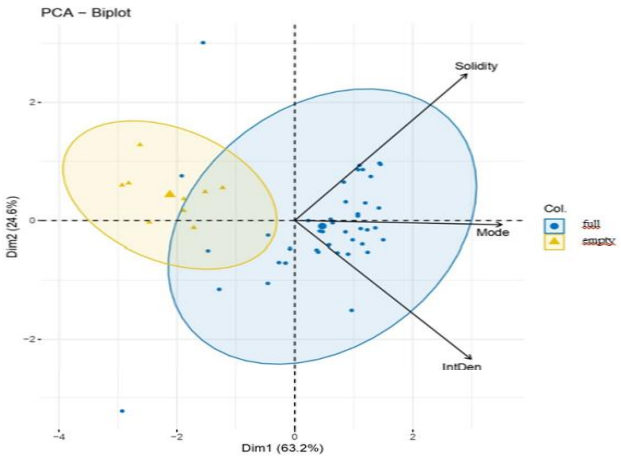
Therefore, the possibility of using the X-ray image analysis technique to assess the viability of papaya seeds is promising. It is an easy, fast, and accurate method in which the seed can be examined individually in radiographic images and selected for its viability in a few minutes.

In embaúba seeds (PUPIM *et al*., 2008) and pumpkin seeds (CARVALHO *et al*., 2009), lots with a greater number of full seeds have a higher number of normal seedlings. Marcos-Filho *et al*. (2010) showed that there is the relationship between the internal filling of the seeds and the development of castor bean seedlings (*Ricinus communis* L.), where seeds with 80% internal filling produce larger seedlings when compared to seeds with 75% internal filling. In addition, the results of this study confirm that the internal morphology can indicate the seed viability potential, confirming the results reported in *Platypodium elegans* seeds (SOUSA *et al*., 2008).

In castor bean seeds, Kobori, Cicero and Medina (2012) concluded that the X-ray test efficiently assesses the seeds' physical and physiological quality through the internal morphology of the seeds. Until recently, the number of empty seeds was determined by cutting them at the end of the germination test. With the advent of the X-ray technique, these seeds can be detected and discarded early, thus not compromising the quality of the seed lot.

The results presented here confirm that the seeds' internal morphology can indicate their viability and that the identification and removal of empty seeds can promote the improvement of the physical and physiological quality of lots. In addition, the Mode, IntDen, and Solidity parameters obtained from the automated analysis of radiographs of papaya seeds are promising to infer about the viability of the seeds. They can be recommended for the preliminary evaluation and decision-making regarding the disposal of seeds, optimizing this process, and reducing production costs.

Figure 3 - Biplot graph relating three variables and two seed categories (full and empty) of papaya seeds



CONCLUSIONS

1 - The use of X-ray images is a promising technique to identify the viability of papaya seeds;

2 - The analysis of radiographic images is a simple, fast, and efficient method to provide information on seed viability and generate parameters related to its germination capacity. The Mode, IntDen, and Solidity parameters can be used to predict the viability of papaya seeds.

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