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Artigos

Image analysis using X-ray to evaluate seed quality of *Anadenanthera peregrina* (L) Speg

Análise de imagem utilizando raios X para avaliação da qualidade de sementes de *Anadenanthera peregrina* (L) Speg

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ABSTRACT

Solutions based on image analysis are a growing trend towards improving the quality of forest seeds. This study aimed to associate the information obtained through the processing of radiographic images with the physiological potential of *Anadenanthera peregrina* seeds. Ten seed lots from different mother trees were submitted to automated X-ray analysis to obtain variables related to seed morphology and tissue integrity. Then, the seeds were evaluated for their physiological potential through the variables germination, normal seedlings, germination speed index (GSI), seedling length, uniformity index, and vigor index. The results showed that there was a relationship between the variables obtained with the X-ray analysis and those of the physiological potential of the seeds. Most of the lots with low physiological potential had lower tissue density, circularity, and a higher positive skewness value. The X-ray technique can be considered valid to establish a relationship between the physical and physiological quality of seeds, however, it seems to be more efficient to identify seeds with low physiological potential.

Keywords: Germination; High throughput image analysis; Native species; Vigor

RESUMO

Soluções baseadas em análise de imagens são uma tendência crescente para melhorar a qualidade das sementes florestais. Este estudo teve como objetivo associar as informações obtidas por meio do processamento de imagens radiográficas com o potencial fisiológico de sementes de *Anadenanthera peregrina*. Dez lotes de sementes de diferentes matrizes foram submetidos à análise automatizada de raios X para obtenção de variáveis relacionadas à morfologia da semente e integridade dos tecidos. Em seguida, as sementes foram avaliadas quanto ao potencial fisiológico por meio das variáveis germinação, plântulas normais, índice de velocidade de germinação (IVG), comprimento de plântulas, índice de uniformidade e índice de vigor. Os resultados mostraram que houve relação entre as variáveis obtidas com a análise radiográfica e as do potencial fisiológico das sementes. A maioria dos lotes com baixo potencial fisiológico apresentou menor densidade de tecido, circularidade e maior valor de assimetria positiva. A técnica de raios X pode ser considerada válida para estabelecer uma relação entre a qualidade física e fisiológica das sementes, porém, parece ser mais eficiente para identificar sementes com baixo potencial fisiológico.

Palavras-chave: Germinação; Análise de imagens de alto rendimento; Espécies nativas; Vigor

1 INTRODUCTION

Anadenanthera peregrina (L.) is an arboreal Fabaceae, belonging to Mimosoideae subfamily. This arboreal species is native to Brazil and has a wide geographical distribution across semideciduous forests and cerrado transition areas. Its importance is related to its use for medicinal, ornamental, woody, and food use, and recently for its potential to recover degraded areas (SARTORI *et al*., 2014; MOTA *et al*., 2017; FERNANDES *et al*., 2018).

Anadenanthera peregrina is mainly propagated by seeds. In this sense, seeds with high physical and physiological quality increase the likelihood of successful forest planting, especially in the critical stage of seedling establishment (SOUZA *et al*., 2017). Therefore, it is essential to carry out efficient quality control of forest seeds to identify seeds with high quality for later selection and planting, or seeds of low quality for disposal. The germination test is the official procedure for assessing the physiological potential of forest seeds (BRASIL, 2013). However, over the last few years, other tests have been proposed to complement the information provided by the germination test, many of them looking for faster and non-destructive procedures that provide additional information about the seed quality (FARINHA *et al*., 2018; MEDEIROS *et al*., 2018; MUKASA *et al*., 2019; PINHEIRO *et al*., 2020).

Among the available techniques, the X-ray test has been efficient to evaluate seed the intern morphology of a variety of forest species. Recent studies have shown the possible relationship between the physical variables obtained through X-rays and the germination and performance of seedlings, as in seeds of *Acca sellowiana* O. Berg (SILVA *et al*., 2013), *Terminalia argentea* Mart. (GOMES *et al*., 2014), *Platypodium elegans* Vog. (GOMES *et al*., 2016), *Senna multijuga* Rich. (MARCHI; GOMES-JUNIOR, 2017), *Moringa oleifera* Lam. (NORONHA *et al*., 2018), *Leucaena leucocephala* Lam. (MEDEIROS *et al*., 2018), *Moquiniastrum polymorphum* Less. (FARIA *et al*., 2019), and others.

The X-rays can exhibit the structural integrity of seeds through the difference among tissue density, and then identify damages and embryo abnormalities (XIA *et al*., 2019). However, it is necessary to automate these determinations to avoid misleading interpretations, and to obtain faster, reliable, and more accurate analyses (MEDEIROS *et al*., 2018). As the result, several studies use an open-access software ImageJ (SCHNEIDER *et al*., 2012) to process radiographic seed images (MEDEIROS *et al*., 2018; NORONHA *et al*., 2018; PINHEIRO *et al*., 2020).

The growing demand for seedlings of native arboreous species to recover degraded areas has been boosting the application of these new techniques. However, there is still little information about the emerging techniques to evaluate seed quality of *Anadenanthera peregrina.* Therefore, this study aimed to associate the information obtained through the processing of radiographic images with the physiological potential of *Anadenanthera peregrina* seeds.

2 MATERIAL AND METHODS

2.1 Site and plant material

Seeds of *Anadenanthera peregrina* were obtained from fruits collected from 10 mother trees, constituting 10 lots. Each tree was randomly selected in an experimental area of the Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais, Brazil. Fruits were harvested from each mother tree, and seeds were manually extracted and placed to dry naturally for 120 hours until achieving hygroscopic balance (approximately 13% of moisture content).

2.2 X-ray test: automatic analysis of radiographs

The seeds were subjected to analysis of their internal tissues by the X-ray technique. 100 seeds of each treatment (10 lots from 10 trees) were organized orderly in groups of ten seeds and attached by an adhesive paper. The radiographic images were generated using the Faxitron equipment, model MX-20 (Faxitron X-ray Corp. Wheeling, IL, USA). The equipment was adjusted to a voltage of 23 kV and the seeds were exposed to radiation for 10 seconds, at a focal distance of 41.6 cm. The image contrast was calibrated to 1872 (width) x 1907 (center). The images were saved in Tagged Image File Format (TIFF) and then analyzed.

Automated images analysis were taken using the free software ImageJ® (https://imagej.nih.gov/ij/download.html), performed by IJCropSeed macro (MEDEIROS *et al*., 2020a). The variables below were obtained:

Area (pixel): area inside the polygon defined by the perimeter;

Circularity: obtained through the equation: Circularity = $4 \cdot \pi \cdot \text{Area}/\text{Perimeter}^2$

Aspect ratio: obtained through the equation: Aspect ratio = major axis/minor axis

Relative density (gray.pixel^{-1}): defined as the average of the gray values of all pixels in the selected area;

Integrated density (gray.pixel_area.pixel^{-1}): the sum of the pixel values in the image or the selection;

Skewness: defined as the third-order moment about the mean.

2.3 Physiological analyses

After obtaining the radiographic images, the seeds were tested for their physiological quality. The germination test was conducted on rolls of germination paper (Germitest®) moistened with water in the amount of 2.5 times the dry paper weight, and kept in a germinator at 25 °C for 10 days. Germination (root protrusion) and normal seedlings were counted daily. Then, the percentages of root protrusion (root greater than 2 mm), normal seedlings, germination speed index (GSI), uniformity index, and vigor index were calculated according to the formulas described by Silva *et al.* (2019). After the last counting of the germination test, the seedling length was measured, and the results were expressed in mm seedling^{-1}.

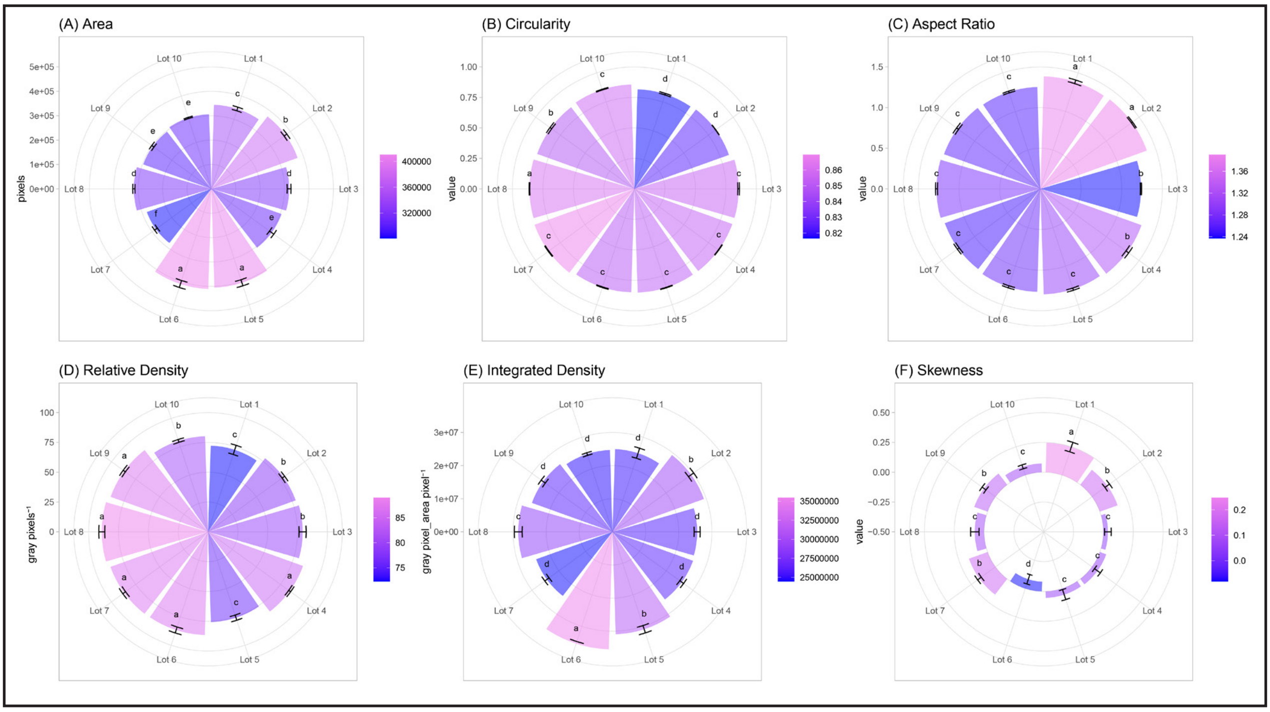
2.4 Experimental design and statistical analysis

The experiment was conducted in a completely randomized design with five replications for each lot. Statistical analysis was performed with the analysis of variance. The normal distribution of the error and the homogeneity of the variances were verified by the Shapiro-Wilk test. Treatment means were compared using the Scott-Knott test ($p \leq 0.05$). The mean values of the radiographic image analysis data and the physiological tests of each treatment were used to perform the Principal Component Analysis (PCA), where an "n x p" matrix was obtained, where "n" corresponds to the number of treatments (n = 10) and "p" the number of variables analyzed (p = 12). The eigenvalues and eigenvectors were calculated from the covariance matrices and plotted on a two-dimensional graph (category ordering diagram and correlation circle), generated from the Factoextra package (Kassambara and Mundt, 2016). The software R 4.0.0 (R CORE TEAM, 2019) was used in all analyses.

3 RESULTS AND DISCUSSION

The physical analyses obtained by the X-ray test showed significant differences among the lots. Overall, it is noteworthy that lot 6 had the highest values of area (Figure 1A) and tissue density (relative and integrated) (Figure 1D and E). On the other hand, lots 1 and 2 presented the greatest values of aspect ratio (Figure 1C), and lot 1 had the highest value of skewness (Figure 1F). Regarding the circularity (Figure 1B), the seed lots showed no remarkable differences compared to other analyzed variables.

Figure 1 – Physical analyses obtained through radiographic images of 10 seed lots of *Anadenanthera peregrina*

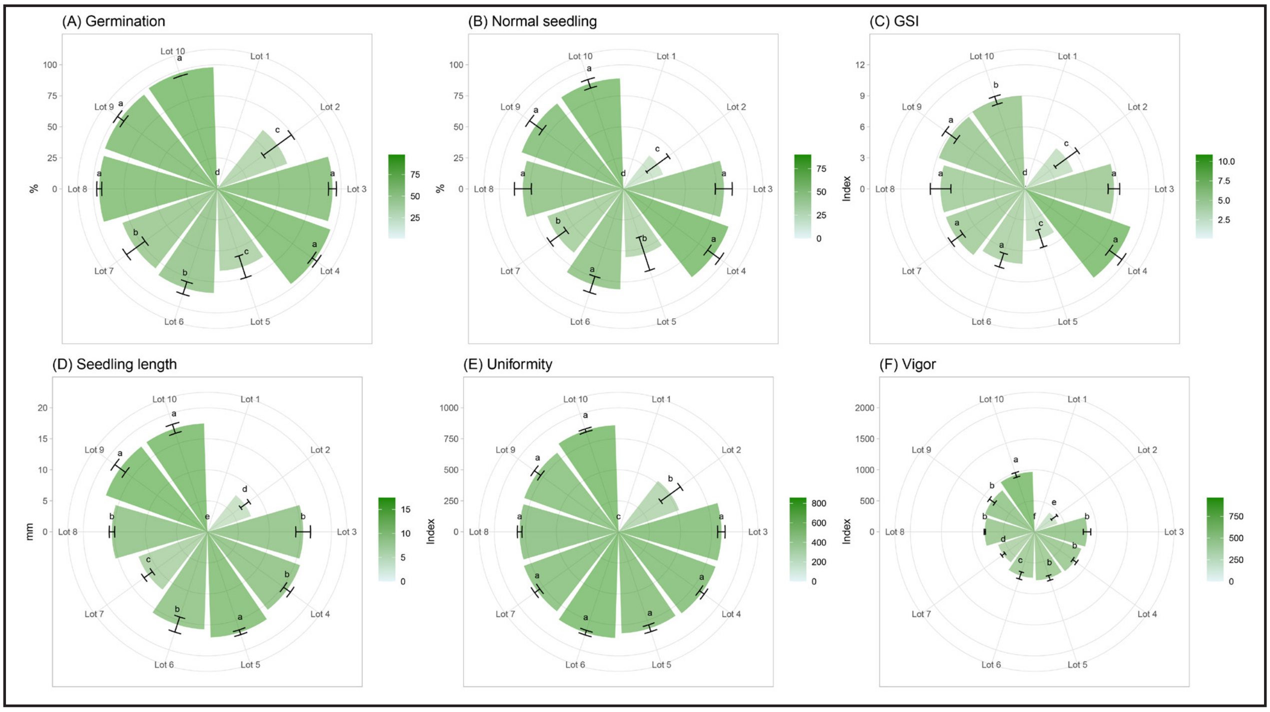


Source: Authors (2020)

In where: *Means followed by different letters are significantly different at 5% probability ($p < 0.05$) by the Scott-Knott test. Bars represent the standard deviation.

Based on the physiological analyses, significant differences were detected among the lots. Lot 1 showed the lowest performance in all germination and vigor variables analyzed, reaching even null values (Figure 2). Similar behavior was observed in lot 2, which had lower values of germination (Figure 2A), normal seedlings (Figure 2B), seedling length (Figure 2D), and uniformity and vigor index (Figure 2E and F) than the other lots. In contrast, lots 9 and 10 had overall the highest physiological quality, with greater values of germination (Figure 3A), normal seedlings (Figure 2B), GSI (Figure 2C), seedling length (Figure 2D), and vigor index (Figure 2F). The uniformity index showed no differences among the lots, except for lots 1 and 2, which had the lowest values (Figure 2E). In a general context, the seed lots can be categorized into three vigor levels, which lots 9 and 10 had high vigor, lots 1 and 2 had low vigor, while lots 3, 4, 5, 6, 7, and 8 showed an intermediate physiological quality (Figure 2).

Figure 2 – Physiological analyses obtained through radiographic images of 10 seed lots of *Anadenanthera peregrina*



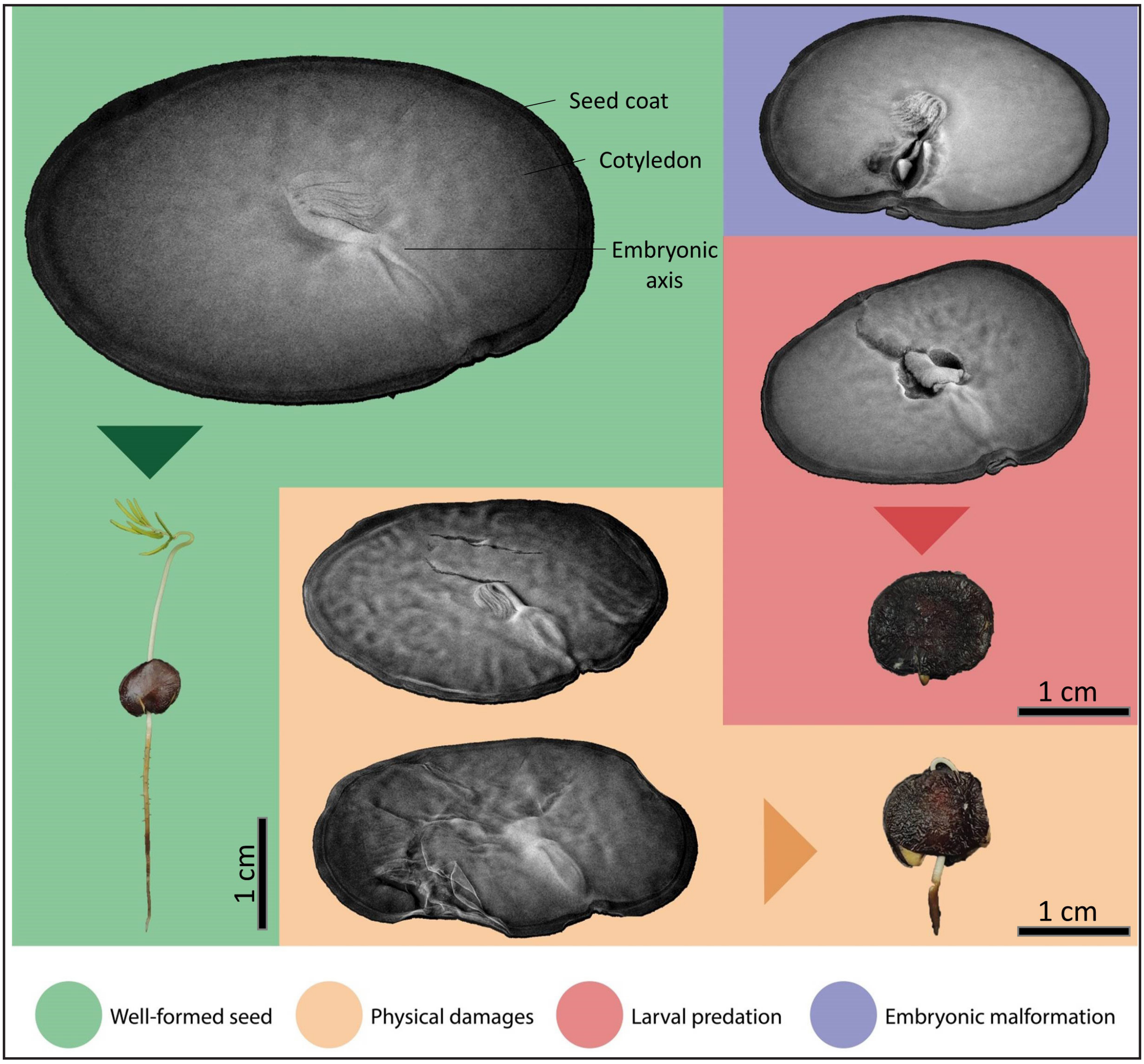
Source: Authors (2020)

In where: *Means followed by different letters are significantly different at 5% probability ($p < 0.05$) by the Scott-Knott test. Bars represent the standard deviation.

Due to the aforementioned data, the relation between the physical and physiological attributes of *Anadenanthera peregrina* seeds is mainly observed in low-quality seeds (lots 1 and 2), which presented the highest values of aspect ratio and skewness (Figure 1C and F); lower tissue density (Figure 1D and E), and low values of germination (Figure 2A and B), GSI (Figure 2C), seedling length (Figure 2D), uniformity index (Figure 2E), and vigor index (Figure 2F).

Figure 3 shows the tendency in the relation between the physical and physiological quality of *Anadenanthera peregrina* seeds. In general, well-formed seeds (well-formed and dense tissues), generate normal and vigorous seedlings (green color). Seeds with physical damage (such as cracks) generate abnormal and low vigorous seedlings (orange color). Seeds that do not germinate, showing a malformation in the embryo (blue color), and larval predation, mainly when it occurs directly in the embryonic axis (red color) (Figure 3).

Figure 3 – Three-dimensional projections of radiographs of *Anadenanthera peregrina* seeds with enhancement texture applications. Seeds with different internal characteristics and some of their respective seedlings and non-germinated seeds



Source: Authors (2020)

The X-ray technique has been efficient for detecting internal damages (such as cracks and larval predation) in forest seeds. These findings were recently reported on species such as *Piptadenia gonoacantha* (PINHEIRO *et al*., 2020), *Arctostaphylos pungens*, *Juniperus deppeana* (RUBALCAVA-CASTILLO *et al*., 2020), and *Pinus* spp. (ARKHIPOV *et al*., 2020). In this study, the X-ray test results are consistent with other studies that evaluated forest species, such as *Leucaena leucocephala* (MEDEIROS *et al*., 2019), *Moquiniastrum polymorphum* (FARIA *et al*., 2019), and *Moringa oleifera* (NORONHA *et al*., 2019). On the whole, all these studies found a direct relation between physical and physiological seed attributes. Furthermore, tissue density was highly correlated to the physiological seed quality of these species.

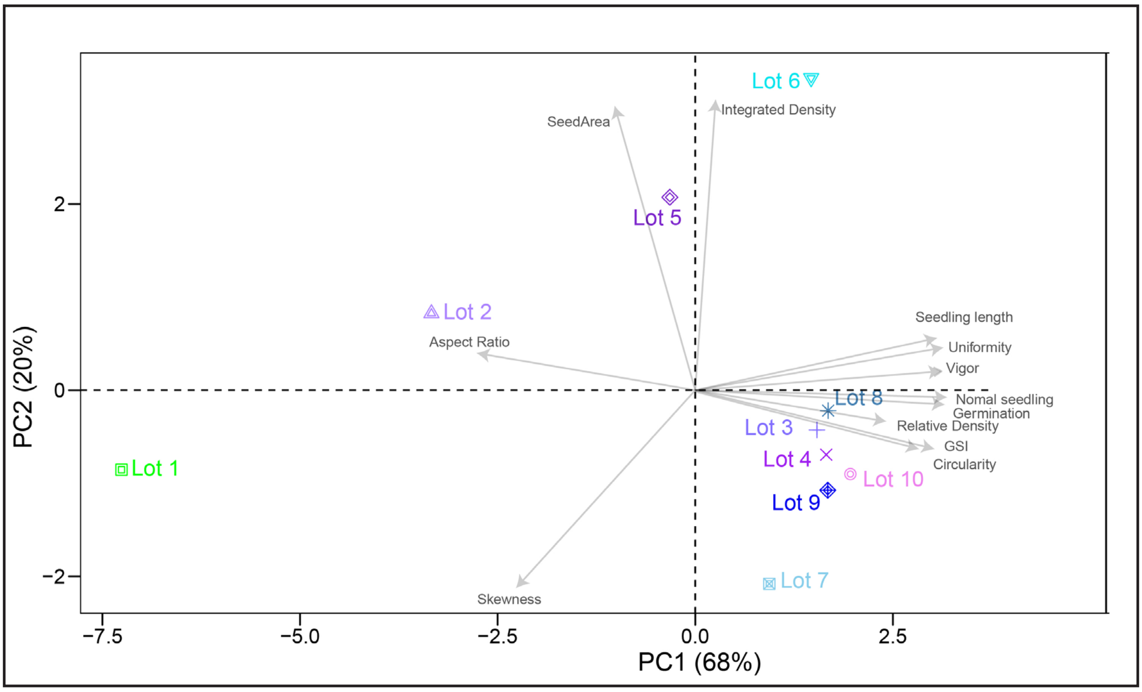
According to Medeiros *et al*. (2020b), variables such as relative density, integrated density, and seed filling indicate the integrity level of seed tissues. Since seed tissues are well-formed and have no damage, the X-ray photons have greater resistance to pass through the seed, whereas damaged tissues offer less resistance. Moreover, a lower tissue density can be related to a reduced amount of reserves, such as proteins, lipids, and starch and an embryonic malformation, which directly contributes to decreasing seed germination and vigor (OHTO *et al*., 2018; ZHAO *et al*., 2018; DOLL *et al*., 2020). In this context, the mobilization of reserves is essential to improve seed germination and vigor, as recently reported in *Erythrina velutina* seeds under thermal stress conditions (FELIX *et al*., 2020).

Most studies that evaluate forest seeds observe the relationship between physical and physiological attributes even in high vigor seeds. However, it is important to emphasize that high vigor lots (lots 9 and 10) did not have any close relationship between the physical and physiological quality of seeds. Although these lots showed high germination and vigor (Figure 2), they did not show higher values of tissue density (Figure 1D and E). Moreover, taking lot 3 as an example, which presented low tissue density (especially integrated) (Figure 1D) and high germination (Figure 2A and B). Even though it is widely recognized the use of the X-ray technique to establish a relation between the physical and physiological seed quality attributes; nevertheless, our findings showed that this relation seems to be more efficient for low vigor seeds of *Anadenanthera peregrina.* As a result, skewness seems to have a good relation with the physiological quality of low vigor seeds of *Anadenanthera peregrina*, in which lot 1 (0% germination) presented higher values for this variable. The seed skewness, as well as tissue density, is related to the integrity of seed tissue. Its magnitude indicates the symmetry of the roughness peaks and troughs about the mean line and is equal to zero in case of perfect symmetry (MEDEIROS *et al*., 2020a). Therefore, the highest value of the seed skewness observed in lot 1 seems to be related to the embryonic malformation of these seeds.

These results of our study could indicate that there is genetic diversity among the *Anadenanthera peregrina* lots. Rosa *et al*. (2020) validated an X-ray methodology for internal and densitometric analysis of *Lecythis pisonis* seeds that demonstrated a high genetic divergence among matrices. According to these authors, these findings are important and could be applied in genetic breeding programs, whereby a wide diversity enables the selection of superior genotypes by segregating successive generations.

The principal component analysis (PCA) summarized the obtained results, in which the sum of components 1 (PC1) and 2 (PC2) explained 88% of the total variability of the data (Figure 4).

Figure 4 – Biplot graph of the principal component analysis obtained from variables related to the physical and physiological attributes of *Anadenanthera peregrina* seeds



Source: Authors (2020)

In where: PC1 - Principal Component 1; PC2 - Principal Component 2.

Lots 1 and 2 (low physiological quality) were in negative scores of PC1 and opposite to the vectors of physiological quality (germination and vigor), and some vectors of physical quality such as circularity and relative density. Furthermore, these lots (1 and 2) were near to the aspect ratio and skewness vectors and ensure its relations with the low quality of *Anadenanthera peregrina* seeds. Otherwise, lots 9 and 10 (high germination and vigor), and lots 3, 4, 5, 6, 7, and 8 (intermediate vigor) were in the positive scores of PC1, near to the germination, normal seedlings, GSI, uniformity, vigor, tissue density, and circularity vectors.

In summary, despite presenting the potential for the analysis of *Anadenanthera peregrina* seeds, the X-ray technique may show limitations for this species (and not always be related to germination and vigor), especially in lots of higher physiological quality. These results may provide support for future studies, such as the use of artificial intelligence techniques using radiographic images for seed classification of *Anadenanthera peregrina*.

4 CONCLUSIONS

The seed X-ray technique combined with the automated analysis of radiographs is efficient to evaluate the internal morphology and physiological quality of *Anadenanthera peregrina* seeds, mainly in low-quality lots. This technique has also the potential to identify internal damages and embryonic malformation.

REFERENCES

ARKHIPOV, M. V.; PRIYATKIN, N. S.; GUSAKOVA, L. P.; KARAMYSHEVA, A. V.; TROFIMUK, L. P.; POTRAKHOV, N. N.; BESSONOV, V. B.; SHCHUKINA, P. A. Microfocus x-ray method for detecting hidden defects in seeds of woody forest species and other types of vascular plants. **Technical Physics**, [s.l.], v. 90, n. 2, p. 324–332, 2020.

BRASIL. Ministério da Agricultura Pecuária e Abastecimento. **Instruções para análise de sementes de espécies florestais**. Ministério da Agricultura Pecuária e Abastecimento. Brasília: MAPA/ACS, 2013. 97 p.

DOLL, N. M.; ROYEK, S.; FUJITA, S.; OKUDA, S.; CHAMOT, S.; STINTZI, A.; WIDIEZ, T.; HOTHORN, M.; SCHALLER, A.; GELDNER, N.; INGRAM, G. A two-way molecular dialogue between embryo and endosperm is required for seed development. **Science**, [s.l.], v. 367, n. 6476, p. 367: 431–435, 2020.

FERNANDES, H. E.; SANTANA, T. F.; CABRAL, K. P.; ERASMO, E. A. L.; SOUZA, P. B. Avaliação dos diferentes níveis de sombreamento na germinação de sementes de *Anadenanthera peregrina* (L.) SPEG. **Biodiversidade**, [s.l.], v. 17, n. 3, p.62-70, 2018.

FARIA, J. C. T.; MELO, L. A.; ASSUMPÇÃO, C. R. M.; BRONDANI, G. E.; BREIER, T. B.; FARIA, J. M. R. Physical quality of seeds of *Moquiniastrum polymorphum*. **Brazilian Journal of Biology**, São Carlos, v. 79, n. 1, p. 63–69, 2019.

FARINHA, A. O.; BRANCO, M.; PEREIRA, M. F. C.; AUGER-ROZENBERG, M. A.; MAURÍCIO, A.; YART, A.; GUERREIRO, V.; ROQUES, A. Micro X-ray computed tomography suggests cooperative feeding among adult invasive bugs *Leptoglossus occidentalis* on mature seeds of stone pine *Pinus pinea*. **Agricultural and Forest Entomology**, [s.l.], v. 20, n. 1, p. 18–27, 2018.

FELIX, F. C.; MEDEIROS, J. A. D.; FERRARI, C. S.; PACHECO, M. V.; TORRES, S. B. Molecular aspects during seed germination of *Erythrina velutina* Willd. under different temperatures (Part 1): reserve mobilization. **Journal of Seed Science**, Londrina, v. e202042029, 2020.

GOMES, K. B. P.; MARTINS, R. D. C. C.; MARTINS, I. S.; JUNIOR, G. G. Avaliação da morfologia interna de sementes de *Terminalia argentea* (Combretaceae) pelo teste de raios X. **Revista Ciência Agronômica**, [s.l.], v. 45, n. 4, p. 752–759, 2014.

GOMES, K. B. P.; MATOS, J. M. M.; MARTINS, S.; MARTINS, R. D. C. C. X-ray test to evaluate the physiological potencial of *Platypodium elegans* Vog . seeds (Fabaceae). **Scientia Agropecuaria**, Trujillo, v. 7, n. 3, p. 305–311, 2016.

KASSAMBARA, A.; MUNDT, F. **Factoextra: extract and visualize the results of multivariate data analyses**. R Package Version 1, 2016.

MARCHI, J. L.; GOMES-JUNIOR, F. G. Utilização de técnicas de análise de imagens para determinação do tamanho do embrião em sementes de *Senna multijuga* (Rich.) e sua relação com a germinação e o vigor. **Journal of Seed Science**, [s.l.], v. 39, n. 1, p. 13–19, 2017.

MEDEIROS, A. D.; ARAÚJO, J. O.; LEÓN, M. J. Z.; SILVA, L. J.; DIAS, D. C. F. S. Parameters based on X-ray images to assess the physical and physiological quality of *Leucaena leucocephala* seeds. **Ciência e Agrotecnologia**, Lavras, v. 42, n. 6, p. 643–652, 2018.

MEDEIROS, A. D.; SILVA, L. J.; PEREIRA, M. D.; OLIVEIRA, A. M. S.; DIAS, D. C. F. S. High-throughput phenotyping of brachiaria grass seeds using free access tool for analyzing X-ray images. **Anais da Academia Brasileira de Ciências**, Rio de Janeiro, v. 92, supl. 1, 2020b.

MEDEIROS, A. D.; SILVA, L. J.; SILVA, J. M.; DIAS, D. C. F. S.; PEREIRA, M. D. IJCropSeed: An open-access tool for high-throughput analysis of crop seed radiographs. **Computers and Electronics in Agriculture**, [s.l.], v. 175, 105555, 2020a.

MEDEIROS, A. D.; ZAVALA-LEÓN, M. J.; ARAÚJO, J. O.; PEREIRA, M. D.; DIAS, D. C. F. S.; SILVA, L. J. Relationship between internal morphology and physiological quality of *Leucaena leucocephala* seeds using image analysis. **Revista** Árvore, Viçosa, v. 43, n. 2, e430208, 2019.

MOTA, G. S.; SARTORI, C. J.; MIRANDA, I.; QUILHÓ, T.; MORI, F. A.; PEREIRA, H. Bark anatomy, chemical composition and ethanol-water extract composition of Anadenanthera peregrina and *Anadenanthera colubrina*. **PloS one**, [s.l.], v. 12, n. 12, e0189263, 2017.

MUKASA, P.; WAKHOLI, C.; MO, C.; OH, M.; JOO, H.-J. J.; SUH, H. K.; CHO, B.-K. K. Determination of viability of Retinispora (Hinoki cypress) seeds using FT-NIR spectroscopy. **Infrared Physics and Technology**, [s.l.], v. 98, p. 62–68, 2019.

NORONHA, B. G.; PEREIRA, M. D.; MEDEIROS, A. D. Incremento de área em sementes de moringa durante a embebição por meio da análise de imagens. **Ciência Florestal**, Santa Maria, v. 29, n. 1, p. 221-232, 2019.

NORONHA, B. G.; MEDEIROS, A. D.; PEREIRA, M. D. Avaliação da qualidade fisiológica de sementes de *Moringa oleifera* Lam. **Ciência Florestal**, Santa Maria, v. 28, n. 1, p. 393-402, 2018.

OHTO, M. A.; STONE, S. L.; HARADA, J. J. Genetic control of seed development and seed mass. **Annual Plant Reviews**, [s.l.], v. 27, p. 1–24, 2018.

PINHEIRO, D.T.; CAPOBIANGO, N. .; MEDEIROS, A. D.; CAIAFA, K. F.; DIAS, D. C. F. S.; PINHEIRO, A. L. Assessment of the physical and physiological quality of *Piptadenia gonoacantha* seeds (Mart.) J. F. Macbr. using image analysis. **Revista** Árvore, Viçosa, v. 44, e4426, 2020.

PINHEIRO, D. T.; MEDEIROS, A. D.; ZAVALA-LEÓN, M. J.; DIAS, D. C. F. S.; SILVA, L. J. Physical and physiological quality of Jatropha curcas L. seeds at different maturity stages using image analysis. **Spanish Journal of Agricultural Research**, [s.l.], v. 18, e0206, 2020.

R CORE TEAM. **R Development Core Team. R: A Language and Environment for Statistical Computing**. 2019.

ROSA, T. L. M.; ARAUJO, C. P. DE; KAMKE, C.; FERREIRA, A.; FERREIRA, M. F. DA S.; OLIVEIRA, J. P. B.; SCHMILDT, E. R.; LOPES, J. C.; MENGARDA, L. H. G.; OTONI, W. C.; SANTOS, A. R.; ALEXANDRE, R. S. Sapucaia nut: Morphophysiology, minerals content, methodological validation in image analysis, phenotypic and molecular diversity in *Lecythis pisonis* Cambess. **Food Research International**, [s.l.], v. 137, 109383, 2020.

RUBALCAVA-CASTILLO, F. A.; SOSA-RAMÍREZ, J.; LUNA-RUÍZ, J. J.; VALDIVIA-FLORES, A. G.; DÍAZ-NÚÑEZ, V.; ÍÑIGUEZ-DÁVALOS, L. I. Endozoochorous dispersal of forest seeds by carnivorous mammals in Sierra Fría, Aguascalientes, Mexico. **Ecology and Evolution**, [s.l.], v. 10, n. 6, p. 2991–3003, 2020.

SARTORI, C. J.; MORI, F. A.; VALLE, M. L. A.; MENDES, L. M.; PROTÁSIO, T. P. Rendimento gravimétrico em taninos condensados nas cascas de *Anadenanthera peregrina* em diferentes classes diamétricas. **Cerne**, Lavras, v. 20, n. 2, p. 239–244, 2014.

SCHNEIDER, C. A.; RASBAND, W. S.; ELICEIRI, K. W. NIH Image to ImageJ: 25 years of image analysis. **Nature Methods**, [s.l.], v. 9, n. 7, p. 671–675, 2012.

SILVA, L. J.; MEDEIROS, A. D.; OLIVEIRA, A. M. S. SeedCalc, a new automated R software tool for germination and seedling length data processing. **Journal of Seed Science**, Londrina, v. 41, n. 2, p. 250–257, 2019.

SILVA, V. N.; SARMENTO, M. B.; SILVEIRA, A. C.; SILVA, C. S.; CICERO, S. M. Avaliação da morfologia interna de sementes de acca sellowiana O. Berg por meio de análise de imagens. **Revista Brasileira de Fruticultura**, Jaboticabal, v. 35, n. 4, p. 1158–1169, 2013.

SOUZA, P. B.; COSTA, W. S.; PINHEIRO, A. L.; COELHO, D. J. S. **Ecologia, Manejo, Silvicultura e Tecnologia de Espécies Nativas da Floresta Atlântica**. Viçosa: UFV, 2017. 80 p.

XIA, Y.; XU, Y.; LI, J.; ZHANG, C.; FAN, S. Recent advances in emerging techniques for non-destructive detection of seed viability: A review. **Artificial Intelligence in Agriculture**, [s.l.], v. 1, p. 35–47, 2019.

ZHAO, M.; ZHANG, H.; YAN, H.; QIU, L.; BASKIN, C. C. Mobilization and Role of Starch, Protein, and Fat Reserves during Seed Germination of Six Wild Grassland Species. **Frontiers in Plant Science**, [s.l.], v. 9, 234, 2018.

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