Spanish Journal of Agricultural Research
18 (3), e0206, 11 pages (2020)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2020183-16028
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)

RESEARCH ARTICLE

OPEN ACCESS

Physical and physiological quality of *Jatropha curcas* L. seeds at different maturity stages using image analysis

Daniel T. Pinheiro (Pinheiro, DT), André D. Medeiros (Medeiros, AD), Manuel J. Zavala-León (Zavala-León, MJ), Denise C. F. S. Dias (Dias, DCFS) and Laércio J. da Silva (Silva, LJ)

Federal University of Viçosa, Agronomy Dept., Av. PH Rolfs s/n, 36570-900, Viçosa, Minas Gerais, Brazil.

Abstract

Aim of study: To assess the potential of automated X-ray image analysis to evaluate the physical characteristics of *Jatropha curcas* seeds, and to relate the parameters obtained with the physiological quality of the seeds harvested at different maturity stages.

Area of study: Experimental area of Agronomy Department, Federal University of Viçosa (UFV), Brazil.

Material and methods: The fruits were harvested from 20 plants, based on the external skin color (green, yellow, brownish-yellow and brown). The study was performed by automated and visual analysis of radiographic images of the seeds, in which measurements of tissue integrity, density and seed filling were performed. Seed dry matter, germination and seedling growth were also analysed.

Main results: Variables obtained through automated analysis of radiographic images correlated significantly with all physiological variables ($r > 0.9$), as well as visual image evaluations ($r > 0.75$). The seeds extracted from green fruits presented lower tissue integrity and lower physiological quality. Radiographic analysis was efficient for monitoring *J. curcas* seed quality at different maturity stages. Morpho-anatomical parameters obtained from X-ray analysis were highly correlated with seed physiological attributes.

Research highlights: It is important to develop and improve methodologies based on lower-cost techniques, such as X-ray analysis. In this context, we verified that X-ray images can be used for monitoring *J. curcas* seed filling and maturation. Radiographic images of seeds can be analyzed automatically with ImageJ software. Internal morphology and physical characteristics of seeds have relationship with their physiological quality.

Additional key words: high throughput image analysis; seedling growth; X-ray.

Abbreviations used: DMS (damaged seeds); GER (germination); GI (growth index); GSI (germination speed index); HPQ (seeds with high physical quality); IntDens (integrated density); MFS (malformed seeds); NS (normal seedlings); RelDens (relative density); SDM (seedling dry matter); SeeDM (seed dry matter); SeedF (seed filling); SL (seedling length); UI (uniformity index); VI (vigor index).

Authors' contributions: This work was carried out in collaboration among all authors. DTP: designed the research, carried out the experiment and wrote the first draft of the manuscript. ADM and MJZL: performed the laboratory and statistical analysis. DCFSD and LJS: advised and helped on all steps, including English review. All authors read and approved the final manuscript.

Citation: Pinheiro, DT; Medeiros, AD; Zavala-León, MJ; Dias, DCFS; da Silva, LJ (2020). Physical and physiological quality of *Jatropha curcas* L. seeds at different maturity stages using image analysis. Spanish Journal of Agricultural Research, Volume 18, Issue 3, e0206. https://doi.org/10.5424/sjar/2020183-16028

Received: 14 Nov 2019. **Accepted:** 17 Sep 2020.

Funding agencies/institutions	Project / Grant
Coordenação de Aperfeiçoamento de Nível Superior – Brasil (CAPES)	Finance Code: 001
Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)	
Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).	

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Daniel T. Pinheiro: pinheiroagroufv@gmail.com

Introduction

Jatropha curcas L. produces seeds with high oil content. Among its main uses are biodiesel production (Kamel *et al.*, 2018), antibacterial and antifungal action (Haq *et al.*, 2019; Hu *et al.*, 2019) and, to a lesser extent, the production of by-products such as cosmetics, biopesticides, fertilizers and others, which enhance the potential of this species on the world agricultural scenario (Montes & Melchinger, 2016; Mazumdar *et al.*, 2018).

For this species, seminal propagation is common, which demands seeds with high physiological potential. An important factor related to seed quality is the ideal time for harvest, which is directly related to the maturation process. The maturation process is characterized by embryonic formation, accumulation of reserves and acquisition of other functional characteristics including germination, desiccation tolerance, seed dormancy and longevity (Devic & Roscoe, 2016; Leprince *et al.*, 2016).

In many species, the ideal time to harvest is defined by the coloring of the fruit skin. Research results based on physiological and biochemical evaluations indicate that *J. curcas* seeds should be harvested when the fruits reach yellow and brownish-yellow color (Kaushik, 2003; Silva *et al.*, 2012, 2017, 2018). However, analyses related to tissue density and other physical analyses could complement these results and contribute to elucidate the maturation process from a more efficient perspective, as has recently been observed in pepper (Medeiros *et al.*, 2020a) and tomato seeds (Borges *et al.*, 2019).

In this context, the application of techniques such as X-ray analysis (Medeiros *et al.*, 2020b), computerized seedling evaluation (Castan *et al.*, 2018), multispectral and hyperspectral image analysis (Xia *et al.*, 2019), microtomography (Gomes-Junior *et al.*, 2019), magnetic resonance (Melchinger *et al.*, 2017) and others, offer a new form of high throughput phenotyping of seed characteristics.

In general, the main benefits of these analyses are being non-destructive, non-subjectivity, fast and efficient in predicting attributes directly correlated with seed quality (Brunes *et al.*, 2016; Nielsen *et al.*, 2017; Medeiros *et al.*, 2018; Leão-Araújo *et al.*, 2019; Medeiros *et al.*, 2020b). However, these techniques need a high initial investment to acquire the equipment, such as microtomography and magnetic resonance. Thus, it is important to develop and improve methodologies based on lower-cost techniques, such as X-ray analysis. In addition, X-ray analysis brings additional advantages over the techniques of multispectral analysis, as they allow obtaining information about internal morphology characteristics and their structural integrity (Arkhipov *et al.*, 2019). It is known that the X-ray analysis is efficient for evaluating the viability of J. curcas seeds (Pinto *et al.*, 2009; Medeiros *et al.*, 2020b), but the improvement of this technique, such as developing automated analysis of the radiographs, could be useful to assess the physical quality and to obtain parameters related to the physiological quality of the seeds. This technique could be used in quality control programs of seed companies.

Thus, the aim of this work was to evaluate the potential of automated X-ray image analysis to assess the physical characteristics of *J. curcas* seeds, and to relate the parameters obtained with the physiological quality of the seeds harvested at different maturity stages.

Material and methods

Local and plant material

Jatropha curcas L. seeds were obtained from approximately 500 fruits, harvested from 20 similar plants randomly selected in an experimental are of the Agronomy Department, at Federal University of Viçosa, in 2018/2019, in Viçosa, Minas Gerais State, Brazil (20°46'01.7"S42°52'05.6"W). The fruits were harvested based on their external skin color: green (L=53.87±12.35; a=-18.66±1.47±1.96; b=42.99±16.39), yellow (L=67.29±1.96; a=-5.44±4.5; b=57.99±3.77), brownish-yellow (L=46.49±1.85; a=7.36±0.15; b=32.67±0.78) and brown (L=20.08±6.2; a=2.19±1.63; b=5.15±4.9). Each fruit color was considered a stage of development, according to Silva *et al.* (2017). The number of days after anthesis to reach each stage of development was estimated based on the mean number of days spent for a sample set of fruits, which corresponded to approximately 50, 60, 70 and 80 days after anthesis to reach the green, yellow, brownish-yellow and brown stage, respectively (Fig. 1).

The seeds were manually extracted from the fruits, washed in running water and dried in laboratory for 96 hours until reaching the equilibrium moisture level (~ 8%). To determine seed dry matter, four replications of 25 seeds were weighed, placed to dry for 24 h at 105 ºC and weighed again, with the difference between wet and dry weight defined as the dry matter content of the seeds. The remaining seeds were placed in paper bags and kept at 20 ºC until the other evaluations.

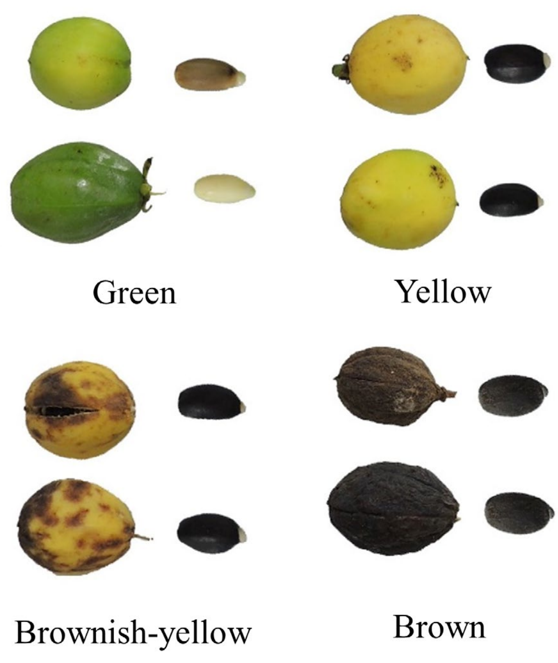

Figure 1. Visual aspect of *J. curcas* L. fruits (left) and seeds (right) at different maturity stages based on the fruit skin color.

X-ray images and automated analysis of radiographs

In this study, 200 seeds from each treatment were subjected to the analysis of their internal tissues by X-ray technique. For that, the seeds were fixed in groups of six seeds in an orderly and equidistant manner on adhesive paper. This procedure allowed the individual identification of each seed in the subsequent analyses. Thus, the images of these seeds were obtained by an X-ray equipment, model Faxitron MX-20 (Faxitron X-ray Corp. Wheeling, IL, USA), adjusted to a voltage of 23 kV, a radiation of 10 seconds, a focal length of 41.6 cm, at contrast 16383 (width) × 3124 (center). The radiograph images were saved in Tagged Image File Format (TIFF). The images were analyzed with the free software ImageJ® (https://imagej.nih.gov/ij/download.html), with the aid of the PhenoXray macro (Medeiros *et al.*, 2020c). The sequential analysis performed by the macro consisted of scale calibration (adopted 59,6003 pixels.mm^{-1}), opening of monochromatic images, segmentation and extraction of different tissue integrity descriptors of each seed contained in the image. The following parameters were assessed:

- Relative density (RelDens), gray pixel^{-1}: defined as the average of the gray values of all pixels in the selected area.
- Integrated density (IntDens), gray mm pixel^{-1}: defined as the sum of the pixel values in the selection.
- Seed Filling (SeedF), %: obtained by dividing the area with gray levels above the defined threshold by the total area of each seed.

Visual analysis of the radiographs

Radiographic images were also visually evaluated and each seed was individually labeled in three classes: 1) Seeds showing high physical quality (HPQ) – the internal structures of these seeds were well-developed and did not presented any damages; 2) Damaged seeds (DMS) – seeds presenting internal physical damage, mainly insect attack; and 3) Malformed seeds (MFS) – empty seeds and seeds showing impairment in embryo or endosperm development.

Physiological analysis

After obtaining the radiographic images, the seeds were tested for their physiological quality. Each seed was traced to individually associate the x-ray data with its physiological quality. For this purpose, four repetitions of 50 seeds, arranged in the same order of the radiographs, were submitted to the germination test. The seeds were distributed on germination paper towels (Germitest®), moistened with water equivalent to 2.7 times the dry paper mass, and kept in a germinator at 25 ºC for 12 days (Oliveira *et al.*, 2014). The number of seeds germinated (seeds showing radicle greater than 2 mm) and the number of normal seedling (seedling with health growth showing primary leaves, hypocotyl and radicle well-formed) was obtained daily. Using the daily data, the germination speed index (GSI) (Maguire, 1962) was calculated. The length of the hypocotyl and the radicle of each normal seedling were measured using image analysis. The seedlings were photographed, and the images were manually analyzed in the software ImageJ®. The germination data and seedling length data were processed using the package SeedCalc of the software R (Silva *et al.*, 2019). The parameters generated by the SeedCalc were mean seedling length (mm seedling^{-1}), growth index, uniformity index and vigor index. These indexes were calculated as described below:

— Growth index:

$$\text{Growth index} = [(\text{mean(h)} \times \text{wh}) + (\text{mean(r)} \times \text{wr})]$$

where mean(h) and mean(r) are the arithmetic means of shoot length and root length, respectively; wh and wr are adjustable weights in the formula for shoot and root, however, with reference values of 10 and 90, respectively (Sako *et al.*, 2001).

— Uniformity index:

$$\text{Uniformity Index} = \left[1 - \frac{\sum_{i=1}^{n}|Xi - \bar{X}|}{n \times X}\right] \times 1000 - \left[n_{dead} \times \left(\frac{50}{n_{total}}\right)\right]$$

where Xi is the length of the seedling analyzed, X is the mean length of seedlings of the seed lot analyzed, *n* is the variable of total number of seedlings evaluated, ndead is the number of ungerminated or dead seeds present, and ntotal is the total number of seedlings (Castan *et al.*, 2018).

— Vigor index:

$$\text{Vigor index} = (\text{Growth} \times \text{wg}) + (\text{Uniformity} \times \text{wu})$$

where Growth is the growth index, and Uniformity is the uniformity index chosen by the user; wg and wu are adjustable weights in the formula for growth and uniformity, however, with standard values of 70 and 30, respectively (Sako *et al.*, 2001).

After these analyses, the seedlings were dried in a forced air circulation oven at 65 ºC until weight stabilization, with subsequent weighing in precision balance to determine the dry matter (mg seedling^{-1}).

Experimental design and data analysis

The experiment was conducted in a completely randomized design, with four replications. Data were subjected to analysis of variance, after normal distribution of error and homogeneity of variances were verified by the Shapiro-Wilk and Bartlett tests, respectively. The averages of the treatments were compared by Tukey's test ($p \leq 0.05$). Pearson's correlation coefficients (r) were calculated with the data obtained in all evaluations for all treatments. In addition, the average values of radiographic image analysis data and physiological tests of each treatment were used to perform Principal Component Analysis (PCA). The software R 3.6.1 (R Core Team, 2018) was used for statistical analysis.

Results

When compared to seeds obtained from green fruits, it is possible to observe a significant increase in the dry matter of seeds obtained from yellow, brownish-yellow and brown fruits. Significant higher dry matter content was observed in seeds obtained from yellow-brown fruits, while there was a reduction in the dry matter of seeds from brown fruits (Fig. 2).

Based on the physical analyses of seed tissue integrity, performed by automatic analysis of digital radiographs, it was observed that seeds obtained from green fruits were significantly lower in relative density (Fig. 3A), integrated density (Fig. 3B) and seed filling (Fig. 3C). The seeds obtained from fruits with yellow, brownish-yellow and brown skin color did not differ from each other for these parameters (Fig. 3).

Considering the seeds showing HPQ (Fig. 3D), the lowest value was observed for seeds extracted from green fruits. Also, no significant differences in the number of damaged seeds were observed between yellow, brownish-yellow and brown treatments (Fig. 3E). As observed for the lower values of seed density and seed filling for the green fruits, visual analysis of radiography showed that the green fruits had a higher amount of malformed seeds (Fig. 3F).

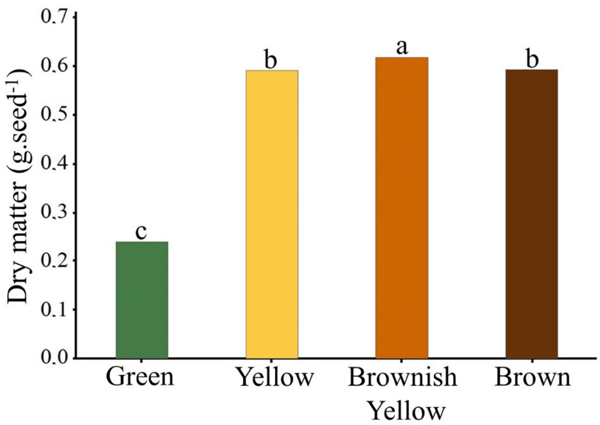

Figure 2. Dry matter content of *J. curcas* seeds extracted from fruits at different maturity stages. Means followed by the same letter do not differ from each other by the Tukey test (p <0.05).

Similarly to the physical analyses, the seeds obtained from the green fruits had lower physiological quality (Fig. 4). The seeds extracted from yellow, brownish-yellow and brown fruits did not differ from each other for germination (Fig. 4A), and all of the others seed quality parameters (Fig. 4).

The radiographic images of *J. curcas* seeds were used to associate each seed with their respective seedlings (Fig. 5). As was observed in the physical (Fig. 3) and physiological (Fig. 4) analyses, there was higher proportion of empty seeds in green fruits, which directly prevented the formation of normal seedlings, besides impairing germination (Fig. 5A). The seeds obtained from yellow (Fig. 5B) and brownish-yellow (Fig. 5C) fruits were well-formed, presented lower damage incidence, what resulted in normal and vigorous seedlings. Although well-formed, seeds extracted from brown fruits (Fig. 5D) presented greater damage intensity, mainly injured tissue caused by stink bugs (data not shown).

The variables obtained through the automated analysis of the radiographic images (relative density, integrated density and seed filling) correlated positively and significantly with all physiological variables (r > 0.90). Similar results were observed for the parameters obtained in the visual analysis of the radiographies (number of seeds showing high physical quality and damaged seeds) (r > 0.75), with exception for the number of malformed seeds, which in turn presented a significant negative correlation with all variables analyzed (r > -0,78) (Fig. 6).

In the PCA analysis, the sum of the first two components (PC1 and PC2) explained 99.1% of the total data variability (Fig. 7A). The green fruits treatment was located in the negative score of component 1 (PC1) (Fig. 7A), mainly due to the greater number of MFS (Fig. 7C) observed for this maturity stage. The yellow, brownish-yellow and brown fruits were located in the positive score of PC1 (Fig. 7A), which correlated positively with the vectors of physical (gray and blue colors) and physiological (beige color) attributes (Fig. 7B).

The brownish-yellow fruits located near the seed dry matter (SeeDM), automated-radiograph (gray color) and physiological (beige color) vectors. It is in accordance with what was observed for these parameters, *i.e.*, the seeds extracted from this stage of maturity showed greater dry matter content (Fig. 2). In general, the brown fruits treatment was located near the DMS vector (PC2+; blue color), as its seeds were generally well-formed or presenting damages (Fig. 4E and Fig. 5D).

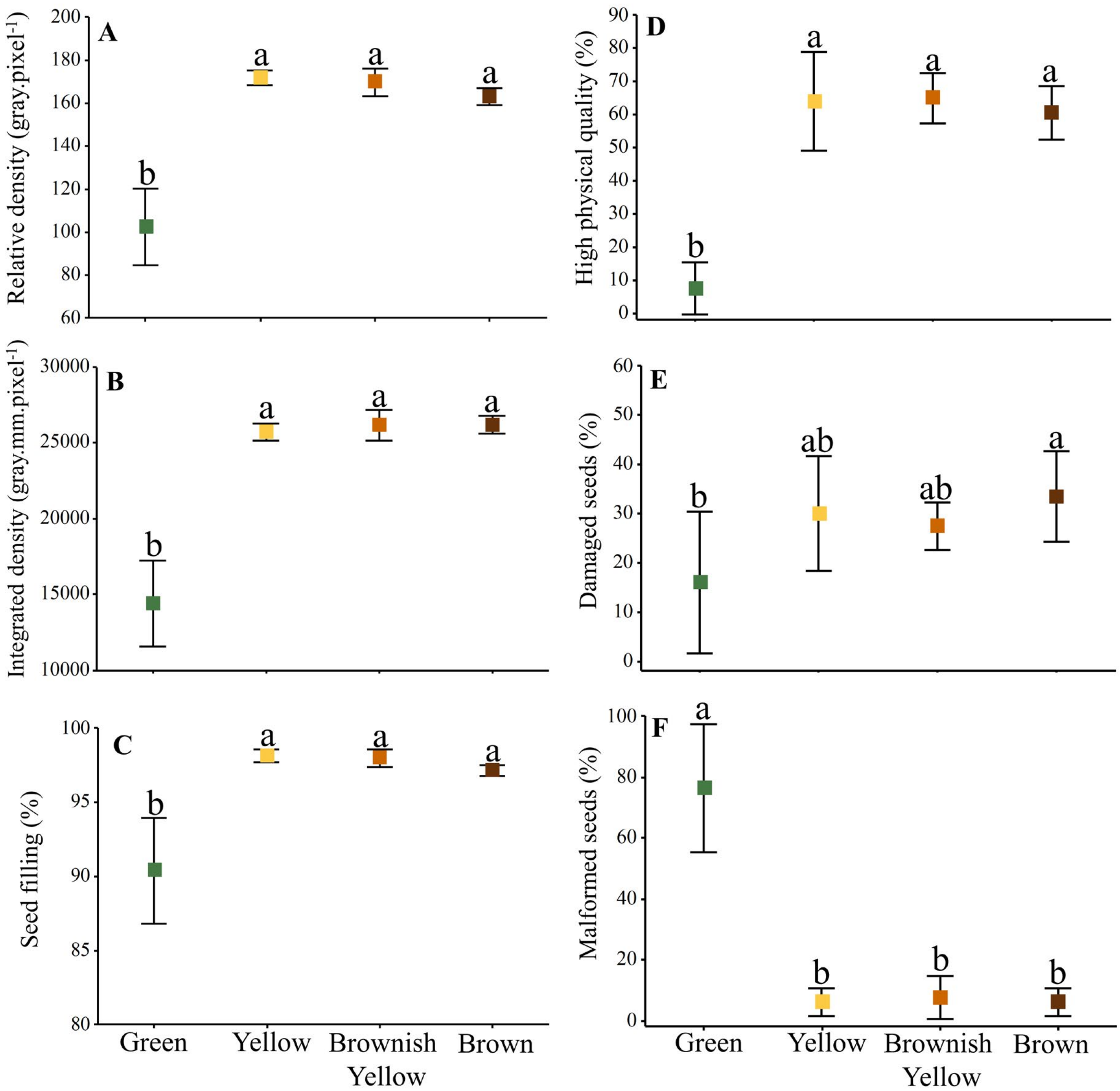

Figure 3. Physical characteristics of *J. curcas* seeds extracted from fruits at different maturity stages. The relative density (A), integrated density (B), and seed filling (C) were obtained by automated analysis of seed radiographs. The number of seeds showing high physical quality (D), damaged seeds (E) and malformed seeds (F) were obtained from the visual analysis of seed radiographs. Means followed by the same letter do not differ from each other by the Tukey test (p <0.05). Bars represent the confidence intervals.

Discussion

In the present study, the physical quality of *J. curcas* seeds, harvested at different stages of fruit maturity, was evaluated through X-ray image analysis, visually and automatically. Also, the parameters obtained with the x-ray images were correlated with the physiological quality of individual seeds.

During the seed maturation process, there is significant increase in dry matter of the seeds due to deposition of reserves (Bewley & Nonogaki, 2017). In the present study, the greater values of seed dry matter were observed for seeds extracted from yellow and brownish-yellow fruits (Fig. 2). Thus, it was possible to confirm that *J. curcas* seed physiological maturity occurs between yellow and brownish-yellow stages (Silva *et al.*, 2012). In addition, it can be stated that the seeds obtained from the green fruits did not complete the physiological maturation process. These seeds presented lower dry matter content, lower tissue density (Figs. 3A and 3B), lower seed filling (Fig. 3C) and higher proportion of malformed seeds (Fig. 3F).

The parameters Relative Density, Integrated Density and Seed Filling are associated with the gray values of the pixels in the images. As the X-rays can be transmitted, reflected or absorbed, the lower the tissue density, the higher the transmittance and the lower the absorption of these rays (Rahman & Cho, 2016). Thus, high gray values

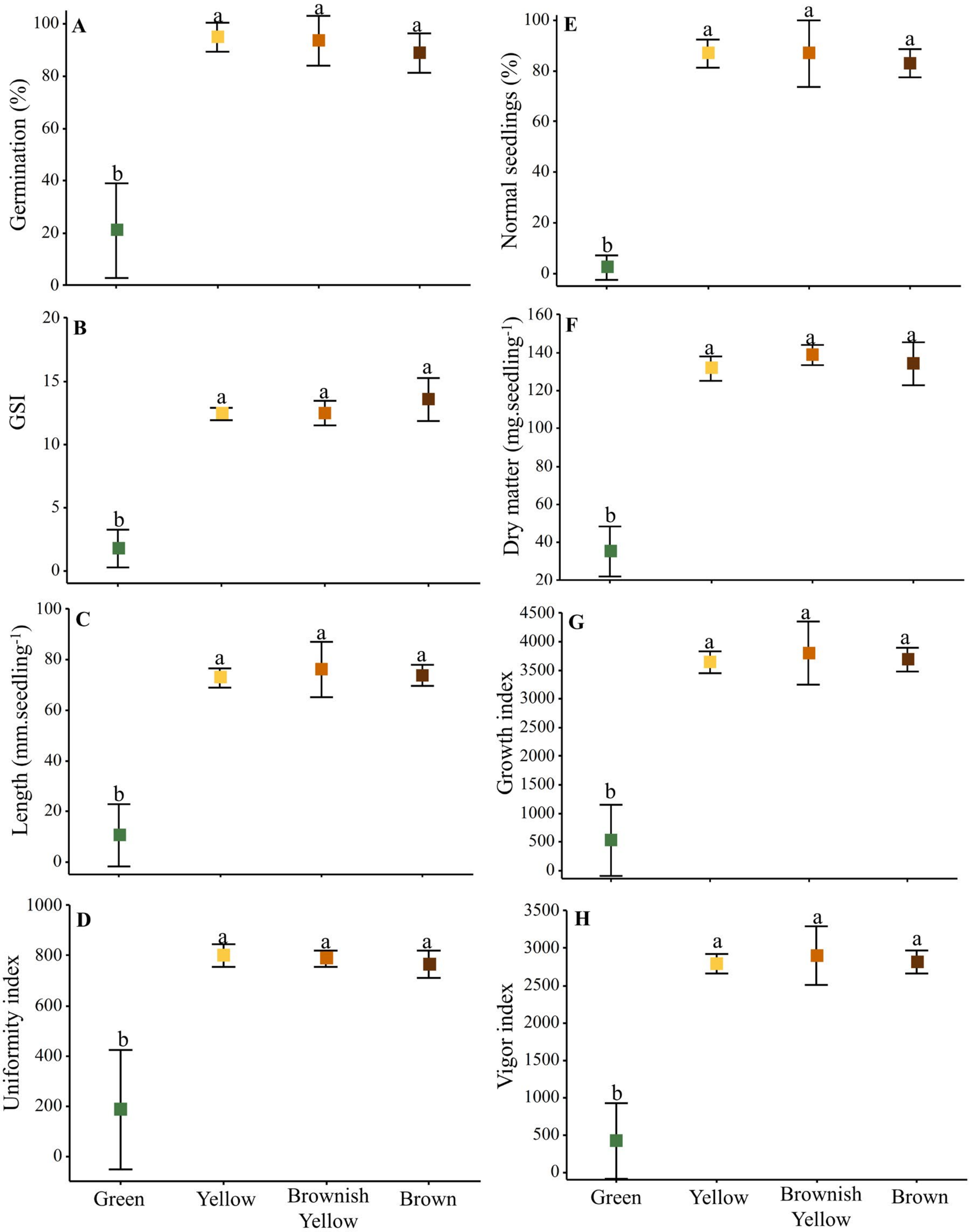

Figure 4. Physiological parameters assessed in seeds of *J. curcas* extracted from fruits at different maturity stages. Germination (A), GSI (B), seedling length (C), uniformity index (D), normal seedling (E), seedling dry matter (F), growth index (G) and vigor index (H). Averages followed by the same letter do not differ from each other by the Tukey test (p <0.05). Bars represent the confidence intervals.

mean high tissue densities and low gray values, low tissue densities. In the present work, these parameters were efficient to reflect differences in tissue density between seeds of the different maturity stages (Fig. 3). It has also been reported in other species such as *Ricinus communis* (Kobori *et al.*, 2012), *Brachiaria* sp. (Antony *et al.*, 2017; Jeromini *et al.*, 2019) and *Leucaena leucocephala* (Medeiros *et al.*, 2018), that these parameters can be used

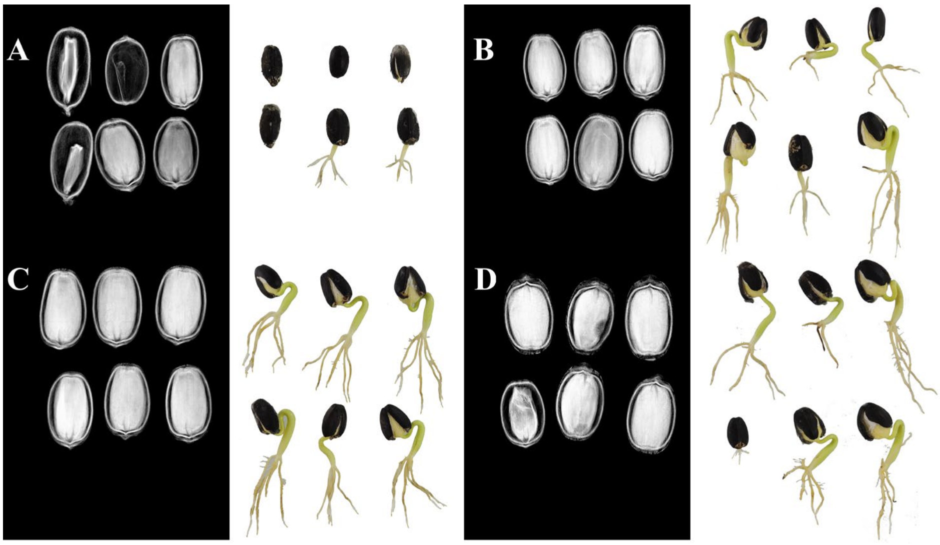

Figure 5. Radiographic images of *J. curcas* seeds with their respective seedlings or non-viable seeds. The seeds were extracted from green (A), yellow (B), brownish-yellow (C) and brown (D) fruits.

to evaluated the maturity degree and are related to the physiological quality of the seeds.

Other image analysis techniques, such as multispectral images, also provide important information for analyzing seed quality (El-Mesery *et al.*, 2019). In maize seeds, this technique was efficient for identifying physical damage, reaching an accuracy greater than 83% (Sendin *et al.*, 2018). However, the multispectral techniques, although providing information about the seeds in the visible and non-visible spectrum (ultraviolet and infrared), do not allow the visualization of the internal morphology of the seeds. In this sense, the X-ray technique is one of the few capable of accessing the visualization of internal morphology, allowing easy identification of physical damage and embryonic malformation (Arkhipov *et al.*, 2019).

The visual analysis of X-ray images has been used previously to assess the physical quality of *J. curcas* seeds (Medeiros *et al.*, 2020b). Although this analysis is very efficient, as was shown in the present work (Fig. 3), it is subjective and take long time to be performed, considering its application by seed companies that deal with many seed lots. As it can be observed for the correlation analysis (Fig. 6), the seeds showing high physical quality (positive) and the malformed seeds (negative) presented high correlation with all the automated-generated parameters. Thus, the automated analysis of x-ray imagens of *J. curcas* seeds can be recommended and is efficient and can replace the visual analysis.

J. curcas seeds obtained from green fruits are known to have lower physiological quality as well as lower oil content and dry mass when compared to later stages of maturity (Silva *et al.*, 2012). Also, the seeds extracted from yellow and from brownish-yellow fruits have greater physiological quality. However, this is the first work that relates the physiological potential of *J. curcas* seeds of different maturity stages with physical attributes, as seed filing, relative density and integrated density, assessed by X-ray test. Previous work have shown the efficiency of

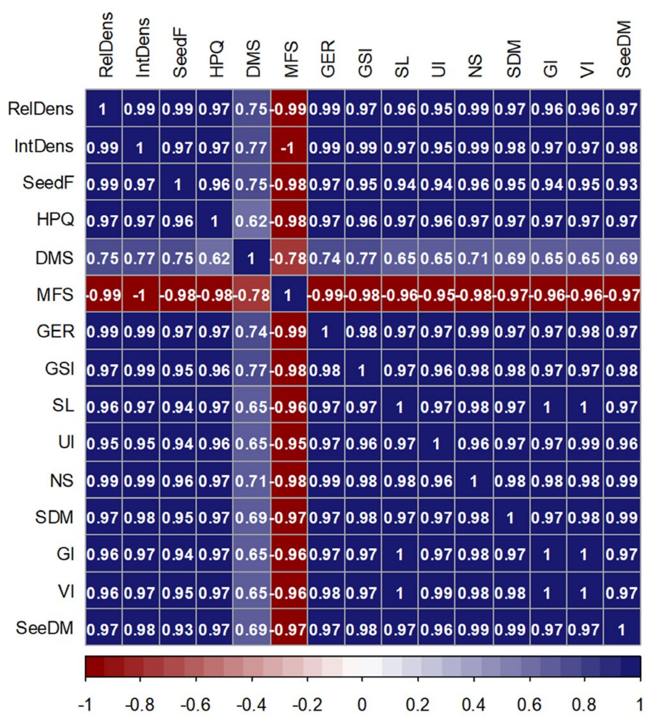

Figure 6. Pearson's correlation coefficients between variables obtained by radiographic image analysis and physiological quality of *J. curcas* seeds. RelDens = relative density; IntDens = integrated density; SeedF = seed filling; HPQ = seed showing high physical quality; DMS = damaged seeds; MFS = malformed seeds; GER = germination; GSI = germination speed index; SL = seedling length; UI = uniformity index; NS = normal seedlings; SDM = seedling dry matter; GI = growth index; VI = vigor index; SeeDM = seed dry matter.

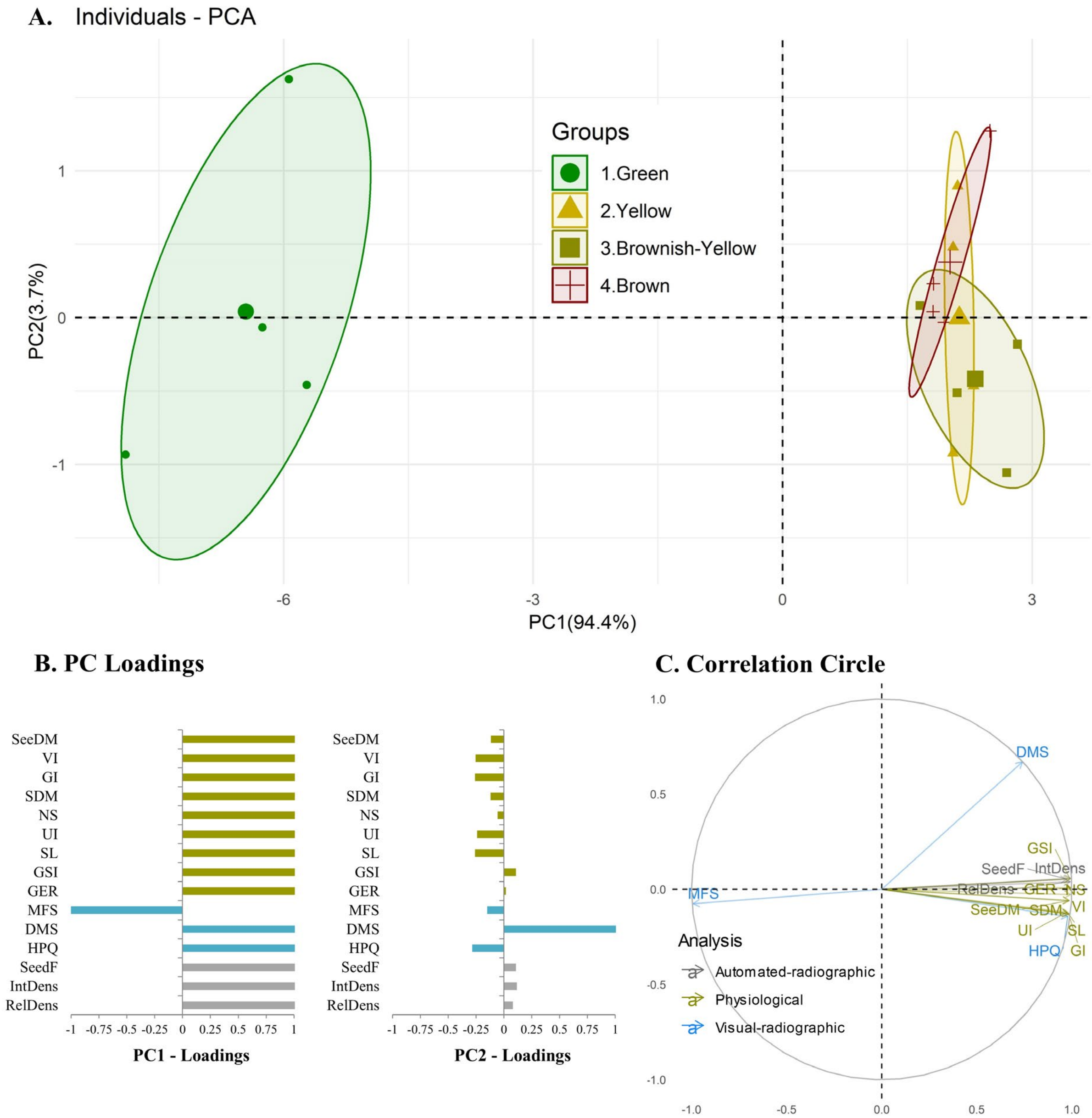

Figure 7. PCA analysis of the parameters obtained for *J. curcas* seeds extracted from fruits harvested at different maturity stages. Ordering diagram (A), PC loadings (B) and circle of correlations (C) of the first two components (PC1 and PC2). *Automated analysis* of radiographs: RelDens = Relative density; IntDens = Integrated Density; SeedF = Seed Filling. *Visual analysis of radiographs*: HPQ = seeds showing high physical quality; DMS = damaged seeds; MFS = Malformed seeds. *Physiological analysis*: GER = Germination; GSI = Germination speed index; SL = Seedling length; UI = Uniformity Index; NS = Normal seedlings; SDM = Seedling dry matter; GI = growth index; VI = vigor index; SeeDM = Seed dry matter.

x-ray analysis to predicting seed performance, as for tomato (Borges *et al.*, 2019) and pepper (Medeiros *et al.*, 2020a). These authors have also found a good relationship of the parameter seed filling with the seed physiological quality, which enables a good perspective to use this parameter to rapidly and efficiently predict the seed physiological quality using x-ray images.

Seed physiological maturity occurs when the seed reaches maximum dry matter (Bewley & Nonogaki, 2017). However, the maximum seed quality does not always correspond to this event. In this sense, although seeds of brownish-yellow fruits presented higher dry matter content (Fig. 2), they did not differ from seeds of yellow and brown fruits regarding germination and vigor (Fig. 4). Silva *et al.* (2012) observed that *J. curcas* seeds obtained from brown fruits presented similar germination to those obtained from yellow and brownish-yellow fruits, but with lower vigor in relation to these early stages of development. It is important to point out that these authors evaluated, besides seedling analysis, other vigor tests such as electrical conductivity, seedling emergence and accelerated aging. In addition, it is reported that *J. curcas* seeds obtained from brown fruits had lower storage potential when compared to those obtained from yellow and brownish-yellow fruits (Silva *et al.*, 2017, 2018). Thus, both the reduction in seed dry matter (Fig. 2) and the higher incidence of damage (Fig. 3E) observed in brown fruit seeds indicate that, although not showing significant reduction in seedling performance (Fig. 4), they show reduction in seed vigor.

The negative correlations observed between the number of MFS and the other variables reinforce the direct effect of embryonic malformation on the physiological quality of *J. curcas* seeds, especially for seeds obtained from green fruits. In the PCA analysis, the green fruits scores located near the MFS vector. As seeds extracted from green fruits did not complete the process of physiological maturation (Silva *et al.*, 2017, 2018), this greater number of malformed seeds is explained.

Otherwise, the seeds extracted from brown fruits exceeded the physiological maturity point (indicated by the reduction of seed dry matter). These fruits tended to have a higher amount of DMS (Fig. 3E; Fig. 7), which is related to the longer time of exposure to environmental conditions after maturation, such as relative humidity, temperature alternance, and insect attack. These conditions contribute to the intensification of seed deterioration, leading to a reduction in seed vigor (Rao *et al.*, 2017). Among these factors, high temperatures and relative humidity can be considered the main factors that contribute to the deterioration process. In this context, the seed deterioration is mainly related to the increase in the respiratory rates of seeds, contributing to the reactive oxygen species (ROS) production. In excess (above basal levels), the ROS contributes to the degradation of reserves and cellular compounds, such as lipids and proteins (Mhamdi & Van Breusegem, 2018).

In summary, the image analysis, both automated and visually, were efficient to characterize *J. curcas* seeds at different maturity stages. The parameters obtained showed high correlation with seed germination and vigor. In addition, this work presents a high throughput methodology for radiographic image analysis that can be widely used for phenotyping of seed physical attributes. In future works, these results may be used for the development of models based on artificial intelligence, aimed at predictive classification of *J. curcas* seeds and other species in relation to physiological quality based solely on the image analysis.

In conclusion, high throughput analysis of *J. curcas* seed radiographs using the macro PhenoXray developed for ImageJ is a suitable tool for monitoring the seed quality. *J. curcas* seeds obtained from green fruits have lower tissue density and lower physiological quality comparing to the other maturity stages. Seeds obtained from brown fruits have high physiological performance but showed higher tendency for presenting physical damages. The morpho-anatomical parameters of the seeds obtained by the x-ray image analysis are highly correlated to their physiological quality.

References

Antony E, Sridhar K, Kumar V, 2017. Effect of chemical sprays and management practices on Brachiaria ruziziensis seed production. F Crop Res 211: 19-26. https://doi.org/10.1016/j.fcr.2017.06.009

Arkhipov MV, Priyatkin NS, Gusakova LP, Potrakhov NN, Gryaznov AY, Bessonov VB, Obodovskii AV, Staroverov NE, 2019. X-ray computer methods for studying the structural integrity of seeds and their importance in modern seed science. Tech Physics 64: 582-592. https://doi.org/10.1134/S1063784219040030

Bewley JD, Nonogaki H, 2017. Seed maturation and germination. Ref Mod Life Sci 2017: 1-9. https://doi.org/10.1016/B978-0-12-809633-8.05092-5

Borges SRS, Silva PP, Araújo FS, Souza FF, Nascimento WM, 2019. Tomato seed image analysis during the maturation. J Seed Sci 41: 22-31. https://doi.org/10.1590/2317-1545v41n1191888

Brunes AP, Araújo AS, Dias LW, Villela FA, Aumonde TZ. 2016. Seedling length in wheat determined by image processing using mathematical tools. Rev Ciênc Agron 47: 374-379. https://doi.org/10.5935/1806-6690.20160044

Castan DOC, Gomes-Junior FG, Marcos-Filho J, 2018. Vigor-S, a new system for evaluating the physiological potential of maize seeds. Sci Agric 75: 167-172. https://doi.org/10.1590/1678-992x-2016-0401

Devic M, Roscoe T, 2016. Seed maturation: Simplification of control networks in plants. Plant Sci 252: 335-346. https://doi.org/10.1016/j.plantsci.2016.08.012

El-Mesery HS, Mao H, Abomohra AEF, 2019. Applications of non-destructive technologies for agricultural and food products quality inspection. Sensors 19: 846. https://doi.org/10.3390/s19040846

Gomes-Junior FG, Cicero SM, Vaz CMP, Lasso PRO, 2019. X-ray microtomography in comparison to radiographic analysis of mechanically damaged maize seeds and its effect on seed germination. Acta Scient Agron 41: e42608. https://doi.org/10.4025/actasciagron.v41i1.42608

Haq A, Siddiqi M, Batool SZ, Islam A, Khan A, Khan D, Khan S, Khan H, Shah AA, Hasan F *et al.*, 2019. Comprehensive investigation on the synergistic antibacterial activities of *Jatropha curcas* pressed cake and seed oil in combination with antibiotics. AMB Expr 9: 67. https://doi.org/10.1186/s13568-019-0793-6

Hu P, Wu L, Hollister EB, Wang AS, Somenahally AC, Hons FM, Gentry TJ, 2019. Fungal community structural and microbial functional pattern changes after soil amendments by oilseed meals of *Jatropha curcas* and *Camelina sativa*: A microcosm study. Front Microbiol 10: 537. https://doi.org/10.3389/fmicb.2019.00537

Jeromini TS, Martins CC, Pereira FECB, Gomes-Junior FG, 2019. The use of X-ray to evaluate *Brachiaria brizantha* seeds quality during seed processing. Rev Ciênc Agron 50: 439-446. https://doi.org/10.5935/1806-6690.20190052

Kamel DA, Farag HA, Amin NK, Zatout AA, Ali RM. 2018. Smart utilization of jatropha (*Jatropha curcas* Linnaeus) seeds for biodiesel production: Optimization and mechanism. Ind Crops Prod 111: 407-413. https://doi.org/10.1016/j.indcrop.2017.10.029

Kaushik N, 2003. Effect of capsule maturity on germination and seedling vigour in *Jatropha curcas*. Seed Sci Technol 31: 449-454. https://doi.org/10.15258/sst.2003.31.2.21

Kobori NN, Cicero SM, Medina PF, 2012. Teste de raios X na avaliação da qualidade de sementes de mamona. J Seed Sci 34: 125-133. https://doi.org/10.1590/S0101-31222012000100016

Leão-Araújo ÉF, Gomes-Junior FG, da Silva AR, Peixoto N, Souza ERB, 2019. Evaluation of the desiccation of campomanesia adamantium seed using radiographic analysis and the relation with physiological potential. Agron J 111: 592-600. https://doi.org/10.2134/agronj2018.05.0302

Leprince O, Pellizzaro A, Berriri S, Buitink J, 2016. Late seed maturation: drying without dying. J Exp Bot 68: 827-841. https://doi.org/10.1093/jxb/erw363

Maguire JD, 1962. Speed of germination—Aid in selection and evaluation for seedling emergence and vigor. Crop Sci 2: 176-177. https://doi.org/10.2135/cropsci1962.0011183X000200020033x

Mazumdar P, Singh P, Babu S, Siva R, Harikrishna JÁ, 2018. An update on biological advancement of *Jatropha curcas* L.: New insight and challenges. Renew Sustain Energy Rev 91: 903-917. https://doi.org/10.1016/j.rser.2018.04.082

Medeiros AD, de Araújo JO, Zavala-León MJ, Silva LJ, Dias DCFS, 2018. Parameters based on x-ray images to assess the physical and physiological quality of *Leucaena leucocephala* seeds. Ciênc Agrotec 42: 643-652. https://doi.org/10.1590/1413-70542018426023318

Medeiros AD, Zavala-León MJ, Silva LJ, Oliveira MAS, Dias DCFS, 2020a. Relationship between internal morphology and physiological quality of pepper seeds during fruit maturation and storage. Agron J 112: 25-35. https://doi.org/10.1002/agj2.20071

Medeiros AD, Pinheiro DT, Xavier WA, Silva LJ, Dias DCFS, 2020b. Quality classification of *Jatropha curcas* seeds using radiographic images and machine learning. Ind Crop Prod 146: 112162. https://doi.org/10.1016/j.indcrop.2020.112162

Medeiros AD, Silva LJ, Pereira MD, Oliveira, MAS, Dias, DCFS, 2020c. High-throughput phenotyping of brachiaria grass seeds using free access tool for analyzing X-ray images. An Acad Bras Ciênc 92 (Suppl.1): e20190209. https://doi.org/10.1590/0001-3765202020190209

Melchinger AE, Munder S, Mauch FJ, Mirdita V, Böhm J, Mueller J, 2017. High-throughput platform for automated sorting and selection of single seeds based on time-domain nuclear magnetic resonance (TD-NMR) measurement of oil content. Bio Eng 164: 213-220. https://doi.org/10.1016/j.biosystemseng.2017.10.011

Mhamdi A, Van Breusegem F, 2018. Reactive oxygen species in plant development. Development 145: dev164376. https://doi.org/10.1242/dev.164376

Montes JM, Melchinger AE, 2016. Domestication and breeding of *Jatropha curcas* L. Trends Plant Sci 21: 1045-1057. https://doi.org/10.1016/j.tplants.2016.08.008

Nielsen MS, Damkjær KB, Feidenhans'l R. 2017. Quantitative in-situ monitoring of germinating barley seeds using X-ray dark-field radiography. J Food Eng 198: 98-104. https://doi.org/10.1016/j.jfoodeng.2016.11.011

Oliveira GL, Dias DCFS, Hilst PC, Silva LJ, Dias LAS, 2014. Standard germination test in physic nut (*Jatropha curcas* L.) seeds. J Seed Sci 36: 336-343. https://doi.org/10.1590/2317-1545v36n31015

Pinto TLF, Marcos-Filho J, Forti VA, Carvalho C, Gomes-Junior FG, 2009. Avaliação da viabilidade de sementes de pinhão manso pelos testes de tetrazólio e de raios X. Rev Bras Sementes 31: 195-201. https://doi.org/10.1590/S0101-31222009000200023

Rahman A, Cho BK, 2016. Assessment of seed quality using non-destructive measurement techniques: A review. Seed Sci Res 26: 285-305. https://doi.org/10.1017/S0960258516000234

Rao NK, Dulloo ME, Engels JM, 2017. A review of factors that influence the production of quality seed for long-term conservation in genebanks. Genet Resour Crop Evol 64: 1061-1074. https://doi.org/10.1007/s10722-016-0425-9

R Core Team, 2018. R Development Core Team. R A Lang. Environ Stat Comput 55: 275-286. http://www.R-project.org.

Sako Y, McDonald MB, Fujimura K, Evans AF, Bennett MA, 2001. A system for automated seed vigour assessment. Seed Sci Technol 29: 625-636.

Sendin K, Manley M, Williams PJ, 2018. Classification of white maize defects with multispectral imaging. Food Chem 243: 311-318. https://doi.org/10.1016/j.foodchem.2017.09.133

Silva LJ, Dias DCFS, Milagres CC, Dias LAS, 2012. Relationship between fruit maturation stage and physiological quality of physic nut (*Jatropha curcas* L.) seeds. Ciênc Agrotec 36: 39-44. https://doi.org/10.1590/S1413-70542012000100005

Silva LJ, Dias DCFS, Oliveira GL, Silva-Júnior RA, 2017. The effect of fruit maturity on the physiological quality and conservation of *Jatropha curcas* seeds. Rev Ciênc Agron 48: 487-495. https://doi.org/10.5935/1806-6690.20170057

Silva LJ, Dias DCFS, Sekita MC, Finger FL, 2018. Lipid peroxidation and antioxidant enzymes of *Jatropha curcas* L. seeds stored at different maturity stages. Acta Sci Agron 40: 34978. https://doi.org/10.4025/actasciagron.v40i1.34978

Silva LJ, Medeiros AD, Oliveira MAS, 2019. SeedCalc, a new automated R software tool for germination and seedling length data processing. J Seed Sci 41: 250-257. https://doi.org/10.1590/2317-1545v42n2217267

Xia Y, Xu Y, Li J, Zhang C, Fan S, 2019. Recent advances in emerging techniques for non-destructive detection of seed viability: A review. Artif Int Agr 1: 35-47. https://doi.org/10.1016/j.aiia.2019.05.001