Original Research Article

Image Analysis in the Evaluation of the Physical and Physiological Quality of Jiló (*Solanum gilo*) Seeds During Development

ABSTRACT

Currently, the analysis of seed images has shown to be effective for the evaluation of the physical and physiological quality of seeds, besides being a non-destructive method. The objective of this study was to evaluate the free internal area of jiló (*Solanum gilo*) seeds by analyzing X - ray images and correlating the results with the germination of seeds at different maturation stages at 35, 40, 45 days after anthesis and fruits at 45 days after anthesis with 7 days at rest (45 DAAr). Seeds with free internal area ≤ 10% produced normal seedlings whereas seeds with an average free area above 10% produced abnormal or non-germinated seedlings. Seeds of fruits harvested at 45 DAA and seven days at rest showed a smaller internal free area and a formation of 100% of normal seedlings. The analysis of the radiographic images of jiló seeds allows the measurement of the free internal areas, as well as the determination of the relation between these and the germination.

Keywords: Germination; ImageJ®; Physiological maturity; Seed development; X-ray.

1. INTRODUCTION

Originating from India and introduced in Brazil by slaves, jiló (*Solanum gilo* Raddi) is a tropical heat-demanding vegetable. It belongs to the Solanácea family and its fruits are of light green or dark green color when immature, becoming orange reddish when ripe [1].

Jiló is a vegetable of great acceptance in the market, mainly in the southeast region, but, in general, it is still a species that require further studies, mainly in the area of seeds [2].

Several authors describe the importance of high quality of this seed as determinant in the production of the plant in the field. The degree of maturation of the seeds influences its quality, and the immature seeds have low vigor and germinative Power,[3,4,5]

In the present study, the analysis of seed and seedling images has been shown to be efficient for the evaluation of the physiological and physical components of seeds.

Seed analysis is considered a constantly evolving dynamic activity, characterized by continuous improvements in process development and standardization. For Guedes et al.[6], the standardization of these methods should be constantly re-evaluated through the application of reference tests, alternative tests and the determination of new methodologies.

Studies on seed analysis using radiographic images are a relatively recent alternative to classify the various aspects of seeds, such as their internal morphology, mechanical damage, insect damage, among others. In this sense, the capture and processing of the radiographed image has allowed the establishment of relations between integrity, morphology and determination of the physiological potential of the seeds [7].

Thus, one of the basic requirements for the identification of problems associated with the physiological potential of seeds is the investigation of their internal morphology. Studies aimed at evaluating the internal morphology of seeds have been performed using the image analysis technique. Among the methods used for this purpose, the X - ray test stands out [8].

In addition to all of these possibilities of use, the X - ray test has been successfully performed to relate the internal morphology of the seed with the germination or morphology of tomato seedlings [9], sweet pepper [10,11],Eggplant [5], melon, pumpkin and watermelon [8], *Acca sellowiana* [12], pumpkin [13].

However, in most cases, this classification is performed visually, and parameters that are more precise are needed to develop more consistent models of evaluation to define categories of extension of embryonic development or free space within the seeds [7].

Thus, the objective of this research was to relate the internal morphology of jiló seeds (*Solanum gilo*) at different stages of maturation with germination through radiographic images.

2. MATERIAL AND METHODS

2.1. Seed Collection Location

Research was carried out at the Center for the Development and Technology Transfer of UFLA at the Hortiagro Sementes Company, Municipality of Ijaci, MG, and in the Central Seed Laboratory of the Department of Agriculture of the Federal University of Lavras.

At the first stage of the study in a greenhouse, jiló seedlings were formed for the installation of the experiment and seed production. The seeds were sown on commercial substrate in 128-cell trays. The seedlings were transplanted into a greenhouse when they showed four definite leaves.

During the flowering phase, after emasculation and manual pollination to produce hybrids, the flowers were labeled daily on the day of anthesis until the attainment of the required number of fruits to guarantee enough seeds for all the analyzes of each treatment .

Six commercial genetic materials, JIL 001, JIL 006, JIL white and cultivar JIL - 005 (pollen donor) and two hybrids H1 and H2, from the crosses between JIL - 005 (♂) X JIL - 001 (♀) and JIL-005 (♂) X JIL-006 (♀) were used. The crosses were conducted manually, after the emasculation of the female parentals.

Fruit harvesting was performed at 35, 40, 45 days after anthesis (DAA) of the first flowering. Half of the number of fruits harvested at 45 DAA was put to rest for seven days (45 DAAr) in a cool and ventilated place, totaling 24 treatments (six genetic materials and four stages of fruit development).

The experiment was installed in a randomized block design (RBD) with 24 treatments and three replications, where each plot was composed of 1 row of 6 meters long with 10 plants, spaced 1 meter between rows and 0.60 m between plants.

2.2. Laboratory Experiments

After manual extraction, the seeds were dried in an air circulation oven at 35 ° C until approximately 8% water content, where the image and physiological analysis tests were performed. For the radiographic analysis, four replicates of 50 seeds of each treatment were used, placed on a transparent adhesive tape (double face) and fixed in transparent plastic slide; the seeds of each treatment were numbered according to the position occupied in the slide, so that they could be identified in subsequent determinations.

The plastic slide was placed inside the X-ray digital equipment, Faxitron® brand, model MX-20 DC-12, and subject to radiation for 12 seconds at 26 kV. Then, the generated images were saved to the computer's hard disk for analysis. The seeds were removed from the transparent slide and transferred to acrylic boxes, gerbox type, being arranged in the same order as they were in the X - ray images. After this procedure, they were conducted to the germination test.

The germination test was carried out on two sheets of blotting paper moistened with distilled water in the proportion equivalent to 2.5 times the mass of the paper and the seeds distributed in gerbox in the same positions where they were in the radiographic images and kept in germinating chambers BOD type under alternating regime of temperature and light, being 20 ° C / 16 hours in the dark and 30 ° C / 8 hours in the presence of light. Counting was done 14 days after sowing [14].

Normal (PN), abnormal (PA) and dead seed (SM) seedlings were photographed using a Canon SX50® digital still camera. Images were saved to the computer's hard drive for further analysis.

2.3. Measurement of the free internal area of jiló seeds

The images of the radiographed seeds were saved in the JPEG format and analyzed with the ImageJ® software [15], adapting the analysis methodology previously used in other studies to measure leaf area and internal area of seeds, Silva et al.[12], according to example shown in Fig. 1.

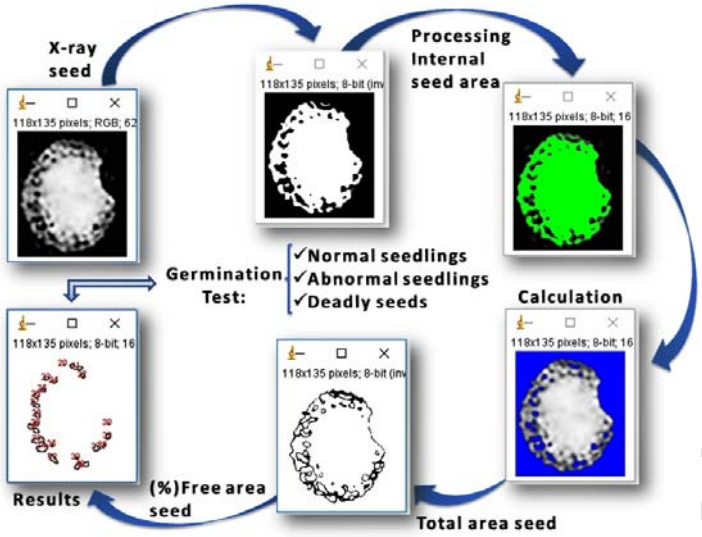


Fig. 1. General representation of jiló seed image analysis, from radiographic image to the attainment of free internal area and germination tests.

The steps of the analysis in the ImageJ® program were taken as follows: image opening, and its conversion to grayscale type in 8 bits. Then, the area of interest for image analysis and calibration was selected. In this study, the value in squared millimeters (mm^2) was considered for each image as a reference, which was 231 X 210 mm. For the choice of parameters to be measured, perimeter, standard deviation and fraction area were chosen as object area, which represents the free internal areas of the seeds. The color adjustment was performed to separate the areas of interest from the other constituents of the image. And finally, parameters were measured and results obtained. All procedures were performed manually for each seed.

The free area of the internal seed cavity in each treatment (n = 200) was calculated by means of the ImageJ® software in four replicates of 50 seeds. After the attainment of radiographic images, 3D images and their histograms of jiló seeds harvested at the different stages of maturation were processed with ImageJ® software.

2.4. Statistical analysis

For the analysis of variance in the germination test and in the image analysis of the seeds, the statistical program Sisvar was used [16]. For the comparison between the means, the Scott-Knott [17] test was employed, at 5% probability.

3. RESULTS AND DISCUSSION

3.1. Radiographic images and free internal area of seeds

Through the analysis of the radiographic images, it was possible to see the internal area of the jiló (*Solanum gilo*) seeds and to identify different percentages of free internal areas in different stages of maturation. The free areas inside the seeds were visualized in all genetic materials.

The overall mean of the free internal areas of all treatments, with 25, 18, 6 and 4%, for seeds harvested at 35, 40, 45 DAA and 45DAAr (seeds harvested at 45 DAA and left at rest for seven days), respectively, are shown in Fig. 2.

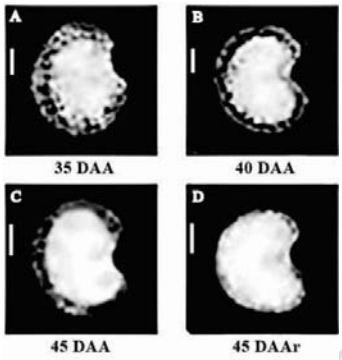


Fig. 2. **Sequence of radiographic images of jiló seeds during the maturation process, A (35 DAA), B (40 DAA), C (45 DAA), and D (45 DAAr). Bars are 5 mm.**

The shade found in radiographic imaging is defined according to level of radiation absorption in different regions of the seed, which is determined by the thickness, density and composition of the seed tissues [18,19]. Thus, seeds that do not have embryonic tissue, caused by the absence of resistance to the passage of X - rays, provide dark images.

After the attainment of the radiographic images, 3D images and their histograms on jiló seeds harvested at different maturation stages were visualized with the aid of ImageJ® software (Fig. 3).

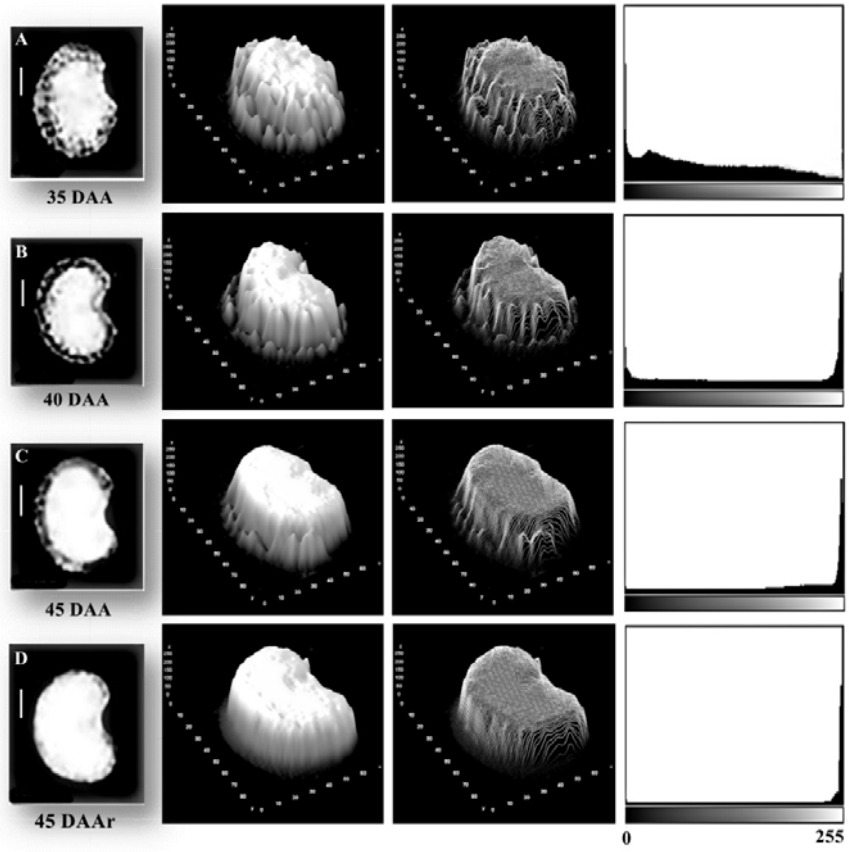


Fig. 3. Radiographic images of jiló seeds at different maturation stages (35, 40, 45 DAA and 45 DAAr), and their respective 3D images and their histograms in pixels (0 to 255). Bars are 5 mm.

According to (Fig. 3), in the initial stages of seed maturation (35 and 40 DAA), the radiographic images (A and B) present larger dark areas, with a larger number of cavities in the 3D images, and the peaks in histograms occur on the left side, with darker shades (larger free internal areas).

In seeds harvested at 45 DAA and 45 DAAr, the radiographic images (C and D) are clearer and have smaller free internal areas. From the 3D images in these stages, the number of cavities in the seeds (seed filling) decreased, and peaks in histograms moved to the right (seeds with smaller free areas).

Additional research showed the efficiency of the X - ray image analysis for the evaluation of the internal area of canafistula seeds [20], sweet pepper [10], eggplant [5], melon, pumpkin, and watermelon [8] and pumpkin [13].

The analysis of the radiographic images with the help of the ImageJ® software enabled the measurement of the free internal areas of the seeds and, thus, allowing the evaluation of the relation between the free internal area of the seeds and the germination.

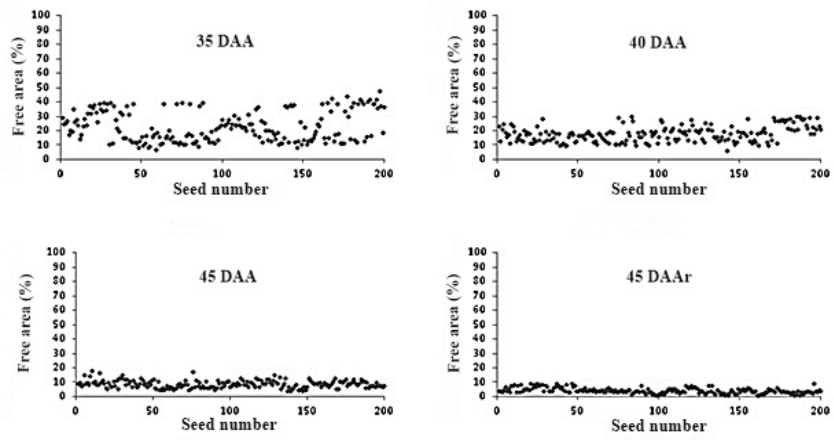
This result is important because the software reported in the literature for this type of analysis are Tomato Analyzer® [7] and Image ProPlus® [11,5], whereas in the modern literature review, no image

163 analysis study was performed on jiló (S*olanum gilo*) seeds using ImageJ® software, but in *Acca*
164 *sellowiana* seeds [12].

165 In light of the foregoing, the software can be an alternative for research on different seed species in
166 this area of study, since methods that use capture and image processing at high-speed are the most
167 advanced techniques that can provide a high level of efficiency for analysis of seed quality [21].

168 Based on the results obtained in the image analysis with the ImageJ® software, there was a
169 progressive decrease of the free internal area of seeds in all genetic material with the process of seed
170 maturation.

171 General average distribution of the free area within each seed (n = 200) of the genetic material JIL
172 white, JIL 001, JIL 005, JIL 006, H1 and H2 are shown in Fig. 4.



173

174 **Fig. 4. Average free internal area (%) of each jiló seed (*Solanum gilo*) in a sample of 200 seeds,**
175 **representing all the genetic material at 35, 40, 45 DAA and 45 DAAr.**
176

177 According to Carvalho and Nakagawa [25], the more distant from the anthesis the more fruits are
178 harvested, the heavier the seeds will be. Miranda et al. [22], studying the maturation of eggplant fruits,
179 concluded that there was no difference in the dry weight gain between seeds harvested at 50 DAA in
180 relation to seeds harvested at 60 DAA. Oliveira et al. [23], working with sweet pepper seeds,
181 concluded that at 55 DAA, the seeds had reached physiological maturity, and this was the point where
182 the seeds reached a higher dry mass, coinciding with the best harvesting point.

183 Thus, with the advancement at the stage of maturation of jiló seeds, there was an increase of dry
184 mass and consequent decrease of free internal area. The evaluation of the internal seed morphology is
185 essential both for the characterization of poorly studied species and for the improvement of the quality
186 of seed lots, with regards to their physical and physiological attributes, since information on the
187 existence of defective and empty seeds is desirable because they may influence germination results
188 [24].
189
190

3.2. Germination test

It was possible to relate the results of the germination test with the percentage of free internal area of the seeds, normal and abnormal seedlings and dead seeds. In seeds of all genetic material harvested at 35 DAA, germination did not occur with 100% of dead seeds and with a mean free area within the seed of 25%.

At 40 DAA in seeds of the genetic material JIL white, JIL 001, JIL 005 and JIL 006, there was a mean percentage of free area inside the seed of 18%, and average germination percentage of 10%, without the formation of normal seedlings, with an average of 21% of abnormal seedlings and 75% of dead seeds. Whereas for the hybrids H1 and H2, at 40 DAA, germination of 35 and 25% occurred, respectively, however, with a low percentage of normal (15 and 5%) and abnormal (10 and 20%) seedlings.

At 45 days after anthesis (45 DAA), there was seed germination percentage of white JIL, JIL 001, JIL 005 and JIL 006, H1 and H2 of 80, 86, 55, 84, 98 and 96%, respectively with the same percentage of normal seedlings, no formation of abnormal seedlings and 20, 14, 45, 16, 2 and 4% of dead seeds. Whereas seeds originating from fruits harvested at 45 DAAr showed a mean percentage of free internal area of 4%, and 100% of germination of normal seedlings, except for the JIL white cultivar, with 95% germination.

Seeds from fruits harvested at 45 DAAR of cultivars JIL white, JIL 001, JIL 005 and JIL 006 were statistically superior to seeds harvested at (35, 40 and 45 DAA). In these seeds, higher percentages of germination and the lowest percentages of free internal area were found, except in seeds of hybrids H1 and H2, in which there were no statistical differences of the percentage of germination at 45 DAA and 45 DAAr.

There was a direct relationship between the germination and the free area inside the seeds, since there was a greater germination in seeds with a smaller free internal area. The lowest values of free internal area and higher germination were found in seeds of hybrids H1 and H2 from the 45 DAA stage (Table 1).

Table 1. Free internal area in the jiló seed (FA (%)), germination (G), normal seedlings percentage (NS), abnormal seedlings (AS), and dead seeds (DS).

JIL White					
Stage	FA (%)	G (%)	NS (%)	AS (%)	DS (%)
35 DAA	32 d	0 c	0	0	100
40 DAA	24 c	0 c	0	25	75
45 DAA	10 b	80 b	80	0	20
45 DAAr	5 a	95 a	95	0	5
CV[1]	8.1	9.4			
JIL 001					
Stage	FA (%)	G (%)	NS (%)	AS (%)	DS (%)
35 DAA	22 d	0 d	0	0	100
40 DAA	17 c	10 c	0	20	80
45 DAA	10 b	86 b	86	0	14
45 DAAr	5 a	100 a	100	0	0
CV[1]	6.7	5.7			
JIL 005					
Stage	FA (%)	G (%)	NS (%)	AS (%)	DS (%)
35 DAA	26 d	0 c	0	0	100
40 DAA	21 c	0 c	0	25	75
45 DAA	10 b	55 b	55	0	45
45 DAAr	5 a	100 a	100	0	0
CV[1]	13.3	12.7			
JIL 006					
Stage	FA (%)	G (%)	NS (%)	AS (%)	DS (%)
35 DAA	25 d	0 d	0	0	100
40 DAA	18 c	9 c	0	17	83
45 DAA	9 b	84 b	84	0	16
45 DAAr	4 a	100 a	100	0	0
CV[1]	7.5	5.3			
H1					
Stage	FA (%)	G (%)	NS (%)	AS (%)	DS (%)
35 DAA	22 c	0 c	0	0	100
40 DAA	13 b	35 b	15	10	75
45 DAA	4 a	98 a	98	0	2
45 DAAr	3 a	100 a	100	0	0
CV[1]	14.7	4.6			
H2					
Stage	FA (%)	G (%)	NS (%)	AS (%)	DS (%)
35 DAA	23 d	0 d	0	0	100
40 DAA	15 c	25 c	5	20	75
45 DAA	5 b	96 a	96	0	4
45 DAAr	3a	100 a	100	0	0
CV[1]	6.6	7.2			

Means followed by the same lower case letters in the columns do not differ by the Scott-Knott test at 5% probability. [1]Coefficient of variation.

Similar results related to the reduction of free area and the increase in germination originating from fruits harvested at different maturation stages in sweet pepper species were found by Oliveira et al. 23, Carvalho and Nakagawa [25], Nakada et al. [26], and Santos et al. [27].

In general, for all genetic materials studied (JIL white, JIL 001, JIL 005, JIL 006, H1 and H2), fruits harvested at 35 DAA with mean free area ≥ 22% did not germinate and 100 % of the presented dead seeds.

Seeds from fruits harvested at 40 DAA with an average free area of ≥ 15% produced only abnormal seedlings for JIL white, JIL 001, JIL 005, JIL 006 and with a mean percentage of 80% of dead seeds. Where as for the seeds of hybrids H1 and H2 germination at 40 DAA, however very low and with the presence of abnormal seedlings.

Seeds from fruits harvested at 45 DAA, with an average free area of ≤ 10% produced on average 84% of normal seedlings, and there was no formation of abnormal seedlings. Where as seeds of fruits harvested at 45DAA and resting for seven days showed 100% normal seedlings and an average free area of 4%.

It stands out that the presence of abnormal seedlings for genetic materials, JIL white, JIL 001, JIL 005, JIL 006, at 35 and 40 DAA, may be directly related to seed immaturity and the high percentage of free area within these. This can be found in the evaluation of X - ray images, together with the ImageJ® program, and also associated to the results of physiological quality.

In a similar study, in sweet pepper seeds, when the free space between the embryo and the endosperm was superior to 2.7%, that is, seeds with endosperm area and embryo inferior to 97.3%, there was a reduction of germination with the increase in the percentage of abnormal or non-germinated seedlings [11].

In several researches, it has been reported that partially formed seeds were not able to provide normal seedlings in the germination test, such as *Cecropia pachystachya* Trec. [28], Peruvian pepper [29], sweet pepper [11], eggplant [5], watermellon [8], pumpkin [13].

However, for many species of fleshy fruits, fruit harvesting followed by a resting period for 7 or 10 days positively interferes with seed quality [30,31].

The analysis of radiographic images of jiló seeds (*Solanum gilo*), with the aid of ImageJ® software, allowed the measurement of free internal areas at different stages of maturation, as well as the determination of the relationship between those and germination. Seeds of fruits harvested at 45 DAA and seven days at rest showed a smaller free internal area and a formation of 100% of normal seedlings.

4. CONCLUSIONS

The analysis of radiographic images of jiló seeds (Solanum gilo), with the aid of ImageJ® software, allow the measurement of free internal areas at different stages of maturation, as well as the determination of the relationship between those and germination. The seeds of fruits harvested at 45 days after anthesis and seven days at rest (45DAAr) present a smaller free internal area and a formation of 100% of normal seedlings, being this the ideal harvesting point.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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