Original research papers

Monitoring the maturation of crambe seeds using X-ray image analysis

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ABSTRACT

The application of modern and efficient techniques to access seed physical and physiological information has contributed to new advances in the agricultural sector. The objective of this study was to evaluate morphometric parameters obtained from the X-Ray analysis using crambe seeds harvested at different maturation stages and to relate them to seed physiological potential. Seeds harvested at different maturation stages were evaluated using X-Ray test, germination, first germination count, germination speed index, root protrusion velocity and accelerated aging. The Principal Components Analysis was applied to data collected. The results demonstrated that the X-Ray analysis allowed to visualize internal morphology of crambe seeds. Differences between seeds harvested at different maturation levels were observed. Correlations between physical variables were verified, such as seed area with germination (r = 0.96). The use of X-Ray analysis combined with semi-automated analysis is efficient to differentiate crambe seeds in different maturation stages. The morphometric parameters are related to the physiological quality of crambe seeds.

Keywords: Crambe abyssinica Hochst; seed technology; image analysis; physiological quality.

1. INTRODUCTION

Improvement of image analysis algorithms combined with optical sensing technologies have a great potential as efficient and promising methods in the field assessment for seed quality [1].

Electromagnetic radiation based on image analysis in non-visible spectral bands, such as X-ray, has been featured for several applications in the agricultural field [2]. The principle of X-ray imaging technique is based on selective passage of radiation through the object, which reveals its internal density variations, so image data are acquired and analyzed to obtain internal structural information of the studied object [3].

Recent studies have demonstrated the efficiency of X-ray technique for application in various studies with seeds [4–7]. However, the radiographs analysis may be compromised when radiographic images are evaluated by human visual, since the process is slow and can generate interpretation errors [6]. Thus, the development of methodologies based on computer vision that make less subjective and more accurate analysis would contribute significantly to the seed area.

Crambe (*Crambe abyssinica* Hochst) is a rustic plant, adaptable to diverse soil and climatic conditions. Due to its high oil content in the seeds, crambe stands out as an important raw material in biodiesel production, chemical products and insulating fluids for electricity industry [8]. This crop is sexually propagated, therefore, the production of good quality seeds is essential to supply the market. Because of its ununiform fruiting, an important aspect that must be taken into

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considered during seed production is to determinate the appropriate seed spot or interval. Thus, efficient monitoring of morphometric variables, such as level of seed filling and integrity of their internal tissues could help in decision making at the correct harvest time.

Therefore, the objective of this study was to evaluate morphometric parameters obtained from the X-ray analysis using crambe seeds harvested at different maturation stages and to relate them to seed physiological potential.

2. MATERIAL AND METHODS

2.1 Location and characterization of seed

The seeds were produced in the experimental field of “Universidade Federal de Viçosa”, located in Minas Gerais, Brazil (20 ° 45'30.7 "S and 42 ° 52'15.8" W, altitude of 684 m), in 2018. The region climate is Cwb type, mesothermic humid with rainy summers and dry winters, according to Köppen classification. The soil in which the crop was cultivated was classified as Yellow Red Argisol type. Seeds were harvested at different maturation stages based on the external pericarp color as shown in Fig. 1.

Fig. 1. Criteria used to categorize seeds based on pericarp color.

The study analyses were conducted at the Seed Analysis and X-Ray Analysis Laboratories, both belonging to the Universidade Federal de Viçosa.

2.2 Physical and physiological quality assessment

The seeds of each maturation stage were submitted to the following physical and physiological analysis according to the tests described below:

2.2.1 Physical analysis

X-Ray test: to analyze the internal seed morphology, four replicates of 50 seeds for each maturation stage were used. The seeds were fixed orderly on adhesive paper and then placed inside the Faxitron digital X-ray equipment MX-20 (Faxitron X-ray Corp. Wheeling, IL, U.S.A). To generate the radiographic images, the equipment was configured to a exposure time of 10 seconds radiation, a 23 kV voltage, a focal length of 41.6 cm and a calibrated image contrast of 13917 (width) x 5374 (center). The digital images were generated and saved in a TIFF format and then analyzed as semi-automated form using ImageJ® software according to methodology proposed by Medeiros et al. [6]. The area variables (mm^2), perimeter (mm), roundness, solidity, relative density (gray $pixel^{-1}$) and integrated density (gray mm^2 $pixel^{-1}$) were calculated for seed and embryo, as well as seed filling percentage.

2.2.2 Physiological analysis

Germination test: performed after obtaining the X-ray images, using same seeds and arranged in a same way that they were X-rayed. For the test, Gerbox® transparent plastic boxes (11 x 11 x 3 cm) were used, with two germination papers on its bottom, which were wetted with distilled water, using the proportion of 2.5 times dry paper. Then, they were placed at temperatures of 25 ° C in a germination chamber. The evaluations were made according to the Rules for Seed Analysis [9] and the results recorded in germination percentage (normal seedlings).

First germination count: performed along with germination test, counting the seedlings normal number on the seventh day after assembling the test [9].

Germination speed index and Radicle protrusion speed: performed along with the germination test through daily counting of the normal seedlings and radicle protrusion, respectively, after sowing. The indexes were calculated using equation proposed by Maguire [10].

Accelerated aging test: seeds of each treatment were arranged in a single layer on a stainless steel screen inside plastic boxes (11 x 11 x 3.5 cm), containing 40 ml of distilled water. These boxes were then held at 41 ° C for 48 hours. Then, a germination test was performed, as previously described. Evaluations were made five days after sowing and the results were expressed as normal seedlings percentage [11].

2.3 Experimental Design and Statistical Analysis

The experiment was conducted in a completely randomized design with four replicates. The data were submitted to analysis of variance, after verification of the normality assumptions and variances homogeneity, by the tests, Shapiro-Wilk and Bartlett, respectively. Lots means were compared by Tukey's test ($p < 0.05$) and submitted to Pearson's correlation analysis. The correlation significance was evaluated by the t test ($p < 0.05$). Data analysis was performed using statistical software R 3.5.2 [12]

3. RESULTS AND DISCUSSION

The X-ray equipment configurations used in this study were suitable for generating a good quality radiographic images. Seed exposure to 23kV radiation for 10 s allowed visualization of internal crambe seeds morphology and pericarp, empty cavities and embryo identification (Fig. 2).

Fig. 2. Radiographic images of crambe seeds

In a study carried out with *Acca sellowiana* [13], pepper [14], sesame seeds [15] and cashew achenes [16], it was also noticed that using a voltage lower than 25 kV is sufficient to generate radiographic images suitable for parameters analysis related to the internal seeds morphology.

Through a simple visual analysis of the analyst it was possible to identify poorly filled seeds or embryos with a low tissue density. However, semi-automated X-Ray analysis using ImageJ® software was performed, aiming to make this analysis process less subjective, fast and able to quantify non-perceptible variations with human eye, such as gray density. The semi-automated analysis variables are presented in Table 1.

Table 1. Morphometric and tissue density variables obtained from radiographs analysis of crambe seeds

Treatment	Area mm^2	Perimeter mm	Circularity	Solidity	Relative density gray $pixel^{-1}$	Integrated density gray $pixel^{-1}$	Fillness %
				Seed			
Green	6.44 c	9.64 b	0.89 a	0.988 a	87.39 ab	571 b	45 a
Green-yellow	7.77 ab	10.82 a	0.84 abc	0.982 b	90.14 a	708 a	44 ab
Yellow	7.91 a	10.96 a	0.83 bc	0.982 b	83.54 ab	672 a	42 b
Golden	8.10 a	11.01 a	0.84 ab	0.983 b	81.93 b	671 a	39 c
Brown	7.15 b	10.77 a	0.79 c	0.978 c	67.64 c	495 b	35 d
Fc	19.14*	10.89*	9.06*	23.65*	27.58*	16.14*	67.64*
CV	4.16	3.23	2.81	0.15	4.04	7.03	2.38
				Embryo			
Green	2.92 bc	8.14 ab	0.56 b	0.941 bc	148.86 ab	439 b	-
Green-yellow	3.46 a	8.22 a	0.65 a	0.956 a	151.36 a	527 a	-
Yellow	3.41 a	8.26 a	0.64 ab	0.953 ab	146.5 ab	504 ab	-
Golden	3.25 ab	8.45 a	0.59 ab	0.943 b	149.49 ab	488 ab	-
Brown	2.56 c	7.57 b	0.56 b	0.929 c	140.12 b	361 c	-
Fc	17.77*	5.27*	4.84*	12.9*	3.78*	17.44*	-
CV	5.75	3.58	6.31	0.63	3.04	6.78	-

* Significant by F test ($p < 0.05$). Means followed by the same letter in the column did not differ by Tukey's test ($p < 0.05$). Fc = F calculated. CV = coefficient of variation

It was observed that the morphometric parameters evaluated in the whole seed indicated that green seeds had a smaller area and perimeter, with a greater circularity (not different from Green-yellow and Golden seeds) and solidity (indicative of irregular borders). The embryo size ranged from 2.92 to 3.41 mm^2 on average, showing statistical differences between treatments. The others variables describing whole seed and embryo size and shape also detected significant variations between treatments.

These variables related to morphometric characters have a great importance for breeding programs and may correlate with physiological attributes. In studies with broccoli, Abud et al. [4] observed that seeds area and circularity correlated significantly with seedlings length, indicating a possibility of using these variables to infer data on physiological quality.

For relative density parameter, it was observed that seeds harvested in brown stage had low gray levels, indicating less dense or more damaged tissues. This may be explained possibly because these seeds were exposed more time in the field, so that deterioration tended to be greater. The gray level in the embryo differed only Green-yellow from Brown seeds.

The whole seeds integrated density, obtained from the product between relative density and seed area, indicated that Green and Brown seeds showed no differences among them, but presented the lowest values in relation to the others treatments. However, when considering only the embryo, Brown seeds differed from the others lots, being characterized by lower value for this characteristic.

On the other hand, the seeds fillness indicated that Green seeds had a larger area occupied by the embryo, not differing from Green-yellow seeds. In general seed filliness decreased with the maturation advancement. This may be related to the higher water content contained in seeds with Green pericarp and less pericarp lignification. As the pericarp tends to become more rigid (increased lignin content) and the embryo loses water, thereby increasing empty cavity within the seed.

Studies carried out with Jiló (*Solanum gilo*) and papaya (*Carica papaya*) seeds, showed that the radiographic images analyzed also allowed the evaluation of internal seed morphology at different maturation stages, contributing to seed lots quality improvement and allowing to identificate seeds with a greater potential to germinate [17,18].

The evaluation of physiological aspects is essential to determine the best time to harvest seeds, since seed germination and vigor are the main beacons for selecting seeds according their quality. Table 2 shows the variables obtained with seed physiological characterization.

Table 2. Physiological variables evaluated in crambe seeds with different maturation stages

Treatment	Germination %	First germination count	Germination speed index	Radicle protrusion speed	Accelerated aging %
Green	14.5 c	8.5 c	0.81 c	1.37c	11.0
Green-yellow	26.0 abc	14.0 abc	1.42 bc	2.04 bc	11.0
Yellow	30.0 ab	22.5 ab	1.91 ab	2.63 b	12.0
Golden	35.5 a	23.0 a	2.20 a	3.87 a	16.5
Brown	20.0 bc	10.5 bc	1.15 bc	1.67 bc	10.0
F	9.29*	5.79*	10.27*	17.42*	0.62^{ns}
CV	21.44	35.62	23.4	20.37	53.43

* Significant ^{ns} Non-significant by F test (p <0.05). Means followed by the same letter in the column did not differ by Tukey's test (p <0.05). Fc = F calculated. CV = coefficient of variation

It is possible to observe that none of the treatments reached the minimum standard for crambe seeds commercialization in Brazil, which is 60%. This low germination may have occurred due to fungus contamination, since seeds were harvested and stored with a high humidity for 30 day period, without fungal treatment. This was confirmed by a high sporulation degree seen during the experiment conduction. However, the lots were accommodated under the same conditions, in other words, the factor acted in a generalized way, thus allowing to make a comparison between treatments.

Even with low germination, it was still possible to identify differences between treatments for the majorities of physiological variables evaluated, except for accelerated aging test. In general, the Golden seeds showed the highest performance for most of variables, while seeds harvested with Green or Brown pericarp, mostly, achieved the lowest performance for these evaluated characteristics.

Fig. 3. shows the multivariate analysis of principal components. Component 1 comprised 52.5% of the variability contained in the data and component 2, 35.4%, explaining a total variation of 87.9%. Thus, it was possible to reduce from 18 dimensions to only two that explained a significant percentage of observations.

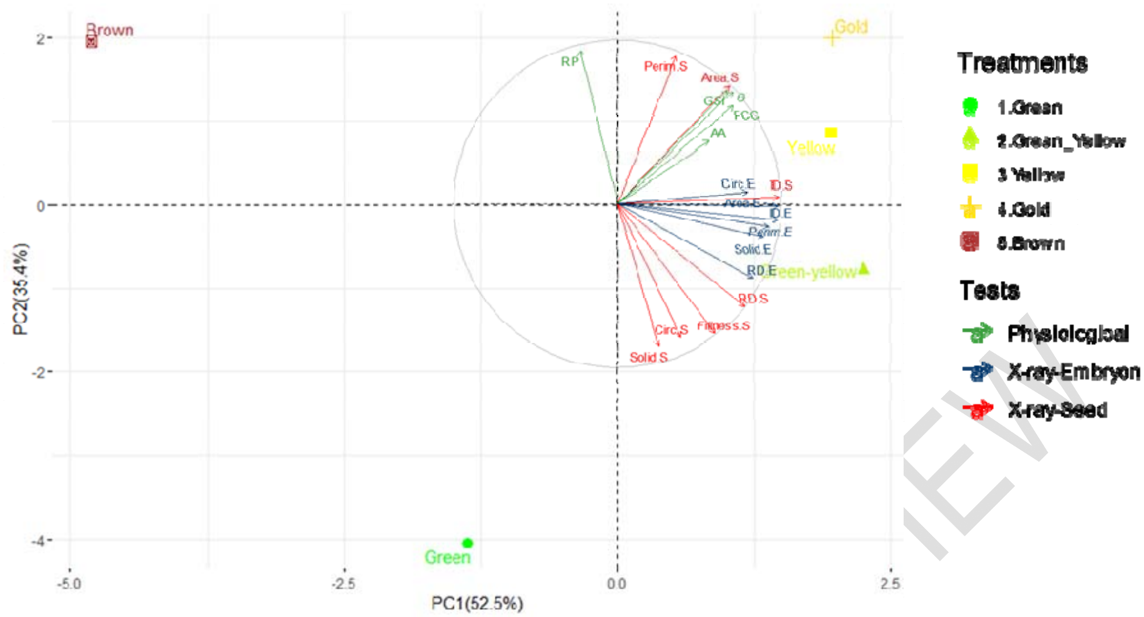

Fig. 3. Biplot obtained by grouping the variables related to the physical and physiological seed characteristics of crambe seeds with different maturation levels

Perim. = perimeter, Circ. = circularity, Solid. = solidity, RD = relative density, ID = integrated density, G = germination, FCG = first germination count, GSI = germination speed index, RP = radicle protrusion speed, AA = Accelerated aging

It was observed that seeds harvested with gold and yellow pericarp staining were close to the vectors of physiological quality (e.g. germination, first germination count, germination speed index and accelerated aging), indicating that seeds of these groups presented higher physiological potential, since each vector points to the direction in which the characteristic value has the maximum increase through the ordering diagram. In contrast, green and brown seeds were distant and opposite to vectors of physiological quality, confirming the lower physiological potential of these treatments.

In addition, it was observed that some physical variables, such as area and perimeter, showed significant correlations with germination (r = 0.96, 0.83), as well as with other physiological attributes. Medeiros et al. [6] in their research with leucine seeds, found strong correlations between morphometric parameters perimeter and circularity with physiological attributes, the higher the perimeter and the lower the circularity, the greater the germination and vigor of the seeds.

In view of the presented results, it was demonstrated that the proposed methodology is efficient to monitor the maturation of crambe seeds, providing a fast and reliable information on morphometric characters, being a highly applicable tool. However, further research needs to be done to fully validate the methodology and to relate physically to physiological parameters more safely.

4. CONCLUSION

The use of X-ray analysis combined with the semi-automated analysis is efficient to differentiate crambe seeds with different stages of maturation. The morphometric parameters are related to the physiological quality of crambe seeds.

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