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Effectivity of X-ray test to evaluate the physiological quality of sesame seeds due to fruits position at the plant

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ABSTRACT

The IAC-Ouro sesame cultivar has recently expanded to Brazilian Northeast, but the studies related to physiological quality and vigor of seeds produced under these conditions are scarce yet. Due to difference in fruits maturity from different parts of the plant, the aim of this study was to relate X-ray images with the seed maturation degree and evaluate the quality of seeds from fruits located on the lower, middle and upper third of the stem. We growth the sesame in an experimental area and harvest the fruits from different positions at 103 days after sown. Seeds were taken out from fruits and evaluated through the following tests at laboratory: germination test, germination first count, germination speed index and germination average time, dry mass of 1,000 seeds, seedling dry mass, electrical conductivity and X-ray test. The seeds of fruits from lower plant third showed similar quality to the middle plant third, while the upper plant third fruits provided seeds less vigorous. The X-ray test was efficient in evaluating the degree of maturation of seeds, indicating a better quality of seeds from the lower and middle third of the plant, verified by more radiopacity. The fruits from the bottom of stem develop first, and therefore, are the first to mature, which can be harvested early, without loss of seeds quality and vigor.

Key words: germination; seeds maturation; *Sesamum indicum* L.; vigor

Efetividade do teste de raios x para avaliação da qualidade fisiológica de sementes de gergelim em função da posição dos frutos na planta

RESUMO

A cultivar de gergelim IAC-Ouro recentemente tem se expandido para o Nordeste brasileiro, mas são escassos os estudos relacionados qualidade fisiológica e vigor das sementes produzidas nessas condições ambientais. Devido à diferença em maturidade dos frutos nas diferentes partes da planta, objetivou-se relacionar as imagens de raio x com o grau de maturação das sementes de gergelim e avaliar a qualidade das sementes oriundas de frutos localizados no terço inferior, mediano e superior do caule. Usou-se o delineamento inteiramente casualizado. As plantas foram cultivadas na área experimental da Universidade Federal do Ceará (UFC) e a colheita dos frutos de diferentes partes do caule foi realizada aos 103 dias após o plantio. As sementes foram retiradas dos frutos e submetidas aos seguintes testes em laboratório: porcentagem de germinação, primeira contagem, índice de velocidade e tempo médio de germinação, massa seca de 1.000 sementes, massa seca das plântulas, condutividade elétrica e teste de raios x. As sementes dos frutos do terço inferior apresentaram qualidade semelhante às do terço mediano, enquanto que as do terço superior se mostraram menos vigorosas. O teste de raios x mostrou-se eficiente na avaliação do grau de maturação das sementes, evidenciando uma melhor qualidade das sementes oriundas dos terços inferior e mediano, verificada através de um maior grau de radiopacidade, o qual pode ser relacionado a menor qualidade das plântulas formadas (PN e MSP), sendo possível empregar essa técnica na avaliação da qualidade do lote de sementes.

Palavras-chave: germinação; maturação de sementes; *Sesamum indicum* L.; vigor

Introduction

Sesamum indicum L., popularly known as sesame, is a species belonging to the Pedaliaceae family that presents great economic potential due to its possibilities of exploration, both in the national and international market. In Brazil, sesame has a growing market for exploration of its grains and oil, which can be used in various food and herbal products (Queiroga et al., 2010). Thus, with the increase in production of this oilseed, there is a natural high demand for quality seeds.

Sesame crops represent an alternative of income for producers in the Brazilian Northeast, for its tolerance to the edaphoclimatic conditions of the region, and for its feasibility of production by small and large farmers (Silva et al., 2016). The IAC – Ouro cultivar presents the main advantages for natural consumption, and is present in several products of the food and baking industry. Its seeds have a composition of 52% oil, 20% protein, 18% carbohydrates, 5% fiber, besides calcium, phosphorus, iron, potassium, sodium, magnesium and sulfur. After extracting the oil, the bran or flour has about 40% protein. The oil has a high percentage of oleic fatty acid; it is therefore a protein- and mineral-rich food (Embrapa, 2008).

The production of high quality sesame seeds is influenced by several factors, among which the harvest stands out. According to Beltrão & Vieira (2001) the determination of the ideal seed harvesting time is of fundamental importance because the longer the plant stays in the field after complete maturation, the greater is the loss during harvesting. These authors affirm that the determination of the harvesting time of dehiscent sesame is not easy, due to the uneven maturation of the capsules, typical of plants of indeterminate growth.

According to Carvalho and Nakagawa (2012), variations in the moment of physiological maturity occur as a function of the position of the fruit in the plant, and maturity of the field or plant should be characterized considering the proportion of fruits that reach a given characteristic related to the physiological maturity of the seed. Queiroga et al. (2012) say that the cutting season of sesame plants is generally determined by maturation of the fruits of the lower stem. Fruits that are located at the base of the stem are those that develop first, and are therefore the first to reach maturity, and thus initiate the process of dehiscence, which is the natural opening of the capsules.

The evaluation of physiological quality is an important aspect to be considered in a seed production program. In this context, physiological quality is maximal if germination and vigor reach maximum values (Hölbig et al., 2013). Thus, vigor tests provide additional information, indicating possible differences between lots that have similar germination percentages.

In recent years, X-rays have been used for seed evaluation as a relevant tool for various purposes in seed technology, such as visualization of insect damage and mechanical injury, detection of abnormalities in embryos and for determination of seed development staging (Battisti et al., 2000). Among the advantages of this test to infer the degree of maturation of the seeds, we can highlight that it is a simple and easily reproducible technique that is not influenced by the environment. Moreover, the images obtained can be cataloged, archived and later used to define non-subjective, standardized, and therefore more accurate evaluation criteria than those based on human visual perception (Menezes et al., 2005).

The goal of this work was to compare x-ray images with the degree of maturation of sesame seeds and to evaluate the quality of fruit seeds located in three parts in the stem: lower third, middle third and upper third.

Materials and Methods

Basic seeds from the Instituto Agronômico de Campinas (IAC), IAC-Ouro cultivar, were sown on December 11, 2013, in the experimental area for seed production in the agricultural year of 2014, in the Agricultural Sector of the Federal University of Ceará (UFC). The area is located at latitude 3° 44'S, and longitude 38° 33' W, 19.5 m altitude, in the municipality of Fortaleza/CE, which according to Köppen's classification has a type AW ' climate, described as rainy tropical climate.

According to the classification of Embrapa (2006), the soils used in the experimental area are Red Yellow Argisols. Fertilization consisted of the application of 40 t/ha of cattle manure, as recommended by Pereira et al. (2002). Prior to sowing, soil samples were collected at 0-20 cm depth and physical-chemical analyses were performed at the UFC Soil Analysis Laboratory. The characteristics investigated were: pH (H_2O) = 6.40; P = 4.00 mg kg; O.M. = 6.4 g kg; K + = 0.09 cmolc kg; Ca^{2+} = 0.9 cmolc kg; Mg^{2+} = 1.00 cmolc kg; H^+ + Al^{3+} = 0.33 cmolc kg, and the physical characteristics were: soil density = 1.36 g.cm^3, and particle density = 2.61 g.cm^{-3}.

Cattle manure was also evaluated, presenting Organic Carbon = 116.74 g/kg, Nitrogen = 7.91 g/kg and C/N = 14.6. This material was subsequently "tanned" and placed in furrows of 10 cm depth at the moment of sowing.

The harvest was carried out at 103 days after planting, in which 20 plants were selected at random in the useful area, cut at the level of insertion of the first fruits (30 cm high in relation to the ground). After cutting the plants, stems were divided into three equal parts, to cover maturation stages based on the external morphology of the capsules, considering green, yellowish green and yellowish colorations corresponding to the upper, middle and lower third in the plant, respectively (Figure 1).

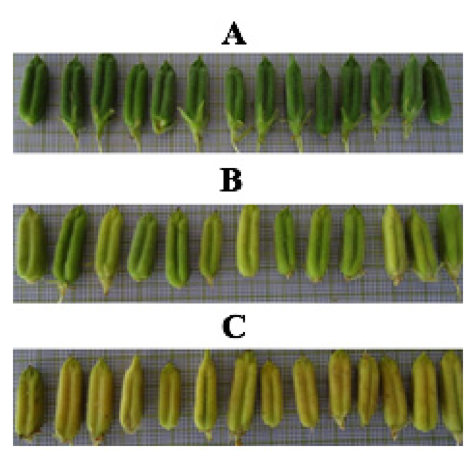

Figure 1. Sesame fruits, IAC-Ouro cultivar, collected from capsules of the upper (A), medium (B) and lower (C) third of the stem.

Three treatments were studied:

1. Seeds originating from capsules of the lower third in the plant;
2. Seeds originating from capsules of the middle third in the plant;
3. Seeds originating from capsules of the upper third in the plant.

Once the fruits of the main and secondary stems were removed and classified, the moisture content of the seeds was determined by the greenhouse method at 105 ± 3ºC for 24 hours (Brasil, 2009) with two subsamples of 500 seeds per treatment; the results were expressed in percentages. The fruits were kept in an electric dryer with forced air circulation at 30-35°C for four days until the fruits from the middle and lower third were dry and open, and those of the upper third were closed. After drying the capsules, the seeds reached approximately 12% of moisture (wet basis), and were submitted to the sieving and ventilation processes, and were then packed in a plastic bag and sent to the seed analysis laboratory to perform the following evaluations: 1,000 seed dry mass (SDM): using four replications of 1,000 seeds per treatment which were dried at 105 ± 3ºC for 24 hours (Brasil, 2009) and weighed on a Gehaka® analytical balance, with 0.01 precision, and the mean values were expressed in grams.Percentage of germination (% G): 200 seeds were used in four replicates of 50 seeds, seeded in germitest paper moistened with distilled water in the proportion 2.5 times the dry paper weight, organized in Petri dishes, which were kept in a BOD-type germination chamber at 25°C under constant light. Evaluations were carried out after the second day (first germination count-FGC) until the sixth day of germination, counting the percentages of normal seedlings (Brasil, 2009). Germination speed index (GSI) and the Mean Germination Time (MGT): these were calculated along with the germination test, where GSI was obtained using the formula described by Maguire (1962): $GSI = G1/N1 + G2/N2 + G3/N3 + ... + Gn/Nn$; and MGT by the formula proposed by Labouriau (1983): $t = \Sigma ni * ti/\Sigma n$ days, where: t = mean germination time; ni = number of seeds germinated in a given time interval; n = total number of germinated seeds; ti = days of germination. Dry mass/seedling (DMS): conducted according to Nakagawa (1994), with determination on the sixth day after sowing, after drying in an oven at 70°C for 72 hours, with results expressed in mg/seedling.

Electrical conductivity (EC): four subsamples of 50 seeds were weighed and soaked in 200 mL plastic cups containing 50 mL of distilled water, kept in BOD at 25°C for a period of eight hours (Torres et al., 2009). Conductivity readings were then performed on a Marconi® MA-521 conductivity meter, and the results expressed in $\mu S\ cm^{-1}\ g^{-1}$ of seeds.

As for radiographic analysis, four replicates of 50 seeds per treatment were used. These were fixed in transparent slides with the aid of double-sided adhesive tapes in order to obtain images using a digital x-ray apparatus, model HPMX-20, with an intensity of 22 KV and a mean exposure time of 12 seconds. Then, the morphological characteristics were evaluated by means of the radiographic images of the seeds, and seeds were classified according to the Rules for Seed Analysis (Brasil, 2009) into the following types: full seed, containing all the tissues essential for germination; empty seed containing less than 50% of the tissues; and physically damaged seed, with cracked or broken coat.

The experiment had a completely randomized design with three treatments and four replications. Data of the studied variables were submitted to analysis of variance and the means of the treatments were compared by the Tukey test at 5% of probability.

Results and Discussion

The freshly harvested seeds of the upper third of the stem showed high moisture content, indicating that they had not completed their maturation (Table 1). In contrast, seeds of the middle and lower thirds presented percentages of humidity within the range of physiological maturation, corresponding to 30 to 50% humidity, as Carvalho and Nakagawa (2012) point out.

Regarding the moisture content of seeds after being submitted to drying (Table 1), adequate values were found for the standardization of the physiological tests, which is below 15%.

The variables germination percentage, first germination count, germination speed index and mean germination time did not differ statistically to the 5% probability level between seeds from the different parts of the plant, presenting similar physiological quality. As for dry mass/seedling, 1,000 seed dry mass and electrical conductivity, there was a significant difference at the 5% significance level between the different positions of the fruits on the stem of the plant (Table 2). These results are in line with those obtained by Machado et al. (2010)

Table 1. Moisture content of freshly harvested and dried sesame seeds, IAC-Ouro cultivar, from capsules in three positions in the plant (lower, middle and upper third).

Treatments	Moisture content of the seeds (%)	
	Freshly harvested	After drying
Lower third	30.19	9.26
Middle third	28.93	12.26
Upper third	68.15	14.04

Table 2. Mean values and significant minimum differences (SMD) for the variables germination percentage (% G), first germination count (FGC), mean germination time (MGT), germination speed index (GSI), normal seedlings (NS), dry mass of seedlings (DMS), 1,000 seed dry mass (SDM) and electrical conductivity (EC) of sesame seeds, IAC-Ouro cultivar, from three positions of capsules in the plant.

Treatments	G (%)	FGC (%)	MGT (days)	GSI	NS (%)	DMS ($mg\ seedling^{-1}$)	SDM (g)	EC ($\mu S\ cm^{-1}\ g^{-1}$)
Lower third	98.5 a	74.5 a	2.30 a	22.37 a	95.33 a	3.46 a	3.63 a	265.22 a
Middle third	99.0 a	80.5 a	2.23 a	23.00 a	93.33 a	3.21 b	3.50 b	277.79 a
Upper third	95.5 a	76.5 a	2.26 a	22.05 a	82 b	2.62 c	2.66 c	444.53 b
DMS	3.55	13.44	0.14	1.49	4.8	0.15	0.08	21.58

* Means followed by the same letter in the column did not differ from each other at 5% probability by the Tukey test.

in which the position of the fruits in the castor bean did not influence the germination percentage of castor bean seeds (*Ricinus communis* L.), but differed from those observed by Sbirciog (2015) in which a negative correlation was observed between the position of the fruit in the plant and the quality of bell pepper seeds (*Capsicum annuum* L.), where the more superior positions of fruits in the stem were related to lower germination rates, because fruits of the base reach complete physiological maturity before fruits of the apex.

Similar germination values of seeds from different positions in the plant stem may be related to the drying procedure to which the immature seeds were submitted, as it was observed by Oliveira et al. (2010) in cotton seeds (*Gossypium hirsutum* L.). It is probable that drying was responsible, at least in part, for the good physiological quality. The acquisition of drying tolerance by immature seeds seems to be a complex phenomenon involving interactions of metabolic and/or structural adjustments, which allow cells to withstand losses of large amounts of water with a minimum damage (Carvalho & Nakagawa, 2012; Oliveira et al., 2012).

The analysis of germination speed of sesame seeds from the different evaluated treatments showed a high GSI, whereas the values of MGT were low, around two days, showing a similar germinative performance of seeds. This also explains the absence of a significant difference in FGC. Borghetti & Ferreira (2004) state that when seed germination occurs clustered in time, germination has a low variance. With regard to SGI, the higher its value, the higher is the germination speed, which allows inferring that the seed lot is more vigorous.

Table 2 shows that seeds from capsules of the middle and lower thirds have higher percentage of normal seedlings than those from the upper third. This result can be related to the fact that seeds from upper third of sesame plants, unlike the others, did not reach the physiological maturation, which can be verified by the high moisture content of the immature seeds (Table 1).

As for the dry mass of the seedling, there was a significant difference between mean values of the three treatments (Table 2). In the lower third, where there are mature capsules, seeds gave rise to seedlings that had a greater accumulation of dry matter (3.46 mg seedling^{1}). Similar results were observed by Mengarda & Lopes (2012) where chilli pepper seeds (*Capsicum frutescens*) from fruits collected in the basal region (C3R3) also provided the best initial development of seedlings with respect to root length, and fresh and dry mass of seedlings. These results corroborate those of Carvalho & Nakagawa (2012), who state that seeds that are not completely mature can be germinate, but they do not result in seedlings as vigorous as those harvested at the ideal harvest point because mature seeds present physical and physiological development that guarantees them maximum vigor. However, according to these authors, seeds from fruits at different maturation stages in the same plant undergo different environmental conditions, resulting in a heterogeneous lot in terms of vigor.

As regards the 1,000 seed dry mass, it was observed that mean values of the middle and lower third differed from each other and were significantly higher when compared to the mean of the upper third because there was a higher accumulation of dry matter in the seeds, 3, 50 g and 3.63 g, respectively, which were reached at 103 days after sowing (Table 2). These values can be considered high when compared with the data reported by Lago et al. (1994), who found the maximum dry weight of sesame seeds, IAC-Ouro cultivar, at 105 days after emergence, reaching a 1,000 seed dry mass of 2.51 g. The differences of these results may be related to the environmental conditions to which the seeds were submitted during their maturation, since according to Perry (1972), physiological characteristics of the seed are determined by the genotype and modified by the environment.

Heavier seeds have well-formed embryos and more reserves, being therefore more vigorous and giving rise to better developed seedlings. According to Queiroga et al. (2010), in relation to the mass of 1,000 sesame seeds, values above three grams meet the requirements of the market, a fact that was observed for seeds from the middle and lower thirds of the plants in the present study. Santos et al. studied the dry matter content of *Jatropha curcas* L. seeds and also observed significant differences between the mean dry mass values of 1,000 seeds of the different maturation stages of the fruits of this oilseed.

Regarding electrical conductivity, seeds from the upper third differed significantly from the other treatments because they presented higher leaching of electrolytes after the eight hours of imbibition (Table 2). In contrast, seeds from the middle and lower third presented lower values for this variable, indicating lower solute release and, therefore, higher membrane integrity. This fact is possibly correlated with the maturity of these seeds, since, according to Abdul-Baki & Baker (1973), the non-uniform maturation of sesame dehiscent plants results in the production of immature seeds in the apical part that release more leachates because they are in the stage of development and maturation. In addition, the high mean value of the electrical conductivity, 444.53 Ms cm^{-1} g^{-1}, found in sesame seeds from capsules of the upper third means that these seeds are much deteriorated and with low vigor (Oliveira & Gomes Filho, 2010). These results agree with those observed by Silva et al. (2017) who evaluated the physiological quality of sesame seeds, BRS - Seda cultivar, as a function of organic fertilization and position of the fruits in the plant, and concluded that seeds from the upper third were less vigorous in the electrical conductivity test when compared to seeds from the middle and lower thirds.

In the X-ray test, it was observed that seeds of the different treatments were well formed and classified as full seeds (Figure 2). However, seeds of the upper third of the plant had a higher degree of radio-luminescence, that is, were darker when compared to the seeds of the middle and lower third of the plant. This is indicated by a higher degree of radiopacity determined by the light color, evidencing a higher tissue density. According to Pupim et al. (2008), dark areas in x-rays correspond to seed tissue areas in which there is X-ray penetration, whereas whites represent the denser parts of the seed.

The correspondence between the X-ray images and the results of the germination pattern test indicated that seeds from capsules of the upper third of the plant (full and with lower opacity) had a lower percentage of normal seedlings (82%),

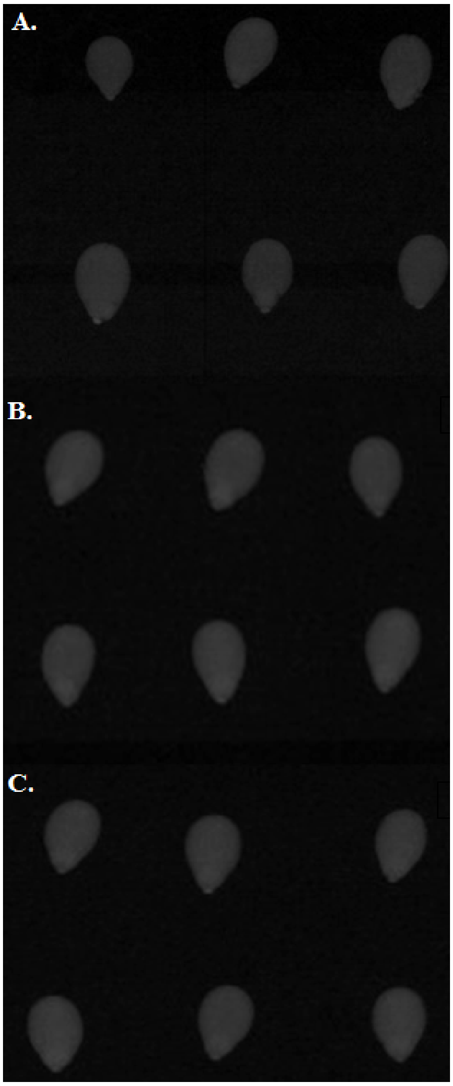

Figure 2. X-ray image of seeds from the upper (A), medium (B) and lower (C) thirds of sesame plants, IAC-Ouro cultivar

while seeds from capsules of the middle and lower thirds (full and with higher opacity) resulted in 93 and 95% of normal seedlings, respectively (Table 2). Similarly, in their analysis of radiographic images of castor bean seeds (*Ricinus communis* L.), Kobori et al. (2012) observed that the majority of the seeds that resulted in normal seedlings came from seeds classified as full and opaque and partially full and opaque. These results are due to different levels of maturation, for seeds from the upper third of the plant were harvested before reaching the maximum dry matter (Table 2), making it possible to distinguish seed lots at the level of vigor.

On the other hand, the X-rays did not allow the determination of the level of development of internal structures of the seeds due to the impossibility of their visualization resulting from their high oil content, typical of oleaginous plants such as sesame. Indeed, according to Beltrão & Vieira (2001) oleaginous plants have about 50% of lipids in their reserve tissue. Similar results were also obtained by Pupim et al. (2008) in X-ray images of embaúba (*Cecropia pachystachya*) seeds. Thus, the structures of the embryo could not be identified because the seeds had the same degree of radiopacity.

Conclusions

Sesame seeds (*Sesamum indicum* L.) from fruits of the lower third of the stem presented similar quality to those of the middle third, while those of the upper third presented lower quality, indicating that green fruits containing these seeds can be collected in advance without risk of loss of vigor of seeds;

The X-ray test was efficient for the evaluation of the degree of seed maturation, evidencing a better quality of seeds from the lower and middle thirds of the stem as indicated by a higher radiopacity, which can be related to the lower quality of the seedlings formed (PN and MSP). Thus, it is feasible to use this technique in the evaluation of seed lot quality;

The fruits of the stem base are the ones that develop first, being therefore the first to reach maturity, and can be collected in advance without loss of quality and vigor of seeds.

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