

## Morphological study of squash seeds at different stages of maturation through the use of X-ray<sup>1</sup>

Estudo morfológico de sementes de abóbora em diferentes estágios de maturação por meio do uso de raios-X

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**ABSTRACT** - Analysis of images has been recently used in order to both assess seed quality and elucidate some aspects of seed physiology, including X-ray tests and other resources. The objective of this study was to examine whether the X-ray may be successfully used to detect physical abnormalities, and relate their occurrence with physiological potential of tetsukabuto type squash seeds. Fruits were harvested at different maturation stages (15, 30, 45, 60 and 75 days after anthesis - DAA) and temporarily or not stored for 20 days. Seeds were radiographed using an intensity of 25 kV for 40 seconds and then germinated under alternating temperatures of 20-30 °C. After four and eight days, percentages of normal and abnormal seedlings and dead seeds were recorded. Results of both counts were evaluated comparatively with images of the internal morphology of each seed, obtained by means of X-ray test. The radiographic analysis is effective in detecting full, malformed and empty squash seeds. This confirmed information provided by the germination test, in which seeds harvested from 60 DAA and stored for 20 days were better in physiological potential.

**Key words:** *Cucurbita maxima*. *Cucurbita moschata*. Germination. Seed internal morphology.

**RESUMO** - A análise de imagens tem sido usada recentemente para avaliar a qualidade das sementes e elucidar alguns aspectos da fisiologia das sementes, incluindo testes de raios X e outros recursos. O objetivo deste estudo foi examinar se o raios X pode ser utilizado para detectar anormalidades físicas e relacionar sua ocorrência com o potencial fisiológico de sementes de abóbora tipo tetsukabuto. Os frutos foram colhidos em diferentes estádios de maturação (15, 30, 45, 60 e 75 dias após a antese - DAA) e armazenados por 20 dias. As sementes foram radiografadas com intensidade de 25 kV por 40 segundos e em seguida foi realizado um teste de germinação em alternância de 20-30 °C. Após quatro e oito dias, foram registrados percentuais de plântulas normais e anormais e sementes mortas. Os resultados de ambas as contagens foram avaliados comparativamente com imagens da morfologia interna de cada semente, obtidas por meio de teste de raios X. A análise radiográfica é eficaz na detecção de sementes de abóbora inteiras, malformadas e vazias. A utilização da técnica de raios X é eficiente na detecção de sementes cheias, mal formadas e vazias de abóbora, e ainda confirma os resultados obtidos nos testes fisiológicos, onde as sementes provenientes de frutos colhidos aos 60 DAA e armazenados por 20 dias apresentaram uma qualidade superior.

**Palavras chaves:** *Cucurbita maxima*. *Cucurbita moschata*. Germinação. Morfologia interna da semente.

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## INTRODUCTION

Evaluation of seed maturation process provides important information, since long period between physiological maturity and harvest, may result in physiological potential losses due to adverse climate conditions, attack by insects and microorganisms that affect the late and intensity of seed deterioration. The time of seed physiological maturity varies significantly depending on the prevailing environmental conditions, species and cultivars, and in some cases may be associated with changes in the morphological characteristics of fruits and seeds. In other situations, especially in fleshy fruits such as squash and other cucurbits, seed maturation process might be influenced by a fruit temporary storage period after harvest, before seed extraction. There are reports indicating that seeds from fleshy immature fruits such as squash, cucumber and eggplant may present comparable physiological quality to the seeds extracted from ripe fruits, when these immature fruits are conveniently stored for a certain period before seed extraction (PEREIRA *et al.*, 2014; SILVA *et al.*, 2017).

Besides the results from germination and vigor tests, information on the internal morphology, occurrence of damages, evolution of seed development, evaluation of the progress of differentiation of embryonic tissues and its relationship with the tissues' reserves could deeply help in this studies (CRAVIOTTO *et al.*, 2002); this should be performed by X-ray analysis. This technique consists in radiograph the seeds to study its structure and the internal morphology, and thus establish relations with seed performance. The efficiency of this analysis has been proved in several studies, and is used to identify both full and empty seeds, as well as damaged seeds (GOMES JÚNIOR, 2010). This technique has also been used to relate the internal morphology of seed in germination or seedling morphology such as in tomato (BORGES *et al.*, 2019) and bell pepper (GAGLIARD; MARCOS-FILHO, 2011) seeds. The X-ray test has been considered effective to assess seed quality because its accuracy. Also, it does not causes mutations in the seed due to the low dosage of radiation absorbed during the process and also, not affect germination. Consequently, seeds subjected to this type of analysis may be incubated, allowing the establishment of relations between its internal morphology and germination. The images obtained could be cataloged, archived and used later for defining a nonsubjective evaluation criteria, also standardized and therefore more accurate than those based on the human vision (INTERNATIONAL SEED TESTING ASSOCIATION, 2004; MENEZES; CÍCERO; VILLELA, 2005).

The use of image analysis to evaluate seed physiological potential is still very limited, but advances in the knowledge of this technique may be useful

to improve the development of seed quality control programs. Thus, this study aimed to analyze X-ray images to identify internal morphology characteristics during squash seed development. This species was used as a model, and the information obtained in this study may be extended or used for other cucurbit species or even for other vegetable crops.

## MATERIAL AND METHODS

The research was carried out in the Seed Analysis and Image Analysis laboratories, in the Plant Production Department of the "Luiz de Queiroz" School of Agriculture, University of São Paulo, and in the Seed Laboratory of Embrapa Hortaliças, Brasília - DF, from January to February 2013. 'Jabras' squash hybrid seeds resulting from *Cucurbita maxima* (female parent) x *Cucurbita moschata* (male parent), were produced in a protected area at the Embrapa Hortaliças experimental field in Brasília, DF, in the period from May to October 2012, through manual pollination. Fruits were harvested at 15, 30, 45, 60 and 75 days after anthesis (DAA), in order to obtain materials with differences in physiological potential. In each stage, thirty fruits were harvested, fifteen of which were stored for twenty days in plastic boxes at room temperature; in the others, seeds were extracted immediately after harvest. Seeds were then washed in tap water and surface dried at room temperature for 24 hours. Afterwards, seeds were dried under air circulation, at 32 °C, for 48 hours and then processed conditioned using a pneumatic separator, to eliminate lighter impurities. This same procedure was adopted for seeds from stored fruits. After seed drying and processing, the following determinations were made:

**a) Seed moisture content:** evaluated by the oven method, at 105 °C, with two replicates for each treatment (harvesting season x storage), according to the Rules for Seed Analysis (BRASIL, 2009).

**b) X-ray test:** carried out with five replicates of twenty seeds per treatment. Seeds were placed in wells of an acrylic plate and numbered according to the position occupied, so that they could be identified during subsequent measurements. The acrylic plate containing the seed was placed directly on a film of X-ray (Kodak Min-R 2000 EV, size 18x24 cm). The images were obtained with an intensity of 25 kV and 40 seconds of exposure, using the X-Ray equipment Faxitron, model MX-20. The revelation was made in a Hope X-Ray, Micromax Model 319 processor. The images of X-ray film were digitized by a scanner Uma x, Power Model Look 1100, in order to enlarge and check them in a computer. After developing the radiographs, seeds were classified according to their

internal morphology: a) number of filled seeds: those with normally structured vital areas (essential tissues); b) number of malformed seeds: those with vital poorly structured areas; c) number of empty seeds.

c) **Germination test:** conducted with four replicates of 50 seeds for each treatment, on germitest paper towel rolls, moistened with water equivalent to 2.0 times the mass of the dry paper, under alternating temperatures of 20 °C - 30 °C, under light). Seed counts were performed at four (first germination count) and eight days after the test, by computing the percentages of normal seedlings, according to the criteria established by the Rules for Seed Analysis (BRASIL, 2009). The results were expressed in average percentage per treatment.

The tests were carried out in a completely randomized design with four replications in a factorial arrangement of 5 x 2 (five harvest dates and two periods of fruit storage). Data were subjected to the analysis of variance and the mean comparison performed by Tukey test ( $p \leq 0.05$ ). After analysis of variance, regression analysis by PROC REG procedure of the SAS computer program (SAS 9.1.3, 2000-2004) was performed.

## RESULT AND DISCUSSION

Seed moisture content (SMC) after drying varied from 6 to 9% (Figure 1). SMC is an important factor in the X-ray studies, affecting the quality of the image obtained, since in general, the lower the water content, the higher its optical density, facilitating differentiation of the internal structures by means of radiography. The adjustment of the X-ray unit depends on the thickness, density and composition of the seed and the

apparatus used (INTERNATIONAL SEED TESTING ASSOCIATION, 2004). In the present study, the period of exposure to radiation of 40 seconds and the intensity of 25 kV allowed adequate visualization of squash seeds on radiographs. Under these conditions, it was possible to visualize the development of the internal seed structure. The gray levels observed in the analysis of radiographic images are defined according to the level of radiation absorption in different seed regions. Thus, seeds that have no embryonic tissue, due its low resistance to the passage of X-rays, provided dark images.

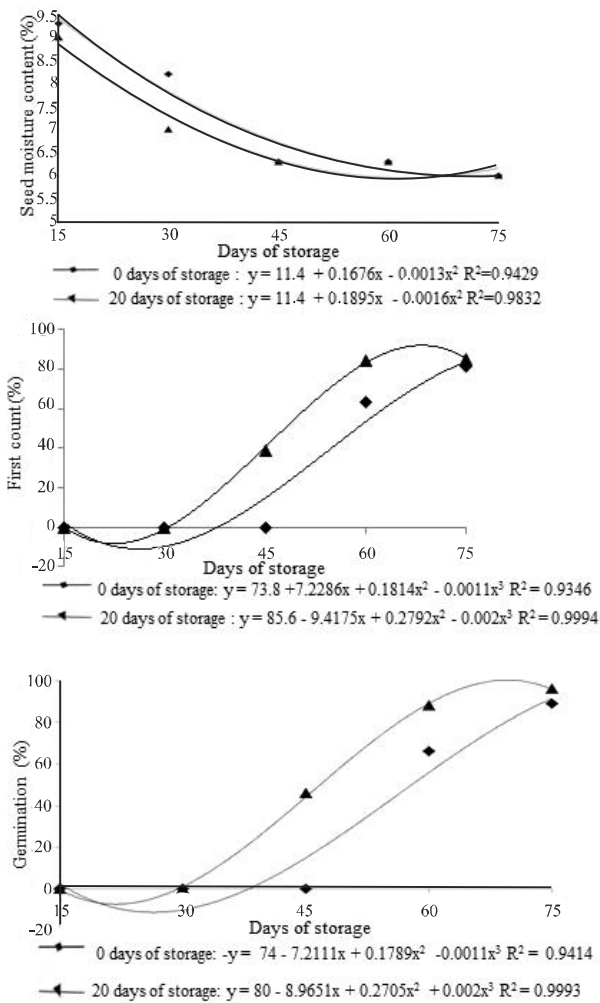
Only seeds from fruits harvested at 75 DAA, and stored for 20 days showed no empty seeds. Regarding to malformed seeds, only those derived from fruits harvested at 30 DAA showed no such abnormality, since all these seeds were classified as empty (Table 1). The full (completely developed) seeds were observed in various treatments. As expected, the longer the fruit maturity, the higher number of full seeds.

In order to obtain reliable results, it is necessary to establish a relationship between seed structure, observed on radiographs, with respective seedlings or dead seeds. As observed at the by first count and percentage germination results (Figure 1), the treatment with the highest percentage of full seeds showed better physiological potential as compared to other treatments. The maximum first count (85%) was obtained in seeds obtained from fruits harvested at 75 DAA whereas the maximum germination (97%) was obtained in seeds from fruits harvested at 75 DAA (Figures 1B, 1C). Similar results were also observed in squash seeds, where plots with the highest number of full seeds showed higher germination (CARVALHO *et al.*, 2009). These authors also observed that physically damaged seeds interfered negatively in germination.

**Table 1** - Number of full seeds (FS), malformed seeds (MS) and empty seeds (ES) of 'Jabras' squash from fruits harvested at 15, 30, 45, 60 and 75 days after anthesis (DAA) and stored for 0 and 20 days

Fruits (DAA)	Extraction (Days after harvest)	FS (%)	MS (%)	ES (%)
15	0	0	0	100
15	20	0	49	51
30	0	0	56	44
30	20	5	80	15
45	0	12	85	3
45	20	64	36	0
60	0	68	32	0
60	20	79	18	3
75	0	76	23	1
75	20	84	16	0

**Figure 1** - Moisture content (A), first count (B) and germination (C) of ‘Jabras’ squash hybrid seeds from fruits harvested at 15, 30, 45, 60 and 75 days after anthesis (DAA) and stored for 0 and 20 days

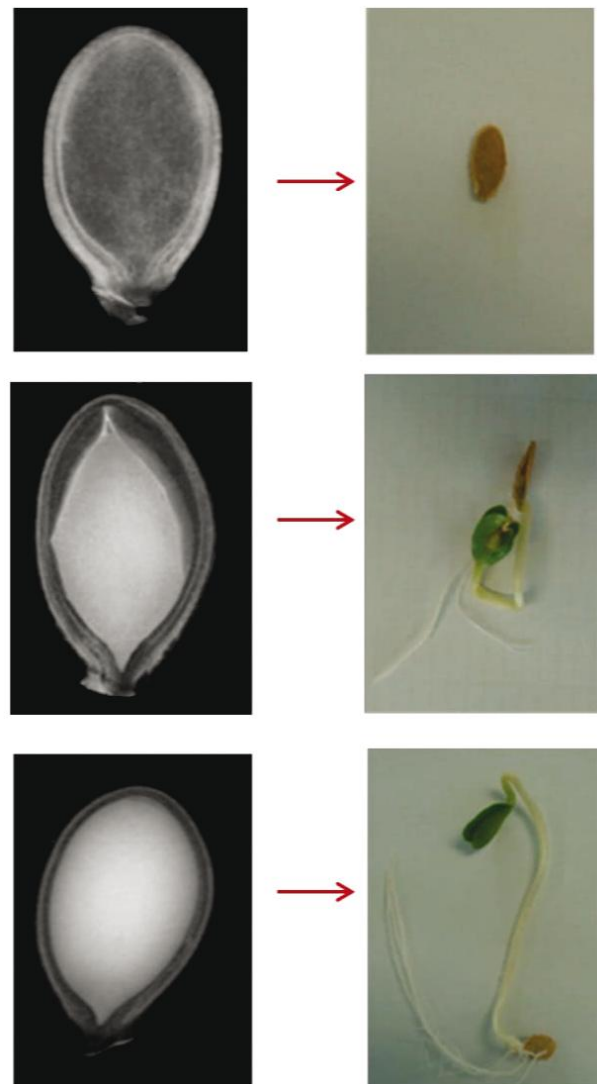


In the present study, the results obtained in germination test were consistent with the seed classification in X-ray analysis; however, the presence of normal and abnormal seedlings was also verified in full seeds. Using X-ray it is possible to visualize the seed internal morphology or even to observe other problems such as mechanical or insect damages and the presence of advanced tissue deterioration. However, some well-formed seeds may produce abnormal seedlings or dead seeds, since the X-ray test does not allow the detection of certain physiological problems, including, for example, intermediate stages of the decay process, which could be affecting the germination process (CARVALHO; ALVES; OLIVEIRA, 2010).

Most seeds classified as full yielded normal seedlings whereas most of those classified as malformed yielded abnormal seedlings. In contrast, 100% of the empty seeds did not germinate (Figure 2). Similar results were also obtained in seeds of sorghum (JAVORSKI; CICERO, 2017) and squash (SILVA *et al.*, 2014).

Thus, seeds from fruits harvested from 60 DAA and stored for 20 days showed higher number of filled seeds, indicating a higher physiological potential compared to other treatments. Silva *et al.* (2015) observed that the squash seed physiological maturity

**Figure 2** - ‘Jabras’ squash hybrid seeds classified as empty (above), malformed (center) and full (below) by the X-ray test, and their seed and seedlings after the germination test, showing respectively non germinated seed, abnormal seedling and normal seedling



occurred also in fruits harvested at 60 days after anthesis and stored for 20 days. During this period, seeds reached the maximum dry matter, maximum germination and vigor, and a high concentration of LEA proteins. These findings corroborated the present conclusion.

## CONCLUSION

The internal morphology of seeds may be indicative of their potential germinability, and the use of the technique of X-ray proved to be efficient to identify this attribute in squash seeds.

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