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Non-destructive assessment of sweet basil (*Ocimum basilicum* L.) seeds quality

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ABSTRACT: Sweet basil (*Ocimum basilicum* L.) seeds exhibit heterogeneity due to the characteristic flowering and pollination process of this species. Consequently, the necessity for research geared toward establishing effective methodologies for identifying seeds capable of germinating and with potential for producing vigorous seedlings becomes apparent. This study was undertaken with the aim of assessing the relationship between internal morphology, as evaluated through radiographic image analysis, and the germination performance of sweet basil seeds. Radiographs of seeds from eight lots of the cultivar *Alfavaca Basilicão* were examined. The length of seedling originated from radiographed seeds was obtained through computerized image analysis using SVIS® software and the results were compared with the radiographic images. Radiographs allowed visualization of the internal morphology of sweet basil seeds, enabling the identification of empty seeds, malformed seeds, seeds with tissue deterioration and mechanical damage. The analysis of radiographic images substantially contributed to establishing a cause-and-effect relationship between the physical integrity of tissues and the germination performance of the seeds, underscoring the potential for selecting seeds that will contribute to enhanced lot quality.

Index terms: germination performance, image analysis, SVIS®, X-ray.

RESUMO: As sementes de manjericão (*Ocimum basilicum* L.) são desuniformes, decorrente do processo de florescimento e polinização característico da espécie. Diante desse cenário, surge a necessidade de se realizar pesquisas voltadas para o estabelecimento de métodos eficazes para a identificação de sementes aptas a germinar e com potencial para originar plântulas vigorosas. O presente trabalho foi realizado com o objetivo de avaliar a relação entre a morfologia interna, por meio da análise de imagens radiográficas, e o desempenho germinativo de sementes de manjericão. Foram avaliadas radiografias de sementes de oito lotes do cultivar Alfavaca Basilicão. O comprimento das plântulas provenientes das sementes radiografadas foi obtido pela análise computadorizada de imagens, utilizando-se o *software* SVIS®, sendo os resultados comparados com as imagens radiográficas. Por meio das radiografias foi possível visualizar a morfologia interna das sementes de manjericão, bem como detectar sementes vazias, malformadas, com tecidos deteriorados e danos mecânicos. A análise de imagens radiográficas permitiu estabelecer uma relação de causa e efeito entre a integridade física dos tecidos com o desempenho germinativo das sementes, com potencial para a seleção de sementes que irão compor lotes de melhor qualidade.

Termos para indexação: desempenho germinativo, análise de imagens, SVIS®, raios X.

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INTRODUCTION

Native to Southeast Asia and Central Africa, sweet basil (*Ocimum basilicum* L.) stands out as the most cultivated vegetable in the Lamiaceae family. It is present in several regions of the world under temperate, subtropical, hot and humid conditions, and can be cultivated throughout the year, as long as the plants are protected from frost (Favorito et al., 2011). In Brazil, the species was introduced by European immigrants, finding in the national territory edaphoclimatic conditions conducive to its cultivation.

Sweet basil is rich in essential oil, which can be extracted from the branches, leaves, and inflorescences of plants, consisting of phytochemicals with proven antioxidant, anticancer, antiviral, and antimicrobial action (Bravo et al., 2021), commercially valued by the pharmaceutical, cosmetic, and food industries (França et al., 2017). Despite the growing demand for medicinal, aromatic and spice plants, Brazil faces significant challenges due to the lack of development of new technologies in cultivation. In addition, the national production chain of these species is still unstructured, resulting in dependence on seed imports. In the case of basil, only 85 seed production fields were recorded, which together totaled a production of 1.7 tons (Brasil, 2023).

The Ministry of Agriculture, Livestock and Food Supply (MAPA) requires a minimum germination percentage of 60% for the commercialization of sweet basil seeds in the national territory (Brasil, 2019). This requirement reflects the difficulty in producing these seeds on a commercial scale, due to the characteristics of the species. According to Brasil (2009), sweet basil seeds are botanically described as indehiscent dried fruits of the carcerule type; the fruits are small and light, with an individual mass ranging from 0.28 to 1.8 mg (Patel et al., 2015), composed of a black pericarp and the embryo, without the presence of endosperm (Zhou et al., 2022). Sweet basil plants are allogamous and show uneven flowering, resulting in different stages of development in the same inflorescence (Brito et al., 2010). In addition, its life cycle can be annual or perennial, depending on the environmental conditions of the growing site and on the agronomic characteristics of the cultivar (Favorito et al., 2011). Because of these complexities, a lot may contain seeds with different degrees of tissue formation, abnormalities and damage that can influence germination performance.

The radiographic image analysis technique has been used and improved for detailed evaluation of the internal morphology of seeds of different species, making it possible to identify morphological and structural abnormalities of the internal tissues, quickly and non-destructively (Rahman and Cho, 2016; França-Silva et al., 2023). This method is based on exposing the seeds to an X-ray source, consisting of electromagnetic waves within the range of 0.01 to 10 nm and to a photosensitive film or digital sensor (Kotwaliwale et al., 2014). When passing through the seeds, part of the radiation is attenuated due to the radiodensity of the tissues, resulting in the generation of a latent image, characterized by the variation of the shades of gray, resulting from the differential absorption of energy (Medeiros et al., 2018). Seeds made up of well-formed tissues, without damage and with greater filling, have greater resistance to the passage of X-ray, imprinting light gray shades on radiographs (Bianchini et al., 2021).

Although radioactivity associated with X-ray may pose potential risks, the reduced doses of radiation used in the test offer the advantage of enabling detailed assessment of the internal morphology of seeds, without compromising their germination capacity (Ahmed et al., 2020a; Hong et al., 2023), allowing other tests to be performed with the same sample. Thus, the morphological alterations identified in the radiographs can be associated with the subsequent germination performance of the seeds, by means of physiological tests. Studies with seeds of different species, such as *Solanum pennellii* Corr. (Pessoa et al., 2023), *Brassica oleracea* var. *capitata* L., *Raphanus sativus* var. *radicula* Pers. and *Lepidium sativum* L. (Musaev et al., 2022), *Crambe abyssinica* Hochst. ex R.E. Fr. (Ribeiro et al., 2021) and *Citrullus lanatus* (Thunb.) Matsum. & Nakai (Ahmed et al., 2020b), when relating the physical integrity of the internal tissues and the germination performance, found the efficiency of using radiographic images for non-destructive evaluation of the quality of these seeds.

The X-ray test can contribute to significantly improving the germination performance of lots, due to the possibility of identifying and discarding seeds with abnormalities in their morphology (Guedes et al., 2014), representing a potential strategy to improve the quality of a seed lot; however, the methodology of the X-ray test is not standardized for sweet basil seeds. Thus, the present study was carried out with the objective of evaluating the internal morphology of sweet basil seeds, through the analysis of radiographic images, and its relationship with the germination performance of the radiographed seeds.

MATERIAL AND METHODS

The experiment was carried out at the Image Analysis and Seed Analysis laboratories of the Department of Crop Science at the *Escola Superior de Agricultura "Luiz de Queiroz"*, *Universidade de São Paulo* (Esalq/USP), Piracicaba, SP, Brazil. Sweet basil seeds of the cultivar *Alfavaca Basilicão*, free of chemical treatment, acquired from companies that sell vegetable seeds, represented by eight lots, were used.

First, seed water content was determined by the oven method. For that, two samples of approximately 0.5 g of seeds for each lot were kept at 105 ± 3 °C for 24 hours and the results were expressed as percentages (wet basis), according to the Rules for Seed Testing (Brasil, 2009).

X-ray test: 200 seeds from each lot were divided into eight groups of 25 seeds and fixed on a double-sided adhesive tape on a transparent acetate slide, keeping the ventral side facing down; each seed was numbered according to its position on the slide, for proper identification in later determinations. The plastic slide containing the seeds was placed inside the Faxitron® Cabinet X-Ray system, MX-20 DC-12 model, connected to a Core 2 Duo computer (3.16 GHz, 3 GB RAM, 160 GB HDD), at a distance of 11.4 cm from the radiation source. To obtain the radiographic images, the system was configured with an intensity of 28 kV, 8.5 seconds of exposure and a contrast of 1286 (width) x 4427 (center). The images were saved in TIFF (Tagged Image File Format) and stored in a specific folder on the computer's hard drive. Then, the radiographed seeds were subjected to the computerized analysis of seedling images.

Computerized analysis of seedling images: maintaining the numerical identification of the X-ray test, the seeds (in groups of 25) were distributed in two parallel rows in the upper third of two sheets of blotting paper, previously moistened with water in an amount equivalent to 2.5 times the mass of the substrate, placed in transparent plastic boxes (11 x 11 x 3.5 cm). The containers were arranged in a BOD germination chamber forming an angle of 60° to 70° with the horizontal, remaining under alternating regime of temperature and light (20 °C/16 h and 30 °C/8 h). After four days, the seedlings (normal and abnormal) and the dead seeds were transferred to a blue EVA (Ethyl Vinyl Acetate) sheet, and the images were captured with an HP Scanjet 200 scanner, installed in an inverted position inside an aluminum box (60 x 50 x 12 cm), adjusted to 100 dpi resolution and coupled to a Core i7 computer (3.50 GHz, 16 GB RAM, 1 TB HDD). The images were processed by SVIS® software, and the individual length of each seedling (cm) was obtained by converting 1 pixel into 0.0254 cm (Hoffmaster et al., 2003).

The data from the X-ray test and the computerized analysis of seedling images were subjected to descriptive statistical analysis. The results were evaluated by means of the relationship between the image observed on the radiograph of each seed and its respective germination performance (germination and seedling length). The total number of intact seeds and seeds with internal morphology alterations, identified by the analysis of the radiographs, as well as normal seedlings, abnormal seedlings and dead seeds were calculated as percentages of occurrence for each lot.

RESULTS AND DISCUSSION

Prior knowledge of the internal morphology of seeds before the acquisition and processing of radiographic images is a fundamental requirement to ensure correct identification of regions affected by different types of damage, as well as their implications for germination and subsequent seedling development. The main components of sweet basil seeds were identified from intact seeds, observed with the aid of a stereoscopic magnifying glass (Figures 1a and 1b); the pericarp completely surrounds the embryo, and it is possible to locate the cotyledons, the plumule, and the hypocotyl-radicle axis.

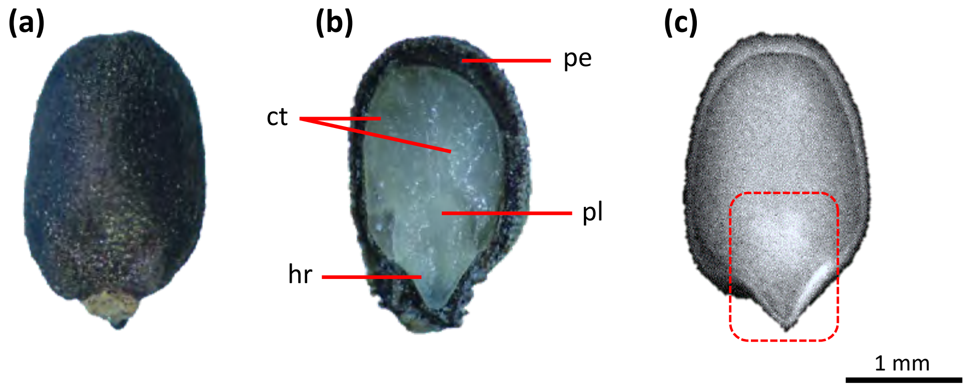

Figure 1. Basic components of a sweet basil seed. External morphology (1a), longitudinal section (1b) and radiographic image (1c). pe – pericarp; ct – cotyledon; pl – plumule; hr – hypocotyl/radicle axis. Red rectangle indicates the region of the embryonic axis.

Exposure of the seeds to a radiation intensity of 28 kV for 8.5 seconds, together with water contents ranging from 6.1% to 7.0%, made it possible to obtain radiographic images with sufficient quality to visualize the internal morphology. The embryonic axis is a region with lighter hue, located in the lower portion of the seed, and it is not possible to clearly distinguish its individual components (Figure 1c), which may be related to the overlapping of tissues with similar density, since a significant part of the embryonic axis is surrounded by cotyledons. According to Abud et al. (2018), tissues with similar optical densities tend to produce radiographic images with lower grayscale contrast, affecting the clarity in the visualization of the internal structures of the seed. This usually occurs due to the amount of radiation absorbed by the seeds, which in turn varies according to the ionizing wavelength and the characteristics inherent to each species, such as chemical composition, thickness and density of the tissues (Brasil, 2009).

By means of the radiographic images, it was possible to verify the occurrence of different categories of seeds, based on the physical integrity of their tissues: intact, with deteriorated tissues, mechanical damage, empty and malformed (Table 1). Most of the seeds were intact, with percentages ranging from 76.5% (Lot 1) to 97.5% (Lot 7), occurring less frequently in lots 1, 2 and 8, which had the lowest germination percentages. The initial expectation was that physically intact seeds (Figures 2A, 2B and 2C) would germinate and produce only normal seedlings (Figure 2a). However, abnormal seedlings (Figure 2b) and dead seeds (Figure 2c) were also observed in this category, although in reduced proportions. According to Ahmed et al. (2020a), improper seed handling or chemical changes resulting from prolonged storage periods can affect the germination process, even if the seeds do not show visible damage on radiographs.

The presence of deteriorated tissues was the morphological alteration most frequently observed in sweet basil seeds (Table 1), reaching an overall mean percentage of 7.1% of incidence, accounting for the highest proportion of abnormal seedlings and dead seeds (5.9%). This result is probably related to the size and amount of reserves that sweet basil seeds have, since, according to Selvi and Saraswathy (2017), small seeds have a lower amount of reserves, being prone to marked deterioration after physiological maturity.

The radiation from X-ray easily passes through the regions of the seed made up of deteriorated (less dense) tissues, identified on radiographs by darkened spots printed on the images (Bianchini et al., 2021). Seeds in which deterioration occurred at the opposite end of the embryonic axis (Figure 2D) developed normal seedlings, and it was possible to observe the lesion in the seedling cotyledon (Figure 2d). When the deterioration reached an extensive area in the central region of the cotyledons (Figure 2E), abnormal seedlings were produced, either without the aerial part (Figure 2e), or that did not germinate. According to Nonogaki et al. (2010), the deterioration that occurs in the intermediate portion of the reserve tissue can impair the germination capacity of the seed, due to the difficulty in the translocation of nutrients for the development of the embryonic axis. Seeds that showed deterioration in the embryonic axis (Figure 2F) did not germinate (Figure 2f), probably due to embryo death.

Table 1. Relationship between the categories identified by the X-ray test and the germination of eight lots of sweet basil seeds.

Lot	GE	Categories					Total	GE_{IN}
		IN	DT	MD	EM	MF		
		%						
1	NS	53.0	1.0	0.0	0.0	0.0	54.0	69.3
	AS	13.0	2.5	2.0	0.0	0.0	17.5	
	DS	10.5	9.5	5.0	0.0	3.5	28.5	
2	NS	60.0	3.0	0.0	0.0	0.0	63.0	72.7
	AS	11.0	2.5	3.0	0.0	0.0	16.5	
	DS	11.5	6.5	1.5	0.5	0.5	20.5	
3	NS	80.5	1.5	0.5	0.0	0.0	82.5	90.4
	AS	5.5	0.5	4.0	0.0	0.0	10.0	
	DS	3.0	2.5	1.5	0.0	0.5	7.5	
4	NS	82.0	1.5	0.0	0.0	0.0	83.5	92.6
	AS	4.5	1.5	1.5	0.0	0.0	7.5	
	DS	2.0	6.0	0.0	0.0	1.0	9.0	
5	NS	80.0	0.5	0.0	0.0	0.0	80.5	88.4
	AS	8.0	3.0	1.0	0.0	0.0	12.0	
	DS	2.5	4.5	0.0	0.0	0.5	7.5	
6	NS	89.0	0.5	1.0	0.0	0.0	90.5	97.3
	AS	2.0	0.5	1.5	0.0	0.0	4.0	
	DS	0.5	2.5	1.0	0.5	1.0	5.5	
7	NS	96.5	0.0	0.5	0.0	0.0	97.0	99.0
	AS	0.5	0.5	0.0	0.0	0.0	1.0	
	DS	0.5	0.0	0.0	0.5	1.0	2.0	
8	NS	71.0	1.5	0.0	0.0	0.0	72.5	84.0
	AS	8.5	3.0	5.5	0.0	0.0	17.0	
	DS	5.0	1.5	4.0	0.0	0.0	10.5	
Mean		87.6	7.1	4.2	0.2	1.0	77.9*	86.7

GE = germination; NS = normal seedlings; AS = abnormal seedlings; DS = dead seeds; IN = intact seeds; DT = seeds with deteriorated tissues; MD = seeds with mechanical damage; EM = empty seeds; MF = seeds with malformed embryo; GE_{IN} = germination of the fraction of seeds classified as intact; *value referring to the number of normal seedlings.

Mechanical damage was identified in all lots, in a smaller proportion than deteriorated tissues, but with greater potential for interference in the germination process, since of the 4.2% of seeds in this category, only 0.2% originated normal seedlings (Table 1). The losses caused by this type of damage varied according to the region affected and the intensity of the injury. Surface cracks on the cotyledons (Figure 2G) resulted in normal seedlings (Figure 2g); lesions on the region of the embryonic axis (Figures 2H, 2I and 2J) caused loss of seed viability, resulting in abnormal seedlings, with partial (Figure 2h) or total (Figure 2i) rupture of the hypocotyl-radicle axis and dead seeds (Figure 2j).

According to Arruda et al. (2016), the location and extent of injuries should be considered during the analysis of radiographic images, as seeds with cracks, ruptures, or loss of part of the cotyledons can germinate and originate

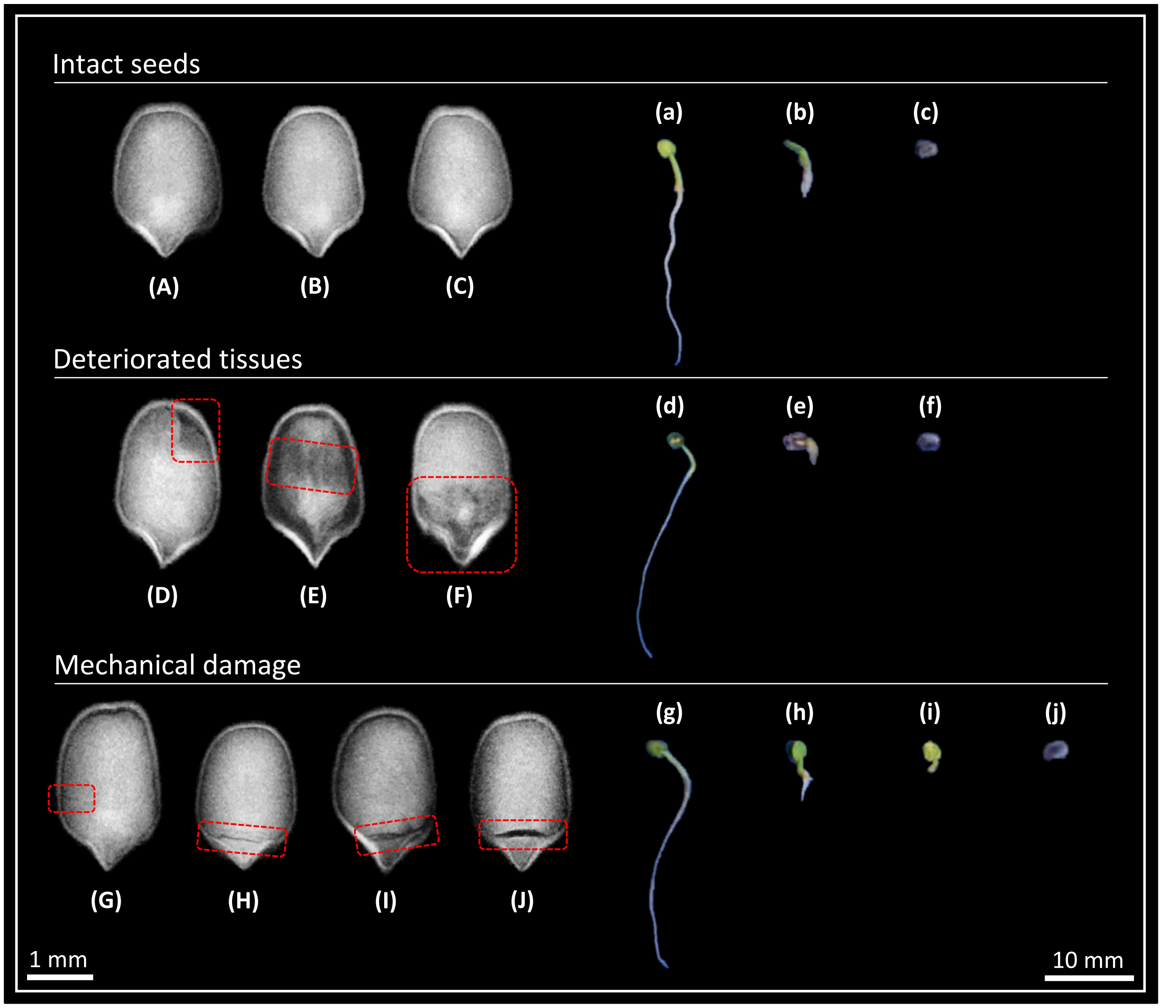

Figure 2. Radiographic images of intact sweet basil seeds (2A, 2B and 2C), with deteriorated tissues (2D, 2E and 2F) and mechanical damage (2G, 2H, 2I and 2J) and their respective normal seedlings (2a, 2d and 2g), abnormal seedlings (2b, 2e, 2h and 2i) and dead seeds (2c, 2f and 2j).

normal seedlings, as long as the lesions do not compromise regions vital to the germination process. Rego et al. (2023) reported that damage that is superficial or opposite to the embryonic axis of *Vigna unguiculata* (L.) Walp. seeds tend not to compromise germination. However, when the lesions affect the embryonic axis, they result in the development of abnormal seedlings or death of the seeds. A similar finding was reported by Medeiros et al. (2020a), who observed that damaged *Cucumis melo* L. seeds originated normal seedlings, as long as the integrity of the embryonic axis was not compromised. On the other hand, according to Marcos-Filho (2015), even when the damage does not result in immediate loss of viability, it can cause negative effects on vigor and increase the susceptibility of seeds to deterioration caused by the action of microorganisms, resulting in the reduction of seed storage potential.

The presence of empty seeds was found only in lots 2, 6 and 7, in proportions lower than 1%, while seeds with malformed embryos occurred in almost all lots, reaching a maximum value of 3.5% in Lot 1, without the occurrence of germinated seeds in these categories (Table 1). Empty seeds were characterized on radiographs by the pronounced contour of the pericarp and by the completely darkened internal cavity (Figure 3a). In the category of malformed embryos, abnormalities such as atrophy (Figure 3b) and irregular shape (Figure 3c) of the embryo, atrophied embryonic axis (Figure 3d), and partial formation of the cotyledons (Figures 3e, 3f and 3g) were detected, the last one being the most frequent in the lots evaluated. Martins et al. (2008) reported a higher occurrence of empty seeds in *Ocimum*

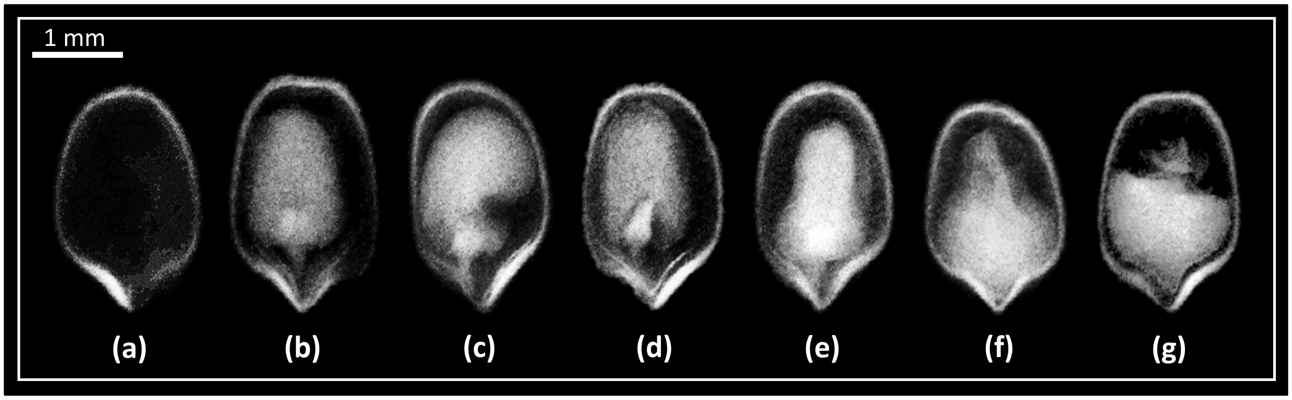

Figure 3. Radiographic images of sweet basil seeds with empty internal cavity (3a) and malformed embryos (3b, 3c, 3d, 3e, 3f and 3g).

gratissimum L. seeds, with no germination of empty seeds. Results obtained with *Bauhinia scandens* L. and *Crotalaria juncea* L. seeds showed that seeds with malformed embryos, depending on the severity and location of the affected tissues, may eventually originate normal seedlings, but with less development of the embryo parts (Arruda et al., 2016; Jeromini et al., 2021).

The overall average percentage of normal seedlings increased from 77.9% to 86.7% when excluding empty, malformed, deteriorated and mechanically damaged seeds, and this increase had a greater impact on lots with higher occurrence of abnormalities (Table 1); in Lot 1 there was an increase of 15.3 percentage points, while in Lot 7 the increase was 2 percentage points. This result reinforces the strong correspondence between the physical characteristics identified through the analysis of the radiographs and the germination of the seeds, already observed by other authors (Al-Turki and Baskin, 2017; Musaev et al., 2022; Hong et al., 2023).

The better performance of the lots when seeds with some type of abnormality were eliminated was also observed in relation to seedling length, especially in lots with a higher percentage of seeds with deteriorated tissues, mechanical damage, empty and malformed (Figure 4). In Lot 1 (with a higher incidence of problems detected on radiographs), the average length of the seedlings increased from 1.54 cm to 1.99 cm, when only intact seeds were considered. This result corroborates Noronha et al. (2018), who mentioned that embryonic damage and malformations may be responsible for low germination and reduction in seedling length. The existence of a close relationship between the integrity of the internal morphology, observed by the X-ray test, and the germination performance of seed lots is supported by previous research (Abud et al., 2018; Borges et al., 2019; Medeiros et al., 2020b; Ribeiro et al., 2021), ratifying the results obtained for sweet basil seeds in this study.

Lots with higher occurrence of damage (1, 2 and 8) originated seedlings with shorter length, even when only seedlings from intact seeds were considered (Figure 4). This result shows that the physical integrity of the tissues observed in the radiographs is not the only factor that interferes with the physiological quality, so the germination performance of a lot may be overestimated. Thus, the X-ray test should not be adopted as the only approach to evaluate the quality of seed lots. In this context, the information from the X-ray test can complement the results of physiological tests, since factors such as the level of metabolic activity and latent infections caused by microorganisms are not determined through the analysis of radiographic images (Van der Burg et al., 1994).

Sweet basil plants have uneven flowering, so a high occurrence of problems related to heterogeneous seed development was expected. However, this expectation did not materialize in the lots evaluated. In addition, although the changes in the internal morphology of the seeds were easily observed during the evaluations, it was not possible to establish a relationship between the categories identified by the X-ray test with aspects such as shape or color, characteristics often associated with the degree of maturation of the seeds (Miranda et al., 2017; Trancoso et al., 2021).

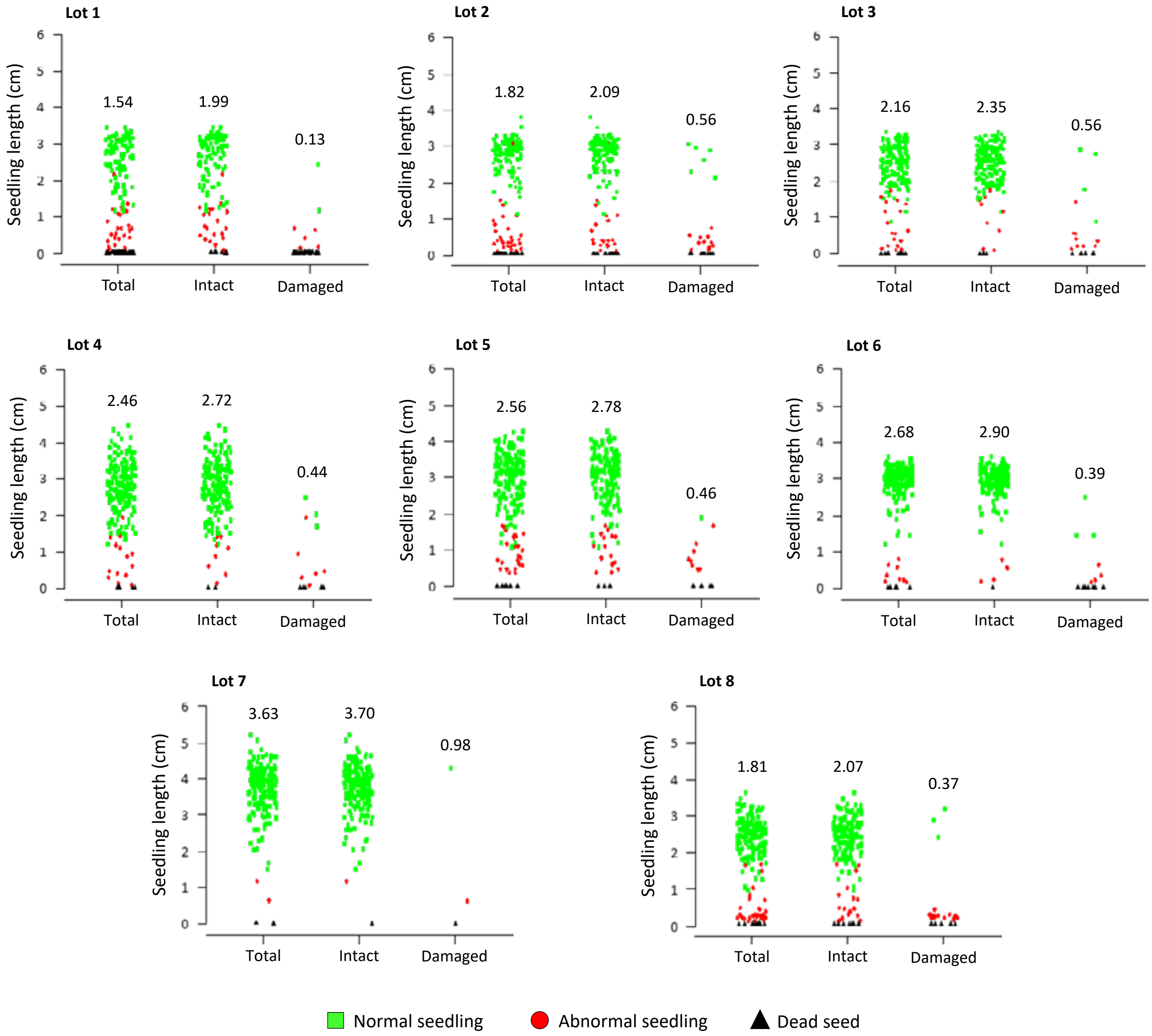

Figure 4. Average seedling length of eight lots of sweet basil seeds, considering the total number of seeds evaluated in comparison to seeds classified as intact and damaged by the analysis of radiographic images.

In summary, the analysis of radiographic images of sweet basil seeds allowed proving the existence of a causal relationship between a possible lower germination performance and the physical integrity of the internal tissues of the seeds, ratifying the use of the X-ray test as an additional valuable tool, potentially capable of improving the quality of seed lots.

CONCLUSIONS

Analysis of radiographic images can be used in the non-destructive evaluation of the internal morphology of sweet basil seeds and allows the efficient establishment of a cause-and-effect relationship between the physical integrity of the tissues and the germination performance of the seeds, through the detection of internal damage and embryonic malformations.

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