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REVIEW

Advances in imaging technologies for soybean seed analysis

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ABSTRACT: Among grain-producing species, soybean is one of the most important commodities, with increasing demand for production in coming years. Evaluation of soybean seed quality is fundamental for ensuring maximum germination and yield potential. Therefore, effective methods are necessary for examining different properties associated with physical-chemical, physiological, and seed-health changes that affect seed quality. This review focuses on the fundamental principles and on the application of techniques of radiographic imaging, magnetic resonance imaging, multispectral imagining, chlorophyll fluorescence imaging, and infrared thermography to evaluate changes related to loss of soybean seed quality, such as mechanical injury, injury caused by insects, embryonic malformation, and incomplete maturation. Computerized seedling image analysis is also presented for evaluation of seed lot vigor. The examples presented here show the potential of these image analysis techniques for identifying different types of injuries and increasing the efficiency of in-house quality control programs in soybean seed production companies.

Index terms: *Glycine max* (L.) Merrill, image spectroscopy, optical sensing, seed technology.

RESUMO: Entre as espécies produtoras de grãos, a soja é uma das *commodities* mais importantes, com demanda crescente por produção nos próximos anos. A avaliação da qualidade da semente de soja é fundamental para garantir o máximo potencial de germinação e produtividade. Portanto, métodos eficazes são necessários para examinar diferentes propriedades associadas a alterações físico-químicas, fisiológicas e na sanidade das sementes que afetam a qualidade das sementes. Esta revisão enfoca os princípios fundamentais e a aplicação de técnicas de imagem radiográfica, ressonância magnética, imagem multiespectral, imagem de fluorescência de clorofila e termografia infravermelha para avaliar alterações relacionadas à perda de qualidade da semente de soja, como injúrias mecânicas, injúrias causadas por insetos, malformação embrionária e maturação incompleta. A análise computadorizada de imagens de plântulas também é apresentada para avaliação do vigor do lote de sementes. Os exemplos aqui apresentados mostram o potencial dessas técnicas de análise de imagens para identificar diferentes tipos de injúrias e aumentar a eficiência dos programas internos de controle de qualidade nas empresas produtoras de sementes de soja.

Termos para indexação: *Glycine max* (L.) Merrill, espectroscopia por imagem, detecção ótica, tecnologia de sementes.

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INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] seed production is prominent among agribusiness economic activities, since soybean seeds are the raw material essential for establishing soybean grain production fields. Brazil is currently the world's largest producer of this leguminous crop; in the 2020/21 crop year, 10.4 million tons of seeds were produced (Brasil, 2021) and 135 million tons of grain were harvested (Conab, 2021).

Due to the vast spread of soybean growing in the world, its economic importance, and its sensitivity to biotic and abiotic adversities, investments have been made in research and development to ensure high yields and seed quality (Bradbeer, 1988; Belluzzo et al., 2014). To detect and ensure the quality and certification of seeds for sale, the methods traditionally used, though standardized, are destructive, laborious, time-consuming, and subjective, and they require prior preparation of the seed samples (Carvalho et al., 2019; França-Silva et al., 2019; Gomes-Junior, 2019).

The emergence and development of technologies based on use of computational resources and optical sensors make it possible to overcome the limitations of the destructive conventional methods of seed quality analysis. Computational techniques can be complementary alternatives for non-destructive and rapid analysis and reanalysis of seed quality. Different parameters obtained from seeds (size, shape, texture, color, chemical constitution, tissue density, metabolic activity, etc.) may be related to their physical, physiological, genetic, and/or health characteristics (Chen et al., 2002; Huang et al., 2015; Castan et al., 2018; Gomes-Junior, 2019).

Considering the diversity and technological expansion for non-destructive evaluation of seed quality, this review was developed focusing on techniques of two-dimensional and three-dimensional (X-ray micro-computed tomography) radiographic images, magnetic resonance imaging, spectral imaging, chlorophyll fluorescence imaging, infrared thermography, and computerized seedling image analysis, which are potentially viable for detecting and evaluating seed parameters that may be related to seed quality, with a special emphasis on the soybean species.

IMAGING TECHNOLOGIES APPLIED FOR SOYBEAN SEEDS

Radiographic imaging and micro-computed tomography

The radiographic image is obtained from the interaction of electromagnetic waves of X-rays with the material, with wavelengths ranging from 0.01 nm to 10 nm (X-rays) and energy from 0.12 to 120 keV (Kotwaliwale et al., 2014; Xia et al., 2019). The penetrating power of the X-rays are affected by the structure, composition, and density of the sample, which causes part of the radiation to be absorbed and transmitted in different proportions, culminating in the formation of a latent two-dimensional (2D) image. The image appears in gray levels, in which light regions correspond to the densest areas of the sample, where the X-rays are more absorbed; the dark regions refer to the areas of the sample with lower density, where the X-rays are less absorbed (Bino et al., 1993; ISTA, 2021).

The X-ray technique allows non-destructive observation of changes in the density of the sample that may be related to its structure; this technique is ideal for evaluation of different materials, such as seeds from different plant species. Simak and Gustafsson (1953) were pioneers in the use of the X-ray technique for evaluation of the morphology of *Pinus sylvestris* L. seeds, and they identified abnormalities in the embryo that were directly related to decline in germination of the species. Furthermore, they found that by using a low dose and a short exposure time, the radiation emitted was not sufficient to bring about genetic mutations and negatively affect seed germination (Simak and Gustafsson, 1953; Carvalho et al., 2009).

Officially, the use of the low energy X-ray test for evaluation of seed physical quality came to be recommended by the International Seed Testing Association - ISTA in 2004, proving to be a fast and non-destructive method directed to analysis of seed structure. It allowed detection of seeds that were filled, empty, malformed, mechanically damaged, and/or infested or damaged by insects and fungi (ISTA, 2004). This review shows that the X-ray technique is promising for detecting soybean seeds with mechanical damage (Figures 1a and 1b), weathering damage arising from continuous cycles of absorption and loss of water by the seeds in the post-maturity production field (Figure 1c), damage caused by insects (Figure 1d), malformed seeds (Figure 1e), and seeds with retracted cotyledons, forming an open inner space (Figure 1f). These changes in their different forms are related to loss of properties of a physical, physiological, and seed-health nature that constitute seed quality and can directly affect establishment of the initial stand of soybean plants in the field, compromising yield and the level of economic return (Flor et al., 2004; Pinto et al., 2009; 2012; Chelladurai et al., 2014).

Although the radiographic image analysis technique allows detection of different types of damage that can be related to loss of soybean seed viability, it is not possible to precisely evaluate the depth and extent of the damage, since it is a transmissible method and the radiographic image is a single 2D projection of the seed (Gomes-Junior, 2019). For example, from analysis of the radiographic image of Figure 1d, it is not possible to identify if the damage resulting from insects occurred in only one of the cotyledons of the seed or in both, much less identify the depth of this damage. Given this situation, the use of tomographic methods, such as X-ray micro-computed tomography makes analysis of radiographic images valid and effective for precise detection of the extent and depth of damage in soybean seeds.

X-ray micro-computed tomography is a technique that uses a microfocus X-ray source, a rotation system, and a detector consisting of sensors, like those of the charge-coupled device (CCD) type, that scans in a line of X-ray photons to reproduce a radiographic image of expanded size (Chen et al., 2013). In the microtomographic image, the X-ray photons directed at an object in rotation are attenuated by its density and chemical composition, and the remaining radiation is detected by the CCDs and transformed into various 2D projections (attenuation profiles), each one related to the different points of the radiographed object. Joining the 2D images (arising from the process of mathematical reconstruction of the 2D projections) generates a high-resolution three-dimensional (3D) image (sections in the coronal, transaxial, and sagittal directions) of at least 50 micrometers, which provides details of the internal microstructure of the object (Gomes-Junior and Van Duijn, 2017; Hanna and Ketcham, 2017; Cengiz et al., 2018; Porsch, 2020).

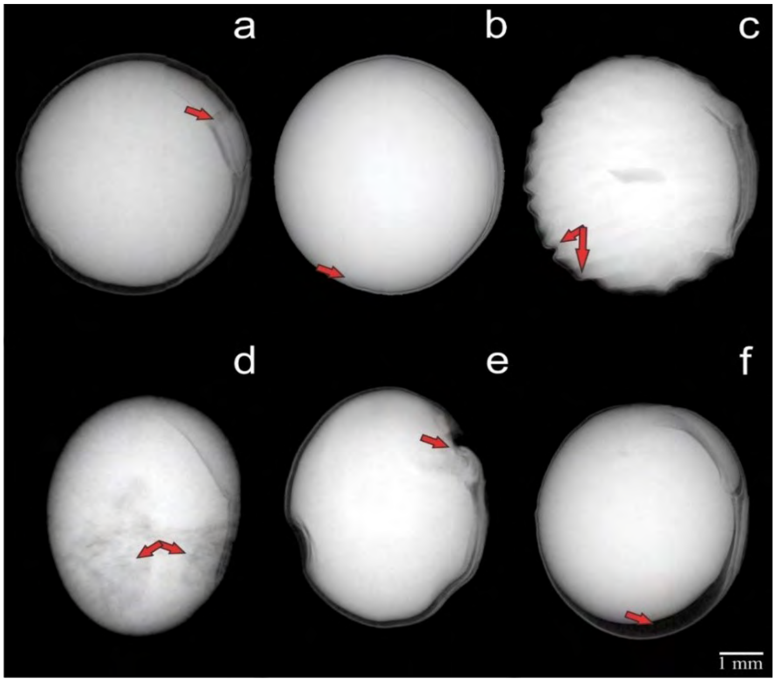

Figure 1. Radiographic images of soybean seeds with (a) mechanical damage in the hypocotyl-radicle axis region, (b) microcrack in the cotyledonary region, (c) wrinkling in the seed coat, cotyledons, and axis of the embryo resulting from weathering damage, (d) spots on the cotyledon(s) resulting from insect attacks, (e) malformation of the embryonic axis, and (f) retraction of the cotyledons, with formation of free space between the cotyledons and the seed coat.

Due to its non-destructive characteristics, computerized X-ray tomography has been used in studies involving seeds, allowing the outside and inside microstructure of seeds to be visualized and characterized with a high degree of resolution and contrast. These microstructures include the characteristics of the hilum and the kinetics of common bean (*Phaseolus vulgaris* L.) seed hydration (Gargiulo et al., 2020), phenotyping of quinoa (*Chenopodium quinoa*. Willd.) seeds (Gargiulo et al., 2019), density and volume of the inner structures of maize (*Zea mays* L.) seeds (Guelpa et al., 2016), mechanical damage in maize seeds in association with germination performance (Gomes-Junior et al., 2019), and porosity in *Pinus* sp. (Ham et al., 2017) seeds, as well as infestation and damage from coffee berry borer (*Hypothenemus hampei* Ferrari) in coffee (*Coffea canephora* Pierre) (Alba-Alejandre et al., 2018).

In soybean seeds, X-ray micro-computed tomography shows great potential for use, especially for characterization and identification of mechanical damage and damage caused by insects. However, this technique has not been widely used in evaluating changes in soybean seeds that may be related to germination performance. This paper shows the viability of computerized X-ray tomography for evaluating soybean seeds with mechanical damage (Figure 2a) and those damaged by insects (Figure 2b). The radiographic image of the seed shown in Figure 2a is the same seed shown in Figure 1b. As highlighted above, as radiographic imaging is a transmissible method, it corresponds to a single 2D projection of the seed tissues, which limits visualization of the microcrack in the cotyledonary region. However, X-ray micro-computed tomography allowed the inner morphology of the seed to be inspected in different sections, such that visualization of the microcrack became clear, as observed in the coronal and transaxial sections of this seed (Figure 2a). In addition, it revealed that the microcrack occurred in only one of the cotyledons, and it even allowed the depth of this damage to be characterized, as can be observed in the coronal section (Figure 2a1).

In the case of Figure 2b, which represents a seed with damage caused by insects, the radiographic image identified the regions attacked by the insect (spots with darker shades of gray, indicated by the arrow). However, the X-ray micro-computed tomography sections allowed clearer visualization of the damage than the radiographic image. Clear differences in the degree of attenuation of the X-rays (gray levels) were observed between the healthy regions and those

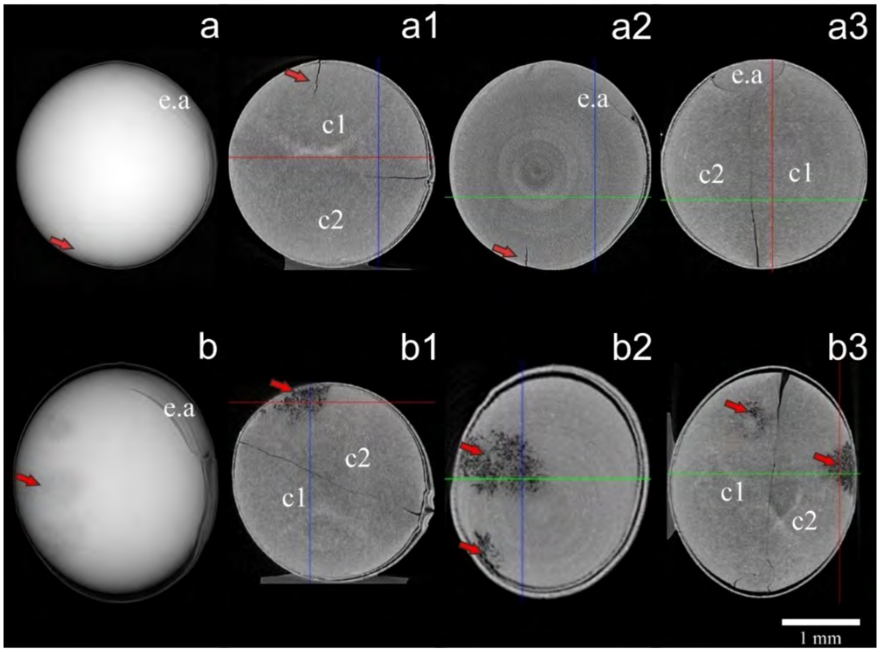

Figure 2. Two-dimensional (2D) radiographic images of soybean seeds with a microcrack (a) and damaged by insects (b) and X-ray micro-computed tomography images of the same seeds radiographed and visualized in 2D sections in the coronal (a1 and b1), transaxial (a2 and b2), and sagittal (a3 and b3) planes; the areas with injuries are indicated with red arrows. c1 and c2 – cotyledons 1 and 2, respectively; e.a – embryonic axis.

damaged by the insect (Figures 2b1, 2b2, and 2b3). Analysis revealed that the damage occurred in both cotyledons, according to the 2D sagittal section (Figure 2b3). Furthermore, analysis of 2D sections from microtomography allowed the depth of the damage (c2 in Figures 2b1 and 2b3) and its spatial distribution (Figure 2b2) to be characterized.

Magnetic resonance imaging

The magnetic resonance image is based on the physical properties of the nuclei of determined chemical elements of a sample under analysis, such as ^{1}H, ^{13}C, ^{19}F, ^{23}Na, ^{31}P, and ^{39}K, such that exposure of these nuclei to a strong magnetic field leads to excitation of these elements through pulsed radiofrequency waves. When the radiofrequency pulse is disconnected, the excess energy released by the nuclei emits signals that are captured by a coil and are converted into an image using computational systems and mathematical modeling (Hage and Iwasaki, 2009; Mazzola, 2009; Borisjuk et al., 2012; Munz et al., 2016).

Among these different atomic species, the most used is the hydrogen nucleus (^{1}H) for acquisition of images, due to its great abundance in living organisms and also since it has ½ spin, which generates only two energy levels, facilitating definition of the procedures for obtaining images (Smith and Ranallo, 1989). Thus, most of the studies on magnetic resonance imaging have been performed from hydrogen protons of the water molecule or from lipid molecules present in plant tissues. They are monochromatic images to which a color map can be added and whose gray levels vary according to the spin density in certain areas of the sample, to the water/lipid concentration in the tissues (the more concentrated, the greater the intensity of the signal) and/or to the times of relaxation of the tissues of the sample (Magalhães, 1999; Mazzola, 2009; Borisjuk et al., 2012; Munz et al., 2016).

Since magnetic resonance imaging is a non-invasive and non-destructive technique, studies conducted in the laboratory have indicated its viability for evaluating the physical-chemical state of plant samples and relating such characteristics to plant metabolism (Forato et al., 2009; Munz et al., 2016). For example, the effects of imbibition were investigated on germination of seeds of pepper (*Capsicum annuum* L.) (Foucat et al., 1993), wheat (*Triticum aestivum* L.) (Gruwel et al., 2004), and soybean (Koizumi et al., 2008), lipid deposition in soybean (Borisjuk et al., 2005) and cotton (*Gossipium* sp.) seeds (Horn et al., 2012), as well as evaluation of the metabolism of rapeseed (*Brassica napus* L.) seed germination (Munz et al., 2017). In light of the above, this paper shows that the use of magnetic resonance imaging was effective for evaluating the hydration of soybean seeds and identifying changes in seed tissues related to deterioration (Figure 3). The images acquired in soybean seeds hydrated until reaching 18% water content (wet basis) showed signal hyperintensity (high brightness) in the form of branching, characterizing the cotyledon veins (Figure 3b). Secondary veins and a central vein directed toward the embryonic axis are seen. This result represented a promising basis for studies on soybean seed physiology, since magnetic resonance imaging is a non-destructive and non-invasive procedure, making it possible to perform germination tests with the seeds analyzed. Although radiographic analysis is also a non-destructive method, it did not allow identification of these cotyledonary veins (Figure 3a), since it is based only on the density and chemical composition of the tissues for image formation. Furthermore, magnetic resonance imaging allowed identification of regions in the seeds related to the injuries caused by insect attack (Figure 3d), which were not easily seen on the radiographic image (Figure 3c).

Image spectroscopy

Recently, imaging techniques based on spectroscopy have been widely used for evaluation of seed quality. This method can have two configurations regarding the images generated: characterized as multispectral imaging when acquired in discrete spectral bands and as hyperspectral imaging when acquired in narrow and contiguous bands forming a continuous spectrum.

The hyperspectral image sensors can provide information that may range from the ultraviolet region to the near-infrared range (380 to 2500 nm) of the electromagnetic spectrum, as well as spatial and textural data, which allows a complete and reliable analysis of the intrinsic properties and morphological characteristics of the digitalized object

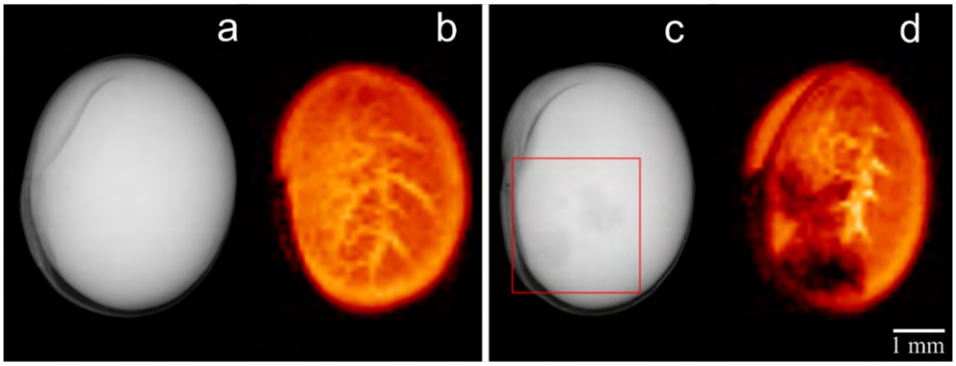

Figure 3. Radiographic images of soybean seeds without damage (a) and seeds damaged by insects (c) with 9% water content, and magnetic resonance imaging (in false color) of the same seeds (b; d) after hydration until reaching 18% water content. In the radiographic image, the spots in slightly darker shades of gray delimited by the red square (c) and black shades (d) are tissues with lower metabolism (dead tissues) resulting from damage caused by insects and, consequently, lower intensity of the magnetic resonance signal.

(Kandpal et al., 2016). As a result of the possibility of covering the entire near-infrared range, the hyperspectral imaging technique allows measurement of the chemical properties of the seeds. This technique has been effectively used for characterization of viable and inviable soybean seeds (Baek et al., 2019).

In spite of the viability of multispectral imaging in evaluation of physical-chemical characteristics associated with seed quality, studies using hyperspectral imaging still predominate in the literature. That is because the main objective of the use of hyperspectral imaging is identifying specific wavelengths for establishing a multispectral imaging system as an essential part of a computer-integrated machine-vision system for different applications (Sendin et al., 2018), which would mean less time for image acquisition and processing.

The multispectral imaging technique uses a remote sensing system with LED (Light Emitting Diode) or laser type lighting of electromagnetic spectra from the ultraviolet radiation band (≥ 200 nm) to the near-infrared range (≤ 1000 nm), with the use of discrete filters (generally from 3 to 20 non-contiguous bands). When that light is directed on the target object, it is reflected and converted by a CCD monochromatic sensor into spectral images that represent specific physical-chemical properties of the material analyzed (Boelt et al., 2018; ElMasry et al., 2019; França-Silva et al., 2020).

The multispectral imaging technology has been used to evaluate different species of seeds, such as to relate seed size and embryo length with seed germination in spinach (*Spinacia oleracea* L.) (Shetty et al., 2012), to estimate the viability of castor bean (*Ricinus communis* L.) seeds (Olesen et al., 2015), to differentiate tomato (*Solanum lycopersicum* L.) cultivars (Shrestha et al., 2015), to identify surface mechanical damage in beet (*Beta vulgaris* L.) seeds (Salimi and Boelt, 2019), to detect the *Drechslera avenae* (Eidam) Sharif fungus in black oat (*Avena strigosa* Schreb) seeds (França-Silva et al., 2020), and to detect the *Fusarium pallidoroseum, Rhizoctonia solani*, and *Aspergillus* sp. fungi in cowpea (*Vigna unguiculata* (L.) Walp) seeds (Rego et al., 2020).

Since the multispectral imaging technique is viable for detecting and estimating distinct parameters related to the quality of various species of seeds, this paper demonstrate that the use of this technology was also effective for detecting *Cercospora kikuchii*, insect damage, ruptures in the seed coat, and surface mechanical damage in soybean seeds (Figure 4). Based on conventional images obtained in the visible red-green-blue (RGB bands) spectrum, some darker spots (red arrows) are observed on the seed coat with *C. kikuchii* (Figura 4a), but they were clearer with the use of multispectral imaging (Figure 4a1). In a similar way, the change in color observed in the seed coat related to the area damaged by insect attack (Figure 4b) became more perceptible after the acquisition of multispectral imaging (Figure 4b1).

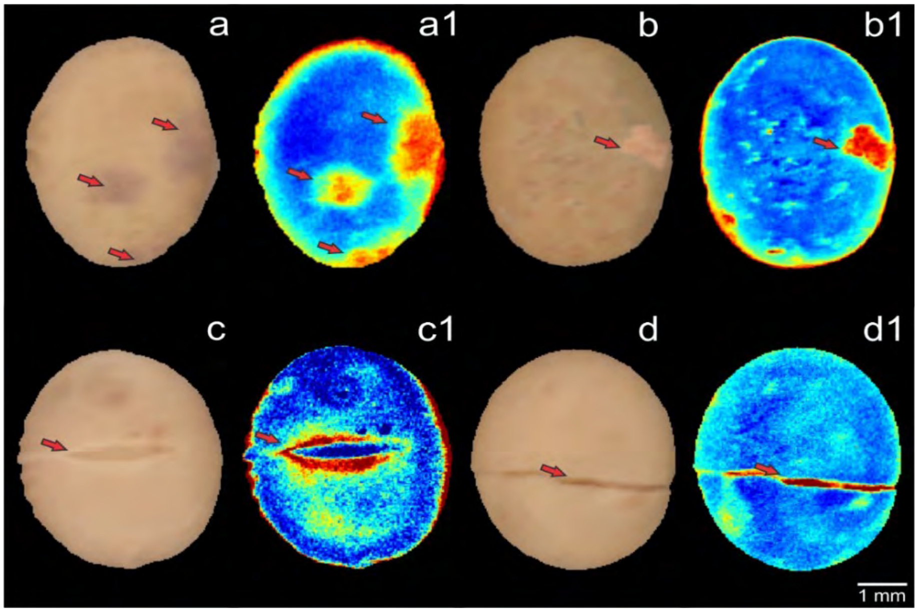

Figure 4. Red-green-blue (RGB) image (a) and multispectral image (a1) of soybean seeds with purple spot (*C. kikuchii*), seed with insect damage observed in RBG image (b) and multispectral image (b1), rupture in the seed coat of soybean observed with RGB image (c) and multispectral image (c1), and visible mechanical damage in the seed coat in the RGB image (d) and multispectral image (d1). In the multispectral images, the false colors red, orange, and yellow indicate the damaged regions (arrows), while the false color blue represents the region of the seed without damage.

The rupture of the seed coat of soybean seriously affects its physiological potential. This defect has genetic causes and is related to meteorological conditions during seed formation and maturation. Multispectral imaging was also effective in identifying this problem in soybean seeds, with greater effectiveness in visualization in relation to the RGB image (Figure 4c and 4c1). This was also seen in identification of surface mechanical damage, characterized by ruptures in the seed coat of soybean (Figures 4d and 4d1).

The use of image spectroscopy for evaluation of soybean seeds makes it possible to identify intact seeds, damaged seeds, and other impurities in the sample. In addition, the seed samples can be reused for conducting other tests (the germination test, for example). That way, the results obtained from multispectral imaging can be associated with the physiological potential of the seeds examined (Olesen et al., 2015; Boelt et al., 2018; ElMasry et al., 2019; Salimi and Boelt, 2019; França-Silva et al., 2020; Rego et al., 2020).

Chlorophyll fluorescence

The chlorophyll fluorescence technique is based on the intrinsic fluorescent properties of the chlorophyll *a* and *b* molecules in living plant tissues that occur after exposure of the plants to the technology of LED or laser lighting, with spectral lengths from 400 nm to 750 nm, CCD monochromatic sensors with different spectral filters, and a detector sensitive to the electromagnetic spectrum (lock-in amplifier) (Jalink et al., 1998; Ooms and Destain, 2011; Wang et al., 2018). After rapid emission of pulses (µs) of light with 430 nm and 660 nm, the chlorophyll *a* molecules are excited and absorb the light energy, where they reach a more excited and therefore less stable state, which leads to maximum fluorescence (Zelitch, 1971). Upon returning to their fundamental state (less energy), the accumulated excess of energy is released by heat dissipation and corresponds to the fluorescence, the signals of which are captured between 660 nm and 730 nm, depending on the equipment, and processed on specific software programs (Jalink et al., 1999; Jalink and Van Der Schoor, 2011).

Detection of chlorophyll fluorescence is a rapid and non-invasive technique that has been effective in evaluating the degree of maturity of kale (*Brassica oleracea* L.) seeds (Jalink et al., 1998; Yadav et al., 2015) in association with physiological potential, in studying the effects of retaining chlorophyll in maturation and storage of *Arabidopsis thaliana* L. seeds (Nakajima et al., 2012), in determining the harvest time of rice (*Oryza sativa* L.) seeds, aiming at storage longevity (Hay et al., 2015), and in evaluating the maturity of tomato seeds (Li et al., 2016).

A frequent problem in soybean seed production fields, especially in tropical regions, is the occurrence of seeds with high retention of non-degraded chlorophyll at the end of maturation, which have lower germination and vigor than those with complete maturity. A pioneering study conducted by Cicero et al. (2009) using a device based on excitation LEDs and optical sensors confirmed the effectiveness of the chlorophyll fluorescence technique for identifying and separating greenish soybean seeds mixed with mature seeds and for enhancing the physiological potential of the seed lots evaluated. Thus, this paper shows the application of the chlorophyll fluorescence imaging technique for detecting greenish soybean seeds (with different rates of retained chlorophyll) and quantifying their fluorescence signals (Figure 5).

The seeds were harvested, and the evaluations were made after three months of storage in a controlled environment (20 °C and 55% relative humidity). The mature seed category (yellow seeds) was denominated Y, and

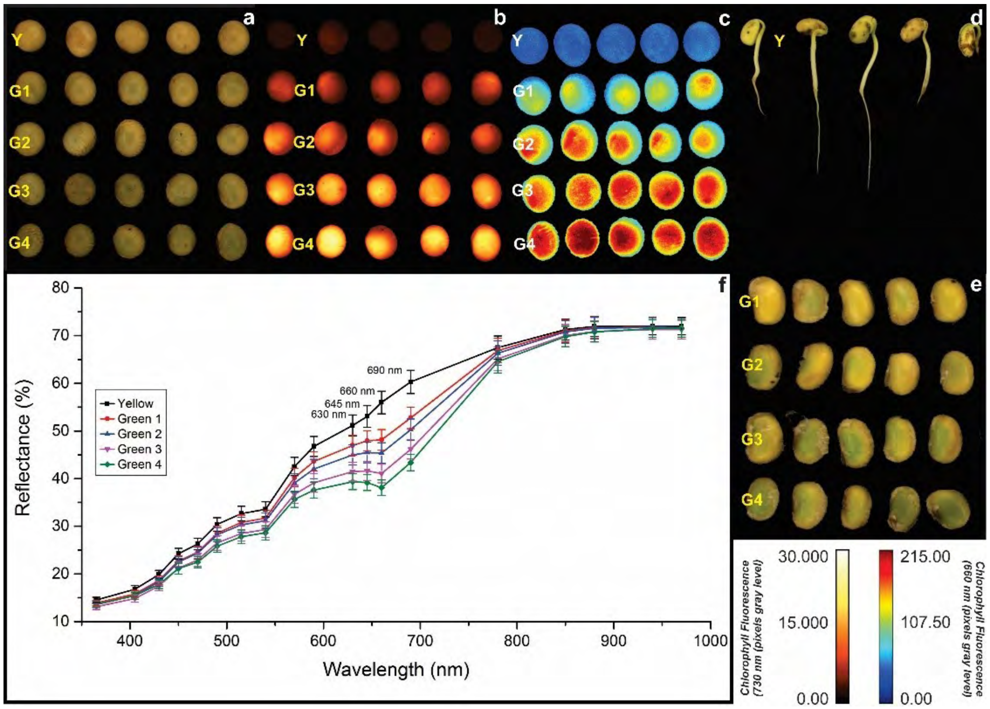

Figure 5. Red-green-blue (RGB) images of soybean seeds of the cultivar SYN 1163 RR with different levels of retained chlorophyll (a), chlorophyll fluorescence images of the same seeds obtained by multispectral equipment operating at the spectral wavelength of 730 nm (b) and 660 nm (c), images of the germination test showing the soybean seedlings coming from the yellow seeds without retained chlorophyll (d), and dead seeds that had chlorophyll retention (e). Spectral differences of the yellow seeds with a mean rate of chlorophyll fluorescence points equal to 4970 (Y) and greenish seeds with mean rates of 11,871 (G1 – Green 1), 17,153 (G2 – Green 2), 24,106 (G3 – Green 3), and 28,424 (G4 – Green 4) (f), with the most expressive differences at the wavelengths of 630 nm, 645 nm, 660 nm, and 690 nm. In the images, the color map was attributed based on the gray levels, where the shades of colors from red to white (730 nm) and from light blue to red (660 nm) represent the greenish seeds with high rates of chlorophyll retention and that emitted greater signals of chlorophyll fluorescence.

the green levels (based on visual analysis) of the seeds were denominated G1, G2, G3, and G4, such that the larger the number with the letter G, the greater the intensity of green (Figure 5a). The chlorophyll fluorescence signals captured by a sensor at the wavelength of 660 nm (Figure 5b) showed higher intensities for the seeds of the G3 and G4 categories, with mean values of the pixels of the seed image corresponding to 24,106 and 28,424 on a scale from 0 (without fluorescence) to 65,535 (maximum fluorescence signal). In a similar manner, the measurements made by a sensor at 730 nm (Figure 5c) showed an increase in chlorophyll fluorescence intensity (blue to red color) in the categories of higher levels of greening.

In summary, the greater the intensity of green in the seed coat, the greater the chlorophyll fluorescence signals and the worse the physiological performance of the seeds. Only the category Y seeds germinated, showing that regardless of the rate of chlorophyll retained in the seed coat, there is total loss of germination after 3 months of storage. These results show the importance of identification and separation of greenish seeds for improving the quality of the seed lots, which can be performed with high effectiveness using the chlorophyll fluorescence image technique. However, as can be observed in Figure 5f, even if a chlorophyll fluorescence imaging system is not available, it is possible to identify the greenish seeds using a sensor that captures the seed reflectance signals operating in the visible band of the electromagnetic spectrum (especially from 630 to 690 nm).

Infrared thermography

Infrared thermography is a technology based on infrared radiation (with wavelengths ranging from 0.74 µm to 1000 µm) emitted by an object that has temperature greater than absolute zero (273.15 °C) (Ishimwe et al., 2014). From a system that uses a thermal infrared camera and digital sensor (CCD) that detects infrared radiation, the invisible radiation emitted by the object is captured in the form of pseudo-color images that correspond to the temperature gradients of the object analyzed, and such pseudo-color images can be converted into thermal images or two-dimensional thermograms. From these images, information from the target object can be extracted and analyzed without establishing contact (Gowen et al., 2010; Al-Doski et al., 2016; Xia et al., 2019; ElMasry et al., 2020).

The thermographic image analysis method has been widely researched for different agricultural applications and has been used to estimate the viability of seeds of lettuce (*Lactuca sativa* L.) (Kim et al., 2013), pepper (Kim et al., 2014), and pea (*Pisum sativum* L.) (Men et al., 2017), to detect *Aspergillus* spp. and *Penicillium* spp. fungi in wheat grain (Chelladurai et al., 2012), to examine infestation by insects in wheat, maize, broad bean (*Vicia faba* L.), and white bean (*Phaseolus vulgaris* L.) grain (Ibrahim et al., 2020), and to discriminate varieties of wheat grain (Manickavasagan et al., 2010).

This paper demonstrates the use of thermographic image analysis to evaluate the imbibition of a sample of soybean seeds with 95% germination before and after artificial aging (96 h at 41°C and 76% relative humidity). The seed imbibition process of the two groups was monitored using a thermographic camera with spectral sensitivity of 7.5-13 µm, image resolution of 320 × 240 pixels, and thermal sensitivity lower than 0.030 °C (30 mK) at 30 °C. Results showed that the aged seeds had a mean surface temperature higher than the non-aged seeds. The thermographic images of Figures 6a and 6a2 correspond to the soybean seeds from the original sample after soaking for 6 h and 12 h in rolls of paper towel at 25 °C. Comparison of these images with those obtained from aged seeds (Figures 6a1 and 6a3) shows that the surface temperature of the aged seeds was approximately 0.5 °C higher, which on the color scale appear predominantly pink and red. These results show the potential of this technique in characterizing soybean seed lots with differences in physiological potential.

This paper also shows the infrared thermography to evaluate images of soybean seeds with damage caused by insects in comparison with images obtained by X-rays and magnetic resonance. Through infrared thermography image analysis, the regions with damage resulting from insect attack had higher temperatures compared to the intact areas of the cotyledon (Figure 6b3), showing that infrared tomography can be used to identify deteriorated regions of the seeds associated with variations in metabolism.

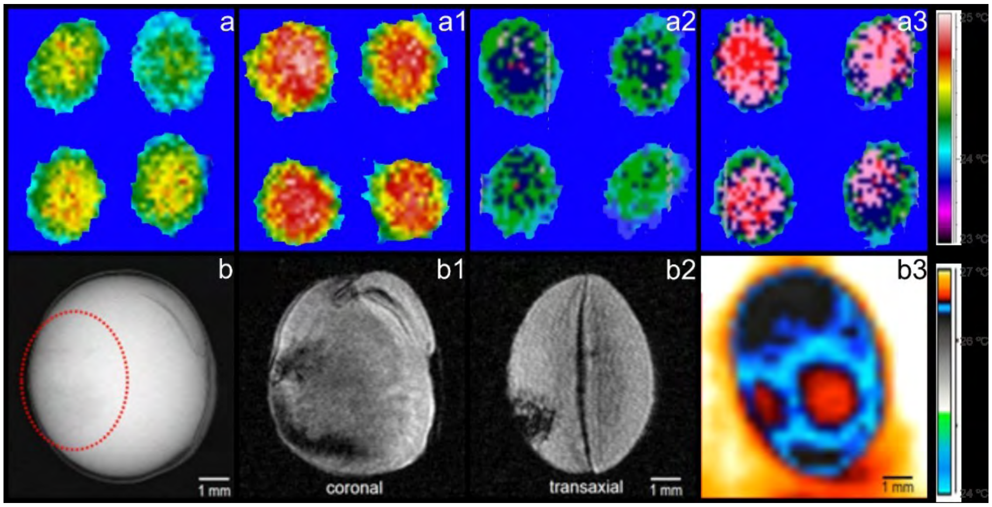

Figure 6. Thermograms of soybean seeds without aging (a and a2) and after artificial aging (a1 and a3) obtained after 6 h (a and a1) and 12 h (a2 and a3) of imbibition at 25 °C. Radiographic image of a soybean seed with insect attack (region defined by the ellipse) (b), and its respective magnetic resonance images in the coronal (b1) and transaxial (b2) sections and thermographic image (b3).

Computerized seedling image analysis

Seed vigor evaluation is fundamental for identification of seed lots with greater potential for establishing adequate plant stand in the field and obtaining high yields. To achieve this goal, information on the physiological potential of the lots tested must be highly consistent. Vigor tests are essential components of quality control programs of seed production companies since they can detect variations in the degree of deterioration of seed lots even when they have similar germination rates (Baalbaki et al., 2009). There has been a significant advance in recent years in the areas of computer science, promoting development of automated procedures for seed vigor analysis. In soybean, an example is digital processing of images associated with machine-learning algorithms, which have been applied to evaluate seed vigor based on measurements of seedling growth.

Computerized systems based on seedling growth, aiming at determination of seed lot vigor, have been ever more frequent in the laboratories of companies that produce and sell soybean seeds. An example of this method is represented by the Seed Vigor Automated Analysis System (*Sistema de Análise Automatizada do Vigor de Sementes*) - Vigor-S (Figure 7). In this analysis system, two rows of ten seeds are distributed on two sheets of moistened paper towel and then covered with a third sheet, which is then made into a roll. The rolls for germination are kept in a seed germinator for 72 h at 25 °C (Figure 7a). After that period, images are captured using a scanner fixed in an inverted position within a metallic box to expedite the image capturing process (Figure 7b). In this step, the seedlings are transferred from the roll of paper towel to a blue-colored background to provide clear contrast, allowing the segmentation process of the seedling images to occur adequately and successful analysis by the software. After digitalization and processing of the images by specific software, the seedling parts are identified and marked (Figure 7c). The system provides data on the length of the hypocotyl, the primary root, and the whole seedling and on the hypocotyl/root ratio, as well as indices of vigor, growth, and uniformity of seedling development, which range from 0 to 1000 and are directly proportional to the vigor of the sample. Studies developed by Rodrigues et al. (2020) showed consistency of Vigor-S analysis in discrimination of soybean seed lots regarding vigor, with results equivalent to the emergence of seedlings in the field and other tests conventionally used for this species, such as accelerated aging and tetrazolium tests.

In addition to ranking the lots in regard to vigor level, computerized seedling image analysis can also be used in monitoring the physiological potential of soybean seed lots during storage, in selection of genotypes with greater

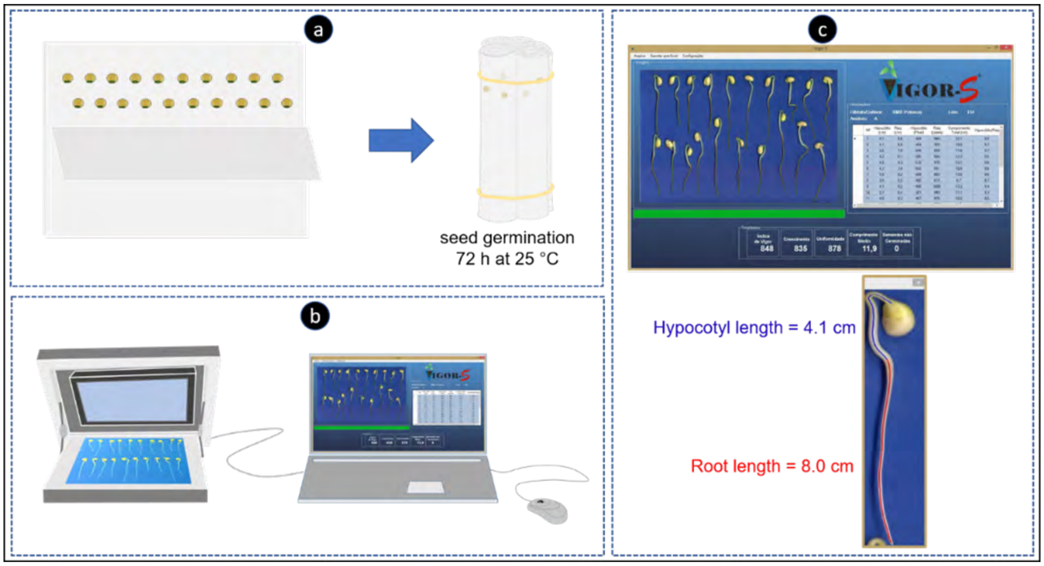

Figure 7. Seed Vigor Automated Analysis System (*Sistema Análise Automatizada do Vigor de Sementes* - Vigor-S) for soybean: representation of the step of setting up the germination test (a), of the devices for capturing (metallic box with scanner) and storage/processing (computer) of the seedling images (b), and of the analysis window, showing soybean seedlings digitalized and processed by the software (c), highlighting the first seedling of the sample with the hypocotyl marked in blue and the primary root in red.

growth rates and greater potential for rapid emergence of seedlings in the field and/or with greater tolerance to high temperatures or water deficit, and in evaluation of the effects of application of biostimulants or of the toxicity of fungicides, insecticides, and herbicides to growth of seedlings or parts of seedlings (Gomes-Junior, 2020). A recent study showed that computerized seedling image analysis is an effective resource with high sensitivity for evaluating the toxicity of the chemical treatment to soybean seeds (Oliveira et al., 2021).

Computerized seedling image analysis has various advantages in relation to other vigor tests. First, in comparison to analysis of seedling growth performed manually with the aid of a ruler, automated analysis allows greater accuracy and speed in evaluations of seedling length, and it reduces the time required for obtaining results. Furthermore, analysis provides standardization among different laboratories and analysts, constituting a significant advantage in relation to vigor tests interpreted only by the analyst; that is, human interference is reduced. The possibility of storage and sharing of information in the form of digital images is another important convenience of computerized seedling image analysis in relation to filing numerical data, as occurs in traditional tests for vigor evaluation.

CONCLUSIONS

This paper evidenced the application of different imaging techniques for characterizing problems associated with the loss of soybean seed quality. Soybean is one of the most important grain-production crops directed toward the oil and meal industry. Thus, setting up the crop with superior quality seeds is fundamental for adequate establishment of stand in the field and for ensuring high grain yields. However, due to its morphological and physiological characteristics, soybean seeds are highly prone to deterioration and sensitive to environmental adversities during maturation and to inappropriate practices in harvest, processing, and storage management, as was shown by the occurrence of different types of damage (mechanical, by weathering, by insects) and problems during maturation associated with malformation of the seed and the occurrence of greenish seeds. Consequently, rapid and effective procedures are increasingly necessary for identification of problems associated with loss of seed quality.

Techniques based on the optical sensors described in this review proved to be effective in evaluating soybean seeds and identifying the main problems associated with loss of quality in soybean in both pre- and post-harvest. Imaging in different wavelengths, it was possible to establish more complete analysis of the seeds and to characterize a single problem in different ways, as for example, the occurrence of damage caused by insects visualized by X-ray microtomography, magnetic resonance imaging, multispectral imaging, and infrared thermography. However, it should be noted that the methods used have specific particularities for identifying determined characteristics with greater accuracy and potentialities for improving evaluation of seed quality. Radiographic analysis showed potential for identifying changes in the structure and density of seed tissues. Magnetic resonance imaging can be very useful for identifying physical and metabolic changes. Image spectroscopy can provide important information regarding changes in color and chemical composition associated with loss of seed quality. Chlorophyll fluorescence allowed effective identification of seeds with low physiological potential characterized by greenish shades due to retention of chlorophyll. Infrared thermography is able not only to effectively characterize changes in metabolism associated with insect damage, but can also be an alternative in discriminating seed lots or individual seeds in a population regarding vigor. Finally, the computational vision based on seedling analysis represents an important strategy for increasing the accuracy and efficiency of evaluations of the vigor of soybean seed lots in quality control programs.

In spite of increasing investigation using imaging techniques for evaluating seed quality, with the advantages of being non-invasive, non-destructive, rapid, and high accuracy procedures, there is still the limitation of use of these techniques by the seed industry due to the high cost of the technology and the need for greater efficiency in evaluating high volumes of seeds in a short period of time. Except for computerized seedling image analysis, which uses lower cost equipment and materials, the other image analysis techniques described here have high costs and are currently far out of reach of most seed production companies.

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