Plant Archives Vol. 25, No. 1, 2025 pp. 289-294 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.no.1.043

THE FUTURE OF SEED QUALITY ANALYSIS: MULTISPECTRAL IMAGING AS A NON- DESTRUCTIVE TOOL

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(Date of Receiving-30-11-2024; Date of Acceptance-30-01-2025)

ABSTRACT

The seed production sector as well as agriculture need to upgrade from the classical testing methods to quick and non-destructive methods for evaluating the quality of the products. Spectral imaging modalities, a synergistic integration of spectroscopy and imaging technologies, have emerged to address quality evaluation challenges by setting forward various designs with useful and practical applications in food and agriculture. Multispectral imaging (MSI) systems encompass light sources providing a restricted number of wavelengths, which will dispense the specific spectra. With the advantage of obtaining spatial and spectral data across a wide range of the electromagnetic spectrum, the futuristic multispectral imaging in combination with different multivariate chemometric analysis scenarios has been successfully implemented not only for food quality and safety control purposes, but also in handling critical research challenges in seed science and technology. This review will provide an overview of all current methods for acquiring, processing and reproducing multispectral images for use in a various kinds of seed quality assessment applications.

Key words : Image analysis, Multispectral imaging, Quality analysis, Seed testing, Spectral imaging.

Introduction

Seed quality is a multi-constitute characterization of seeds, which comprise of varietal and analytical purity, germination capacity, vigour, seed health and uniformity. Conventionally, in most of the quality control programmes morphological and phenotypic attributes that are recorded by employing physical and chemical along with visual inspection methods, the biochemical, genotypic and molecular markers are destructive and time consuming. The visual inspection of seeds individually by workers is very monotonous and a time- consuming process and it is sometimes less than satisfactory, which in succession results in inspection errors (Li *et al*., 2018).

In order to improve seed quality analysis both the Association of Official Seed Analysts (AOSA) and the International Seed Testing Association (ISTA) acknowledge the significance of creating new technologies for quick, non-destructive seed quality determination and lower the overall cost of labor-intensive tests (Boelt *et al*., 2018). Recently, imaging techniques are gaining a lot of interest for monitoring and evaluating quality have recently drawn a lot of interest in the seed industry (ElMasry *et al*., 2009). Imaging-based methods can be regarded as effective tools for testing and assessing individual seeds in order to examine the imbibition process, look into the germination capacity, and identify changes in vigor between seed lots, in addition to their excellent ability to assess the overall quality parameters of seed lots (Dell'Aquila *et al*., 2007). Currently, the availability of inexpensive imaging equipment, the increasing processing capacity of modern computers, and rising interest in agriculture are all contributing to the growing use of imaging in many agricultural applications.

Multispectral imaging

Multispectral imaging (MSI) renders calibrated reflectance measurements at several 'discrete' wavelength bands distributed over a wide spectral range, from ultraviolet (UV) to near infrared (NIR) region.

Multispectral imaging of seeds is done by imaging their surface reflectance at selected wavelengths from 365 to 970 nm. It measures the reflectance at fewer (<50) and wider discrete wavelengths (10-50 nm). The subsequent multispectral image is a stack of numerous greyscale sub-images representing the precise relative light reflectance at numerous non-overlapping "discrete" wavelength bands spanning a range beyond human vision. Consequently, the process of obtaining a multispectral image is sometimes referred to as multi-channel imaging due to its ability to record picture data at particular multi-wavelengths throughout the electromagnetic spectrum, which offers the details needed for the identification and characterization of the constituent parts of the seeds under study (Panagou *et al*., 2014). According to variations in spectral signature and common morphological characteristics, this technology may be used in quality assessment of different agricultural products.

Methods of image acquisition

All spectral imaging systems, must collect data from target objects in the spatial and spectral domains, thus making it critical to provide an acquisition mechanism that allows the system to simultaneously scan the sample in both directions and record light intensity at each pixel at all wavelengths. On the basis of applications and the accessible optical devices, there are three methods of acquiring spectral imaging, viz. point scanning (whiskbroom scanning), line scanning (push-broom scanning) or area scanning (wavelength or plane scanning) (ElMasry *et al*., 2019).

The majority of applications use area scanning with a charged coupled device (CCD) imaging sensor and sequentially illuminating the seeds with LEDs in the desired waveband to acquire images. These wavebands must to be carefully selected in order to correspond with the application or research subject (Sendin *et al*., 2018). But a majority of MSI applications employ the same multipurpose MSI technology (use specific 19 bands), where the company specifies the spectral range and wavebands.

In spite of the methods of acquisition, a three-dimensional block of data known as the "data cube" or "spectral cube" Rijk is created at the conclusion of the scanning procedures for the complete target sample. It has two spatial dimensions (x, y) and one spectral dimension (ë). The first spatial direction is represented by the i index, the second spatial dimension by the j index, and the spectral dimension by the k index.

Illumination-based MSI systems

An array of twelve LEDs with various nominal emission wavelengths operates this demonstrative device. A computer using a USB link may automatically regulate the emission intensity of LEDs that are installed on a printed circuit board (PCB) (Cumpson *et al*., 2016). Using a preprogrammed microcontroller, each LED is sequentially turned on for a predetermined amount of time during image acquisition and timed with the camera trigger. As a consequence of turning on the LEDs one at a time, the monochromatic camera captured 12 separate greyscale photos, which were then combined to create a single multispectral image. The VideometerLab Company (Videometer A/S, Hørsholm, Denmark) currently manufactures similar designs that follow the same principle

VideometerLab Instrument

This device comprises of a 5 mega pixel CCD camera that is positioned within the topmost layer of an integrating sphere. The sphere is coated with a highly white paint that diffuses light, and narrowband high-power LEDs are positioned at the rim to provide uniform and diffuse lighting of the sample at the bottom port of the sphere reflection. Sequential strobes are produced by the LEDs at the following 19 wavelengths: 375, 405, 435, 450, 470, 505, 525, 570, 590, 630, 645, 660, 700, 780, 850, 870, 890, 940 and 970 (with a backlight at 625 nm below the sample holder). The diodes emit a narrow band of light confirming that only light of the specific wavelength is present when an image is acquired. These designs have been extensively used for various purposes, including quality tests of numerous products as well as seed science and technology (Panagou *et al*., 2014). This kind of LED illumination-based multispectral imaging system has received a lot of attention lately because of its affordability, quick controlled switching capability, and durability (Jaillais *et al*., 2015).

Workflow of MSI system

The workflow for MSI of seeds in general encompasses the following six steps:

(1) Preparation of sample

(2) Calibration of MSI system

(3) Acquisition of multispectral images

(4) Segmentation of ROIs

(5) Feature extraction from the segmented ROIs

(6) Analysis of the extracted features

Preparation of sample : Multiple seeds can be imaged simultaneously, due to the spatial nature of the multispectral images. Seeds are placed in a Petri dish by placing them equidistant from each other. Double-sided tape can be used to secure seeds in the Petri dish to keep them from moving (Rego *et al*., 2020). When placing the seeds, it is important to consider which side is most relevant for the application and thus should be facing the imaging sensor and in applications where multiple sides are equally relevant images from multiple sides can be achieved by imaging each seed multiple times (Salimi *et al*., 2019). In situations where the seeds don't need any extra preparation or manual evaluation, a conveyer belt can be utilized to automate the imaging procedure.

Calibration of MSI System: The MSI system must be calibrated prior to image acquisition which includes, both a radiometric calibration and a geometric calibration, which is achieved by imaging calibration targets with well-known reflectance and geometry (Hu *et al*., 2020). Calibration is carried out by using the three consecutive plates: a white plate for background correction, a dark plate for reflectance correction, and a dot plate for geometric pixel position alignment calibration before a light setup calibration.

Acquisition of multispectral images : The MSI system is prepared to image the prepared samples after calibration. A measurement's result is a multispectral image, or "data cube", made up of W × H pixels × C channels, where W and H stand for width and height of the image, respectively, and C channels are included in each pixel to represent the discrete multispectral bands. Pixel values indicate the chemistry above and below the surface of a seed in the tiny area that the pixel covers when a pixel position intersects with a seed (Hansen *et al*., 2016).

Segmentation of Regions-of-interest : The multispectral images consist of ROIs along with background items like the conveyer belt, Petri dish, background material, and other inert stuff. The segmentation process involves extracting the ROIs from the image and separating them from the background items. Using a simple threshold in a single channel, a sum of the channels, or on a score image produced CDA or PCA, segmentation can frequently be completed with a high contrast background material and enough space surrounding each seed.

Feature Extraction from the segmented ROIs : The characteristics, which comprise four classes based on how they characterize the seed and connect to the multispectral image, are reflectance, color, shape, and texture. They are usually extracted from the whole seed, but they can also concentrate on a particular area of the seed, particularly the endosperm region (De la Fuente *et al*., 2017). The reflectance and color features indicate the intensity of each of color or reflectance of the seed and are related to the spectral dimension (C). In order to extract a trimmed mean (Olesen *et al*., 2015) or a ratio of pixels over a specified threshold (Boelt *et al*., 2018), the reflectance features either treat the wavebands separately by obtaining first-order derivatives from the raw wavebands (Shetty *et al*., 2012) or combine them using a CDA transformation. On the other hand, the color features extract first-order characteristics from a well-defined color space, such as CIELAB (Shrestha *et al*., 2015), by combining wavebands that overlap with the human visual spectrum. Shape features are produced from the binary picture created during segmentation and are related to the spatial dimensions (W × H). They consist of basic descriptors like area, breadth, and length (Liu *et al*., 2014), as well as more intricate ones like elliptical fitting parameters and similarity to well-known basic forms like circles, ellipses and rectangles. Through the quantification of the spatial change in intensity across the seed, the texture features integrate the spectral and spatial dimensions. Changes in color in the seed surface pattern and slight variations in the surface structure (hills and valleys) can both contribute to this spatial variation in intensity.

Somewhat depending on the application, different features can be derived. The reflectance properties, as well as some color and texture features are used in all applications pertaining to the presence of fungi. Conversely, varietal purity applications almost exclusively use form, color, and reflectance parameters; Reflectance is preferred in applications pertaining to seed viability and vigor, and color and form are less important.

Analysis of the extracted features : A descriptive statistic and data modeling are frequently included in the multivariate data analysis of the extracted features. In MSI, a variety of linear and non-linear techniques have been applied to data modeling. The most popular techniques are partial least squares, support vector machines (SVM), PCA, CDA and to a lesser extent, neural networks and k-nearest neighbors.

Applications of MSI for seed quality assessment

This technique has demonstrated encouraging outcomes in identifying many attributes related to seed quality. By the way, using this technology to evaluate seed quality is mostly dependent on having a thorough grasp of the principles of the technology and knowing how to connect criteria related to seed quality with the data found in the images.

Varietal discrimination and seed purity : Multispectral imaging has been used in a number of species, including rice (*Oryza sativa* L.), soybeans, and tomatoes (*Solanum lycopersicum* L.) (Boelt *et al*., 2018). Subsequent research on pepper (*Capsicum annuum* L.) and alfalfa has been documented (Yang *et al*., 2020; Li *et al*., 2020).

A recent study evaluated the genetic diversity in a group of pigmented rice accessions from the Philippines using MSI by Mbanjo *et al*. (2019). The research found colored rice accessions, which are a useful genetic resource for enhancing commercial rice types in the future.

Vrešak *et al*. (2016) used a VideometerLab multispectral imaging system with 19 bands (375–950 nm) to acquire multispectral images of seed samples for the purpose of differentiating wheat and triticale varieties.

A genebank provided twelve different geographically derived alfalfa cultivars (Medicago sativa L.) by Yang *et al*. (2020). To categorize cultivars, several multivariate data analyses were performed. The accuracy of categorization was only 42-44% when morphological features were used alone; however, when spectral features were included, the accuracy increased to 92-23%.

Presence of Inert Matter and other seeds : According to Sendin *et al*. (2018), MSI can be used to identify plant debris and other crop seeds in white maize (*Zea maize* L.). Seeds of several crop species were accurately classified such as sunflower (*Helianthus annuus* L.), sorghum (*Sorghum bicolor* L.), wheat (*Triticum aestivum* L.) and soybean. Plant debris was also classified with 100% accuracy.

The distinction of sweet clover (*Melilotus* ssp.) in alfalfa with a classification accuracy of >99% by MSI was recently reported by Hu *et al*. (2020).

Prediction of pest and mechanical damage : The identification of the wheat grain moth (*Sitotroga cerealella*) has been tested using X-ray and MSI by França-Silva *et al*. (2020). While the study demonstrated the potential of MSI for recognizing eggs on the seed surface.

Recently, research on maize, sweet corn, and soybeans shows that damaged seeds are more likely to result in abnormal seedlings (Chomontowski *et al*., 2020). Salimi *et al*. (2019) conducted a study that demonstrated the potential of MSI in categorizing different forms of damage without the need for further analytical assessment. 82% total accuracy in damage class differentiation was made possible using a classification model based on surface features generated from MSI and multivariate data processing.

Prediction of seed viability and vigour : Olesen *et al*. (2015) demonstrated a strong association between the outcomes of tetrazolium tests and MSI, and they were able to identify live castor bean (*Ricinus cummunis* L.) seeds with 92% accuracy.

Moreover, Liu *et al*. (2019) used both spectral and morphological parameters in MSI to find a good prediction accuracy (91-92%) for high-quality watermelon (*Citrullus lanatus* Thunb.) seed in two different types.

A study predicting spinach germination ability by Shetty *et al*. (2012) also supports the high divergence between viable and non-viable seed in mean intensity reflection in the wavelength interval 375–970 nm, with the highest difference in the NIR-regions.

In order to identify hard seeds, Hu *et al*. (2020) used MSI to analyze the seeds of six different Fabaceae species. When paired with multivariate data analysis, MSI can detect hard seeds for three species (sweet clover, alfalfa, and galega (*Galega officinalis* L.) with accuracy ranges of 88–92%; for the remaining three species, the results are ambiguous. In comparison to non-hard seeds, hard seeds in all three species under investigation displayed a higher reflectance.

In cowpea (ElMasry *et al*., 2019) and spinach (Olesen *et al*., 2011), single seed NIRS spectroscopy and MSI have been used to evaluate viability following artificial seed aging or controlled degradation. Following artificial aging of both seed lots, two lots of spinach with viability percentages of 90% and 97% were selected for single seed NIRS analysis (Olesen *et al*., 2011). Cowpea was artificially aged in four treatments (with aging intervals of 24–96 hours) to produce variance in germination performance (ElMasry *et al*., 2019).

The physiological potential of *Jatropha curcas* L. seeds is strongly correlated with both MSI and X-ray measurements, according to a recent study described by Bianchini *et al*. (2021). The researchers examined both viability and vigor, and they discovered that reflectance data at the 940 nm NIR wavelength had an accuracy of 96%.

Detection of fungal infection and Seed Health : Using a visual scoring system as a reference, Weng *et al*. (2020) artificially inoculated rice with uninfected seeds of *Ustilaginoidea virens*. Nevertheless, a PCA found it challenging to distinguish between the healthy and slightly diseased seeds.

Olesen *et al*. (2011) used a multispectral imaging system (395–970 nm) to distinguish between healthy and infected seeds with different Fusarium strains (*Verticillium* spp., *Fusarium* spp., *Stemphylium botryosum*, *Cladosporium* spp. and *Alternaria alternate*). This resulted in an accuracy of 26–88% for separating seeds that were not infected from those that were infected with *Fusarium* spp.

Sendin *et al.* (2018) employed a multispectral imaging system with 19 discrete wavelengths in the UV, visible and NIR ranges (375–970 nm) of another investigation to identify various abnormalities in maize kernels. Exceptional classification accuracy was demonstrated by the PLS-DA model, which ranged from 83% to 100%.

Conclusion

The robust integration between spatial imaging, spectroscopy, and chemometrics tools makes the technique an ideal tool for studying various morphological, physicochemical, and physiological properties of seeds. This remarkable ability has encouraged researchers to collaborate together to create quick, precise and affordable spectrum devices that can be used in the grain and seed industries. Based on the applications that have been presented and examined, MSI has the potential to be used for assessing seed quality, especially for components related to surface structure and chemical composition, seed color, morphology and size, the detection and characterization of fungi, insect damage, varietal purity and elements related to seed growth and quality issues are all possible using the nondestructive, dependable and quick tool. It is anticipated that the multispectral imaging technique can be transferred from lab settings to real-world applications in the form of real-time seed monitoring systems that fulfill the demands of contemporary industrial control and sorting systems, provided that all limitations and difficulties encountered by this technology are carefully considered. This objective will advance and this technology will become more appealing for potential uses in quality control and automatic seed inspection as a result of the declining cost and rising speed and power of computer hardware and artificial intelligence.

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