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Image Analysis: A Modern Approach to Seed Quality Testing

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Authors' contributions

This work was carried out in collaboration between all authors. Authors Hemender and SS designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors VSM and AB reviewed and edited the manuscript. Author Jitender managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Image analysis is a state-of-the-art technique for seed quality testing. This tool provides vast usage in evaluation of various physiological and morphological characteristics of the seed with a more comprehensive perception. It is based on the extraction of numerical data from a captured image for characteristics like colour, size, shape of seed and seedlings and their subsequent processing with the help of suitable computer software. Speedy analyses, cost-effectiveness, automatic nature and user-friendly environment for work are some important advantages of Image Analysis over other conventional techniques. Numerous software have been developed for application in different fields of seed science research like germination studies, vigour estimation, varietal identification and purity testing etc. and most of these showed their potential adoption in the future as such or with some required transformations.

Keywords: Image analysis; seed testing; seed morphology; computer software; automation.

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1. INTRODUCTION

Seeds are the beginning and the end of most agricultural practices. The characteristics of seeds like their physiology, morphology, genetic purity, biochemistry and molecular biology are critically important for their success in the field. Quality of seed is very crucial for healthy and vigorous seedlings, good plant stand in field and ultimately a good crop harvest and yield. The fundamentals of seed quality are genetic purity, physical purity, germination, vigour and disease-free status. In commercial seed chain, this quality is maintained by following standard seed testing procedures by means of which we measure the viability and all the physical and physiological factors that regulate the performance of seeds. Seed testing is basically done for the evaluation of a seed lot and it tells us about its seedling emergence potential. But these testing procedures have their limitations, like most of these are time consuming, labor intensive and sometimes the results are not reproducible in actual field conditions. An implication of new techniques for testing of seeds should be focused and also attention is being laid at international level for the development of appropriate methods like image analysis of seed and other plant organs, bio-chemical and molecular markers.

Image analysis technique (machine vision system) is among such systems that offer the prospect that researchers will be able to study seed morphology, its anatomical activity during germination and growth of germinated seedlings more closely and hence, increase accuracy in studying various processes related to the seed. The term "image analysis" refers to the extraction of numerical data from an acquired image. Machine Vision System is basically a computerized apparatus designed for Image Analysis (IA) which functions similar to the human observations. Fundamental approach in this technique is acquisition of data (shape, size, colour etc.) via a video or still camera followed by analysis of these data using suitable computer software [1].

Image Analysis shows many important advantages over manual techniques. It provides rapid analysis as compared to any of the conventional methods. Seeds are not subjected to any kind of treatment or damage. Once the system that works has been designed then the whole process can be automated. Imaging software provides an increasingly interactive and

user-friendly environment to work. After the initial outlay for equipment and research unlike other systems, IA has very few additional costs.

2. METHODS AND SOFTWARES USED IN IMAGE ANALYSIS

An automated image analysis system basically contains a digital camera or a flat-bed scanner to capture the images of the seed. These images are then processed using a suitable computer software package producing numeric data which are then used for further statistical analysis. This basic methodology varies with some minor modifications when used for different purposes *i.e.* germination studies, vigour assessment, grading and sorting etc. (Fig. 1). However, when this technique is applied for varietal identification or characterization studies, seed coat colour acquisition is also important because sometimes varieties show significant and characteristic seed colour differences among them due to developmental physiology. There are numerous softwares which have been used in different image analysis studies (Table 1).

In a study by Wiesnerova and Wiesner [2], Flax cultivars were characterized by quantitation of seed color for identification purpose. L^* , a^* , b^* coordinates of CIELAB color space [3,4] were calculated from original RGB color channels of seed digital images. For seed shape quantitation, the four measures (seed area, seed perimeter, seed mean chord, seed MinFeret) were calculated from seed images by LUCIA 3.52 software package (Nikon Europe B.V., The Netherlands) using following methods:

2.1 Seed Area (Ar)

It is defined by the number of pixels in the seed blob, which is considered as an important seed size standard.

2.2 Seed Perimeter (Pr)

It can be calculated from seed projections in directions of 0° , 45° , 90° , and 135° on the basis of the longest axis of a seed according to the Crofton formula:

$$Perimeter (Pr) = \frac{\pi_*(Pr_0 + Pr_{45} + Pr_{90} + Pr_{135})}{4}$$

Where, Pr_0 , Pr_{45} , Pr_{90} and Pr_{135} are seed projections in directions of 0° , 45° , 90° , and 135° respectively

Table 1. Various softwares used in the image analysis studies

	Name of software	Crop	Parameters studied	References
1.	LUCIA 3.52 software package	Flax, Lentil	Seed area, perimeter, mean chord, MinFeret	Wiesnerova and Wiesner [2], Firatligil-Durmuş et al. [5]
2.	KS-400 V.3.0	Vetch, Pea	Seed morphometric and colorimetric features (Varietal identification)	Grillo et al. [6], Smykalova et al. [7]
3.	Delta-T© image analysis system having software 'winDIAS'	Mustard, Oat	Characterization by measuring variation in seed morphology	Vijaya Geetha et al. [8], Sumathi and Balamurgan [9]
4.	ImageJ software	Sunflower	X and Y position of the inertia centre and curve length	Ducournau et al.[14]
5.	Matrox image processing board	Lettuce, Sorghum	Germination studies	Howarth and Stanwood [12]
6.	ImageTool v.3.0 software	<i>Medicago sativa</i> , <i>Onobrychis viciifolia</i>	RGB intensities of seed images	Behdari et al. [11]
7.	Seed Vigor Imaging System (SVIS®).	Various crops	Analysis of seedling images, providing indexes of growth, uniformity and vigor	Sako et al. [15]

2.3 Seed Meanchord (Cm)

It expresses the middle value of a secant size measured in the direction of 0°, 45°, 90°, and 135° degrees based on the longest axis of a seed according to the following formula:

$$\text{Meanchord (Cm)} = \frac{4 * \text{Area}}{Pr_0 + Pr_{45} + Pr_{90} + Pr_{135}}$$

Where, Pr₀, Pr₄₅, Pr₉₀ and Pr₁₃₅ are seed projections in directions of 0°, 45°, 90°, and 135° respectively.

2.4 Seed Min Feret (Fm)

It is a minimum value among all possible Feret diameters. Feret diameter for an angle α equals to the length of the seed projection for α where $\alpha \in (0, 180)$. In order to keep calculations in a real time, Fm was calculated by LUCIA software on a decimal scaling of $\alpha = 0^\circ, 10^\circ, 20^\circ, 30^\circ, \dots, 180^\circ$.

Same software was used by Firatligil-Durmuş et al. [5] for characterization of shape and geometrical properties of Lentil seeds. Grillo et al. [6] inspected Vetch seeds for varietal identification by analyzing acquired images with

the help of software package KS-400 V.3.0 (Carl Zeiss, Vision, Oberkochen, Germany). Smykalova et al. [7] also used this method for analysis of morphometric and colorimetric features of seeds of Pea varieties and discriminate among the varieties regarding their cropping localities. Delta-T© (Delta- Instrumental Device- Cambridge, UK) image analysis system is an another example which was used by Vijaya Geetha et al. [8]; Sumathi and Balamurugan [9] with the help of custom written software 'WinDIAS' [10] for characterization of mustard and oat respectively by measuring variation in seed morphology. Behdari et al. [11] used ImageTool V.3.0 software point tools to extract, independently, the RGB components of the pixels representing the seed images taken by a digital camera and then manually the intensity of each color component was measured. The relation between the RGB density values and the vigor of *Medicago sativa* and *Onobrychis viciifolia* seeds was modeled by combining information obtained from germination experiments and image analysis.

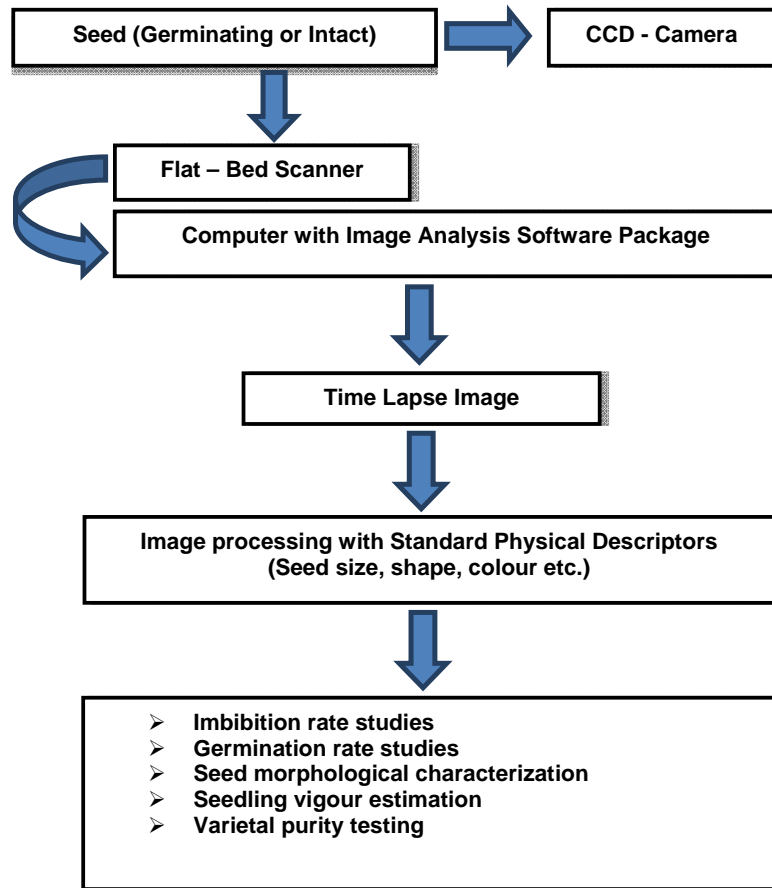


Fig. 1. Basic design of image analysis system [Partially adopted from Dell’Aquila (61)]

Various other softwares have also been used for seed germination monitoring, vigour estimation and in priming studies. With advancement in computer technology, a machine vision system was developed at the National Seed Storage Laboratory (USDA-ARS, Fort Collins, USA). This system was divided into two basic units: the biological system that allowed seed germination in a chamber with controlled environmental conditions like humidity, temperature and lighting, and the other unit having computer vision system. The vision system consisted of a charged coupled device (CCD) camera, a Matrox image processing board (Matrox Electronic systems Ltd., Québec, Canada), and a personal computer. Growth parameters of germinating seeds and seedlings were automatically assessed by this system. The values obtained were further analyzed for their association with imbibition time and statistically compared with the results of a standard germination test [12]. Later on, a more improved biological system, having temperature and photoperiod control,

connected with a computer unit was developed to assess root elongation rate in Rice (*Oryza sativa* L.) and Sorghum (*Sorghum bicolor* Moench.) on an hourly basis [13].

A prototype was developed to study the germination rate of sunflower by Ducournau et al. [14] which was able to provide full informative data. Since then, this prototype has been improved which resulted in broad species coverage and rapid analysis of larger samples. Now, Jacobsen tables are also made associated with this type of vision machines which allow seed germination with continuous watering and accurate control of temperature conditions. Four calibrated cameras were used to analyse large samples (up to 400 seeds per camera) and image analysis was improved to record germination parameters of individual seeds. Further analysis is performed with ImageJ software in order to separate seeds from the background, and to record significant parameters such as area, X and Y position of the inertia centre and

curve length. This system also names and stores images from each camera in separate directories according to the background information to ensure complete traceability of the experiments. Various experiments were performed, using a range of magnifications, to test and validate the system with various species like small seeded crops such as oilseed rape to large seeds like sunflower or maize for a more pragmatic approach.

There was a major breakthrough in automated seed vigour testing with the development of Seed Vigor Imaging System (SVIS[®]). This system was developed at the Ohio State University [15] and has been proposed as an alternative to traditional vigor tests [16-22] in various crops. The software enables the analysis of seedling images by providing indices of growth, uniformity and vigor, a rapid and objective measurement of seed quality [23] and avoids human error during the evaluations [17].

A new technology has also been developed known as dynamic speckle, or biospeckle. Basic principle behind this technique is an optical phenomenon produced when living materials, such as biological tissues, are illuminated by laser light. The term 'speckle' means a random granular pattern resulted from diffusive reflection of highly coherent light beam (laser) on a surface having complicated structure. Features of seed and other plant tissues like root, shoot etc., acquired by imaging, can be amplified and assessed by their speckle activity using appropriate algorithms [24]. This technique has proved to be a potential non-destructive methodology to assess bean (*Phaseolus vulgaris* L.) seed viability. The technique has also been applied to detect the presence of fungi colonies on bean seed coats [25].

3. APPLICATIONS IN SEED SCIENCE RESEARCH

3.1 Varietal Identification

Image analysis has a potential use in identification and characterization of different genotypes in various crops. It can also be used for testing of distinctness of a new variety. With the help of this technique researchers are able to study seed surface features more closely and can differentiate among various lots accurately. Morphological, colorimetric and textural characterization measures of many types of grains have been reported for both cereals and

legumes, in order to identify and classify grain types [26-34] as well as for wild species [35-41]. Dell'Aquila et al. [42] studied perimeter, length and area of white cabbage seeds during imbibition phase and have concluded image analysis as a potential technique for measuring variations in seed physical parameters due to various biological changes during seed germination. Sahoo et al. [43] observed varietal discrimination of sunflower seeds using machine vision approach while Anouar et al. [44] used this technique for grouping four types of carrot seeds based on their seed size. Varietal identification and characterization using image analysis is well documented for various crops like sorghum [45], lucerene [46], castor [47], sesame [48], phaseolus [30], mustard [8] and oat [9].

3.2 Role of Edge Detection in Varietal Identification

The character set mostly used in cultivar purity testing and their characterization are geometrical features of the seed like area, perimeter, diameter, length, width, roundness etc. Edge detection is one of the most commonly used operations in image analysis for measuring these features accurately because edges form the outline of an object. Edge is the boundary between an object and the background, and between overlapping objects. This implies that if the edges in an image can be identified accurately, all of the objects can be located and basic properties of area, perimeter and shape can be measured.

Since computer vision involves the identification and classification of objects in an image, edge detections forms an essential tool of the system. There are many ways to perform edge detection. Various edge detection algorithms have been developed in the process of finding the perfect edge detector. However, the most may be grouped into two categories, gradient and Laplacian. The gradient method detects by looking for the maximum and minimum in the first derivative of the image, while Laplacian method searches for zero crossings in the second derivative of the image to find edges [49]. Canny [50] proposed a method that was widely considered to be the standard edge detection algorithm. In regard to regularization Canny saw the edge detection as an optimization problem. He considered three criteria desired for any edge detector: good detection, good localization, and only one response to a single edge. Then he developed the optimal filter by maximizing the

product of two expressions corresponding to two former criteria (i.e. good detection and localization) while keeping the expression corresponding to uniqueness of the response constant and equal to a pre-defined value.

3.3 Seed Vigor Estimation

Vigor tests can provide information closer to what occurs during seedling establishment in the field, because they are based on physiological and biochemical characteristics sensitive to changes in seed physiological potential before those identified in the germination test. Seed vigor tests are based on different concepts, such as the resistance to stress, speed of germination, membrane integrity and seedling development [51]. Image analysis technique is found to be very effective for estimation of seed vigour. In this technique, the determination of the vigor is performed more quickly and without the direct human interference. It was used initially by McCormac et al. [52] developing an automatic system for the determination of the average length of the primary roots of tomato seedlings. Later on, Sako et al. [15] developed "Seed Vigor Imaging System[®]" (SVIS) to assess lettuce seed vigor by capturing images of three-day old seedlings and determination of vigor and uniformity indices, hypocotyl and primary root length, and root/hypocotyl ratio. This software is found to be a promising tool for vigor estimation providing a rapid, accurate and objective measurement of seedlings [23] and avoids human error during the evaluations [17].

3.4 Seed Vigor Imaging System[®] (SVIS)

This software was developed at Ohio State University/USA. The software performs the digital processing of images of scanned seedlings. Besides providing average values of seedling length it also establishes vigor and uniformity indices. It was initially used to assess lettuce seed vigor and successfully adapted in various crops till date. In this system, germination is performed in paper-towel rolls, with four replications of 25 seeds usually. Normal seedlings from each replicate were transferred from the germination paper to a sheet of black cardboard to provide contrast between the seedlings and the black background during the capture of images by a scanner machine. Scanned images are analyzed and after the digital processing of the images and individual analysis of each seedling root/hypocotyls axis, the numerical values of the vigor index and of

uniformity of seedling development are calculated by the software, which may vary from 0 to 1000. The assessment of the seedling length is performed by positioning the cursor over each seedling, automatically obtaining its length (mm), results are expressed as mean values for each variable [21]. An important aspect worth mentioning here is the use of SVIS[®] is not just restricted to direct comparison of the vigor of different lots through the analysis of seedlings growth analysis, but it can also provide a chance to use this system as a complement to other tests [53].

Contreras and Barros [54] utilized SVIS for lettuce seed vigor analysis and also showed a high correlation with seedling emergence in a greenhouse, in addition to the other vigor tests examined. Marcos-Filho [55] concluded that the SVIS[®] results for ten seed lots of two melon cultivars correlated better with the seedling emergence and speed of emergence at 12 and 18°C compared to other tests of vigor. Dias *et al.* [56] compared this method with other conventional vigor tests in bell pepper seed and revealed that SVIS[®] gives sufficient sensitivity for potential physiological assessment compared to the results provided by traditional vigor tests and fulfills the requirement for an efficient, rapid, cost-effective and standardized method allowing comparisons among laboratories and avoids the influence of human interpretations of the results. Other comparative studies had also been done on various crops like in cucumber [19], sunhemp [57], tomato [58], common bean [59] with the help of this software. In another study by Alvarenga and Marcos-Filho [22], it was concluded that the cool germination, accelerated aging (traditional and with saturated salt), field seedling emergence and seedlings length (SVIS[®]) are adequate parameters for assessing vigor of cotton seeds during storage, and that both accelerated aging and SVIS[®] (vigor index and seedlings length) present enough sensitivity to identify changes on seed vigor during storage. Brancalion et al. [60] used image analysis to evaluate different priming treatments on *Guazuma ulmifolia* (Malvaceae) seeds by utilizing SVIS[®] and found this method efficient for seedling analysis.

3.5 Germination and Viability

Image analysis can be a significant system to monitor phases of seed germination in controlled environment and the changes associated with it can be assessed accurately; thus helps in seed

viability and germination studies. The assessment of RGB index of each individual seed within a large seed sample may allow the development of non-destructive methods for sorting seed sub-samples with different germination capability [61].

Li et al. (California State Polytechnic University) applied computer vision in lettuce seeds to detect germination. In this study, two steps were involved, first is image acquisition and second is image analysis which uses image skeletonization and other image processing algorithms. Seed images are converted to binary images followed by extraction of seed skeletons, which are used to calculate the changes in seed length. Other parameter such as area is also calculated. Seed area and length changes towards radicle emergence over time are used to identify the onset of germination. With the help of this technique, germinated and non-germinated seeds can be easily distinguished and moreover, seed characteristics such as radicle length and seed area can also be measured. Behtari et al. [11] used image analysis system for predicting germination of *Medicago sativa* and *Onobrychis viciifolia* seeds. In this study, images were processed by a computer to generate numerical red-green-blue (RGB) density values. These density values were significantly correlated with germination and it is concluded that the RGB values of the density-imaged seed tests are non-destructive, practical, and can distinguish between high- and poor-quality seed lots.

During germination, the moisture content of the seed increases as water is imbibed during 1st phase of germination, as well as, the seed length, width and thickness also increase linearly with moisture content with approximately the same proportionality [52,42]. These basic findings allowed the design of a computer-aided image analysis system devoted essentially to monitor seed image features during swelling and subsequent germination [62].

3.6 Seed Processing

Geometric features of seed are very important in the design of processing equipments for handling, harvesting, transporting, cleaning, separating, packing and processing of seeds. Machine vision or image analysis can be a faster, non-destructive alternate to the traditional sizing equipment currently used in the seed industry. Digital image analysis technique has been developed and used to determine the physical

dimensions of seeds and grains of various crop. Sakai et al. [63] revealed that a computer vision system is highly reliable in evaluating the shape and size of rice seeds. Shahin and Symons [26] developed a machine vision system for grading lentils based on flat-bed scanner, to assess seed size distribution using image analysis. To identify Sicilian landraces and Canadian cultivars of lentil, Venora et al. [29] implemented a method to evaluate morphometric and colorimetric features of the seeds. A similar method was then applied to identify and classify seeds of beans [30] and lentil landraces [64]. Likewise, Wiesnerová and Wiesner [2] used an image analysis system to measure seeds shape and colour in order to describe flax cultivars. Several similar applications have been published by many authors, proving that image analysis is really a helpful tool to identify and classify various kind of seeds. Smykalova et al. [7] measured morphometric and colorimetric features by image analysis and used to identify and discriminate between the varieties of pea. In 2012, Mandal et al. [65] developed a low-cost image analysis technique using a flatbed document scanner and MATLAB software for determination of seed dimensions. The technique showed strong correlation between the image analysis and experimental data of length and width of corn, baby corn, pigeon pea, soybean and paddy seeds. Arya and Lehana [66] developed a new seed analyzer which can analyse physical parameters with great accuracy and efficiency thus provides robust, fast and fully automatic algorithm.

4. CONCLUSION

There are a number of standard procedures for seed quality evaluation and sorting which are mainly based on the assessment of various physical, morphological and physiological properties of seeds but in recent past, a strong need was felt for the development of more accurate, quick and non-destructive methods of seed quality evaluation. Machine vision or computerized image analysis system is found to be very convenient method for seed related studies as it is free from human errors, more rapid and provides close analysis of seeds and germinating seedlings. The declining cost and increasing capability of computer hardware of image processing and its integration with controlled environmental condition systems are other advantages associated with this technique. An additional advantage of this method is that the images, vigor indices and other information

pertaining to the specific seed lot/type are stored and a database can be developed for future reference. Assessing the RGB index of each individual seed within a large seed sample helps in the development of non-destructive and more efficient methods for sorting seed subsamples with different germination capabilities. Data obtained through this technique can further be processed statistically and displayed graphically, and a database may be developed to integrate image analysis data with taxonomic and biomorphological features of plant species. New algorithms and hardware architectures have been developed, and the availability of appropriate image analysis soft-ware tools made this approach highly useful for present and future prospectus of seed quality research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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