# Review

# Seed image analysis: its applications in seed science research

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#### **Abstract**

The paper reviews basics in computer-aided image analysis, which are contributing to improving insight of seed morphology and biology, in terms of seed quality and germination and various aspects of seed image analysis like image acquisition and pattern recognition. Under separate headings, it deals the means by which digital images are acquired and processed and how imaging technology is applied in seed science research in terms of varietal identification, characterization, germination, moisture, grading and sorting by analysis of seed size, shape and color parameters. The final section deals with the future development of the new technique and its integration in multifaceted plant biology systems.

Keywords: Seed, Image analysis, Seed science and Applications

## INTRODUCTION

# **Seed Image Analysis**

The primary bases for defining the seed quality are purity, germination and freedom from diseases. Distinguishing a variety on the basis of classical taxonomic approach is highly difficult because of time consuming, labor intensive and expensive. By increasing the private companies involvement in plant breeding implies the better and precise varietal description and protection through various plant breeders' rights and related schemes. The assessment of varietal identity and purity is also contributed by the seed certification procedures. In addition to these, for the proper implementation of PVP and PBR programs in India, DUS characters are going to play a crucial role. Implication of new techniques for addressing a particular variety can be focused and also attention is being laid at international level for the development of suitable lab techniques like image analysis of seed or plant organs, bio chemical and molecular markers. Image analysis technique (machine vision system) is one of such systems offers the prospect that researchers will be able to study seed surface features more closely and hence increase the available character set. Thus it has potential use in a wide range of tasks such as determining the cultivar identity of seed lots and testing of the distinctness of new cultivars for the award of breeders' right and cultivar registration (Keefe and Draper, 1986). Seed analysis is becoming increasingly important, both for quality control in seed production and for harvest classification. Particularly for research purposes like seed phenotyping, the more information can be extracted from the seeds the better. Fast and easy to achieve image-based measurements thus can provide data correlating with genetic properties of germination and growth performance.

Machine Vision System is a computerized tool for Image Analysis (IA). It functions being similar to the human observations. Machine vision refers to the acquisition of data (shape, size, etc.) via a video camera or similar system and the subsequent computer analysis of these data following suitable processing. The term "image analysis" has also been used in this context, but it more strictly refers to the extraction of numerical data from an acquired image. The colour, size, shape characteristics of plant products, and their capability to produce digital images suitable for further processing make modern image acquisition techniques highly adaptable tools. Bio-morphological seed features may be analyzed by computer-aided image analysis systems and data quickly processed and stored in the hard disk, plotted or statistically elaborated (Dell'Aquila, 2004). These data include relationships among seed size and shape and growth time-course, and understanding of

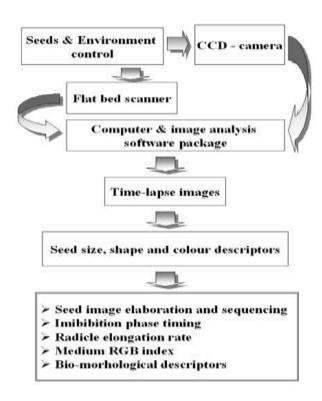


Fig. 1: Scheme of image analysis systems (Adapted from Dell'Aquila, A., 2006).

growth patterns that produce curvature and inflection points. The importance of objective methods in analyzing growth is to be emphasized, because subjective monitoring can mask morphological variation when plant structures, with dimensions in the scale of millimeters or microns, pass from a steady state to a proliferating one (Silk, 1984).

Image Analysis is generally recognized as having many important advantages over other manual techniques. It is non-invasive and in the case of seeds they are not subjected to any kind of treatment. 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. The speed of analysis is much higher than any of the conventional methods.

# Aspects of Computerized Machine Vision System in Seed Science and Technology

According to Draper and Keefe (1988), the basic elements of a machine vision system are image capture, via a video camera / other electronic system (e.g. a charge-coupled device), analogue to digital conversion of the image data, computerized manipulation of image data termed as image processing, image analysis which means extraction of information from the processed image and pattern recognition, to sort and compare objects (e.g., varieties), computerized decision making and presentation of results, including assessments of statistical significance and an automated or robotic mechanism for movement of the sample (or the camera), so that operator intervention is minimized (desirable for a useful practical system). MV technique has two important aspects, Image Analysis (IA) and Pattern Recognition (PR). Standardization of IA programs for characterization of variety is initiated with measurement of seed, proceeded in sequence to plant organs, to an extent where the analyst feels capable in varietal distinctness. IA is well suited to the acquisition of large amounts of quantitative data concerning seed/plant morphology & this information could be used to support that a variety is new in context of proposed variety licensing and registration whereas Pattern Recognition (PR) is not variety identification. After measuring a sample of seed / cotyledon / leaf / flower all statistical techniques employed for the analysis of IA data & interpretation of results are studied under pattern recognition. For instance, the environment may affect the seed character like length and width. Therefore, for the reliability in results, it is advisable to include such selected parameters in varietal identification program, which are least affected by the environment. In standardization of

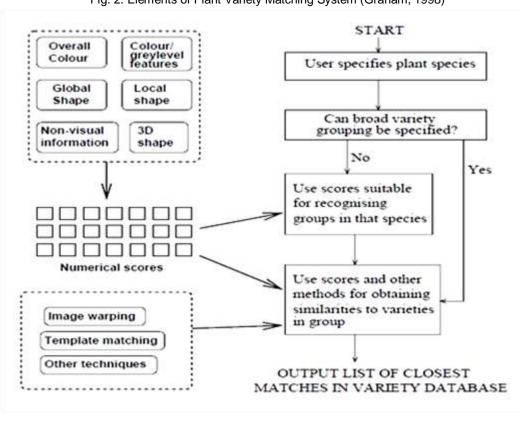


Fig. 2. Elements of Plant Variety Matching System (Graham, 1998)

PR methods through seeds they are viewed both from above (seed sitting crease down) and/or from the side (point of contact).

According to Horgan *et al.* (1998), the real use in variety identification, machine vision system need to deal with many crop species, use many aspects of plant appearance, be easily extended to include an increasing set of varieties, exploit new developments in machine vision and integrate with non-visual information.

# APPLICATIONS IN SEED SCIENCE RESEARCH

### **DUS Testing**

Assessing the appearance of plants is an important botanical skill, with many applications, ranging from simple recognition to plant health diagnosis. One application is Distinctness, Uniformity and Stability (DUS) testing, where new varieties are compared to establish differences from existing varieties before they are given official recognition. The aim is to identify consistent differences in respect of one or more characters between existing and potential new varieties. Any aspect of visual appearance is a potential character. Traditionally, differences in appearance can be assessed by measurement or subjective scoring. The latter can be used for simple size and shape measurements, such as lengths and their ratios. The latter is used for more subtle differences that would be more difficult or cumbersome to

measure. It suffers the inevitable drawbacks that it is tedious to do and subject to inconsistency between observers and over time. There have been a number of attempts to use automatic methods to assess plant appearance. These have ranged from straightforward use of colour meters (McMichael, 1994) to the use of image analysis to extract shape features (Keefe and Draper, 1988). The development of non-destructive methods for the evaluation of cereal grain varieties has important implications for the food processing industry. The described experiment investigated 11 varieties of spring and winter wheat of different quality class. The analysis was performed on images acquired from a flatbed scanner interfaced to a PC. Kernel images were digitalized at high resolution (2673 X 4031) with 24-bit depth and 400 dpi. The variables input into the statistical model were the textures of single kernel projections. Textures were computed separately for seven channels (R, G, B, Y, S, U and V). The results were examined with the application of discriminant analysis and neural networks. The accuracy of texture based classification of 11 wheat varieties reached 100%. The experimental design which yielded the most satisfactory results comprised texture measurements from the combined area of 20 kernels and variables from seven channels input into the neural network. The final classification quality was not affected by the year of cultivation, moisture content or grain variety. (Piotr, 2011).

#### Varietal Identification

Seed identification processes by specialized technicians is slow and somewhat subjective and give results which may be difficult to quantify for both business and technological purposes. Therefore it is important economically and technically to implement repeatable and quick automated methods to identify and classify seeds. Crop varieties provide the necessary options to growers, processors and consumers. In the case of crops such as wheat, where end use is dependent on use of a specific variety, identification of that variety is crucial. Variety identification is also important for plant breeders and geneticists. Visual assessments made using color, size, shape and texture are simple, but they can be highly subjective. Reliable visual evaluation needs experience and expertise. An objective method will help in reducing the subjective nature of this visual assessment. Automatic systems can be based on seed images, from which the characteristics for the classification, such as size, shape, colour and texture, can be obtained quickly. Digital image analysis offers an objective and quantitative method for estimation of morphological parameters. This process uses digital images to measure the size of individual grains and mathematically extract features and shape related information from the images. With the evolution of imaging and computing hardware, several imaging systems were developed for characterization and classification of wheat varieties in US, UK, Canada, and Australia. These have been extensively reviewed by algorithms Sapirstein (1995).Image analysis implemented by classification methods, appear to be useful for automatic seed identification (Granitto et al., 2003: Bacchetta et al., 2008). Colour, shape and size of whole seeds and their spots of some Italian landraces of bean (Phaseolus vulgaris L.) were measured using a specifically developed macro, based on image analysis library KS-400 V 3.0. Statistical classifier to identify the cultivars was obtained using the image analysis data elaborated with the Linear Discriminant Analysis algorithm (Venora et al., 2007 and 2009). One of the most contributions in varietal identification is the identification of Fabaceae family through image analysis system. The Fabaceae or legume family has 686 genera and more than 17,600 species. Fruit data (157 characters) were collected for 669 genera, seed data (127 characters) for 655 genera and distributional data (six characters) for all genera. Final data matrix had 105,033 items of fruit data, 83,185 items of seed data and 4,122 items of distributional data. Approximately 1,360 images were attached to the genera and 205 images to individual characters. The database in combination with the Intkey software program will facilitate: 1) identification of legume seeds and fruits to genus through interaction between users and the database on microcomputers with characteristics of the user's choice; and 2) retrieval of information concerning seeds, fruits and distribution of legume genera, adapted

to the specific needs of each user. Users are introduced to the database and software and instructed in their use. (Kirkbride et al., 2004). Several textural feature groups of seeds images were examined to evaluate their efficacy in identification of nine common Iranian wheat seed varieties. On the whole, 1080 gray scale images of bulk wheat seeds (120 images of each variety) were acquired at a stable illumination condition (florescent ring light). Totally, 131 textural features were extracted from gray level, GLCM (gray level cooccurrence matrix), GLRM (gray level runlength matrix), LBP (local binary patterns), LSP (local similarity patterns) and LSN (local similarity numbers) matrices. The average classification accuracy of 98.15% was obtained when top 50 of all selected features were used in the classifier. The results confirmed that LSP, LSN and LBP features had a significant influence on the improvement of classification accuracy compared to previous studies. (Alireza et al., 2012).

#### Characterization

Success is based on the perfect imagination about the image descriptors of the object. These descriptors are defined by mathematical expressions and successfully transferred into the computer programs. 1) Characters: All those shape descriptors, which can be defined in mathematical expressions. 2) Variable: There environmental influence on the characters; therefore a average of variation within a character between objects of optimum sample size is taken as a reliable shape descriptors. 3) Parameter: The value of character obtained by the average of 'variable' is regarded as 'parameter'. 4) Database: The image is directly converted in the binary image by thresholding (Grey level). The information can only be extracted concerning the boundary of object in an image. The boundary line of the object is described by Freeman Chain code Technique. The images of the objects being examined are stored as pixel array, which is then used to extract mathematically complex descriptors of the object. To describing the shape of object, aberrant such as area, perimeter by counting pixels, length, width by X,Y Cartesian coordinates and the angles can be obtained by Tangent function. A reference cultivar needs to be identified so that the characters can be compared with that of test cultivar. The reference cultivar can be obtained successfully by "Nearest Neighborhood method". The parameters of a cultivar under test are compared with that of reference. The characters of cultivar are classified in database for the purpose of uniformity testing. Possible contaminants / objects, which are not uniform in shape, can be identified within few minutes.

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identity of seed lots and testing of the distinctness of new cultivars for the award of breeders' right and cultivar registration (Keefe and Draper, 1986). Machine vision has been utilized for cultivar description, characterization and identification of varieties using seeds and plant parts (Draper and Travis, 1984; Van de Vooren et al., 1991) measured pod length and width using image analyzer and compared with manual measurement in French bean. Image analysis system was used by Aquila et al. (2000) to measure area, perimeter and length of white cabbage seeds in order to monitor changes in seed physical parameters during imbibition and suggested that image analysis techniques have high potential in seed biology studies. With the aid of digital imaging software (Veho<sup>™</sup>), 10 seed morpho-metric traits were digitally measured and/or calculated from captured images of 17 inbred lines drawn from the Striga - resistant tropical maize population. All the parameters showed positive correlation except embryo angle, seed shape factor and circularity index. Seed area, length, perimeter and flatness index contributed the largest variability within the population (eigenvectors = 0.332, 0.328, 0.323 and 0.318), respectively, suggesting their potential usage for cultivar discrimination by digital imaging of maize seeds. Dendrogram constructed from the SLCA showed four major clusters of the inbred population. The clusters constitute a classification tool for future evaluation of genetic purity of test inbred and F1 hybrid samples within Striga resistant maize population. They concluded that the applicability of digital imaging for analysis of seed genetic quality and will ultimately improve the hybrid seed sector in Nigeria (Daniel et al. 2012).

#### Germination

Seed germination has intrigued the human activity since the late Neolithic age, because of practical reasons becoming a milestone in the 'agriculture framework' (Evenari, 1984). The application of computational techniques to the study of seed germination covers three aspects: computer-assisted image analysis systems, descriptive simulation modeling, and combined relation modeling between morphological changes and biological processes. A digital image of a plant seed can be regarded as a two-dimensional object which can be measured in size, shape and color density during the development stage of germination by computer image analysis technology. Seed changes its biological structure passing from a quiescent stage to a proliferating one, and any morphological variation can be associated with the corresponding variation of seed geometry and color space components. Future perspectives are addressed to design comprehensive biological models that may contribute to studying seed quality patterns possibly integrated with those proposed for other biological systems (Aquilla, 2009).

#### Moisture

Moisture content is the most vital factor influencing physical and mechanical properties of cereal crop seeds. For example, an increase in moisture content leads to an increase in the major, minor and intermediate diameters of groundnut kernel (Hossain et al. 1998); increase in all linear dimensions, projected area and volume of mature okra seeds (Calisir et al. 2005); increase in length, width, thickness, arithmetic mean diameter, geometric mean diameter, sphericity, volume and surface area of popcorn kernels (Karababa, 2006), sorghum seeds (Mwithiga and Sifuna, 2006) and guinoa seeds (Vilche et al, 2003). Manickavasagan et al. (2008) used monochromatic images to determine the moisture content of Canadian wheat. Similar experiments were performed by Tahir et al. (2007) who identified the properties of wheat and barley kernels supporting the determination of the grain's moisture content based on camera images. Grain with a moisture content of 12%, 14%, 16%, 18% and 20% was classified with more than 90% accuracy.

# Vigor Assessment

Vigour is the ability of a seed lot to establish normal (or usable) seedlings under diverse production environments. Vigour tests are essential for seed production companies to evaluate and develop production and post-harvest techniques, to make inventory management and sales decisions and to justify premium prices. Vigour tests are also essential for commercial growers who need to have the means to independently assess seed vigour, to justify buying premium-priced seed and to have confidence in the performance of their crops (Karlovich, 1998). Therefore, it is important that standard seed vigour tests become available for flower seed producers and consumers. The International Seed Testing Association (Perry, 1981) and Association of Official Seed Analysts (AOSA, 1983) have published guidelines for vigour testing in seeds, which have been updated by Hampton and TeKrony (1995). Use of computer-aided image analysis of seedling size overcomes many of the limitations that occur during manual vigour tests (McDonald et al. 2001). Image analysis provides rapid measurement of an object's physical characteristics and allows quantitative, objective observation (Kranzler, 1985). Several commercial systems use some form of computer-aided analysis of digital images to evaluate seedling growth as a measure of seed vigour. These include the Paradigm Seed lot Vigour Assessment System (McNertney, 1999) and the Ball Vigour Index (Conrad, 1999) that use a CCD video camera to capture images of seedlings. Subsequently, Geneve and Kester (2001) and Sako et al. (2001) developed similar systems that capture images using a flat-bed scanner. Software has been developed to evaluate seedling growth and in most cases a vigour index has been computed based on seedling growth,

growth uniformity and germination percentage (Conrad, 1999; McNertney, 1999; Sako *et al.*, 2001).

# **Sorting and Grading**

A number of techniques for seed quality evaluation and sorting are based on the detection of various physical and physiological properties of seeds, and, more recently, the greatest efforts have focused on producing sophisticated non-destructive methods. The declining cost and increasing speed and capability of computer hardware of image processing and its integration with controlled environmental condition systems have made computer vision more attractive for use in automatic inspection of crop seeds. New algorithms and hardware architectures have been developed, and the availability of appropriate image analysis soft-ware tools suggests that the use of machine vision systems is becoming convenient in a seed biology laboratory. A raisin sorter has been designed and fabricated based on machine vision. This system is composed of conveyor belt, lighting box, controlling and processing system unit and sorting unit. The algorithm segmentation scheme described is a novel and simple approach to robustly segment an image of raisin into desired, undesired and background regions. By using this accurate algorithm, one can study all pixels of a digital image and obtain the necessary features. Finally they concluded that by a suitable HSI color space values raisins are graded it two classes, the overall precision or correct classification rate of this system was estimated as 93.3 percent and the devised machine vision and algorithm for grading raisins is quite general and can be easily adapted for grading other granule agricultural products (Mahdi et al., 2010)

#### Prediction of kernel weight

Image analysis is a well-established complement of grain morphology characterization. The image analysis technique allows the enhancement of images, as well as the identification and automatic isolation of particles for further study. In addition, it is a rapid and time-saving technique that allows for the acquisition of quantitative data that could be very difficult or even impossible to obtain otherwise. Recent researches on the inspection of cereal kernels by image analysis, computer vision or microscopic techniques have been reported (Edney et al., 2002; Brosnan and Sun, 2004). A large number of these studies used image analysis techniques for the classification and identification of cereal varieties or kernel quality (Shouche et al., 2001; Nielsen, 2003). For instance, Nielsen (2003) proposed a method using Grain check (Foss Electric AS Hillerod, Denmark) and Perten Single Kernel Characterization System4100 (Perten Instruments Inc., USA) to evaluate malting barley quality of 25 barley varieties by image analysis, Principal Component Analysis (PCA), and multivariate Partial Least Squares (PLS). Reasonable PLS regression models were obtained for the structural and physical part of the malting quality and established the hardness of the barley kernels as the most important variable in malting performance. In addition, Garcila del Moral *et al.* (1998) studied embryo size and kernel volume by image analysis in order to evaluate the malting quality of barley kernels. The results showed the nitrogen content as a good predictor of grain protein content, embryo size and grain volume, and the grain protein content as the only practical predictor of malt extract.

#### CONCLUSION

Image analysis, with its ability to emulate human intelligence in handling visual data, is an important technology that will find many applications in modern varietal identification and seed certification. It is already extremely powerful and will continue to improve, following developments in computing technology along with the sensory and central processing elements. In the context of seed production and processing image analysis techniques are capable of coping with the variability and therefore have wide-ranging applications in seed industry. A major research area is how to encapsulate, represent and implement an artificial version of human intelligence. If this can be done, many processes, which have defined automation, will become the subjects of automatic systems using image analysis as a major method of control. Considerable work is being done in the field of image processing for different applications of the agriculture and seed industry, particularly more in varietal identification and quality. The accuracy of the technique has been reported to be in the range of 70 - 99%. However, the development in image acquisition, pattern recognition and decision making as well as improvements in software and hardware will help to improve the system assuming better return on investment and reduced costs.

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