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REVIEW ARTICLE

Computerised seed imaging: a new tool to evaluate germination quality

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ABSTRACT

The paper reviews developments in computer-aided image analysis, which are contributing to improving insight of seed morphology and biology, in terms of seed quality and germination. Under separate headings, it treats the means by which digital images are acquired and processed, and how imaging technology is applied in monitoring seed imbibition, radicle tip elongation rate, or quality testing and sorting by analysis of seed size, shape, and colour parameters. The final section deals with the future development of the new technique and its integration in multifaceted plant biology systems.

Key Words: *image analysis systems; seed imbibition; radicle; elongation; colour index sorting; crop seeds*.

INTRODUCTION

One of the major requirements in developing machine vision systems for analysing and sorting plant products (e.g., seeds, fruits, or vegetables) is the ability to analyse an image accurately and quickly. Various methods (e.g., use of CCD-camera, flat bed scanner, X-ray scanning, or NMR imaging) can be used to obtain seed images showing external or internal features of certain quality factors, such as size, shape, colours, and defects (Chen and Sun, 1991).

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 analysed by computer-aided image analysis systems and data quickly processed and stored in the hard disk, plotted or statistically elaborated (Dell'Aquila, 2004a). These data include relationships among seed size and shape and growth time-course, and understanding of growth patterns that produce curvature and inflection points. The importance of objective methods in analysing 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). Seeds have a three-dimensional shape, while captured images displayed on monitor or in printed page are in two-dimensional (2-D) format (Loomis et al., 1999). The digital seed image can be assumed as a 2-D object having both dimensions placed along the orthogonal axes of a Cartesian plane. As a result, several descriptors of seed size (e.g., metrical measurements), shape (e.g., numerical factors normalised on the basis of those calculated at the steady state of the seed), and Red-Green-Blue (RGB) colour component density of the individual seed can be easily estimated. Obviously, any selection of algorithms, as well as the creation of macros to allow fast data processing, should be implemented to calculate any variation of external morphology when seeds are subjected to imbibition process.

One of the practical applications of image analysis is to assist researchers and seed analysts in monitoring seed swelling and viability and, so, to overcome some operative limitations of the standard germination test, as approved by ISTA (2005) and AOSA (1993). In addition, the assessment of RGB index of each individual seed within a large seed sample may allow the development of non-destructive methods in sorting seed sub-samples with different germination capability (Dell'Aquila, 2006). These implications suggest computer-aided image analysis to be a promising technique that should be employed in setting the first approach to seed morphology investigation. In this review the development of the methodology integrated with testing standard methods of seed quality to highlight future prospects in seed biology study is reported.

IMAGE ACQUISITION AND PROCESSING SYSTEMS

Generally, an image analysis system includes two sets of components: a) support unit where seeds are placed, which can be set up by a common scanner flat bed or a complex system where seeds can germinate under controlled environmental conditions; b) a computer imaging unit and a software package for image capturing and processing (Figure 1). The scanner technology is low cost and has been widely applied in producing seed catalogues. A flat bed scanner (Hewlett Packard ScanJet 6300C, USA) at 1200 dpi resolution to capture high quality lettuce (*Lactuca sativa* L.) and cucumber (*Cucumis sativus* L.) seed and seedling images was utilized in the development of image libraries, used to study botanical structures for educational purpose as well as to share images via Internet (McDonald et al., 2001). Computer seed imaging was also used in creating digital seed catalogues in three-dimensions, capturing sequences of photographs of the seed with a digital camera or a video-camera from various angles on a commercial computer in Macintosh environment (Loomis et al., 1999).

Interactive models of a 'virtual seed' from onion (*Allium cepa* L.), ragweed (*Ambrosia trifida* L.), carrot (*Daucus carota* L.), and wild carrot can better reproduce the main features of the seed using QuickTime VR object movie technology (QuickTime, 2005) and may integrate printed 2-D images and interactive viewing with a personal computer (Sako et al., 2001a). Scanner image capture technique was used also in seed quality testing. Images of 3 days older lettuce (Sako et al., 2001b) and soybean (*Glycine max* Merrill.) seedlings were acquired with a special scanner contained in a metal box and processed with an appropriate software package (Hoffmaster et al., 2003). The generated image analysis parameter values represented collectively a vigour index based on morphological features. A demonstration of the new vigour test made on several crops can be found on the web page http://www.cse.ohio-state.edu/~fujimura/seed/. A similar method to capture digital images of seedlings grown in Petri dishes was also developed to asses vigour of different lots of small sized Impatiens wallerana, petunia (*Petunia* x *hybrida*), lisianthus (*Eustoma grandiflorum* L.), cauliflower (*Brasica oleracea* L.), tomato (*Lycopersicon esculentum* L.), pepper (*Capsicum*

annuum L.), vinca (*Catharanthus roseus* L.), and marigold (*Tagetes patula* L.) seeds (Geneve and Kester, 2001; Oakley et al., 2004). To measure root length and diameter of switchgrass (*Panicum virgatum* L.), fescue (*Lolium arundinaceum* L.), orchardgrass (*Dactylis glomerata* L.), and clover (*Trifolium repens* L.), a more sophisticated system was designed using digital images captured with combined scanner and digital camera (Zobel, 2003). More recently, scanner technology has been used also to capture highly defined coloured images of lentil (*Lens culinaris* Medik.) seeds subjected to deteriorating conditions, in order to analyse the change of RGB colour components of the intact seed coat associated to the loss of germination quality (Dell'Aquila, 2006).

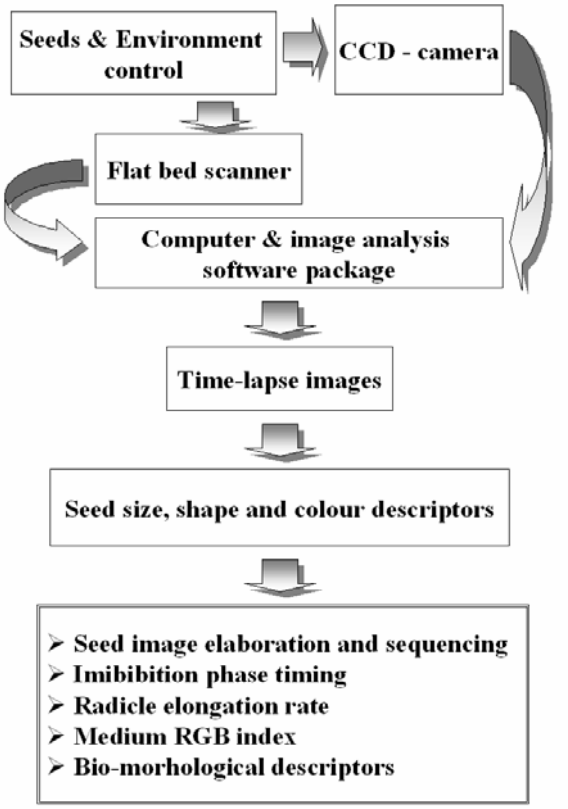

Figure 1. Scheme of image analysis systems.

In the two last decades, the advent of computer-aided data acquisition by video camera, coupled with image processing and analysis, has allowed to capture time-lapse image sequences and to quantify several morphological features, necessary for germination and vigour testing. Preliminary design of an image analysis system suitable for rapid wheat (*Triticum aestivum* L.) image capture and processing followed by data statistical analysis included a mobile camera gantry and a microcomputer running the CP/M® (Digital Research Inc.) operating system (Keefe and Draper, 1988). Image analysis functions were controlled by customized software, written in a modified version of Microsoft® (Microsoft Corp., USA). Once the final binary image (e.g., the silhouette) was processed, a wide range of parameters could be automatically estimated, namely area, perimeter, length, width, the location on the monitor display, x and y coordinate describing the position of the object center in pixel, and the diameters of various orientations. In order to integrate the slant-broad test, used for the measurement of seedling root lengths after a number of growth days (Jones and Cobb, 1963), an image analysis system was designed utilizing a moveable gantry that allowed the video camera to scan the length of one group of five carrot or lettuce seedlings at a time

(McCormac et al., 1990). The system was also used to identify and characterise wheat cultivars on the basis of differences in seed morphology (Keefe and Draper, 1986; Keefe, 1990). The machine vision system used was Quantimet Q10 (Cambridge Instruments Quantimet 10, UK) running custom written software (Modified Microsoft Basic). Seed images were captured with a black and white video camera, so that a binary image (512 x 480 pixels) of the seed silhouette could be stored in the computer memory. With the increasing potential of computer technology, a machine vision system was developed at the National Seed Storage Laboratory (USDA-ARS, Fort Collins, USA) with the objective to introduce new technology in preserving seed germplasm (Howarth and Stanwood, 1993a, b). The designed system was divided into two basic units: the biological system that allowed seed germination in a chamber with controlled humidity, temperature and lighting, and the computer vision system. The vision system consisted of a black and white charged coupled device (CCD) camera with 50 mm lens, a Matrox image processing board (Matrox Electronic systems Ltd, Québec, Canada), and a personal computer-based 386 and 486 systems operating in 3.11 Windows environment. The root length for each seed of lettuce and sorghum was automatically assessed by the machine vision system to be plotted versus imbibition time and statistically compared with the results of a standard germination test made by a seed analyst (Howarth and Stanwood, 1993b). Later on, a more complex biological system, combining temperature and photoperiod control, coupled 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 (Iijima et al., 1998).

Interest focused on the setting up of an image analysis system to measure linear dimensions of seeds with the aim to correlate their change with water uptake process. Seeds of winter wheat and oat (*Avena sativa* L.) with 9.5-10.5% moisture content (mc) were subjected to a "moistening" procedure to reach gradually 21-22% mc or to a "drying" procedure from 21-22% mc to original hydration state (Kruse, 2000). For image acquisition a black and white CCD-camera was used with a resolution of 756x 581 pixel and equipped with a macro-objective: a common 386-computer and the commercial software ImageProPlusTM (Media Cybernetics, USA) with specially designed macros were used. The entire imbibition process leading to germination of *Brassica* seeds was monitored firstly by McCormac and Keefe (1990), using the computerised image analysis system Quantimet Q10. The automated recording of the visible increase in seed size was successfully used as an alternative to measurement of seed weight to follow the course of imbibition. Later on, new experimental evidence was produced on the versatility of image analysis in studying the imbibition process in white cabbage (*Brassica oleracea* L.) seeds by Dell'Aquila et al. (2000). To have a clear view of the seeds using a video camera, the seeds were imbibed in a plastic Petri dish containing low concentrated polymerised agarose placed on a transluminator with fluorescence light. The computer unit consisted of a CCD-camera with a Nikon 55 mm lens, a Sun 4/110 SPARC computer and a frame-grabber (DT 1451, Data Translation, Marlboro, USA). The measurement software (Scil-Image, TNO-TPD, Delft, The Netherlands) determined area, perimeter, width and length as descriptors of seed size changes during swelling (Table 1). The environment system was further improved in the laboratory of image analysis of the Institute of Plant Genetics of National Research Council (IGV-CNR, Bari, Italy). A thermostatic chamber was designed to include a CCD-camera, timer depended lighting system, and a holder with Petri dish containing polymerised agarose (0.1% w/v) added with high concentrated NaCl (0.44M), for salt stress imbibition trials on white cabbage seeds (Dell'Aquila, 2003). Alternatively, this environment unit was also used to evaluate the germination of broccoli (*Brasica oleracea* L.) and radish (*Raphanus sativus* L.) seeds under different regimes of temperatures (Dell'Aquila, 2005). Time-lapse images of 9-12 seeds were captured every hour. The computer unit was standardized using a Silicon Video 2112 CMOS and a PIXCI D2X imaging board (EPIX, USA), a 55mm telecentric lens, a Pentium III in 98 or XP Windows environments and the software package ImagePro-Plus TM v 4.5. The video signal from the camera

was digitised to give a binary image of 1288x1032 pixels. Since a seed during the imbibition process can be compared to an object changing size and shape, firstly image segmentation was carried out to streamline the process of object contour identification and to overcome the shadow effect, which could interfere with RGB component intensity, when the image was acquired with full colour option (Figure 2). A macro created with Image-pro's macro language (IPBasic, Media cybernetics, USA) was used for object number counting, image analysis parameter measuring, data transfer to Microsoft® Excel worksheet, and classification of defined seed number by selected ranges of medium RGB index values (Table 1).

Table 1. Image analysis parameters (adapted from Dell'Aquila, 2004a).

Parameter	Unit	Definition
Seed area	mm^2	The area of the polygon that defines the seed's outline
Seed perimeter	mm	The length of outline of each seed
Seed length	mm	The diameter along the major axis of the seed
Seed width	mm	The diameter along the minor axis of the seed
Radicle length	mm	The distance between the point of the seed coat in which radicle protrusion occurs and the radicle tip
Roundness Factor		The circularity of the seed, calculated by the formula: $Perimeter^2 / 4 \pi$ Area.
Aspect		The ratio between the major axis and the minor axis of the ellipse equivalent to the object (i.e., having similar area of the seed).
Medium RGB index	value ranging between 0-255	The medium Red-Green-Blue (RGB) value calculated by the formula: (R value+ G value +B value/ 3)

Image analysis systems have been applied also to automatically test germination percentage in a large population of seed. Van der Heijden et al. (1999) used a system controlled by a single computer program to study germination time courses in tomato, lettuce, arabidopsis, and cabbage seeds under different temperature and water stress conditions. The time of radicle tip emergence was used to plot precise germination curves for the thermal time models (Bradford, 1996) and to predict seed vigour. A more sophisticated image acquisition system was developed to capture images of different trays, containing plugs in which lettuce, cauliflower, and tomato seeds were grown for subsequent transplanting (Ureña et al., 2001). Once the CCD-camera was positioned over a given tray, a label placed on the tray surface containing the serial number in bar code form was read.

Then, an image of the tray was obtained, allowing the examination of the cells to search for germinated seeds; the degree of seedling development was classified using fuzzy logic and processed data on germination percentage and length of each seedling represented indices of germination quality. More recently, Ducournau et al. (2005) elaborated new algorithms based on the idea that the emergence of a radicle tip at a defined time results in a modification of the binary images. The system was tested to study germination of sunflower (*Helianthus annuus* L.) seeds, and detailed germination curves were obtained allowing a perfect fitting in a probit model.

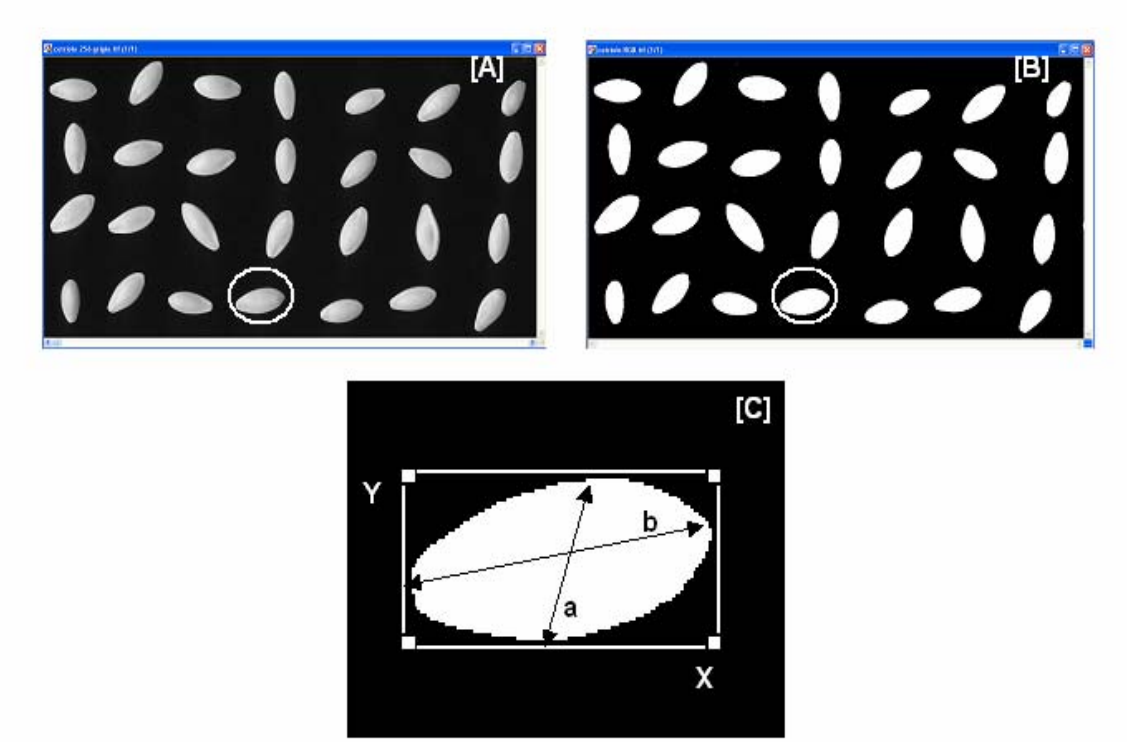

Figure 2. Digital image of cucumber seeds (**A)**; binary image obtained by segmentation of Figure 2A (**B**); definition of parameters related to seed size and shape to compute morphological features (a – width; b – length) (**C)**.

APPLICATION IN MONITORING SEED IMBIBITION PHASES

The sequence of time-lapse captured seed images can represent an 'image print' of seed swelling and growth, which can be measured by means of image analysis parameters. In small seeded *Brassica* species swelling process was recorded as the increase in seed volume and the rate of imbibition of individual seeds was correlated with subsequent seedling root growth on slantboards (McCormac and Keefe, 1990). The morphology and shape of the *Brassica* seed make it suitable for 2-D measurements, assuming that each seed approximates a sphere and that linear expansion is similar along both dimensions. This work hypothesis was used to study by image analysis the imbibition process of different varieties of *Brassica* seeds, imbibed with 0.1-0.5 agarose in a Petri dish at 25°C (Dell'Aquila et al., 2000; Dell'Aquila, 2003, 2004a, b, and 2005). In the case of twelve seeds with 100% germination, the swelling process could be monitored by measuring the increase in seed size (area, perimeter, width and length) and shape (roundness factor and aspect; see also Table 1). Twelve distinct curves of area increase were obtained (Figure 3A), and the same was performed for the others seed size descriptors. The patterns resembled the triphasic curve of water uptake (Bewley, 1997): the first phase (Phase I) of rapid increase was completed at approximately 6 h, followed by a second phase (Phase II) of little apparent area change, which terminated at 10–20 h imbibition. A rapid increase of area values characterized the beginning of the third phase (Phase III), coinciding with a visible radicle protrusion from the seed coat. Despite high final germination, a large variation in time of Phase II was detected by image analysis, which provided useful information on single seed performance within a seed population. When the imbibition process was monitored using the roundness factor, its increase was not comparable to that of the area (Figure 3B). As expected, it was found that the first phase of non-apparent change in roundness factor from the start of imbibition to radicle protrusion coincided with Phase I and II of seed area increase, followed by a second phase of rapid in-

crease. In the latter phase, which corresponded to Phase III of seed area increase, distinct curves were obtained for each individual seed. The image analysis system was applied also to other species (Dell'Aquila, 2004a, b, 2005), including lentil, pepper (*Capsicum annuum* L.), tomato, radish, lettuce, and carrot. A database was developed at the IGV-CNR (Bari, Italy) to store information including the images of several crop seeds and image analysis characteristics collected from a 2-D imaging system. These data have been recently published on the following web site: http://germimaging.ba.cnr.it.

More detailed experiments on the effectiveness of water uptake measurement by seed size change using image analysis system were carried out on wheat and oat seeds (Kruse, 2000). In broccoli seeds, the rate of fresh weight increase, measured during the first phase of water uptake, was determined using the gravimetric method, and values were correlated with those of the corresponding seed size change parameters (Figure 4). The slope of linear regression between seed area and fresh weight increase suggests that seed area was the most sensitive image analysis parameter in monitoring seed hydration (Dell'Aquila, 2004a). Phase II, characterized by little change in water content, is to be considered of primary importance in initiating radicle emergence (Bradford, 1990). The image analysis technique was applied to test the reliability of measurements and the sensitivity of seed linear dimension parameter (the most used was seed area) to monitor any modification of the germination process. Exposure of broccoli seeds to –2MPa NaCl treatment during imbibition led to an increasing reduction in germination (Dell'Aquila, 2003). When seeds were transferred to NaCl, the increase in area slowed down; upon stress removal a remarkable increase in seed area was detected during the first hour of water imbibition. These findings suggest that rapid image processing and recording of seed germination and size may represent an innovative technique for an accurate determination of any variation of seed hydration status. Also the effect of different regimes of temperatures on the germination of broccoli and radish seeds (Dell'Aquila, 2004b, 2005) has been monitored by image analysis system. Obviously, low (15°C) and high (30-35°C) temperatures delay Phase III of rapid area increase corresponding to radicle tip protrusion, while the extent of Phase I and II of area increase changed with temperature shift-up and -down treatments.

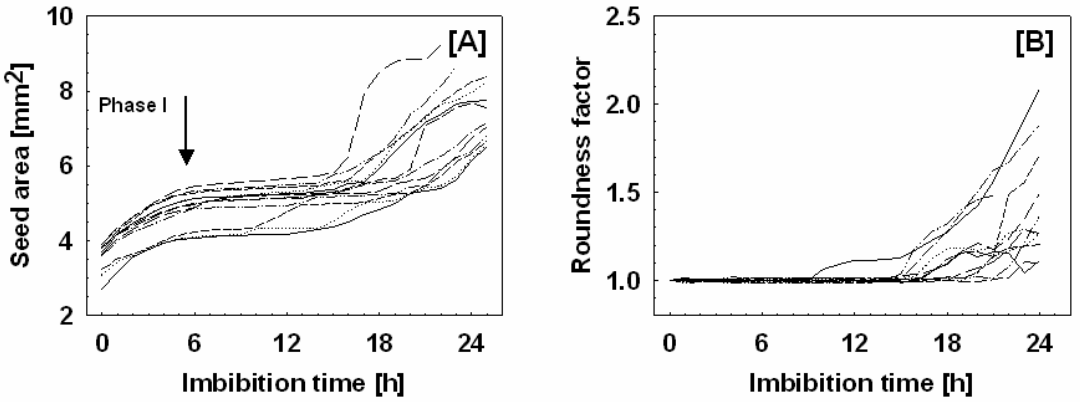

Figure 3. Time courses of area (**A**) and roundness factor (**B**) increases in twelve broccoli seeds over 24h imbibition (adapted from Dell'Aquila, 2004a).

The last phase of rapid area increase, mostly due to protrusion of the radicle tip and its growth, corresponded to Phase III of fast resumption of water uptake. The rate of increase in seed area may be correlated with the corresponding radicle elongation rate (Table 1), for a single germinated seed, when 'visible germination' can be assessed in a germination test. Highly significant linear regression lines may be established in *Brassica*, radish, lentil, lettuce,

pepper, tomato, and carrot seeds (Dell'Aquila, 2004a, b). This trend was also observed in broccoli and radish seeds (Dell'Aquila, 2005) imbibed under different regimes of temperatures, and a close correlation was established between radicle elongation rate and seed area increase rate, calculated for each temperature, as shown by monoexponential curves in broccoli and radish seeds (Figure 5).

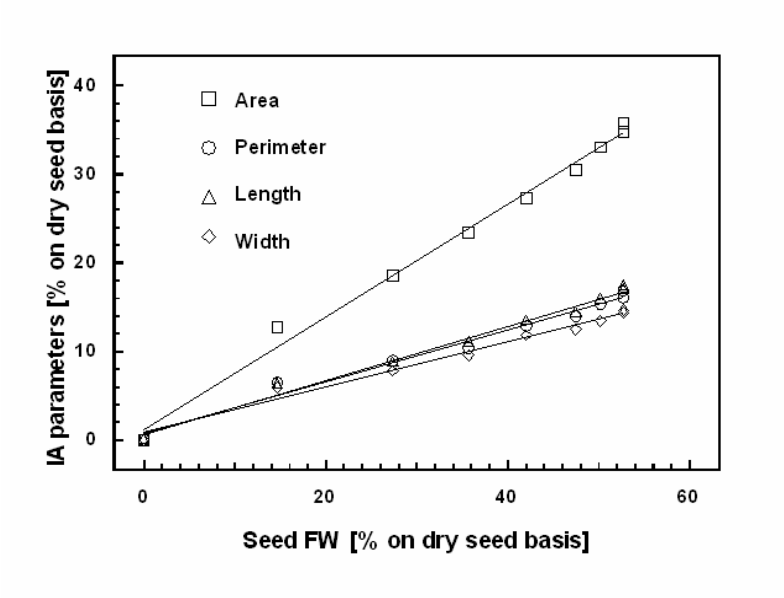

Figure 4. Linear regression lines between seed fresh weight (FW) and image analysis parameters in broccoli seeds. Correlation coefficients are significant at $P \leq 0.001$ (adapted from Dell'Aquila, 2004a).

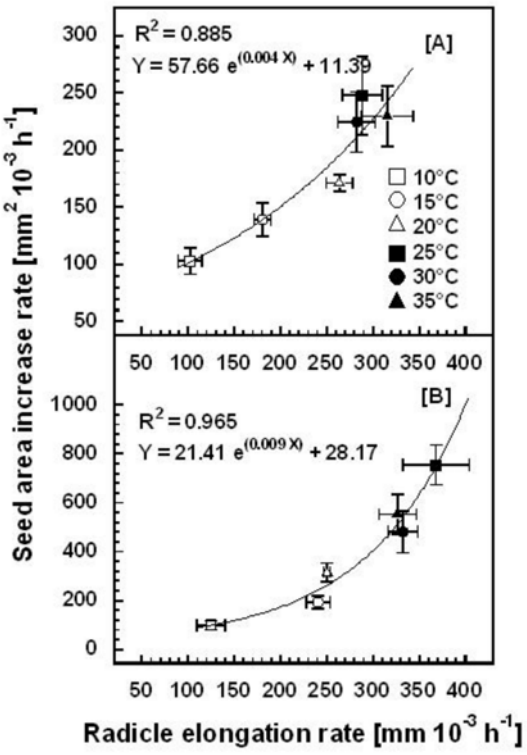

Figure 5. Relationship between seed area increase and radicle elongation rates over a 10-35°C temperature range in broccoli (**A**) and radish (**B**) seeds (adapted from Dell'Aquila, 2005).

APPLICATION IN DETERIORATED SEED QUALITY TESTING AND SORTING

A number of methods for seed quality evaluation and sorting have been recently developed, mainly based on the detection of various physical and chemical properties that correlate well with certain vigour and germination parameters (McDonald, 1998). Lipid peroxidation and the production of free radicals may be the main cause of seed deterioration.

In addition, non-enzymatic reactions, such as Amadori and Maillard reactions, reduce sugars or protein amino groups to form fructosyl derivates or glycated proteins, whose interaction produce polymeric brown products (Wettlauer and Leopold, 1991; Sun and Leopold, 1995). As a result of these deteriorating effects, the visible physical change is the discoloration or browning of the seed coat. The effect has been described in legumes, where colour change can be quite heterogeneous within a seed sample, and seeds that maintain their original colour at full maturity tend to preserve high vigour (Priestley, 1986). New computer-aided image analysis may be extended to the analysis of Red-Green-Blue (RGB) colour components of 2-D seed images. Since all visible colours can be represented with varying combinations of these primaries (Fairchild, 1998), related colour mapping is represented by a numeric range of RGB values (from 0-0-0 for black colour to 255-255-255 for white colour). The method is based on measuring the medium RGB index (Table 1) of single seeds, which are then separated into three fractions, each having a different RGB range. This method was applied to lentil seeds, which were deteriorated under controlled conditions of moisture content and temperature and sorted in three fractions with distinct germination potential over the entire period of ageing (Dell'Aquila, 2006). The border of the three fractions was chosen from the unaged seed lot according to the method used in sorting cabbage seeds with chlorophyll fluorescence marker. Two sub-samples were taken, one sub-sample with high and medium colour density containing the majority of seeds with high germination percentage and the other sub-sample with low colour density that covered the remaining seeds with low germination percentage (Jalink et al., 1998; Dell'Aquila et al., 2002). In this way, a non-destructive method was developed in sorting aged lentil, cucumber, lettuce, and tomato seeds (Figure 6). Discrepancies may derive from different regimes of deterioration conditions, such as low seed moisture content and storage temperature, that could affect in different ways the 'browning effect', therefore the choice of RGB marker thresholds may be adapted to a wide range of storage conditions.

CONCLUDING COMMENTS

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 software tools suggests that the use of machine vision systems is becoming convenient in a seed biology laboratory.

The speed of operation of a machine vision system must allow rapid image processing and recording of measurements. Data may be further processed statistically and displayed graphically, and a database may be developed to integrate image analysis data with taxonomic and bio-morphological features of plant species. So this integrated system can represent a new approach to understand seed biology and quality, and it includes: 1) operative system modelling and automation, 2) digital imaging, 3) data collection and integration with those obtained from standard seed quality tests (ISTA rules, 2005), and 4) new experimentation and hypothesis. The ultimate goal is not the management of system data, but their use for the development of mathematical models to describe and predict seed germination and

quality as well as to extend seed analyst ability to control operations automatically. For the design of these models, there is a remarkable demand for new software tools that allow more flexible and valid methods of analysing and visualising multifaceted biological systems (Girke et al., 2003; Gutiérrez et al., 2005). Genomics and proteomics together with digital imaging can result in a novel trait in economically important crop plants involving a new combination of technologies and a new 'vision' for biology systems in plant research.

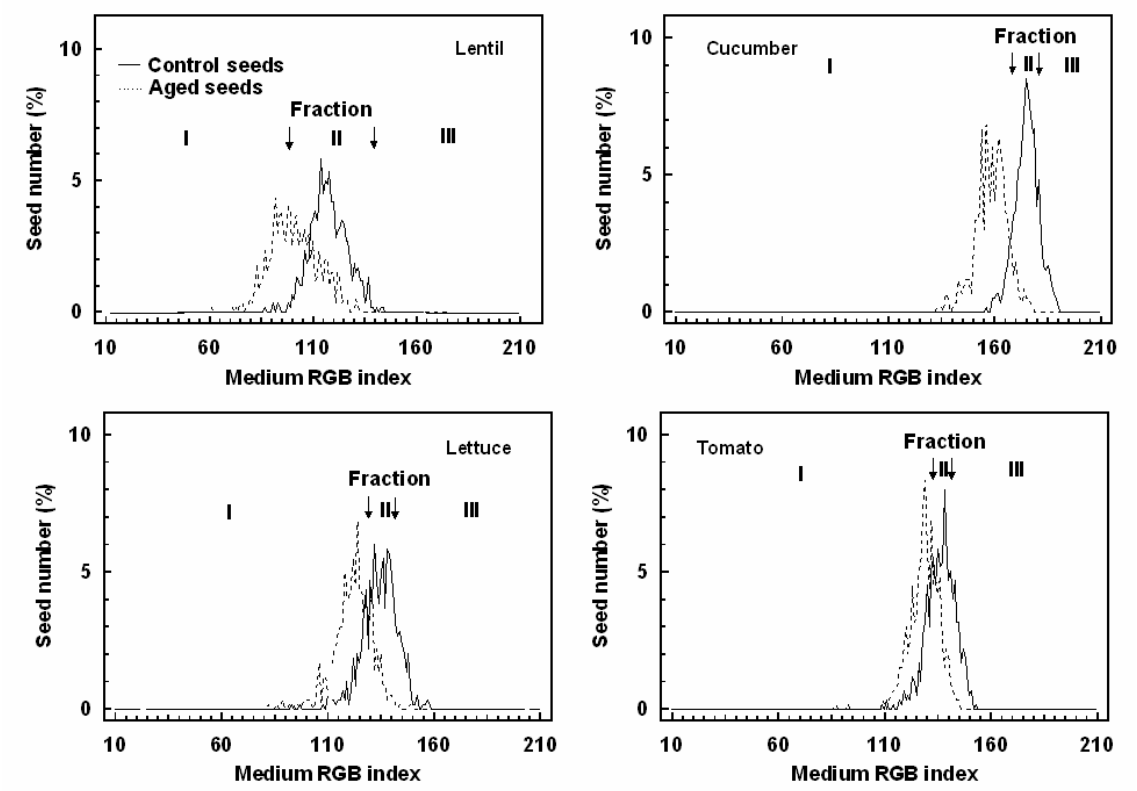

Figure 6. Distribution histograms of medium RGB index values in control and aged lentil, cucumber, lettuce and tomato seeds. Ageing was carried out under 16% SMC and 40°C for 10 days. Arrows indicate the RGB value thresholds in sorting seeds in Fraction I with low germination and in Fraction II and III with high germination (adapted from Dell'Aquila, 2006).

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