IMAGING TECHNIQUES TO ENHANCE THE PRESERVATION AND UTILIZATION OF SEED GERMPLASM

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ABSTRACT

Several image processing techniques are being developed at the National Seed Storage Laboratory to enhance the preservation and utilization of seed germplasm. Two projects investigate the potential of this technology in controlling and providing additional information in germination testing, seedling growth rate analysis and tetrazolium testing. Another thrust of the research has been to develop an image-based database. Investigations in this study concentrate on the development of features which describe physical characteristics of seed. An object-oriented database was developed within the Windows 3.1 operating environment. This database is unique because it is capable of displaying many types of information, for example seed images, graphs, or color pads. Machine vision and image analysis have shown promise and many new applications of this technology are being developed.

Additional index words: **machine vision, seed vigor, tetrazolium testing seed characteristics, slant board.**

INTRODUCTION

The United States National Plant Germplasm System (NPGS), which includes the National Seed Storage Laboratory (NSSL), is responsible for the long-term preservation, collection, propagation and distribution of plant genetic resources (Shands, 1990). NPGS collections contain major agricultural crops such as corn, wheat, rice, and other plant collections such as vegetables and ornamentals. These collections contain wild species as well as developed cultivars. Most of this germplasm is held as seeds.

The record keeping for germplasm preservation is a never ending task. Agronomists, plant pathologists, plant breeders, conservationists and many other scientists are faced with the challenge of creating, updating, and maintaining germplasm databases. The Germplasm Resources Information Network (GRIN) database was developed in order to utilize and maintain germplasm. The task of maintaining this database is the responsibility of the National Germplasm Resources Laboratory of the United States Department of Agriculture, Beltsville, MD. Some of the information within GRIN describes physical characteristics of the seeds. These characteristics include hundred seed weight, shape, color, and texture. With the exception of hundred seed weight, all of these characteristics involve subjective evaluations. In many accessions genetic variability is repre-

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sented by differences in seed size (length, width, and thickness), color, texture, etc. These differences are not recorded in GRIN. Much of this variability could be expressed by color images and other extracted features.

Seed deterioration is another problem facing the NPGS and NSSL. This has plagued mankind since the cultivation of plants began and has been a major concern for the NSSL. The main objective at NSSL is the long-term preservation of plant germplasm for the United States. Plant germplasm is stored at sub-zero temperatures, and the questions often asked are "How long can a seed sample be stored under these conditions and maintain viability?" and "Why do these seeds deteriorate?" Obviously, it is important to measure the viability of the seeds both before and during storage. Before seeds are placed into storage, the initial viability is determined. During storage, seeds samples are tested to determine the rate of deterioration and if regeneration is needed.

Seed vigor and germination tests have traditionally been used to determine deterioration of seed samples. A seed vigor test describes the seed potential to emerge rapidly and produce a mature crop under certain field conditions (Heydecker, 1972). The test must be performed in an objective manner to maintain a common language between all testing entities. Although AOSA (1988) attempts to provide guidelines for testing seeds, it also requires subjective interpretation of the results. This can cause discrepancy among experienced analysts.

The current seed viability tests are labor intensive and subjective. The standard germination test (AOSA, 1988) determines the percent germination of a seed sample, and takes 4 to 28 days or longer to complete. If the dormancy of the seeds is not overcome, percent germination will not reflect the true viability. Tetrazolium (TZ) testing can be used to detect viability of dormant seeds. Results from TZ tests often can be obtained within 24 hours. However, due to the high level of subjectivity in assessing color development, the TZ test cannot replace the standard germination test. Machine vision is a tool that might assist researchers and seed analysts to measure and predict seed viability.

There have been numerous studies on the use of machine vision for improving agriculture product quality and most has concentrated on color and blemish recognition. Several researchers have worked on detecting bruises on apples (Taylor et al., 1984; Taylor and Rehkuglar, 1985; and Brown et al., 1974). Computer Recognition System developed a orange grading system which sorted oranges into three classes (Harris, 1988). Howarth (1991) developed a machine vision system to grade fresh market carrots. This system identified blemishes caused by rot but also graded the carrots based on several shape parameters.

Many image processing applications have been developed to measure seed characteristics. Churchill et. al. (1990) developed a machine vision system to measure seed dimensions of tall fescue, orchardgrass and perennial ryegrass. Texture and color image processing techniques were developed to identify weed seeds (Petersen and Krutz, 1992). Feature extraction techniques using fractals were used to describe the shape of ear corn (Panigrahi and Misra, 1990). Image analysis has been identified as a tool that could be used for taxonomy (Draper and Travis, 1984). Most recent attempts to measure seed shape by machine vision have primarily concentrated on specific varieties or on classifying the difference between a small group of varieties.

McCormac et al. (1990) investigated image analysis for measuring the root length of lettuce using a slant board test. However, several problems were identified with this technique. First, root length was only measured after the slant board test was completed. Secondly, only linear length was measured; therefore, the true length of the root may not have been measured.

The objectives of the computer imaging research at the NSSL are to: 1) increase the efficiency and accuracy of evaluating stored seed germplasm, 2) reduce the amount of personnel resources needed to monitor seed viability, and 3) provide information about seed accessions that is not currently available and to make this information readily available to users of germplasm.

IMAGE PROCESSING SYSTEM[2]

The basic components of a computer imaging system consist of a imaging sensor, an image frame grabber, a micro-computer, and a video monitor. The imaging sensor converts electromagnetic energy into an analog signal. The most common sensor is a CCD (Charged Coupled Device) camera. The image frame grabber converts the analog signal from the imaging sensor into a digital signal. The digital signal is stored in a buffer on the frame grabber and can be accessed via the computer data bus. The digital signals are organized into a matrix of pixels. The value at each pixel is relative to the reflected light at a specific location within the sensor's field of view. This is generally a value between 0 and 255; 0 corresponds to no light reflected or black and 255 corresponds with a maximum reflectance or white.

The computer host systems in the NSSL imaging laboratory are PC-based 386 and 486 systems. The two image frame grabbers utilized are a Matrox 640 PIP and a Matrox MVP-AT. The Matrox 640 PIP image processing board processes and stores gray scale images in a 640 × 480 array. The black and white camera connected to this board is a Cohu camera with a 50 mm lens. Also included with this camera setup is a microscope adapter. The Matrox MVP-AT is a multiuse frame grabber. This board can be configured as either a color or gray scale imaging board. In the color mode, the array size of the board is 512 × 480 and in the gray scale mode, the array size can be set in several different sizes: 4 – 512 × 480 to 1 – 1024 × 1024. Two color cameras can be utilized with this board, a Cohu color camera and a Sony 3 CCD color video camera. The color Cohu camera, like the gray scale camera, accepts 'C' mount lenses. A 50mm lens was used. The Sony camera used a bayonet mount, and a telephoto lens (7.5 to 90 mm) was used. A microscope adaptor was available for use with an Olympus microscope.

The two operating environments were DOS 5.0 and Windows 3.1. Most of the developmental work was done in the Windows 3.1 environment and once a concept was developed, it was ported into the DOS 5.0 environ-

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[2] Mention of company or trade name does not imply endorsement of the products by the USDA-ARS. It is for purpose of description only.

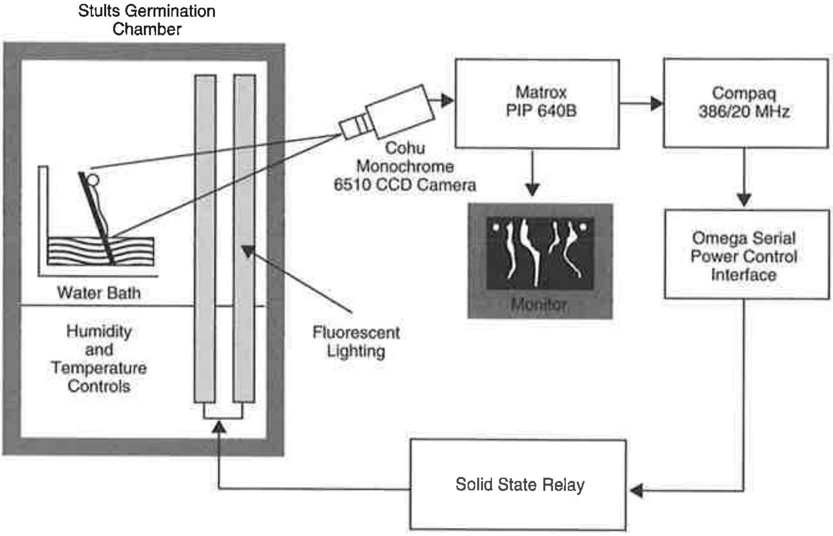

Figure 1. Schematic of machine vision system for root length measurement.

ment in which many of the imaging operations were performed on the imaging boards and at fast rates. The developmental package used within the Windows 3.1 environment was OPTIMAS 3.1. This package was useful for applying different filtering and other image processing techniques. The OPTIMAS software also included an Analytical Language for Images (ALI) which was used to write macros. Turbo C++ by Borland was used in the Windows 3.1 environment to develop Windows application for color image processing. In the DOS 5.0 environment, Microsoft C version 5.0 was used to implement algorithms used to make image measurements.

IMAGING PROJECTS

The four major projects at the NSSL are: 1) to develop a machine vision system to measure seedling growth rate and determine if this information can be used to predict seed viability, 2) to develop a machine vision to assist the seed analyst to perform a non-subjective tetrazolium viability test, 3) to develop image processing and feature extraction techniques to measure the physical characteristics of seeds, and 4) to develop an image-based database for plant germplasm. An outline of the concepts for these projects and the latest advances follow.

Seed Growth Rate Test

The objectives of this study were 1) to develop feature extraction techniques needed to measure seedling growth rate, 2) to develop a machine vision system capable of monitoring seedling growth rate, and 3) to determine the reliability of the machine vision system. A machine vision system was developed to measure root growth rate over the entire germination period. The system schematic is shown in Figure 1. A Cohu black and

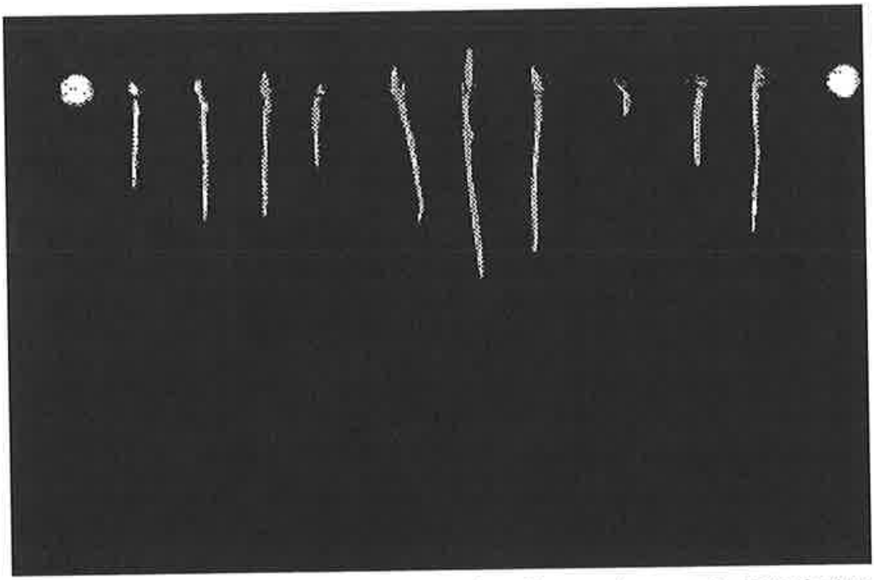

Figure 2. A typical image obtained during testing of ten sorghum seeds. Note the two pins seen as round white objects. The sorghum seeds were planted in a row between these pins. Roots are shown attached to the blotter paper extending down from the pin location and the stem portion extends up from the pin location.

white camera was set outside a Stults germination chamber. This chamber provided the necessary environmental conditions to run a standard germination test. The chamber remained dark throughout the test except when images were obtained during which lights remained on for approximately 30 seconds every hour. The images were stored on the hard disk and analyzed later. An example of an image is shown in Figure 2.

The steps to locate and measure the root growth included calibration, location, and measurement. Two calibration measurements were made in each image, positional and size. As shown in Figure 2, two circular positioning pins were used to perform the calibration measurement. These circular pins were located, and the position of the pins and distance between the two pins were used for calibration. Once the pins were located, roots were located and the length was measured. This was completed for all images and the growth rate curves for lettuce and sorghum are shown in Figure 3. These measurements compared similar to human measurements. The average error between the two measurement systems were -0.13 cm for the lettuce test and -0.07 cm for the sorghum test. For more detail on the procedures and results, see Howarth and Stanwood (1992a).

This system analyzes ten seeds every germination period. An automated system has been developed and is under construction which will present 50 slant boards to the camera. This will allow 500 seeds to be analyzed every germination period. This will make this system attractive to the NSSL research and preservation units and to the seed industry.

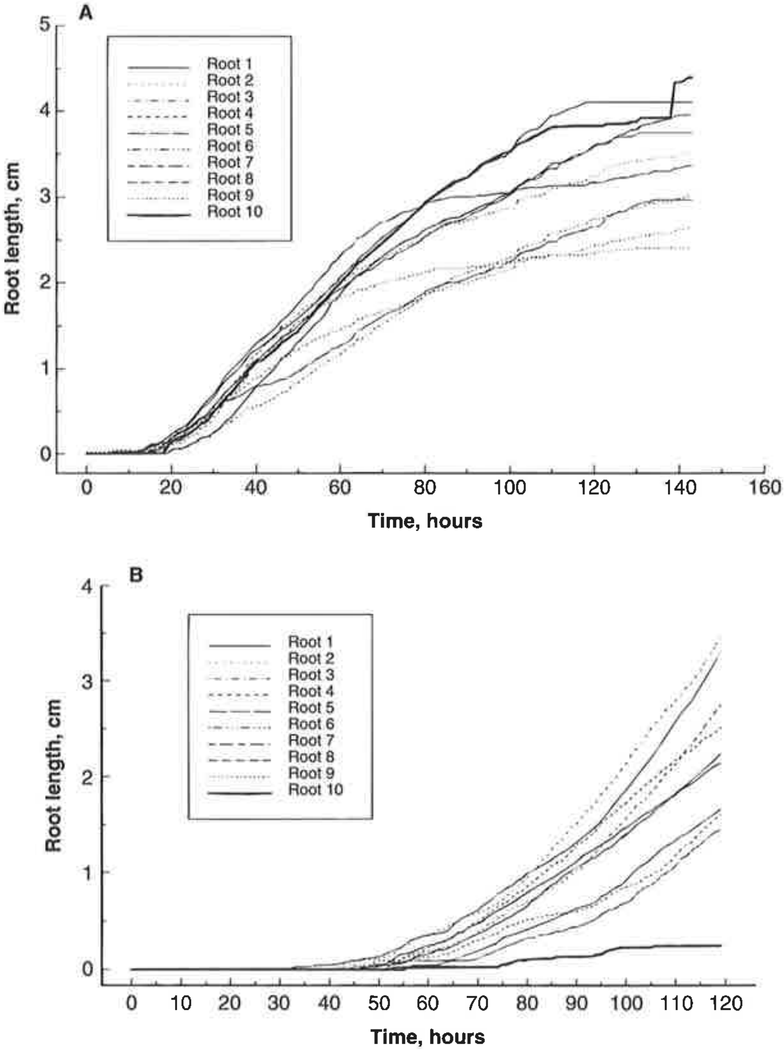

Figure 3. Illustration of ten root growth rate curves measured by the machine vision system: A) lettuce and B) sorghum.

Tetrazolium Seed Viability Test

In order to automate the TZ viability test, it was necessary to develop color feature extraction and classification techniques to automate the classification of TZ stained corn seeds. A machine vision system was developed using the color image processing system and is fully described in Howarth and Stanwood (1992b). The ratio between stained area and total corn area was used as the primary feature. The stained area was located and measured by subtracting the red image plane from the green plane.

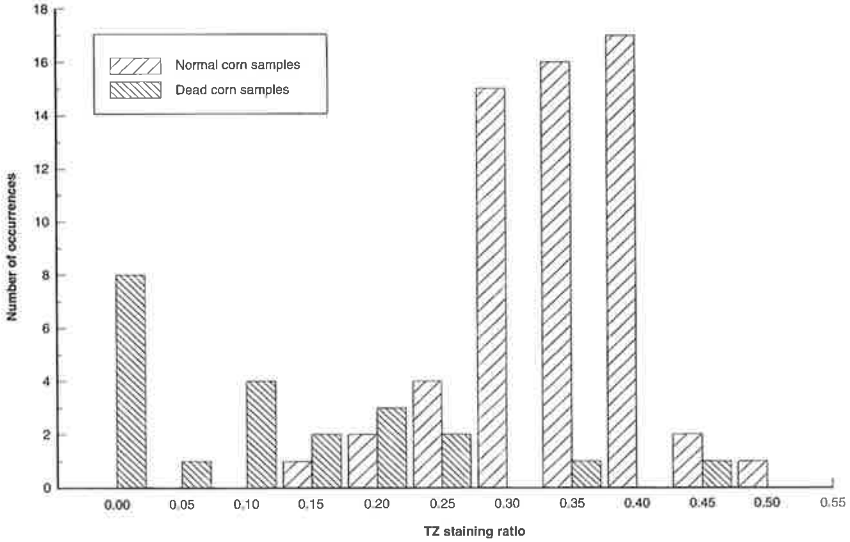

Figure 4. TZ staining ratio histogram showing the distribution of normal and abnormal or dead corn kernels.

This produced an enhanced image which was thresholded. Thresholding is a procedure in which an optimum intensity value (also called the threshold) is selected. Then, the intensity value of all pixels within the image are compared to the threshold value. All intensity values above the threshold are classified object and value below the threshold value are classified background. This transformed image is now considered a binary image. Then, this binary image was organized using the connected components algorithm. The total corn area was segmented from the background by thresholding the intensity of the image. The intensity is the average of the red, green and blue (RGB) image planes. The classification histogram was constructed from the training data and is shown in Figure 4. This histogram shows the distribution of normal and abnormal or dead corn kernels. Based on this information, a Bayes classifier was developed. Overall, the misclassification rate for the training tests was 8 of 80 samples (10.0%). For the validation set, 7 samples were mis-classified out of 67 (10.4%). However, all these errors were Type II errors. The Type II error in this case is the dead or abnormal corn kernels that were incorrectly classified normal. This was caused by the machine vision not being robust enough to identify special cases. These cases were when a specific and important region within the stained embryo was not stained. By automatically identifying and locating these non-stained regions, the current system could reach its full potential.

Physical Characterization of Seeds

Much of the seed characteristic information within the GRIN database is based on subjective evaluation. A large degree of variability can be repre-

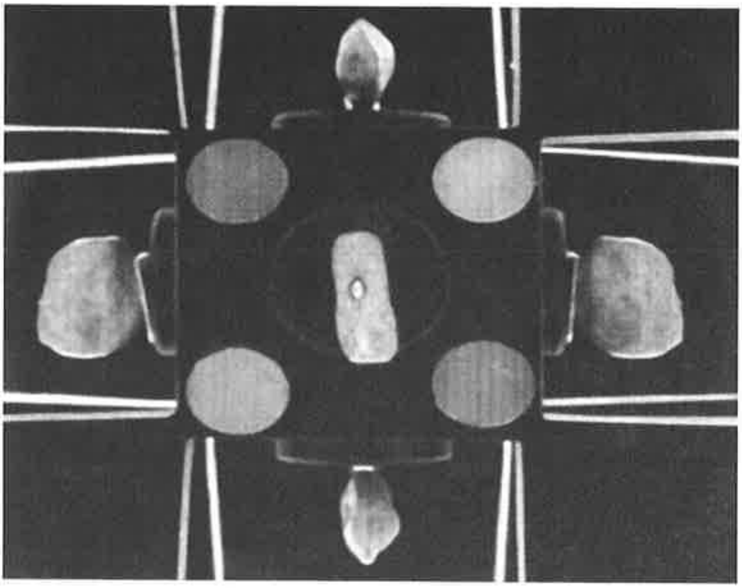

Figure 5. Sample image obtained from the 3-D imaging system. The center image represents the top view of the seed. The top and bottom images are side views of the seed. The right and left images are the end views of the seed.

sented within a seed sample. Therefore, a tool was needed to evaluate physical characteristics of seed in a non-subjective procedure. As mentioned earlier, many image processing applications have been developed to measure seed characteristics; however, all have concentrated on two dimensional images. In many cases, some characteristics can be obscured or hidden from view using only two dimensional images.

In order to collect three dimensional information, a machine vision system was developed to measure this information with a two dimensional imaging system. A mirror mount was constructed and this mount was fabricated from aluminum so that four mirrors could be position in pyramidal fashion about a seed positioning stand. The mirrors are positioned at 45° incident to the imaging plane. An example of an image obtained from this system is shown in Figure 5. As shown in Figure 5, the center corresponds to the top of the seed and about the center are four reflected images of the two side and two end views of the seed.

Three color pads also shown in Figure 5 were used to locate area of interest (AOI) regions and to spacially calibrate the images. These pads were located using a circle detection algorithm (Howarth and Stanwood, 1992c). Once these color pads were located, five AOI regions were located and several size, shape and color features were calculated from each region. The size features were length, width, and height. The shape features were form factor, circularity, and centroidal profile. The color features were the RGB statistics (i.e. average and standard deviation) and

RGB histograms. For more specific information regarding this work, refer to Howarth and Stanwood (1992C).

Image-Based Plant Germplasm Database

Traditional approach to database programming and management has revolved around the textual data with which the program and management environment must deal. Recent interest has focused on non-textual information (i.e. images, graphs, etc.). Virtually all proposals from the database community for management of non-textual information use object-oriented techniques (Grosky and Mehrotra, 1989). To be able to handle images, graphics, and other non-textual information, it appears that an object-oriented database environment is the most efficient and attractive technique.

Object-oriented programming is a paradigm which is distinguished by its representation of the relationship between data and programs (Engel et al. 1989). Traditional programming languages like FORTRAN and C operate in a procedural environment. In this procedural environment, there is typically a main function and additional functions that are called from the main function (Pappas and Murray, 1992). This is commonly referred to as the top-down approach. An object-oriented program is constructed from a group of related "objects". These objects form a hierarchy which makes an object-oriented program more modular; therefore, new objects and methods can be added with relatively few unanticipated side effects (Fernhout, 1989).

Where traditional databases require all data elements to have similar formats, an object oriented database can tailor data structures to describe the information being stored. This allows the database to contain a variety of information, from text to graphics to computer simulations (Beck, 1989). Therefore, this type of database has the ability to use an object-oriented programming language to describe different data structures by defining classes that are individually tailored to the data (Freeman and Field, 1992).

An object-oriented color image database of plant germplasm was developed in the Windows 3.1 operating environment. The images were displayed as full color (over 16 million colors) bitmaps using a super VGA card and monitor. The information within this database included the images, passport and seed characteristics downloaded from GRIN, and physical characteristic seed data collected from the 3-D imaging system. Several types of objects were created to handle this variety of data. These included objects to handle and display bitmaps, graphical and textual information, and to generate and display color pads and representative shape figures. Presently, 33 accessions of *Phaseolus*, 20 accessions of endangered species seed, and several other samples of various seeds have been input into this database. This database may provide curators, researchers and plant breeders with a useful tool for visually inspecting current seed of plant germplasm.

FUTURE DIRECTION

During the last few years, the projects discussed have provided the NSSL with a sound foundation for future development. Several areas must be investigated so that the current projects may reach full potential.

Discussions with other researchers at the NSSL and Colorado State University have begun to expand the scope of the imaging research.

For current and ongoing projects, several major concerns that will be addressed are as follows. The first projects slated for the next year is the testing of the SGRobot, which is the automated system for measuring seedling growth rate. Furthermore, tests are being designed to understand the biological meaning of different root growth rates and to determine the link between seed growth rate, seed vigor, and seed longevity. The TZ project will be improved to distinguish special cases of staining. This, along with testing of many cultivars of corn, will enhance the current system to provide seed analysts with a tool to quickly measure seed viability. For the seed characterization project, it is important to develop a system which is more robust and more tolerant of positional bias. This would alleviate positional error and simplify image acquisition. Other important aspects of this system that will be investigated are improvements in the lighting and focusing systems to help to reduce overall system error. To realize the full potential of the image-based database, several problems will need to be addressed. First, a technique to compress the images must be incorporated into this database, and, secondly, the method of distribution must be addressed. As these operations are installed, plant breeders, seed scientists, and other researchers can began to use this system and comment on its abilities. Their comments will be useful for development of future versions.

One of the future imaging research projects slated is pollen germination counts. The current procedure is very time consuming and causes eye fatigue. An imaging system could help to count germinated and non-germinated pollen. As well, a technique to observe and measure germinating pollen tubes non-invasively will also be investigated.

The imaging technology shows enormous potential for the enhancement and development of non-invasive biosensors. These sensor can be used as tools to perform tedious and subjective tasks and to help unlock the mystery of seedling vigor.

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