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Application Of Image Processing For Seed Quality Assessment: A Survey

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ABSTRACT

This paper presents the review of methods used in seed technology that can be efficiently implemented using machine vision system. The methods discussed in this paper are seed vigor testing, seedling growth rate measurement, measure of germination index, etc. The recent development of computer-aided image analysis techniques for monitoring seed germination and vigor has been done. Seedling growth rate is thought to be a sensitive measure of seed vigor, but is difficult to incorporate in routine vigor testing because it has been too labor intensive to periodically evaluate seedling growth over time. This paper studies various computer-aided techniques which reduces the labor input required to evaluate seedling growth rate and increases the accuracy of these measurements. Several theories of seed testing are also briefly mentioned.

1. INTRODUCTION

Seed is the most basic entity of agriculture, which governs the quality and yield of its production. Without good seeds the investment on fertilizers, water, pesticides, and other inputs will not be worth. It is necessary to improve the quality of seed for ensuring the high efficiency, quality and productivity of agriculture production. Seed vigor is the important index of measuring seed quality. A seed vigor test can reflect the potential of seed in the field or the seed under storage. Seed vigor testing is a very comprehensive method of assessing seed lot quality.

1.1 Basics of Seed Technology

Seed testing is the cornerstone of all other seed technologies. Seed testing is used for control of quality parameters during seed handling, and test results are submitted to customers as documentation on seed quality. It is the means by which the quality of seed can be measured and viability of seed is ensured. Seed testing is determining the standards of a seed lot namely physical purity, moisture, germination, vigor and thereby enabling the farming community to get quality seeds.

In seed evaluation, germination test indicates the ability of seed to produce a normal plant under favorable conditions. Since germination tests are carried out under optimal germination conditions, the test results express the germination potential under ideal conditions of temperature, moisture and light. In many instances, seed lots of apparently equal quality as indicated by germination percentage will produce largely different responses in field emergence. Clearly, a germination test alone is not enough to assess seed quality, vigor test is also required. Germination test and seed vigor tests have traditionally been used to determine deterioration of seed samples. Standard germination percentage and the seed vigor of the seed lot can be used to measure seed lot quality.

1.2 Germination Test

Germination is defined as "the emergence and development of seedling to a stage where the aspects of its essential structures indicate whether or not it is able to develop further into a plant under favorable conditions in the soil' *(ITSA 1996)*. Germination is normally carried out in germination cabinets under

controlled environment. The conditions prescribed by *ISTA* include the following variables:

- Temperature (level and regime, e.g. constant day and night or fluctuating)
- Light (+/- light or period of day/ night cycles)
- Substrate (sand, top of sand, top of paper, between paper and pleated paper)

The *ISTA* rules also indicate days of first and last count to standardize the duration of the test period. Germinated seeds are counted regularly during the prescribed germination period from the indicated 'first count' to 'final count'. The final test result is grouped into the following classes:

1. *Normal germinants:* The cumulative number of seeds which have developed into seedlings of normal and healthy appearance with all essential structures of a seedling. This also includes seedlings where possible damage is caused by secondary infection.
2. *Abnormal germinants:* The cumulative number of seeds which have germinated during test period but in which seedlings show abnormal or unhealthy appearance e.g. lacking essential structures such as cotyledons, or being discolored or infected by seed borne pathogens.
3. *Ungerminated seeds:* Seeds which have not germinated by the end of test period. These are grouped into following subclasses:
   a. Hard seeds, which are seeds that remain hard because they have not imbibed.
   b. Fresh seeds, which are seeds that have not germinated although they appear firm and healthy.
   c. Dead seeds, which are seeds that are soft, or showing other signs of decomposition.
   d. Other seeds, e.g. empty seeds.

The final evaluation of the germination test is reported as germination percentage or germination capacity, which counts 'normal germinants'.

1.3 Seed vigor test:

Vigor testing is an important component of seed testing because it's more sensitive test than germination, and because loss of vigor may be noted much earlier than loss of germination. Seed vigor directly determines the emergence potential of a seed lot and physical and genetic aspects of seeds also affect their storage, processing and transport. *AOSA* defines seed vigor as 'those seed properties that determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions'. Both the *AOSA* (1983) and *ISTA* (1999) Vigor Testing Handbooks provide specifications for seed vigor tests based on determining a seed lot's vigor that may include measures of speed and uniformity of seedling growth. Vigor testing predicts the general ability of a seed lot to germinate normally over a range of adverse conditions. Seed vigor has been determined by both germination rate and seedling growth rate. Germination rate measures the speed of germination which is commonly represented by time to 50% germination, while seedling growth rate is evaluated on a real time basis by measuring rate of elongation of the radical per unit time. Some of the methods for seed vigor testing are summarized below:

i. Germination percentage of normal seedlings after imposition of stress – such as cold or accelerated aging,
ii. Biochemical tests – such as tetrazolium or electrolyte conductivity, and
iii. Seedling growth.

A vigor test cannot replace a germination test but rather supplements it with more information about seed quality.
Naturally, the germination test will almost always have the higher result because the evaluation parameters are more forgiving than those used in the vigor test; so the germination test tells producers how their seed will perform in optimum conditions.

2. RELATED WORK

All the tests explained above are normally performed manually. These tests are generally costly, time consuming, and the results of tests may vary from laboratory to laboratory. So there is a need of objective system which will provide consistent results.
A machine vision system will be able to perform the tests fast, consistent and it will also reduce the human interference. The work related to application of machine vision in seed testing is explained below:

Y. Sako, et.al. developed a system for automated seed vigor assessment. This system contains a flatbed scanner which is used to capture the images of seedlings; this scanner is interfaced with computer. The images obtained were processed by computer to calculate the vigor index based on sample mean of various statistics acquired from morphological features of the image seedlings. The system was tested for lettuce seedlings grown in dark for three

days. The vigor index computed by system was compared with vigor index computed manually using individual seedling measurements. In best case, the percentage difference between manual and computer determinations of the vigor index was only 0.99% for lot 1. In worst case, percentage difference was 14.71% for lot 9. These values were much acceptable than the variation in results from laboratory to laboratory.

A. L. Hoffmaster, et.al. proposed an image processing computer application to automatically assess the vigor of three-day-old soybean seedlings. An image of soybean seedling was captured using flatbed scanner. The soybean seedlings were segmented from the background and converted into various digital formats. These representations were used to segment the seedlings into normal and abnormal categories. The normal seedlings are further processed to perform length measurement. Using skeletonization, a 1- pixel wide summary structure of the seedling were obtained. To calculate actual length of the seedlings, the cotyledon portion of the seedling skeleton is removed. After removing the cotyledons, skeletons were further processed to calculate length of seedling. The weighted sum of these length measurements along with the speed and uniformity of growth values produces the vigor index representing the vigor of the seedling.

Seed vigor is an important parameter in determining seed quality. Seed vigor tests are generally useful for evaluation of large seeded agronomic crops which are not useful for smaller seeded vegetable and flower species. Seedling growth rate can also be used to access vigor in seeds. For this purpose, Robert L. Geneve, et.al. designed a system to evaluate early seedling growth rate using flat-bed scanner. A flat-bed scanner is used to obtain the digital images of seedling emergence and transparent medium is used to facilitate scanning. This system was tested for six small seeded species (cauliflower, tomato, pepper, impatiens, vinca, and marigold). Twelve seeds per species were sown in plastic Petri dishes containing either one piece of blue blotter, two pieces of germination paper or one piece of a clear, uncoated cellulose film. Before placing seeds on film, water was added to the Petri dish. Each species were evaluated after 2 days of radicle emergence. Digital image analysis of these images was done. Three analysts separately evaluated same set of seeds. Scans were made for seeds on the cellulose film through the Petri dish with the lid removed. Comparison of all the results was done which shows there was no statistical difference between the blotter paper and cellulose film for tomato, pepper, impatiens, vinca, and marigold. Cauliflower seedling length was greater on paper than cellulose film and blotter. These results indicated that the cellulose medium could be a suitable substitute for germination paper and blue blotter when imaging for vigor assessment. The length or area of each seedling was computed by computer-aided analysis using available software (MacRhizo). This software was able to measure root length and not customized for measuring individual seedlings.

K. Oakley, et.al. developed a system for computer-aided image analysis of digital images to evaluate seedling growth as a measure of seed vigor. A flat-bed scanner was used to capture the images of seedlings. Similarly, software was developed to calculate seedling growth and in most cases a vigor index has been calculated based on seedling growth, growth uniformity and germination percentage. Vigor index for six seed lot has been measured based on cotyledon area of seedlings produced in plug under controlled environmental conditions. For 25 seeds standard germination test was conducted by placing in four replicate of plastic petri dishes containing one piece of blue blotter with 5.25 mL of water sealed with Parafilm and placed in germination chamber held at 23^{o}C with 40µmol.s^{-1}m^{-2} from cool white fluorescent lamps. After 18 days from sowing, normal seedlings were calculated. This is repeated for each seed lot. Saturated salt accelerated aging vigor tests were conducted using one-hundred seeds of each seed lot for 48h. Germination percentages were evaluated using standard germination conditions immediately after saturated salt accelerated aging. Seed moisture content on a fresh weight basis was measured before and after accelerated aging. Each vigor test was repeated. Seedling growth rate was measured using four replicates containing nine seeds per seed lot. Petri dishes containing one piece of clear, uncoated cellulose film are used for this test. Precautions are taken in order to avoid contamination. Before placing seeds on the film water was added to dish. Petri dishes were sealed with Parafilm and placed in single germination chamber held at constant 23^{o}C with 40µmol.s^{-1}m^{-2} from cool white fluorescent lamps. The images of Petri dishes were captured using a flat-bed scanner provided with base and top lightning. Petri dishes were scanned with lids on once every 24 h from 4 to 7 days after imbibition. Using above procedures seedling length, area and growth rate were measured over a four day period. The results of all experiments showed that all the six *Impatiens* seed lots had high standard germination percentage greater than 96%. Computer-

aided measurement of seedling length statistically identified three levels of seedling vigor, one high performing seed lot, two seed lots with moderate vigor and three low vigor seed lots. It also shows that seedling area measurements were highly correlated to seedling length and therefore ranked seed lots by vigor in the same order as seedling length. Seedling growth rate for length or area was linear over the first seven days for each seed lot regardless of vigor. Seedling growth rate was similar to vigor levels computed by the saturated salts accelerated aging test and vigor index. Errors in computer-aided seedling lengths were evident when hypocotyls hook was not fully open. These errors could be reduced using a slant board for growing seedlings.

McCormac et al. developed an image analysis system for measuring the root length of lettuce using a slant board test. But this method has some errors such as root length was only measured after the slant board test was completed, only linear length was measured which may cause false measurement, and the length was measured from a stationary position. This position was the same for all seedlings. It is possible that this starting position was not the point separating root and hypocotyl. This source of error may cause error in measurement of vigor information. In order to remove these errors M. S. Howarth et.al introduced a system for measurement of seedling growth rate by machine vision. This system is divided into two parts: the biological system and the computer vision system. The biological system consists of a germination chamber with controlled environmental conditions and the computer vision system consists of an image acquisition system, image processing software, etc. Temperature of the germination chamber was set at approximately 23^0C and humidity was controlled using a drip system at approximately 90% RH. Inside germination chamber, the seeds were germinated on blue blotter paper and mounted on a slant board at 70^0angle. This slant board was placed in a water bath. The entire germination chamber was enclosed so that no ambient light could enter the chamber. The camera was mounted outside the germination chamber and was covered with black fabric to eliminate ambient light. The images of germinated seeds were acquired after 1 hour interval in order to monitor the growth rate of each seed. After image acquisition, these images were processed by using image processing software for root length calculation. This system was tested for seed samples of lettuce *(Lactuca sativa)* and sorghum *(Sorghum bicolor).* In each test, three runs of 10 seeds each were conducted. Images were collected every hour and saved to the hard disk over the entire test. Each test was conducted for 144 hours. The results showed for the lettuce test, the overall average error was -0.13 cm with a standard deviation of 0.08 and for sorghum, the overall average error was -0.07 cm with a standard deviation of 0.08. The errors between manual and machine vision measurement was very small hence this can be used to estimate seed vigor.

The integration of the computer-aided image analysis techniques with the standard germination test is described by Dell'Aquilla. This system is designed to investigate the potential of new technique in monitoring seed imbibition and germination performance of a seed sample. The imaging project covers three major objectives: i) the development of a computer-aided image analysis system to monitor seed imbibition, ii) the integration of germination test with seed image processing under a wide range of environmental conditions, such as NaCl stress (applied to broccoli), different temperature regimes (applied to broccoli and radish) or following controlled deterioration (applied to broccoli), iii) the definition of image analysis parameters in assessing early radicle elongation. The changes in the seed size (area, perimeter, length, and width) and the seed shape (roundness factor) is monitored for broccoli, radish, lentil, lettuce and carrot. Results showed that image analysis system is versatile and also provides the easiness of investigation of germination behavior.

Many computer-aided systems have been developed to evaluate seed germination rate based on seed area and seedling length as described earlier. In order to increase the throughput of germination testing, Chao Li, et.al. presented an approach that differentiates germinated seeds from non-germinated ones based on changes in seed length and area. In this approach, image analysis of seed images captured at regular predefined interval is done. This approach used Canny edge detection as well as Hough line transform to separate seeds from colored background, then distance transform is done to find skeletons of seed, and finally connected component labeling is used to measure length of seed skeletons. Two lettuce seed genotypes from a recombinant inbred line (RIL) population of lettuce, family 231 and 67.2 were used for experimentation. For each genotype, 4 replicates with 16 seeds each were placed in germination boxes that contained solidified agar (1.6%) as germination medium. Sterilization of seed surface is done in sodium hypochlorite solution (1.6%, 15min) to prevent fungal infection. The germination boxes were placed in incubator maintained at 20^oC and continuous red light is provided by red LEDs (660 nm). Germination events

were photographed at 2h intervals beginning 12h and ending 24h after imbibition. Each germination box maintained relatively high germination rate over 90%.

Dell'Antonio described the perspectives of digital imaging technology in relation also to descriptive modeling and tracking simulation with the aim to advance this technique as a promising tool in studying the 'seed germination system'.

3. CONCLUSION

This paper describes the survey of different machine vision techniques for seed quality evaluation. It describes how the imaging technology is applied in monitoring seed imbibition, germination behavior and analysis of seed size, shape parameters. Recently, the greatest efforts have focused on producing non-destructive methods with capability of computer hardware of image processing and its integration with controlled environmental condition systems. 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.

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