



X-Ray Based Germination Test of Cotton Seed

Dr. Sandeep V. Rode

Department of Electronics & Telecommunication
Sipna College of Engineering & Technology, Amravati.
Maharashtra, India

Ms. Mrinal R. Sawarkar

Department of Electronics & Telecommunication
Sipna College of Engineering & Technology, Amravati.
Maharashtra, India

Abstract: In this paper we used digital image processing for germination from X-ray images of cotton seeds. Germination is the process by which a plant grows from a seed. The methods discussed in this paper are internal damages, embryo, and percentage of germination of cotton seeds. The recent development of digital image processing techniques for monitoring cotton seed germination has been done. X-ray analysis can be used to determine the quality of cotton seeds, showing the cause of bad germination. X-ray images provide information on the internal structure and morphology of seeds, mechanical damage, and percentage of empty and filled seeds, micro fractures, possible embryo deformations and insect infestation. This paper various digital image processing techniques which reduces the labour input required to evaluate seedling growth rate and increases the accuracy of these measurements. X-ray photographs were prepared with Faxitron 43805N X-ray Film exposed at 10keV, 2mA (soft X-ray) at a distance of 35cm from the X-ray source for 2min. X-ray photographs were evaluated based on embryo morphology and the presence of endosperm. Embryo morphology was scored as either normal or abnormal.

Keywords: Germination Test, X-ray radiography, Digital image processing, MATLAB.

I. INTRODUCTION

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.

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". Germination is normally carried out in germination cabinets under controlled environment.

II. 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.

The traditional way to conduct the germination test is based on human abilities, it takes times and high labor to conduct the germination test in the seed quality control process. In agriculture, the germination rate describes how many cotton seeds of a particular plant species, variety or seed lot are likely to germinate over a given period. It is a measure of germination time course and is usually expressed as a percentage, e.g., an 85% germination rate indicates that about 85 out of 100 seeds will probably germinate under proper conditions over the germination period given.

The germination rate is useful for calculating the cotton seed requirements for a given area or desired number of plants. In seed physiologists and seed scientists "germination rate" is the reciprocal of time taken for the process of germination to complete starting from time of sowing. The number of seed able to complete germination in a population is referred as germination capacity. There are three types of seed root structure. A. Normal Seedling, B. Abnormal Seedling, C. Dead Seedling.



Fig 2.1 Normal Seedling of Cotton Seed.



Fig. 2.2 Abnormal Seedling of Cotton Seed.

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, 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 [1].

A. L. Hoffmaster, 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 [2].

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The integration of the computer-aided image analysis techniques with the standard germination test is described by Dell'Aquila. This system is designed to investigate the potential of new technique in monitoring seed imbibitions 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 imbibitions, 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 radical 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 [4].

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 [5].

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°C 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° angle. This slant board was placed in a water bath [6].

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 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 [7]. 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 [8]. 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°C 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 [9]. 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' [11].

III. MATERIAL & METHODOLOGY

The research paper was conducted in laboratory of Seed Testing, Nagpur and Seed Technology Research Unit, Department of Botany, College of Agriculture, Akola. Take a 25 cotton seed replications samples were placed using X-ray and numbered according to their position to allow their identification for the subsequent tests, and take an X-ray of cotton seeds using X-ray machine and obtained the X-ray film. The cotton seeds were removed from the X-ray plate and transferred individual rows, numbered in the same order as reported in the X-ray images, and then tested for germination.

This test was conducted with four replications of 25 cotton seeds, which were placed on double-sided tape on a plastic transparent sheet; the seeds were numbered according to their position on the sheet, so that they could be identified in subsequent measurements. Then, the transparent sheet was placed inside a Faxitron MX-20 DC-12 digital X-ray system, connected to a Core 2 Duo computer (3:16 GHz, 2 GB RAM, 160 GB Hard Disk) and 17-inch MultiSync® LCD1990SX monitor, and kept 28.6 cm apart from the source of radiation emission for 20 seconds. After the images were generated, they were saved on a hard disk drive for subsequent analysis. The seeds were removed from the sheet and transferred to an acrylic tray with individual cells, and numbered in the same order they had in the X-ray images. After that, the seeds were submitted to the germination test, as described below. Previously numbered cotton seeds were distributed on two sheets of paper towel moistened with an amount of distilled water equivalent to 2.5 times the weight of the dry substrate and covered with another paper towel sheet.

It was performed on a paper towel substrate moistened with distilled water in the ratio of 1:2.5 (paper: water). The previously numbered cotton seeds were distributed on one sheet of paper towel (in the upper third of the substrate to enable the development of individual seedlings) and covered with another sheet. The wrapped seeds were placed in the incubator at 25 °C and the evaluation was performed on the seventh day after sowing.

The measurement of free space within the seed and, for that matter, embryo size can be determined by analysis of X-ray images with the software. While carrying out studies with cotton and pumpkin seeds, found that the software was sensitive to consistently evaluate the degree of development of seed embryos and suggested that the

procedure is promising for seeds of other species with similar structure to those studied in their research. Likewise, were successful in using the software for analysis of the internal morphology of cucumber seeds. However, this method has been studied for an only few species to date, and further research is needed on the subject. In this sense, the objective of the present study to verify the relationship between the internal morphology of cotton seeds and germination, using the X-ray tests and computer-aided X-ray imaging analysis with the software and check if this relationship changes after the seeds are stored for a particular period of time under different environmental conditions.

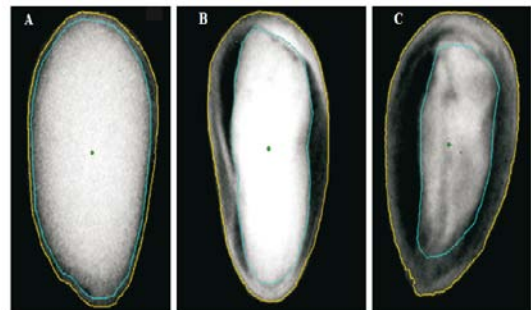


Fig 3.1 .Analysis of seed X-ray images (20 kV / 20 s) of cotton seeds, Values obtained for "Pericarp Area" = 0.14 (A), 0.43 (B) and 0.59 (C), corresponding to the ratios embryo/internal seed cavity of 86%, 57% and 41%, respectively.

System conceptual diagram

The system is imitating the abilities of the classification of the germination of the seeds. Fig. 1 shows the overview of the system conceptual diagram. The operation of the system is divided into two phases: 1) the preparation of the system data set for training data set and testing data set, and 2) the preparation of the system data set for testing the system on another data set of unseen or unknown images. The two phases has to be operated in order to observe its performance and accuracy. However, the second phase has to be used to validate and evaluate the system efficiently.

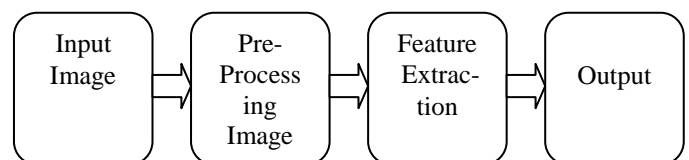


Fig. 3.2 Conceptual Diagram of System

- **Input Test Image**

Take an input seed image from seed lot by using digital camera.

- **Image Enhancement**

Image enhancement is the process of adjusting digital images so that the results are more suitable for display or further image analysis. For example, you can remove noise,

sharpen, or brighten an image, making it easier to identify key features.

- Pre-process Images

Basically the images which are finding during image acquisition may not be directly suitable for detection and sorting purposes, such as weather conditions, noise and poor resolution. Because of some factors images and unwanted background etc. we always try to approve the well-known techniques. The steps involved in pre-processing are:

- Input image
- Removing the background
- RGB to Gray conversion
- Gray to Binary conversion Unsharp filtering
- Boundary removal

- Feature Extract

Feature extraction methods extract the distinct features from the images like edges, corner, which can be used to match or discriminate image from other similar image. In developed an algorithm for extraction of color features from bulk seed samples. Generally, the color, shape, and texture features extracted from the image to recognize an image.

System Structure flow Chart

The structure chart elaborates on how each model works is shown in Fig. 2. The system consists of five main process modules: 1) image acquisition, 2) image preprocessing, 3) feature extraction, 4) Germination test and 5) Display results. Each process module has the following details.

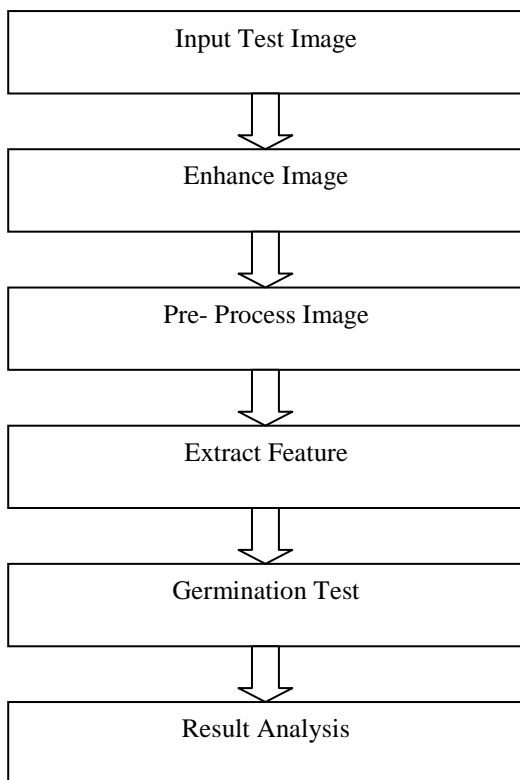


Fig.3.3 Data flow Diagram of System

VI. ANALYSIS

In this paper we are generating the result in three parameters as follow

1. Germination Test
2. Graphical Representation
3. Mean and Entropy of Seed Images

1. Germination Test Result





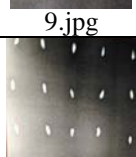
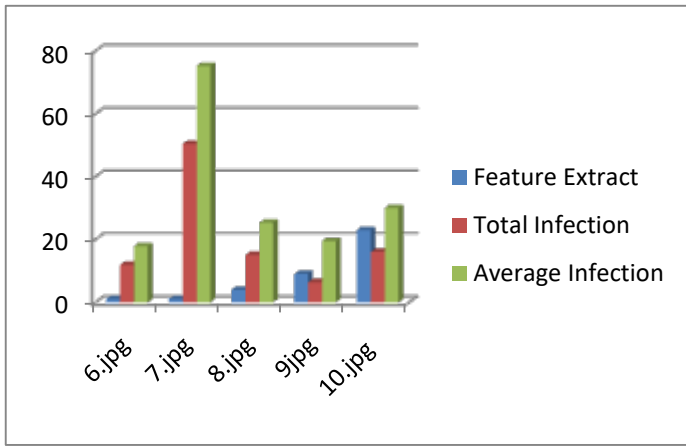
X-Ray Image	Features Extracted	Total Infection	Average Infection	Germination
 6.jpg	1	11.87	17.82	Good Germination
 7.jpg	1	50.35	75.20	Bad Germination
 8.jpg	4	15.099	25.32	Average Germination
 9.jpg	9	6.42	19.46	Good Germination
 10.jpg	23	16.07	30.03	Good Germination

Table 4.1 Result of Germination Test Using X-ray Images

2. Graphical Representation

4.2 Feature Extract vs Total infection vs Average Infection



Graph 4.2 Feature Extract vs Total infection vs Average Infection

3. Mean and Entropy of Seed Images

Images	6.jpg	7.jpg	8.jpg	9.jpg	10.jpg
Mean of an original image	62.3 91	143. 28	135. 187	144. 82	134.40 9
Mean of an Enhance image	85.1 35	139. 33	127. 46	150. 91	121.34
Entropy of original image	6.92 8	7.71 8	7.76 6	7.51 8	7.53
Entropy of Enhance image	7.61 75	7.82 5	7.82 5	7.82 6	7.69
Standard deviation of original image	47.6 6	65.3 39	58.0 4	48.3 69	58.593
Standard deviation of Enhance image	68.3 9	77.2 65	67.0 36	62.4 6	70.43

Table 4.3 Comparison of mean, entropy and standard deviation

V. CONCLUSION

X-ray imaging analysis revealed the internal structure of the seeds and morphology of seeds, mechanical

damage, and percentage of empty and filled seeds, micro fractures, possible embryo deformations and insect infestation. Cotton seed images obtained by X-ray analysis were sharp and it was possible to identify associations between internal morphology and germination potential. Appropriate image analysis software was effective in quickly and accurately assessing the parameters necessary for a quantitative analysis, providing a potential non-destructive method for seed quality testing and germination test.

VI. REFERENCES

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