MEASUREMENT OF SEEDLING GROWTH RATE BY MACHINE VISION

M. S. Howarth, P. C. Stanwood
MEMBER
ASAE

ABSTRACT. Seed vigor and germination tests have traditionally been used to determine deterioration of seed samples. Vigor tests describe the seed potential to emerge and produce a mature crop under certain field conditions and one measure is seedling growth rate. A machine vision system was developed to measure root growth rate over the entire germination period. The machine vision measurement technique was compared to the manual growth rate technique. The vision system provided similar growth rate measurements as compared to the manual growth rate technique. The average error between the system and manual measurement was –0.13 cm for the lettuce test and –0.07 cm for the sorghum test. This technique also provided an accurate representation of the growth rate as well as percent germination.

Keywords. Machine vision, Seeds, Vigor.

The deterioration of a seed sample is a problem that has plagued man since he began planting the first gardens. At the National Seed Storage Laboratory in Fort Collins, Colorado, the main goal is the long-term preservation of plant germplasm for the United States. Plant germplasm is stored at near- and sub-zero temperatures, and the questions continuously asked are "How long can a seed sample be stored under these conditions and maintain viability?" and "What is the deterioration of these seeds?". Another important goal is to catalog plant germplasm for plant breeders and plant biotechnology in general.

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 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 Association of Official Seed Analysts (AOSA, 1988) attempts to provide guidelines for testing seeds, it also requires subjective interpretation of the results. This can cause discrepancy between experienced analysts. A practical vigor test should also include the following characteristics: reproducible, interpretable (i.e., correlated with emergence under certain field conditions), rapid, objective, simple, and economically practical.

Seedling growth rate is a commonly used test to measure vigor. This test as described in the AOSA (1983) is performed in part as a germination test. Once the seedling has germinated for the suggested period of time, either linear growth or dry weight is measured. The major problems associated with this test are (1) time consumption, (2) likelihood of seedling elongation variation between cultivars, and (3) temperature and moisture affects during germination.

Keeping these vigor test requirements in mind, a machine vision inspection approach to this problem could eliminate some of the limitations which currently exist and provide an objective inspection system. Vision systems work nearly continuously with a consistent level of accuracy and throughput. A system could be developed for placement in a controlled environment, thus allowing temperature and humidity to be controlled at suggested conditions. Human involvement during the test procedure also would be minimized, therefore eliminating such problems as eye fatigue, decision differences between inspectors, etc. The current seedling growth rate test measures root length at the end of the germination period and the measurement is strictly a straight length. Root growth could be monitored over the entire germination period with a machine vision system. The actual length of the root could be easily measured and shape features of the root could be evaluated. This may lead to a more robust test to determine the degree of deterioration of a seed sample.

There have been numerous studies on the use of machine vision for agriculture. Most of this work has concentrated on color and blemish recognition. Several researchers have worked on detecting bruises on apples (Taylor et al., 1984; Taylor and Rehkugler, 1985; and Brown et al., 1974). Computer Recognition System developed an orange grading system which sorted oranges into three classes (Harris, 1988). Howarth (1991) developed a machine vision system to grade fresh market carrots as acceptable or cull. This system not only identified blemishes caused by rot but also graded the carrots based on several shape parameters.

McCormac et al. (1990) investigated the use of an image analysis system for measuring the root length of lettuce using a slant board test. Root length was calculated...
by measuring the vertical distance from the zero axis which was manually located to the root tip. The root tip was detected by the imaging analyzer by single level thresholding. It was determined that the machine vision based system performed equally as well as the manual method. However, several problems were found to exist with this measurement 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 seedling root may not have been measured. Third, 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. Since the method of human measurement was identical, the error produced by these measurement characteristics were not realized. This source of error could potentially misrepresented vigor information.

Since machine vision systems have potential to provide consistent measurement of all needed parameters in a non-invasive environment, the objectives for this project are as follows: (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. Development must include illumination, image acquisition, image processing, and pattern recognition requirements; and (3) to determine the reliability of the machine vision system as compared to human inspection performance.

MACHINE VISION MEASUREMENT SYSTEM
MACHINE VISION SYSTEM CONFIGURATION

The machine vision system was divided into two basic systems: the biological system and the computer vision system. The schematic of this vision system is illustrated in figure 1. The vision system consisted of a Cohu black and white camera with a 50-mm lens, a Matrox 640 PIP image processing board, and a Compaq 386/20MHz computer. The camera was mounted outside of the germination chamber and was covered with black fabric to eliminate ambient light. The major component of the biological system was a Stults seed germination chamber in which environmental conditions were controlled. Temperature was set at approximately 23°C. Humidity was controlled through the use of a drip system at approximately 90% relative humidity. Lighting was provided by four fluorescent bulbs mounted horizontally two on each side. These lights were controlled by the computer system. Within the germination chamber, seeds were germinated on blue blotter paper and mounted on a slant board at a 70° angle. This slant board was placed in a water bath. The entire germination chamber was enclosed so that no ambient light could enter the chamber.

IMAGE ACQUISITION

The image acquisition software was written in a macro language through the Optimas 3.10 image processing software. First, initial information was input by the user which defined the image filenames, the acquisition frequency and the duration of the test. Next, a loop was initiated so that at the pre-defined interval (acquisition frequency) the following steps were conducted. The lights and camera were turned on. After a short delay of approximately 10s, the image was grabbed and the lights turned off. The images were then saved to the hard disk drive.

SYSTEM CALIBRATION

Two system calibrations were needed for size and position. These were both performed by positioning two pins into the slant board 10.0 cm apart in the horizontal direction (see fig. 2). The distance between the two pins was used to calibrate the vision system from pixels to centimeters. These pins were also used to position the seeds. Seeds were planted exactly along an imaginary line between the pins. By locating the center of these pins, a position adjustment was made in both the x and y directions.

In order to locate the center of the two pins, the Sobel edge detector (Levine, 1985) was implemented within a window containing the top portion of the image. For all pixels exceeding the threshold level in the edge-enhanced image, two points are plotted in the direction of the edge.

Figure 2-A typical image obtained during testing of ten lettuce seeds. Note, the two pins seen as round white objects. The lettuce seeds were planted on 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 locations.

Figure 1-Root length measurement machine vision system schematic.
gradient at a distance d. One point is plotted on the inside of the edge and the other on the outside as illustrated in figure 3. The edge gradient points form a circular pattern around the pins and a tight cluster at the center of the pins see figure 4.

A simple cluster seeking algorithm was applied to the edge gradient points which located the pins centers (Tou and Gonzalez, 1974). This algorithm is based on arranging patterns using a distance measure. Since all points are located in the x, y coordinate space, the Euclidean distance was used and is defined as follows:

\[ D_{ij} = \sqrt{(p_i - z_i)\cdot(p_i - z_j)} \]  

where

\[ p_i = \text{the } ith \text{ pattern vector or edge gradient point} \]
\[ z_i = \text{ith pattern center vector} \]
\[ t = \text{transpose} \]

First, the cluster center \( z_l \) is assigned to the first gradient point \( p_l \) where \( p_l = \{X_l\ Y_l\} \) and a non-negative threshold \( T \) is chosen. By inspection, \( T \) was chosen to be 3. Next, the distance \( D_{21} \) is computed between \( p_2 \) and \( z_l \). If \( D_{21} \) exceeds \( T \), a new cluster is formed. This cluster center is \( z_2 \) which equals \( p_2 \). However, if \( D_{21} \) is less than or equal to \( T \), \( p_2 \) is assigned to \( z_l \) and \( z_l \) is recalculated as the average of all its members. This algorithm was applied to each pattern.

Once all points were assigned to clusters, the two largest clusters defined the center of the two positioning pins. Using these two locations, each image was calibrated for size and position. The distance between the two pins was 10.0 cm; therefore, size calibration to convert pixels to em was the ratio between 10.0 cm and the Euclidean distance between pins in pixels. The positional calibration involved aligning each image with the first image. Equation 2 was used to adjust the x and Y coordinates for each image:

\[ X_{adj, i} = \frac{1}{2} ((X_{h,1} - X_{i,1}) + (X_{h,2} - X_{i,2})) \]
\[ Y_{adj, i} = \frac{1}{2} ((Y_{h,1} - Y_{i,1}) + (Y_{h,2} - Y_{i,2})) \]  

where \( X_{h,j} \) and \( Y_{h,j} \) are the x, y location for the center of the jth pin in the first image and \( x_{i,j} \) and \( y_{i,j} \) are the x, y location for the center of the jth pin in the ith image.

The Sobel threshold and the distance threshold, \( T \), were chosen based on the ability of the Sobel operator and cluster seeking technique to locate the center of each calibration pin successfully. This algorithm was employed on several images with and without seeds with root growth. By inspection, the Sobel threshold was set to an enhanced intensity value of 387 and the distance threshold was set to three pixels.

ROOT LOCATION AND MEASUREMENT

Before the roots were located and measured, the original image was filtered with a horizontal edge filter. This is identical to the horizontal component of the Sobel operator. The result was scaled to range between -255 and 255. The row of pixels between the two pin centers was run length encoded to locate each root. Run length encoding (RLE) is a data compression algorithm which encodes runs of black and white pixels in a binary image (Horn, 1986); From these runs, transitions were extracted and a root was defined as two successive transitions enclosing white pixels. The horizontal edge threshold was set to a value of 40 by inspection. Once a root was located, the starting coordinate was computed as the midpoint between two transitions enclosing white pixels and was stored. Next, the length of the roots present was measured by RLE succeeding lines within a small window about the last midpoint located. The window enclosing the root tip was defined as ±6 pixels in the horizontal direction and in relation to the previous root tip location. This value was chosen by inspection. For sorghum and lettuce, the root is most likely to grow vertically down the slant board; therefore, it was sufficient to approximate the horizontal change as being small. And since the roots were evenly spaced, this minimized the chance of roots crossing. As long as the root was present, the length was computed as the Euclidean distance between the midpoint between first and last transitions within the current tip window and the midpoint from the previous row. Once no transitions were present, the previous root midpoint location was defined as the root tip for the current image.
Each image was searched for the number of roots until all roots were located. Once all roots were located, only the root lengths were measured for each root. As images were being evaluated, the length of each root was printed to a file. Following completion of the last image, a root length correction was calculated. This correction adjusted each root length due to the vertical offset between the two calibration pins. This correction was the vertical distance between the root starting location and the point directly above and on the imaginary line between calibration pins. Each root starting and ending coordinates, length and corrected length were printed upon conclusion.

SYSTEM TESTING

The machine vision measurement system was tested twice. The first test was conducted on a relatively small seed sample, lettuce (Lactuca sativa), and the second test on a larger seed sample, sorghum (Sorghum bicolor). Thirty seeds in each test were analyzed. Three runs were conducted for each test in which 10 seeds were planted. This was done to maintain good spatial resolution of approximately 0.02 cm/pixel.

The two tests were conducted as vigor tests which are outlined by AOSA standards (AOSA, 1983). The tests were conducted in complete darkness with the exception that lights were turned on once every hour for less than 30 s. Temperature and humidity were controlled by the germination chamber at approximately 23°C and 90% RH, respectively. Each test was run for 144 hours.

Images were collected every hour and saved to the hard disk over the entire test. Following, the root length was measured by the procedure previously described. Three seed analysts measured the root length for each seed from the computer screen to compare with the computer measurements to minimize manual contact with the seed sample. The root length was measured using a metric ruler. Each analyst randomly selected images and measured the length of each root present. The images were randomized to alleviate measurement bias between images. After measurement, all human and computer measurements were converted to cm using the distance between positioning pins to calculate the conversion ratio and were compared.

RESULTS AND DISCUSSION

The root length measurement system collected three runs of images for both lettuce and sorghum seeds. Following image acquisition, the root length for each seed was computed by the machine vision system and the seed analysts. Figures 5 and 6 illustrate machine vision results for a sample of lettuce and sorghum seeds, respectively. In figure 5, the lettuce starts to grow after about 18 h. Growth is rapid for approximately 60 h and then the root growth tapers off. For sorghum in figure 6, growth initiates after about 30 h. At the end of the test, the sorghum roots were still growing. The machine vision system measured all germinating roots. In one instance, a group of pixels that were not associated with a root was measured. This was determined to be the result of a root moving. The section of code which identifies the presence of old and new roots classified a group of noisy pixels as a root.

The machine vision system was compared with the seed analyst results. During testing, the seed analysts experienced several measurement errors. They had difficulty measuring curved roots and experienced errors in recording data. For the three seed analysts, approximately 190 measurement errors were recorded for the lettuce test and 142 for the sorghum test. The average error between analysts and machine vision system for the two tests are shown in figures 7 and 8. In figure 7, the average error for the lettuce test at the end of the testing time dropped back to zero. This seems to be in response to the halt in growth rate. From observations of figures 7 and 8, it appears that as the growth rate increases the error also increases.

The average error results for each test are shown in table 1. 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 average error for the second run of the lettuce test was much lower than the other two runs. This was due to the low germination rate of these 10 seeds. The germination rate for the second run was 80% and three additional seeds germinated but grew less than 1.0 cm. For runs one and three, germination was 100% and 80%, respectfully. All roots that germinated in these runs grew more than 1.0 cm.

Another parameter that the machine vision system computed was percent germination. For the lettuce test, the
percent germination was 86.7% and 96.7% for the sorghum test. Although the percent germination was high, three roots were considered defective in the lettuce test and seven roots in the sorghum test were defective. From previous germination tests, normal germination for the sample of lettuce seed was 86% and was 88% for the sorghum seed. Normal germination is the percentage of seed which produce seedlings with both shoots and roots fully developed.

CONCLUSIONS

A machine vision system was developed to measure root growth rate. The system was tested using two different crops, Lactuca sativa and Sorghum bicolor. The system was compared to a manual measurement technique and the system performed well. The average error observed for the lettuce test was -0.13 cm and was -0.07 cm for the sorghum test. Only one measurement error was recorded with the machine vision system and that was easily diagnosed as the measurement of a non-root. The average time required to measure 10 roots per image was approximately 20 s. Since images were obtained one per hour, this was well within the acceptable limit of one hour. Percent germination was measured as 86.7% for the lettuce test and 96.7% for the sorghum test which compared favorably to standard germination tests. The error reported between the manual and machine vision system measurements was small enough so that the machine vision system could be used to estimate seed vigor. As well, the machine vision system offers labor saving qualities which makes this system appealing.

Before further development can be initiated, basic research into the usefulness of the growth rate curves will need to be conducted. Future plans include enhancing the system so that more than 10 seeds can be analyzed during a test.

REFERENCES

AOSA. 1983. Seed vigor testing handbook.

Table 3. Average error between seed analyst and machine vision system

<table>
<thead>
<tr>
<th>Seed Sample</th>
<th>Run</th>
<th>No. of Seeds</th>
<th>Average Error (cm)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactuca sativa</td>
<td>1</td>
<td>10</td>
<td>-0.21</td>
<td>0.13</td>
</tr>
<tr>
<td>(Lettuce)</td>
<td>2</td>
<td>10</td>
<td>-0.03</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>-0.14</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
<td>-0.13</td>
<td>0.08</td>
</tr>
<tr>
<td>Sorghum bicolor</td>
<td>1</td>
<td>10</td>
<td>-0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>(Sorghum)</td>
<td>2</td>
<td>10</td>
<td>-0.08</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>-0.05</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
<td>-0.07</td>
<td>0.08</td>
</tr>
</tbody>
</table>