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Machine Vision Technique for Rapid Measurement of Soybean Seed Vigor

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Abstract

Purpose: Morphological properties of soybean roots are important indicators of the vigor of the seed, which determines the survival rate of the seedlings grown. The current vigor test for soybean seeds is manual measurement with the human eye. This study describes an application of a machine vision technique for rapid measurement of soybean seed vigor to replace the time-consuming and labor-intensive conventional method. **Methods:** A CCD camera was used to obtain color images of seeds during germination. Image processing techniques were used to obtain root segmentation. The various morphological parameters, such as primary root length, total root length, total surface area, average diameter, and branching points of roots were calculated from a root skeleton image using a customized pixel-based image processing algorithm. **Results:** The measurement accuracy of the machine vision system ranged from 92.6% to 98.8%, with accuracies of 96.2% for primary root length and 96.4% for total root length, compared to manual measurement. The correlation coefficient for each measurement was 0.999 with a standard error of prediction of 1.16 mm for primary root length and 0.97 mm for total root length. **Conclusions:** The developed machine vision system showed good performance for the morphological measurement of soybean roots. This image analysis algorithm, combined with a simple color camera, can be used as an alternative to the conventional seed vigor test method.

Keywords: Image analysis, Machine vision, Seed viability, Seed vigor, Soybean seed

Introduction

Improving crop yield and production stability is important in satisfying the rising demand of human consumption due to the current increasing world population and rapid economic growth of developing countries. Seed quality is one of the most important factors in crop production and should be considered at the first stage of crop cultivation.

Germination testing, which is defined by the International Seed Testing Association (ISTA), is one of the primary means of evaluating seed quality and reflects a seed's ability to produce normal seedlings. A germination test is a common method of determining the percent germination, dormancy, and overall viability of seeds. It is the most consistent way to assess viability. It is important to monitor viability, since non-viable seeds may not be apparent at other stages of processing. The seed germination rate of a specific seed lot is a key indicator as to how that seed will perform in the field.

The vigor test is an important step of seed quality testing. Seed vigor directly determines the emergence

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potential of a seed lot as well as physical and genetic aspects of seeds that affect their storage, processing, and transport. The ISTA Vigor Testing Handbook (1999) provides specifications for seed vigor tests based on the 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 is determined by both the germination rate and seedling growth rate. The germination rate measures the speed of germination, which is commonly represented by the time to reach 50% germination, while the seedling growth rate is evaluated on a real-time basis by measuring the rate of elongation of the radicle per unit time. The germination and vigor tests are usually labor-intensive and time-consuming processes. Therefore, there is a need to develop a new detection technology to alleviate this challenge.

Machine vision or computer vision is an artificial intelligence technique that simulates human vision. This technology uses image acquisition, digital image processing, analysis, and image recognition of multidimensional data from the real world to obtain information about objects. A typical machine vision system consists of four basic components: a computer, a camera or sensor, a lens, and an illumination system with a digitizer. A machine vision system should be capable of identifying and grading seeds based on external features that can be detected in an image such as size, shape, color, and texture. Machine vision has been successfully used to assess seeds of a range of crop and non-crop species.

The objective of this study was to develop an image acquisition system for measuring morphological features of soybean seed vigor and to construct a low-cost machine vision system capable of rapid measurement. The performance of the developed algorithm and system were verified with conventional visual inspection.

Materials and Methods

Sample

Soybean seed samples were purchased from the Danong Corporation in South Korea. Seventeen soybean seeds were used for the measurement of total length, primary length, surface area, and diameter daily for seven days. A germination paper was moistened to sow the seeds and then pinned on a black board. The samples were maintained in a humidity chamber for seven days. The temperature and humidity in the chamber were approximately 25 °C and 90%, respectively. The seed vigor test was carried out according to ISTA guidelines (ISTA, 1999).

Image acquisition system

A CCD camera (Microsoft LifeCam Studio, Microsoft, Redmond, WA, USA) was used to obtain images of the samples. The camera was placed at the top of the imaging acquisition system (Fig. 1). The camera provides a USB interface to transfer data and uses 5 W power from a computer. The distance between the object and the camera was set to 0.4 m, and the autofocus function was used. The light source was 7.2 W white light-emitting diodes (LEDs) that were oriented horizontally on either side of the system. The samples were located at the bottom and camera resolution was set at 1920 × 1080. After image acquisition, image analysis was performed using MATLAB (R2016a, MathWorks, Natick, MA, USA). The computer vision software, which was based on a graphical user interface (GUI), was developed in-house for soybean seed image acquisition and processing.

Image processing

The procedure for image processing is shown in Figure 2. The obtained image was divided into three channel images of red, green, blue, and the blue image was selected for further image processing for root detection. The blue image was converted to a binary image and then a thinning algorithm was applied to obtain thinned images of roots. The various morphological parameters of roots such as primary length, total length, surface area, and diameter were extracted from the binary and thinned image.

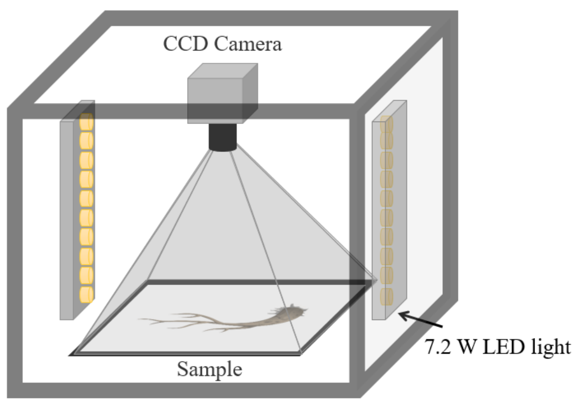

Figure 1. Schematic of imaging acquisition system.

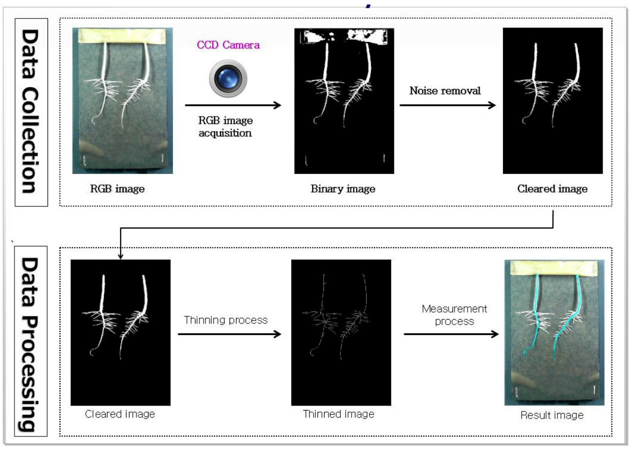

Figure 2. The procedure of data collection and processing.

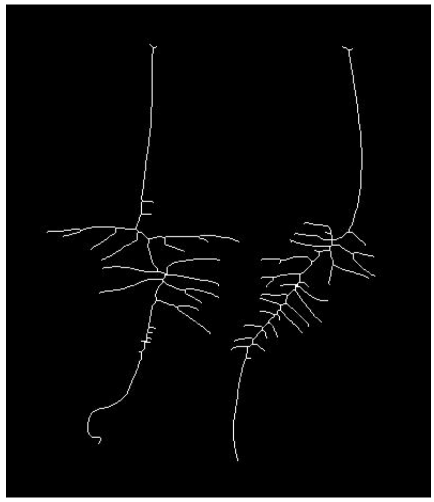

Figure 3. The final thinned image.

P_8	P_1	P_2
P_7	P_0	P_3
P_6	P_5	P_4

Figure 4. Central pixel and its eight neighbor pixels.

Measurement of seed vigor

A series of root samples with different lengths and thicknesses were used and evaluated during the seven days of the experiment. The morphological characteristics of the root samples were measured by both the computer vision system and the manual measurements method.

The roots grown from the 17 soybean seeds used in this experiment were manually measured for primary root length and total root length using a vernier caliper with an accuracy of 0.02 mm. All parts except the seed coat were regarded as roots, and the root of the part connected to the sporoderm was set as the initial point. The experiment was carried out for seven days, and the obtained number of morphological values was 119 (17 samples × 7 days).

Measurement of primary length and total length of root

Image thinning refers to the continuous removal of the outer layer of pixels from a binary image while maintaining connection between the pixels. The final thinned image of a root, a root skeleton image, is a sequence of single pixels (Fig. 3).

The length of a root was measured by multiplying the distances between the pixels in the root skeleton image by a calibration coefficient. The measurement of root length depends on the pixel connections in the eight neighboring pixels (Fig. 4). Pixel connections were counted in two categories based on the distance between neighboring pixels: (1) the distance between the central pixel and the adjacent pixels in the horizontal and vertical directions is 1 (P_0–P_1, P_0–P_3, P_0–P_5, and P_0–P_7 in Fig. 4), and (2) the distance between the central pixel and the diagonal pixels is $\sqrt{2}$ (P_0–P_2, P_0–P_4, P_0–P_6, P_0–P_8 in Fig. 4). The emergence of adjacent pixels and diagonal pixels, noted by direction codes N_{adj} (number of adjacent pixels) and N_{dia} (number of diagonal pixels), were calculated by mathematical statistical methods. Accordingly, the length of the root was calculated by adding N_{adj} and N_{dia} and multiplying the total by a calibration coefficient, B. The equation (1) is as follows:

$$L = (\sqrt{2}\, N_{dia} + N_{adj}) \times B \tag{1}$$

where B is the calibration coefficient for estimating the actual length of the root. It was calculated using a reference black square (size 10 mm × 10 mm).

Measurement of the total surface area and diameter

The total surface area of the root was calculated based on the binarized image. The total surface area of the root was estimated by the following equation (2):

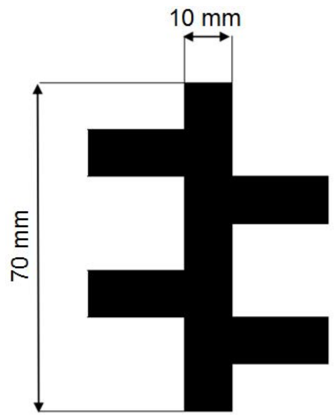

Figure 5. Reference pattern image for total surface area confirmation.

$$S = A \times \Sigma N_a \tag{2}$$

where A is the calibration coefficient of area and N_a is the number of pixels in the binarized image of the root. The calibration coefficient of area (A) is the ratio between the actual and estimated areas and was calculated using a reference pattern image designed and printed for this experiment (Fig. 5). The reference image was obtained for pre-experiment variable estimation and used before measuring the samples.

The average diameter (D) of the root was obtained by dividing the area by the length calculated above using the following equation (3):

$$D = \frac{S}{L} \tag{3}$$

where S is total surface area of the root and L is the total length of the root.

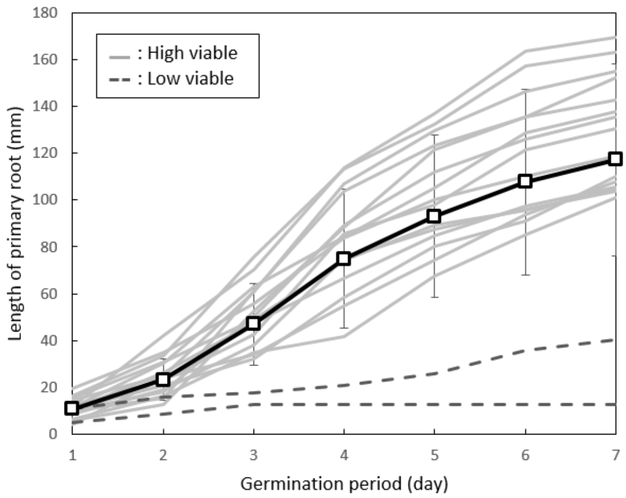

Figure 6. Growth of primary roots by germination period.

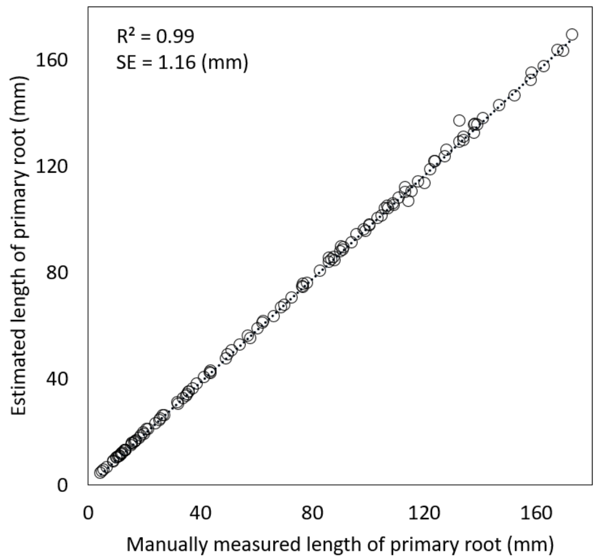

Figure 7. Scatter plot of manually measured and estimated length of primary roots.

Results and Discussion

Length of primary root

According to ISTA standards, there are several methods to measure seed vigor, including aging, cold, conductivity, seeding performance, and tetrazolium tests. The length of the main root is a very important indicator of seed vigor. In this study, the root length, area, and diameter were selected as indicators of seed vigor, and these morphological characteristics were calculated by image processing.

The length of the primary root was calculated using the thinned image. Figure 6 shows the lengths of the primary roots for all 17 samples over seven days (gray lines). Of the total 17 samples, 15 samples grew well throughout the seven days (high viability; solid lines) and the length of the primary roots of the remaining 2 less-viable samples were less than 50 mm (low viability; dashed lines). The thick black line indicates the average values of the samples each day, with the error bar indicating the standard deviation. This result shows that there was a significant difference in the vitality of individual seeds.

Figure 7 shows the relationship between the manually measured and estimated lengths of the primary roots. The coefficient of determination (R^2) was very high at 0.999, and the standard error (SE) was 1.16 mm. This result shows that the developed system is capable of accurate measurement of primary root length.

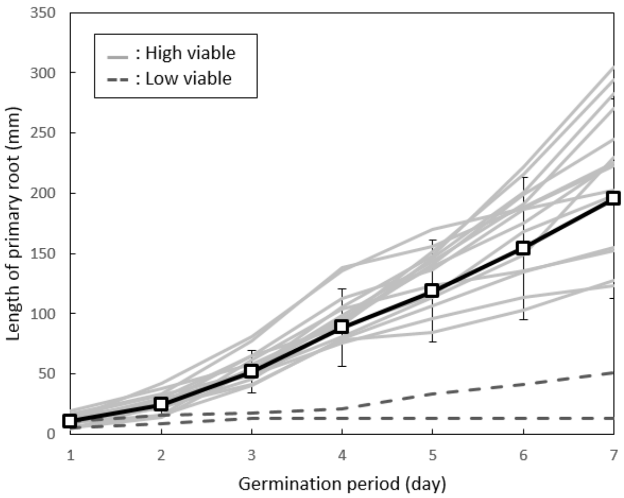

Figure 8. Growth of total roots by germination period.

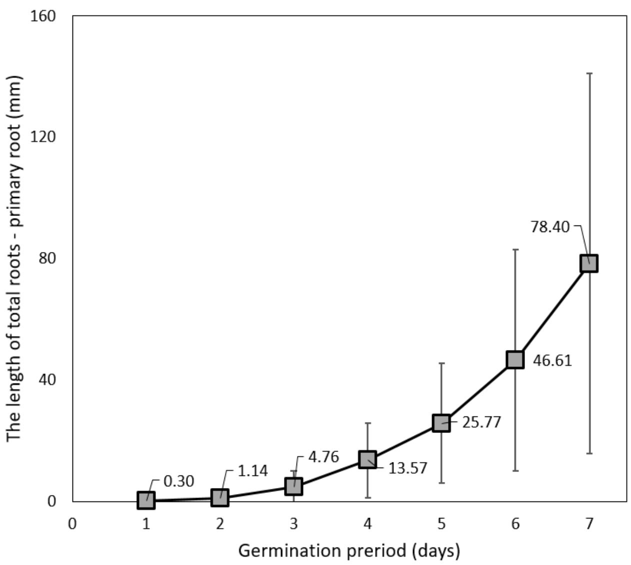

Figure 9. Differences between the length of total and primary roots with germination period.

Length of total roots

As seedlings grow, many rootlets appear in addition to the primary root. These rootlets can be used as important information in testing the seed vigor because they show the plant's immediate reaction to its growth environment. In this study, the length of the total roots was estimated using thinned root images, an approach that had not been tried in previous research. Figure 8 indicates the lengths of the total roots for all 17 samples for all seven days.

The shape of the graph was similar to that in Figure 6, with larger values because it shows the length of the primary roots plus the length of the rootlets. Figure 9 is a graph showing the difference between the length of primary and total roots throughout the germination period. Root length increased over time throughout the germination period. The deviation of the rootstock (the difference between total and primary roots) also increased, indicating that it could be used as an indicator of seed viability.

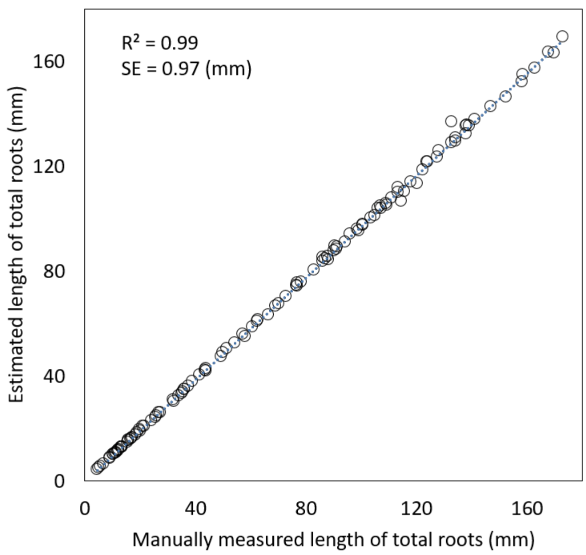

Figure 10. Scatter plot of manually measured and estimated length of the total roots.

The length of the rootlets showed a sharp increase after the fourth day. The average deviation of the rootstock on the third day of germination was 4.76 mm, but the average deviation of the rootstock on the seventh day of germination was 78.4 mm, more than 16 times that of the third day. The standard deviation of the rootlet length was also increased, indicating that it was another significant piece of information about seed vigor that the length of primary roots did not contain.

The manually measured and image-estimated lengths of the total roots are shown in Figure 10. The R^2 and SE were 0.999 and 0.97 mm, respectively. The estimated lengths of the total roots were strongly correlated with manual measurement values, indicating that the developed system is suitable for the measurement of the length of the total roots of soybean seeds.

Total surface area and average diameter of roots

Figure 11 and Figure 12 show the total surface area and average diameter of roots with germination period, respectively. The total surface area had a positive correlation of 0.974 with the length of total roots. The

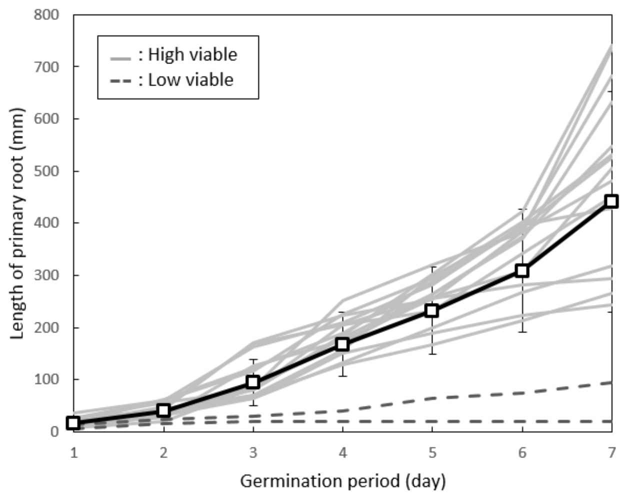

Figure 11. Growth of total surface area by germination period.

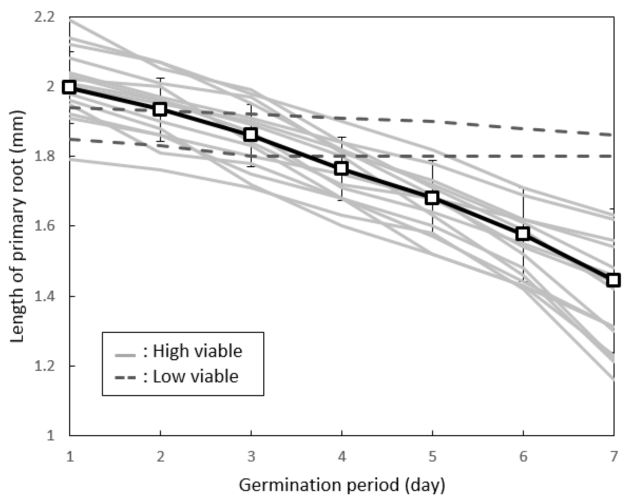

Figure 12. Growth of average diameter of roots by germination period.

total surface area can be used to predict the weight of the germinating sample as well as to measure its water absorption capacity.

We also obtained the average diameter of roots using equation 3. The average diameter decreased from the second day of the germination period (Fig. 12). This is because of the increase in the lengths of small rootlets. In Figure 12, the decrease in the average diameter of roots of the two seedlings with lower viability is less substantial than that of the other samples. This result shows that the average diameter of roots and seed vigor are closely related.

Conclusions

A low-cost machine vision system was developed for rapid and objective measurement of soybean seed vigor. In order to evaluate the performance of the developed imaging system and algorithm, the measured and estimated lengths of primary and total roots of soybean seeds during germination were compared. The standard errors (SE) for length of primary and total roots were 1.16 mm and 0.97 mm, respectively, which shows high accuracy. In addition, the total surface area and the average diameter of roots were closely related to the seed vigor. Previous studies used primary root length and uniformity to calculate a seed vigor index using machine vision techniques. In this study, the three morphological characteristics, root length, area, and diameter, which were not used previously, were closely related to seed vigor. This result can further improve the reliability of the measurement of seed vigor using machine vision because the existing seed vigor index can now be further refined.

Future studies need to consider 3-dimensional bending of roots during germination to improve the accuracy of measurements of the morphological characteristics of roots.

The experimental results of this study demonstrate that the machine vision technique has good potential for predicting germination ability of soybean seeds. A low-cost color imaging system using morphological image analysis could be used for industrial application in fast and cost-effective seed sorting and phenotyping.

Conflict of Interest

The authors have no conflicting financial or other interests.

Acknowledgments

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