A System for Automated Seed Vigor Assessment

Y. Sako[1], M. B. McDonald[2], K. Fujimura[1], A. F. Evans[2], and M. A. Bennett[2]

[1]Department of Computer and Information Science
[2]Department of Horticulture and Crop Science
The Ohio State University, Columbus, OH 43210-1086

Abstract

Seed vigor testing provides valuable information for assessing seed lot quality. However, most vigor tests have not experienced widespread use because of their subjectivity, high cost and variability in test results from laboratory to laboratory. This report presents a system for automated seed vigor assessment that is objective, economical and easy to perform. The system interfaces an inverted flatbed scanner that captures digital images of germinating seedlings to a computer. The images are processed by the computer to generate numerical values that collectively represent the quality of a seed lot (vigor index) based on sample mean of various statistics acquired from morphological features of the imaged seedlings. The system was tested on lettuce seedlings grown for three days in the dark. The results indicated that the imaging system accurately quantified seed vigor to yield reproducible, objective vigor assessments.

Introduction

Vigor testing is important because it ranks the performance of a seed lot against other seed lots when planted in the field. Various specifications for seed vigor testing exist in handbooks (AOSA, 1983; ISTA, 1987). While some of these tests are used commercially, most vigor tests are considered time consuming, costly, and produce variable test results; traits that have hindered their widespread use. An objective, reproducible, speedy and economical method of vigor testing that directly determines the speed and uniformity of seedling growth is desirable.

McCormac, et al. (1990) discussed an automated system for assessing vigor of lettuce (*Lactuca sativa* L.) seed lots. By growing the seedlings using a slant-board test (Smith et al., 1973) in which seeds were planted on a blotter and grown vertically in the dark, a video camera was able to capture 500 x 500 gray-scale images of five germinating seedlings. After capturing the root length of individual seedlings, the average length was used to assess seed vigor since this parameter correlates with field emergence and lettuce head size at harvest (Wurr and Fellows, 1985). Commercial seed vigor assessment systems also exist to determine seed vigor such as the Ball Vision Index (Conrad, 1997) and Paradigm System (McNertney, 1999), but these systems only examine seedling parts such as cotyledon area (Ball Vision Index) or root length (Paradigm System). The objective of this study was to establish an imaging system that could capture images of seedlings from the side, enabling simultaneous measurements of both hypocotyls and radicles that provided accurate and reproducible seedling measurements for vigor assessment.

Materials and Methods

Seeds were planted and seedlings imaged (Figure 1) as described by McDonald, et al. (2001). After seedling images were acquired, they were processed by software developed in this study that quantified the quality of the seed lot given a set of images, each containing 50 seedlings (Figure 2). The general flow of the software was the following: Regions that represented seedlings were extracted from the image by thresholding (Gonzalez and Woods, 1992). For illustration, a magnified portion of Figure 2 is shown in Figure 3. Figure 4 illustrates the result of thresholding the image in Figure 3. Since seedling regions extracted may contain noise, the noise was removed by discarding regions that were too small in size. The image was further enhanced by performing median filtering which is a noise removal operation that smoothes out jagged edges of seedling regions. Next, seedling skeletons were found by performing skeletonization (Hilditch, 1969) on the seedling region (Figure 5). To enable measurements of individual seedlings when overlapping occurred, the skeleton for each seedling was separated from others by adapting simulated annealing (Kirkpatrick, et al., 1983). After seedling separation, the separation between the hypocotyls and radicles was based on the presence of root hair formation for each seedling that allowed the lengths of the hypocotyl and radicle to be computed for each seedling.

After hypocotyl and radicle measurements were made for all seedlings, the results were combined into a vigor index as follows:

$$Vigor = w_G * growth + w_u * uniformity,$$

$$Growth = min(w_h * l_h + w_r * l_r, 1000),$$

$$Uniformity = max(1000 - (w_{sh} * s_h + w_{sr} * s_r + s_{total} + w_{sr/h} * s_{r/h}) - w_d * numdead, 0)$$

Where l_h and l_r were the sample means of the hypocotyl and radicle lengths, respectively, s_h, s_r, s_{total}, and $s_{r/h}$ were the sample standard deviations of the hypocotyl length, radicle length, total length, and the ratio of the hypocotyl and radicle lengths, and the *w*'s represented associated weights with the parameters being multiplied.

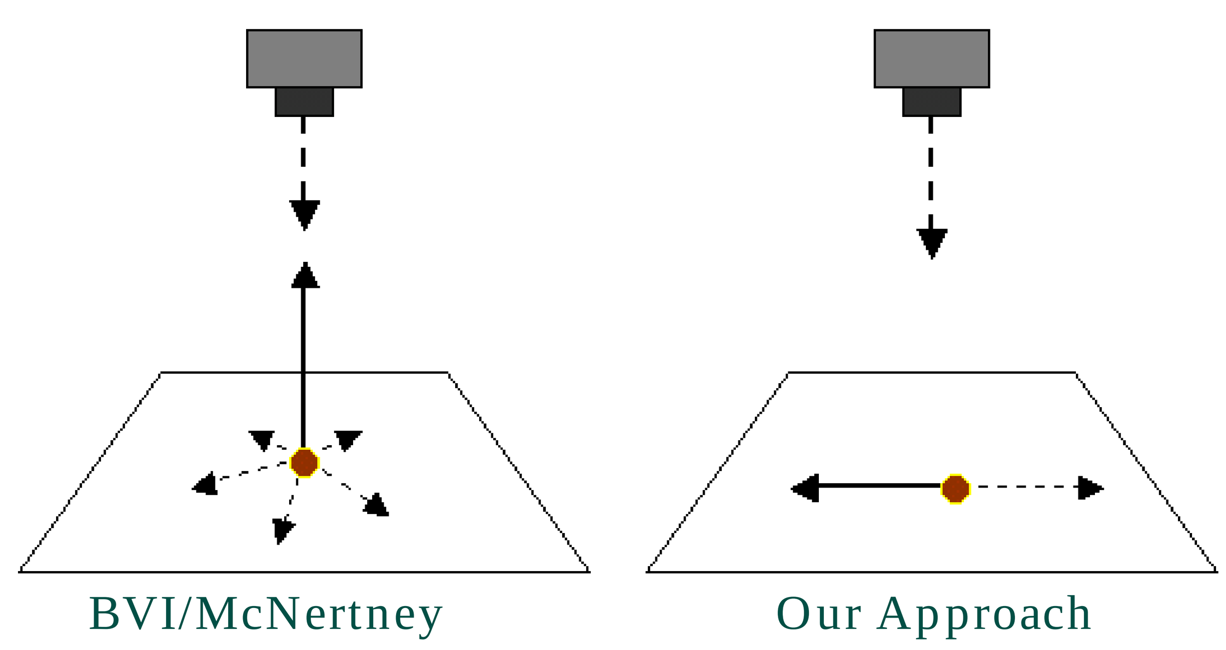

Figure 1. The imaging approach used in this study where seedlings are germinated at an 85° angle from horizontal to acquire both radicle and hypocotyl images compared to the Ball Vision Index (BVI) and McNertney imaging systems that only detect cotyledon or radicle growth, respectively.

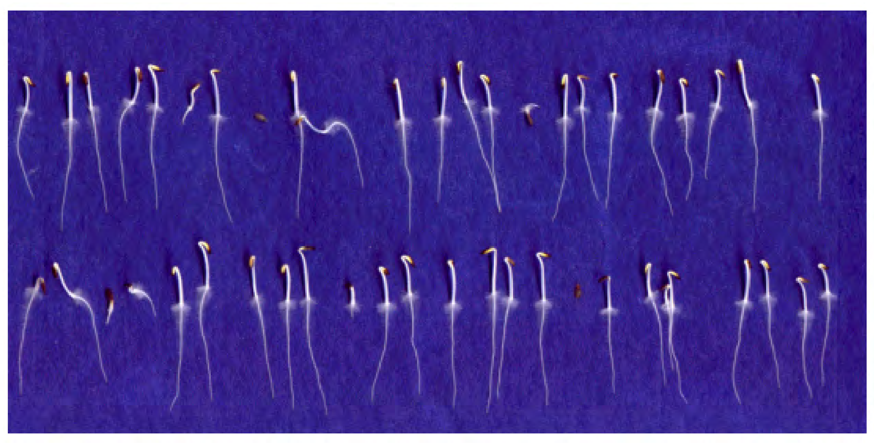

Figure 2. Digital image of 50 lettuce seedlings germinated on a blotter and used for subsequent automated seed vigor index analysis.

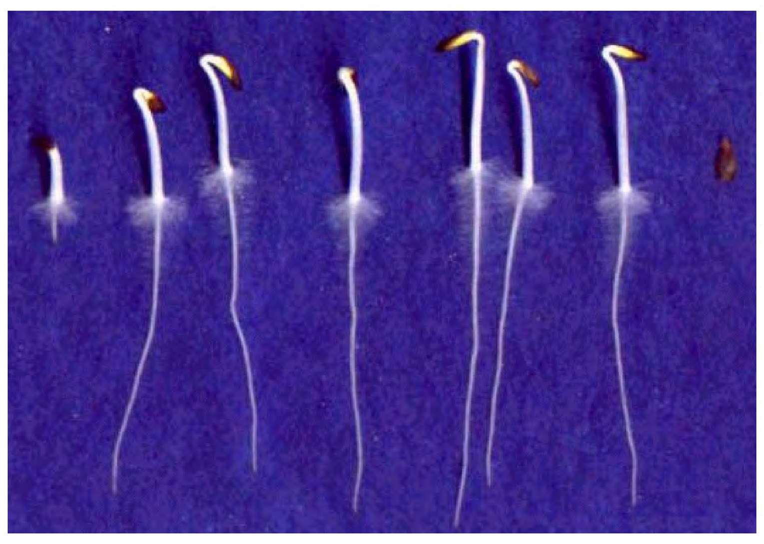

Figure 3. A magnified portion of Figure 2 to illustrate subsequent software analyses.

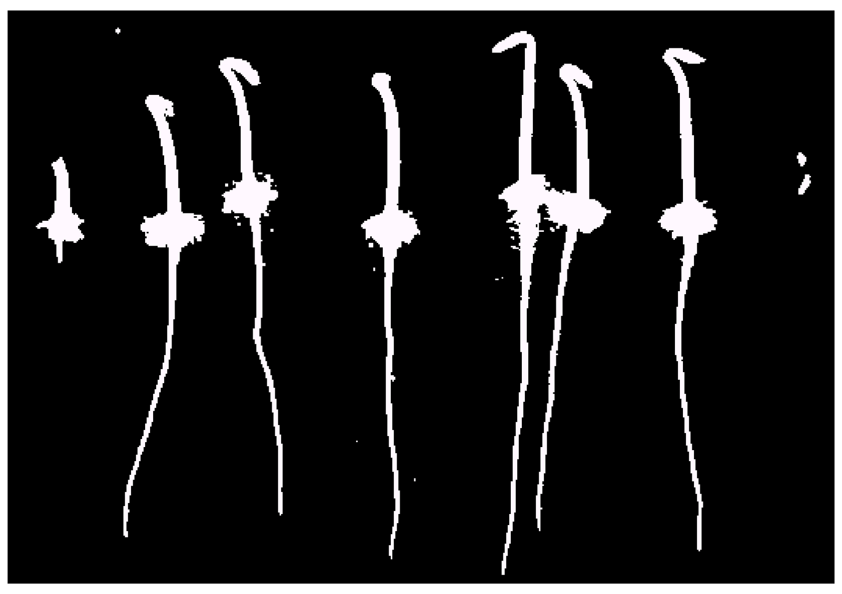

Figure 4. The result of thresholding Figure 3 to acquire only black and white information.

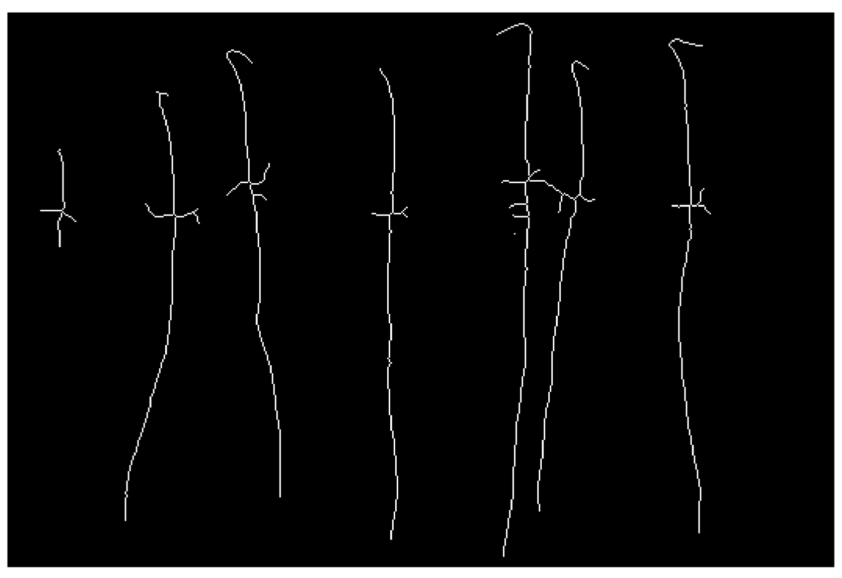

Figure 5. Skeletonization of seedlings in Figure 6.

The vigor index was divided into growth and uniformity parameters with each component having a minimum value of 0 and a maximum value of 1000. The vigor index ranged from 0 to 1000 since it is a weighted average of the components, where weights range from 0 to 1 and sum to 1. Because uniformity is a component of the vigor index, a uniformly dead sample can have a uniformity of 1000, and thus obtain a relatively high vigor index. Since this is not desirable, a penalty term in uniformity was introduced so that, when a sample has all dead seeds, the uniformity becomes 0 instead of 1000. The penalty term appears as w_d * *numdead,* where w_d is the penalty per seed and *numdead* is the number of dead seeds.

Results and Discussion

The vigor assessment system was used to numerically evaluate four lettuce seed lots with different levels of vigor (Figures 6 and 7). The software marked hypocotyls in red and radicles in green. Accuracy of the system was tested by comparing the vigor index against a vigor index computed manually using individual seedling measurements.

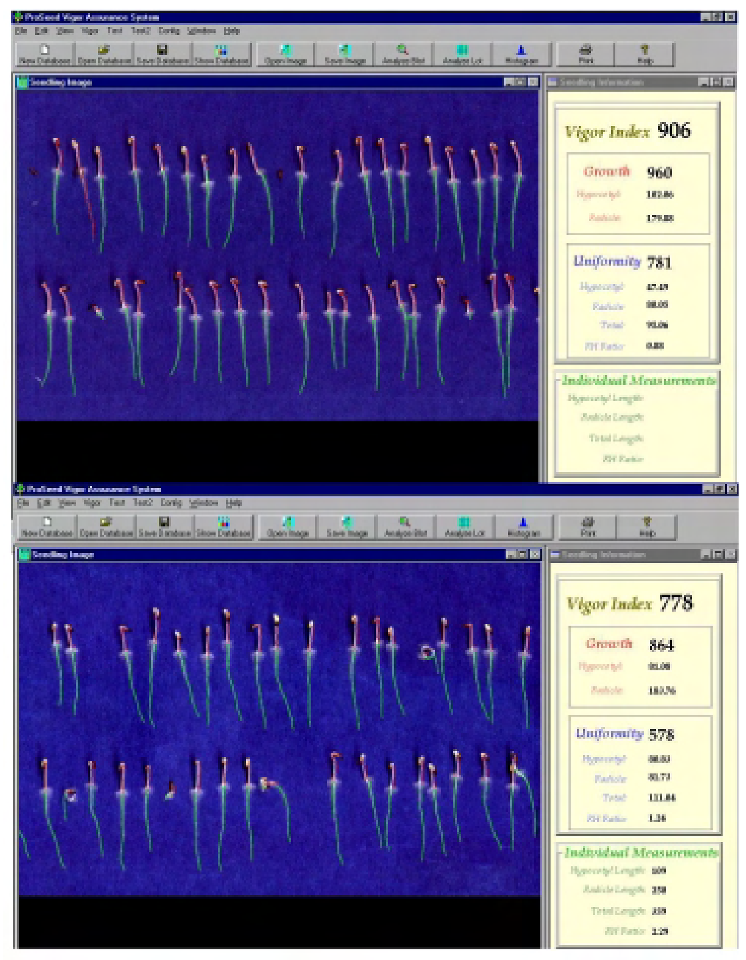

Figure 6. The growth, uniformity and vigor index values computed by the automated seed vigor analysis software for two lettuce seed lots considered high in vigor.

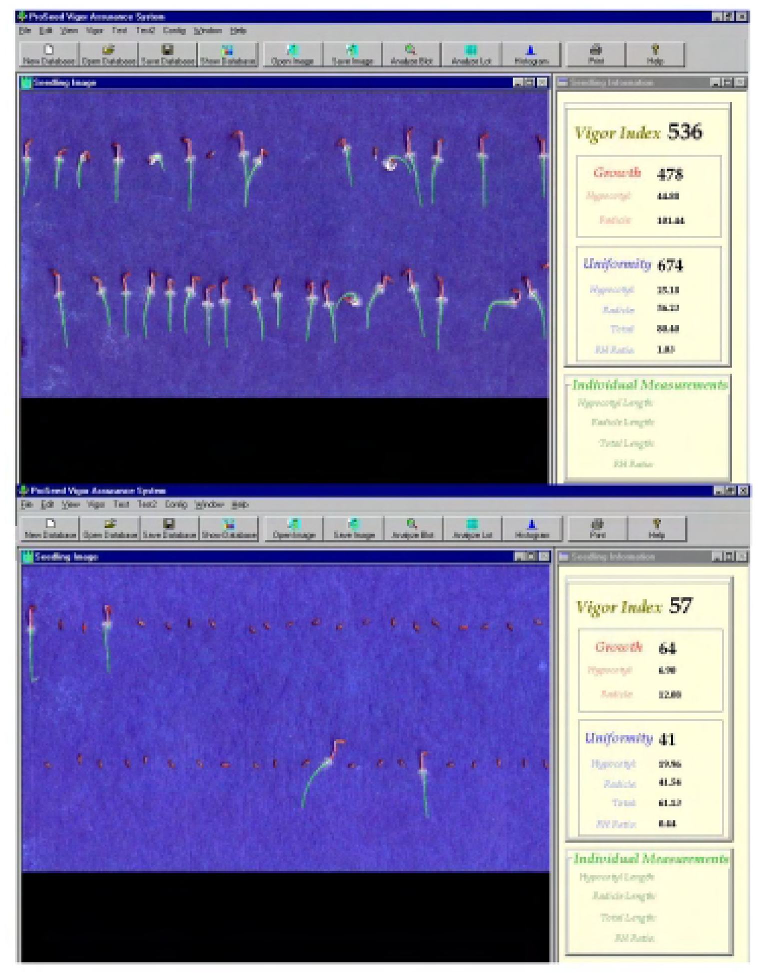

Figure 7. The growth, uniformity and vigor index values computed by the automated seed vigor analysis software for two lettuce seed lots considered low in vigor.

Table 1 shows that, in the best case, the percentage difference between manual and computer determinations of the vigor index was only 0.99% for lot 1. In the worst case, the percentage difference was 14.71% for lot 9. These values are likely less than those obtained by seed analysts from differing laboratories.

Table 1. Manual and automated seed vigor assessment software (S/W) values for speed (average hypocotyl and radicle length) and uniformity (standard deviation of hypocotyls, radicle, total seedling length, and radicle/hypocotyl ratios) for the four lettuce seed lots illustrated in Figures 6 and 7.

	Manual	S/W	% diff	Manual	S/W	% diff	Manual	S/W	% diff	Manual	S/W	% diff
Lot			1	7			13			9		
Avg. Hypo. Len	70.15	74.84	6.69%	70.95	84.64	19.30%	38.53	45.3	17.57%	4.37	5.5	25.86%
Avg. Rad. Len	171.95	174.32	1.38%	171.36	171.26	-0.06%	118.25	118.26	0.01%	11.54	12.08	4.68%
S.D. Hypo. Len.	30.95	35.16	13.60%	30.74	39.85	29.64%	17.53	20.21	15.29%	15.01	19.13	27.45%
S.D. Rad. Len.	75.09	82.68	10.11%	73.09	84.06	15.01%	50.51	56.44	11.74%	39.63	41.54	4.82%
S.D. Total. Len.	103.72	114.68	10.57%	102.8	110.17	7.17%	66.9	74.57	11.46%	54.63	60.48	10.71%
S.D. R.H. Ratio	1.14	1.01	-11.40%	0.83	0.85	2.41%	1.17	0.94	-19.66%	0.73	0.44	39.73%
Growth	780	810	3.85%	783	851	8.68%	488	522	6.97%	50	57	14.00%
Uniformity	542	495	-8.67%	542	650	19.93%	635	623	-1.89%	0	0	0.00%
Vigor	708	715	0.99%	710	790	11.27%	532	552	3.76%	34	39	14.71%

For this study, lettuce was selected as a model species because lettuce seedlings produce well-defined hypocotyls and radicles that are straight. Furthermore, it has been reported that embryo elongation and germination rate are good predictors of lettuce seed vigor (Hacisalihoglu, et al., 1999). Thus, this automated system to detect speed and uniformity of lettuce seedling growth is tailored to identify seed vigor for this crop. For the system to be more general and useful, extensions to feature extractions for other important agricultural crops such as corn (*Zea mays* L.) and soybean (*Glycine max* [L.] Merrill) will be necessary and are currently under study.

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