COMPUTER IMAGING TO IMPROVE SEED QUALITY DETERMINATIONS

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Summary

Seed analysis requires specific evaluation skills such as a thorough knowledge of crop and weed seed morphology for a purity test and an ability to discriminate normal and abnormal seedlings in a germination test. Inexpensive flat bed scanners can capture high quality seed and seedling images that can be stored in comprehensive image website libraries or shared via the internet for proper interpretation of specimens.

Seed vigor testing provides valuable information for assessing seed lot quality. However, vigor testing has not experienced widespread use because of its labor intensiveness, high cost, and variability in test results from laboratory to laboratory. An automated seed vigor assessment system is presented that is objective, economical, and easy to perform. The system interfaces a flat bed scanner that captures digital images of germinating seedlings to a computer. The images are processed by a computer to generate numerical values that collectively represent the quality of a seed lot (vigor index) based on various statistics acquired from morphological features of the imaged seedlings. These statistics include the sample mean of hypocotyl and radicle lengths, and sample standard deviation of the hypocotyl length, radicle length, total length (hypocotyl length plus radicle length), and radicle-to-hypocotyl-length ratio that indicate speed and uniformity of seedling development. The system was tested on lettuce seedlings grown for three days in the dark. The results indicated that the imaging system accurately quantified these parameters to yield reproducible, objective vigor assessments.

Introduction

Seed analysis is a subjective skill relying on the knowledge and expertise of the analyst. As a result, seed testing organizations regularly conduct referee and ring testing programs where seed analysts evaluate the same seed samples to assure themselves that results are standardized from laboratory to laboratory. Standardization of seed testing is vital to ensure the orderly and global marketing of seeds. To assist standardization, seed analysts have traditionally relied on educational media such as reference texts, handbooks, manuals, and herbaria. A major fault of these current identification systems is the inability to share actual seed and seedling specimens with those more

skilled in their evaluation. Advancements in computer technology offer the promise of 1) easily and inexpensively digitizing seed and seedling images using flat bed scanners and 2) rapidly conveying digitized images of seeds and seedlings via e-mail attachments or visitations at websites where specimen libraries are maintained

Seed vigor is a quantitative and qualitative value that describes the quality of a seed lot and can be based on sampled observations of seedling growth. Traditionally, seed analysts determine seed vigor by visual inspection of the speed and uniformity of seedling growth or manual measurements of certain seed/seedling features. Various specifications for seed vigor testing exist, including those listed in the Association of Official Seed Analysts' (AOSA) Seed Vigor Testing Handbook (1983) and the International Seed Testing Association (ISTA) Seed Vigor Testing Handbook (1987).

Vigor testing is important because it ranks the potential field performance of seed lots. As a result, vigor testing is valuable to growers who want to know the quality of a seed lot before purchase and distributors who can use vigor testing for quality assurance purposes. Although vigor testing provides useful information, most vigor tests are time consuming, costly, and produce variable test results from laboratory to laboratory. These undesirable traits have prevented the widespread, standardized use of seed vigor testing. An objective, reproducible, speedy, and economical method of vigor testing would make seed vigor testing more reliable and useful to seed users. Previous applications of computer imaging in seed biology have been reported, primarily in root analysis. Numerous publications exist for measuring root lengths of various crops by computer imaging (Dowdy, Nater, and Dolan, 1995; Dowdy, Smucker, Dolan, and Ferguson, 1998; Rasband and Bright, 1995; Tagliavini, Veto, and Looney, 1993; Tanaka, Yamauchi, and Kono, 1995). These studies used general image processing software such as NIH-Image (Rasband, 1991), which is public domain software developed by the National Institute of Health. Use of such general image processing software may be sufficient for the purpose of simple root measurements, but writing custom software is inevitable for more complicated tasks such as seed vigor testing.

McCormac, Keefe, and Draper (1990) discussed an automated system for assessing vigor of lettuce (*Lactuca sativa* L.) seed lots. By growing the seedlings using a slant-board test (Jones and Cobb, 1963; Smith, Welch, and McCoy, 1973) in which seeds were planted on a blotter and grown vertically in the dark, a gray-scale video camera was able to capture 500x500 gray-scale images of germinating seedlings. Thresholding was performed on the image to extract seedling silhouettes, and a reference line, or a sowing line, was manually placed on the blotter to measure the lettuce seedling root length. However, they were able to capture only five seedlings per image due to limitations imposed by their imaging device. Thus, they arranged seedlings into groups of five, and moved the video camera to capture images of each group. After obtaining the root length of individual seedlings, the average length was used to assess seed vigor because this parameter has been shown to correlate with field emergence and lettuce head size at harvest (Smith, Welch, and McCoy, 1973); results later confirmed by 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). However, the seedling attributes captured by these systems are limited indicators of seed vigor since they are examining only seedling parts such as cotyledon area (Ball Vision Index) or root length (Paradigm System). The objectives of this study were to 1) develop an imaging platform that could be adapted in a routine seed testing laboratory at little cost to enhance the standardization of purity and germination testing and 2) establish an imaging platform that could capture multiple images of seedlings from the side, enabling simultaneous measurements of both hypocotyls and radicles, that then can be further processed by more advanced computer methods to minimize human intervention and increase accuracy and reproducibility in seedling measurements for vigor assessment.

Materials and Methods

For seed/seedling identification, the following procedures were used:

Seeds. Seeds were placed directly on a flat bed scanner (Hewlett Packard ScanJet 6300C), covered with a green cellophane paper to provide uniform contrast, and captured at maximum resolution (1200 dpi).

Seedlings. Lettuce (*Lactuca sativa* L.) and cucumber (*Cucumis sativus* L.) seeds were germinated on two saturated blue blotters (Anchor Paper Co., St. Paul, MN) in the lids of 15 x 23 mm (6 x 9 inch) plastic boxes. The seeds were vacuum planted in two horizontal rows of 25 seeds each for lettuce, 5 cm from the top and 5 cm from the bottom of the blotters, and one horizontal row of 15 seeds for cucumber. After planting, the plastic boxes containing the seeds were placed at an angle of 85° from horizontal in a germinator set at 20°C for lettuce and 20-30°C for cucumber. After 3 days, the plastic lid containing the seedlings was placed on a drawer that was closed under a scanner (UMAX Astra 2000, Fremont, CA) attached upside-down to the top of a metal box and the images captured at 1200 dpi for photographic reproduction (Figure 1).

Computer. The computer used was a Dell Dimension XPST (Austin, TX) 700 MHz, Pentium III with 256 MB SDRAM memory and a 20.4 GB hard drive.

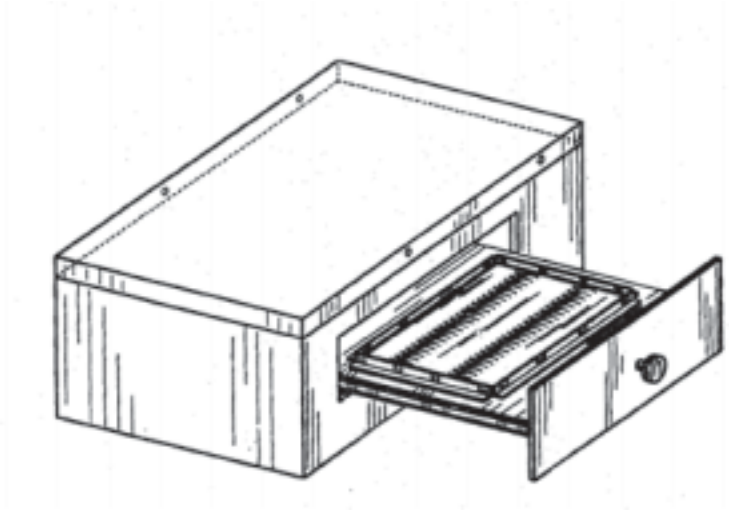

Figure 1. Diagrammatic representation of metal box containing an enclosed scanner affixed upside-down to the lid of the box. Germinated seedlings are placed on the drawer of the box, the drawer closed, and the scanner images the seedlings.

For the lettuce seed vigor determinations, the following procedures were used:

Germination.

1. Two blue blotters (15 x 22 cm, Anchor Paper Co., St. Paul, MN) were saturated with distilled water.

2. The blotters were placed inside the top of a plastic box (15 x 23 x 4 cm, Model 600-C, Pioneer Packaging, Dixon, KY).

3. The seeds were placed on a vacuum planting plate (Hoffman Manufacturing, Albany, OR) designed to plant two rows of 25 seeds each (total of 50) per box, 5 cm from the top and 5 cm from the bottom of the blotters.

4. Excess seeds were removed from the vacuum planting plate and the seed planted on top of

the saturated blotters.

5. The seeds were firmly pressed into the blotters using a flat plastic plate to ensure that they remained on the blotter when placed vertically.

6. The plastic box was closed and a rubber band placed around the box to prevent the box from opening while simultaneously minimizing moisture loss.

The box was placed upright, 85 0 from horizontal, in a germinator (SG 30, Conviron Environmental Limited, Winnepeg, Manitoba) at 20 0C (ISTA, 1999) for three days. No light was used in the chamber to minimize phototropic bending of the seedlings to the light.

Image acquisition. A flatbed scanner was used to acquire seedling images. The flatbed scanner offered several advantages over other imaging devices, such as a video camera or a digital camera (McDonald, Evans and Bennett, 2001). Other approaches to image acquisition have created a special closed environment for the imaging system containing lighting equipment and an imaging device approximately one meter above the seedlings (Conrad, 1997; McNertney, 1999).

Software processing of the images. After seedling images were acquired, they were processed by software developed in this study. The overall goal of the software was to quantify the quality of the seed lot given a set of images, each containing 50 seedlings. A sample image is shown in Figure 2.

For more detailed descriptions of the software employed in this study, refer to Sako (2000). The general flow of software operation was the following. First, regions that represent seedlings were extracted from the image (i.e., separated from the background). This was done by a technique

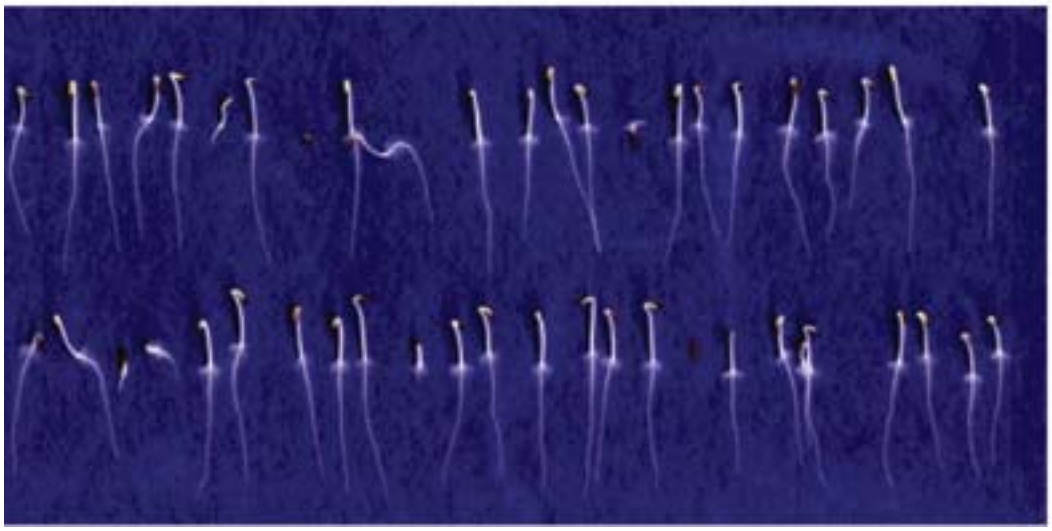

Figure 2. A typical seedling image.

called 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 (in terms of the number of pixels each region occupies). The image was further enhanced by performing median filtering that is a noise removal operation which preserves most edges. In this case, median filtering was used to smooth out jagged edges of seedling regions. Without this smoothing operation, the next step of skeletonization yields poor skeletons leading to inaccurate results. Next, seedling skeletons were found by performing skeletonization (Hilditch,

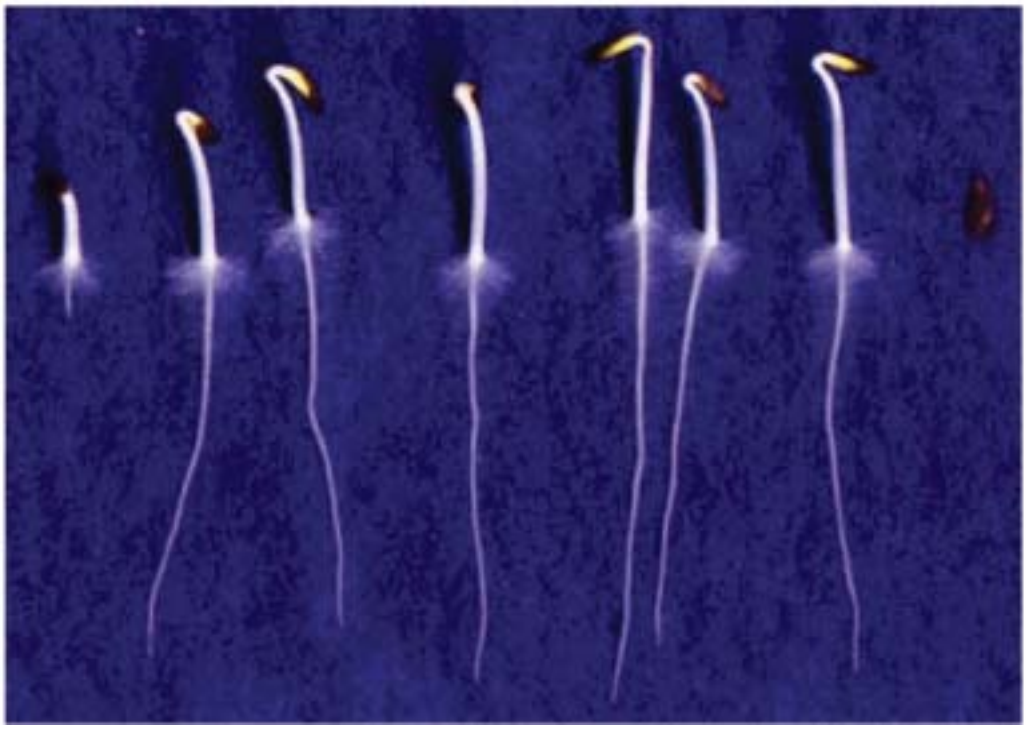

Figure 3. A portion of a seedling image magnified for illustration.

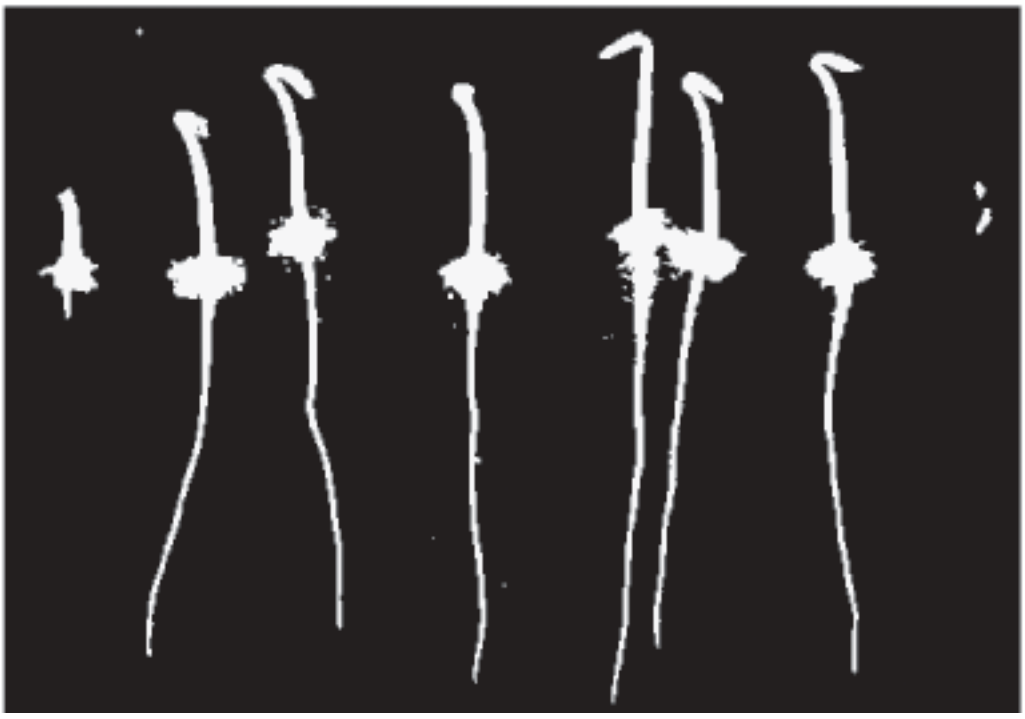

Figure 4. Result of performing thresholding on Figure 3.

1969) on the seedling region. Skeletonization is an operation in which a region is iteratively decreased from outside until the region becomes line segments with a pixel width of one, while resulting pixels remain connected (Figure 5).

When a seedling region contains multiple seedlings, performing skeletonization on the region resulted in skeletons of overlapped seedlings. Typically, seedling roots overlapped, which makes measurement of an individual seedling length difficult. To enable measurements of individual

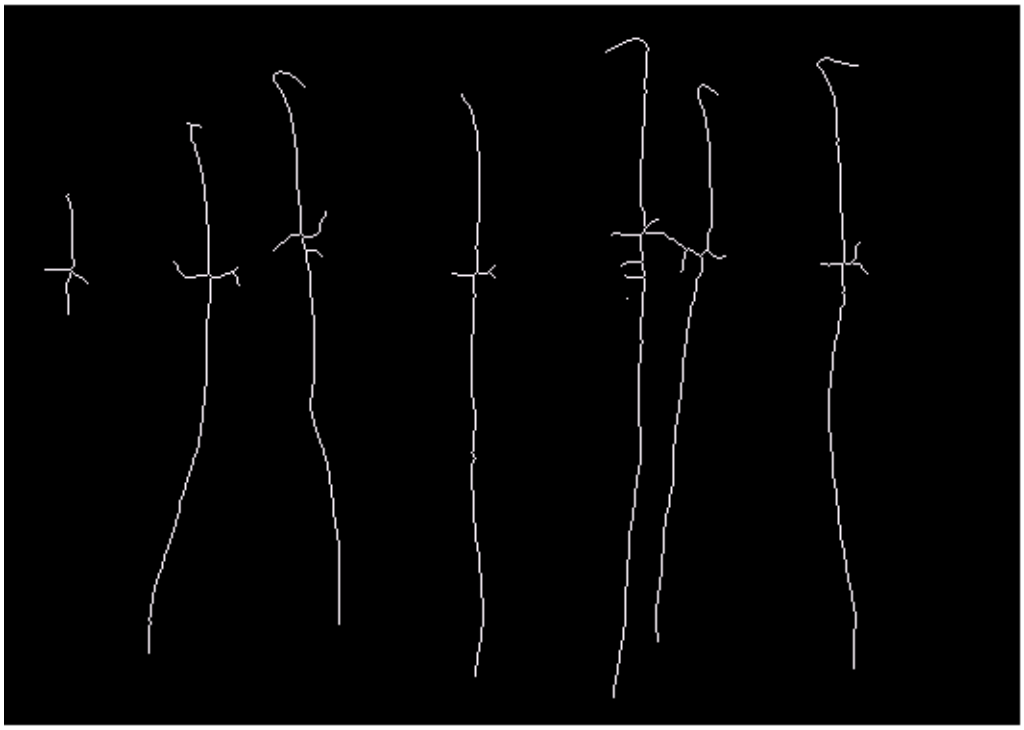

Figure 5. Result of performing skeletonization on Figure 4.

seedlings, the skeleton for each seedling was separated from others by adapting the technique of simulated annealing (Kirkpatrick, et al., 1983). Simulated annealing is a stochastic, global minimization/maximization method. After seedling separation is performed, the separation between the hypocotyl and the radicle was based on the presence of root hair formation for each seedling. Based on the separation point and the skeleton, the lengths of the hypocotyl and the radicle could be computed for each individual seedling.

Vigor Index. After measurements of the hypocotyl and the radicle were made for all seedlings, the results were combined to obtain a vigor index. The vigor index was defined as follows:

$$vigor = w_G * growth + w_U * uniformity ,$$

$$growth = \min(w_h * \overline{l_h} + w_r * \overline{l_r}, 1000) ,$$

where and were the sample means of the hypocotyl length and the radicle length, respectively, sh, sr, stotal, and sr/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.

The vigor index was divided into growth and uniformity parameters because seed analysts often examine these parameters to determine seed vigor. Each component had 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. Weights can be adjusted by the software to favor either growth or uniformity. Ideally, high vigor seedlings should be long and exhibit high uniformity in the seed lot. 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 in uniformity as wd*numdead, where wd is the penalty per seed and numdead is the number of dead seeds.

Results and Discussion

Seed/Seedling Imaging

Images of seeds (Figure 6 and at www.ag.ohio-state.edu/~seedbio/seed_id) could be captured in less than one minute. For seedling images, seeds were germinated in a near vertical position (85o) on standard blue blotters. This approach provided visibility of the entire shoot and root on a uniform plane against a contrasting color. Examples of lettuce and cucumber seedlings are shown in Figure 7.

Not all scanners examined functioned in this upside-down orientation, so it is important to select a scanner that can operate in this position. This approach is superior to inverting seedlings on a traditional flat bed because the seedlings sometimes fall from the blotter or move so that features

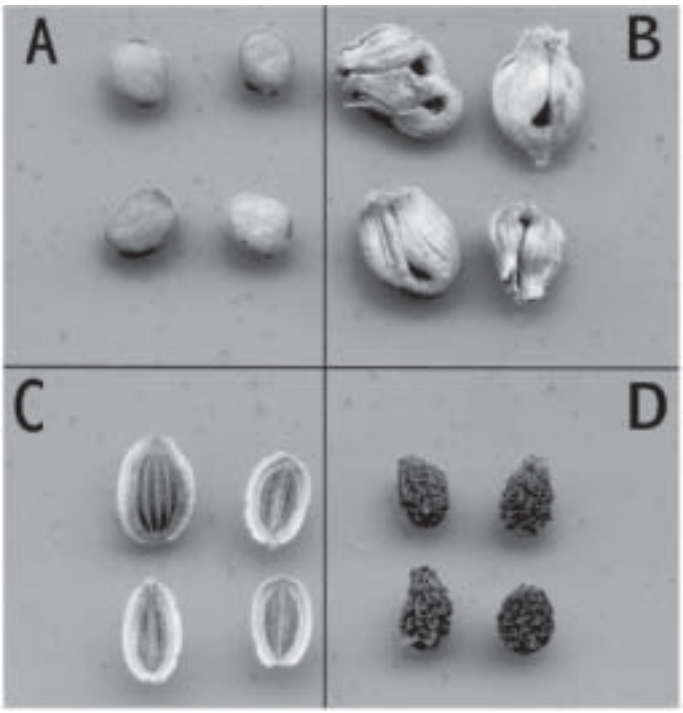

Figure 6. Examples of seeds scanned by an HP ScanJet 6300C flat bed scanner. A. Radish (Rhaphanus sativus), B. Buffalograss (Buchloe dactyloides), C. Dill (Anethum graveolens), D. Meadowfoam (Limananthus alba). Also check www.ag.ohio-state.edu/~seedbio/seed_id.

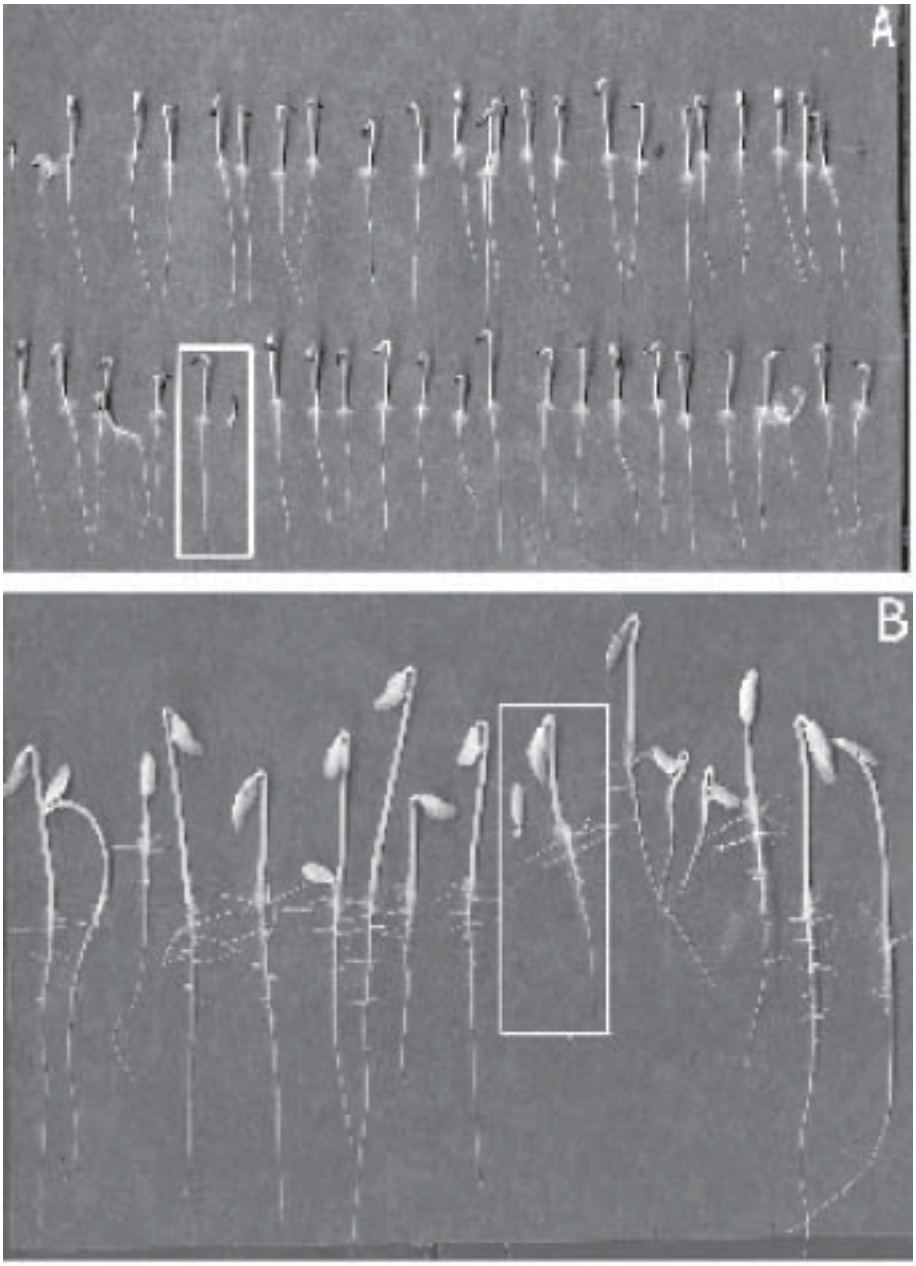

Figure 7. Examples of lettuce (A) and cucumber (B) seedlings scanned in an enclosed metal box by an inverted UMAX Astra 2000 flat bed scanner. Note seedlings in white box to be cut and pasted in Figure 4.

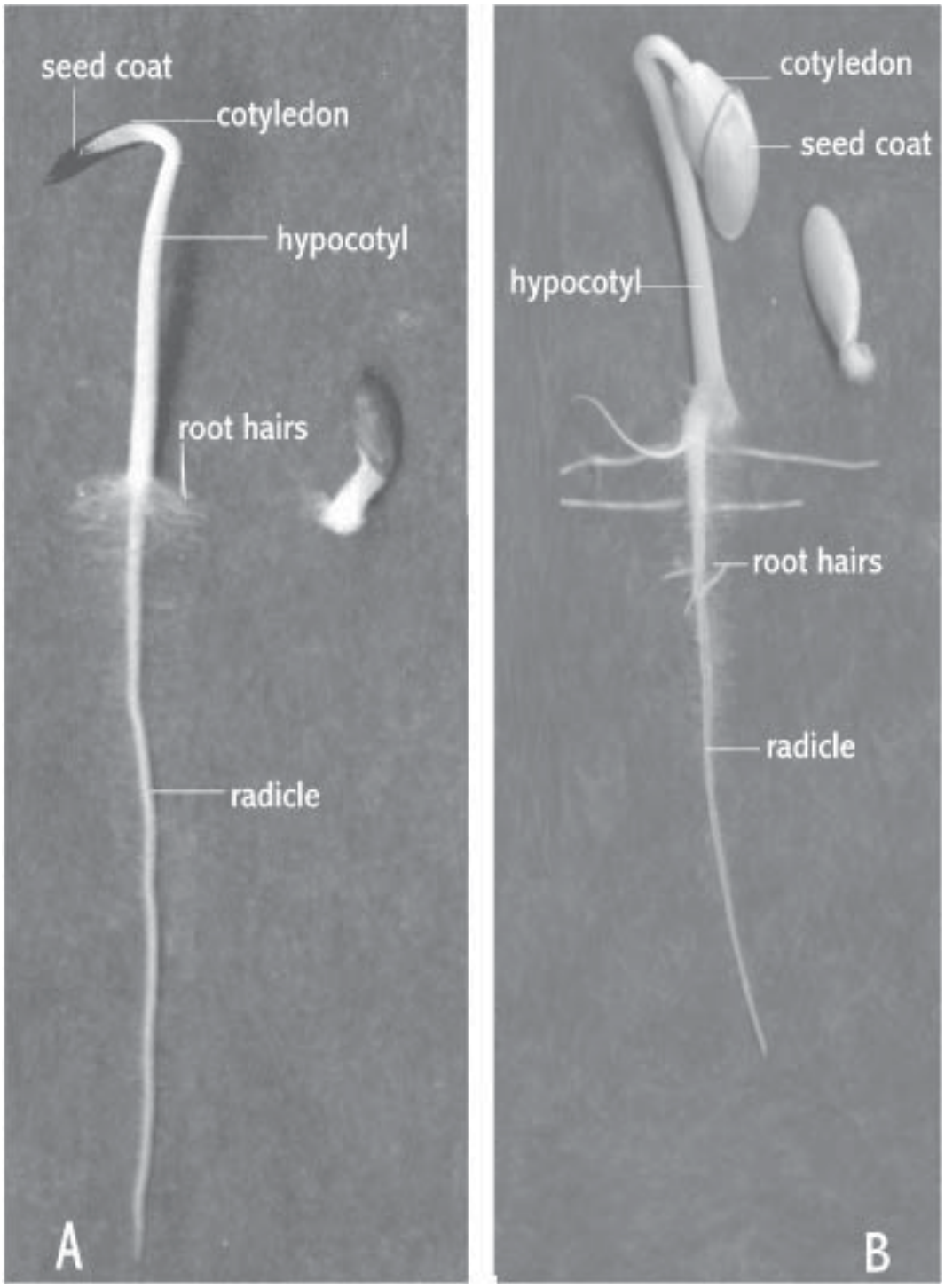

Figure 8. Normal and abnormal lettuce (A) and cucumber (B) seedlings cut and pasted from a germination blotter in Figure 7 and labeled according to anatomical structure.

become obscured. A further advantage is that root hairs and other delicate structures are not disturbed. It should be emphasized that this scanner possessed a depth of field of at least 1.0 cm ensuring that all seedling structures remained in focus.

Once the seedlings are scanned and digitized into high definition (1200 dpi) files, they can be cut and pasted for direct comparison(s) of normal and abnormal seedlings, labeled for structure identification, or saved as compressed JPEG file e-mail attachments for confirmation of normal/abnormal categorization by other seed analysts. Figure 8 illustrates examples of normal and abnormal lettuce and cucumber seedlings cut and pasted from one germination blotter.

Seed Vigor Testing

The vigor assessment system was used to numerically evaluate four lettuce seed lots with different levels of seed vigor (Figures 9 and 10). The screenshots do not show the entire images the software examined due to the scale at which they were displayed on the monitor (thus, less than 50 seedlings are shown for each image, but the entire images are used for the vigor index computation). The software marked hypocotyls in red and radicles in green. For most seedlings, these features were detected correctly. However, some errors occurred (e.g., the second seedling from the left in the top row in Figure 9: top, the entire seedling was marked as hypocotyl since the radicle was not detected due to insufficient root hair formation). Figure 10: bottom shows looped seedlings whose features were not correctly extracted. The vigor index and its constituents are shown on the right hand side of each screenshot (Figures 9 and 10).

Accuracy of the system was tested by comparing vigor index (and its constituents) that were computed from individual seedling measurements given by a human and a computer. Manual measurement of each seedling was performed by displaying the seedling image on the computer screen and having a human trace the seedling parts via mouse clicks. Table 1 shows the sample mean of hypocotyl and radicle lengths, and sample standard deviation of the hypocotyl length, radicle length, total length (hypocotyl length plus radicle length), and radicle-to-hypocotyl-length ratio for four seed lots tested. Also, the overall growth and uniformity components and the overall vigor index are shown as derived from these numbers. The numbers are shown for the manual and software measurements, and the percentage difference computed by 100*(software measurement - actual measurement) / (actual measurement). In the best case, the percentage difference between manual and computer determinations in the vigor index was only 0.99%. In the worst case, the

Table 1. Comparison (% difference) of results obtained via manual/software (S/W) measurements for four (1, 7, 13, and 9) seed lots.

	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%

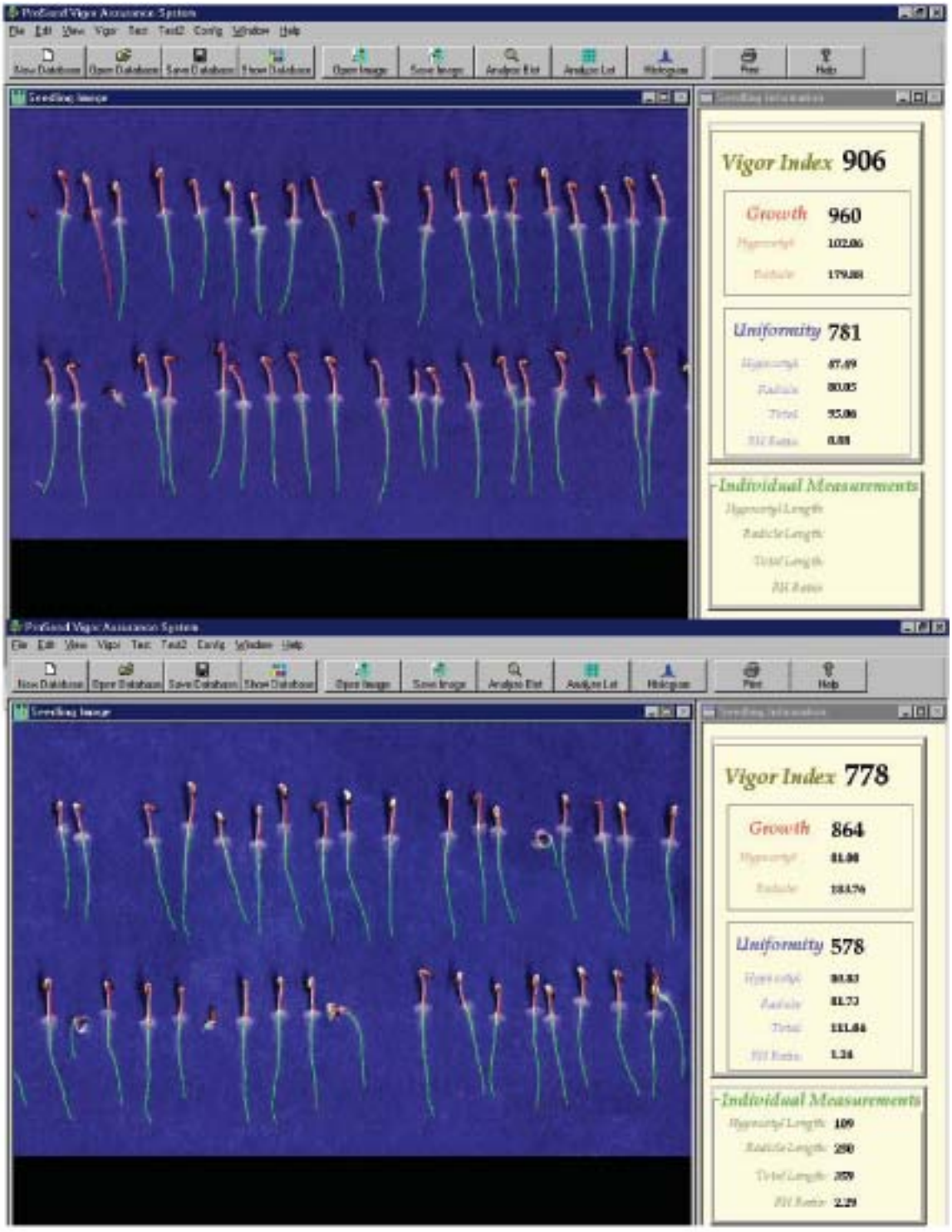

Figure 9. Vigor testing results 1.

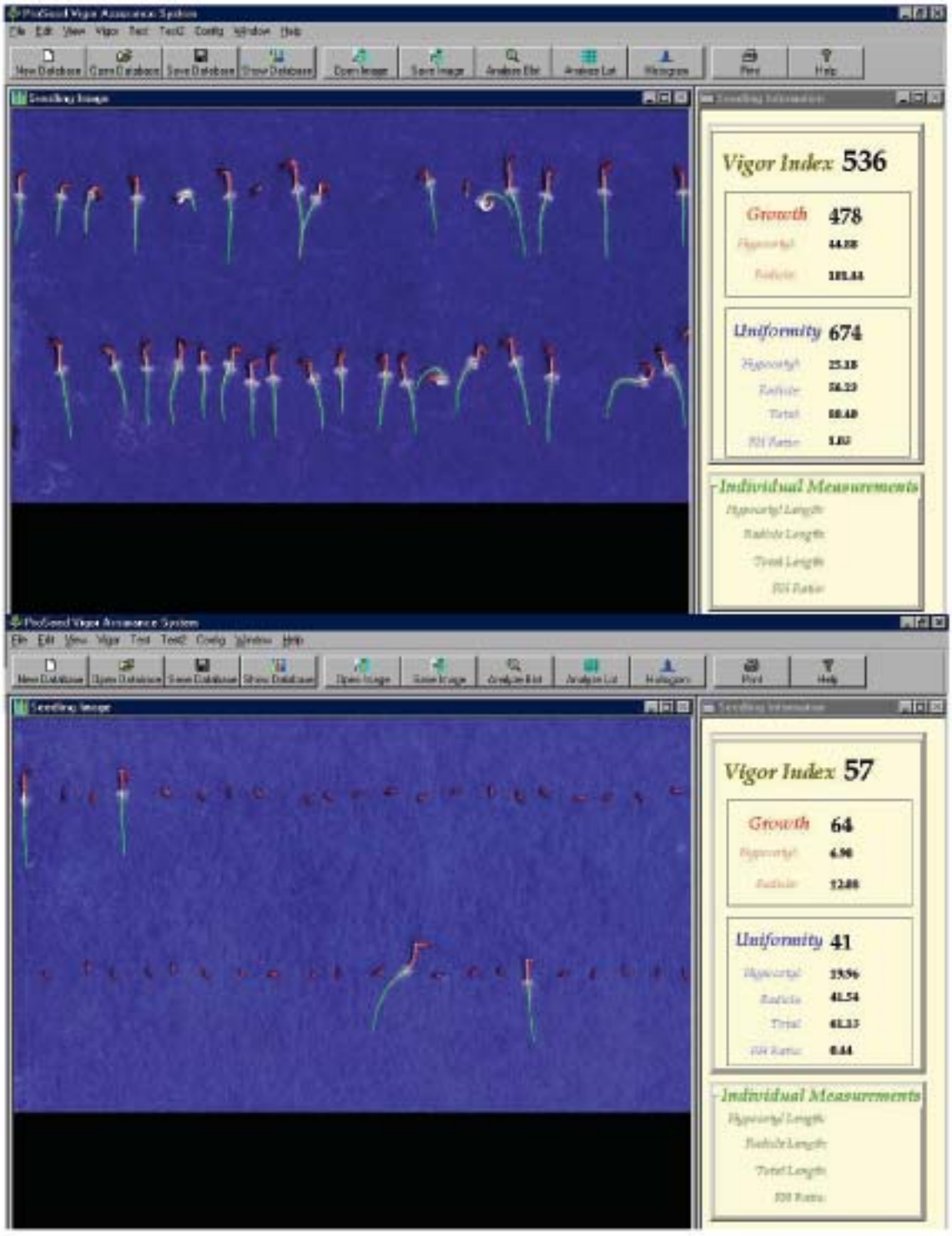

Figure 10. Vigor testing results 2.

percentage difference was 14.71%. Although the percentage difference was 14.71% for the worst case, the vigor index for the manually-derived (34) and software-derived (39) were extremely close. In such a low vigor seed lot, the difference of 5 is negligible. Seed analysts from differing laboratories undoubtedly would acquire more variation in results than depicted in this objective, reproducible approach to seed vigor testing.

Shapes of seedlings vary considerably from species to species (AOSA, 1992). Some species develop a single root, while others develop multiple roots. Other species develop seedlings that curl, while some develop seedlings that are straight. For the purpose of this study, lettuce was selected as a model species because lettuce seeds typically produce seedlings that are straight with only one primary root. Also, lettuce has seedling structures that are well defined: hypocotyl (shoot) and radicle (root). Furthermore, it has been reported that both embryo elongation and germination rates are good predictors of lettuce seed vigor (Hacisalihoglu, et al., 1999). Although this system was developed specifically for lettuce, it serves as a foundation for building automated vigor assessment systems for other species with similar seedling structure.

The vigor index equation has many weights that can be adjusted, but in order to define a standard and to make comparisons meaningful, a set of weights must be determined. A reasonable set of weights can be found based on the field performance data using machine learning methods (Mitchell, 1997). For example, if there are 100 seed lots, software analysis can be performed on the seed lots to measure the means and standard deviations of various parameters in the vigor equation, then the seeds can be planted in the field and the field emergence rate (what percentage of seeds emerged from the soil) is recorded. Each seed lot can be associated with a number (i.e., its field emergence rate), and weights adjusted accordingly so that the ranking given by the field emergence is retained when the seed lot's vigor index is computed. Alternatively, average and inverse of standard deviation length of the plants grown in the field can be measured. Then, the weights are adjusted so that the growth and uniformity components correlate with the average and inverse of standard deviation from the field data.

The development of the system demonstrated here would make vigor testing results more accessible and reliable since the results are objective, reproducible, speedy, and economical. However, the method described here is directly applicable only to lettuce and perhaps other species similar to lettuce. For the system to be more general and useful, extensions to the system should be considered. Obvious extensions of the automated vigor assessment system would be to enable feature extraction for other important agricultural crops such as corn (Zea mays L.) and soybean (Glycine max [L.] Merill) and flower seeds such as impatiens (Impatiens walleriana Hook). Specifically, algorithms for reliably detecting cotyledons, extracting hypocotyls/radicles, and detecting primary path (and secondary paths) may have to be developed and implemented specifically for each species. Once such algorithms are developed for a number of species, these may generally be applied to other species with similar seedling development, enabling the use of this vigor assessment system for a wide variety of crops.

This study has demonstrated that 1) scanners permit an economical and high quality digitization of seed and seedling images, 2) captured images can be stored to develop a comprehensive library of seeds and/or normal seedlings for future education of seed analysts, 3) compressed images can be promptly†"shipped" via the internet to others for comparison, and 4) seedling images captured in this way can be analyzed for speed and uniformity of growth using appropriate software.

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