COMPUTER IMAGING TO ASSESS SEED GERMINATION PERFORMANCE

M. B. MCDONALD

Department of Horticulture and Crop Science

Ohio State University

Columbus, OH 43210

United States of America

ABSTRACT

Seed vigor testing provides valuable information for assessing seed lot quality. However, vigor testing has not experienced widespread use because it is labor intensive, high in cost, and test results are often variable from laboratory to laboratory. An automated seed vigor assessment system is presented for lettuce (*Lactuca sativa* L.), soybean (*Glycine max* [L.] Merr.) and maize (*Zea mays* L.) 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 (vigor index) that collectively represent the quality of a seed lot 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, soybean and maize seedlings grown for three days in the dark. The results indicated that the imaging

system accurately quantified th0se parameters to yield reproducible, objective vigor assessments.

INTRODUCTION

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) Vigor Testing Handbook (1983) and the International Seed Testing Association (ISTA) Seed Vigour Testing Handbook (1995).

The objectives of this study were to 1) develop an imaging platform that could be rapidly adapted in a routine seed testing laboratory at little cost to enhance the standardization of vigor testing and 2) establish an imaging platform that could capture multiple images of lettuce, soybean and maize seedlings enabling simultaneous measurements of hypocotyl, radicle, and overall seedling length that could be further processed by more advanced computer methods to minimize human intervention and increase accuracy and reproducibility in seedling measurements for vigor assessment.

METHODS

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

Germination

Germination procedures for lettuce (Sako, et al., 2001), soybean (Hoffmaster, et al., 2003) and maize (Hoffmaster, et al., 2005) were as previously described.

Image Acquisition

A flatbed scanner was used to acquire all seedling images. The flatbed scanner offered several advantages over other imaging devices, such as a video camera or a digital camera (McDonald, et al., 2001).

Software Processing of the Images

After seedling images were acquired, they were processed by software developed by Sako (2000) for lettuce, by Hoffmaster (2002) for soybean and by Hoffmaster, et al. (2005) for maize. Based on the separation point and the skeleton for lettuce, the lengths of the hypocotyl and the radicle could be computed for each individual seedling. In the case of soybean, root hairs were not present to mark the separation point so the entire seedling length was computed. For maize, the entire growth of the seedling was determined (primary, seminal roots and coleoptile).

Seedling Length Determinations

Lettuce

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 for lettuce was defined as follows:

$$vigor = w_G * growth + w_U * uniformity \text{ ,}$$

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

$$uniformity = \max(1000 - (w * s_h + w * s_r + w * s_{total} + w * s_{r/h}) - w_d * numdead, 0) \text{ ,}$$

where $\overline{l_h}$ and $\overline{l_r}$ were the sample means of the hypocotyl length and the radicle length, 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.

Soybean

Before processing, soybean seedlings were classified into alphabetical types based on characteristics of their skeletons. The six types recognized by the program were the I, T, Y, P, A, and M-type seedlings (Hoffmaster, 2002; Hoffmaster, et al., 2003).

Maize

Maize seedlings possess a differing root structure that is composed of multiple roots (seminal and primary roots) connected to each seed compared to soybean seedlings. The multiple roots present connection problems to the seed because of shadows and create an overlap problem determining which roots belong to which seeds. To address these issues, a different approach was used for maize compared to lettuce and soybean.

As a first step, maize seeds were identified based on their yellowish-red color. To accomplish this, a synthesized image of maize seeds was used as a training image. In SUV color space, the range of s-, u-, and v-value colors that maize seeds fall into was established. The RGB maize image was then transformed into SUV color space.

Roots were separated by their white color against the brown background of the germination paper. When disconnected roots were encountered, the root was assigned to the seed that was nearest to it.

RESULTS AND DISCUSSION

Lettuce

The vigor assessment system was used to numerically evaluate four lettuce seed lots with different levels of seed vigor (Figure 1). The software marked hypocotyls in red and radicles in green. For most seedlings, these features were detected correctly. 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 (Smith, et al., 1973; Wurr and Fellows, 1985; Hacisalihoglu, et al., 1999). Both Penaloza, et al. (2005) and Contreras and Barros (2005) utilized the SVIS imaging system and showed that the results correlated with lettuce seedling emergence in the greenhouse. 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.

Soybean

By using the program on a sample image of 50 soybean seedlings at three days, the following pictorial results were obtained (Figure 2). A green line overlaid on each normal seedling reflected the length measured for each seedling. Abnormal seedlings were marked in red. The speed and uniformity of growth values and the vigor index were shown in the output window below the seedling image (Figure 2). Correlation coefficients have been run on standard germination, early growth rate, accelerated aging, cold test, field emergence, and SVIS 3-day vigor, SVIS 3-day uniformity, SVIS 3-day growth and accelerated aging SVIS vigor, growth and uniformity for 169 soybean seed lots. All vigor tests except accelerated aging vigor, accelerated aging uniformity, accelerated aging growth and standard germination were not significantly different in predicting field performance (Hoffmaster, et al., 2005).

Through the development of this soybean system, a fast, objective, and reproducible method to perform a seed vigor test on soybean seedlings was achieved. For every input example presented to the software for processing, a seed vigor index was derived in less than 20 seconds. This is a significant advancement compared to manually measuring the seedlings, which may take up to 30 minutes. Another advantage of this soybean imaging system is objective and reproducible results.

Even though the developed system was designed specifically for soybean seedlings, it shows promise for other seedlings with similar structure. The principles of the soybean system were also applied to three-day-old cotton (*Gossypium hirsutum* L.) and impatiens (*Impatiens walleriana* Hooker f.) seedlings to produce vigor indices and/or length measurements with success (Hoffmaster, 2002).

Maize

Maize seedlings were imaged at 3-days germination followed by an SVIS analysis (Figure 3). The results demonstrated that the nearest neighbor connection and overlapping algorithms successfully attached roots and separated overlapping seedlings.

Maize seedlings pose a differing challenge to software development of seedling imaging compared to soybean seedlings. They possess both seminal and primary roots that often grow horizontally separated by a seed producing a coleoptile (Figure 3). This creates three dilemmas. First, the size of the seed and the number of roots create shadows that make connection of the roots to the correct seed difficult. Second, the horizontal nature, particularly of the seminal roots, causes an overlapping syndrome that makes it difficult to ascertain which root belongs to which seed. Third, the coleoptile must be measured as a part of the growing seedling, but it is separated in distance from the roots by a substantial seed. These three dilemmas were solved by developing two principal mathematical solutions. The first involved connecting the seedlings using nearest neighbor analysis that was applied both to the roots and the coleoptile. Overlapping of seedling parts is undesired in image analysis. One approach to solving this problem was to simply germinate the seeds and acquire images of the seedlings before they grew to a stage where overlap occurs. The second approach has been to develop an algorithm using angular features of the roots that minimized overlap when it occurred. This area of software development is continuing.

CONCLUSION

In conclusion, the research presented here advances the use of computer software to simplify seed technology tasks. The work of Sako et al. (2001) towards lettuce seedlings was extended to support more complicated seedlings such as soybean (Hoffmaster, et al., 2003) and maize (Hoffmaster, et al., 2005). The soybean and maize systems analyzed images of three-day-old seedlings and derived length measurements for each seedling that were combined into a 0 to 1000 index representing the overall vigor of the seed lot. The vigor index was designed to reflect the speed and uniformity of growth measures that indicated the seedlings' potential for rapid and uniform emergence upon planting in the field. Through extensions of the principles of these lettuce, soybean and maize systems, other seed vigor assessment systems for different crops can be developed.

ACKNOWLEDGEMENTS

Funding for this research was provided by the Ohio Agricultural Research and Development Center Research Enhancement Competitive Grants Program and the American Seed Research Foundation. Salaries and additional research support were provided in part by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University. Journal article no. _________.

REFERENCES

Association of Official Seed Analysts. (1983). *Seed Vigor Testing Handbook. Contribution No. 32*. Lincoln, NE. 89pp.

Contreras, S. and Barros, M. (2005). Pruebas de vigor en semillas de lechuga y su correlacion con emergencia [Vigor tests on lettuce seeds and their correlation with emergence]. *Ciencia E Investigacion Agraria* 32, 3-11.

Hacisalihoglu, G., Taylor, A.G., Paine, D.H., Hilderbrand, M.B. and Khan, A.A. (1999). Embryo elongation and germination rates as sensitive indicators of lettuce seed quality: Priming and aging studies. *HortScience* 34, 1240-1243.

Hoffmaster, A.L. (2002). An Automated System For Seed Vigor Testing Three-Day-Old Soybean Seedlings. MSc thesis, The Ohio State University, Columbus, OH, USA.

Hoffmaster, A.L., Fujimura, K., McDonald, M.B. and Bennett, M.A. (2003). An automated system for vigor testing three-day-old soybean seedlings. *Seed Science and Technology* 31, 701-713.

Hoffmaster, A.F., Xu, L., Fujimura, K., McDonald, M.B., Bennett, M.A. and Evans, A.F. (2005). The Ohio State University Seed Vigor Imaging System (SVIS) for soybean and corn seedlings. *Journal of Seed Technology* 27, 7-24.

International Seed Testing Association. (1995). *Handbook of Vigour Test Methods*. (eds. Hampton, J.G. and TeKrony, D.M.). Zurich, Switzerland. 117pp.

McDonald, M.B., Evans, A.F. and Bennett, M.A. (2001). Using scanners to improve seed/seedling evaluations. *Seed Science and Technology* 29, 683-689.

Penaloza, P., Ramierz-Rosales, G., McDonald, M.B. and Bennett, M.A. (2005). Lettuce (*Lactuca sativa* L.) seed quality evaluation using seed physical attributes, saturated salt accelerated aging and the Seed Vigor Imaging System. *Electronic Journal of Biotechnology* (in press).

Sako. Y. (2000). Systems for seed vigor assessment and seed classification. MSc thesis, The Ohio State University, Columbus, OH, USA.

Sako, Y., McDonald, M.B., Fujimura, K., Evans, A.F. and Bennett, M.A. (2001). A system for automated seed vigour assessement. *Seed Science and Technology* 29, 625-636.

Smith, O.E., Welch, N.C. and McCoy, O.D. (1973). Studies on lettuce seed quality: II. Relationship of seed vigor to emergence, seedling weight and yield. *Journal of the American Society for Horticultural Science* 98, 552-556.

Wurr, D.C.E. and Fellows, J.R. (1985). A determination of the seed vigour and field performance of crisp lettuce seedstocks. *Seed Science and Technology* 13, 11-17.

FIGURES

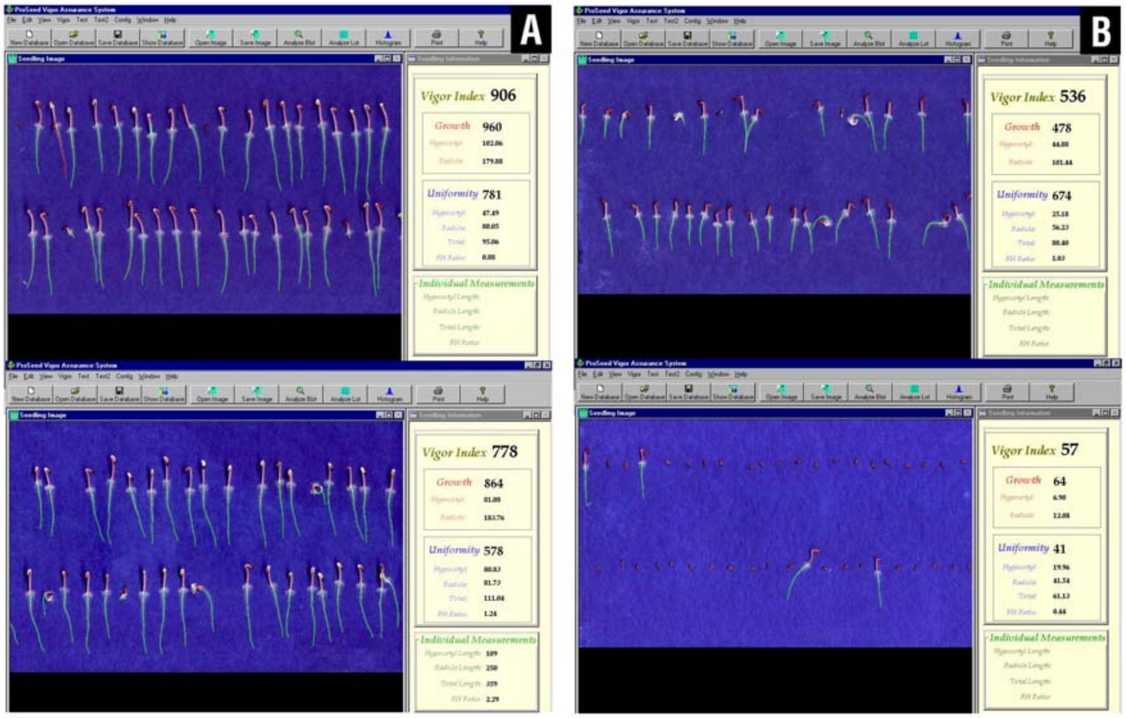

Figure 1. Vigor testing results for lettuce seeds.

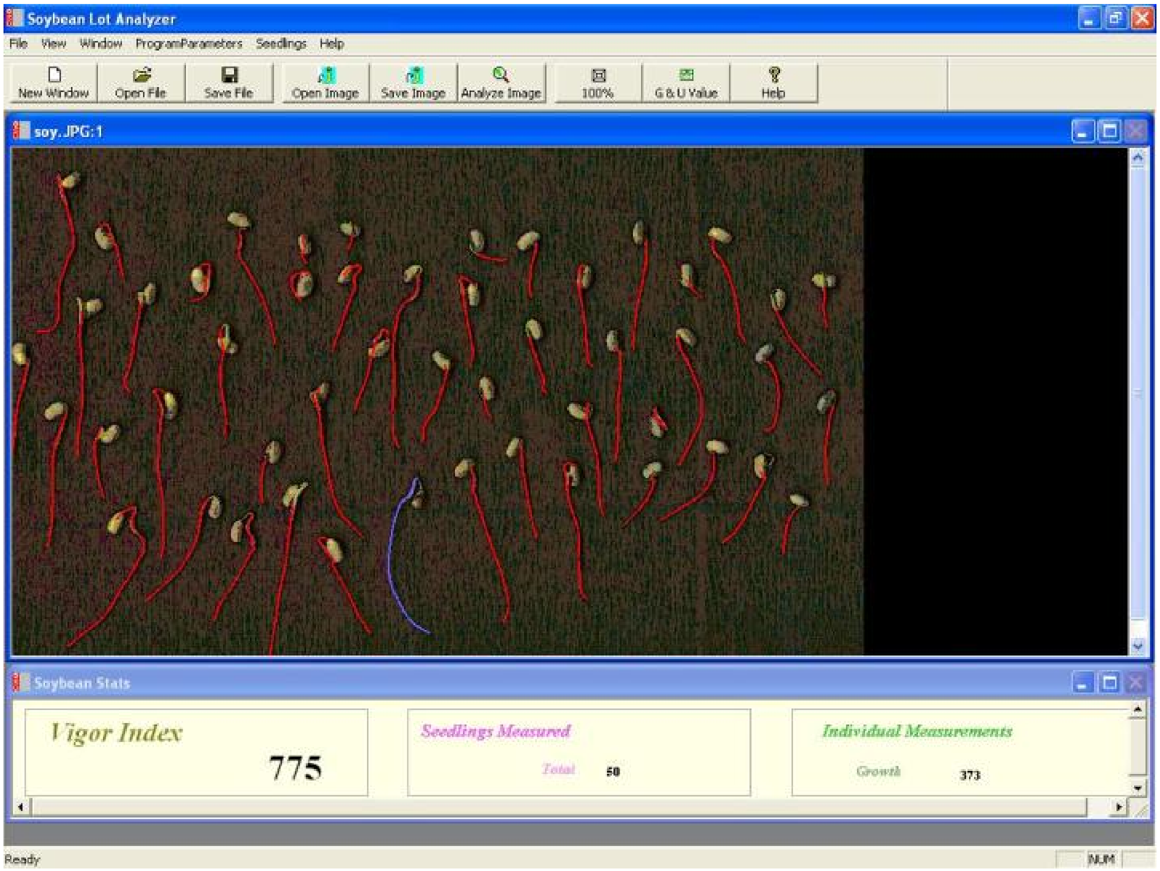

Figure 2. Vigor test results for soybean seeds.

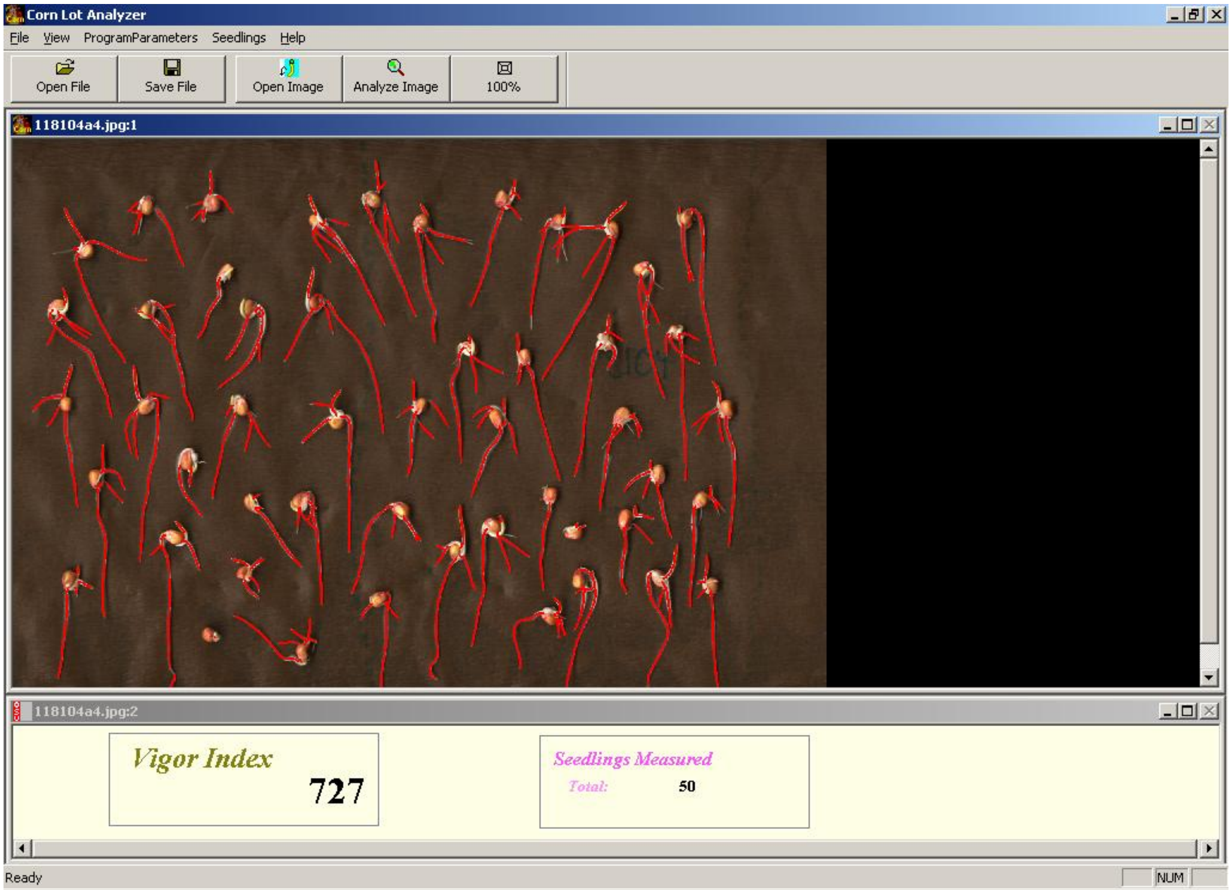

Figure 3. Vigor test results for maize seeds.