From Forest Nursery Notes, Summer 2008

135. Vigor testing in small-seeded horticultural crops.. Geneve, R. L. Acta Horticulturae 782:77-82. 2008.

Vigor Testing in Small-Seeded Horticultural Crops

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Keywords: accelerated aging, germination, biochemical, standard germination, flower seeds, vegetable seeds, cold test

Abstract

Vigor is an important characteristic of seed lots and can be defined as the inherent ability of seeds within a seed lot to establish normal (or usable) seedlings under diverse growing environments. Vigor is a complex seed attribute determined by plant genetics, the seed production environment and post-harvest processing and handling. Seed vigor is routinely evaluated for commercial seed lots, but vigor testing can be more challenging in small-seeded horticultural crops compared to larger-seeded agronomic crops. This manuscript provides an overview of selected vigor testing methods suitable for small-seeded horticultural crops.

INTRODUCTION

The relatively high initial cost of horticultural seeds has led growers to employ precision seeding and transplant production systems to maximize seedling stands. This places a high reliance on high quality seeds for maximal seedling emergence and uniformity. Specialization has led to increased capital investment in modern greenhouses, automated seeders and sophisticated transplanting robots (Styer and Koranski, 1997). This has challenged the seed industry to provide seeds that perform under these demanding production systems (Karlovich, 1998).

The ultimate goal of seed testing is to provide useful information on a seed lot's quality. This is accomplished through standard germination and vigor testing. Standard germination evaluates the seed's ability to produce a normal seedling under near-optimal germination conditions (International Seed Testing Association, 1999) and therefore does not always reflect future emergence in the field or greenhouse. Seed vigor testing attempts to identify "those seed properties which determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions" (Association of Official Seed Analysts, 1983). It becomes apparent that seed lots with comparable standard germination percentages can vary widely in their vigor and that vigor testing can often provide a better predictive measure of seedling emergence.

Vigor testing is well established in agronomic crops where only a handful of important species dominate commercial production. However, vigor testing can be more challenging for the horticultural industry because of the hundreds of species grown. In addition, direct transfer of vigor testing methodology from agronomic to horticultural crops is not always possible, especially in small-seeded vegetable and flower seeds.

METHODS OF SEED VIGOR TESTING

Guidelines for vigor testing were first described by ISTA in 1981 (Perry, 1981) and AOSA in 1983. These methods have subsequently been revised by Hampton and TeKrony (1995). They group vigor tests into three categories that include: 1) single tests based on germination behavior; 2) multiple testing procedures; and 3) physiological or biochemical indices of vigor (Table 1).

Single Vigor Tests

Single seed vigor tests measure standard germination or seedling growth often after a stress imposition. Vigor tests that evaluate standard germination after stress

include accelerated aging, saturated salts accelerated aging, controlled deterioration, cold tests, and germination across a thermal gradient table (Bennett et al., 2004; Geneve, 2005).

Accelerated aging and cold tests have been used most successfully with large-seeded crops. However, several small-seeded crops have been effectively evaluated for vigor using standard accelerated aging methods. Examples include canola (*Brassica napus*) and onion (*Allium cepa*). For canola, the test was run at 42°C for 48 hours and was effective for determining vigor in seeds from different harvest dates (Elias and Copeland, 2001). For carrot (*Daucus carota*), accelerated aging effectively evaluated seed vigor in seeds produced at elevated temperature (Elballa and Cantliffe, 1996).

However, for most small-seeded horticultural crops accelerated aging imposes too severe a stress to be a useful testing method. Two alternative tests for small-seeded crops are saturated salts accelerated aging (Jianhua and McDonald, 1996) and controlled deterioration (Matthews, 1980) because they limit seed hydration during the imposition of heat stress (Geneve, 2005).

Saturated salts accelerated aging suspends seeds over a salt solution rather than water as in standard accelerated aging. It has proven useful for flower crops like impatiens (*Impatiens wallerana*) and pansy (*Viola* × *wittrockiana*) (Jianhua and McDonald, 1996; Oakley et al., 2004) and vegetables such as lettuce (*Lactuca sativa*) and onion (Bennett et al., 2004).

Controlled deterioration exposes seeds to high temperature (40 or 45°C) for a short duration (24 or 48 hours) after the moisture content have been raised to approximately 20%. Powell and Matthews (1981) showed the utility of controlled deterioration on vigor testing in onion, lettuce, cabbage and Brussels sprouts (*Brassica oleracea*).

Seedling growth tests include measures of time to radicle protrusion (germination speed), seedling growth rate after radicle protrusion, and sorting seedlings into strong or weak growing categories (i.e. grow-out tests).

AOSA (1983) considers germination speed as an indicator of seed vigor. Caution must be used when calculating a single value to represent germination speed (Brown and Mayer, 1986). The most common measures are T_{50}, which determines the time to 50% germination in the population of germinating seeds, and mean time to germination (Maguire, 1962). Similar values to time to radicle protrusion can be calculated for seedling emergence in greenhouse studies. Single values for seedling emergence rates are rarely calculated (Finch-Savage, 1991), rather data are recorded daily to determine emergence curves (Samfield et al., 1991) or total emergence percentage after completion of the experiment (Fay et al., 1993).

In contrast to germination or seedling emergence rates, which are measures of time, seedling growth rate is a determination of growth parameters such as length, area, or dry weight at periodic times after radicle protrusion. Seedling growth rate is a sensitive measure of seed vigor (Woodstock, 1969), but is difficult to incorporate in routine vigor testing because it is too labor intensive to periodically evaluate seedling growth over time. Therefore, seedling growth rate has been often estimated by measuring seedling size after a set time. Radicle length measured at discrete intervals under controlled conditions has been used successfully to test for seed vigor in a number of small-seeded vegetable crops including carrot, lettuce (McCormac et al., 1990; Smith et al., 1973), cauliflower, onion and leek (Finch-Savage, 1986). Radicle length or growth rate using a slant-board test was also correlated with field emergence in these crops. The method employs germination of seeds on a slant board so that straight seedlings are obtained that are subsequently hand measured by an analyst as described for lettuce (Smith et al., 1973). This test is used commercially for several small-seeded horticultural crops.

An alternative to hand measurements is the use of computer-aided analysis for seedling size calculated from digital images. Several systems have been developed using CCD digital video camera prior to computer analysis of radicle length (McCormac et al., 1990; McNertney, 2004). An alternative to CCD digital video cameras is the use of flat

bed scanners to capture digital images of seedlings (Dell'Aquila et al., 2000; Geneve and Kester, 2001; Sako et al., 2001). The flat bed scanner provides excellent resolution of small seeds that are currently difficult or impossible to achieve with digital video cameras. For example, lisianthus (*Eustoma*) which is one of the smallest seeds grown by the bedding plant industry was easily captured by a flat bed scanner (Geneve and Kester, 2001) and seedling length accurately measured. Oakley et al. (2003) showed that seedling size measured by either length or area at a specific time from digital images was similar to other vigor indices for each seed lot. Similar results have been shown for lettuce (Sako et al., 2001) suggesting that computer-aided image analysis could satisfy the requirements for a quality vigor test across a range of small-seeded crops (Geneve et al., 2006).

Commercial flower producers and brokers rely heavily on seedling emergence studies conducted under greenhouse or growth chamber conditions to evaluate seed vigor. Usable seedlings are evaluated under conditions similar to those used by commercial seedling plug growers. Ball Seed Company has developed a computer-aided analysis system that uses digital images of flower crop seedling emergence to provide a quantitative measure of usable seedlings (Conrad, 2004). A CCD digital video camera images plug-grown seedlings under controlled environments and determines a vigor index based on germination percentage and cotyledon area.

Multiple Vigor Tests

Multiple testing addresses the premise that seed vigor is a complex response to variable environments and that seeds in a seed lot may not respond the same to each of those environments. For example, Dutt et al. (2007) showed that germination speed in individual impatiens seeds did not correlate with their subsequent seedling growth rate after germination. They concluded that these two assessments were measuring different aspects of seed vigor in the same seed lot. Therefore, multiple testing using several different vigor tests can provide a better prediction of field emergence as shown for pepper (Trawatha et al., 1990).

Biochemical Vigor Tests

Biochemical tests include various measures of metabolic activity in seeds. These include aspects related to respiration (i.e. ATP synthesis, tetrazolium staining), membrane repair (i.e. electrolyte leakage) and volatile production (i.e. ethylene). Although several of these tests have shown good correlation with seed vigor (Cantliffe, 1981; Siriwitayawan et al., 2003) only electrolyte leakage measured by conductivity tests and tetrazolium have been used extensively to evaluate commercial seed lots.

CONCLUSIONS

From the previous discussions, it is apparent that vigor testing for small-seeded horticultural crops presents numerous challenges to the seed analyst. No one single test is likely to be appropriate for the large number of species important to the horticultural industry. In general, saturated salt accelerated aging would seem the most adaptable stress test because many seed laboratories currently have the facilities to conduct accelerated aging for agronomic seeds. Controlled deterioration would also be suitable, but care must be taken to obtain appropriate seed moisture content.

Computer-aided capture and analysis of seed germination and seedling growth is an emerging technology that could prove very useful for comparing seed lots for vigor. It has the advantage of using standard germination conditions already established for most crops and measuring established parameters related to seed vigor (i.e. seedling growth and uniformity).

ACKNOWLEDGEMENTS

Paper number 07-11-005 of the Kentucky Experiment Station.

Literature Cited

Association of Official Seed Analysts. 1983. Seed vigor testing handbook. Contribution No. 32. Association of Official Seed Analysts, Lincoln, Nebraska.

Bennett, M.A., Grassbaugh, E.M., Evans, A.F. and Kleinhenz, M.D. 2004. Saturated salt accelerated aging (SSAA) and other vigor tests for vegetable seeds. Seed Technol. 26:67-74.

Brown, R.F. and Mayer, D.G. 1986. A critical analysis of Maguire's germination rate index. Journal of Seed Technol. 10:101-110.

Cantliffe, D.J. 1981. Vigor in vegetable seeds. Acta Hort. 111:219-226.

Conrad, R. 2004. Ball seed vigor index. Seed Technol. 26:98-103.

Dell'Aquila, A., van Eck, J.W. and van der Heijden G.W.A.M. 2000. The application of image analysis in monitoring the imbibition process of white cabbage (*Brassica oleracea* L.) seeds. Seed Sci. Res. 10:163-169.

Dutt, M. and Geneve, R.L. 2007. Time to radicle protrusion does not correlate with early seedling growth in individual seeds of impatiens and petunia. J. Amer. Soc. Hort. Sci. in press.

Elballa, M.M.A. and Cantliffe, D.J. 1996. Alterations of seedstalk development, seed yield, and seed quality in carrot by varying temperature during seed growth and development. J. Amer. Soc. Hort. Sci. 121:1076-1081.

Elias, S.G. and Copeland, L.O. 2001. Physiological and harvest maturity of canola in relation to seed quality. Agron. J. 93:1054-1058.

Fay, A.M., McDonald, M.B. and Still, S.M. 1993. Vigor testing of *Rudbeckia fulgida* seeds. Seed Sci. and Technol. 21:453-462.

Finch-Savage, W.E. 1986. A study of the relationship between seedling characters and rate of germination within a seed lot. Ann. Appl. Bio. 108:441-444.

Finch-Savage, W.E. 1991. Development of bulk priming/plant growth regulator seed treatments and their effect on the seedling establishment of four bedding plants. Seed Sci. Technol. 19:477-485.

Geneve, R.L. 2005. Vigor testing in flower seeds. p.311-332. In: M.B. McDonald and F. Kwong (eds.), Flower Seeds, Biology and Technology. CAB International, London.

Geneve, R.L., Dutt, M. and Downie, A.B. 2006. Development of a sequential digital imaging system for evaluating seed germination. p.315-323. In: S. Navie, S. Adkins and S. Ashmore (eds.), Seeds: Biology, Development and Ecology. CAB International. London.

Geneve, R.L. and Kester, S.T. 2001. Evaluation of seedling size following germination using computer-aided analysis of digital images from a flat-bed scanner. Hort. Sci. 36:1117-1120.

Hampton, J.G. and TeKrony, D.M. 1995. Vigor testing methods. 3rd edition. Intern. Seed Testing Assoc. Zurich.

International Seed Testing Association. 1999. International rules for seed testing. Seed Sci. Technol. 27:1-299.

Jianhua, Z. and McDonald, M.B. 1996. The saturated salt accelerated aging test for small-seeded crops. Seed Sci. Technol. 25:123-131.

Karlovich, P.T. 1998. Flower seed testing and reporting needs of the professional grower. Seed Technol. 20:131-135.

Maguire, J.D. 1962. Speed of germination – aide in selection and evaluation for seedling emergence and vigor. Crop Sci. 2:176-177.

Matthews, S. 1980. Controlled deterioration: a new vigour test for crop seeds. p.647-660. In: P.D. Hebblethwaite (ed.), Seed Production, Butterworths, London.

McCormac, A.C., Keefe, D.P. and Draper, S.R. 1990. Automated vigour testing of field vegetables using image analysis. Seed Sci. Technol. 18:103-112.

McNertney, D.C. 2004. The paradigm SVA system. Seed Technol. 26:92-97.

Oakley, K., Kester, S.T. and Geneve, R.L. 2004. Computer-aided digital image analysis of seedling size and growth rate for assessing seed vigour in impatiens. Seed Sci. and Technol. 32:907-915.

Perry, D.A. 1981. Handbook of vigour test methods. Intern.l Seed Testing Assoc., Zurich, Switzerland.
Powell, A.A. and Matthews, S. 1981. Evaluation of controlled deterioration, a new vigour test for small seeded vegetables. Seed Sci. and Technol. 9:633-640.
Sako, Y., McDonald, M.B., Fujimura, K., Evans, A.F. and Bennett, M.A. 2001. A system for automated seed vigour assessment. Seed Sci. and Technol. 29:625-636.
Samfield, D.M., Zajicek, J.M. and Cobb, B.A. 1991. Rate and uniformity of herbaceous perennial seed germination and emergence as affected by priming. Journal J. Amer. Soc. Hort. Sci. 116:1-13.
Siriwitayawan, G., Downie, A.B. and Geneve, R.L. 2003. Ethylene evolution is positively correlated with seed vigor in sweet corn and tomato seed lots with differing vigor levels but similar germination capacity. J. Amer. Soc. Hort. Sci. 128:608-614.
Smith, O.E., Welch, N.C. and Little, T.M. 1973. Studies on lettuce seed quality: I. Effect of seed size and weight on vigor. J. Amer. Soc. Hort. Sci. 98:529-533.
Styer, R.C. and Koranski, D.S. 1997. Plug and transplant production. Ball Publishing, Batavia, IL.
Trawatha, S., Steiner, J. and Bradford, K. 1990. Laboratory vigor tests to predict pepper seedling field emergence performance. Crop Sci. 30:713-717.
Woodstock, L.W. 1969. Seedling growth as a measure of seed vigor. Proceedings of the Intern. Seed Testing Assoc. 34:273-280.

Tables

Table 1. Categories of seed vigor arranged according to the germination parameters used to evaluate the seed lot.

Vigor test category	Vigor test	Unit of measure
Biochemical	1. Tetrazolium	Tetrazolium uses topology of red stain in embryo.
	2. Electrolyte leakage	Electrolyte leakage uses electrical conductivity (μmhos/g).
	3. ATP	ATP is a measure of energy availability.
	4. Ethylene	Ethylene production is associated with germination and correlates to vigor.
Germination percentage	1. Abnormal seedlings	Percentage of normal seedlings under standard germination conditions. Some studies only report radicle protrusion percentage. Some tests impose a stress (temperature and/or moisture) prior to standard germination. Thermal gradient germination uses variable temperature during germination rather than standard germination conditions. Natural aging uses K_i from models for seed deterioration in storage.
	2. Cold test	
	3. Thermal gradient germination	
	4. Aging tests	
	a. Controlled deterioration	
	b. Accelerated aging	
	c. Saturated salt accelerated aging	
	d. Natural aging	
Germination speed	1. Germination speed	T_{50}; mean time to germination. Expressed as unit of time (days or hours) to reach 50% radicle or seedling emergence.
	2. Seedling emergence	
Seedling growth	1. Seedling size	Linear (cm) or area (mm^2) after a specified time or rate calculated over time (cm or mm^2) per unit time (hour).
	2. Seedling growth rate	
	3. Vigor index	Vigor index uses growth plus a measure of uniformity.