Saturated Salt Accelerated Aging (SSAA) and Other Vigor Tests for Vegetable Seeds

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Abstract

Obtaining uniform stands of vegetable crops in field or greenhouse environments is often challenging. Successful germination and seedling establishment is usually linked to several important factors, including (1) the use of high vigor seed; (2) planting at proper soil depths; (3) sowing into well prepared seedbeds with calibrated seeders and good seed singulation; (4) field selection and planting date decisions; (5) suitable cultivars, and (6) seed treatments. The emphasis of this presentation will be on seed vigor tests for vegetable seeds, yet all the factors listed above are closely tied to the concept of vigor. Definitions of vigor (Hampton and TeKrony, 1995) mainly describe the associated seedling establishment consequences of seedlots which differ in quality. The objectives of this manuscript are to (1) review the benefits of quality vegetable seeds; (2) discuss the connection of seed production and seed enhancement variables to seed vigor, and (3) summarize current and emerging vigor tests used for vegetable crop species.

Introduction

The world's vegetable species are numerous, and the consumption of vegetables is essential for optimal human health. Recent FAO statistics show an average per capita vegetable consumption of 86 kg/year, and usage is increasing worldwide (Rubatzky and Yamaguchi, 1997). Most vegetable crops are propagated by seed, with a wide range of morphology and seed sizes. This diversity of seed types is an obvious challenge for everyone involved in the crop production cycle, yet also contributes to the dynamic and scientifically based nature of the global vegetable seed industry.

Benefits of high quality vegetable seed include (1) rapid and uniform germination; (2) seedlings and plants better able to withstand environmental stresses; (3) desired plant population targets reached; and (4) more uniform crop maturity and increased harvest efficiency. Germination testing can provide reasonable estimates of stand establishment when planting conditions are optimal. Vigor testing is important and essential since it ranks the potential field performance of seedlots over a broader range of

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stresses and field (greenhouse) conditions. Vigor tests in general are expected to

- provide a more sensitive index of seed quality than the germination test
- provide a consistent ranking of seedlot performance
- be objective, rapid, simple, and economically feasible
- be reproducible and interpretable (McDonald, 1980; Perry, 1984)

Vigor test methods typically fall into one of three major categories: (1) stress tests, (2) seedling growth and evaluation tests, and (3) biochemical tests. Seed vigor is a broad concept, and many scientists have argued that no single test is likely to rank lots for all possible seed and environment combinations. The production of normal and various abnormal seedling classes is a common feature of many stress and seedling growth tests. Most vigor tests are relatively time-consuming and costly compared to standard germination tests (Sako *et al.*, 2001). The abundance of vegetable seed enhancements (e.g. priming, coatings, pelleting) and combination with added PGR's or other compounds can add to the cost and interpretation needed. For example, biological control organisms present a new type of seed enhancement and treatment for many species, including vegetable crops (Bennett, 1998; Warren and Bennett, 1999). The effect of some biocontrol agents and PGR seed applications (e.g. GA's) will need to be considered in seed health testing and vigor assessments (Hampton and TeKrony, 1995).

Enhanced vegetable (and flower) seed often ends up in greenhouse transplant production systems, which may require the use of different vigor tests than seed intended for field sowing (Taylor *et al.*, 1998). Seed quality can be described and tested in many ways (genetic purity, physical purity, germination, vigor, enhancements), but it starts for a given seedlot with seed production.

Influence of seed development and production on seed vigor

Maximum seed vigor development for soybeans and seed of most nonfleshy fruited species occurs at the stage of physiological maturity (Fig. 1). Seed germination (viability) and ability to produce normal seedlings can occur soon after fertilization, but seed vigor is more closely linked to maximum seed dry weight accumulation. Vigor is also at its peak for a shorter time frame than normal seedling or viability rating (Fig. 1), which illustrates the need for careful seed production, handling/cleaning, and storage practices. Fleshy-fruited (wet-seeded) species such as tomato (*Lycopersicon esculentum* Mill.) and those of the Cucurbitaceae family are often harvested based on fruit color. Some recent work by Ramirez-Rosales *et al.* (2001) suggests that seed quality development in high lycopene tomato genotypes may differ compared to traditional cultivars. Mature red fruit stage may be too advanced for best seed quality of high lycopene lines. Seed quality differences linked to seed production conditions are also common, as shown for six onion (*Allium cepa*) seedlots in Table 1. Broad differences in germination and vigor among seedlots of the same cultivar, let alone species, are important factors in selecting subsequent seed enhancement procedures (such as pelleting and priming). (Caseiro *et al.*, 2002).

Vigor tests for vegetable species

Biochemical tests (conductivity, TZ, etc.), stress tests (cold, accelerated aging), and seedling growth tests have been studied for a wide range of vegetable crop species. Garden pea (*Pisum sativum* L.) is often vigor tested using the conductivity test. The test is fast, non-subjective, and repeatable, and has been extensively correlated with field emergence results (Powell, 1986). Low quality seeds leach exudates more than high quality seeds during the initial hours of imbibition. Initial seed moisture must be adjusted to a range of 10-14% before seeding to ensure precision in conductivity tests, since very dry seeds (<10% SMC) will have much higher conductivity values than seeds of the same seed lot with higher SMC. Seed leakage can be influenced by (1) seed maturity, (2) degree of seed aging and (3) levels of physical damage. Recent spinoffs of the conductivity test include more sensitive measurement of specific compounds in the seed leachate. Potassium leakage (Marcos-Filho, 1998), amino acid leakage (Taylor *et al.*, 1995), and sinapine levels (Hill *et al.*, 1988) have shown promise as seed vigor tests for vegetable species. Seedling growth and evaluation tests include (1) the first count of a standard germination test; (2) seedling growth rate; and (3) seedling weight tests. Precision for all types of vigor tests is essential for reproducibility and standardization. Temperature and seed moisture during testing are most critical for success. This is also true for the common stress tests (cold, AA) used widely for corn (*Zea mays* L.), cotton (*Gossypium* spp.), soybean (*Glycine max*) and other important crops (TeKrony and Spears, 2001).

Cold tests determine the ability of seeds to germinate and develop normal seedlings after exposure to cold, moist conditions in the presence of soil pathogens. Many different procedures have been developed by various testing labs (e.g. saturated cold test, soil and sand mixes) which make standardization difficult. Germination chambers must be maintained at ±0.3 C for precision in any of the stress tests (AA, controlled deterioration, cold) to avoid fluctuations in results. It is important to note that most incubators used for the standard gemination test cannot be used for vigor tests. Water-jacketed chambers are best for precise temperature control. Vigor test committees of AOSA and ISTA will continue to summarize results of equipment evaluations and provide lists of recommended equipment for specific tests (TeKrony and Spears, 2001).

The accelerated aging test (AA) was initially developed to determine seed storage potential, but has evolved into a commonly used and important seed vigor indicator for many large-seeded crops. The AA test exposes seed to high temperatures (40-45° C) and high relative humidity (>95% RH) for 48 to 96 hours prior to germination testing. Use of the AA for a large seeded vegetable species, such as sweet corn, may be compromised by the anatomical and compositional differences of *su, sugary enhancer (se),* and *shrunken 2 (sh2)* genotypes. Pericarp damage and pathogen levels in (or on) *sh2* seed are especially troublesome (Borowski *et al.,* 1991; Parera *et al.,* 1996). Use of a saturated salt accelerated aging (SSAA) test is hypothesized to more accurately evaluate sweet corn seeds. The SSAA test should (1) reduce water uptake, (2) minimize microflora growth, and (3) slow overall seed deterioration, thereby allowing a more precise and repeatable measurement of sweet corn seed vigor. Results with the SSAA test have been promising with a small seeded flower species, *Impatiens* (Jianhua and McDonald, 1996). Lab studies examined several *se* and *sh2* sweet corn cultivars (Tables 2 and 3). An inner tray aging apparatus was used as described in Jianhua and McDonald (1996). Seeds were placed on wire mesh in a single layer with the solutions (water for AA; NaCl for SSAA) beneath them. Seeds were aged for 72 h at various test temperatures (41, 43, 45 or 47° C). Seeds were then evaluated for percentage of normal and abnormal seedlings after 7 d at 25° C (Tables 2 and 3). Cold tests were also conducted on these seed lots.

Percent normal germination was above 90% for all but two *sh2* cultivars (Bennett *et al.*, 2001). Standard AA test results provided a broader range of normal seedlings (40-100%) vs. the SSAA test (71-99%) at the standard temperature of 41° C. By using a test temperature of 43° C, however, the normal seedling range was virtually identical for AA and SSAA (24-98% vs. 25-99%). Coefficient of variation (CV) values increased as test temperature increased from 41-43° C, (average of 12.8 vs. 29.6), and SSAA CV values were consistently lower at all four temperatures in our study than those for the AA tests (Tables 2 and 3).

One expected result of this experiment, that the SSAA technique would reduce seed microflora growth due to the lower RH levels using NaCl solution, was not noticeable as the seeds were removed after the initial 72 h temperature stress. This may be due to the excellent seed treatments which were commercially applied to our seed samples. Labs which test seed directly from production fields would likely observe a reduction in microflora growth using the SSAA technique.

Lab studies in 2001 examined 12 *sh2* sweet corn cultivars (Table 4). Standard germination results show percent normal seedlings above 80% for all cultivars. SSAA and AA results for 41°C resulted in normal seedings above 75% for all but one cultivar. Percent abnormal and dead seedlings increased when seeds were aged under the higher temperatures of 43 and 45°C. With most cultivars, percent normal seedlings were generally higher from SSAA compared to AA tests (Table 4).

The *sh2* mutant of maize increases sucrose and reduces starch levels in developing endosperm. A rapid rate of water uptake caused by the elevated concentration of osmotic solutes in *sh2* and other sweet corn seeds may affect membrane integrity during imbibition (Parera *et al.*, 1996). This rapid uptake of water by sweet corn seed may also limit the ability of standard AA tests (~100% RH) to accurately assess seed vigor differences.

A separate study assessing sweet corn water uptake showed that seeds of all cultivars and endosperm types reached 27-37% seed moisture content (SMC) in standard AA conditions (H2O only), or roughly 2x the SMC observed for seeds subjected to the SSAA (Table 5) (Barr and Bennett, 1998).

The thermogradient (TG) table is another useful seed testing device for temperature stress and seedlot germination profiles. Standardization or official use of the TG table is not as advanced as the other tests discussed above. It is commonly used, however, in many seed company and university laboratories, and produces a quick assessment of germination rates across 10 temperature bands. The TG table can be very useful in quality control programs for seed enhancement companies and labs (Khan, 1992).

As illustrated in the schematic from Miles (1985), vigor change can occur rapidly once seed is in storage (Figure 1). Vigor tests are expected to rank lots in seed deterioration patterns, and seed labs must also store enough seed of control samples for each species tested. The primary purpose of control samples is to provide an instant quality control program, enabling seed analysts to detect any changes in testing procedures (TeKrony and Spears, 2001). These samples should be included routinely in every vigor test run as blind checks.

Computer imaging technology also has enormous potential for application to vigor testing. Key attributes of vigor tests (objective, rapid, consistency, reproducible, economical) are well suited to automated computer systems. Important strides in image acquisition, image analysis software, and vigor index development have been made by several public and private research groups in recent years (Sako *et al.*, 2001). Computer assisted applications for seed quality assessment, seed marketing, and germplasm development will be powerful additions to future gains in seed science and technology. With the wide range of available tests, it is important to recall that values from any seed vigor test are relative, not absolute. Proper interpretation and use of the results require the addition of seed analyst, seed industry and seed consumer experiences in the lab, field, and greenhouse environment. Continued attention to improved seed production practices, seed biology research, and vigor testing protocols will pay dividends in the form of optimal vegetable seed quality.

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Table 1. Results of germination test (%), speed of germination index, saturated salt accelerated aging (SSAA) (%), speed of germination index on SSAA and seed moisture content of six lots of hybrid onion seeds, cv. 'Granex 33'.

	Parameters				
Lots	Germination[1] (%)	Speed of Germination Index	Saturated Salt Accelerated Aging (%)	Speed of Germination Index after SSAA	Moisture Content (%)
1	78 b[2]	5.46 b	18.0 a	1.28 a	7.9
2	87 a	7.39 a	11.0 bc	1.03 ab	9.1
3	91 a	8.13 a	17.0 ab	1.38 a	8.5
4	41 c	2.47 c	1.0 d	0.05 c	8.8
5	79 b	5.82 b	1.0 d	0.06 c	8.5
6	87 a	7.30 a	9.0 c	0.59 b	8.5
C.V	5.65	9.67	23	46.5	

[1] Data were arc sin (√x /100) transformed prior to statistical analysis

[2] Treatment means separated by Tukey's test. $P \leq 0.05$.

Table 2. Standard germination, cold test results, and percentage germination for *se* sweet corn (*Zea mays* L.) after 7 days at 25° C following accelerated aging (AA) or saturated salt AA (SSAA) in saturated NaCl (76%) solutions for 72 h at 41, 43, 45 0r 47° C (2000).

Se	Std	Cold	-----41°C-----		-----43° C----		-----45°C----		----47°C-----	
cvs.	germ	test	SSAA	AA	SSAA	AA	SSAA	AA	SSAA	AA
SF64Y	98	93	81	90	75	75	82	57	51	15
SF73Y	100	43	98	97	95	83	80	31	51	0
Swt cheeks	100	79	97	92	89	83	89	63	79	38
HMX5349	94	94	97	94	91	98	92	89	88	58
LSD (0.05)	NS	10.6	7.8	8.1	12.7	11.4	11.3	11.1	10.9	8.9
CV	8.3	37.2	10.8	14.9	27.3	31.9	40.2	65.9	10.9	38.7

Table 3. Standard germination, cold test results, and percentage germination for *sh2* sweet corn (*Zea mays* L.) after 7 days at 25° C following accelerated aging (AA) or saturated salt AA (SSAA) in saturated NaCl (76%) solutions for 72 h at 41, 43, 45 0r 47° C (2000).

Sh2	Std	Cold	-----41°C-----		----43° C----		-----45°C----		----47°C-----	
cvs.	germ	test	SSAA	AA	SSAA	AA	SSAA	AA	SSAA	AA
710A	99	82	88	86	73	67	55	43	1	12
GSS3857VP	98	18	71	84	25	39	33	11	0	0
HMX6383S	99	31	96	87	75	71	49	74	25	44
HMX8392S	97	69	99	93	93	83	72	64	24	25
LSD (0.05)	NS	10.6	7.8	8.1	12.7	11.4	11.3	11.1	10.9	8.9
CV	8.3	37.2	10.8	14.9	27.3	31.9	40.2	65.9	10.9	38.7

Table 4. Standard germination, saturated salt accelerated aging (SSAA) and accelerated aging (AA) for sh2 sweet corn (*Zea mays* L.) seedlots - 2001.

		Standard germination			**-----------------41C------------------**						**-----------------43C------------------**						**-----------------45C------------------**					
					SSAA			**AA**			**SSAA**			**AA**			**SSAA**			**AA**		
sh2 Cultivars	**Seed Source**	**N***	**A***	**D***	**N**	**A**	**D**	**N**	**A**	**D**	**N**	**A**	**D**	**N**	**A**	**D**	**N**	**A**	**D**	**N**	**A**	**D**
8102R	AC	98	1	1	98	1	1	97	2	1	96	2	2	92	4	4	95	3	2	88	4	8
ACX 946	AC	85	9	6	76	6	18	80	4	16	74	6	20	75	6	19	64	8	28	55	11	34
BSS 0977 VP	Syngenta	94	4	2	85	7	8	80	14	6	76	10	14	74	16	10	40	14	46	60	19	21
Candy Corner	HM	97	2	1	99	0	1	96	3	1	93	6	1	95	2	3	89	5	6	69	11	20
GS 277A	ST	90	4	6	84	10	6	88	6	6	72	10	18	84	6	10	55	20	25	70	10	20
PS 8201	PS	94	5	1	96	4	0	89	6	5	93	3	4	90	4	6	92	0	8	67	10	23
ACX 945 (ID-lot 70532)	AC	95	2	3	97	1	2	90	4	6	88	7	5	86	7	7	92	4	4	33	22	45
ACX 945 (Idaho)	AC	94	4	2	97	3	0	94	3	3	92	4	4	96	2	3	95	3	2	90	4	6
ACX 945 (Chile-lot 90951P)	AC	93	4	4	94	2	4	89	4	7	94	2	4	86	3	11	89	2	9	72	10	18
ACX 945 (Chile-70951P-KB)	AC	92	4	4	96	2	2	93	2	5	91	2	7	77	11	12	85	5	10	57	13	30
ACX 817 (Chile-lot 70953)	AC	84	8	8	76	8	16	72	10	18	30	27	43	35	18	47	54	22	24	31	19	50
ACX 817 (Chile 0953Q-KB)	AC	87	10	3	90	5	5	46	14	40	18	32	50	23	25	52	62	20	18	22	24	54
LSD (0.05)		4.9	4.1	3.8	4.5	3.0	3.6	6.0	3.6	4.5	5.8	4.0	5.4	6.5	3.7	5.3	8.3	4.1	7.0	9.5	6.9	8.9
CV		5.9	93.8	11.0	8.3	89.8	12.3	14.4	10.3	13.2	26.5	12.8	12.3	24.3	10.7	12.0	22.1	10.6	10.7	31.2	73.0	72.2

*N=normal seedlings; A=abnormal seedlings; D=dead seedlings.

Table 5. Seed moisture content (fr. wt. basis) results for six sweet corn seedlots following AA and SSAA (41° C for 96 h)

Cultivar	H2O	KCl	NaCl
Confection (*sh2*)	31.6	14.5	14.7
Skyline (*sh2*)	29.4	13.3	13.3
Starship (*sh2*)	30.7	14.5	14.6
Tuxedo (*se*)	37.5	17.1	16.4
Sundance (*su*)	36.6	16.2	15.9
SX-696 (*su)*	27.0	15.0	15.0

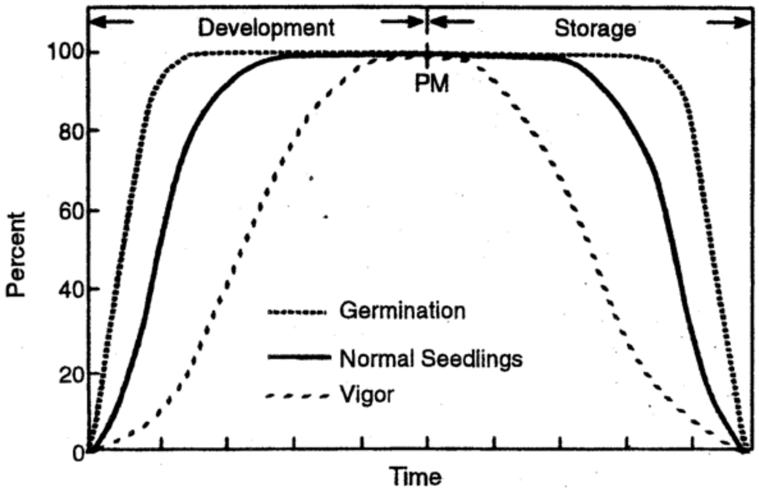

Figure 1. Pattern of soybean seed quality increase during development and decrease during storage for desiccated seed (Miles, 1985).