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RESEARCH ARTICLE

Lettuce (*Lactuca sativa* L.) seed quality evaluation using seed physical attributes, saturated salt accelerated aging and the seed vigour imaging system

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Abbreviations: SSAA: Saturated Salt Accelerated Aging
SVIS: Seed Vigour Imaging System
CD: controlled deterioration
AA: accelerated aging

High seed quality is essential for optimum stand establishment in lettuce. As a result, it is necessary to have seed vigour tests that permit rapid, objective and accurate evaluation of seed quality. This study evaluated physical and physiological seed quality components of four seed lots of six lettuce varieties obtained from a commercial company. Seeds were evaluated for seedling emergence under greenhouse conditions, standard germination, seed physical aspects, the Saturated Salt Accelerated Aging (SSAA) test and the Seed Vigour Imaging System (SVIS). Results indicated that large-seeded lettuce varieties had higher percentage germination, higher SSAA values, higher SVIS index and more rapid and uniform greenhouse emergence. Black- seeded lettuce varieties possessed higher seed quality and less fungal invasion when evaluated by the SSAA test. The SVIS index significantly correlated with SSAA values and seedling emergence under greenhouse conditions suggesting it can be used as a measure of seed vigour. It is concluded that the SSAA and SVIS tests are practical and accurate determinants of lettuce seed quality and distinguish between high and poor quality lettuce seed lots.

Seed quality evaluation can be conducted by physical and physiological vigour tests (McDonald, 1999) that provide information on the potential behaviour of a seed lot under greenhouse and field conditions. However, to date, there is no standard vigour test that compares laboratory and greenhouse performance and rapidly and objectively

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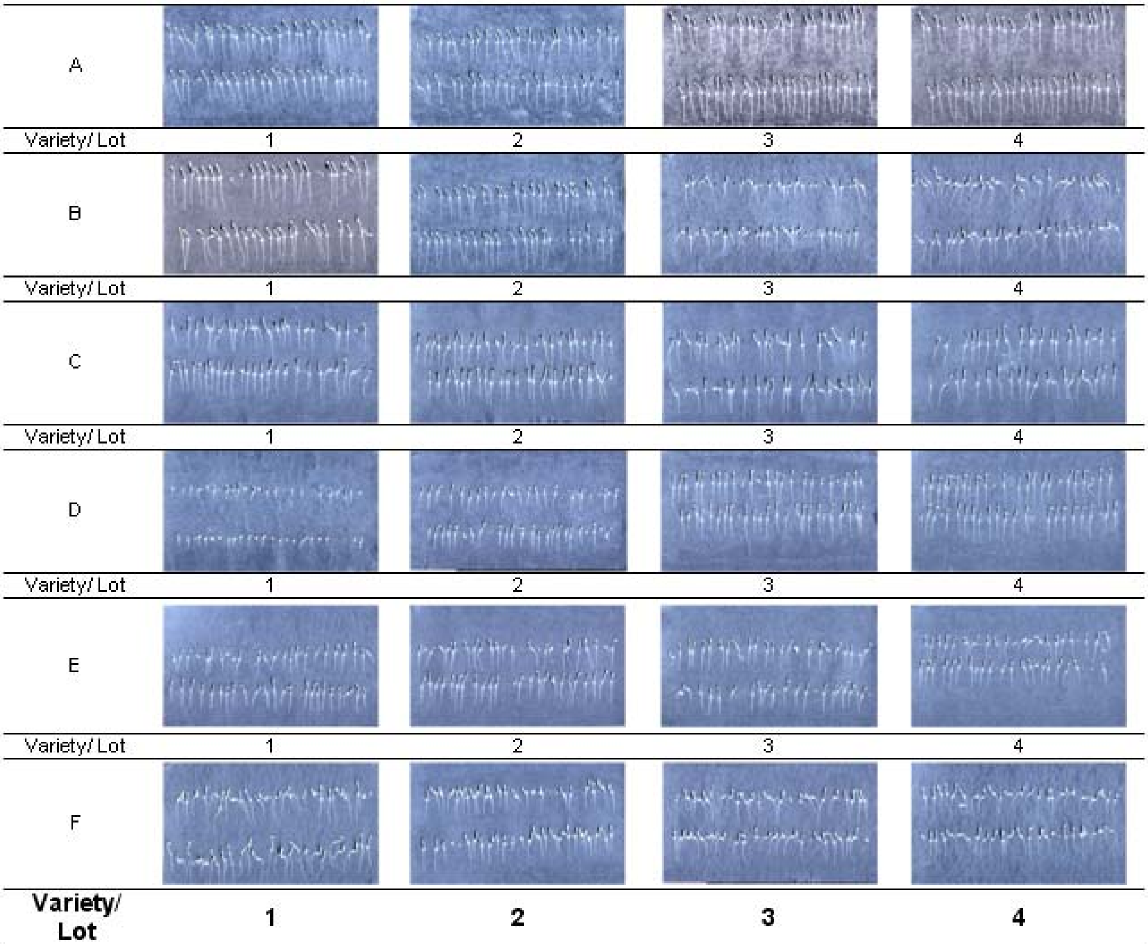

Figure 1. Images of vigor index test obtained from different lettuce varieties (A-F) with seed lots (1-4) obtained under different growing conditions.

assesses seed quality differences among lettuce seed lots. The standard germination test continues to be the most common measure of seed quality in lettuce and other vegetable crops. However, this test is conducted under ideal laboratory conditions seldom encountered in the field. As a result, germination results do not necessarily correlate with field performance. In addition, this test requires seven days (ISTA, 1999) before a determination of seed germination is possible. Ideally, seed quality tests efficiently differentiate between poor and good seed lots in a short period and produce data that correlate with stress planting conditions (Trawatha et al. 1990). The Saturated Salt Accelerated Aging (SSAA) test (Jianhua and McDonald, 1996; McDonald, 1999) and Seed Vigour Imaging System (SVIS) (Sako et al. 2001; Hoffmaster et al. 2003) have been proposed as alternatives to traditional vigour tests.

The SSAA test was first proposed as a vigour test for small-seeded species by Jianhua and McDonald (1996) and has been successfully used for vegetable and flower seed crops (Marcos Filho, 2004). A contrary report was presented for carrot, lettuce and broccoli (Ribeiro and Carvalho, 2001). The SSAA test also determined seed storage potential in wheat (*Triticum aestivum* L.) and provided more standardized results than the accelerated aging (AA) test (Meriaux et al. 2004). Traditional vigour tests, such as the accelerated aging (AA) test, were developed especially for soybean (*Glycine max* [L.] Merr.) and maize (*Zea mays* L.) seeds to determine storage potential (ISTA, 1995). The controlled deterioration (CD) test is used for small-seeded crops such as rye (*Secale cereale* L.) (Steiner and Stahl, 2002) and other crops (ISTA, 1995). In the AA test, relative humidity in the aging chamber is nearly 100% as the seeds are aged, which may not reflect natural storage deterioration processes. In the CD test, the increase in seed moisture prior to the test is difficult to control (ISTA, 1995; McDonald, 1999). To address these issues, the SSAA test was proposed (Jianhua and McDonald, 1996) in which seeds are subjected to temperatures of ~ 42ºC and differing relative humidities that vary depending on the salt selected. For example, a saturated NaCl solution produces 76% relative humidity (Jianhua and McDonald, 1996) that reduces the rate at which small-seeded crops deteriorate compared to the 100% relative humidity in the traditional AA test. Thus, SSAA more closely mimics low seed moisture content stress conditions encountered during storage. The benefits of using this test in small-seeded species have been reported for impatiens (*Impatiens wallerana* Hook) and pansy (*Viola tricolor* L.) (Jianhua and McDonald, 1996; McDonald, 1999) as well as other crops (Marcos Filho, 2004). However, to our knowledge, the utility of this as a vigour test has not been reported for lettuce seeds.

The SVIS is a vigour test that evaluates lettuce seed performance by computer images of three-day-old seedlings (Sako et al. 2001). In SVIS, seeds are germinated at 25ºC in a near vertical orientation. The resulting seedlings are scanned and their length and uniformity analyzed using software that computes an overall vigour index (Sako et al. 2001). This vigour test provides a rapid (3 days) and objective measurement of lettuce seed quality. An additional advantage of SVIS is that the images and vigour indices are stored and a data base developed for future reference. Although SVIS provides these advantages in seed quality evaluation compared to traditional vigour tests, there still is no information comparing SVIS against other vigour tests or greenhouse performance in lettuce.

Physical characteristics of seeds have also been used to distinguish varieties within species (Dehghan-Shoar et al. 1998; Illipronti et al. 1999; Keefe, 1999). For example, the effect of seed coat thickness on germination performance in

Table 1. *A. hypogaea*[1] sequences with putative annotation to transcription factors, proteins involved in signal transduction and stress response, which could be used as markers in peanut.

Variety	Lot	Length (mm)	Width (mm)	Area (mm^2)	Volume (mm^3)	Colour	Germination (%)
A	1	2.78	0.87	1.90	1.00	24.064	93
	2	2.71	0.83	1.67	0.76	23.269	91
	3	2.77	0.90	1.80	0.85	20.724	99
	4	2.67	0.84	1.70	0.80	19.199	98
	Variety	**2.73 d**	**0.86 d**	**1.78 c**	**0.85 b**	**21.814 f**	**95 a**
B	5	3.35	1.06	2.73	1.61	33.310	97
	6	3.36	1.10	2.85	1.76	32.996	94
	7	2.65	0.88	1.78	0.85	26.860	90
	8	3.05	1.07	2.51	1.51	35.588	98
	Variety	**3.10 c**	**1.01 c**	**2.47 b**	**1.43 b**	**32.189 d**	**95 a**
C	9	3.06	0.97	2.37	1.38	28.040	95
	10	3.89	1.30	3.27	1.42	25.462	96
	11	3.76	1.15	3.30	2.15	41.445	96
	12	3.73	1.75	3.58	2.65	23.331	100
	Variety	**3.61 a**	**1.29 a**	**3. 13 a**	**1.90 a**	**29.570 e**	**97 a**
D	13	3.08	0.91	2.21	1.10	41.835	81
	14	2.97	0.88	2.11	1.07	47.747	91
	15	2.85	0.88	1.96	0.95	47.714	90
	16	2.84	0.84	1.87	0.87	47.659	91
	Variety	**2.94 c**	**0.88 d**	**2.04 b**	**0.99 b**	**46.239 b**	**88 b**
E	17	2.97	0.87	2.09	1.07	38.919	90
	18	3.10	0.90	2.14	1.04	41.837	88
	19	3.00	0.86	2.03	0.97	40.406	76
	20	2.60	0.74	1.50	0.60	39.954	78
	Variety	**2.92 c**	**0.84 d**	**1.94 c**	**0.92 b**	**40.279 c**	**83 c**
F	21	3.55	1.18	3.17	2.14	55.723	98
	22	3.05	0.96	2.21	1.16	48.570	93
	23	3.51	1.11	3.01	1.87	48.109	94
	24	3.55	1.13	3.05	1.90	48.168	94
	Variety	**3.42 b**	**1.09 b**	**2.86 b**	**1.77 b**	**50.143 a**	**95 a**

[1]Values in the columns followed by different letters showed statistical differences, LSD ($P < 0.05$).

watermelon (*Citrullus lunatus* Matsum and Nakai) has been reported (Duval and NeSmith, 2001). Colour of the seed coat has also been associated with seed quality: dark-seeded species having better seed quality than white-seeded species (Karivarhadaraaju et al. 2001). Seed size is another physical aspect associated with seed germination performance and root and hypocotyl development (Liu et al. 1993; Soltani et al. 2002). In maize, larger seeds generally have higher germination and vigour compared to smaller seeds (Batistella et al. 2002). Thus, physical seed traits may influence subsequent seed performance.

This study was conducted to characterize the physical attributes of lettuce seeds and to compare these parameters against percentage germination, SSAA, SVIS scores and seedling emergence under greenhouse conditions.

MATERIALS AND METHODS

Table 2. Percentage normal, abnormal, and dead seeds following a standard germination test, SSAA test after 72 hrs, and the SVIS vigour index for six lettuce varieties each containing four seed lots produced under different field conditions.

Variety	Lot	Percent germination						SVIS Vigour Index
		Standard			SSAA 72 h			
		Normal	Abnormal	Dead	Normal	Abnormal	Dead	
A	1	93	6	1	94	4	2	827
	2	91	7	2	94	4	2	808
	3	99	1	0	99	1	0	783
	4	98	1	1	86	11	3	862
	Variety	**95 a**	**4 b**	**1 c**	**93 a**	**5 c**	**2 b**	**820 c**
B	5	97	3	0	85	14	1	801
	6	94	4	2	90	6	4	727
	7	90	6	4	65	25	10	766
	8	98	2	0	98	2	0	861
	Variety	**95 a**	**4 b**	**1 c**	**84 a**	**12 b**	**4 b**	**789 c**
C	9	95	4	1	81	15	4	926
	10	96	3	1	93	1	6	935
	11	96	2	2	92	1	7	971
	12	100	0	0	90	10	0	916
	Variety	**97 a**	**2 b**	**1 c**	**89 a**	**7 bc**	**4 b**	**937 a**
D	13	81	13	6	0	11	89	605
	14	91	7	2	71	18	11	872
	15	90	8	2	65	28	7	928
	16	91	4	5	84	6	10	958
	Variety	**88 b**	**8 a**	**4 b**	**55 b**	**16 b**	**29 a**	**841 bc**
E	17	90	6	4	51	40	9	822
	18	88	8	4	73	20	7	969
	19	76	11	13	68	24	8	818
	20	78	8	14	54	25	21	796
	Variety	**83 c**	**8 a**	**9 a**	**61 b**	**27 a**	**12 b**	**851 b**
F	21	98	1	1	95	4	1	964
	22	93	4	3	86	10	4	803
	23	94	4	2	84	13	3	952
	24	94	5	1	79	18	3	942
	Variety	**95 a**	**4 b**	**1 c**	**86 a**	**11 b**	**3 b**	**915 ab**

Values in columns followed by different letters showed statistical differences, Tukey ($P \leq 0,05$).

Table 3. Percentage of emerged seedlings at the stage of expanded cotyledon and useful plants obtained under greenhouse conditions from six lettuce varieties with four seed lots obtained from different production conditions.

Variety	*Lot*	*Emerged seedlings*	**Seedlings with expanded**	**Useful plants**
A	1	93	58	99
	2	94	46	99
	3	88	44	98
	4	42	20	99
		89 ab	**47 a**	**99 ab**
B	5	83	52	98
	6	66	32	90
	7	75	43	96
	8	79	47	96
		76 c	**43 ab**	**95 c**
C	9	91	51	98
	10	91	52	96
	11	83	52	100
	12	93	59	100
		89 b	**53 a**	**99 ab**
D	13	73	1	100
	14	89	64	90
	15	90	58	100
	16	95	62	100
		87 b	**46 ab**	**98 bc**
E	17	94	43	100
	18	90	34	100
	19	85	29	100
	20	88	28	100
		89 b	**33 b**	**100 a**
F	21	98	54	100
	22	93	42	100
	23	95	46	100
	24	98	44	100
		96 a	**46 ab**	**100 a**

Values in columns followed by different letters showed statistical differences, LSD ($P < 0.05$).

Plant material

Four different seed lots each from six different lettuce varieties making a total of 24 treatments were obtained from a commercial lettuce seed company.

Determination of physical characteristics

Samples of 200 seeds each were physically characterized by software developed by Sako et al. 2001 using computer images generated by a scanner. The variables determined were area, volume, length, width, length/width ratio and colour intensity. Seeds were scanned by an inverted scanner adapted to acquire images at a resolution of 100 dpi.

Seed quality

Standard germination. Four replications of 50 seeds for each seed lot were planted on disposable Petri plates (9 cm diameter) containing two blotters (Anchor Paper Co., St. Paul, MN) moistened to saturation with double distilled water. Plates were placed in a germination chamber at 20 ±

1ºC for 7 days. Normal and abnormal seedlings were assessed for percentage germination (ISTA, 1999) and dead seeds recorded.

Saturated Salt Accelerated Aging (SSAA). Two samples of 200 seeds for each treatment were placed on a screen inside a plastic box (Jianhua and McDonald, 1996). Forty ml of a saturated NaCl solution were poured into each plastic box. Boxes were placed in a CO_2 incubation chamber maintained at 41 ± 1ºC for 48 and 72 hrs. Seeds were evaluated for germination as described previously.

Seed Vigour Imaging System (SVIS). Two rows of 25 seeds each were planted on two blue blotters that were placed in plastic boxes (15 x 23 x 4 cm, Model 600-C, Pioneer Packaging, Dixon, KY) and inserted in a germination chamber at 20 ± 1ºC at an inclination of ~125 degrees in relation to the base of the chamber in the absence of light in order that seedlings grew parallel to the blotter. Four replicates of similar characteristics were used. Seedlings were scanned three days after planting (Figure 1) and a vigour index determined with computer software developed by Sako et al. (2001). This software determines a vigour index in a scale from 0 to 1000 based on speed of growth and uniformity of lettuce hypocotyl and radicle length. Both the growth speed and uniformity were weighted at 50% each.

Seedling emergence. Four replications of 50 seeds per treatment were placed in 200 cell trays filled with the cultivation media Metromix 360 (Scott-Sierra, Marysville, OH). Trays were placed in a greenhouse maintained at ~24 ± 1ºC. Percentage emergence was determined at 8 and 15 days after planting. Emergence was defined as appearance of the cotyledons above the Metromix 360. Seedlings with expanded cotyledons were also recorded. Percentage of normal seedlings was determined 20 days after planting as useful plants (plugs).

Statistical analysis

Data were analyzed by ANOVA in a completely randomized design with four replications. Mean comparisons were conducted using LSD at an alpha level of 0.05. Pearson correlation analysis was also performed on some of the variables.

RESULTS

Seed characterization and germination percentage

The six lettuce varieties showed physical seed differences, particularly variety C, which had the largest values for width, area and volume, and varieties A and E with the smallest values for length, width and area. The combined variables, such as area and volume, did not permit an individual characterization of the varieties. Colour intensity was unique from the other physical aspects evaluated that distinguished the varieties. Within the varieties, both dark- (varieties A, B, and C) and white-coated (varieties D, E, and F) seeds were present (Table 1).

Seed volume and germination showed a non-significant correlation (data not shown). Some varieties, such as A and C, with the smallest and largest seeds, respectively, had similar germination percentages. Seed coat colour showed that dark-seeded varieties (A, B, and C) and one white-seeded variety (F) had the highest germination percentages (Table 1).

Varieties D and E showed the lowest percentage of normal seedlings after the 72 hrs SSAA test indicating they were the lowest in seed vigour (Table 2). These D seeds had early stages of a disease that apparently was promoted by the deterioration conditions (Table 2). Similar results were found among the varieties that showed the greatest germination percentages (varieties A, B, C and F) and their ability to respond to deterioration caused by the 72 hrs SSAA test (Table 2). Varieties D and E had white seed coats; variety E had small seeds (Table 1). The SSAA test increased the percentage of abnormal seedlings for all varieties except variety D (Table 2).

The SVIS index contains both growth and uniformity parameters that showed variety C and F possessed the highest seed vigour of all lettuce varieties studied and these were also associated with seed physical traits (Table 1), germination, SSAA response, and rapid and uniform seedling growth (Table 2, Figure 1). In some cases, the others varieties attained high standard germination percentages or high germination after SSAA as shown for varieties A and B, but these varieties had lower growth and uniformity of both the radicle and hypocotyl (Figure 1) and, resultantly, lower SVIS scores (Table 2).

The agronomic significance of the SVIS index was more closely related to the number of emerged seedlings, seedlings with expanded cotyledons and useful plants (Table 3). Variety F produced the most emerged seedlings. Variety B produced the fewest emerged seedlings and useful plants. Overall, both the 72 hrs SSAA test and the SVIS index were significantly correlated with the different greenhouse stages until useful plants were obtained (Table 4), but only the SSAA test was significantly correlated with germination (Table 4). This is demonstrated by the lack of significance in the correlation between vigour index, SSAA and plug stages (Table 4). Even though both tests, germination and SVIS index, subject lettuce seeds to similar temperature conditions, they demonstrate that fast and uniform emergence and seedling development are more sensitive measures of seed vigour than percentage germination alone.

During transplant production, under greenhouse conditions for 20 days, seed health issues associated with seed quality did not affect seedling emergence (Table 3), while growth rate and uniformity as determined by SVIS were affected (Table 2).

DISCUSSION

Seed characterization and germination percentage

The use of differing seed size physical parameters as discrimination criteria for seed among varieties and different species has been previously reported (Dehghan-Shoar et al. 1998; Illipronti et al. 1999; Keefe, 1999). Nerson (2002) showed that small muskmelon seeds had the lowest percentage germination, emergence, and the lowest seedling growth demonstrating that there is an association between seed physical parameters and seed quality.

Seed colour was another physical trait that differed among the lettuce varieties permitting them to be classified as white- and dark-seeded genotypes (Table 1). Similar responses have been described in watermelon (Duval and NeSmith, 2001) and for other species (Karivarhadaraaju et al. 2001).

The germination results of this study are different from those reported by Liu et al. (1993) in tomato (*Lycopersicon esculentum* L.) and by Batistella et al. 2002 in maize. The lack of relationship between seed size and germination could be explained by the more important influence of embryo size or weight; in general, larger seeds have larger embryos, which is associated with increased germination (López-Castañeda et al. 1996). Further, Mian and Nafziger (1994) showed that different seed sizes influence the water potential of winter wheat seeds and, therefore, the speed of germination. That difference can also affect the uniformity of germination and subsequent seedling development (Seiwa and Kenji, 2000) that causes a higher germination percentage in seeds with greater size, even though this may not necessarily occur with smaller seeds (Liu et al. 1993; Adkins et al. 1996; Duval and NeSmith, 2001).

Standard germination and vigour tests

The lowest germination percentages were observed in white-coated and small seeds (Table 2 and Table 4), findings consistent with those of Karivarhadaraaju et al. 2001 and Liu et al. 1993.

The SSAA test caused a decrease in percentage germination with a consequent increase in abnormal seedlings, except in those varieties (D, E) that exhibited fungal diseases. These varieties possessed white seed coats and this effect was especially apparent for variety D (Table 2).

The humidity and temperature conditions caused by the SSAA test favoured the development of the fungal inoculum present on/in the seed, and this fungal growth was not as apparent in the germination test. Seed health has been included among the factors that directly determine seed vigour (Dharam and Maheshwari, 2002) or indirectly due to the effect of toxic metabolites on germination (Asalmol et al. 2001). Seed susceptibility to pathogens is also linked to seed colour, with contradictory information between our results and those of others (Jamadar et al. 2001).

The possible effect of seed size on germination is associated with the length of the structures that form the seedling, but not necessarily with the subsequent biochemical conversion of storage reserves into

Table 4. Correlation coefficients among germination, 72 hrs SSAA test, emerged seedlings, useful plants, SVIS, and seedlings with expanded cotyledons in six varieties of lettuce.

Vigor test	Germination	SSAA 72 hrs	Emerged seedlings	Useful plants	SVIS
SSAA 72 hrs	0,406* (4,31)				
Emerged seedlings	0,123 n.s. (1,20)	0,292* (2,96)			
Useful plants	0,094 n.s. (0,92)	0,413* (4,40)	0,496* (5,54)		
SVIS	0,168 n.s. (1,65)	0,372* (3,98)	0,342* (3,53)	0,258* (2,59)	
Seedlings with expanded cotyledons	0,364* (3,79)	0,384* (4,03)	0,457* (4,98)	0,407* (4,32)	0,369* (3,85)

*: significant correlation ($P < 0.05$)
( ) Student's-t test statistic; $t_{0,975\ (94)}$ =1.98

germinating tissues (Soltani et al. 2002). Thus, differences in seed vigour among seed lots should also be found in an SSAA test that measures the degradation of the seed (Hacisalihoglu et al. 1999).

As seen in Figure 1, among the six varieties evaluated, there were differences in seedling length and uniformity. The various stages of emerged seedlings and useful plants were not correctly determined by percentage germination results, but were correctly identified by the SSAA test (Table 5 and Table 6). This result emphasizes the necessity of providing a form of stress to identify varying aspects of seed vigour (Hacisalihoglu et al. 1999).

The correlations between SSAA and the standard germination test (Table 4) do not support the findings of Adkins et al. 1996 that single vigour tests are not adequate for determining seed vigour. We observed that both high and low quality lettuce seed lots could be accurately identified by the SSAA test (Table 2).

The appearance of the seed and its germination with the stages leading to seedling growth can be correctly identified by the SSAA test. In addition, speed and uniformity of seedling growth in the greenhouse can also be identified with a SVIS index. Both vigour tests appear to be methods that allow the early prediction of lettuce seedling establishment in the greenhouse (Trawatha et al. 1990).

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