RESEARCH PAPER

Vigor Tests on Lettuce Seeds and Their Correlation With Emergence

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Abstract

S. Contreras and M. Barros. Vigor tests on lettuce seeds and their correlation with emergence. The main objective of this study was to evaluate some of the available seed vigor tests to predict lettuce (*Lactuca sativa* L.) seedling emergence. Eight lettuce seed lots were evaluated by the following laboratory vigor tests: germination (GT), accelerated ageing (AA), saturated salt accelerated aging (SSAA), conductivity (EC), and digital image analysis (IA). Also evaluated were the percentage (EP) and speed (ES) of the seed lots emergence under three conditions: seedling trays (ST) filled with a mixture of peat (80%) and perlite (20%), boxes with a clay loam soil (S-boxes), and boxes with a mixture of clay loam soil (66%) and sand (34%; SS-boxes). Correlation coefficients among the laboratory and emergence test results were calculated and significant differences were found. The correlation coefficients between EC results and each emergence parameter were not significant, while AA results were only significantly correlated with the emergence percentage for SS-boxes. GT values and emergence results were significantly correlated in all cases. SSAA results were equally or more positively correlated than GT results with the EP and, for the three sowing conditions, they were more correlated with the ES than GT values. Vigor index results from the IA were significantly correlated with both emergence parameters, and its correlation with emergence on ST (EP and ES) was greater than GT values. The results of this study showed that SSAA and IA were the best laboratory tests for lettuce seed vigor evaluation, especially for seed lots to be used for plug seedling production.

Key words: Accelerated aging, conductivity, emengence, seed vigor, vigor index.

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INTRODUCTION

The seed market has experienced important changes such as commercial globalization and an increase in economic value during the last decades (Contreras, 2002). Among the most important crops that have been commercialized for seed purpose, the demand of high value crops such us vegetable and flower crops have considerably increased (McDonald, 1998). Consequently, consumers are requesting higher quality standards and producers are trying to satisfy these demands. The germination test is widely accepted and frequently used as quality indicator of a seed lot. However, under field/greenhouse conditions the germination test has overestimated the performance of the seeds because it is performed under optimal conditions for each species. Furthermore, it is inadequate for discriminating between seed lots in regards to the speed and uniformity of seed germination (McDonald, 1980; Copeland and McDonald, 2001). In order to overcome these inconveniences, the seed vigor concept is proposed. Seed vigor was defined by AOSA (1983) as "those properties of seeds that

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determine the potential for rapid, uniform, emergence and development of normal seedlings under a wide range of field conditions".

Among the characteristics expected from a vigor test are (McDonald, 1980; Bennett, 2002): 1. To provide a more sensitive seed quality index than the germination tests, 2. To provide a consistent index for the relative classification of seed lots according to their performance, 3. Objective, fast, simple and economically feasible, 4. Repeatable and easy to interpret, and 5. Correlate with the seed performance in the field. The vigor tests that have been proposed can be grouped in three categories (Bennett, 2002): 1. Stress tests (e.g. cold test, accelerated aging). 2. Biochemical tests (e.g. electric conductivity, tetrazolium test). 3. Germination evaluation and seedling growth tests (e.g. first count of the germination test, normal seedling emergence in peat, image analysis). The use of most of these tests has been extensively evaluated in corn and soybean crops. However, reports of its use in vegetable and/or ornamental crops are scarce with inconsistent results (Fay *et al.*, 1993; Jianhua and McDonald, 1996; Panobianco and Marcos-Filho, 2001; Bennett, 2002; Sako *et al.*, 2002).

Lettuce (*Lactuca saltiva* L.) is one of the most important horticultural species worldwide. In Chile, it is the third vegetable crop with 6.103 ha planted in 1999-2000 (ODEPA, 2003). Planting can be done by direct seeding or by transplant of seedlings. Lately the specialized production of seedlings in trays has increased. For a successful production using this technology, a high percentage of emergence in a fast and uniform way is required. Therefore the seed vigor is crucial. The objective of this study was to evaluate different tests for determined the vigor in lettuce seeds and correlate the results obtained with the emergence of seedlings under different planting conditions.

MATERIALS AND METHODS

Eight lots of lettuce seeds (A to H) provided by Seeds Seminis Sudamérica (Santiago, Chile) were used. The lots corresponded to four different botanical varieties: A and B were *L. sativa* var. *capitata*, C and D were *L. sativa* var. *acephala*, E and F were *L. sativa* var. *crispa*, G and H were *L. sativa* var. *longifolia*. Each lot underwent the germination, vigor and emergence tests described below.

Germination test (GT). Two hundred seeds of each lot were arranged on pleated paper (Microtox N° 20, 12 µm), moistened previously with 45 ml of distilled water, placed in covered plastic trays. Each tray, one per lot, was placed in a germination chamber at 20 ºC with alternate cycles of light (16 h) and darkness (8 h). This procedure was repeated twice for each lot. Evaluations were made 4 and 7 days later, considering as germinated only normal seedlings that were free of necrotic spots or showed very incipient cotyledon necrosis. This criterion was more rigorous than the one established by ISTA (1999), where seedlings with cotyledons up to 50 % necrotic are considered normal. Data from the first count of this test was analyzed separately. These values were used to estimate the speed of germination of the seed and were used as seed vigor index (Copeland and McDonald, 2001).

Accelerated aging test (AA). This was performed by adapting the methodology described by Perry (1984). Seeds (0.25 g, approximately 240 seeds) of each lot were distributed on a plastic mesh support, avoiding the superposition of seeds. Each support was introduced in plastic jars (8.5 cm wide and 8 cm high) with 200 ml of distilled water, the seeds remained approximately 2 cm above the level of liquid. The jars were tightly sealed. Eight jars, one per lot, were placed in a rectangular plastic box (52 x 35 x 20 cm) with 4 L of distilled water, closed and incubated in a growth chamber at 40°C (±2 ºC) for 72 h before jars were removed and opened. A seed sample of 200 seeds per support underwent the germination test following the method described previously. This experiment was repeated twice.

Saturated salt accelerated aging test (SSAA). The procedure used was similar to the one described for the test AA, except that each jar was treated with 75 g of NaCl, in order to keep the solution saturated at the aging temperature.

Conductivity test (EC). Essentially, the method described by Perry for beans (1984), with minor modifications, was used. With these purpose four repetitions of seeds of 0.1 g (about 100 seeds) from each of the eight seed lots were immersed in 50 ml of distilled water for 24 h at 20 ºC before to separate seeds and determined the electric conductivity of the liquid phase. Furthermore, a control containing only distilled water was included for each trial. The real conductivity of each solution was calculated by subtracting the control value from the conductivity determined for each solution from seed lots.

Image analysis test (IA). The IA tests of seed samples from each of the eight lettuce seed lots were done in Ohio State University, Department of Horticulture and Crop Science, as described by Sako *et al.* (2001). The procedure consisted in the analysis of the scanned image of 50 seedlings, distributed in two rows over blue germination paper of 15 x 22 cm, taken after 3 days of germination at 20 ºC. With a computer software, the image of the hypocotyl was differentiated from that of the radicle and the seedlings, measuring the length of each structure. Based on the growth of the seedlings, the software computes a growth index and a uniformity index, both varying from 0 to 1000. Finally the program gives a vigor index which corresponded in this study to the sum of 70% of the growth index and 30% of the uniformity index. Four repetitions of 50 seeds each per seed lot were done.

Emergence tests in seedling trays (ST). Planting took place in March, 2003, in plastic trays of 128 cells (one seed per cell), using as substrate a mixture of 80% peat (Sunchine PreMix N°6) and 20% of perlite (Harbolite). Each seed lot was planted on two trays, placed under the protection of a black plastic mesh of 60% covering. At 7, 11 and 14 days after planting (DAP) the emerged seedlings (expanded cotyledons) of each tray were counted.

Emergence tests in boxes. In April of 2003, planting was done in 40 x 45 x 19 cm boxes filled with two different substrates and placed in a greenhouse. Three half filled boxes were used, with a mixture of clay soil and sand in a 2:1 ratio (SS-boxes), and three boxes with only clay soil (S-boxes). Each box was planted with 40 seeds of each lot randomly distributed 4-cm-space rows. At 7, 11 and 14 DAP the seedlings that emerged (expanded cotyledons) were counted.

With the number of seedlings that emerged during each of the emergence tests, the emergence speed (ES) was calculated, according to Hall and Wisner (1990):

$$ES = \frac{\text{PE at C 1}}{\text{N° of days at C 1}} + \frac{\text{PE at C2} - \text{PE at C1}}{\text{N° of days at C 2}} + \ldots + \frac{\text{PE at C “n”} - \text{PE at C “n-1”}}{\text{N° of days at C “n”}}$$

where: PE= emerged seedlings, and C= count.

Statistical analysis. The analysis of proportion (Mead *et at.*, 1993) was used to analyzed data obtained on the germination tests (GT, AA and SSAA tests) and emergence tests (%) that presented a binomial distribution. Confidence intervals were established (95%) and according to its comparison were determined the differences among lots in each test. Analysis of variance was used for the EC, IA and ES tests. When significant difference among lots was detected ($p<0.05$), the least significance difference test (LSD, p= 0.05) was used. To determine the relationship between the laboratory tests and emergence, the coefficient of correlation was determined (Mead *et al*., 1993).

RESULTS AND DISCUSSION

Significant ($p <0.05$) differences were obtained among seed lots for each of the tests evaluated. Based on these results, seed lots A and G were the best and seed lot D was the worst. When the results were statistically analyzed significant differences were obtained within lots of the same lettuce cultivar (Table 1).

Most of the time, seed emergence was higher than the germination obtained for the same seed lot (Table 1). This can be explained by the fact that the criterion of normality used in the GT tests in this study did not recognize as germinated those seedlings having incipient necrotic lesions in their cotyledons. This becomes obvious when considering the germination of normal seedlings plus the abnormal ones (Table 1). When comparing the emergence values of the three planting conditions; the least favorable media was the media of the S-boxes. The emergence of the ST was similar to the emergence obtained on the SS-boxes. The ES values are also shown in Table 1, for a better characterization of the vigor of seed lot and to complement the emergence value obtained. On this occasion the values of ES for the ST and the boxes can not be directly compared, because the difference in the number of seeds used. If comparing the ES between boxes, one can observe that all the lots took more than twice the time to emerge in S-boxes than in the SS-boxes, corroborating the fact that S-boxes were the worst emergence conditions. Regarding the comparison between lots, for the ST as well as the SS-boxes, the classification in general was similar to what GT originated. Concerning to S-boxes, a clear differentiation between lots was not possible, probably due to the unfavorable environment and the greater variability originated.

Table 2 shows the results of the different vigor tests. Except for EC and IA, these values were the percentage of germination, so its interpretation is relatively simple. In EC, the results represent the electric conductivity of the solution in which the seeds were covered, expressed in $\mu S \cdot cm^{-1} \cdot g^{-1}$ and were inversely related to the seed vigor. This was rather difficulty to interpret which is a disadvantage for this type of test (McDonald, 1980). The vigor index, values between 0 and 1000 (Sako *et at*., 2001), obtained from IA also had different interpretation. The higher the number the greater the growth and uniformity of the seedlings, therefore, higher seed vigor. In this sense, as opposed to the value of conductivity, it is easily interpreted by consumers and users in general and should not pose any difficulty. Regarding the classification of the different tests applied to the seed lots, overall it was similar to GT. However, there were some discrepancies that will be discussed later.

Table 1. Germination and emergence indexes of the lots under different sowing conditions (ST: speedling trays with a peat + perlit mix. SSB: boxes with clay loam soil + sand mix. and SB: boxes with clay loam soil).

Seed	Germination[1] (%)		Emergence (%)			Emergence speed		
Lot	Normal[2]	Total[2]	ST[2]	SSB[2]	SB[2]	ST[3]	SSB[3]	SB[3]
A	96 a	100 a	98 a	99 a	83 a	17.8 ab	5.5 a	12.7 a
B	78 b	89 d	88 bc	83 bc	83 a	15.7 cd	4.6 bc	11.8 a
C	66 c	95 c	80 c	88 b	74 a	14.5 d	4.8 b	11.4 a
D	13 e	82 d	66 d	71 cd	55 b	11.6 e	3.6 d	8.0 a
E	72 bc	88 d	86 bc	81 bc	73 a	15.4 cd	4.0 cd	9.3 a
F	37 d	68 e	63 d	59 d	50 b	10.0 e	2.4 e	5.8 a
G	96 a	99 ab	98 a	99 a	83 a	17.8 a	5.6 a	11.2 a
H	42 d	97 bc	92 b	78 bc	77 a	16.3 bc	4.0 cd	10.5 a

1 Normal= % of normal seedlings; total= % of normal and abnormal seedlings.

2 Values of a same column followed by the same letter did not show significant differences according to analysis of proportions with 95% of confidence.

3 Values of a same column followed by the same letter did not show significant differences according to LSD test ($p= 0.05$).

Table 2: Different vigor tests' results by which were evaluated the lettuce seeds (ST: germination, AA: accelerated ageing, SSAA: salt saturated accelerated ageing, EC: conductivity, AI: image analysis).

Lot	1st count GT[1] %	AA[1] %	SSAA[1] %	EC[2] $\mu S \cdot cm^{-1} \cdot g^{-1}$	AI[2] Vigor Index
A	96 a	46 b	88 b	10,2 cd	495 c
B	72 b	6 d	53 c	16,6 e	401 c
C	59 c	0 f	46 cd	9,3 c	446 c
D	6 e	1 ef	12 f	17,3 e	293 d
E	65 bc	17 c	27 e	8,0 b	426 c
F	30 d	5 de	8 f	8,0 b	133 e
G	95 a	71 a	98 a	4,3 a	753 a
H	30 d	4 de	40 d	11,1 d	601 b

[1] Values of a same column followed by the same letter did not show significant differences according to analysis of proportions with 95% of confidence.
[1] Values of a same column followed by the same letter did not show significant differences according to LSD test (p= 0.05).

The correlation between the laboratory tests and the parameters of emergence in the different sowing conditions is shown in Table 3. Contrary to what would have been expected, GT presented relatively high correlations in all the situations, being these at least significant ($p< 0.05$). There are two reasons to explain what was observed, the first is the criterion of normality used in this study, that by being more demanding than the ISTA norm, it would have evaluated better the vigor of the seeds. The second reason is the big difference in quality between the lots studied, favoring the correlation between what was observed in GT and the emergence of seeds. In the first count of GT the correlations were significant in all situations and similar to those of GT.

Table 3. Correlation coefficients among emergence parameters and laboratory tests (GT: germination. AA: accelerated ageing. SSAA: salt saturated accelerated ageing. EC: conductivity. AI: image analysis).

Emergence parameter[1]	GT	1st count GT	AA	SSAA	EC	AI
Emergence (%)						
Seedling tray	*0,82**	*0.80**	*0.67*	*0.87***	*-0.32*	*0.88***
SS-box	*0.85***	*0.86**	*0.73**	*0.93***	*-0.31*	*0.81**
S-box	*0.83**	*0.81**	*0.52*	*0.84***	*-0.17*	*0.81**
Emergence speed						
Seedling tray	0.82*	0.80*	0.66	0.88**	-0.28	0.89**
SS-box	0.82*	0.82*	0.70	0.93**	-0.22	0.81*
S-box	0.75*	0.74*	0.42	0.82*	-0.00	0.72*

[1] SS: clay loam soil + sand; S: clay loam soil.
*. ** Significant with $p< 0.05$ y $p< 0.01$. respectively.

As expected, the results of germination after the AA and SSAA tests (Table 2) presented a marked decrease, being more noticeable in AA. Here, one could also see the development of fungi, mainly of the genus *Aspergillus* and *Penicillium*, on the seeds of most lots, confirming what other authors observed regarding aging under conditions of saturation of humidity, would cause such a severe deterioration in small seeds that it would be difficult to classify and evaluate them later

(Jianhua and McDonald, 1996; Wang *et al*., 1996). In addition to the inconvenient in applying the AA test, the correlation between its results and emergence was relatively low in all situations and was only significant for emergence in SS-boxes (Table 3). Thus, AA proved to be a poor alternative for vigor evaluating in lettuce seeds. In the SSAA test, the addition of NaCl would enable approximately 76 % of humidity conservation around the seeds controlling its rate of water absorption (Jianhua and McDonald, 1996). This change in the aging conditions, aside from preventing fungi formation would allow a more homogeneous and controlled deterioration of the seeds, improving the precision of this test when used to evaluate small seeds (Jianhua and McDonald, 1996; Panobianco and Marcos-Filho, 2001; Bennett, 2002). In fact, one can see a greater sensitivity in the classification of seed lots with SSAA, showing differences between lots that were not detected with the other tests (Table 2). As opposed to what was observed for the AA test, SSAA presented similar or higher correlations to GT in all situations; highly significant ($p<0.01$) except in ES in the S-boxes, where it was significant. The advantages observed in the AASS test in this study, agree with other authors in small seeds (Jianhua and McDonald, 1996; Bennett, 2002). This test offers an interesting alternative to evaluate the vigor of lettuce seeds.

The classification of seed lots in the EC test presented some differences with the other tests; for instance, the quality of lot A was lower than lot F and there were not difference between lots E and F (Table 2). Moreover, the correlation between the results of EC and seed emergence was always not significant (Table 3). Similar lack of specificity was reported by Panobianco and Marcos-Filho (2001) for tomato. These authors considered that the problem with the use of this test is that the compounds leaked from the seeds are unknown. Another difficulty of the EC test is the presence or absence of fungicides on the surface of the seeds (Zhang and Hampton, 1999) which can result in variations in the results, causing readings of high conductivity and making lots seem weak when they really are not. In this study, only lots A, B, C, G and H were treated with thiram, this could explain for example that lot A were classified as of low vigor by EC (Table 2); yet , this does not account for the lack of differences between the lots E and F (both untreated and with very different characteristics of germination and emergence). Hence given the results of this study, the EC does not seem a feasible alternative to evaluate the vigor of lettuce seeds.

In general, the classification of the seed lots with the test of IA resulted similar to that of GT and the tests of aging, but it had differences regarding to lot H classification (Table 2). In this case, H is evaluated as one of the best lots, getting closer to the results of emergence. The explanation of the abovementioned is that this test does not consider the abnormality of the seedlings due to necrosis of the cotyledons. This would represent an inconvenient just if this kind of abnormality alters the behavior of the seedlings in the initial states of emergence. This could represent a problem of certain lots of seeds that is not being considered in the IA. The problem refers only to this type of abnormality; because deficient growth of the radicula and /or the hypocotylous would be considered by the test (Sako *et al.*,2001). Regarding the correlations between the vigor index of IA and the emergence of the seeds (Table 3), these were in general high and always significant. Of special interest are the correlations with emergence (% and ES) in the seedling trays, which were highly significant, greater than those of GT and as good as the ones of SSAA. Given the growing importance of this way of producing seedlings, the use of IA is a promising alternative for lettuce seed vigor evaluation. In addition, this test considered the use of only 200 seeds (versus 400 of the GT and SSAA), and has the advantage of being faster (3 versus 7 and 10 days of the GT and SSAA, respectively) and objective (the seedlings are evaluated by a computer software).

Based on the results of this study, the tests of SSAA and IA are interesting alternatives for lettuce seed vigor evaluation, especially for plug seedling production. However, it is necessary to specify the effect of cotyledonal necrosis on the subsequent development of the lettuce seedlings. It is possible that there also exists a correlation between the results of these tests and the emergence of other horticultural and ornamental species of economic importance, an aspect that requires verification.

RESUMEN

El objetivo de este estudio fue evaluar algunas de las pruebas de vigor disponibles en la predicción de emergencia en plántulas de lechuga (*Lactuca sativa* L.). Ocho lotes de semillas fueron evaluados por las siguientes pruebas de laboratorio: germinación (PG), envejecimiento acelerado (EA), EA con saturación de sales (EASS), conductividad eléctrica (CE) y análisis de imagen (AI). También se evaluó el porcentaje (PE) y velocidad (VE) de emergencia de los lotes en tres condiciones: bandejas speedling (BS) con una mezcla de turba (80%) y perlita (20%), cajones con suelo franco arcilloso (CS) y cajones con una mezcla de suelo franco arcilloso (66%) más arena (34%, CSA). Se establecieron las correlaciones entre los resultados de estas pruebas y las de laboratorio. La correlación entre los resultados de CE y los parámetros de emergencia no fue significativa en ninguna de las condiciones, mientras que EA solo lo fue con el PE de CSA. La correlación de la PG fue significativa en todos los casos. Los resultados de la EASS estuvieron tanto o más correlacionados que los de PG con PE y, para las tres condiciones, se correlacionaron mejor con la VE que los de PG. Resultados del AI se correlacionaron en forma significativa con PE y VE, presentando mejor correlación con la emergencia en BS que la PG. De acuerdo con los resultados, EASS y AI fueron las mejores pruebas de laboratorio para evaluación de vigor en semillas de lechuga, especialmente cuando la semilla es usada para producción de plántulas en bandeja.

Palabras Clave: Conductividad, emengencia, envejecimiento acelerado, vigor en semillas.

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