

## ALTERNATIVE METHODOLOGIES TO TEST SEED VIGOR IN LETTUCE

Aline Klug Radke<sup>1</sup>, Bruna Barreto dos Reis<sup>2</sup>, Andreia da Silva Almeida<sup>3</sup>, Géri Eduardo Meneghello<sup>4</sup>, Lilian Madruga de Tunes<sup>5</sup>, Francisco Amaral Villela<sup>6</sup>

1 Doutoranda no Programa de Pós Graduação em Ciência e Tecnologia de Sementes da Universidade Federal de Pelotas, Bolsista CNPq-Brasil (alinekradke@hotmail.com) Pelotas-Brasil

2 Mestranda no Programa de Pós Graduação em Ciência e Tecnologia de Sementes da Universidade Federal de Pelotas

3 Doutora, bolsista PNPd – CAPES Programa de Pós Graduação em Ciência e Tecnologia de Sementes da Universidade Federal de Pelotas

4 Engenheiro agrônomo, Doutor, Programa de Pós Graduação em Ciência e Tecnologia de Sementes da Universidade Federal de Pelotas

5 Professora, Doutora, Programa de Pós Graduação em Ciência e Tecnologia de Sementes da Universidade Federal de Pelotas

6 Professor, Doutor, Programa de Pós Graduação em Ciência e Tecnologia de Sementes da Universidade Federal de Pelotas

Recebido em: 30/09/2014 – Aprovado em: 15/11/2014 – Publicado em: 01/12/2014

### ABSTRACT

Lettuce is one of the most popularly consumed vegetables in the world and in Brazil is produced year round, in various regions that differ markedly in their climate characteristics. This work aimed to adapt the test of accelerated aging to assess the physiological potential of lettuce seeds. Five seed lots were sampled and the seeds subjected to the germination test, emergence speed index, seedling emergence and accelerated aging. For the latter test four periods were assessed, i.e. 24, 48, 72 and 96 hours, applying the standard methodology that uses water and saturated and unsaturated sodium chloride (NaCl) solutions. The combination of 41°C and 24 hours exposure proved the most efficient to rank seed lots according to their vigor. Also, the exposure time of 72 hours, whether for the conventional accelerated aging test or the alternative unsaturated salt solution, proved to be efficient.

**KEYWORDS:** Accelerated aging; *Lactuca sativa* L.; Physiological potential; Salt solution.

## ALTERNATIVAS METODOLÓGICAS NOS TESTES DE VIGOR EM SEMENTES DE ALFACE

### RESUMO

A alface, certamente é uma das hortaliças mais populares e consumidas no Brasil e em grande parte do mundo, sendo cultivada durante o ano todo, em diferentes regiões do país abrangendo diferenças climáticas. No presente trabalho objetivou-se adequar a metodologia do teste de envelhecimento acelerado para avaliação do potencial fisiológico de sementes de alface. Foram utilizados cinco lotes de sementes e submetidos aos testes de germinação, índice de velocidade de emergência, emergência de plântulas e envelhecimento acelerado, empregando-se

os períodos de envelhecimento de 24, 48, 72 e 96 horas, na metodologia tradicional com água e com o uso de soluções: saturada e não saturada de cloreto de sódio (NaCl). O teste de envelhecimento acelerado a 41°C por 24 horas, nos métodos analisados, é o mais eficiente para a estratificação dos lotes em diferentes níveis de vigor. O período de 72 horas, tanto pelo método de envelhecimento acelerado tradicional como no de solução salina não saturada foi eficiente.

**PALAVRAS-CHAVE:** Envelhecimento acelerado; *Lactuca sativa* L.; Potencial fisiológico; Solução salina.

## INTRODUCTION

Lettuce (*Lactuca sativa* L.) is an annual, native temperate plant belonging to the Asteraceae and certainly one of the most popular and commonly consumed vegetables in Brazil and worldwide (HENZ & SUINAGA, 2009). In Brazil lettuce can be grown year round and in various different regions of the country, covering climatic differences and consumption habits (NASCIMENTO, 2002).

Seed vigor evaluation is an essential part of any program of seed quality control, providing helpful information to detect and solve problems at different levels of the production process, as well as on seed performance (COSTA et al. 2008). The germination test, however, standardized and universally accepted as the official procedure, is conducted in the laboratory under highly favorable conditions for the crop, generally overestimating the physiological potential of seed lots. However, when conditions deviate from the ideal, in the field or after a period of storage, lots with similar germination percentage may show different performances, due to differences in vigor levels (LIMA & MARCOS FILHO, 2011). It is therefore increasingly necessary to improve testing capacity for the assessment of seed vigor, especially with regard to consistency of results and if possible, reducing the time required for the test (PEREIRA et al., 2011).

Among the tests used to assess seed vigor, accelerated aging is one of the most studied and recommended for several agricultural crops. This test is based on the enhancement of the rate of seed deterioration by exposing them to high levels of temperature and moisture, respectively. Both, temperature and moisture are considered the most relevant environmental factors controlling the rate of seed deterioration (MARCOS FILHO, 1999).

There are several gaps in the assessment of the physiological potential of seeds from vegetable crops, including lettuce, and the accelerated aging test has been acknowledged for its precision in assessing seed vigor from various cultivated species, being able to provide information with a high degree of consistency (TUNES et al. 2013). However, there are factors which can affect the results, so it is necessary to adjust the methodology to obtain consistency (TORRES et al., 2009). Therefore, alternatives to driving testede accelerated aging have been studied, since according to the solution used specific humidities can be obtained, leading to a decrease in the intensity and rate of water uptake by seeds, culminating in a lesser degree of deterioration and less variation the results (JIANHUA & McDONALD, 1996; TUNES et al., 2012).

The present work aimed to adapt the principles of the accelerated aging test to assess the physiological quality of lettuce seed.

## **MATERIALS AND METHODS**

Five lots of lettuce seeds from cultivar Regina were sampled to determine their levels of quality through the following tests:

**Moisture content:** Two sub-samples for each seed lot were oven dried at  $105^{\circ}\text{C} \pm 3^{\circ}\text{C}$  for 24 hours, in compliance to the Brazilian rules for Seed Testing – RAS (BRASIL, 2009).

### **Germination**

Conducted with four sub-samples of 50 seeds per lot, laid evenly over two foils of blotting paper in plastic boxes of the gerbox type. The paper foils were moistened with distilled water at a volume equivalent to 2.5 times their dry weight. The germination boxes were put in a germination chamber at  $25^{\circ}\text{C}$  and the seedling count was performed at the fourteenth date, expressing results as mean percentage of normal seedlings per lot (BRASIL, 2009).

### **Greenhouse emergence**

Four 50-seed sub samples were distributed in polystyrene trays with individual cells filled with commercial substrate (Plantmax®). Emergence counts were recorded daily, up to the fourteenth day after sowing on seedling tips  $\geq 1$  cm, yielding results expressed as percentage of emerged seedlings (NAKAGAWA, 1999).

### **Greenhouse speed of emergence index**

This test was undertaken parallel to the greenhouse germination test, comprising the daily count of emerged seedlings up to the fourteenth day after sowing. For each subsample the SGI was calculated as the number of emerged seedlings per day divided by the respective number of days passed since sowing (MAGUIRE, 1962).

After the determination of the initial seed quality, lots underwent conventional and modified aging tests, as described below:

### **Accelerated aging (conventional)**

Conducted with the use of gerbox plastic containers with individual compartments (mini chambers) filled with 40mL of water and fitted with an aluminum screen, onto which seeds were spread evenly to form a uniform layer.

### **Accelerated aging (unsaturated salt solution) – SSNS**

Performed similarly to the conventional accelerated aging test, but substituting water by 11 mL of NaCl solution (11g NaCl in 100 mL of water), thus creating an environment with 94% moisture (ÁVILA et al., 2006)

### **Accelerated aging (saturated salt solution) - SSS**

Similar to the SSNS method it differs on the solution concentration, which is now of 40 mL NaCl (40 g NaCl in 100 mL water), creating an environment of 76% moisture, as adapted from the method described by JIANHUA & MCDONALD (1996).

For all methods of accelerated aging periods of 24, 48, 72 and 96 hours were tested, keeping the boxes in a BOD chamber at  $41^{\circ}\text{C}$ . After each aging period four replicates of 50 seeds per lot were subjected to the germination test, following the methodology described previously for the latter.

Parallel to the accelerated aging testing, seed moisture content was determined for each of the four aging periods, to check for the uniformity of the test environment, as recommended by MARCOS FILHO (1999).

Data recorded as percentage were transformed to  $\arcsin \sqrt{x/100}$  prior to statistical analysis. The experimental design was completely randomized and treatment means were compared by the Tukey test at 5% probability; correlation coefficients were compared by the method of Pearson at the 1% and 5% probability levels.

## RESULTS AND DISCUSSION

The results for moisture content, germination, speed of seedling emergence index and seedling emergence in greenhouse show differences on the initial seed quality of lettuce seed lots (Table 1). Seed moisture ranged from 6.05% to 6.14% remaining below the tolerated levels (MARCOS FILHO, 1999), which guaranteed the accuracy of the test results as the initial moisture levels were considered uniform for all samples.

The germination test showed no differences among lettuce seed lots and seed moisture averaged 6.11% across lots, the largest difference being of 0.16 percentage points. These results certify the reliability of the vigor tests, since samples that show large differences in moisture levels may alter the accuracy of results, whereas uniform levels favor the consistency of results under standard procedures (VIEIRA & KRYZANOWSKI, 1999). Seedling emergence and speed of emergence index tests yielded differences on the levels of vigor among lots, which could not be detected by the germination test. This was an important factor justifying the execution of vigor tests parallel to those of germination.

Results for seedling emergence and emergence speed index (Table 1) showed that seed lots 4 and 5 yielded vigor levels beyond 75%, which resulted in better stands.

**TABLE 1.** Moisture content (MC), germination (G), seedling emergence (SE) and emergence speed rate (ESR) from lettuce seed lots. UFPel, Capão do Leão, RS. 2014.

LOTS	MC	G (%)	SE (%)	ESR
1	6,14	90a	51c	8,5b
2	6,05	89a	54c	7,1c
3	6,11	91a	65b	7,8c
4	6,21	88a	75a	11,1a
5	6,05	90a	78a	11,6a
CV (%)		7,28	5,19	6,29

\*Means within columns followed by the same letter do not differ by the Tukey test at the 5% level.

Table 2 shows results for the average initial moisture content of seeds and after accelerated aging testing, with and without the salt solution. Aged lettuce seeds showed increase on their moisture contents relative to starting levels with increasing length of the tested period, irrespective of the method used (Table 2).

The moisture content of seeds exposed to saturated salt solution was lower and more uniform. Salt solutions reduced the moisture uptake rate of lettuce seeds during the accelerated aging tests compared to the conventional method; similar results were observed by COSTA et al. (2008) in seeds of cabbage, cauliflower, and

broccoli, by TORRES & BEZERRA NETO (2009) in seeds of annatto, by ALVES et al. (2012) in seeds of eggplant and by TUNES et al. (2013) in parsley seeds.

According to TORRES & MARCOS FILHO (2001), saturated salt solutions promote a reduction on the rate of water uptake by small seeds during the period of aging. This allows hypothesizing that conditions for accelerated aging with salt solutions promote less drastic effects since lower moisture levels are reached, implying that the degree of seed deterioration would be attenuated relative to that of seeds tested under the conventional method. TORRES et al. (2009) also states that the use of saline contributed to slow the absorption of water by the seed during aging thereof. Another advantage for saturated salt solutions is that they promote lower moisture values, generally inadequate to allow for fungi development during the test. Similar observations were reported by AVILA et al. (2006) in radish seeds, by PEREIRA et al., (2011) in coriander seeds, ALVES et al. (2012) in seeds of eggplant.

**TABLE 2.** Moisture levels of lettuce seeds after four exposure periods to the conventional accelerated aging (CAA), unsaturated salt solution (USS) and saturated salt solution (SSS) tests. UFPel. Capão do Leão, RS. 2014.

Lots	CAA				USS				SSS			
	Time (hours)				Time (hours)				Time (hours)			
	24	48	72	96	24	48	72	96	24	48	72	96
1	10,31	15,03	27,33	44,03	8,11	12,31	14,13	19,51	10,91	13,04	14,21	18,31
2	9,15	16,29	26,91	45,01	8,16	13,51	13,11	18,06	10,14	13,08	12,79	17,46
3	7,89	15,22	27,09	43,80	7,93	12,03	12,68	18,05	9,33	12,27	12,60	18,10
4	7,91	13,44	24,33	33,13	6,83	9,21	11,21	14,42	7,14	10,13	11,01	13,34
5	7,12	14,12	24,13	37,31	6,95	10,07	10,96	13,26	7,78	10,35	10,15	12,25

All the accelerated aging tests identified seed lots 4 and 5 as the ones with the highest vigor levels (Table 3), and seed lots 1, 2 and 3 with the lowest vigor. We found agreement between both greenhouse tests, i.e. seedling emergence speed index and seedling emergence (Table 1).

A negative relationship between exposure time to accelerated aging and subsequent germination was observed, coinciding with findings in peas by NASCIMENTO et al. (2007). A drastic fall on germination levels was observed for the conventional accelerated aging test, when comparing the longest period (96 hours) with the 24-hour exposure treatment (Table 3). Germination percentages for the conventional accelerated aging test yielded extremely low values of normal seedlings, thus showing that the procedure of exposing seeds for 96 hours is inadequate for lettuce seeds. Similar results were reported by LIMA & MARCOS FILHO (2011) that in cucumber seeds, TUNES et al. (2011) for coriander seeds and TUNES et al. (2013) for parsley seeds. This effect probably is due to the high level of moisture achieved by seeds after aging. This finding makes it relevant to adjust the salt concentration to the moisture level reached by the seeds during the test, so that the stress levels make it possible to rank seed lots without drastically reducing the germination percentage after completing the accelerated aging process (ÁVILA et al., 2006).

**TABLE 3.** Germination percentages of lettuce seed lots after four periods of exposure to the conventional accelerated aging (CAA), unsaturated salt solution (USS) and saturated salt solution (SSS) tests. UFPel. Capão do Leão, RS. 2014.

Lots	CAA				USS				SSS			
	Time (hours)				Time (hours)				Time (hours)			
	24	48	72	96	24	48	72	96	24	48	72	96
1	42c	27c	22c	07b	58c	63b	45c	32b	58c	59b	49c	44b
2	40c	38b	28c	16b	51c	67b	40c	41b	55c	68b	56b	41b
3	61b	40b	33b	10b	70b	63b	57b	39b	73b	65b	59b	56a
4	74a	57a	44a	28a	80a	75a	65a	50a	81a	77a	66a	56a
5	77a	49a	46a	25a	82a	81a	68a	66a	80a	79a	70a	64a
CV (%)	11,13				7,66				10,95			

\*Means within columns followed by the same letter do not differ by the Tukey test at the 5% level.

For the three accelerated aging tests (Table 3) it can be observed that the period of 24 hours was more efficient to rank the lots, as shown by the seedling emergence test, while the period of 72 hours resulted adequate for the conventional procedure as well as for the unsaturated salt solution. Work developed by TORRES (2005) found that both the conventional procedure and the saturated salt solution, with exposure time of 72 hours at 41°C, were sensitive enough to evaluate the physiological potential of seeds.

Our results indicate that there are tests available capable of efficiently ranking vigor in lettuce seed lots. The exposure period of 24 hours in the accelerated aging tests ranked seeds under different vigor levels, ranking seed lots 1 and 2 as those with the lowest vigor, lot 3 with medium vigor and seed lots 4 and 5 as those with the best seed quality.

### CONCLUSION

Of all the methods tested, accelerated aging with an exposure period of 24 hours at 41°C is the most efficient to rank lettuce seed lots according to their vigor levels. The 72-hour period for both the conventional accelerated aging method and the unsaturated salt solution, also showed efficiency.

### REFERENCES

ALVES, C. Z.; GODOY, A. R.; CANDIDO, A. C.S.; OLIVEIRA, N. C. Qualidade fisiológica de sementes de jiló pelo teste de envelhecimento acelerado. **Ciência Rural**, v.42, n.1, p. 58-63, 2012.

ÁVILA, P.V.; VILLELA, F.A.; ÁVILA, M.S.V. Teste de envelhecimento acelerado para avaliação do potencial fisiológico de sementes de rabanete. **Revista Brasileira de Sementes**, v. 28, n. 3, p. 52-58, 2006.

BRASIL. Ministério da Agricultura e da Reforma Agrária. **Regras para análise de sementes**. Brasília: SNDA/DNPV/CLAV, 395p. 2009.

COSTA, C.J; TRZECIAK, M.B; VILLELA, F.A. Potencial fisiológico de sementes de brássicas com ênfase no teste de envelhecimento acelerado. **Horticultura Brasileira**, v. 26, n. 2, p. 144-148, 2008.

HENZ, G. P.; SUINAGA, F. **Tipos de Alface Cultivados no Brasil**. Brasília, DF: Embrapa Hortaliças. Comunicado Técnico, 75, 2009.

JIANHUA, Z.; McDONALD, M.B. The saturated salt accelerated aging test for small seeds crops. **Seed Science and Technology**, v. 25, n. 1, p. 123-131, 1996.

LIMA, L.B.; MARCOS-FILHO, J. Procedimentos para condução de testes de vigor baseados na tolerância ao estresse térmico em sementes de pepino. **Revista Brasileira de Sementes** [online], v. 33, n. 1, p. 45-53. 2011. ISSN 0101-3122. Disponível em: <<http://dx.doi.org/10.1590/S0101-31222011000100005>>. Acesso em: 20 de mai. 2014.

MAGUIRE, J.D. Speed of germination – aid in selection and evaluation for seedling and vigour. **Crop Science**, v. 2, n. 1, p. 176-177, 1962.

MARCOS FILHO J. Teste de envelhecimento acelerado. In: KRZYZANOWSKI, F.C; VIEIRA, R.D; FRANÇA NETO, J.B (eds). **Vigor de sementes: conceitos e testes**. Londrina: ABRATES. p.3.1-3.24. 1999.

NAKAGAWA, J. Testes de vigor baseados no desempenho das plântulas. In: KRZYZANOWSKI, F.; VIEIRA, R.D.; FRANÇA-NETO, J.B. (Ed.). **Vigor de sementes: conceitos e testes**. Londrina: ABRATES, . cap.2, p.2-24. 1999.

NASCIMENTO, W.M.; FREITAS, R.A.; GOMES, E.M.L.G.; SOARES, A.S. Metodologia para o teste de envelhecimento acelerado em sementes de ervilha. **Horticultura Brasileira**, v. 25, n. 2, p. 205-209, 2007.

PEREIRA, M.F.S; TORRES, S.B.; LINHARES, P.C.F.; PAIVA, A.C.C.; PAZ, A.E.S.; DANTAS, A.H. Qualidade fisiológica de sementes de coentro [*Coriandrum sativum* (L.)]. **Revista Brasileira Plantas Medicinais**, v.13, especial, p.518-522, 2011.

TORRES, S.B; BEZERRA NETO, F. Teste de envelhecimento acelerado para avaliação do potencial fisiológico de sementes de urucum. **Horticultura Brasileira**, v.27, p.055-058, 2009.

TORRES, S.B; OLIVEIRA, F.N; OLIVEIRA, A.K; BENEDITO, C.P; MARINHO, J.C. Envelhecimento acelerado para avaliação do potencial fisiológico de sementes de melão. **Horticultura Brasileira**, v. 27, p. 070-075, 2009.

TORRES, S.B. Envelhecimento acelerado em sementes de pepino com e sem solução salina saturada. **Horticultura Brasileira**, v. 23, n. 2, p. 303-306, 2005.

TORRES, S.B.; MARCOS-FILHO, J. Teste de envelhecimento acelerado em sementes de maxixe (*Cucumis anguria* L.). **Revista Brasileira de Sementes**, v. 23, n. 2, p. 108-112, 2001.

TUNES, L.M; PEDROSO, D.C; GADOTTI, G.I; MUNIZ, M.F.B; BARROS, A.C.S.A; VILLELA FA. Accelerated aging to assess parsley seed vigor. **Horticultura Brasileira**, v. 31, p. 457-460, 2013.

TUNES, L. M.; TAVARES, L. C.; RUFINO, C. D. A.; BARROS, A. C. S. A., MUNIZ, M. F. B., DUARTE, V. B. Accelerated aging of broccoli seeds (*Brassica oleracea* L. var. italica Plenck). **Bioscience Journal**, v. 28, n. 2, 2012.

TUNES, L.M; PEDROSO, D.C; BARBIERI, A.P.P.; CONCEIÇÃO, G.M.; ROETHING, E; MUNIZ, M.F.B.; BARROS, A.C.S.A. Envelhecimento acelerado modificado para sementes de coentro (*Coriandrum sativum* L.) e sua correlação com outros testes de vigor. **Revista Brasileira Biociência**, v. 9, n. 1, p. 12-17, 2011.

VIEIRA, R.D.; KRZYZANOWSKI, F.C. Teste de condutividade elétrica. In: KRZYZANOWSKI, F.C.; VIEIRA, R.D.; FRANÇA NETO, J.B. (Ed.). **Vigor de sementes: conceitos e testes**. Londrina: ABRATES, cap. 4, p.1-26, 1999.