Revista Agrarian
ISSN: 1984-2538

Priming protocols on *Lactuca sativa* seeds evaluated by image analysis

Protocolos de condicionamento fisiológico de sementes avaliados por meio de análise de imagens

Vitor Henrique Vaz Mondo[1], Francisco Guilhien Gomes Junior[2], Silvio Moure Cicero[2], Mark A. Bennett[3], Miller B. McDonald[3]

[1]University of São Paulo (USP/ESALQ), Department of Crop Science, P.O. Box 09, 13418-900, Piracicaba/SP, Brazil; currently at Embrapa Rice and Beans, GO-462, Km 12, Zona Rural, 75375-000 Santo Antônio de Goiás, GO, Brazil. E-mail: vitor.mondo@embrapa.br
[2] University of São Paulo (USP/ESALQ), Department of Crop Science, Piracicaba, SP, Brazil.
[3]The Ohio State University (OSU), Department of Horticulture and Crop Science, Columbus, USA.

Recebido em: 19/09/2012 Aceito em: 07/05/2013

Abstract. Seed priming treatment is seen as a viable and common technology to reduce time between seed sowing and seedling emergence. Normally the priming evaluation is conducted by germination assays, however for vigor analysis of seed lots, more precise, quick and efficient tests are desirable and it could be reach using the image analysis technique. This work aimed to analyze the efficiency of the Seed Vigor Image System (SVIS) evaluating priming protocols for lettuce seeds. Using two varieties, and two lots each, the priming treatments were conduct with PEG 8000 osmotic solution on four different duration, zero, 12, 24 and 36. For physiological potential evaluation, germination, seedling emergence and SVIS tests were conducted. The results obtained in this study have confirmed that *SVIS* is a practical and valuable approach to evaluate the efficacy of priming treatments in *Lactuca sativa* seed lots considered commercially valuable.

Keywords. Lettuce seeds, osmoconditioning, physiological potential, seed quality

Resumo. O condicionamento fisiológico de sementes é visto como uma tecnologia comum e viável para a redução do período entre a semeadura e a emergência das plântulas. Normalmente a avaliação dessa tecnologia é conduzida por testes de germinação, no entanto, para análise de vigor de lotes de sementes, testes mais precisos, rápidos e eficientes são desejáveis e isso pode ser alcançado com aplicação da técnica de análise de imagens. Dessa forma, objetivou-se com o presente trabalho a análise da eficiência do sistema 'Seed Vigor Image System' (SVIS) na avaliação de protocolos para condicionamento fisiológico de sementes de alface. Usando-se dois cultivares de alface e dois lotes de sementes para cada cultivar, os protocolos de condicionamento fisiológico foram conduzidos com solução osmótica de PEG 8000 em quatro diferentes durações, zero, 12, 24 e 36 horas. Para avaliação do potencial fisiológico das sementes, os testes de germinação, emergência de plântulas e SVIS foram conduzidos. Os resultados obtidos nesse estudo confirmaram que o SVIS é uma ferramenta valiosa para a avaliação da eficiência de protocolos de condicionamento fisiológico em lotes de sementes de *Lactuca sativa* com valor comercial.

Palavras-chave. Sementes de alface, condicionamento osmótico, potencial fisiológico, qualidade de sementes

Introduction

Seed priming is defined as a pre-sowing treatment in osmotic solution that allows seeds to imbibe water to proceed to the first stage of germination but prevents radicle protrusion through the seed coat (Heydecker et al., 1973); since most seed embryos are desiccation tolerant up to this developmental stage, the seed advancement in germination process during priming can be arrested by drying (Capron et al., 2000).

Seed priming treatment is seen as a viable and common technology to reduce time between seed sowing and seedling emergence (Capron et al., 2000) resulting in rapid and uniform emergence, high vigor, and better yields mostly in vegetable and flower species (Capron et al., 2000; Fessel et al., 2001) and field crops (Foti et al., 2007) that help justify the additional cost of these treatments (Warren & Bennett, 1997).

Lettuce seeds are commercially primed to prevent the induction of thermo- or photo-inhibition during the germination process and a secondary advantage is a faster rate of radicle emergence across temperatures when seeds are planted, resulting in better production qualities such as less variation in plant size and greater number of cartons per acre at harvest packout (Hill et al., 2008).

Priming evaluation is normally conducted by germination assays (Cabron et al., 2000); however for vigor analysis of seed lots, more precise, quick and efficient tests are desirable. To satisfy these conditions, researchers have used the image analysis technique. As based on the recently available technologies and physiological discoveries, the evaluation methods are constantly improving (Matthews et al., 2012; Koddel et al., 2012), however the variety of models can result in different interpretations of seed vigor.

In this context, Penãloza et al. (2005) and Kikuti and Marcos-Filho (2012) working with lettuce seeds, Mondo et al. (2011) with corn seeds, Silva et al. (2012) with hump seeds, and Chiquito et al. (2012) with cucumber seeds found satisfactory results regarding the use of the Seed Vigor Image System (SVIS) developed by the Ohio State University for seed vigor evaluation, but research works relating the efficiency of seed priming and SVIS are very scarce. Gomes-Júnior et al. (2009) working with this theme found interesting results of the SVIS application to evaluate priming on sweet corn seeds.

The Seed Vigor Image System (SVIS) has been proposed as an alternative to traditional vigor tests (Sako et al., 2001). The system captures images of the germination test conducted at three days at 20ºC in a near vertical orientation, evaluates the seedling's length and estimates an overall vigor (Sako et al., 2001), growth and uniformity index. This vigor test provides a rapid and objective measurement of lettuce seed quality (Penaloza et al., 2005) and does not involve human error in count evaluation, estimates and measurements (Sako et al., 2001; Mondo et al., 2011).

The main purpose of this work was to analyze the efficiency of SVIS to evaluate priming protocols on lettuce seeds.

Material and Methods

Plant material

Seeds from two different lettuce varieties, *'Javelina'* and *'Sharp Shooter'*, two lots each, obtained from a Seminis Inc., located in Oxnard, CA, United States of America, were studied. The lots from each variety were classified as the same size (average seed count between 1,132.20 and 1,223.66 seeds g^{-1} for *'Javelina'*, and between 1,155.75 to 1,198.71 seeds g^{-1} for *'Sharp Shooter'*).

Seed moisture content

Determined by oven drying two seed samples of 2 g from each seed lot at 105°C ± 3°C for 24 hours. Thereafter, the samples were weighted, and the seed moisture was expressed as percentage (Brasil, 2009).

Seed priming treatment

Each seed lot was divided into four portions that constitute a control (zero hours) and three treatments with different priming durations (12, 24 and 36). Priming was conducted with PEG 8000 at 345.33g L^{-1} concentration at an osmotic potential of -1.50 MPa (Villela & Beckert, 2001). Seeds were distributed on two layers of moistened blue blotter paper (Anchor Paper Co., St. Paul, MN) in transparent plastic Petri dishes (8.0 cm diameter) at 2.5 times the paper dry mass on the PEG 8000 solution, and placed in a germination chamber at 20±1ºC with alternate cycles of light (16h) and darkness (8h) for different priming durations. After the treatment, the seeds were immersed in distilled water and superficially dried on paper towels. For the drying process they were placed on a screen inside a transparent plastic box (11 x 11 x 3 cm) with 40 mL of a saturated NaCl solution resulting in 76% of relative humidity (Jianhua & McDonald, 1997), for more 48 hours.

Seed quality evaluation

Standard germination test (G): was used per lot three replicates of 50 seeds, , arranged on two blotter papers moistened with distillated water at two times the weight of the blotters and placed in covered plastic boxes (15 x 23 x 4 cm, Model 600-C, Pioneer Packaging, Dixon, KY). The boxes were placed in a germination chamber at 20±1ºC with alternate cycles of light (16 h) and darkness (8 h). At seven days after the imbibition, the normal seedlings were counted (Contreras & Barros, 2005).

Saturated salt accelerated aging (SSAA): this test was applied only for seed lots characterization and was conducted with 150 seeds for each treatment which were placed on a screen inside a transparent plastic box (11 x 11 x 3 cm) and 40 mL of a saturated NaCl solution were poured into each plastic box (Jianhua & McDonald, 1997). Boxes were placed in incubation chamber maintained at 41±1ºC for 72 hours (Peñaloza et al., 2005). Seeds were evaluated by germination tests as described previously.

Emergence tests in seedling trays: 50 seeds in three replicates were sown in plastic trays of 200 cells (one seed per cell), using as substrate a mixture of 80% peat (Sunchine PreMix N°6) and 20% of perlite (Harbolite) and irrigated. Each tray was placed under the protection of a black plastic mesh of 60% covering. At seven (SE7D) and fourteen (SE14D) days after planting (DAP) the emerged seedlings (expanded cotyledons) of each tray were counted.

Seed vigor image system (SVIS): the SVIS test was conducted at Ohio State University, Department of Horticulture and Crop Science, as described by Sako et al. (2001) and applied by Contreras & Barros (2005) and Peñaloza et al. (2005). In transparent plastic boxes (15 x 23 x 4cm, Model 600-C, Pioneer Packaging, Dixon, KY), 50 seeds were sown on two blue blotters papers and placed in a germination chamber at 20±1ºC with alternate cycles of light (16 h) and darkness (8 h), at an inclination of ~125 degrees in relation to the base of the chamber in order that seedlings grew parallel to the blotter. The procedure consists in the analysis of the scanned image of 50 seedlings, taken after 3 days of germination, with computer software, measuring the length of each seedling. Based on the growth of the seedlings, the software computed a growth index and a uniformity index, both varying from 0 to 1000. Finally the program calculated a vigor index which corresponded in this study to the sum of 70% of the growth index and 30% of the uniformity index.

Statistical analysis

The experimental design was a complete randomized with three replicates and the data was analyzed graphically and compared based on mean standard errors.

Results and Discussion

Characterization for 'Javelina' and 'Sharp Shooter' varieties seed lots

Commercially, seed companies do not apply priming protocols on seed lots with low physiological potential. According to physiological potential results (Table 1) obtained for seed characterization, the seed lots used in this work had high quality, showing high germination percentages and vigor for both varieties, what justifies their use on priming treatments. The seed moisture contents varied from 13.4 and 8.5% for *'Javelina'* and from 7.5 to 6.8% for *'Sharp Shooter'* varieties, before priming, and after 12h imbibition was around 26% for both varieties increasing for each treatment and, reaching 40.7% for lot A and 34.6% for lot B, for *'Javelina'* and, 28.9% for lot A and 33.3% for lot B, for *'Sharp Shooter'* after 48 h priming (Table 2). After drying, the moisture content was close to the initial seed moisture content (data not shown).

Table 1. Percentage of normal seedlings obtained from standard germination test (G), salt saturated accelerated aging (SSAA), seedling emergence at 7 days (SE7D) and seedling emergence at 14 days (SE14D) for *'Javelina'* and *'Sharp Shooter'* varieties seed lots before priming.

Lot	*'Javelina'*				*'Sharp Shooter'*			
	G	SSAA	SE7D	SE14D	G	SSAA	SE7D	SE14D
	------------ (%) ------------				------------ (%) ------------			
A	93	97	90	91	93	90	89	89
B	93	94	91	96	96	92	90	94

Revista Agrarian
ISSN: 1984-2538

Table 2. Seed Moisture Content (%) after different priming durations (h), for *'Javelina'* and *'Sharp Shooter'* seeds.

Lot	*'Javelina'*				*'Sharp Shooter'*			
	0h	12h	24h	36h	0h	12h	24h	36h
	------------ (%) ------------				------------ (%) ------------			
A	13.4	26.2	34.7	40.7	7.5	25.4	31.4	28.9
B	8.5	26.3	28.9	34.6	6.8	26.1	33.7	33.3

Physiological potential for 'Javelina' and 'Sharp Shooter' varieties seed lots after priming

It was possible to observe for both seed lots a slightly, but significant, increase on germination percentage (Figure 1A) after priming treatments, being more evident on 24 and 36h treatments. The same results were found for seedling emergence after 7 days (Figure 1B) and 14 days (Figure 1C).

For *'Sharp Shooter'* variety the germination test didn't show significant increases on percentage of normal seedlings, even though being possible to identify tendencies of increase for both seed lots when compared to control. Comparing the treatments by the seedling emergence test, the results started to be clearer, and was possible to see at 7 days (Figure 2B) and 14 days (Figure 2C) that treated seeds had higher vigor than control. Also, for lot A, a better result was observed after 36h priming on both seedling emergence tests, becoming the best priming duration. However, even though lot B showing after 7 days that primed seeds had better physiological potential than non-treated seeds, wasn't possible to concluded a superior priming duration.

It is well known that different varieties and different lots of the same variety could have different responses for priming treatments and it was observed for both *'Javelina'* and *'Sharp Shooter'* varieties in this study. Different osmotic solutions can be used for seed priming and, according to Taylor et al. (1998), what could result on different characteristics and levels of efficiency. Normally, priming treatments increase germination percentage (Khan et al.; 1980) or keep it unchanged for high quality seed lots. Based on this, was possible to understand the priming performances for *'Sharp Shooter'* variety, where each seed lot answered differently for priming duration. This situation reinforces the importance of having an efficient, sensitive and rapid test to evaluate the better protocol to conduct a priming treatment on lettuce seeds.

Analyzing the seed lots by SVIS results, *'Javelina'* lot A showed no differences on uniformity (Figure 1D) among treatments, however, lot B had a slightly decrease from control to 36h priming for lot B. For growth (Figure 1E), lot A continued to have good results after priming, being the 36h treatment the one with better results, and lot B continued with a decrease on seedling development after priming.

Both values, uniformity and growth, are components for the vigor index, calculated by 30% from uniformity and 70% from growth. Considering this, the vigor index (Figure 1F) showed similar results from both indexes, showing priming benefits for *'Javelina'* lot A and a decrease on seed physiological performance for lot B.

Those results were similar from the others physiological tests, germination and seedling emergence tests, for lot A, showing a good correlation among tests and supporting SVIS as good test to evaluated priming protocols, but showed different performances for lot B seeds in different tests. Guilhien et al. (2009) had similar results evaluating priming protocols for sweet corn seeds with SVIS. The authors, like the results found in this study, found different performances from seed lots when applied priming treatments. For 'Sharp Shooter' variety SVIS results, the uniformity index (Figure 2D) confirmed the same results from germination, showing no differences among treatments for both seed lots, A and B. However, when compared by growth index (Figure 2E), even though no significant differences where observed, graphically was possible to find priming benefits on treatments 36h for lot A and 24h for lot B. This is an interesting finding considering the importance of sensitiveness for evaluating tests. Using the vigor

Revista Agrarian
ISSN: 1984-2538

index (Figure 2F), constituted by uniformity and growth indexes, no differences could be observed.

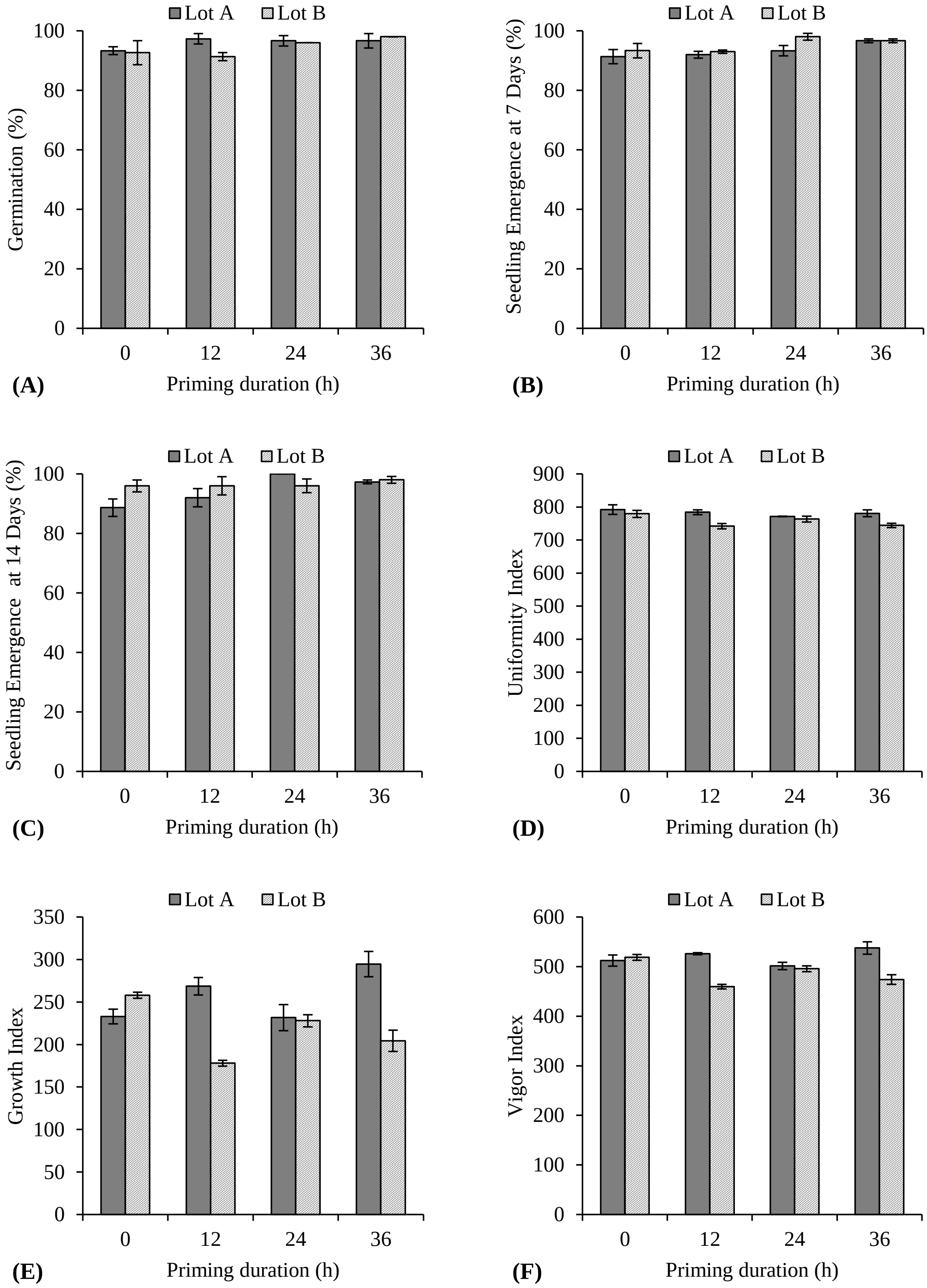

Figure 1. Standard germination test, seedling emergence at 7 days, seedling emergence at 14 days, uniformity index, growth index and vigor index results for 'Javelina' variety seed lost after different priming durations.

Revista Agrarian
ISSN: 1984-2538

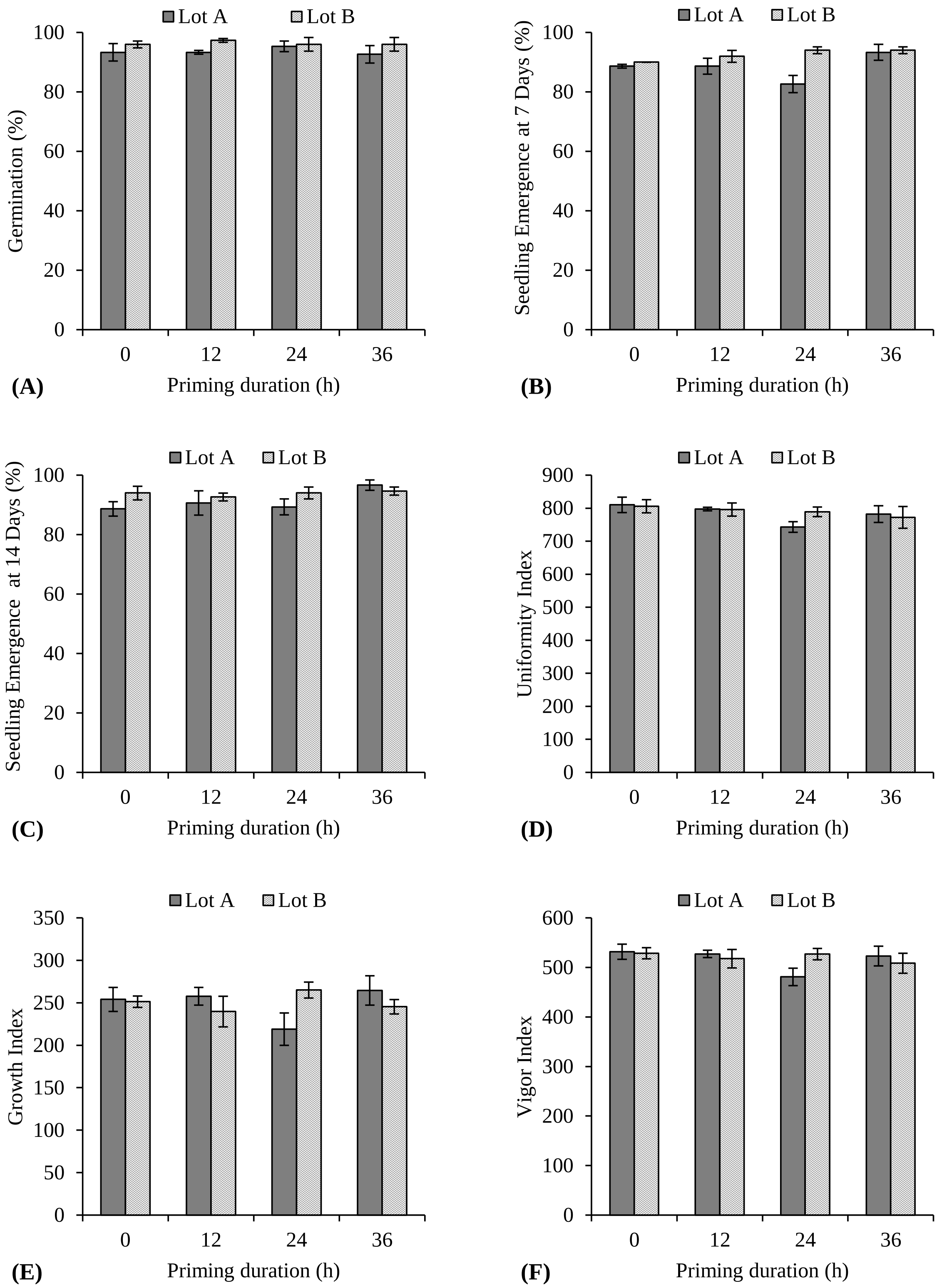

Figure 2. Standard germination test, seedling emergence at 7 days, seedling emergence at 14 days, uniformity index, growth index and vigor index results for 'Sharp shooter' variety seed lost after different priming durations.

Revista Agrarian
ISSN: 1984-2538

In this study, the Seed Vigor Image System vigor indexes, and also its component, growth index, produced interesting results, and could be compared to germination and seedling emergence results. Those findings document this as a rapid seed vigor test that can identify the efficacy of priming treatments, providing advantages such as rapid measurement of seed quality, storage of images for later retrieval, and development of a data base for priming efficacy of differing seed lots for future reference.

There is considerable interest in developing methods and equipment that enable a rapid and automated evaluation of seed quality (Van der burg, 2009) and, despite the reliability of available seed vigor tests, there are still opportunities to improve or develop new methods (Marcos-Filho et al., 2009). According to Pinto et al. (2007) and Santorum et al. (2013), there is an increasing need of efficient methods to assess seed physiological potential of seeds, which enable decision making regarding the collection, processing, storage and marketing of the crop, and the attributes presented in this study demonstrate that SVIS is an enhancement over traditional vigor tests, mainly, because it can bring results in three days, instead of 14 in a seedling emergence test, reducing considerably space, manual labor and human interpretation errors on evaluations.

Conclusions

The results obtained in this study have confirmed that *SVIS* is a practical, valuable and fast approach for evaluating and defining priming protocols in *Lactuca sativa,* constituting an alternative tool on seed technology.

Acknowledgements

We express our appreciation to Andrew F. Evans and Elaine M. Grassbaugh for guidance in the laboratory evaluations used in this study and to the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)* (Brazilian Research Foundation) for grant funds supporting VHVM.

References

BRASIL. **Rules for seed analysis**. Ministry of Agriculture, Livestock and Food Supply. Brasília: MAPA/ACS, 2009.

CAPRON, I.; CORBINEAU, F.; DACHER, F.; JOB, C.; CÔME, D.; JOB, D. Sugar beet seed priming: effects of priming conditions on germination, solubilization of 11-S globulin and accumulation of LEA proteins. **Seed Science Research**, v.10, p.243–254, 2000.

CHIQUITO, A.A.; GOMES-JUNIOR, F.G.; MARCOS-FILHO, J. Assessment of physiological potential of cucumber seeds using the software Seedling Vigor Imaging System® (SVIS®). **Journal of Seed Science**, v.34, p.255-263, 2012.

CONTRERAS, S.; BARROS, M. Vigor tests on lettuce seeds and their correlation with emergence. **Ciencia e Investigación Agraria**, v.32, p.3-10, 2005.

FESSEL, S.A.; VIEIRA, R.D.; RODRIGUES, T.J.D.; FAGIOLI, M.; PAULA, R.C. de. Efficiency of the osmotic osmopriming on lettuce seeds. **Journal of Seed Science**, v.23, p.128-133, 2001.

FOTI, R.; ABURENI, K.; TIGERE, A.; GOTOSA, J.; GERE, J. The efficacy of different seed priming osmotica on the establishment of maize (*Zea mays* L.) caryopses. **Journal of Arid Environments**, v.72, p.1127–1130, 2008.

GOMES-JÚNIOR, F.G.G.; MONDO, V.H.V.; CICERO, S.M.; McDONALD, M.B.; BENNETT, M.A. Evaluation of priming effects on sweet corn seeds by SVIS. **Seed Technology**, v.31, p.95-100, 2009.

HEYDECKER, W.; HIGGINS, J.; GULLIVER, R.L. Accelerated germination by osmotic seed treatment. **Nature**, v.246, p.42–44, 1973.

HILL, H.; BRADFORD, K.J.; TAYLOR, A.G. Primed lettuce seeds exhibit increased sensitivity to moisture during aging. **Acta Horticulturae**, v.782, p.135-141, 2008.

JIANHUA, Z.; McDONALD, M.B. The saturated salt accelerated aging test for small-seeded crops. **Seed Science and Technology**, v.25, p.123-131, 1997.

KHAN, A.A.; PECK, N.H.; SAMIMY, C. Seed osmoconditioning: physiological and biological changes. **Israel Journal of Botany**, v.29, p.133-144, 1980.

Revista Agrarian
ISSN: 1984-2538

KIKUTI, A.L.P.; MARCOS-FILHO, J. Seed vigour tests for lettuce seeds. **Horticultura Brasileira**, v.30, p.44-50, 2012.

KODDEL, J.; BUCKEY, W.T.; GROOT, C.C. de; RETIEREL, M.; ZAMORA, A.M.V.; GROOT, S.P.C. A fast ethanol assay to detect seed deterioration. **Seed Science Research**, v.22, p.55–62, 2012.

MARCOS-FILHO, J.; KIKUTI, A.L.P.; LIMA, L.B. Procedures for evaluation of soybean seed vigour, including an automated computer imaging system. **Journal of Seed Science**, v.31, p.102-112, 2009.

MATTHEWS, S.; NOLI, E.; DEMIR, I.; KHAJEH-HOSSEINI, M.; WAGNER, M.H. Evaluation of seed quality: from physiology to international standardization. **Seed Science Research**, v.22, p.69–73, 2012.

MONDO, V.H.V.; DIAS, M.A.N.; McDONALD, M.B. Seed vigour imaging system for two-day-old corn seedling evaluation. **Seed Technology**, v.33, p.191-196, 2011.

PEÑALOZA, P.; RAMIREZ-ROSALES, G.; McDONALD, M.B.; BENNET, M.A. Lettuce (*Lactuca sativa* L.) seed quality evaluation using seed physical attributes, saturated salt accelerated aging and the seed vigour imaging system. **Electronic Journal of Biotechnology**, v.8, p.299-307, 2005.

PINTO, T.L.F.; CICERO, S.M.; FORTI, V.A. Image analysis technique to evaluate weathering damage in soybean seeds. **Journal of Seed Science**, v.29, p.31-38, 2007.

SAKO, Y.; McDONALD, M.B.; FUJIMURA, K.; EVANS, A.F.; BENNETT, M.A. A system for automated seed vigour assessment. **Seed Science and Technology**, v.29, p.625-636, 2001.

SANTORUM, M.; NÓBREGA, L.H.P.; SOUZA, E.G. de; SANTOS, D. dos; BOLLER, W.; MAULI, M.M. Comparison of test for the analysis of vigor and viability in soybean seeds and their relationship to field emergence. **Acta Scientiarum**, v.35, p.83-92, 2013.

SILVA, C.B.; LOPES, M.M.; MARCOS-FILHO, J.; VIEIRA, R.D. Automated system of seedling image analysis (SVIS) and electrical conductivity to assess sun hemp seed vigour. **Journal of Seed Science**, v.34, p.055-060, 2012.

TAYLOR, A.G.; ALLEN, P.S.; BENNETT, M.A.; BRADFORD, K.J.; BURRIS, J.S.; MISRA, M.K. Seed enhancements. **Seed Science Research**, v.8, p.245–256, 1998.

VAN DER BURG, J. **Raising seed quality: what is in the pipeline?** In: Responding to the challenges of a changing world: the role of new plant varieties and high quality seed in agriculture. Rome, Food and Agriculture Organisation. p.177–185, 2009.

VILLELA, F.A.; BECKERT, O.P. Osmotic potentials of aqueous solutions of polyethylene glycol 8000. **Journal of Seed Science**, v.23, p.267-275, 2001.

WARREN, J.E.; BENNETT, M.A. Seed hydration using the drum priming system. **Hort Science**, v.32, p.1220-1221, 1997.