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Controlled deterioration test and use of the Seed Vigor Imaging System (SVIS®) to evaluate the physiological potential of crambe seeds[1]

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ABSTRACT – Vigor tests are important tools for verifying the physiological potential of seed lots; however, various aspects can interfere in the consistency of the test results. The search for less subjective procedures has drawn the interest of researchers. The aim of this study was to adapt the methodology of the controlled deterioration test and verify the efficiency of the Seed Vigor Imaging System (SVIS®) to evaluate the vigor of crambe seeds. Initially, seed moisture content was determined and germination and vigor were evaluated in five seed lots. For the controlled deterioration test, the seed moisture content was adjusted to 18%, 20%, and 22%, and the results were interpreted at four and five days after sowing. The controlled deterioration test should be performed with initial adjustment of seed moisture content to 18%, and the germination test should be evaluated on the fifth day after sowing. SVIS® provided information on the vigor index and the length and uniformity of seedling development. Computerized image analysis is effective in evaluating the vigor of crambe seeds, and the uniformity of seedling development parameter offers the most consistent data.

Index terms: *Crambe abyssinica*, software, seed vigor.

Teste de deterioração controlada e uso do sistema automatizado de análise de imagens de plântulas (SVIS®) na avaliação do potencial fisiológico de sementes de crambe

RESUMO – Os testes de vigor são ferramentas importantes para aferir o potencial fisiológico de lotes de sementes, porém, vários aspectos podem interferir na consistência dos resultados desses testes, tendo a busca por procedimentos menos subjetivos despertado o interesse dos pesquisadores. Este trabalho foi realizado com o objetivo de adequar a metodologia do teste de deterioração controlada e verificar a eficiência do sistema automatizado de análise de imagens de plântulas (SVIS®) na avaliação do vigor de crambe. Inicialmente, cinco lotes de sementes foram submetidos à determinação do teor de água e avaliações de germinação e vigor. Para o teste de deterioração controlada, o teor de água das sementes foi ajustado para 18, 20 e 22%, e a interpretação dos resultados realizada aos quatro e cinco dias após a semeadura. O teste de deterioração controlada deve ser realizado com ajuste inicial do teor de água para 18% e a avaliação da germinação realizada no quinto dia. O SVIS® forneceu informações sobre o índice de vigor, comprimento e uniformidade de desenvolvimento de plântulas. A análise computadorizada de imagens é eficiente para avaliar o vigor de sementes de crambe, sendo o parâmetro uniformidade de desenvolvimento de plântulas o que apresenta dados mais consistentes.

Termos para indexação: *Crambe abyssinica*, software, vigor de sementes.

Introduction

Crambe [*Crambe abyssinica* (Hochst. Ex R. E. Fr.)] is a Brassicaceae traditionally produced in the United States and Europe. The main use of the species worldwide is for production of biomass for animal feed (it has 30-32% crude protein) and for extraction of erucic acid, a monounsaturated omega-9 fatty acid that can be used in a way similar to mineral

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oils, but with the advantage of being easily biodegradable (Desai, 2004). According to Paulose et al. (2010), the crop stands out for high biomass production, short cycle, and hardiness. In Brazil, the crambe crop was introduced at the beginning of the 1990s by the Fundação MS, mainly with a view toward biodiesel production because the seeds have from 26% to 38% oil content (Jasper et al., 2010; Pitol et al., 2010). In spite of the agricultural potential of this species, studies directed to clarification of production characteristics are still rare.

Within this context, one of the main requisites for achieving the maximum productive potential of a crop is obtaining an adequate plant population in the field, which requires the use of high seed quality (Leão et al., 2016). From the physiological point of view, the development of standardized, fast, objective, and reliable methods is fundamental for creating seed quality control programs in seed companies (Marcos-Filho, 2010).

Traditionally, germination and vigor tests are performed to characterize seed physiological potential. The controlled deterioration test is a worthwhile alternative for avoiding the effects of accentuated differences in the water absorption rate of seed lots of the same species, especially when working with small seeds (Dutra and Medeiros-Filho, 2008). In this test, the seed moisture content is standardized to pre-established values before the period of exposure to stress. That way, seeds are artificially aged under high temperatures; however, with similar moisture content (AOSA, 2002; Lima and Marcos-Filho, 2011; Torres et al., 2013).

Studies that evaluate the best procedure for adjusting the initial seed moisture content have been performed in an attempt to refine standardization of the controlled deterioration test (Torres and Paiva, 2009; Silva and Vieira, 2010; Torres et al., 2012). Nevertheless, species or even cultivars may exhibit different responses when subjected to the procedures recommended and used for other species.

At the same time, the development of innovative alternatives has arisen with the aim of obtaining important information that is less subjective and more reliable concerning the seed physiological potential. An important alternative is the Seed Vigor Imaging System, SVIS®, software that allows vigor index and length and uniformity of seedling development to be obtained (Kikuti and Marcos-Filho, 2012).

The methodology of the SVIS® has now been widely used in laboratories and seed companies in the United States, and results have shown that the procedure is promising for evaluation of the seed vigor of various species (Marcos-Filho et al., 2009; Chiquito et al., 2012; Silva et al., 2012; Kikuti and Marcos-Filho, 2013; Caldeira et al., 2014).

Kikuti and Marcos-Filho (2012) evaluated the effectiveness of the SVIS® in lettuce seeds and concluded that the growth index and the primary root length provided consistent results similar to other tests commonly used for this species. Silva et al. (2012) studied the SVIS® for sun hemp seeds (*Crotalaria juncea* L.) and observed that this software is effective in detecting differences in the physiological potential of seed lots and that, associated with the electrical conductivity test, it can provide important information regarding the seed vigor. Evaluation of seed vigor using the SVIS® has also been reported as successful for species such as lettuce (Sako et al., 2001 and Peñaloza et al., 2005), melon (Marcos-Filho et al., 2006), soybean (Hoffmaster et al., 2005 and Marcos-Filho et al., 2009), eggplant (Silva and Cicero, 2014), and sunflower (Rocha et al., 2015).

Thus, procedures effective in discrimination of crambe seed lots would be alternatives for precise identification of seed physiological potential by seed companies. The aim of this study was to evaluate the methodology of the controlled deterioration test and verify the effectiveness of the Seed Vigor Imaging System (SVIS®) in evaluation of the physiological potential of crambe seeds.

Material and Methods

The study was conducted at the Seed Analysis Laboratory, Department of Crop Science, Sao Paulo State University (UNESP), Jaboticabal Campus, SP, Brazil, and at the Image Analysis Laboratory, Department of Crop Science, University of Sao Paulo (USP/ESALQ), Piracicaba, SP, Brazil. Five commercial lots of crambe seeds from FMS Brilhante cultivar were used, produced by the Fundação MS, located in Maracajú, MS, Brazil.

Seeds were placed in woven polypropylene bags and kept in a cold chamber (10 ºC and 50-60% relative air humidity - RH) while conducting the laboratory studies. The experiments were set up in a completely randomized design and the following evaluations were made:

Seed moisture content: determined by the oven method at 105 ± 3 ºC for 24 h (Brasil, 2009) with two subsamples of approximately 2.0 g of seeds for each lot, before and after the controlled deterioration test. The results were expressed as a percentage on a fresh weight basis.

Germination: four replications of 50 seeds per lot were distributed on 300 g of sieved and sterilized sand in plastic germination boxes (11 x 11 x 3.5 cm) and covered by 0.5 cm of sand. The substrate was previously moistened to 70% of field capacity. To moisten the substrate, a KNO_3 solution (0.2%) was used, according to Brasil (2009). The boxes with the seeds were kept under alternating temperature of 20-30 °C and a 12-h photoperiod. Seedling evaluations were carried out according to the criteria established in the Rules for Seed Testing (Brasil, 2009) at four days for the first germination count, and at seven days after sowing for the final germination count. The results were expressed as a percentage of normal seedlings.

Field seedling emergence: four replications of 50 seeds per lot were sown, arranged in an earth plant bed without fertilization in 2.5-meter length rows, spaced at 0.5 m, at a depth of 2 cm. A spray-irrigation system was used. The mean temperature in the environment while the test was conducted was 23 °C, with a minimum of 15 °C and maximum of 32 °C. Evaluations were made at 14 days after sowing, and only the seedlings that had a hypocotyl longer than 1.0 cm were considered; the percentage of normal emerged seedlings was determined.

Controlled deterioration: the seed moisture content was adjusted to 18%, 20%, and 22% using the wet atmosphere method. For that purpose, 5 g samples of seeds from each lot were distributed on a single layer on a stainless steel mesh inside transparent plastic boxes (11 x 11 x 3.5 cm), which contained 40 mL of deionized water. The boxes were closed and kept in a chamber at 20 ºC, and seeds were weighed periodically until they reached the weight corresponding to the pre-established moisture content. The seed moisture content was adjusted according to the equation:

$$FW = [(100 - MCi) \times (100 - MCd)^{-1}] \times IW$$

in which: FW = final weight, IW = initial weight, and MCi and MCd correspond to the initial and desired moisture content, respectively.

After obtaining the desired seed moisture content, the samples were placed in hermetic aluminized plastic bags and kept in a cold chamber (10 ºC and 50-60% RH) for five days allowing hygroscopic equilibrium among the seeds. Then, samples were placed in a bain-marie at 45 ºC for 24 h. After this period, the samples were kept at ambient temperature for 30 minutes. The seeds were then subjected to the germination test, according to the methodology described above, with evaluations on the fourth and fifth day after sowing.

Image analysis (SVIS®): for analysis using the SVIS® software, four replications of 25 seeds were used for each lot. The seeds were placed in two horizontal rows on the upper third of the surface of paper towels that was previously moistened with KNO_3 solution (0.2%) and distributed so that growth of the primary root was turned downward. The towels were rolled up and transferred to a germination chamber at 20-30 °C and kept for three days in continuous light. After this period, the seedlings were arranged on a sheet of black pasteboard set on the surface of an aluminum box (60 x 50 x 12 cm), inside of which was an HP Scanjet 2004 scanner, placed upside down and operated by Photosmart software with 98 dpi resolution. The seedling images were digitalized and then analyzed by the SVIS® software. The vigor index and length and uniformity of seedling development were evaluated. The vigor index was generated by a combination of growth parameters (70% contribution) and seedling uniformity (30% contribution), both based on the maximum possible length of the crambe seedlings at three days after sowing (3 inches = 7.6 cm).

The data were subjected to analysis of variance by the F test. For the controlled deterioration test, the data were transformed in arc sen $\sqrt{x/100}$ allowing for homogeneity of variances. The Tukey test at 5% probability was used for comparison the means; data were presented without transformation. In the end, a simple correlation analysis was made among the uniformity of seedling development and the germination test, first germination count, field seedling emergence, and controlled deterioration test.

Results and Discussion

All seed lots were homogeneous regarding to the initial moisture content, with variation of 0.8 percentages points between the extremes (Table 1). Similar results were obtained from the same cultivar of crambe seeds in the studies of Leão et al. (2016). A small variation in moisture content is important because can ensure efficient standardization of procedures for the controlled deterioration test so that seed lots can reach the predetermined moisture content in similar periods (Silva and Vieira, 2010) and maintain a uniform deterioration process (Dutra and Medeiros-Filho, 2008).

In initial characterization of the seed lots (Table 1), the germination percentage of all the lots was acceptable for the commercialization of crambe seeds, which is fixed at 60% (Brasil, 2013), and they indicated lower performance

Table 1. Initial seed moisture content (MC), first germination count (FGC), germination (G), and field seedling emergence (FSE) in five lots of crambe seeds.

Seed lot	MC	FGC	G	FSE
	%			
1	8.5	76 a	88 a	73 a
2	8.1	54 b	86 a	52 bc
3	8.0	60 ab	85 a	61 ab
4	8.6	66 ab	82 a	44 bc
5	8.2	47 b	60 b	39 c
F test		5.9**	5.7**	9.9**
CV		15.0%	12.7%	16.0%

Mean values followed by the same letter in the column do not differ among themselves by the Tukey test at 5% probability.
** Significant at the level of 1% probability.

for lot 5. The first germination count and the field seedling emergence showed differences in vigor among lots with similar germination. Lot 1 was identified as having the highest vigor, exceeding lots 2 and 4 regarding to the seedling emergence, and lot 2 for the first germination count. Lot 5 showed the worst performance in all these physiological potential evaluations.

The moisture content of the five seed lots was adjusted to 18%, 20%, and 22% before the controlled deterioration test was carried out, with relatively small variation among the lots (Table 2). This fact is of great importance for the reliability of the test results because can ensures a similar deterioration process among the seed lots (Dutra and Medeiros-Filho, 2008). As highlighted by Powell and Matthews (1981), one limitation of the controlled deterioration test as routine test includes the precision required for increasing the seed moisture content.

During the controlled deterioration test, greater sensitivity in differentiating physiological potential of the lots was observed when the seed moisture content was adjusted to 18% and evaluation was carried out on the fifth day after sowing (Table 3). These results were similar to the field seedling emergence, which also identified lot 1 as the most vigorous and lot 5 as the lowest vigor. Evaluation of controlled deterioration on the fourth day was able to separate lots into only two distinct physiological classes with results different from those obtained for field seedling emergence, indicating lower sensitivity for detecting differences in vigor among the seed lots.

Table 2. Adjustment of moisture content for the controlled deterioration test at 45 °C for 24 h in five lots of crambe seeds.

Seed lot	Moisture content (%)		
	18	20	22
1	17.7	19.8	21.2
2	17.2	19.6	22.6
3	17.5	19.6	22.2
4	18.0	20.4	22.0
5	17.9	20.2	21.2
Mean	17.7	19.8	21.9

Similar results, in which adjustment of seed moisture content to values under 20% proved to be promising were obtained for other species. For example, Mendonça et al. (2003) concluded that moisture content adjusted to 18% during the controlled deterioration test is also efficient to evaluate broccoli seed lots vigor. Rossetto et al. (2004) evaluated the controlled deterioration test in peanut seeds with moisture contents of 15% and 20% and observed that adjustment to 15% showed high correlation with other seed vigor tests. However, in contrast, Torres et al. (2012) studied coriander seeds with adjustments to 18%, 21%, and 24% and found that moisture content adjusted to 21% reveals higher effectiveness in separating seed lots into different vigor levels.

In this study, the controlled deterioration test with initial adjustment of moisture content to 20% and evaluation at four and five days after sowing were in contrast with the other evaluations, classifying lot 1 as low vigor (Table 3). However, this test with adjustment of seed moisture content to 22% had a response nearer to the results obtained during initial evaluations (Table 1). Nevertheless, the seed vigor classification was not similar to the field seedling emergence, which exhibited lower sensitivity in ranking seed lots into vigor levels when confronted with the moisture content adjustment of 18% in evaluation on the fifth day after sowing.

Rossetto and Marcos-Filho (1995) demonstrated that the higher the moisture content of soybean seeds, the more damaging the effect of high temperature during the controlled deterioration test. Thus, this does not allow consistent classification of the lots into different vigor levels. In this context, Kikuti and Marcos-Filho (2008) obtained satisfactory results in cauliflower seeds only when the seed moisture

Table 3. Vigor evaluated by the controlled deterioration test in five lots of crambe seeds, with adjustment of the seed moisture content to 18%, 20%, and 22%, at four and five days after sowing.

Seed lot	18%		20%		22%	
	4^{th} day	5^{th} day	4^{th} day	5^{th} day	4^{th} day	5^{th} day
1	68 a	84 a	26 b	59 c	70 a	81 a
2	40 b	53 bc	70 a	88 a	37 b	53 b
3	60 ab	71 ab	68 a	78 ab	49 ab	72 ab
4	39 b	59 bc	52 a	65 bc	40 b	61 ab
5	36 b	46 c	5 c	23 d	43 b	55 b
F test	6.5**	9.0**	41.8**	40.9**	6.5**	4.5**
CV	23.7%	16.2%	19.7%	12.4%	21.6%	17.3%

Mean values followed by the same letter in the column do not differ among themselves by the Tukey test at 5% probability.
** Significant at the level of 1% probability.

content was adjusted to lower values, such as 20% and 22%; satisfactory results were not possible with adjustment to 24%.

After controlled deterioration, the seed moisture content remained near the initially adjusted value (Table 4). This factor is relevant because it indicates the degree of reliability of controlled deterioration test results (Marcos-Filho, 2015).

Table 4. Moisture content of five lots of crambe seeds after the controlled deterioration test at 45 °C for 24 h.

Seed lot	Moisture content (%)		
	18	20	22
1	18.1	19.9	21.9
2	17.9	19.7	21.8
3	18.1	19.8	22.2
4	18.0	19.9	21.9
5	18.2	20.1	21.9
Mean	18.0	19.9	22.0

Image analysis of crambe seedlings by SVIS® (Table 5) allowed efficient identification of seed lots into different vigor levels in a similar manner to other tests. Lot 1 was identified as the highest vigor for all the parameters generated by the software. On the other hand, the seedling length and the vigor index showed no sufficient sensitivity to detect different levels of physiological potential among other seed lots; the best separation of lots occurred only for uniformity of seedling development, which in addition to showing the best performance of lot 1 also highlighted lot 5 as having the lowest potential, as confirmed in the field seedling emergence results.

It is noteworthy that the results presented by use of traditional vigor tests were not able to efficiently separate lot 3. Lot 3 had exhibited a response intermediate to lots 1 and 5 during initial physiological potential characterization of seed lots (Table 1) as well as the controlled deterioration test with the best procedure for testing in crambe seeds, i.e., adjusting the moisture content to 18% and evaluation on the fifth day after sowing (Table 3).

Table 5. Seedling length (cm), vigor index, and uniformity of seedling development from five lots of crambe seeds obtained by the Seed Vigor Imaging System - SVIS®.

Seed lot	Seedling length	Vigor index	Uniformity of seedling development
1	4.6 a	356.5 a	762.7 a
2	4.0 ab	329.7 ab	733.2 ab
3	3.6 ab	277.2 b	591.7 c
4	3.1 b	279.2 b	644.0 bc
5	4.3 ab	303.5 ab	614.2 c
F test	3.9**	6.7**	8.5**
CV	15.0%	8.4%	7.6%

Mean values followed by the same letter in the column do not differ among themselves by the Tukey test at 5% probability.
** Significant at the level of 1% probability.

In contrast, the indexes generated by the SVIS® for uniformity of seedling development were able to separate the seed lots in an effective manner, as can be observed from the digital images of the seedlings (Figure 1). These results are in agreement with those obtained by Silva et al. (2012), who concluded that uniformity of seedling development can provide useful information regarding the degree of deterioration, initial growth potential, and uniformity of seedling emergence in sun hemp. For this parameter, reliable results were also found by Sako et al. (2001), Hoffmaster et al. (2005), and Marcos-Filho et al. (2006), confirming the high potential of the uniformity of seedling development parameter, generated by the SVIS®, to provide information regarding the vigor of seed lots of various species.

The simple correlation coefficients presented in Table 6 reveal that the results of the field seedling emergence exhibited significant correlation only with uniformity of seedling development (r=0.91). The data of first germination count, for their part, presented significant correlation with those of the controlled deterioration test (r=0.83). The strong correlation with vigor tests widely used for various species, demonstrates the possibility of using information from uniformity of seedling development and the controlled deterioration test to evaluate the crambe seed vigor.

Table 6. Simple correlation coefficients (r) between the field seedling emergence (FSE), first germination count (FGC), germination (G), controlled deterioration (CD) and uniformity of seedling development obtained by the Seed Vigor Imaging System - SVIS® (USD).

Test	FSE	FGC	G	CD
FSE	-	-	-	-
FGC	0.44^{ns}	-	-	-
G	0.76^{ns}	0.77^{ns}	-	-
CD	0.70^{ns}	0.83*	0.73^{ns}	-
USD	0.91*	0.20^{ns}	0.58^{ns}	0.45^{ns}

*Significant at the level of 5% probability and ^{ns} not significant.

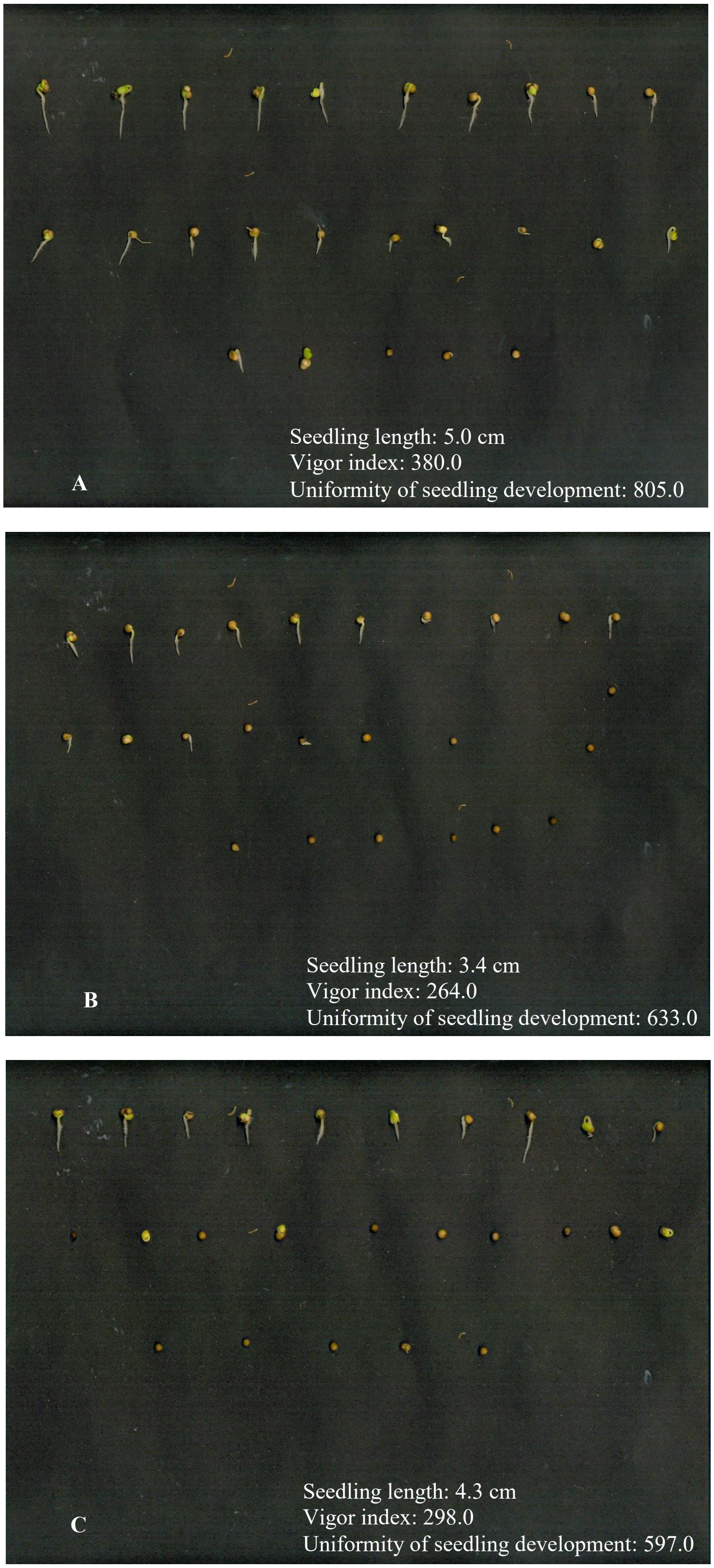

Figure 1. Digital images of crambe seedlings three days after sowing from seed lots of high vigor, intermediate vigor, and low vigor: lots 1 (A), 3 (B), and 5 (C), respectively, analyzed by the Seed Vigor Imaging System (SVIS®).

Conclusions

The controlled deterioration test in crambe seeds should be carried out with 18% moisture content and the germination evaluation on the fifth day after sowing.

Computerized image analysis is effective in evaluating crambe seed vigor, and the uniformity of seedling development parameter presents the most consistent data.

References

AOSA. Association of Official Seed Analysts. *Seed vigor testing handbook*. Ithaca, 2002. 105p. Contribution 32.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Portaria nº 16, de 25 de janeiro de 2013. *Diário Oficial da União*, Poder Executivo, Brasília, DF, 28 jan. 2013. Anexo III. http://www.lex.com.br/legis_24122430_PORTARIA_N_16_DE_25_DE_JANEIRO

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Regras para análise de sementes*. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: MAPA, 2009. 395p. http://www.agricultura.gov.br/arq_editor/file/2946_regras_analise__sementes.pdf

CALDEIRA, C.M.; CARVALHO, M.L.M.; OLIVEIRA, J.A.; COELHO, S.V.B.; KATAOKA, V.Y. Vigor de sementes de girassol pela análise computadorizada de plântulas. *Científica*, v.42, n.4, p.346-353, 2014. http://cientifica.org.br/index.php/cientifica/article/view/556/355

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®). *Revista Brasileira de Sementes*, v.34, n.2 p.255-263, 2012. http://www.scielo.br/pdf/rbs/v34n2/10.pdf

DESAI, B.B. *Seeds handbook*: biology, production processing and storage. 2.ed. New York: Marcel Dekker, 2004. 787p.

DUTRA, A.S.; MEDEIROS-FILHO, S. Teste de deterioração controlada na determinação do vigor em sementes de algodão. *Revista Brasileira de Sementes*, v.30, n.1, p.19-23, 2008. http://www.scielo.br/pdf/rbs/v30n1/a03v30n1.pdf

HOFFMASTER, A.L.; XU, L.; FUJIMURA, K.; McDONALD, M.B.; BENNET, M.A.; EVANS, A.F. The Ohio State University seed vigor imaging system (SVIS) for soybean and corn seedlings. *Seed Technology*, v.27, n.1, p.7-26, 2005. http://seedbiology.osu.edu/People_pdf/miller_ abstractSVIScorn.pdf

JASPER, S.P.; BIAGGIONI, M.A.M.; SILVA, P.R.A.; SEKI, A.S.; BUENO, O.C. Análise energética da cultura do crambe (*Crambe abyssinica* Hochst) produzida em plantio direto. *Engenharia Agrícola*, v.30, n.3, p.395-403, 2010. http://www.scielo.br/pdf/eagri/v30n3/04.pdf

KIKUTI, A.L.P.; MARCOS-FILHO, J. Physiological potential of cauliflower seeds. *Scientia Agricola*, v.65, n.4, p.374-380, 2008. http://www.scielo.br/pdf/sa/v65n4/08.pdf

KIKUTI, A.L.P.; MARCOS-FILHO, J. Seedling imaging analysis and traditional tests to assess okra seed vigor. *Journal of Seed Science*, v.35, n.4, p.443-448, 2013. http://www.scielo.br/pdf/jss/v35n4/05.pdf

KIKUTI, A.L.P.; MARCOS-FILHO, J. Testes de vigor em sementes de alface. *Horticultura Brasileira*, v.30, n.1, p.44-50, 2012. http://www.scielo.br/pdf/hb/v30n1/v30n1a08.pdf

LEÃO, E.F.; SANTOS, J.F.; BARBOSA, R.M.; VIEIRA, R.D. Accelerated ageing as a vigour test for crambe (*Crambe abyssinica*) seeds. *Australian Journal of Crop Science*, v.10, n.5, p.660-665, 2016. http://www.cropj.com/leao_10_5_2016_660_665.pdf

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*, v.33, n.1 p.45-53, 2011. http://www.scielo.br/pdf/rbs/v33n1/05.pdf

MARCOS-FILHO, J. *Fisiologia de sementes de plantas cultivadas*. 2 ed. Londrina: Abrates, 2015. 660p.

MARCOS-FILHO, J. Sistema computadorizado de análise de imagens de plântulas (SVIS®) para avaliação do vigor de sementes. *Informativo Abrates,* v.20, n.3, p.40-44, 2010. http://www.abrates.org.br/downloads/file/22

MARCOS-FILHO, J.; BENNETT, M.A.; McDONALD, M.B.; EVANS, A.F.; GRASSBAUGH, E.M. Assessment of melon seed vigour by an automated computer imaging system compared to traditional procedures. *Seed Science and Technology*, v.34, n.2, p.485-497, 2006. https://doi.org/10.15258/sst.2006.34.2.23

MARCOS-FILHO, J.; KIKUTI, A.L.P.; LIMA, L.B. Métodos para avaliação do vigor de sementes de soja, incluindo a análise computadorizada de imagens. *Revista Brasileira de Sementes,* v.31, n.1, p.102-112, 2009. http://www.scielo.br/pdf/rbs/v31n1/a12v31n1.pdf

MENDONÇA, E.A.F.; RAMOS, N.P.; FESSEL, S.A. Adequação da metodologia do teste de deterioração controlada para sementes de brócolis (*Brassica oleracea* L. - var. Itálica). *Revista Brasileira de Sementes*, v.25, n.1, p.18-24, 2003. http://www.scielo.br/pdf/rbs/v25n1/19625.pdf

PAULOSE, B.; KANDASAMY, S; DHANKHER, O.P. Expression profiling of *Crambe abyssinica* under arsenate stress identifies genes and gene networks involved in arsenic metabolism and detoxification. *BMC Plant Biology*, v.10, p.1-12, 2010. http://bmcplantbiol.biomedcentral.com/articles/10.1186/1471-2229-10-108

PEÑALOZA, P.; RAMIREZ-ROSALES, G.; MCDONALD, M.B.; BENNETT, 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, n.3, p.299-307, 2005. http://www.scielo.cl/pdf/ejb/v8n3/a10.pdf

PITOL, C.; BROCH, D.L.; ROSCOE, R. *Tecnologia e produção*: crambe. Maracaju: Fundação MS, 2010. 60p.

POWELL, A.A.; MATTHEWS, S. Evaluation of controlled deterioration: a new vigour test for small seed vegetable. *Seed Science and Technology*, v.9, n.2, p.633-40, 1981. http://libcatalog.cimmyt.org/download/reprints/96876.pdf

ROCHA, C.R.M.; SILVA, V.N.; CICERO, S.M. Avaliação do vigor de sementes de girassol por meio de análise de imagens de plântulas. *Ciência Rural*, v.45, n.6, p.970-976, 2015. http://www.scielo.br/pdf/cr/v45n6/1678-4596-cr-45-06-00970.pdf

ROSSETTO, C.A.V.; LIMA, T.M.; GUIMARÃES, E.C. Envelhecimento acelerado e deterioração controlada em sementes de amendoim. *Pesquisa Agropecuária Brasileira*, v.39, n.8, p.795-801, 2004. http://www.scielo.br/pdf/pab/v39n8/21741.pdf

ROSSETTO, C.A.V.; MARCOS-FILHO, J. Comparação entre os métodos de envelhecimento acelerado e de deterioração controlada para avaliação da qualidade fisiológica de sementes de soja. *Scientia Agricola*, v.52, n.1, p.123-131, 1995. http://www.scielo.br/pdf/sa/v52n1/19.pdf

SAKO, Y.; McDONALD, M.B.; FUJIMURA, K.; EVANS, A.F.; BENNET, M.A. A system of automated seed vigour assessment. *Seed Science and Technology*, v.29, n.3, p.625-636, 2001. http://www.eurofinsus.com/media/162083/seed-vigor-imaging-system.pdf

SILVA, C.B.; LOPES, M.M.; MARCOS-FILHO, J.; VIEIRA, R.D. Automated system of seedling image analysis and electrical conductivity to assess sun hemp seed vigor. *Revista Brasileira de Sementes,* v.34, n.1, p.55-60, 2012. http://www.scielo.br/pdf/rbs/v34n1/a07v34n1.pdf

SILVA, J.B.; VIEIRA, R.D. Deterioração controlada em sementes de beterraba. *Revista Brasileira de Sementes*, v.32, n.1, p.69-76, 2010. http://www.scielo.br/pdf/rbs/v32n1/v32n1a08.pdf

SILVA, V.N.; CICERO, S.M. Análise de imagens de plântulas para avaliação do potencial fisiológico de sementes de berinjela. *Horticultura Brasileira*, v.32, n.2, p.145-151, 2014. http://www.scielo.br/pdf/hb/v32n2/0102-0536-hb-32-02-00145.pdf

TORRES, S.B.; GOMES, M.D.A.; SILVA, F.G.; BENEDITO, C.P.; PEREIRA, F.E.C.B. Controlled deterioration to evaluate okra seed vigor. *Horticultura Brasileira,* v.31, n.2, p.317-321, 2013. http://www.scielo.br/pdf/hb/v31n2/23.pdf

TORRES, S.B.; DANTAS, A.H.; PEREIRA, M.F.S.; BENEDITO, C.P.; SILVA, F.H.A. Deterioração controlada em sementes de coentro. *Revista Brasileira de Sementes*, v.34, n.2, p.319-326, 2012. http://www.scielo.br/pdf/rbs/v34n2/18.pdf

TORRES, S.B.; PAIVA, E.P. Teste de deterioração controlada para avaliação da qualidade fisiológica de sementes de jiló. *Revista Caatinga,* v.22, n.3, p.35-39, 2009. https://periodicos.ufersa.edu.br/revistas/index.php/caatinga/article/view/905/609