Soybean seed vigor: ethanol quantification test

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Abstract: The objective of this study was to verify the separation of soybean seed lots according to vigor levels by the rapid ethanol test following the adapted ethylometer method. The design used was entirely randomized with four repetitions. The treatments consisted of five soybean seed lots submitted to germination tests, first germination count, accelerated aging, field emergence, electrical conductivity, and ethanol test. To measure ethanol, 50 soybean seeds were placed in plastic bottles containing 50 mL of distilled water and placed in a BOD incubator for different temperatures and exposure times. After the imbibition period, measurements were performed with the aid of an adapted ethylometer. It is possible to quantify ethanol in soybean seed lots using the adapted ethylometer equipment. However, the equipment is not efficient in ranking soybean seed lots at different vigor levels.

Keywords: *Glycine max* L., physiological quality, deterioration, alcohol

Citation: Da Silva, R. N. O., J. A. Cavalcante, G. I. Gadotti, A. B. N. Martins, S. B. Teixeira, R. O. Vergara, and L. V. M. de Tunes. 2021. Soybean seed vigor: ethanol quantification test. Agricultural Engineering International: CIGR Journal, 23 (2):239-246.

1 Introduction

Soybean is a crop that stands out on the world stage due to the great diversity of its by-products and also for its economic importance. The United States currently ranks first in soybean production, followed by Brazil and Argentina. World production of this commodity reached 339.99 million tons in 2017/2018 crop (USDA, 2019). Over 35 million hectares were cultivated in Brazil, which generated approximately 120 million tons in the 2017/2018 harvest (CONAB, 2019).

However, to achieve results of this magnitude, it is essential to use high-quality seeds for crop implantation, as it ensures adequate plant populations even in a wide range of environmental conditions (França Neto et al., 2015). The effects of high-quality seeds on soybean crop establishment, performance, and productivity have been demonstrated in several studies (Bagateli et al., 2019; Cantareli et al., 2015).

In this sense, the quality control of seed companies must be increasingly efficient to serve the consumer market, which requires a fast analysis of the physiological potential of seeds. This information is important to assist the producer in decision-making at different stages in the seed production process (Fessel et al., 2010; Tavares et al., 2016).

Thus, the use of fast tests to evaluate the physiological quality of seeds becomes a powerful tool for seed companies, enabling the disposal of low-quality lots even before processing, avoiding unnecessary expenses with this operation and storage (Carvalho et al., 2009; Cantareli et al., 2015).

Received date: 2020-03-15 **Accepted date:** 2020-09-13

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New alternatives have been sought for fast seed quality assessment in this context, one of them being the ethanol test.

According to the studies cited above, seeds with a higher degree of deterioration release more ethanol than less deteriorated seeds. This higher release of ethanol in seeds with an advanced state of deterioration can be attributed to the damage to mitochondrial membranes. As a result of this damage, there will be decreases in aerobic respiration, ATP production, and increases in the amount of ethanol released (Reedy and Knapp, 1990).

Thus, there is an interest in searching for alternatives to evaluate the physiological potential of soybean seeds in a short time to facilitate the producer decision-making process at the time programming of soybean seed sales and sowing. Research to develop fast, simple, and efficient soybean seed vigor evaluation is of paramount importance.

The objective of this study was to rank soybean seed lots according to vigor levels by the fast ethanol test following the adapted ethylometer method.

2 Material and methods

2.1 Site description and physiological quality

The work was carried out at the Seed Analysis Didactic Laboratory of the "Eliseu Maciel" School of Agronomy of the Federal University of Pelotas, Brazil. The analysis used five lots of soybean seeds from commercial farms produced in the city of Condor (28°07'58.6"S 53°31'33.0"W) in the Rio Grande do Sul, BR.

The experiment was conducted in a completely randomized design with five treatments (lots) and four replications. The seeds were submitted to germination (G), first germination count (FGC), accelerated aging (AA), field emergence (FE), electrical conductivity (EC), and ethanol tests.

The germination test (G) was conducted using 200 seeds per repetition, totaling four repetitions of the test. The seeds were placed in paper moistened with distilled water equivalent to 2.5 times the dry paperweight. Then the rolls were kept in a germinator at 25°C. The evaluation was performed on the eighth day after sowing, considering the percentage of normal seedlings, according to the Rules for Seed Analysis (RAS) (Brasil, 2009).

The first germination count (FGC) was performed together with the germination test. The percentage of normal seedlings was evaluated five days after paper sowing according to the RAS.

The accelerated aging (AA) test was conducted using 50 seeds per repetition. The seeds were distributed in a single layer on a stainless steel mesh screen (gerbox) containing 40 mL of distilled water in the bottom. Immediately after that, the boxes were capped and kept in a BOD incubator, set at 41°C for 48 hours, according to Marcos (1999). According to the RAS, after the aging period, the seeds were submitted to the germination test, being evaluated at five days, considering the percentage of normal seedlings.

The field emergency test (FE) was performed using 50 seeds per repetition, sown in beds with a depth of approximately three centimeters. Seedling counting was performed 21 days after sowing.

To evaluate electrical conductivity (CE), 25 seeds were used by subsamples for each repetition. The seeds were weighed and then immersed in 80 mL of deionized water for 24 hours in a BOD incubator at 25°C, according to the methodology adjusted by Vieira e Krzyzanowski (1999). After the imbibition period, the electrical conductivity was read in the imbibition solution with a digital conductivity meter. Results were expressed as $\mu S\ cm^{-1}.\ g^{-1}$.

2.2 Ethanol test and data analysis

The ethanol test was performed using 200 seeds per repetition, totaling four repetitions. The seeds were placed in 500 mL PET-type plastic bottles, and then 50 mL of distilled water was added to each bottle. Subsequently, the bottles were adequately closed and taken to BOD incubators for different temperatures and exposure periods: 45°C for 8 hours, 40°C for 24 hours. After the soaking period, ethanol measurements were performed with the aid of an adapted ethylometer (model BFD-60, Instruterm), in which a needle attached to the ethylometer was punctured in the bottles thus enabling the measurements; the results are expressed in $mg\ L^{-1}$.

Data were submitted to analysis of variance ($p < 0.05$) and, when significant, means were compared by the Tukey test at 5% probability. The degree of association between the variables was analyzed by Pearson's simple correlation technique (r) at a 5% probability of error.

3 Results and discussion

3.1 Physiological quality

The germination test results (Table 1) found no difference between the lots analyzed. It is important to note that when working with tests that aim to separate lots for vigor, it is essential that germinations are similar between the lots analyzed.Analyzing the first count test data, it was verified that lot 4 presented a lower percentage of normal seedlings, with 80%, differing from lots 1, 2, and 3, which presented average values between 86% to 87% (Table 1). These results can be considered as an indication that the seeds of lot 4 have lower vigor compared to the other lots. One of the deterioration process events is the reduction in germination speed and the decrease of normal seedlings (Delouche, 2002; Toledo et al., 2009).

For the accelerated aging variable (Table 1), it was found that lots 1 and 2 had the highest vigor values, with an average of 83%. Lot 4 showed intermediate behavior, with average vigor of 78%, and lots 3 and 5 were classified as low vigor compared to the other lots, with 74% and 68% averages.

The study of the correlation between vigor tests and field seedling establishment is often used to validate laboratory tests aiming at vigor detection (Braz and Rossetto, 2009; Leal et al., 2012). In this context, field emergence data showed lots 1 and 2 to be the most vigorous (Table 1), obtaining average percentages of 89% and 87% of emerged seedlings. Lots 3 and 4 presented the lowest vigor levels expressed in the present test, presenting values of 74% and 78% of emerged seedlings, respectively.

Analyzing the results of the electrical conductivity test (Table 1), it was observed that lots 1 and 2 presented higher levels of vigor among the studied lots; this is justified by the fact that they presented the lowest values of electrical conductivity. This result corroborates the data obtained previously with first count, accelerated aging and electrical conductivity. Lots 3 and 5 had the lowest vigor levels, as evidenced by the more significant exudate release. Lot 4 did not differ statistically from the high and low vigor lots, presenting an intermediate vigor level compared to the high and low vigor ones.

The electrical conductivity test is based on the fact that seeds, when soaked in water, exude ions, sugars, and metabolites, especially at the beginning of the soaking period, because of the alteration of the integrity of cell membranes. However, in deteriorated seeds, this mechanism or speed of reorganization is absent or inefficient (Bewley and Black, 1994).

Table 1 Germination (G), first germination count (FGC), accelerated aging (AA), field emergence (FE), and electrical conductivity (CE) of five soybean seed lots

Lots	G	FGC	AA	FE	CE
	----------(%)----------		----------(%)----------		-(μS cm^{-1} g^{-1})-
1	91^{ns}	87 a*	83 a	89 a	92.54 b
2	91	87 a	83 a	87 a	91.49 b
3	89	86 a	74 b	74 c	100.72 a
4	88	80 b	78 ab	78 bc	98.42 ab
5	90	84 ab	68 b	79 abc	102.81 a
Average	85	90	79	81	97
CV (%)	2,51	2,14	5,19	6,23	3,61

Note: * Averages followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability.

3.2 Ethanol test

Analyzing the results of the ethanol test, it was verified that there was ethanol production in all soybean seed lots, independent of the different periods and exposure times analyzed (Figure 1). Importantly, when the environment is under anoxia, plant tissues tend to perform alcoholic fermentation, producing ethanol and CO_2 and oxidizing NADH in the process. Alcohol dehydrogenase and lactate dehydrogenase are essential for operating the glycolytic cycle under anaerobic conditions because they recycle NAD^+, reducing pyruvate to ethanol and lactate, respectively. This

process of ethanol accumulation involves NADH oxidation and results in small but essential ATP production for the survival of some species during the absence of oxygen (Taiz and Zeiger, 2017).

However, regarding the stratification of soybean seed lots, it was not possible to rank the lots at different vigor levels. These results may have been influenced during the measurement process since the ethanol test was performed with adapted equipment, which requires adjustments or even the development of specific equipment to perform the test more safely and reliably.

In this perspective, Maciel et al. (2003) highlighted that the development of sensors of this type was significant for many applications because of their advantages such as small size, high stability, sensitivity, and long service life. In addition, these sensors can integrate various types of equipment for various purposes.

However, although the ethanol test did not rank the lots according to vigor levels, it was observed that lots 3, 4, and 5 released more ethanol when subjected to 45°C for 8 hours. Nonetheless, when these same lots were exposed to a longer exposure time to anaerobiosis for a long time (40°C for 24 hours), there was less ethanol release. The other vigor tests ranked lots 3, 4, and 5 as lower quality lots. It should be noted that the study temperatures were higher than what was recommended for the membrane and cardinal degradation of the species by Marcos-Filho (2015).

Cavalcante et al. (2017), when evaluating the ethanol test in ryegrass seeds, found that the most vigorous seeds maintained ethanol production for a longer period because their reserves had higher amounts of carbohydrates in their reserve tissues. In addition, these same authors report that the seeds of lower vigor initially present higher ethanol production but in a short time when compared to vigorous seeds. This fact is possibly related to the integrity of cell membranes, which lose their selective capacity and allow ethanol to be rapidly released from the cytoplasm into the extracellular environment.

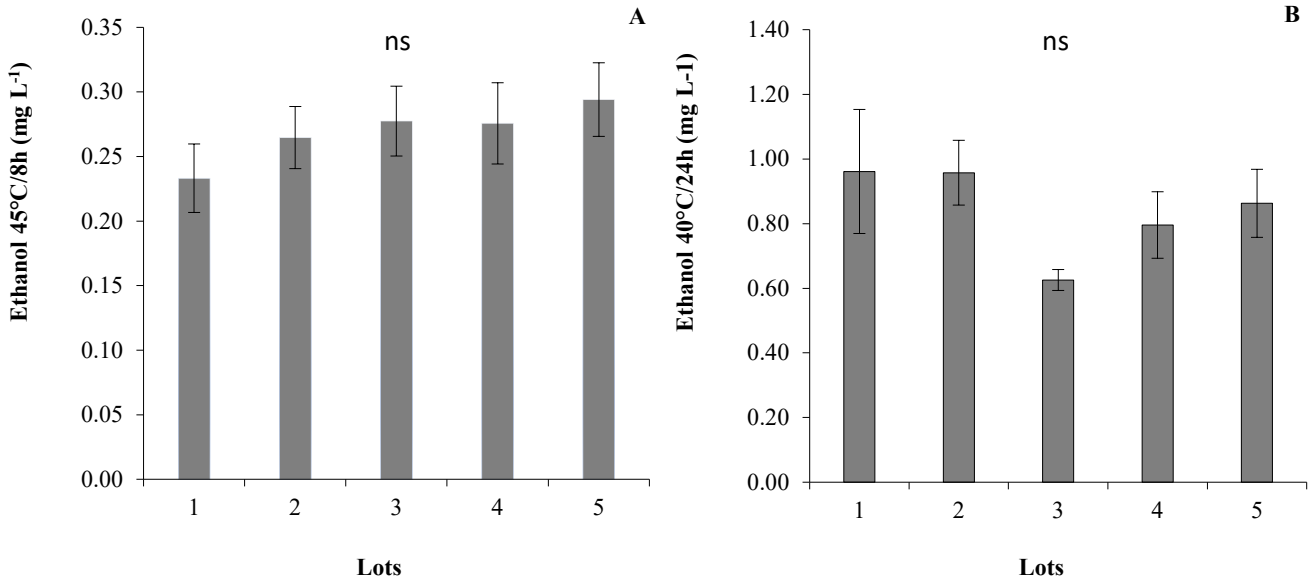

Figure 1 Quantification of Ethanol in soybean seed lots soaked in water at 45°C for 8 h (A) and 40°C for 24 h (B)

3.3 Pearson's correlation

The germination of seeds from lot 1, as well as the first germination count and accelerated aging of seeds from lot 2, were significant according to Pearson's correlation with the ethanol test, in which it was found that germination of lot 1 correlated negatively with the ethanol test at 40°C for 24 h (Figure 2A). For lot 2, a positive correlation was observed for the first germination count (Figure 2B) and negative for accelerated aging (Figure 2C) with the ethanol test at 40°C for 24 h and 45°C for 8 h, respectively.

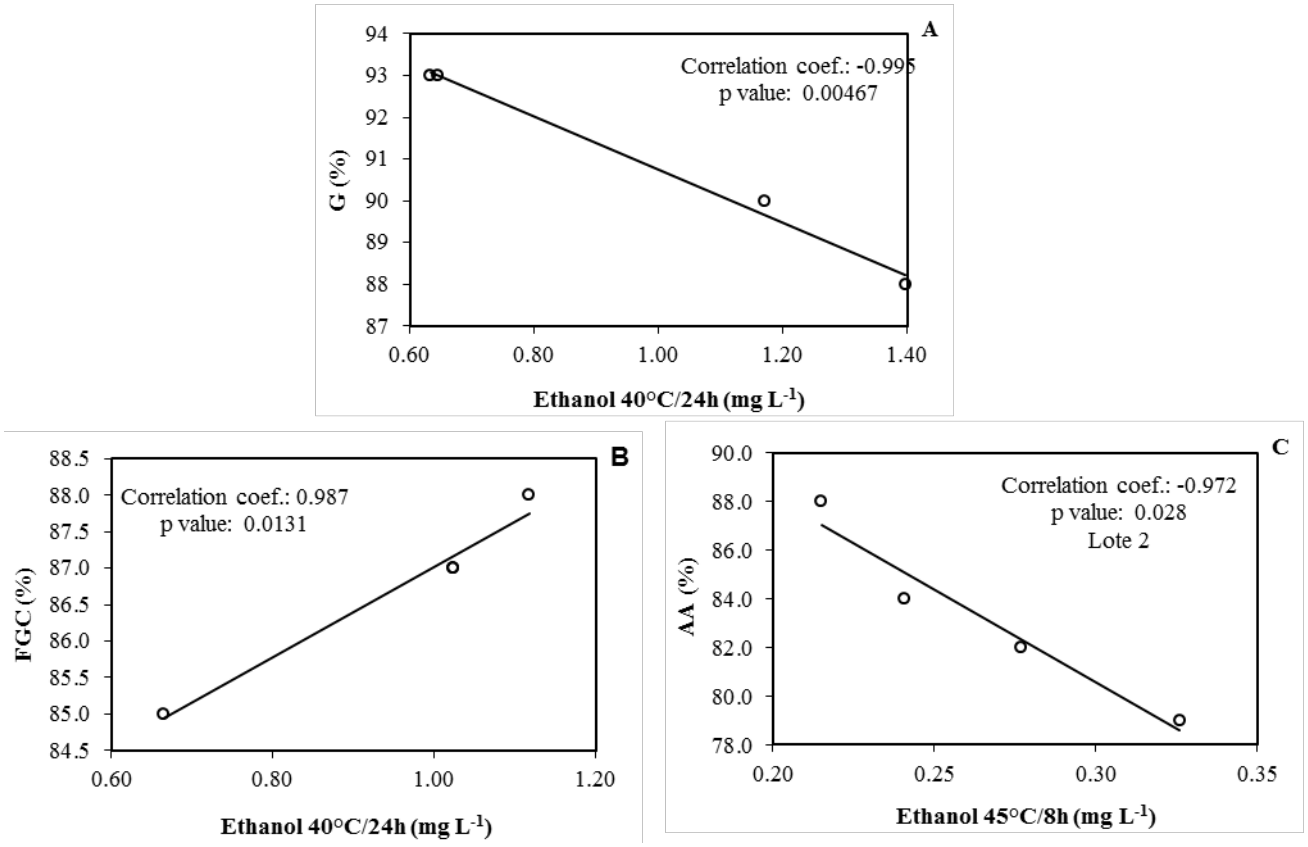

Figure 2 Pearson correlation between variables, germination (A), first germination count (B), and accelerated aging (C) with ethanol test at 40°C for 24 h and 45°C for 8 h for lots of soybean seeds 1 (A) and 2 (B and C)

For lots 4 and 5, a correlation was observed between the first germination count (Figure 3A) and electrical conductivity (Figure 3B) for lot 4, being negative for the first and positive for the second variable with the ethanol test at 40°C for 24 h and 45°C for 8 h, respectively. Lot 5, on the other hand, showed a negative correlation for germination (Figure 3C) and positive for electrical conductivity (Figure 3D), respectively, with 40°C for 24 h and 45°C for 8 h

The positive correlations between electrical conductivity and the ethanol test (Figure 3B and C) are relatively understandable, as seed deterioration is associated with seed exudate concentration in the solution, and these are a reflection of membrane degradation (Copeland and Mcdonald, 1995). It is worth noting that, according to Delouche (2002), membrane damage is the initial event of degenerative changes in seeds. Thus, seeds that have a higher degree of membrane degradation tend to produce higher amounts of ethanol.

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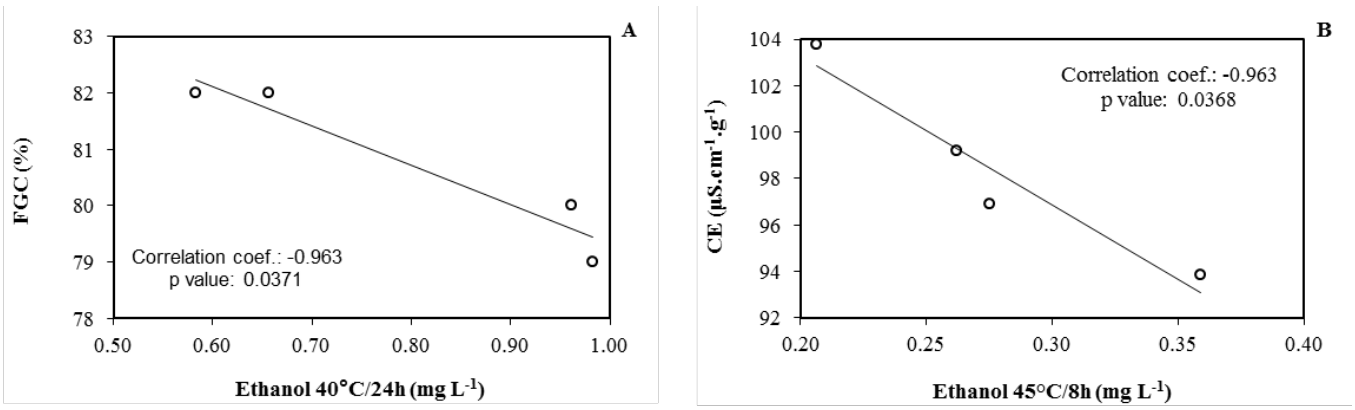

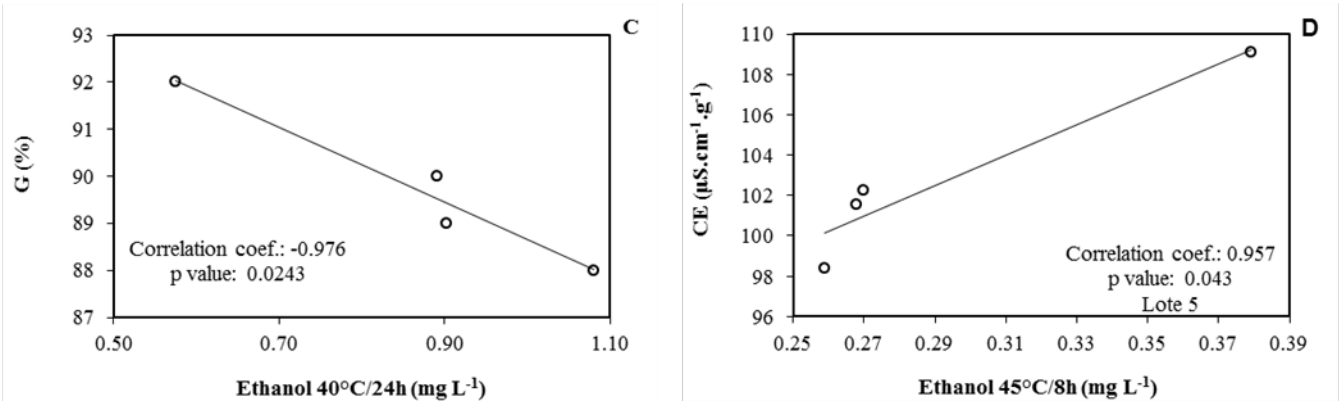

Figure 3 Pearson correlation between the variables first germination count (A), germination (C) and electrical conductivity (B and D) with the ethanol test at 40°C for 24 h and 45°C for 8 h for soybean seed lots 4 (A and B) and 5 (C and D)

3.4 Methodology described for the equipment

The methodology described in this study was created to determine the ethanol content released by soybean seeds to accurately validate the information obtained in conventional tests, considering a significant reduction in the time to obtain the results. However, under the conditions of this work, it was not possible to obtain satisfactory results for soybean seeds, demonstrating a need for further studies related to the ethanol test for this species. So far, there have been studies with canola (Buckley and Buckley, 2009; Buckley and Huang, 2011; Buckley, 2013), cabbage (Bicanic et al., 2003; Rutzke et al., 2008; Kodde et al., 2011), ryegrass (Cavalcante et al., 2017), quinoa (Vergara et al., 2018), corn (Onwimol et al., 2019) and cowpea (Cavalcante et al., 2019).

Therefore, more work is needed with species with a higher chemical composition of carbohydrates than lipids, which may be an essential difference in the test procedure because the syntheses of reserves are different. However, Marinho et al. (2019) concluded that the test evaluation of vigor and tolerance of sweet corn seeds under hypoxia was not efficient for classifying sweet corn seed lots regarding vigor.

Marcos (2015) also argued that natural anaerobiosis depended on the species at the beginning of the imbibition, producing ethanol and lactic acid, which then metabolized lactate to pyruvate and acetate to acetyl CoA, which might have occurred in this species. It is also possible that the equipment only read the high physiological quality of the lots at this stage, not differentiating the lots previously, and that the environment, temperature x time was not efficient for this reading.

Finally, there is a need for further studies and adjustments to the efficiency of such a procedure or more equipment adapted for seed analysis.

4 Conclusions

It is possible to quantify ethanol in soybean seed lots using the adapted ethylometer equipment. However, the adapted equipment is not efficient in classifying soybean seed lots into different vigor levels, which requires adjustments or even the development of specific equipment to perform the test more safely and reliably. This implies that more work is needed with species with a higher chemical composition of carbohydrates than lipids, which may be an important difference in the test procedure because the syntheses of reserves are different, demonstrating a need for further studies of ethanol test for this species.

Acknowledgment

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001 and by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) Project 425399/2016-8.

References

Bagateli, J. R., C. S. Dörr, L. O. B. Schuch, and G. E. Meneghello. 2019. Productive performance of soybean plants originated

from seed lots with increasing vigor levels. *Journal of Seed Science,* 41(2):151-159.

Bicanic, D., S. Persijn, A. Taylor, J. Cozijnsen, B. Van Veldhuyzen, G. Lenssen, and H. Wegh. 2003. Detection of ethanol and acetaldehyde released from cabbage seeds of different quality: laser photoacoustic spectroscopy versus ftir and headspace gas chromatography. *Review of Scientific Instruments,* 74(1): 690-693.

Bewley, J. D. and M. Black. 1994. *Seeds: Physiology of Development and Germination.* **2nd ed**. New York: Plenum Press.

Brasil, Ministério da Agricultura, Pecuária e Abastecimento 2009. *Regras para Análises de Sementes*. Brasília: Mapa/ACS.

Braz, M. R. S., and C. A. V. Rossetto. 2009. Correlação entre testes para avaliação da qualidade de sementes de girassol e emergência das plântulas em campo. *Ciência Rural*, 39(7): 2004-2009.

Buckley, W. T., and K. E. Buckley. 2009. Low-molecular-weight volatile indicators of canola seed deterioration. S*eed Science and Technology,* 37(3): 676-690.

Buckley, W. T., and J. Huang. 2011. An ethanol-based seed vigour assay for canola. *Seed Science and Technology,* 39 (2): 510-526.

Buckley, W. T. 2013. An on-farm ethanol-based color test for canola seed vigor. *Journal of Crop Improvement*, 27(4): 376-390.

Cantarelli, L. D., L. O. B. Schuch, L. C. Tavares, and C. A. Rufino. 2015 Variabilidade de plantas de soja originadas de sementes de diferentes níveis de qualidade fisiológica. *Acta agronómica*, 64(3): 234-238.

Carvalho, L. F., C. S. Sediyama, M. S. Reis, D. C. F. S. Dias, and M. A. Moreira. 2009. Influência da temperatura de embebição da semente de soja no teste de condutividade elétrica para avaliação da qualidade fisiológica. *Revista Brasileira de Sementes*, 31(1):9-17.

Cavalcante, J. A., R. M. Ternus, T. L. Almeida, F. Reolon, A. S. Araújo, G. I. Gadotti, and D. M. Moraes. 2017. Ethanol vigor test to assess physiological quality of annual ryegrass seeds. *Revista Espacios*, 38(47): 10-19.

Cavalcante, J. A., G. I. Gadotti, R. M. Ternus, F. M. Xavier, R. N. O. Silva, P. E. O. R. Eberhardt, A. B. N. Martins, N. F. Hornke, A. S. Mazon, and L. V. M. Tunes. 2019. Evaluation of ethanol preconditioning as a rapid seed vigour and viability test. *Australian Journal of Crop Science*, 13(7): 1835-2707.

Conab. – Companhia Nacional de Abastecimento 2019. *Acompanhamento safra brasileira de grãos,* 6 (7), safra 2018/19, sétimo levantamento.

Copeland, L. O., and M. B. Mcdonald.1995. P*rinciple of Seed Science and Technology*. New York: Chapmen and Hall.

Delouche, J. C. 2002. Germinacion, deterioration y vigor de semilla. *Seed News*, 6(6): 16-20.

Fessel, S. A., M. Panobianco, C. R. de Souza, and R. D. Vieira. 2010. Teste de condutividade elétrica em sementes de soja armazenadas sob diferentes temperaturas. *Bragantia*, 69(1): 207-214.

França Neto, J. B., A. A. Henning, F. C. Krzyzanowski, and I. Lorini. 2015. Diacom: 35 anos de capacitação nos testes de tetrazólio e patologia de sementes de soja. *Revista Seed News*, 19(5): 30-35.

Kodde, J., W. T. Buckley, C. C. Groot, M. Retiere, A. M. V. Zamora, and S. P. C. Groot. 2012. A fast ethanol assay to detect seed deterioration. *Seed Science Research*, 22(1): 55-62.

Leal, C. C. P., S. B. Torres, N. W. Nogueira, V. E. Tomczak, and C. P. Benedito. 2012. Validação de testes de vigor para sementes de rúcula (*Eruca sativa L.*). *Revista Brasileira de Biociências*, 10(3): 421-424.

Maciel, A. P., F. Paro, and E. R. E. Leite. 2003. Longo dióxido de estanho nano estruturado como sensor de nox (nano structured tin dioxide as a nox gás sensor). *Cerâmica*, 49(1): 163-167.

Marcos, F. J. 1999. Teste de envelhecimento acelerado. In *Vigor de Sementes: Conceitos e Testes*, eds. F. M. C. Krzyzanowski, R. D. Vieira, J. B. França Neto. Londrina: Abrates.

Marcos-Filho, J. 2015. *Fisiologia de Sementes de Plantas Cultivadas.* 2nd ed., 660p. Londrina: ABRATES.

Marinho, J. de L., D. S. da Costa, D. U. de Carvalho, M. A. da Cruz, and C. Zucareli. 2019. Evaluation of vigor and tolerance of sweet corn seeds under hypoxia. *Journal of Seed Science*, 41(2): 180-186.

Onwimol, D., T. Rongsangchaicharean, P. Thobunluepop, T. Chaisan, and W. Chanprasert. 2019 Optimization and sensitivity analysis of fast ethanol assay in maize seed. *Journal of Seed Science*, 41(1): 97-107.

Reedy, M. E., and A. D. Knapp. 1990. Ethanol evolution during the early germination of artificially aged soybean seeds. *Journal of Seed Technology*, 14(2): 74-82.

Rutzke, C. F. J., A. G. Taylor, and R. L. Obendorf. 2008. Influence of aging, oxygen, and moisture on ethanol production from cabbage seeds. *Journal of the American Society for Horticultural Science*, 133(1): 158-164.

Taiz, L., E. Zeiger, I. M. Moller, and A. Murphy. 2017. P*hysiology and Plant Development*. **6th ed**. Artmed: Porto Alegre.

Tavares, L. C., A. O. Mendonça, G. I. Gadotti, and F. A. Villela. 2016. Estratégias de marketing na área de sementes. *Arquivos do Instituto Biológico*, 83: 1-9.

Toledo, M. Z., N. R. Fonseca, M. L. Cézar, R. P. Soratto, C. Cavariani, and C. A. Crusciol. 2009. Qualidade fisiológica e armazenamento de sementes de feijão em função da aplicação tardia de nitrogênio em cobertura. *Pesquisa*

AgropecuÁRia Tropical, 39(2): 124-133.

USDA. 2019. *World Agricultural Production*. Washington: USDA.

Vergara, R. O., V. N. Soares, R. N. O. Silva, A. B. N. Martins, G. I. Gadotti, and F. A.Villela. 2018. Teste de quantificação de etanol para avaliação da qualidade fisiológica sementes de quinoa. *Colloquium Agrariae*, 14(2):143-148.

Vieira, R. D., and F. C. Krzyzanowski. 1999. Teste de condutividade elétrica. In Vigor de Sementes: Conceitos e Testes, eds. F. C. Krzyzanowski, R. D. Vieira, and J. B. França-Neto, Londrina:Abrates,1-26. Londrina:Abrates