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Physiological performance of soybean seeds in the accelerated aging test and their germination after several waiting periods

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ABSTRACT: The accelerated aging test is widely used to evaluate the vigor of soybean seeds. Currently, the methodology recommended in the literature is to set up the germination test at most one hour after the seed aging process, which makes it difficult to set up and evaluate a large number of samples. The aim of this study was to assess the effect of delay in setting up the germination test after the process of accelerated aging of soybean seeds, specifically its effect on seed physiological performance. We tested three seed lots of the soybean cultivars BRS 397, BRS 399 RR, BRS 1007 IPRO, BRS 1010 IPRO, and BRS 388, all within the standards required for commercialization. The seed lots were physiologically characterized by evaluation of moisture content, germination, seedling vigor classification, tetrazolium test (viability and vigor) and accelerated aging test. The treatments consisted of hours (0, 1, 2, 3, 4, 5, 6, 7, and 8) of delay in setting up the germination test after accelerated aging. A delay of up to eight hours in setting up the germination test after the accelerated aging process of soybean seeds has no direct relationship with the result of assessment of the physiological performance of the seed lot.

Index Terms: analysis, *Glycine max* (L.) Merrill, seed vigor test.

RESUMO: O teste de envelhecimento acelerado é muito utilizado para avaliar o vigor de sementes de soja. Atualmente, a literatura recomenda a instalação do teste de germinação em, no máximo, uma hora após o processo do envelhecimento das sementes, o que dificulta a instalação e avaliação de grande número de amostras. Dessa maneira, objetivou-se avaliar o efeito do atraso para a instalação do teste de germinação após o processo de envelhecimento acelerado de sementes de soja sobre o seu desempenho fisiológico. Foram utilizados três lotes de sementes das cultivares BRS 397, BRS 399 RR, BRS 1007 IPRO, BRS 1010 IPRO e BRS 388, todas dentro dos padrões exigidos para comercialização. Os lotes foram caracterizados fisiologicamente pela avaliação de suas sementes quanto ao grau de umidade, germinação, avaliação de vigor das plântulas, teste de tetrazólio (viabilidade e vigor) e teste de envelhecimento acelerado. Os tratamentos avaliados foram compostos por tempos (0, 1, 2, 3, 4, 5, 6, 7 e 8 horas) de atraso para a instalação do teste de germinação, após as sementes terem sido submetidas ao teste de envelhecimento acelerado. Os resultados obtidos permitiram concluir que o atraso por até oito horas para a instalação do teste de germinação, após o processo do envelhecimento acelerado de sementes de soja, não tem relação direta com o desempenho fisiológico dos lotes.

Termos para indexação: análise, *Glycine max* (L.) Merrill, teste de vigor.

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INTRODUCTION

Success in obtaining a crop field with an adequate population of plants depends on the use of seeds with high physiological potential, which is evaluated by means of germination and vigor tests. The germination test often used, however, is performed under ideal conditions, and the results obtained do not always reflect the potential of the seed lot under field conditions (Ohlson et al., 2010). In contrast, vigor tests are more sensitive in identifying less advanced stages of seed deterioration, which assists in decision making regarding the destination of seed lots (Pinto et al., 2015). The accelerated aging test is widely used throughout the world to compose quality control programs established by the seed industry, especially for evaluation of the physiological potential of seeds for major cash crops and vegetable crops. It provides valuable information regarding vigor differences among the samples analyzed, storage potential, and seedling emergence in the field. It is a test that has had the validation of the International Seed Testing Association (ISTA) for several years, and it is recommended by the Association of Official Seed Analysts (AOSA, USA). After the tetrazolium test, it is the test most used to evaluate seed vigor, both in the USA and in Brazil (Marcos-Filho, 2020).

The principle of this test consists in increasing the deterioration rate of seeds by exposing them to high temperature and relative humidity, which are considered the environmental factors that most affect the intensity and speed of seed deterioration (TeKrony and Egli, 1995; Marcos-Filho, 2020). It is a vigor test widely used in quality control programs in seed production companies because, in a few days, the storage potential of the processed seed lots can be estimated, and depending on the history of the lot, the performance of these seeds in the field (Marcos-Filho, 2020). In addition, researrchers have evaluated the efficiency of the accelerated aging test to determine the physiological potential of seeds with differente chemical treatments (Santos et al., 2021).

In relation to the methodology of the accelerated aging test, the seeds are to be tested for germination in at most one hour after the end of the aging period, to avoid additional lack of uniformity (Baalbaki et al., 2009; ISTA, 2020; Marcos-Filho, 2020). However, this recommendation is a limiting factor for carrying out the test when tests of many samples must be set up and analyzed. The effect of periods greater than those currently recommended for setting up the germination test should be evaluated, aiming at obtaining more reliable information.

Considering this limitation, the aim of this study was to evaluate the effect of delay in setting up the germination test after the accelerated aging process of soybean seeds regarding seed physiological performance.

MATERIAL AND METHODS

Five soybean cultivars were used (BRS 397, BRS 1007 IPRO, BRS 388, BRS 399 RR, and BRS 1010 IPRO), each one represented by three seed lots that had not received any chemical treatment and that had a germination percentage greater than the minimum established for commercialization of soybean seeds in Brazil (G ≥ 80%), as shown in the results in Table 1. Each seed lot was divided into four replications of 6 kg using a Boerner sample divider. Before the tests were conducted, the seeds were stored in cotton bags in cold and dry storage (10 °C and 50% RH).

The treatments evaluated were composed of hours (0, 1, 2, 3, 4, 5, 6, 7, and 8) of delay in setting up the germination test after the seeds had gone through accelerated aging.

The initial quality of the seed lots was evaluated through the following determinations:

Moisture content: determined by the laboratory oven method at 105 °C (± 3 °C) for 24 hours, according to the Rules for Seed Testing (Brasil, 2009), with two 5-g subsamples of seeds for each lot. The results were expressed in mean percentage (wet basis) per lot. This determination was also made after exposure of the seeds to the conditions of the accelerated aging test for the purpose of evaluating what moisture content the seeds reached after the aging period, since this is an important parameter in checking the stress imposed.

Germination: evaluated in four 50-seed subsamples per replication and per treatment, for a total of 800 seeds sown in rolls of paper towel moistened with water in the amount of 2.5 times the weight of the dry paper, at 25 °C. The results were interpreted according to the criteria established in the Rules for Seed Testing (Brasil, 2009). The results were expressed in mean percentage of normal seedlings for each lot.

Table 1. Initial results obtained in the tests of germination, seedling vigor classification, accelerated aging (A.A.), and tetrazolium (vigor and viability) carried out in soybean seeds of the cultivars BRS 397, BRS 1007 IPRO, BRS 388, BRS 399 RR, and BRS 1010 IPRO, conducted on three seed lots per cultivar.

Cultivar	Lot	Initial moisture content	Germination	Seedling vigor classification		A.A.	Tetrazolium test	
				Strong	Weak		Vigor	Viability
			%					
BRS 397	1	11.1	91 ab	56 a	35 ab	81 b	85 b	92 a
	2	11.1	88 b	59 a	29 b	77 b	87 ab	95 a
	3	11.1	94 a	58 a	36 a	88 a	91 a	96 a
	CV (%)	--	2.6	7.7	10.0	2.7	2.6	2.5
BRS 1007 IPRO	1	11.2	88 a	68 a	20 a	79 b	78 a	93 a
	2	11.1	91 a	76 a	15 a	87 a	79 a	92 a
	3	11.1	90 a	76 a	14 a	86 a	78 a	94 a
	CV (%)	--	2.9	8.0	34.4	3.0	2.7	2.1
BRS 388	1	10.8	91 b	66 a	25 a	81 b	80 a	91 a
	2	10.5	94 ab	69 a	25 a	90 a	85 a	94 a
	3	10.9	95 a	71 a	24 a	90 a	85 a	93 a
	CV (%)	--	1.8	4.5	15.9	3.9	4.1	2.6
BRS 399 RR	1	10.7	95 a	76 a	19 a	83 a	92 a	97 a
	2	10.9	91 b	67 b	24 a	79 a	89 a	95 a
	3	10.8	91 b	71 ab	20 a	81 a	89 a	95 a
	CV (%)	--	1.8	4.7	15.1	4.3	2.9	2.4
BRS 1010 IPRO	1	10.9	94 a	82 a	12 b	87 b	84 b	94 a
	2	11.1	86 b	71 b	15 b	85 b	76 c	87 b
	3	11.0	97 a	76 ab	21 a	93 a	90 a	96 a
	CV (%)	--	1.9	4.8	19.7	2.3	2.6	2.5

Within each cultivar, mean values followed by different letters in the columns differ from each other by Tukey's test at 5% probability. CV: coefficient of variation.

Seedling vigor classification: performed together with the germination test, four 50-seed subsamples were used per replication and per treatment, for a total of 800 seeds evaluated. The normal seedlings were classified as strong (high vigor) or weak (low vigor), the latter being those that had some problem in their structure or that had injuries, but for which the problem was not characterized as an abnormality (Krzyzanowski et al., 2020). The results were expressed in mean percentage of "strong" seedlings.

Tetrazolium: two 50-seed subsamples were used per replication and per treatment, for a total of 400 seeds evaluated. The seeds were pre-conditioned in paper towel previously moistened with water in the amount of 2.5 times the weight of the dry paper and then kept in a seed germinator at 25 °C for 16 h. After that, the samples were placed in plastic cups containing 50 mL of 2,3,5-triphenyl tetrazolium chloride solution at 0.075% and kept in a laboratory oven at 40 °C for three hours for the development of staining (França-Neto and Krzyzanowski, 2018). At the end of that period, the seeds were washed in running water and remained submersed in water in a refrigerator at 5 °C up to the time of evaluation. The test was interpreted according to the seed classification proposed by Moore (1967) and adapted by França-Neto et al. (1988), in which each seed is placed in classes from 1 to 5 when viable, and from 6 to 8 if not viable.

The level of viability is identified according to increasing order of "defects" in the seeds. To estimate the vigor level, the percentage values of seeds included in classes 1 to 3 is taken into consideration (França-Neto and Krzyzanowski, 2018).

Accelerated aging: before carrying out the accelerated aging test, the seeds were pre-conditioned in plastic boxes (11 cm × 11 cm × 3 cm) containing 40 mL of water (to obtain approximately 100% RH) with a metallic screen suspended inside, on which the seeds were placed in a single layer for a period of 16 h at 25 °C (Rossetto et al., 1995; Brasil, 2009; França-Neto et al., 2014), so as to standardize the seed moisture content at 13%, avoiding possible damage through imbibition. After the pre-conditioning, the seeds were found to have reached suitable moisture content, without variation greater than 2.0 percentage points among the samples. Uniformity of the moisture content of the seeds at 13% is essential for standardization of the evaluations and obtaining consistent results. Seed samples with very different moisture contents will absorb water at different speeds, leading to differences in the intensity of deterioration (Marcos-Filho, 1999; 2020). After pre-conditioning, the seeds were kept in the boxes to be aged, since each replication per treatment had already been arranged in separate boxes. Accelerated aging was carried out in a accelerated aging chamber at 41 °C; the boxes containing the seeds were arranged within this chamber, where they remained for 24 hours. At the end of the established aging period, the boxes with the seeds were removed from the aging chamber, kept unopened, where they remained until the time of setting up the germination test. The period of 24 hours of aging was selected as recommended by Costa et al. (1984) and França-Neto et al. (2003), since this test was performed in the soybean sowing season. The germination test was conducted on specific paper toweling, at 25 °C, and evaluation was made on the fifth day after setting up the test; the mean percentage of normal seedlings was calculated for each seed lot. One cultivar was evaluated per day, thus setting up 48 rolls for germination per hour (1 cultivar × 3 lots × 4 replications × 4 subsamples) over nine periods (hours), which allowed the germination test to be carried out in an adequate manner for the aim of the present study. The samples remained under controlled and similar conditions throughout the period of carrying out the experiment.

Analysis of variance was applied to the data of initial physiological quality of the lots evaluated in a completely randomized design using the SASM-Agri software (Canteri et al., 2001). The mean values were compared by Tukey's multiple comparison test ($p \leq 0.05$). Regression analysis was applied to the data of moisture content and of germination obtained for the seeds of each cultivar and respective lots after the different periods of time (hours) until setting up the germination test, using the "reg" procedure of the SAS/STAT system software, Version 9.4© 2016 SAS Institute Inc.

RESULTS AND DISCUSSION

Initial physiological characterization of the seed lots used was performed by the tests of germination, seedling vigor classification, accelerated aging, and tetrazolium (vigor and viability), according to the methods described.

These tests showed that all the lots used in the study exceeded the minimum germination established for commercialization of soybean seeds in Brazil (G ≥ 80%), with values ranging from 86% to 97% (Table 1). In general, all the seed lots had high physiological potential; this evaluation was necessary for the selection of materials that reflect the reality of seed production companies (França-Neto et al., 2019): strong seedling indices determined by the seedling vigor classification test ranged from 56% to 82%; germination after the accelerated aging test ranged from 77% to 93%; vigor determined by the tetrazolium test, from 76% to 92%; and viability, from 87% to 97%.

The regression equations referring to seed moisture content after exposure to the conditions of the accelerated aging test over the different periods (hours) of delay until setting up the germination test are shown in Table 2. For most situations, the regression equations did not show significant linear or quadratic effects (omitted from Table 2), which means that the seed moisture content varied approximately in the same range during the periods of delay in setting up the germination test. Nevertheless, significant values in these coefficients were found in six lots: lots 1 and 2 of the cultivar BRS 1007 IPRO; lots 1, 2, and 3 of the cultivar BRS 399 RR; and lot 2 of the cultivar BRS 1010 IPRO. This means that, in those situations, there was an increase in the seed moisture content during the periods of delay in setting up

Table 2. Regression equations between seed moisture content (%) versus nine periods to begin setting up the germination test (h) in reference to seeds of five soybean cultivars, with three seed lots per cultivar.

Cultivar	Lot	Regression equations: Moisture content (%) × Time (h)
BRS 397	1	$Y = 24.72 + 0.007X$; $R^2 = 0.0069^{ns}$
	2	$Y = 25.09 + 0.043X$; $R^2 = 0.0646^{ns}$
	3	$Y = 24.92 - 0.018X$; $R^2 = 0.0101^{ns}$
BRS 1007 IPRO	1	$Y = 23.63 + 0.173X$; $R^2 = 0.7875^{***}$
	2	$Y = 22.55 + 0.175X$; $R^2 = 0.7516^{**}$
	3	$Y = 24.12 + 0.032X$; $R^2 = 0.0325^{ns}$
BRS 388	1	$Y = 23.99 + 0.114X$; $R^2 = 0.2050^{ns}$
	2	$Y = 22.83 + 0.146X$; $R^2 = 0.4482^{ns}$
	3	$Y = 23.65 + 0.139X$; $R^2 = 0.4037^{ns}$
BRS 399RR	1	$Y = 23.18 + 0.420X$; $R^2 = 0.5436^{***}$
	2	$Y = 22.60 + 0.489X$; $R^2 = 0.7803^{**}$
	3	$Y = 24.42 + 0.190X$; $R^2 = 0.1896^{*}$
BRS 1010 IPRO	1	$Y = 23.83 - 0.0001X$; $R^2 = 0.0000^{ns}$
	2	$Y = 23.37 + 0.133X$; $R^2 = 0.6498^{**}$
	3	$Y = 23.86 + 0.039X$; $R^2 = 0.0741^{ns}$

Levels of significance of the coefficients of determination: ns = not significant; * = significant at 5.0%; ** = significant at 1.0% probability; *** = significant at 0.1% probability.

the germination tests. However, on average, the moisture content for these six lots rose only 1.9 percentage points, that is, from 23.1% to 25.0% (data not shown). For purposes of information, for all the 15 lots evaluated, this parameter rose, on average, from 23.7% to 24.7%, that is, 1.0 percentage point over the eight hours of delay until setting up the tests (data not shown).

Even for the situations in which there was an increase in the seed moisture content, these values were within the limits of tolerance for, according to Marcos-Filho (2015; 2020) and Baalbaki et al. (2009), variations of up to two percentage points in seed moisture content after accelerated aging are considered tolerable regarding the reliability of the results. In the same way, before carrying out the accelerated aging test, it is recommended that there not be differences greater than 2 percentage points in the seed moisture content (Dutra and Vieira, 2004; Coimbra et al., 2009; Marcos-Filho et al., 2009; Silva and Martins, 2009).

In the regression analyses of the germination data over the waiting periods until setting up the test (Table 3), the coefficients of determination (R^2) of the equations were not statistically significant for most of the lots evaluated, except for lots 2 and 3 of the cultivar BRS 1007 IPRO. The values of germination for the three lots of this cultivar are shown in Table 4. In spite of this statistical significance detected among the germination values and for the regression equations in reference to lots 2 and 3, it should be highlighted that statistical differences were not detected among the germination percentages for the periods of 0 to 7 hours of delay in setting up the test (Table 4). Comparing the values obtained after seven hours of delay with those of eight hours of delay, these differences were minimal: 3.0 percentage points for lot 2 (from 81% to 78%) and 1.0 percentage point for lot 3 (from 80% to 79%). It should be highlighted that for

Table 3. Regression equations between the germination percentage (%) versus nine periods to begin setting up the germination test (h) in reference to seeds of five soybean cultivars, with three lots per cultivar.

Cultivar	Lot	Regression equations: Germination (%) × Time (h)
BRS 397	1	$Y = 82.25 + 0.083X$; $R^2 = 0.0160^{ns}$
	2	$Y = 80.44 – 0.467X$; $R^2 = 0.2568^{ns}$
	3	$Y = 85.50 – 0.100X$; $R^2 = 0.0125^{ns}$
BRS 1007 IPRO	1	$Y = 80.97 – 0.017X$; $R^2 = 0.0007^{ns}$
	2	$Y = 87.44 – 0.733X$; $R^2 = 0.5418^{*}$
	3	$Y = 88.78 – 0.933X$; $R^2 = 0.8311^{***}$
BRS 388	1	$Y = 69.86 + 0.450X$; $R^2 = 0.1540^{ns}$
	2	$Y = 89.06 – 0.367X$; $R^2 = 0.2556^{ns}$
	3	$Y = 88.33 + 0.067X$; $R^2 = 0.0167^{ns}$
BRS 399 RR	1	$Y = 81.33 + 0.200X$; $R^2 = 0.0500^{ns}$
	2	$Y = 75.19 + 0.450X$; $R^2 = 0.1443^{ns}$
	3	$Y = 83.81 – 0.517X$; $R^2 = 0.3107^{ns}$
BRS 1010 IPRO	1	$Y = 87.86 – 0.2833$; $R^2 = 0.2643^{ns}$
	2	$Y = 82.94 + 0.033X$; $R^2 = 0.0017^{ns}$
	3	$Y = 83.94 + 0.367X$; $R^2 = 0.2039^{ns}$

Levels of significance of the coefficients of determination: ns = not significant; * = significant at 5.0%; ** = significant at 1.0% probability; *** = significant at 0.1% probability.

most of the lots evaluated, differences were not found among the germination results comparing the periods studied.

Even though some minimal differences were observed among the germination values, using maximum periods of delay for setting up the germination test (eight hours after exposure to the period of accelerated aging), a relationship between germination percentage and the delay (in hours) until setting up the germination test after the period of seed aging performed for 24 hours was not established. However, the period from two to eight hours exceeds the recommendation that, after aging, the germination test should be set up in at most one hour. New tests can be conducted to evaluate the results obtained from seed samples aged for 48 hours, but this result is a good initial indication for taking better advantage of the accelerated aging chamber in seed analysis laboratories used to evaluate vigor in a large number of seed samples.

CONCLUSIONS

The results obtained show that delay of up to eight hours in setting up the germination test after accelerated aging of soybean seeds at 41 °C for 24 hours is not directly related to the physiological performance of the seed lots evaluated.

Table 4. Germination percentage of soybean seeds from three seed lots of BRS 1007 IPRO aged and placed to germinate after nine periods (hours) of delay until setting up the germination test.

Cultivar	Lot	Time (h)									CV (%)
		0	1	2	3	4	5	6	7	8	
BRS 1007 IPRO	1^{ns}	80 a	80 a	82 a	83 a	79 a	81 a	82 a	83 a	78 a	3.47
	2**	85 a	84 ab	86 a	87 a	85 a	84 ab	84 ab	81 ab	78 b	3.50
	3*	87 a	86 ab	86 ab	86 ab	85 ab	84 ab	84 ab	80 ab	79 b	3.37

ns – not significant, ** and * - significant at 1% and 5% probability, respectively. Mean values followed by different letters in the rows differ from each other by Tukey's test at 5% probability. CV: coefficient of variation.

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