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EXUDATE pH AND FLOODING TESTS TO EVALUATE THE PHYSIOLOGICAL QUALITY OF SOYBEAN SEEDS[1]

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ABSTRACT - The present study aimed to assess the variations in the parameters of the exudate pH and flooding tests to evaluate the physiological quality of soybean seeds. We subjected the lots of soybean seeds to germination, emergence, exudate pH, and flooding tests. Further, water content of the seeds, first count of germination, and emergence speed index were also determined. The exudate pH test studied the variations in the soaking period and temperature, while the flooding test assessed the variations in the amount of water and temperature at different periods of immersion. The experiment design was completely randomized. The results were presented as means and were compared using the Scott–Knott test at 5% probability. The Pearson's correlation coefficient between the exudate pH and emergence of seedlings, and also between the flooding test result and emergence of seedlings were calculated. The results of the study indicated that the exudate pH test can be used to evaluate the physiological quality of soybean seeds after 30 min of imbibition at 20 °C. Further, the flooding test was efficient in evaluating the vigor of soybean seeds after 4 h of immersion either in 50 mL of distilled water at 25 °C or in 75 mL of distilled water at 30 °C.

Keywords: *Glycine max*. Viability. Vigor.

TESTE DO pH DO EXSUDATO E ALAGAMENTO PARA AVALIAR A QUALIDADE FISIOLÓGICA DE SEMENTES DE SOJA

RESUMO - O trabalho teve como objetivo estudar variações nos parâmetros envolvidos no teste do pH do exsudato e alagamento para caracterização da qualidade fisiológica de sementes de soja. Inicialmente, foi determinado o teor de água e realizados os testes de germinação, primeira contagem, emergência e índice de velocidade de emergência. No teste do pH do exsudato foram estudadas variações no período de embebição e temperatura; e no teste de alagamento foram estudadas variações na quantidade de água e temperatura. O experimento foi realizado em delineamento inteiramente casualizado com quatro repetições, e as médias comparadas pelo teste de Scott-Knott a 5% de probabilidade. Foi estimado o coeficiente de correlação de Pearson entre os testes do pH do exsudato e alagamento com a emergência de plântulas. O teste do pH do exsudato pode ser utilizado na avaliação do potencial fisiológico de sementes de soja, sendo que este deve ser realizado na temperatura de 20 ºC por 30 minutos de embebição. O teste de alagamento é eficiente na avaliação do vigor de sementes de soja, podendo ser realizado nas combinações 25 ºC/50 mL ou 30 ºC/75 mL, durante 4 h.

Palavras-chave: *Glycine max*. Viabilidade. Vigor.

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INTRODUCTION

The speed at which trustworthy results are obtained is one of the important factors considered while evaluating seed quality. It allows agility in decision making, enables its use on a broader scale, reduces the risks and costs involved in operations, such as harvesting, processing, storage, and marketing (REIS et al., 2010; BARBIERI et al., 2012; HILST et al., 2012). Therefore, rapid tests are essential for the evaluation of viability of soybean (*Glycine max*) seeds as the physiological qualities of the seeds are expressed with greater precision within a certain period.

Despite the differences in their techniques, vigor tests, in general, are intended to detect significant variations in the physiological qualities of marketable lots of seeds. These tests help in classifying seeds based on levels of vigor, especially in the proportion of seedling emergence in the field (MARCOS-FILHO, 2015).

The evaluation of viability of seeds by exudate pH test is inexpensive as well as promising when compared with that of the tetrazolium test. The exudate pH test can be applied easily, and the results obtained are at a faster rate. It avoids the use and/or unnecessary storage of seed lots with lower vigor (RAMOS et al., 2012). However, some researchers have highlighted the importance of developing and/or modifying these rapid vigor tests for different species, since the evaluation of seed quality depends on the efficiency of the procedures involved (LOPES; SILVA; VIEIRA, 2013).

Flooding of seeds is a simple, yet promising test (CUSTÓDIO et al., 2002) to identify seed lots that can withstand injury by rapid imbibition. When seeds are soaked in water, oxygen available to them is greatly reduced, and a large quantity of water quickly enters the seed, thus, injuring the seed by imbibition. The seed with injury has less energy for germination, resulting in lower vigor of the seed.

There is limited information available on vigor tests. There is also a need for standardization of such tests to evaluate the quality of seed lots in a simpler way. In this milieu, the present study was conducted to assess the variations in the parameters of the exudate pH and flooding tests and the combinations of these parameters to differentiate lots of soybean seeds with different levels of vigor.

MATERIAL AND METHODS

The experiment was carried out at the Laboratory of Seed Technology, Federal University of Mato Grosso do Sul (UFMS), Campus of Chapadão do Sul/MS with nine commercial lots of soybean seeds, cultivar Anta 82. The initial evaluation of quality of the seed lots involved the following: determining the water content of the seeds; subjecting the seeds to germination and emergence tests; determining the first count of germination and emergence speed index.

Water content of the seeds was determined after drying the seeds at 105 ± 3 °C for 24 h according to the Rules for Seeds Testing (BRASIL, 2009).

Germination test was carried out in eight replicates with 50 seeds each. The seeds were distributed on germitest papers previously moistened with distilled water, equivalent to 2.5 times the weight of the non-hydrated paper (BRASIL, 2009), in a germinator at 25 °C. Evaluations were carried out on the fifth and eighth day after sowing. First count of germination was carried out in conjunction with the germination test by counting the number of normal seedlings obtained five days after sowing.

Emergence test was carried out in four replicates with 50 seeds each. The seeds were sown in expanded polystyrene trays with commercial substrate, then placed in a greenhouse and irrigated twice a day. Seeds were evaluated ten days after sowing. Emergence speed index (ESI) was calculated in conjunction with the emergence of seedlings according to the formula proposed by Maguire (1962).

For the exudate pH test, the following two solutions were prepared: (1) 1 g of phenolphthalein dissolved in 100 mL of absolute alcohol, and then added to 100 mL of distilled, boiled water (AMARAL; PESKE, 1984) (2) 0.8 g of sodium carbonate in 1000 mL of distilled water and then boiled (CABRERA; PESKE, 2002). Exudate pH test was carried out in four replicates with 25 seeds each. The seeds were individually immersed in plastic cells with 5 mL of distilled water for 20, 30, and 40 min at 20, 25, and 30 °C. After each treatment period, three drops each of phenolphthalein and sodium carbonate solutions were added to the seeds, and then homogenized using a glass rod. The reading was taken immediately upon the contact of the indicator solution with the imbibition solution. The interpretation was based on the coloring of the solution: pink solution indicated viable seeds and colorless solution indicated nonviable seeds.

Four replicates of 50 seeds per treatment were used for the flooding test. Seeds were immersed in distilled water in 200 mL plastic cups, which were then kept in a germinator at 25 and 30 °C in the darkness, and later subjected to germination test (BRASIL, 2009). For the flooding test, variations in the immersion period (4, 8, 12, 16, 20, and 24 h) and amount of water (50 and 75 mL) were evaluated. The water content of the seeds before and after each flooding period and the percentage of normal seedlings that germinated after each treatment were determined.

The experiment design was completely randomized with four replicates. The results were subjected to analysis of variance and compared using the Scott–Knott test at 5% probability. The Pearson's correlation coefficient was calculated between the exudate pH and emergence of seedlings, and also between the flooding test result and emergence of seedlings.

RESULTS AND DISCUSSION

The initial water content of soybean seed lots was not analyzed statistically (Table 1). No variation greater than 2% in the water content between lots was observed in the present study. In tests, such as exudate pH and flooding tests, that expose seeds to hydration, seed samples with sharp variation in the water content are not recommended since dried seeds are subject to further deterioration during the tests. The reason for this deterioration might be the contact water allowing quick imbibition and restarting the metabolism of the seeds in a defective way or causing the cell membranes of the seeds to rupture, thus interfering with the interpretation of the results (MARCOS-FILHO, 2015).

Table 1. Water content (WC), germination (G), first count of germination (FCG), emergence (E), and emergence speed index (ESI) of the nine lots of soybean seeds.

Lots	WC	G	FCG	E	ESI
	%	%	%	%	-
1	8.7	92 a	92 a	86 a	2.75 a
2	8.6	95 a	95 a	66 b	2.00 a
3	8.7	92 a	91 a	50 c	1.50 b
4	8.9	92 a	91 a	71 b	2.25 a
5	8.6	93 a	90 a	86 a	3.00 a
6	8.5	94 a	47 b	73 b	2.00 a
7	8.4	95 a	47 b	65 b	1.75 b
8	7.7	92 a	45 b	77 a	2.25 a
9	8.5	95 a	48 b	29 d	0.75 c

In the columns, means followed by different letters differ by the Scott–Knott test ($P < 0.05$).

No statistical difference in germination between lots was observed in the present study. However, the first count of germination made it possible to separate the seed lots into two distinct groups based on the levels of vigor - lots 1, 2, 3, 4, and 5 had higher means than that of the other lots. For seed vigor tests, the seed lots used should have similar germination potential. They should also be accepted commercially, since the main objective of these tests is to complement the results of germination test and identify quality differences among lots with similar germination potential (MARCOS-FILHO, 2015).

In the seedlings emergence test, seeds are exposed to adverse climatic and soil conditions (LARRÉ; ZEPKA; MORAES, 2007). This allows segregation of seed lots with different levels of vigor (OLIVEIRA et al., 2009). In the present study, seeds lots were separated into four groups based on their levels of vigor: lots 1, 5, and 8 were found to be more vigorous, while lot 9 was found to be least vigorous. Further, the emergence speed indices revealed lots 1, 2, 4, 5, 6, and 7 to be more vigorous and lot 9 to be least vigorous (Table 1).

There was no significant difference in germination between lots in the present study. However, there was significant difference in seedling emergence between lots. This was probably due to the conditions under which the tests were carried out. The germination test was performed under controlled temperature and humidity, while the emergence test was carried out under field conditions.

For vigor tests to be considered efficient, the results of such tests should reflect the proportion of seedling emergence in the field. The tests should estimate the behavior of seed lots after sowing them in a broad range of environmental conditions (ILBI; KAVAK; ESER, 2009).

Test of exudate pH

Table 2 shows the Pearson's correlation coefficients (r) between the seedling emergence and exudate pH. A significant correlation (0.786) was observed after 30 min of imbibition at 20 °C. The exudate pH test also ranked the soybean seed lots similar to that of the seedling emergence test.

In corn seeds, Cabrera and Peske (2002) observed that seed viability estimated by the individual exudate pH test is better achieved after an imbibition period of 20 min at 20 to 25 °C. A study on rapid evaluation of the physiological quality of pea seeds with soaking periods of 15, 30, 45, and 60 min at room temperature (20 °C–25 °C) was conducted by Rech, Villela and Tillmann (1999). Their study concluded that the individual exudate pH test results after 30 min of imbibition allows quick estimation of viability of pea seeds.

Table 2. Correlation coefficient (r) between the seedling emergence and exudate pH at 20, 25, and 30 °C and imbibition periods of 20, 30, and 40 min of the nine lots of soybean seeds.

Temperature (°C)	Imbibition period		
	20´	30´	40´
20	-0.136^{ns}	0.786*	0.177^{ns}
25	-0.147^{ns}	-0.113^{ns}	0.412^{ns}
30	-0.182^{ns}	0.141^{ns}	-0.086^{ns}

*Significant at 5% probability; ^{ns}not significant.

The results of the present study are similar to that of the study conducted by Amaral and Peske (1984). They distinguished viable from nonviable soybean seeds after a 30 min imbibition period at 25 °C using the exudate pH test with the use of a drop each of sodium carbonate and phenolphthalein solutions in 2 mL of exudate of each seed.

Santos et al. (2011) tested soybean seeds soaked in 2 mL of distilled water for 30 min at 25 °C, and then placed one drop each of phenolphthalein and sodium carbonate solutions. The results revealed that the exudate pH test was not efficient in the segregation of seed lots based on their physiological quality. However, this outcome was unlike the observations made in the present study. This variation might be due to the use of 5 mL of distilled water and also the use of three drops each of phenolphthalein and sodium carbonate solutions after the imbibition period in the present study.

The exudate pH test was efficient in quickly estimating the viability of *Araucaria angustifolia* seeds when performed on embryos excised and soaked in distilled water for 30 min (ARALDI; COELHO, 2015). In contrast, Barboza et al. (2014) found no effect of soaking period (20, 40, 60, and 80 min) on the exudate pH test results in *Guazuma ulmifolia* seeds. It should be noted that assessing individual seeds is important considering that there are variations in the quality of seeds within a lot. This might be due to the inability of all the seeds in a lot to reach physiological maturity at the same time, and therefore, do not attain the highest quality simultaneously, which is reflected in the post-harvest viability. Thus, further studies on exudate pH test are recommended in order to observe variations in the results in soybean seeds with different amounts of water, besides the variation caused by the number of drops of phenolphthalein and sodium carbonate solutions.

Flooding test

The water content of soybean seeds after the flooding test is shown in Table 3. Lot 9 had the lowest water content both at 25 and 30 °C after 4 h of immersion. Even after 24 h of immersion in distilled water, the seeds of lot 9 continued to have the lowest water content.

According to Bewley and Black (1994), water uptake by a seed is triphasic. Phase I of water uptake is purely physical, and it depends only on the bond of water to the matrix of the seed. Both live and dead seeds exhibit similar behavior. Phase II is characterized by complete hydration of the seed, which is stabilized by the balance between the osmotic potential and potential pressure. During this phase, the seed absorbs water slowly, and the embryo cannot grow further due to the resumption of growth of the embryonic axis. In phase III, there is increase in the uptake of water, and radicle protrusion is also observed. In the present study, rapid absorption of water by all the nine lots of soybean seeds was observed until 4 h after the immersion of seeds in distilled water. After this period there was stabilization, which was possibly related to phase I of water uptake that probably lasted for a little less than 4 h in soybean seeds.

Table 4 shows the correlation between the flooding test result and seedling emergence. Of the various combinations assessed in the present study, seeds immersed in 50 mL of distilled water at 25 °C for 4 h, 75 mL of distilled water at 30 °C for 4 h, and 50 mL of distilled water at 30 °C for 12 h exhibited significant correlation with the seedling emergence. During the flooding period, the availability of oxygen is reduced and a large quantity of water quickly enters the seed due to the difference in the water potential between the seed interior and the environment. This induces fermentative metabolism and injures the seed by imbibition (COSTA et al., 2008a; COSTA et al., 2008b). The seed with some kind of injury has less energy for germination; such seeds will be negatively affected by the flooding process water, thus lowering the vigor (CUSTÓDIO et al., 2009).

Table 3. Water content (%) of soybean seeds at different periods of immersion.

Lots	4 h	8 h	12 h	16 h	20 h	24 h
25 °C, 50 mL distilled water						
1	53.7	58.9	62.3	60.5	60.6	61.0
2	53.6	58.9	59.8	60.4	60.5	60.6
3	54.0	59.5	61.1	61.5	61.4	62.4
4	53.3	60.9	59.9	60.5	60.9	60.9
5	55.1	61.1	61.9	62.7	63.1	63.1
6	55.5	58.8	59.8	60.0	57.9	60.9
7	55.2	61.4	62.0	62.6	64.6	63.8
8	55.2	61.1	62.2	62.6	63.0	62.9
9	51.7	57.3	58.9	59.2	59.9	60.3
25 °C, 75 mL distilled water						
1	54.1	58.9	59.4	60.1	60.1	60.2
2	54.0	59.4	59.7	60.6	60.6	60.6
3	53.9	61.5	59.7	61.3	61.1	57.8
4	53.5	59.2	59.3	61.2	60.4	60.6
5	56.7	61.9	61.9	62.6	63.3	63.7
6	54.4	58.8	60.6	60.2	60.8	60.5
7	56.9	61.3	62.1	62.7	63.6	62.1
8	55.1	55.3	61.4	61.5	62.4	63.1
9	51.7	58.2	57.9	59.3	58.8	59.3
30 °C, 50 mL distilled water						
1	57.0	59.5	60.3	60.7	61.5	60.3
2	56.6	59.3	60.3	61.1	60.2	60.9
3	56.6	59.7	61.2	61.8	61.2	61.4
4	56.6	59.4	60.9	61.7	61.8	60.7
5	59.2	61.3	62.6	63.6	64.3	63.0
6	56.3	59.8	61.3	62.2	61.4	60.9
7	58.2	62.1	62.6	63.9	63.7	57.2
8	59.1	61.8	62.8	63.3	63.9	63.5
9	54.5	57.7	58.8	59.9	60.3	59.9
30 °C, 75 mL distilled water						
1	56.7	59.0	60.2	60.2	60.5	60.3
2	56.9	59.1	60.3	60.8	60.5	60.7
3	57.0	60.1	61.4	62.0	61.4	61.0
4	56.5	58.8	60.5	61.1	61.4	61.1
5	59.6	61.2	62.7	62.8	63.4	62.4
6	56.5	59.2	61.1	61.1	61.2	60.3
7	58.7	61.2	63.3	63.5	63.7	62.9
8	58.2	60.7	62.4	63.9	63.7	63.5
9	54.4	58.1	59.3	60.3	60.3	59.9

Table 4. Correlation coefficient (r) between the seedling emergence and flooding test result at 25 and 30 °C during different periods of flooding (4, 8, 12, 16, 20, and 24 h) with 50 and 75 mL of distilled water in the nine lots of soybean seeds.

Temperature (°C)	Amount of water (mL)	Flooding period (h)					
			8	12	16	20	24
25	50	0.7723*	0.2712^{ns}	0.1470^{ns}	0.2447^{ns}	0.2325^{ns}	0.2258^{ns}
	75	0.4953^{ns}	0.6383^{ns}	0.4055^{ns}	0.2540^{ns}	0.0008^{ns}	0.6671*
30	50	0.6280^{ns}	0.3471^{ns}	0.7617*	0.3624^{ns}	-0.2208^{ns}	0.4339^{ns}
	75	0.8133*	0.3318^{ns}	0.3003^{ns}	0.4309^{ns}	0.1491^{ns}	-0.0738^{ns}

*Significant at 5% probability; ^{ns} not significant.

The effects of flooding and temperature on seed germination have been observed by Wuebker, Mullen and Koehler (2001). Their study concluded that the seeds are susceptible to loss in their germination capacity when subjected to flooding for 1–48 h. During flooding, the seeds are injured due to rapid imbibition, i.e., large amount of water quickly enter the seeds in response to the difference in water potential between the seed interior and the environment inducing fermentative metabolism instead of aerobic metabolism (CRAWFORD, 1978).

Custódio et al. (2002) evaluated the effects of flooding in bean seeds at 25 °C for 0, 8, 16, 24, 32, 40, and 48 h. The results indicated a decrease in germination of the seed lots by 55% after 8 h of flooding. This confirms that when flooding test is employed in a laboratory, it can be a good indicator to differentiate bean seeds based on their physiological quality. Grzybowski, Vieira and Panobianco (2015) also noted the relevance of flooding test in the characterization of maize seed quality at 25 °C for 48 h.

The study on effect of flooding on seed germination and the establishment of crops under field conditions have indicated that the flooding test can be considered as a quick and an inexpensive selection test, presenting correlation with the emergence of plants in the field. This test can be used in the differentiation of seeds based on their physiological quality (BORGES et al., 2007).

The results of the present study revealed that the flooding the soybean seeds for 4 h either with 50 mL of distilled water at 25 °C or 75 mL of distilled water at 30 °C to be effective in determining the vigor of soybean seeds. Ranking of lots of soybean seeds based on the levels of vigor by this method is similar to that of the emergence test. Furthermore, the test was also effective in identifying the levels of vigor between seeds with similar as well as higher seed germination (above 92%).

CONCLUSIONS

Exudate pH test can be used to evaluate the physiological quality of soybean seeds after 30 min of imbibition at 20 °C.

The flooding test was efficient in evaluating the soybean seed vigor when the seeds were immersed for 4 h either in 50 mL of distilled water at 25 °C or in 75 mL of distilled water at 30 °C.

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