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TEST OF EXUDATE pH IN RICE SEEDS[1]

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ABSTRACT - The objective of this study was to evaluate the efficiency of a pH test of the exudate (with and without tegument) as an indicator of the physiological quality of rice seeds. The experimental design was completely randomized, with seven lots and four replications. The seven lots were assessed for water content and initial physiological quality by means of germination, first germination count, emergence, emergence speed index and tetrazolium tests. The pH test of the exudate was conducted with 100 seeds of each treatment, with tegument and without tegument. The seeds were individually soaked in 2.0 mL distilled water for different periods (20, 40, and 60 minutes) and three temperatures (20, 25, and 30 °C). The results were submitted to the Scott Knott test at 5% probability. The pH test of the exudate is a promising method to evaluate the physiological quality of rice seeds by detecting differences in vigor between marketable lots. This test should be conducted with rice seed without tegument, at 25 °C, with a soak time of 60 minutes.

Keywords: *Oryza sativa.* Physiological quality. Vigor.

TESTE DO pH DO EXSUDATO EM SEMENTES DE ARROZ

RESUMO – O trabalho objetivou avaliar a eficiência do teste de pH do exsudato na qualidade fisiológica de sementes de arroz, com e sem tegumento. Para o teste, o delineamento experimental foi inteiramente casualizado, com sete lotes de arroz e quatro repetições. Os lotes foram submetidos a determinação do teor de água e avaliação da qualidade fisiológica inicial por meio dos testes de germinação, primeira contagem de germinação, emergência, índice de velocidade de emergência e tetrazólio. O teste de pH do exsudato foi conduzido com 100 sementes de cada tratamento, com tegumento e sem tegumento. As sementes foram embebidas individualmente em 2,0 mL de água destilada, por diferentes períodos (20, 40 e 60 minutos) e três temperaturas (20, 25 e 30 °C). Os resultados foram submetidos ao teste de Scott Knott a 5% de probabilidade. O teste do pH do exsudato é promissor para avaliar a qualidade fisiológica de sementes de arroz, detectando diferenças de vigor entre lotes comercializáveis. Este teste deve ser conduzido com sementes de arroz sem tegumento, na temperatura de 25 °C, com tempo de embebição de 60 minutos.

Palavras-chave: *Oryza sativa.* Qualidade fisiológica. Vigor.

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INTRODUCTION

The evaluation of the physiological quality of rice (*Oryza sativa* L.) through the standard germination test is somewhat slow, since the results require between 5 and 14 days, which is a long period for decision making on seed lots. In general, vigor tests aim to identify significant variations in the physiological quality of lots considered suitable for commercialization. These tests help in classifying seeds based on vigor levels, especially seedling emergence ratio in the field (MARCOS FILHO, 2015). Thus, vigor tests that provide results quickly must be developed, since the evaluation of seed quality depends on the efficiency of the procedures involved (LOPES; SILVA; VIEIRA, 2013). The speed at which trustworthy results are obtained is one of the important factors considered while evaluating seed quality. More rapid tests allow agility in decision-making, are usable on a broader scale, and reduce the risks and costs involved in operations, such as harvesting, processing, storage, and marketing (HILST et al., 2012).

From this perspective, the pH test of the exudate stands out, having the characteristics of an ideal test of vigor: practical and low cost, using materials and equipment common in seed laboratories (RAMOS et al., 2012). The exudate pH test can be applied easily and avoids the unnecessary use and/or storage of seed lots with low vigor (RAMOS et al., 2012). The test is based on the integrity of the seed membrane system, assessing the level of deterioration of the seed membranes. Seeds with a high level of deterioration have a higher ion leaching rate, causing the pH of the imbibition medium to change. Sugars, organic acids and ions (including H^+) contribute to the acidification of the medium and cause a decrease in the pH of the seed exudate; the most deteriorated seeds present higher leaching and, consequently, exudates with greater buffering power (CABRERA; PESKE, 2002). On the other hand, the less deteriorated seeds leach less, providing lower buffering power in the imbibition water (PESKE; AMARAL, 1994). Several studies in the literature confirm the efficiency of the pH test of the exudate to evaluate seed vigor as observed in peas (MURCIA; CROVO; CROMENTE, 2018) and soybean (THEODORO et al., 2018).

In view of the above, the objective of this study was to verify the efficiency of the pH test of the exudate to detect variations in vigor in different rice seed lots, with tegument and without tegument.

MATERIAL AND METHODS

The work was carried out with seven rice lots of the BRS Esmeralda cultivar, commercially acquired, which were submitted to water content determination and initial seed quality evaluation. The determination of the water content was carried out using the oven drying method at 105 ± 3 ºC for 24 hours, with two replicates containing approximately 7.0 g of seeds for each lot (BRASIL, 2009).

The germination test was conducted with four replicates of 50 seeds, in a Germitest paper roll, moistened with water in the proportion of 2.5 times the dry paper mass, in a germinator at 25 °C (BRASIL, 2009). The evaluation was performed at 14 days after the test installation, and the results were expressed as the percentage of normal seedlings. The first germination count was performed together with the germination test, in which the percentage of normal seedlings was obtained on the fifth day after the test.

For the emergence test, four replicates of 50 seeds per treatment were used. The seeds were seeded in trays of expanded polystyrene with 200 cells containing commercial substrate. The seeds were kept in a greenhouse and watered twice daily. The counting was performed at 14 days, and the results were expressed as a percentage of normal emerged seedlings. Together with the emergence test, the emergence speed index was evaluated according to the formula proposed by Maguire (1962).

The tetrazolium test was performed using 100 seeds without tegument (lemma and palea) of each lot of rice seeds. The seeds were first wrapped in Germitest paper moistened with distilled water for 16 hours. Subsequently, the seeds were cut in half lengthwise, with only one part being used. Afterwards, the seeds were soaked in 0.1% tetrazolium solution for three hours in the dark and at 35 ºC for coloration. The seeds were classified into viable and non-viable according to the staining presented in the embryonic axis; in this way the percentage of viable seeds was calculated (BRASIL, 2009).

The determination of the water content and germination, first germination count, and tetrazolium test were performed in order to characterize the lots in relation to the initial quality of the seeds. This is necessary in order to reinforce the importance of a fast and practical vigor test that is valuable to those working in the field.

The pH test of the exudate was carried out with four replicates of 25 seeds, totaling 100 seeds per treatment, with and without tegument. These were soaked in 2.0 mL distilled water for three soaking periods: 20, 40 and 60 minutes and three temperatures: 20, 25 and 30 °C.

After the soaking periods, a drop of sodium carbonate solution (0.8 g sodium carbonate diluted in 1,000 mL distilled water and boiled) and one drop of phenolphthalein solution (1.0 g phenolphthalein diluted in 100 mL absolute alcohol and 100 mL distilled and boiled water) were applied (CABRERA; PESKE, 2002). The viability of the

seeds was evaluated by the coloration obtained after the addition of the indicator solutions, in which the pink color indicated viable seeds and the absence of color indicated non-viable seeds. Results were expressed as the percentage of viable seeds.

The experimental design was completely randomized, with seven lots and four replications. The presence or absence of integument, temperatures and imbibition times were analyzed separately, not constituting as factors. The analysis of variance was performed with the aid of the Sisvar program and the means were compared by the Scott-Knott test, at 5% probability.

RESULTS AND DISCUSSION

The difference between the initial seed water content of the seven rice lots was not greater than 1%, within the recommended variation patterns (MARCOS FILHO, 2015). The first count test indicated lots 1, 2 and 4 as those of superior vigor in relation to the other lots (Table 1). In the germination test, lots 1 and 2 presented higher values of germination percentage, compared to the other lots. Seedling emergence and the rate of emergence stratified the plots into three levels of vigor, in which lot 1 showed greater vigor, lots 2, 4 and 5 as intermediate vigor, and lots 3, 6 and 7 as those of low vigor (Table 1). The results obtained in the tetrazolium test indicated lots 1, 2 and 4 as being of high vigor, while lots 3, 5 and 7 were classified as being of intermediate vigor and lot 6 was classified as being of low vigor (Table 1). Even if the strata were stratified into three levels, the results obtained in the tetrazolium test did not resemble those found in the emergence test (Table 1).

Table 1. Water content (WC), first germination count (FGC), germination (G), seedling emergence (SE), emergence speed index (ESI) and tetrazolium (TZ) in seven rice seed lots.

LOTS	WC (%)	FGC (%)	G (%)	SE (%)	ESI -	TZ (%)
1	10.20	67 a	92 a	89 a	4.43 a	89 a
2	10.03	73 a	90 a	83 b	3.15 b	92 a
3	10.86	59 b	80 b	76 c	2.28 c	78 b
4	10.15	71 a	85 b	83 b	3.14 b	88 a
5	10.89	57 b	81 b	80 b	3.08 b	78 b
6	10.06	57 b	80 b	68 c	2.18 c	73 c
7	10.19	60 b	83 b	72 c	2.29 c	78 b
F	-	7.41*	8.46*	16.69*	31.88*	21.94*
CV%	-	7.83	4.19	4.25	9.52	3.75

*Significant by F test at 5% probability. CV - coefficient of variation. Means follow by the same letter in the column do not differ between 5% probability by the Scott-Knott test.

The results (Table 1) verified that the seven seed lots of rice, when submitted to the germination test, presented values within the marketing standard (above 80%). However, the variation found in the emergence test (SE) and emergence speed index (ESI), when compared to the germination test, indicates the need to perform more than one vigor test to infer the physiological quality of the batches. Both the germination test and the first count test were performed under ideal conditions, which rarely occurs in the field. This fact reinforces the importance of vigor tests, which should estimate the behavior of seed lots after sowing in a wide range of environmental conditions (ILBI; KAVAK; ESER, 2009). The pH test of the exudate in the seed of rice with a tegument, conducted at 20 °C (Table 2), was not efficient in identifying differences in vigor between the lots at any of the imbibition times evaluated. At a temperature of 25 °C (Table 2), it presented a significant difference in the imbibition time of 20 minutes, in which lots 1, 2 and 4 were classified as those with high vigor. However, the same ordering of the emergence and ESI tests was not found (Table 1). At 30 °C, the pH test of the exudate (Table 2) showed a significant difference in all imbibition times; however, the stratification was only in two levels of vigor, not resembling the ordination found in the emergence and ESI tests. In

the time of 20 minutes, lots 1, 4 and 5 presented a higher level of vigor. For the 40- and 60-minute imbibition periods, lots 1, 2, 4 and 5 were classified as those of high vigor and the others as low vigor (Table 2).

At all temperatures evaluated in the seed with tegument lots (Tables 2), the pH test of the exudate was not efficient in detecting differences in vigor levels when compared to the emergence test and ESI (Table 1). The pH test is directly related to the number of exudates present in the imbibition solution, thus, the presence of the integument in the seeds during the test can interfere in the imbibition process, generating inconsistent and unreliable results on the physiological quality of the lots.

Table 2. Seed viability of seven rice plots with integument, submitted to pH exudate test at 20, 25 and 30 °C, during different soaking periods.

	20 °C		
Lot	20 min	40 min	60 min
1	98	94	93
2	98	94	93
3	96	94	91
4	98	97	94
5	96	96	91
6	96	94	91
7	97	94	93
F	0.50^{ns}	0.36^{ns}	0.55^{ns}
CV%	3.69	3.64	3.24
Lot	25 °C		
	20 min	40 min	60 min
1	98 a	96	93
2	92 a	93	93
3	95 b	95	92
4	98 a	96	94
5	94 b	93	93
6	92 b	91	90
7	93 b	92	90
F	2.90*	0.83^{ns}	2.40^{ns}
CV%	2.99	3.08	2.10
	30 °C		
Lot	20 min	40 min	60 min
1	98 a	96 a	92 a
2	96 b	93 a	90 a
3	89 b	87 b	86 b
4	99 a	98 a	97 a
5	97 a	93 a	92 a
6	89 b	84 b	82 b
7	91 b	86 b	84 b
F	4.90*	3.83*	3.42*
CV%	4.20	6.12	6.23

*Significant by F test at 5% probability. ^{ns}not significant. CV - coefficient of variation. Means follow by the same letter in the column do not differ between 5% probability by the Scott-Knott test.

In seeds without integument, at 20 °C the pH of exudate test was efficient in stratifying the lots in three levels of vigor, in all the imbibition times (Table 3). After 20 minutes, lots 1, 2, 3, 4 and 5 were classified as high vigor, lot 7 as intermediate and lot 6 as low vigor. In the 40- and 60-minute imbibition periods, lots 1, 2 and 4 were classified as high vigor, lots 3, 5 and 7 as intermediate, and lot 6 as the lowest vigor (Table 3). However, at this temperature (20 ºC) the pH test of exudate failed to produce, in any of the imbibition periods, batch stratification similar to the results found in the emergence and ESI tests.

At 25 °C (Table 3), the pH test of the exudate

showed a significant difference in all imbibition periods, and it was verified that the test stratified the lots at three levels of vigor. In the 20- and 40-minute imbibition periods, lots 1 and 2 were classified as high-vigor, lots 4 and 5 as intermediate, and lots 3, 6 and 7 as low-vigor. After 60 minutes of imbibition, the stratification of the lots occurred in a manner similar to the emergence and ESI tests (Table 1), in which lot 1 was highlighted as high vigor, lots 2, 4 and 5 as intermediate, and lots 3, 6 and 7 as low vigor (Table 3). Similar results were found by Santos et al (2011), setting the temperature at 25 °C during the test of the pH of the exudate in soybean seeds, but with a period of 30 minutes of imbibition.

In comparing our results with those reported in the literature, we note that in experiments on mutamba (*Guazum ulmifolia*) seeds by Barboza et al. (2014), no differences were found between the imbibition periods (20, 40, 60 and 80 minutes) using the same temperature (25 ºC). The results obtained by Matos, Martins and Martins (2009), in *Copaiba langsdorffi* seeds, confirm the efficiency of the pH test of the exudate at 25 °C, but with 30 minutes of imbibition. In soybean seeds, however, it was verified that the exudate pH test can be used to evaluate the physiological potential, which should be carried out at 20 ºC for 30 minutes of imbibition (THEODORO et al., 2018).

At 30 °C (Table 3), there was a difference between the lots at each imbibition time. At 20 minutes, the exudate pH test was sensitive to identify three vigor levels, in which lots 1 and 2 were classified as high-vigor, lots 3, 4, 5 and 7 as intermediate, and lot 6 as low vigor. For the 40- and 60-minute periods, there was stratification in only two levels of vigor, with lot 1 being the most vigorous (Table 3). Thus, at 30 °C, despite stratifying the lots at three levels of vigor, the pH test did not present similar ordering as found in the emergence test, indicating an inability of the test to accurately separate the more vigorous lots from those with low vigor.

Table 3. Seed viability of seven rice lots without tegument, submitted to pH test of the exudate at 20, 25 and 30 °C in different soaking periods.

	20 °C		
Lot	20 min	40 min	60 min
1	94 a	92 a	92 a
2	95 a	89 a	88 a
3	85 a	79 b	78 b
4	90 a	88 a	86 a
5	89 a	83 b	81 b
6	66 c	63 c	61 c
7	78 b	77 b	77 b
F	12.14*	15.81*	15.62*
CV%	6.90	6.06	6.41
	25 °C		
Lot	20 min	40 min	60 min
1	95 a	94 a	90 a
2	91 a	86 a	76 b
3	74 c	62 c	64 c
4	84 b	79 b	77 b
5	82 b	77 b	73 b
6	76 c	62 c	60 c
7	75 c	69 c	68 c
F	15.73*	15.72*	10.23*
CV%	5.00	8.02	0.86
Lot	30 °C		
	20 min	40 min	60 min
1	92 a	86 a	84 a
2	91 a	83 a	82 a
3	82 b	76 a	73 b
4	85 b	77 a	76 b
5	85 b	80 a	74 b
6	76 c	61 b	63 b
7	82 b	77 a	70 b
F	6.46*	7.14*	4.06*
CV%	5.13	7.77	9.47

*Significant by F test at 5% probability. ^{ns} not significant. CV - coefficient of variation.

CONCLUSIONS

The pH test of the exudate shows promise in evaluating the physiological quality of rice seeds, detecting differences in vigor between marketable lots.

The pH test of the exudate should be conducted with rice seed without tegument, at 25 °C and with 60 minutes of imbibition.

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REFERENCES

BARBOZA, V. R. S. et al. Potencial fisiológico de sementes de *Guazuma ulmifolia* Lam. através do teste do pH do exsudato. **Enciclopédia Biosfera**, v. 10, n. 18, p. 2327-2335, 2014.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Regras para análise de sementes**. Secretaria de Defesa Agropecuária. Brasília, DF: MAPA/ ACS, 2009. 395 p.

CABRERA, A. C.; PESKE, S. T. Testes do pH do exsudato para sementes de milho. **Revista Brasileira de Sementes**, v. 24, n. 1, p. 134-140, 2002.

HILST, P. C. et al. Test of exudates color hues for evaluating the physiological potential of coffee (*Coffea arabica* L.) seeds. **Revista Brasileira de Sementes**, v. 34, n. 2, p. 212-217, 2012.

ILBI, H.; KAVAK, S.; ESER, B. Cool germination test can be an alternative vigour test for maize. **Seed Science and Technology**, v. 37, n. 2, p. 516-519, 2009.

LOPES, M. M.; SILVA, C. B.; VIEIRA, R. D. Physiological potential of eggplant seeds. **Journal of Seed Science**, v. 35, n. 2, p. 225-230, 2013.

MAGUIRE, J. D. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. **Crop Science**, v. 2, n. 1, p. 176-177, 1962.

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

MATOS, J. M. M; MARTINS, R. C. C; MARTINS, I. S. Caracterização do teste de pH de exsudato pelo métodos individual para avaliação da viabilidade de sementes de *Copaifera langsdorffi* Desf. **Heringeriana**, v. 3, n. 1, p. 91-97, 2009.

MURCIA, M. L.; CROVO, V. E.; CLEMENTE, N. L. Prueba colorimétrica de pH de exudados para la evaluación de la calidad de semillas de arveja (*Pisum sativum*) de la zona hortícola de Mar del Plata. **Revista de la Facultad de Agronomía**, v. 117, n. 1, p. 171-174, 2018.

PESKE, S. T.; AMARAL, A. S. pH of seed exudate as a rapid physiological quality test. **Seed Science and Technology**, v. 22, n. 3, p. 641-644, 1994.

RAMOS, K. M. O. et al. Electrical conductivity testing as applied to the assessment of freshly collected Kielmeyera coriacea Mart. seeds. **International Scholarly Research Notices Agronomy**, v. 2012, s/n., p. 1-5, 2012.

SANTOS, J. F. et al. Avaliação do potencial fisiológico de lotes de sementes de soja. **Revista Brasileira de Sementes**, v. 33, n. 4, p. 743-751, 2011.

THEODORO, J. V. C. et al. Exudate pH and flooding tests to evaluate the physiological quality of soybean seeds. **Revista Caatinga**, v. 31, n. 3, p. 667-673, 2018.