Methodological adjustments to the tetrazolium test in rice seeds[1]

Ireni Leitzke Carvalho[2], Géri Eduardo Meneghello[2], Lilian Madruga de Tunes[2], Caroline Costa Jácome[3], Vanessa Nogueira Soares[2*]

ABSTRACT - Reducing the execution time of the tetrazolium test is important because it is used for making decisions during the preharvest and this test takes approximately 24 h. Thus, the goal of this research was to study preconditioning and staining periods and concentration of tetrazolium salt, in order to reduce the evaluation time of rice seed viability by tetrazolium test. Three independent experiments were conducted. In the first and second experiments, six rice seed lots from the BR Irga 424 cultivar were used. In the first experiment, different concentrations of tetrazolium salt (0.1, 0.25, 0.5 and 1 %) and staining times (0.5, 1.0 and 2.0 h) were evaluated. In the second one, different hydration periods (0.5, 1.0, 2.0 and 4.0 h) at two temperatures (35 and 40 °C) were tested. In the third one, eight seed lots from the same cultivar were used, and the effectiveness of the modified tetrazolium test in the evaluation of rice seed viability was assesed. It is possible to carried out the tetrazolium test on rice using the hydration of peeled seeds for 1 h at 40 °C and staining for 1 h with a 0.25% salt concentration.

Index terms: physiological quality, seed viability, *Oryza sativa* L.

Ajustes metodológicos para o teste de tetrazólio em sementes de arroz

RESUMO - A redução no tempo de execução do teste de tetrazólio é importante, pois esse teste muito utilizado para tomada de decisão em pré-colheita, consome aproximadamente 24 h. Dessa forma, objetivou-se nesta pesquisa estudar períodos de pré-condicionamento e de coloração e concentração do sal de tetrazólio visando reduzir o tempo de avaliação da viabilidade de sementes de arroz pelo teste de tetrazólio. Foram conduzidos três experimentos independentes, nos experimentos I e II foram utilizados seis lotes de sementes de arroz da cultivar BR Irga 424. No experimento I foram avaliadas diferentes concentrações do sal de tetrazólio (0,1; 0,25; 0,5 e 1%) e períodos de coloração (0,5; 1,0; e 2,0 h). No experimento II foram testados diferentes períodos de hidratação (0,5; 1,0; 2,0 e 4,0 h) em duas temperaturas (35 e 40 °C). No experimento III foram utilizados oito lotes de sementes da mesma cultivar e foi avaliada a eficiência do teste de tetrazólio modificado na avaliação da viabilidade de sementes de arroz. É possível realizar o teste de tetrazólio em arroz utilizando hidratação de sementes descascadas por 1 h a 40 °C e coloração por 1 h com concentração do sal a 0,25%.

Termos para indexação: qualidade fisiológica, viabilidade, *Oryza sativa* L.

Introduction

The tetrazolium test, whose goal is to evaluate seed viability, is much used by companies producing this important agricultural input, mainly to speed up decisions as for the management of fields during pre-harvesting and lots during steps of seed post-harvesting. The use of this test depends on the methodological adjustment for each species, involving the definition of appropriate conditions for preconditioning, preparation, staining and evaluation of seeds (Pinto et al., 2009).

Despite being considered a quick test, significant gains in terms of runtime may be obtained with an increase in the temperature during the preconditioning step, since high temperatures increase the soaking speed of seeds (Costa et al., 2007).

Preconditioning is the longest step of the test; it could be considerably reduced by increasing the soaking temperature, according to the results obtained by Costa et al. (2007), in soybean seeds, and by Chamma and Novembre (2007) in maize seeds. These authors increased to 40 °C the soaking temperature, reducing to 6 and 4 h, respectively, this stage's time. On the other hand, Grzybowski et al. (2012) reduced preconditioning time in barley seeds, using the methodology

[1]Submitted on 09/22/2016. Accepted for publication on 01/24/2017.
[2]Departamento de Fitotecnia ,Universidade Federal de Pelotas, Caixa Postal 354, 96001-970 - Pelotas, RS, Brasil.
[3]Embrapa Clima Temperado, Estação Experimental Terras Baixas, Caixa Postal 403, 96010-971 - Pelotas, RS, Brasil.
* Corresponding author < vnsoares@gmail.com>

of direct immersion in water for 4 h at 20 °C, with longitudinal cut, without increasing the soaking temperature.

The seed hydration step promotes its softening, helping the preparation and the penetration of the tetrazolium solution, as well as activating the enzymatic system, which results in a crisper staining of living tissues. Pre-dampened seeds are generally less susceptible to damages during the preparation for the test; they help the cutting or perforation to expose the embryo to the tetrazolium action. Moreover, the color of pre-dampened seeds is more consistent, which helps evaluating (Moore, 1977).

Staining speed, temperature and tetrazolium solution concentration vary according to the studied species, and they may influence the result of the test. The use of temperature above 30 °C enables obtaining the ideal color in shorter periods (França-Neto et al., 1998; Novembre et al., 2006; Chamma and Novembre, 2007). Moreover, it is possible to reduce the concentration of the tetrazolium solution to 0.075%, in order to evaluate soybean, cotton and wheat seeds (França-Neto et al., 1998; Cervi and Mendonça, 2009 and Carvalho et al., 2013) and to 0.1% in order to conduct the test on barley seeds (Grzybowski et al., 2012).

The reduction in the test duration runtime is important to make decisions, since the traditional tetrazolium test takes about 24 h. Depending on the situation, this period, even if relatively short compared to the germination test, may be excessive and it may hinder making more urgent decisions (Marcos-Filho, 2015). In this context, methodological adjustments may be performed to run the tetrazolium test, considering the involved variables, mainly the ones related to the preparation and preconditioning of seeds before staining, concentration of the tetrazolium salt concentration, period and temperature of exposure to the solution. Thus, the goal of this research was to study options for periods of preconditioning, staining and concentration of tetrazolium salt for staining, in order to reduce the evaluation time of rice seed viability by tetrazolium test.

Material and Methods

The study was divided into three experiments, conducted independently.

Experiment location and used seed material

The work was conducted in the Seed Testing Laboratory of Plant Science Department of the Faculty of Agronomy Eliseu Maciel, University Federal of Pelotas. For experiments I and II, samples from six lots of rice seeds belonging to the BR Irga 424 cultivar were used. In the experiment III, seed samples of eight lots from the same cultivar used in experiments I and II were used; they were selected in order to encompass great amplitude of viability, since a test, to be efficient, must have accuracy and solidity able to identify potential differences in the seed quality.

Initial characterization of samples from seed lots

The seeds used in the three experiments were submitted to water content determination and germination evaluation; on the six lots used in the experiments I and II, the first count germination test were also conducted, according to the methodology described below.

Water content: the used method was in the oven at 105 ± 3 °C, for 24 hours, according to the recommendations from the Rules for Seed Testing (RST) (Brasil, 2009). The results were expressed in average percentage of water content for each lot, on wet basis.

Germination test - it was conducted according to the RST (Brasil, 2009), using 200 seeds per replication, which were divided into four subsamples of 50 seeds, placed on germitest paper that had been moistened with distilled water in the proportion of twice the mass of the dry paper. Paper rolls were placed in a germinator at 25 °C. Counts were performed on day five and fourteen after test installation, counting the percentage of normal seedlings.

First germination count (FGC) - the first germination count was performed together with the germination test and evaluated on the fifth day, when normal seedlings that had already germinated were removed from the substrate.

Experiment I - Evaluation of tetrazolium salt concentration and staining period

Initially, seeds were peeled and immersed in water for 18 hours at 20 °C. After that, they were longitudinally sectioned through the center of the embryonic axis, and discarding approximately 1/2 of the seed width. In this stage, the evaluation of the water content in the peeled seeds was evaluated, according to the methodology described before. After that, 100 seeds, divided into two subsamples of 50 seeds, were placed in plastic cups with 50 mL capacity and immersed in a tetrazolium solution in the concentration of 0.1%; 0.25%; 0.5% and 1%, and taken to a oven at 35 °C for 0.5; 1.0 and 2.0 hours, in the absence of light. At the end of the staining period, seeds were washed under running water and individually evaluated, taking into consideration the intensity and consistency of tissue color, and they were classified following the RST (Brasil, 2009) recommendations for rice, using a stereo microscope, with an increase up to six times. Seeds were classified as viable and non viable, according to the color of the embryo, counting the percentage of viable seeds.

Experiment II - Evaluation of the reduction of hydration periods at two temperatures

In this experiment, seeds were peeled and submitted to different hydration conditions. Five periods were evaluated (0.5, 1, 2, 3 and 4 hours), combined with two hydration temperatures (35 and 40 °C), which were obtained in the oven. Water at room temperature was used. About 130 peeled rice seeds, for each temperature and period, were submerged in approximately 20 mL of distilled water to hydrate, under conditions to be tested. After each hydration period at the respective temperatures, seeds were longitudinally cut, as described in experiment I. Subsequently, they were submitted to staining with a 0.1% tetrazolium solution and kept in a germinator at 35 °C for 2 h, in the dark, according to data obtained in Experiment I. The evaluation procedure was similar to the one from Experiment I.

Experiment III - Effectiveness of the modified tetrazolium test in the evaluation of rice seed viability

In order to perform the tetrazolium test, the most effective procedures were adopted in the Experiments I and II. For situations where there was more than one condition with similar effectiveness, technical criteria were taken into consideration, such as: 1) lower performance time, 2) lower material quantity and 3) better staining condition.

Initially, 130 seeds from each lot were peeled using an MT-88 Suzuki peeling equipment, following the execution and selection procedure described before. After that, two samples of 50 seeds per experimental unit were hydrated for 1 h at 40 °C (conditions obtained in Experiment II) and submitted to a better staining period, which was obtained from Experiment I (1h at 35 °C). The used concentration of tetrazolium salt was 0.25%; this condition provided a more adequate color for the evaluation, according to preliminary tests. After the hydration period, the water content of seeds was determined, according to the methodology described before.

Methodological effectiveness of the tetrazolium test

In the three experiments, the methodological effectiveness of the tetrazolium test was evaluated by the comparison between the results obtained in this test, in the methodologies tested on each experiment, and the result of the germination test, using the formula:

$$\text{Effectiveness TZn} = [1-(|G-TZn|)/G]100$$

Where: G = % of normal seedlings obtained in the germination test; TZ n= % of viable seeds obtained in the tetrazolium test.

Experimental design and statistic analysis

Experiment I and II

Experiments I and II were conducted in completely randomized experimental design, with four replications. Data from the first germination count and germination were submitted to analysis of variance and the averages were compared by Tukey's test at 5% probability. In both experiments, the viability results by tetrazolium test were compared at 5% probability level, by the Tolerance Table 18.16 of the Tetrazolium Test from the RST (2009) to compare test results of average samples from the same lot, analyzed in the same laboratory. In experiment I, for the statistical analysis of effectiveness, a 4x3 factor experiment was considered (salt concentrations x staining time); replications were obtained from each lot. For the statistical analysis of effectiveness in experiment II, a 5x2 factor experiment was considered (time x hydration temperature); replications were obtained from each lot. Data from both experiments were submitted to analysis of variance and, after that, the averages were compared between themselves by Tukey's test at 1% error probability, performing the due splits in case of significant interaction between the studies factors. The SASM-Agri (Canteri et al., 2001) and Winstat (Machado and Conceição, 2003) softwares were used.

Experiment III

Experiment III was conducted in completely randomized experimental design, with three replications. Data obtained in the tests were submitted to analysis of variance and, after that, the averages were compared among themselves by Scott-Knott test at 5% error probability, using the SASM-Agri software (Canteri et al., 2001).

Results and Discussion

Characterization of the six rice seed lots evaluated in experiments I and II

Data from the initial characterization of the rice seed lots used in the Experiments I and II are presented in Table 1. There was similarity in the quality among the lots, highlighted by the results of the FGC and germination tests. The initial water content of the six evaluated lots was similar, varying between 11.4 and 12.4%. In the first germination count test, lot 3 presented higher quality, whereas the other lots did not differ among themselves. In the germination test, lots varied between 92 and 95%, complying with the commercial standard of rice seeds, which is currently 80% (MAPA, 2013).

Table 1. Average values of water content, first germination count (FGC) and germination (G) of six rice seed lots.

Lots	Water content	FGC	G
	----------%----------		
1	12.4	85 b	95 a
2	11.4	88 b	93 a
3	11.6	92 a	94 a
4	12.4	86 b	94 a
5	11.4	85 b	92 b
6	11.8	87 b	94 a
CV (%)		3.54	2.26

Averages followed by the same letter in the column do not differ among themselves by Tukey's test at 5% probability.

Experiment I - tetrazolium salt concentration and staining period

In Figure 1 it is possible to find the color of rice seeds after being exposed to different hydration periods (0.5, 1 and 2h) and concentrations of tetrazolium salt (0.1, 0.25, 0.5 and 1%). It is possible to notice that with a 0.5 h exposure to salt concentrations, the seed color was weak (Figure 1A) and some were not completely stained yet (Figure 1B). However, seeds that remained in the concentrations for 1 or 2 h, obtained a proper color for the evaluation (Figure 1C). Color develops with a variable speed among seeds from different species or even among seeds from a single sample, but this period is generally between 60 and 240 minutes (Marcos-Filho, 2015).

The choice of TZ salt concentration and the incubation time of seeds must be done considering the easiness of differentiating viable and unviable seeds, and the possibility of a better visualization of tissue color disorders. Effective methodologies, using tetrazolium solution in low concentrations, are important to optimize the application of financial resources from the laboratories and to allow analyzing the widest range of samples with the lowest cost (Silva et al., 2013).

Viability results from experiment I, referring to concentration and staining time, are demonstrated in Table 2. A period of 0.5 h was not enough to properly color the seeds, in all analyzed lots, regardless of the salt concentration. On the other hand, it was observed that starting from the 0.25% concentration, results indicated an increase tendency in the viability percentage (Table 2).

In the period of 0.5 h, in the lowest concentration (0.1%), the obtained values were lower in relation to the other concentrations; there was a difference of 31 percent points (pp) compared with the average of the evaluated lots in the highest concentration (1%) of the TZ solution (Table 2). Low concentrations and short time periods resulted in difficulties to evaluate essential structures in sunflower and triticale seeds (Silva et al., 2013; Souza et al., 2010).

The reduction in the concentration of TZ solution used in the staining stage of the test was also suggested for other species; satisfactory results were obtained with concentrations around 0.075% for soybean (França-Neto et al, 1998), cotton (Cervi and Mendonça, 2009), wheat (Carvalho et al., 2013), and 0.1% for barley (Grzybowski et al., 2012). Two factors justify the use of lower concentrations of tetrazolium salt: the high cost of salt and the satisfactory visualization of the living tissues of seeds, which allows observing if there are damages or not, by obtaining a proper color (Santos et al., 2006).

The maximum difference between the germination and tetrazolium test was 2 pp in the staining periods of 1 and 2 h, in all evaluated concentrations and lots (Table 2). This result indicates that the tetrazolium test is as effective as the germination one to evaluate the viability of rice seed lots. In addition to the result similarity, the TZ test is a quick one, considering that it is run in less than 24 hours, whereas the germination test on rice seeds, according to the RST (Brasil, 2009) has its final count after 14 days

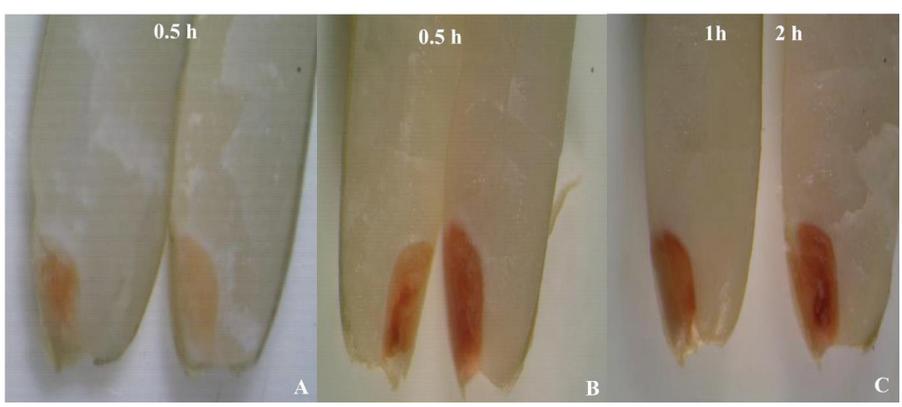

Figure 1. Representation of seeds after staining, in the periods of 0.5 h (A) and (B) and 1 to 2 h of exposure to tetrazolium salt at 0.1% (C).

Table 2. Viability of six rice seed lots (%) by tetrazolium test, according to variations in salt concentrations (SC) and staining time (ST) and germination percentage (G) of rice seed lots.

SC (%)	ST (h)	Lots						
		1	2	3	4	5	6	Average
0.1	0.5	42 b	36 b	37 b	66 b	30 b	65 b	46
	1.0	93 a	93 a	93 a	94 a	92 a	93 a	93
	2.0	93 a	95 a	93 a	94 a	92 a	94 a	94
0.25	0.5	79 b	82 b	56 b	77 b	65 b	69 b	73
	1.0	95 a	91 a	91 a	92 a	93 a	94 a	93
	2.0	95 a	91 a	92 a	92 a	94 a	93 a	93
0.5	0.5	44 b	83 b	84 b	80 b	68 b	68 b	71
	1.0	93 a	92 a	96 a	92 a	89 a	95 a	93
	2.0	93 a	91 a	93 a	92 a	90 a	92 a	92
1.0	0.5	82 b	73 b	91 a	89 a	70 b	70 b	77
	1.0	98 a	93 a	93 a	94 a	91 a	89 a	93
	2.0	97 a	93 a	92 a	94 a	92 a	91 a	93
G (%)		95	93	94	94	92	94	

Averages followed by the same letter in the column do not differ among themselves for each salt concentration tested according Table 18.16 - Tetrazolium Test (viable and non-viable seeds) of the RSA (2009), at 5% probability level.

from the test implementation. The tetrazolium test also appeared to be promising as an alternative method to the germination test on forage grasses seeds (Silveira, 2008; Soares et al., 2016).

As for the methodological effectiveness, it was verified that in staining periods of 1 and 2 h, there was a higher than 97.9% effectiveness in relation to germination for all the studied concentrations, which means that there was a higher than 97% hit in the results of the tetrazolium test, and that there was a lower than 3 pp error (Table 3). Viability results in soybean seeds obtained in the germination and tetrazolium tests must be similar, allowing differences up to 5% among themselves; if there are higher differences, they must be explained (França- Neto et al., 1998). There was a lower effectiveness when the 0.1% concentration of TZ solution with a 0.5 h staining period were used, in relation to the other combinations (Table 3). On the other hand, when TZ concentrations higher than 0.1% were used in the same period, no statistical difference was observed (Table 3).

In order to choose the best method, it was considered that the most effective methodology was the one obtaining higher results as for effectiveness; in this regard, it was observed that starting from 1 h staining there was no statistical difference between methodological effectiveness, regardless of the salt concentration (Table 3). In this case, the most effective methodology is the one that obtained results in the lowest time and concentration, that is, 1 h staining using a 0.1% tetrazolium salt concentration (Table 3).

The results obtained in this experiment highlighted the possibility of using a solution of tetrazolium salt at a lower concentration, for less time. As well as being cheaper, it allows a proper staining of the seed tissue more quickly, with no damages in the evaluation of its viability. Temperature and staining time suggested per species in the RST are considered proper for staining, but must not be considered as absolute because they may vary according to the condition of the seed and the salt purity and as one gets experienced, it is possible to perform the evaluation at an initial color stage (Brasil, 2009). In this regard, with the experience acquired during the period in which the work was conducted, it is possible to say that the use of a lower concentration (0.1%) allowed identifying and evaluating color details of the embryo in the same way as with solutions with higher concentrations.

As for the water content of seeds after the hydration period, the results varied between 25.9 and 28.1%, a quantity that was considered by the authors as sufficient to color living tissues.

Table 3. Effectiveness (%) of the tetrazolium test according to variations in salt concentrations and in the staining period.

Staining period (h)	Tetrazolium salt concentration (%)			
	0.1	0.25	0.5	1
0.5	49.1 bB	77.4 bA	75.5 bA	82.5 bA
1.0	99.1 aA	98.3 aA	98.2 aA	98.2 aA
2.0	98.9 aA	98.2 aA	97.9 aA	98.4 aA
CV (%)	9.25			

Averages followed by the same lowercase letter in the column and the same capital letter on the line do not different among themselves by Tukey's test at 5% probability.

Experiment II - hydration periods at different temperature

It was verified that starting from 2 h hydration at the temperature of 35 °C and 1h at 40 °C, it was possible to evaluate the viability of rice seeds (Table 4). It is important to highlight that in the hydration period of 0.5 h at 35 °C, it was not possible to cut seeds, therefore this condition was considered inappropriate for the preparation of rice seeds (Table 4). Temperatures between 30 and 40 °C are more adequate for the hydration of seeds for the tetrazolium test (Moore, 1977). However, the water content after seed hydration for different periods indicated that, regardless of the hydration period, seeds absorbed more water at 40 °C (Figure 2).

Likewise, Chamma and Novembre (2007) reduced the hydration time for maize seeds using temperatures of 35 or 40 °C and a period of 4 h, obtaining water contents between 19.5 and 21.3%; this indicates the water content of seeds as one of the parameters to perform this test, being able to eliminate the interferences related to substrate variations, such the water quantity available for the seed and the hydration temperature. The water quantity absorbed by brachiaria may be used as a reference parameter for the standardization of the implementation conditions of the tetrazolium test (Novembre et al., 2006).

The soaking period of 1 h at the temperature of 35 °C and 0.5 h at 40 °C was not enough to evaluate the viability of rice seeds (Figure 3). The cut was slow, seeds were more resistant to cutting, causing damages to the embryo and problems referring to its color, hindering the interpretation of the results. On the other hand, it was verified that as seeds remained more time in the soaking, they were less resistant to cutting, helping the evaluation of viability by tetrazolium test (Figure 3). It was observed that in seeds that remained soaked for 0.5 h at 40 °C and 1h for 35 °C (Figure 3A), the staining result was inappropriate, since the seed had a whitish color that created doubts in the evaluation. For the other soaking periods, there was no difference in obtaining the seed color (Figure 3B).

Viability results of the seeds from the six rice seed lots, soaked for periods of 0.5, 1, 2, 3 and 4 h at the temperatures

Table 4. Viability (%) of six rice seed lots by tetrazolium test using different soaking periods at the temperatures of 35 and 40 °C.

Temperature (°C)	Time (h)	Lots						Average
		1	2	3	4	5	6	
35	0.5	0 b	0 b	0 b	0 b	0 b	0 b	0
	1.0	85 b	77 b	86 b	71 b	83 b	81 b	80
	2.0	95 a	93 a	93 a	94 a	97 a	94 a	94
	3.0	95 a	91 a	92 a	93 a	94 a	95 a	93
	4.0	95 a	93 a	94 a	95 a	95 a	95 a	95
40	0.5	68 b	84 b	82 b	78 b	69 b	65 b	74
	1.0	96 a	92 a	93 a	93 a	92 a	97 a	94
	2.0	97 a	93 a	97 a	97 a	92 a	91 a	95
	3.0	96 a	95 a	97 a	97 a	93 a	97 a	96
	4.0	99 a	96 a	99 a	97 a	95 a	94 a	97
Germination (%)		95	93	94	94	92	94	

Averages followed by the same letter in the column do not differ among themselves for each salt concentration tested according Table 18.16 - Tetrazolium Test (viable and non-viable seeds) of the RSA (2009), at 5% probability level.

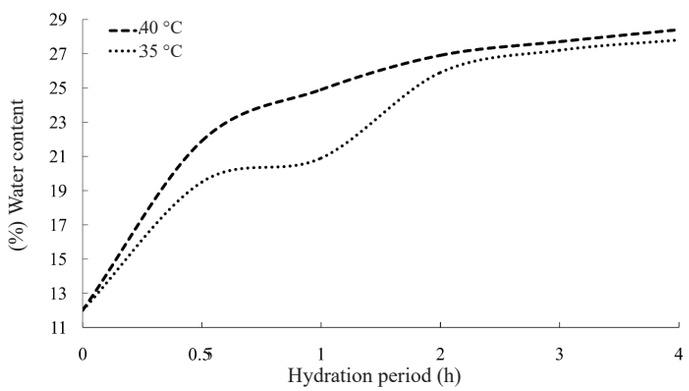

Figure 2. Water content of rice seeds hydrated for 0, 0.5, 1, 2, 3 and 4 hours at temperatures of 35 and 40 °C.

of 35 and 40 °C are presented in Table 4. It was observed that, regardless of the temperature, the soaking period of 0.5 h was not enough to evaluate the viability of rice seeds, since the results were lower than the ones obtained in the germination test (Table 4). This also applies to the soaking period of 1 h at the temperature of 35 °C (Table 4). During these periods, the viability values did not correspond to the ones of the germination test, probably due to the fact that seeds did not absorb enough water to activate the enzymatic system; there was not color or it was inconsistent.

Seeds soaked in the periods from 2 to 4 h at 35 °C had a maximum variation of only three pp in the viability percentages by tetrazolium test, observed in lot 5 (Table 4). This value is within the allowed tolerance among tests from the same laboratory established in the RST for TZ tests (Brasil, 2009). This also applies to the results obtained in lots of seeds soaked in periods from 1 to 4 h at 40 °C where the difference was six pp in lots 3 and 6 (Table 4). It was observed that, on an average, the tetrazolium test produced results that were coherent with the ones obtained by the germination test, with close values, obtaining differences of 3 pp, on an average (Table 4).

The association between germination and viability results by tetrazolium test was verified in cucumber (Lima et al., 2010), maize (Chamma and Novembre, 2007), ryegrass (Silveira, 2008), wheat (Carvalho et al., 2013), triticale (Souza et al., 2010), barley (Grzybowski et al., 2012) and forage grasses (Soares et al., 2016) seeds.

As for the methodological effectiveness, when using the hydration temperature of 35 °C for 1 hour, it was observed that the effectiveness was 85.6%, but in the other hydration periods at this temperature, the effectiveness was higher than 98% (Table 5). However, it is important to highlight that under these conditions, the seed color was weak and inconsistent (Figure 3). The same happened in relation to the temperature of 40 °C in the hydration period of 0.5 h, probably because the water content of seed was lower than 24%, which is considered the ideal condition for staining (Figure 2). As for the other hydration periods, it was verified that the effectiveness of the test at both soaking temperatures was similar (Table 5).

In order to choose the best method, the most effective methodology was the one which had the best results regarding effectiveness. Thus, it was noticed that starting from 2 h hydration at 35 °C and 1 h at 40 °C, there was no significant difference between the treatments (Table 5). The most effective was the one providing the same result in less time.

The reduction in the seed preparation time for the tetrazolium test was also suggested for other species, such as brachiaria (Novembre et al., 2006), tomato (Santos et al., 2007), maize (Chamma and Novembre, 2007) and cotton (Cervi and Mendonça, 2009).

In this context, results indicate that it is possible to

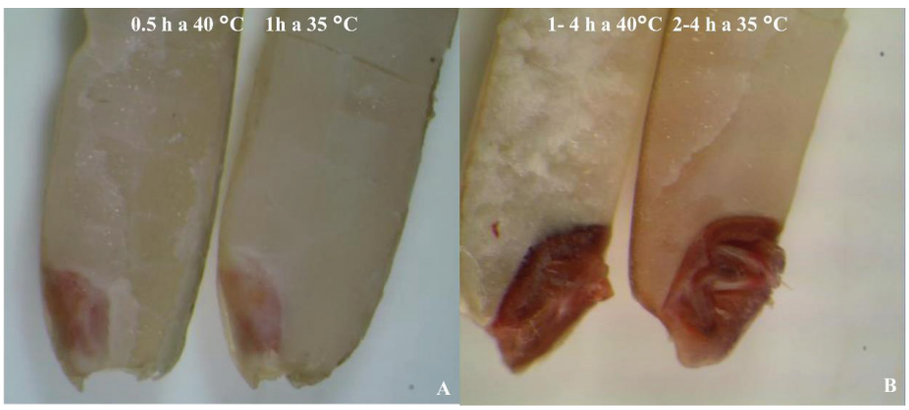

Figure 3. Cuts on rice seeds soaked at different temperatures (35 and 40 °C) and hydration periods and exposed to tetrazolium salt at 0.1%: 0.5 and 1 h hydration at 35 and 40 °C (A) and 1 h at 40 °C (B).

Table 5. Effectiveness of the tetrazolium test (%) according to different soaking periods at the temperatures of 35 and 40 °C.

Hydration temperature (°C)	Time (h)				
	0.5	1	2	3	4
35	0.0 bC	85.6 bB	98.7 aA	98.4 aA	98.9 aA
40	79.2 aB	98.6 aA	98.2 aA	97.9 aA	97.0 aA
CV (%)	3.74				

Averages followed by the same lowercase letter in the column and the same capital letter on the line do not different among themselves by Tukey's test at 5%.

estimate the viability of rice seeds, with a significant reduction in the preparation time of seeds, 1 h at 40 °C and 2 h at 35 °C, in opposition to what was established in the rules for seed analysis, which recommends a minimum period of 18 h in this stage. It is possible to obtain a result in 3 h, considering 1 h to cut, 1 h to hydrate and 1 h for seed staining.

Experiment III

Data about the initial water content of seeds, after removing palea and lemma after hydration, are demonstrated in Table 6. It was verified that the initial humidity among lots varied from 11.3 to 13.2 and the water content of seeds after the hydration period was 26% on average; this is considered important to activate the enzymatic system which is responsible for the color of the seeds (Table 6). In experiment II, water contents from 24% were enough to obtain a proper color.

Table 6. Average data of initial water content, after removing palea and lemma and after preconditioning (hydration).

Lots	Water contents (%)		
	Initial	After removing palea and lemma	After hydration
01	12.8	13.7	25.9
02	12.9	13.6	26.7
03	13.0	13.4	26.6
04	13.0	13.8	25.7
05	13.2	13.4	26.0
06	12.8	13.9	26.9
07	11.4	12.2	25.3
08	11.3	11.6	25.1
Average	12.6	13.2	26.0

Table 7. Viability by germination and tetrazolium test and effectiveness of tetrazolium test, in 8 rice seed lots from the BR Irga 424 cultivar.

Lots	Germination	TZ test	TZ effectiveness
		%	
1	89 a	93 a	96.2
2	75 c	72 c	96.0
3	78 c	75 c	96.6
4	87 a	89 b	96.9
5	83 b	85 b	96.7
6	51 d	62 d	78.6
7	89 a	88 b	97.0
8	74 c	73 c	96.2
Average	78	80	94.3
CV (%)	3.66	4.26	6.13

*Averages followed by the same letter in the column do not differ among themselves by Scott-Knott test at 5% probability.

Data on germination, viability by tetrazolium test (TZ) and effectiveness of the TZ test are presented in Table 7. There was quality distinction among the lots, highlighted by the germination results that varied between 51 and 89%; this is an important condition to comply with the proposal of this experiment. It was verified the analysis of average comparison indicated lot 6 as the one with the lowest germination (51%), and lots 1, 4 and 7 as the ones with the best quality; their germination was between 87 and 89% (Table 7). For most lots, number proximity was observed between the results from the germination and tetrazolium tests, except for lot 6 (Table 7).

By the results, it was verified that there was concordance between the classification obtained by the tetrazolium test using the methodology proposed in this study, and the germination test, except for lots 4 and 7, which even with differences of 2 and 1 pp between the germination and tetrazolium tests, respectively, statistically differed (Table 7). The equivalence between the results was confirmed when the effectiveness formula was applied, which was above 96% for most lots, except for lot 6, where there was a 78.6% effectiveness (Table 7).

The results obtained in experiments I and II were crucial to reduce the total period for the viability evaluation of rice seeds. Using the traditional methodology, results are obtained in 24 hours; however, in this study, equivalent results were obtained in up to 3 hours, considering 1 hour for each step: preconditioning, cut and staining.

In the light of the obtained results, considering that the hydration period factor, combined with the use of less reagents and less staining time for previously peeled rice seeds, are desirable characteristics in running the viability test to obtain results from laboratory analysis. It is recommended, for seeds from this species, to combine temperature and hydration period/tetrazolium salt concentration/staining period of 40 °C/1 h/0.25/1 h at 35 °C, in the absence of light.

Conclusions

It is possible to run the tetrazolium test on rice seeds using the hydration of peeled seeds for 1 h at 40 °C and staining for 1 h with a salt concentration of 0.25%.

References

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

CANTERI, M.G.; ALTHAUS, R.A.; VIRGENS FILHO, J.S.; GIGLIOTI, E.A.; GODOY, C.V. SASM-Agri: Sistema para análise e separação de médias em experimentos agrícolas pelos métodos Scott-Knott, Tukey e Duncan. *Revista Brasileira de Agrocomputação*, v.1, p.18-24, 2001. http://agrocomputacao.deinfo.uepg.br/dezembro_2001/Arquivos/RBAC_Artigo_03.pdf

CARVALHO, T.C.; KRZYZANOWSKI, F.C.; OHLSON, O.C.; PANOBIANCO, M. Tetrazolium test adjustment for wheat seeds. *Journal of Seed Science*, v.35, n.3, p.361-367, 2013. http://www.scielo.br/pdf/jss/v35n3/13.pdf

CERVI, F.; MENDONÇA, E.A.F. Adequação do teste de tetrazólio para sementes de algodoeiro. *Revista Brasileira de Sementes*, v.31, n.1, p.177-186, 2009. http://www.scielo.br/pdf/rbs/v31n1/a20v31n1.pdf

CHAMMA, H.M.C.P.; NOVEMBRE, A.D.L.C. Teste de tetrazólio para as sementes de milho: período de hidratação e de coloração das sementes. *Revista Brasileira de Sementes*, v.29, n.2, p.125-129, 2007. http://www.scielo.br/pdf/rbs/v29n2/v29n2a17.pdf

COSTA, N.P.; FRANÇA-NETO, J.B.; KRZYZANOWSKI, F.C.; HENNING, A.A. *Metodologia alternativa para o teste de tetrazólio em semente de soja- série sementes*. Londrina: Embrapa, 2007 (Circular técnica, 39).

FRANÇA-NETO, J.B.; KRZYZANOWSKI, F.C.; COSTA, N.P. *O teste de tetrazólio em sementes de soja*. Londrina: EMBRAPA-CNPS, 1998. 72p.

GRZYBOWSKI, C.R.S.; OHLSON, O.C.; SILVA, R.C.; PANOBIANCO, M. Viability of barley seeds by the tetrazolium test. *Revista Brasileira de Sementes*, v.34, n.1, p.47-54, 2012. http://www.scielo.br/pdf/rbs/v34n1/a06v34n1.pdf

LIMA, L.B.; PINTO, T.L.F.; NOVEMBRE A.D.L.C. Avaliação da viabilidade e do vigor de sementes de pepino pelo teste de tetrazólio. *Revista Brasileira de Sementes*, v. 32, n.1, p. 60-68, 2010. http://www.scielo.br/pdf/rbs/v32n1/v32n1a07.pdf

MACHADO, A.A.; CONCEIÇÃO, A.R. *Sistema de análise estatística para Windows*. WinStat. Versão 2.0.UFPel, 2003.

MAPA. Ministério da Agricultura, Pecuária e Abastecimento. *Instrução Normativa* n° 45, de 17 de setembro de 2013. 38p. http://www.agricultura.gov.br/vegetal/noticias/2013/09/mapa-atualiza-padroes-de-sementes-de-grandes-culturas

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

MOORE, R. P. *Tetrazolium testing handbook*. Raleigh: North Carolina State University, 1977.

NOVEMBRE, A.D.L.C.; CHAMMA, H.M.C.P.; GOMES, R.B.R. Viabilidade das sementes de braquiária pelo teste de tetrazólio. *Revista Brasileira de Sementes*, v.28, n.2, p.147-151, 2006. http://www.scielo.br/pdf/rbs/v28n2/a20v28n2.pdf

PINTO, T.L.F.; MARCOS-FILHO, J.; FORTI, V.A.; CARVALHO, C.; GOMES-JUNIOR, F.G. Avaliação da viabilidade de sementes de pinhão manso pelos testes de tetrazólio e de raios X. *Revista Brasileira de Sementes*, v.31, n.2, p.195-201, 2009. http://www.scielo.br/pdf/rbs/v31n2/v31n2a23.pdf

SANTOS, S.R.G.; PAULA, R.C.; FOGAÇA, C.A.; MÔRO, F.V.; COSTA, R.S. Viabilidade de sementes de *Sebastiania commersoniana* (Baill.) Smith & Downs (branquilho) – Euphorbiaceae -pelo teste de Tetrazólio. *Científica*, v.34, n.1, p.39-45, 2006. http://www.cientifica.org.br/index.php/cientifica/article/viewFile/27/12

SANTOS, M.A.O.; NOVEMBRE, A.D.L.C.; MARCOS-FILHO, J. Tetrazolium test to assess viability and vigour of tomato seeds. *Seed Science and Technology*, v.35, p.213-223, 2007. https://www.researchgate.net/publication/233640815_Tetrazolium_test_to_assess_viability_and_vigour_of_tomato_seeds

SILVA, R.C.; GRZYBOWSKI, C.R.S.; FRANCA-NETO, J.B.; PANOBIANCO, M. Adaptação do teste de tetrazólio para avaliação da viabilidade e do vigor de sementes de girassol. *Pesquisa Agropecuária Brasileira*, v. 48, n.1, p.105-113, 2013. http://www.scielo.br/pdf/pab/v48n1/14.pdf

SILVEIRA, M.A.M. Teste de tetrazólio como rotina para avaliar germinação em sementes de azevém (*Lolium multiflorum* Lam.). *Pesquisa Agropecuária Gaúcha*, v.14, n.2, p.113-118, 2008. http://www.fepagro.rs.gov.br/upload/1398791787_artigo_05.pdf

SOARES, V.N.; ELIAS, S.G.; GADOTTI, G.I.; GARAY, A.E.; VILLELA, F.A. Can the tetrazolium test be used as an alternative to the germination test in determining seed viability of grass species? *Crop Science*, v.56, n.2, p.707-715, 2016. https://dl.sciencesocieties.org/publications/cs/abstracts/56/2/707

SOUZA, C.R.; OHLSON, O.C.; GAVAZZA, M.I.A.; PANOBIANCO, M. Tetrazolium test for evaluating triticale seed viability. *Revista Brasileira de Sementes*, v.32, n.3, p.163-169, 2010. http://www.scielo.br/pdf/rbs/v32n3/v32n3a18.pdf