Tetrazolium test adjustment for wheat seeds[1]

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ABSTRACT - The assessment of the germination test in wheat seeds varies from 4 to 15 days, because the species normally presents dormancy in freshly harvested seeds. The tetrazolium test can characterize seed viability in less than 24 hours including lots with dormancy seeds. The objective of this study was to develop a practical and efficient procedure for evaluating the viability of wheat seeds using the tetrazolium test. Five seed lots of the BRS 208 cultivar were used, where the following were tested: a) pre-conditioning between moist paper towels or direct immersion in water for 18 hours, at 20 °C; b) longitudinal section of the embryo and the endosperm; c) coloration on paper or by immersion for 2 and 3 hours, at 30 and 40 °C; and d) concentrations of tetrazolium solution at 0.075%, 0.1%, 0.5% and 1.0%. The tetrazolium test may be efficiently used to evaluate wheat seed viability by pre-conditioning the seeds between paper towels (18 hours, at 20 °C) and adopting the following combinations of preparation and coloration: coloration of both halves of the seed on paper (2 hours, at 30 °C), in a 1.0% tetrazolium solution; or coloration of one half of the seed by immersion (3 hours, at 30 °C), in a 0.1% tetrazolium solution; or coloration of one half of the seed by immersion (2 hours, at 40 °C), in a 0.075% tetrazolium solution. This latter procedure is recommended for identifying and discarding lots with lower viability.

Index terms: *Triticum aestivum*, germination, viability.

Adequação do teste de tetrazólio para sementes de trigo

RESUMO - A avaliação do teste de germinação em sementes de trigo varia de 4 a 15 dias, uma vez que a espécie normalmente apresenta dormência em sementes recém-colhidas. O teste de tetrazólio pode caracterizar a viabilidade das sementes em menos de 24 horas, inclusive para lotes com sementes dormentes. O trabalho objetivou propor procedimento prático e eficiente para avaliação da viabilidade de sementes de trigo pelo teste de tetrazólio. Utilizaram-se cinco lotes de sementes da cultivar BRS 208, testando-se: a) pré-condicionamento entre papel toalha umedecido ou por imersão direta em água, por 18 horas, a 20 °C; b) corte em bissecção longitudinal ao longo do embrião e do endosperma; c) coloração sobre papel ou por imersão, durante 2 e 3 horas, a 30 e 40 °C; e d) concentrações de solução de tetrazólio a 0,075%, 0,1%, 0,5% e 1,0%. O teste de tetrazólio pode ser empregado com eficiência para avaliação da viabilidade de sementes de trigo, por meio do pré-condicionamento das sementes entre papel (18 horas, a 20 °C), adotando-se as seguintes combinações de preparo e coloração: coloração das duas metades da semente sobre papel (2 horas a 30 °C), em solução de tetrazólio a 1,0%; ou coloração de uma das metades da semente por imersão (3 horas a 30 °C), em solução de tetrazólio a 0,1%; ou coloração de uma das metades da semente por imersão (2 horas a 40 °C), em solução de tetrazólio a 0,075%, sendo este procedimento indicado para a identificação e descarte de lotes de viabilidade inferior.

Termos para indexação: *Triticum aestivum*, germinação, viabilidade.

Introduction

Wheat is one of the most important cereals grown in the world (Fourar-Belaifa et al., 2011), with China leading the ranking of producers. Brazil ranks 18th, producing 6 mmT (FAOSTAT, 2011), which is insufficient to supply its domestic market.

The productivity of a crop depends directly on plant establishment in the field, which in turn, depends on the growth conditions and especially on the quality of the seeds obtained by the farmer.

Laboratories are able to evaluate wheat seed viability with the germination test, which is widely used in the commercial

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sector and provides a reliable result. However, this test can take up to 15 days for recently harvested wheat seeds (Brasil, 2009), which normally show dormancy (Andreoli et al., 2006). Such dormancy avoids seed germination during the pre-harvest stage (germination on the ear), which can cause significant losses to farmers. On the other hand, in order to make a rapid decision on the destination of lots, it is necessary to have a test, which can evaluate seed viability soon after harvest.

In this context, the tetrazolium test stands out, because it allows a rapid evaluation of seed viability in less than 24 hours and is unaffected by conditions which generally interfere in the germination test, such as the incidence of microorganisms (França-Neto et al., 1998). The most significant advantage of this test in wheat is the possibility of applying it to recently harvested seeds, which do not need to be treated to overcome dormancy before the initial testing.

The evaluation of seed viability by tetrazolium test is routinely used in quality control programs for various species, including soybean (França-Neto et al., 1998), corn (Dias and Barros, 1995; Chamma and Novembre, 2007; ISTA, 2008), watermelon (Bhering et al., 2005), brachiaria (Novembre et al., 2006), tomato (Santos et al., 2007), coffee (Zonta et al., 2009), castor bean (Gaspar-Oliveira et al., 2009), cucumber (Lima et al., 2010), macaw palm (Ribeiro et al., 2010), triticale (Souza et al., 2010), among other important cash crops.

The literature describes different procedures for applying tetrazolium to evaluate wheat seed viability. The Rules for Seed Testing (Brasil, 2009) contain two protocols for pre-conditioning (between paper or directly in water); two types of seed preparation for coloration (longitudinal section along the embryo and ¾ of the endosperm and removal of the embryo); two concentrations of tetrazolium solution (0.5% and 1.0%); as well as the length of time these seeds should remain in the solution, which is variable.

Another recommendation for the test is described by the International Seed Testing Association - ISTA (ISTA, 2008), which indicates two periods of imbibition (4 h and 18 h), two types of seed preparation (removal of the embryo or a longitudinal section along the embryo and ¾ of the endosperm), and one concentration of tetrazolium solution (1.0%).

Thus, it can be seen that the procedures recommended in the literature differ significantly and would need considerable manipulative ability of the analyst in some cases, e.g., for embryo removal. Because there are different protocols with differing levels of difficulty, the tetrazolium test is not routinely adopted by wheat seed producers, and viability is evaluated only by means of the germination test.

A significant loss in the physiological potential of the wheat seeds produced has also been observed due to rainfall in the pre-harvest period, physical injuries during the harvesting process (Farrer et al., 2005), drying (Ghosh et al., 2007) and damage during storage (Spanò et al., 2007; Fourar-Belaifa et al., 2011), since the seeds are stored during the summer months and suffer from the effects of temperature, relative air humidity and storage pests.

Therefore, the objective of the present study was to propose practical and efficient procedures for evaluating the viability of wheat seeds using the tetrazolium test.

Materials and Methods

This research was conducted in the Seed Analysis Laboratory of the Department of Plant Science and Health, Federal University of Paraná, in Curitiba, from June 2010 to April 2011, with five lots of the BRS 208 wheat seed cultivar, which showed distinct physiological qualities (Table 1).

Table 1. Mean data of the seed moisture content and germination of five lots of wheat, cultivar BRS 208.

Lots	Moisture Content	Germination
	..... %	
1	12.7	97 a
2	12.4	91 ab
3	12.6	90 ab
4	12.7	89 b
5	12.4	85 b
C.V. (%)	-	5.71

Means followed by the same letter in the column do not differ according to Tukey's test ($p \leq 0.05$).

The replications were organized as follows: first, the seed sample from each lot was homogeneized in a division centrifuge, based on the criteria established in the The Rules for Seed Testing (Brasil, 2009). At the end of the homogenization, each sample was divided into four subsamples (repetitions) of similar weight using the division centrifuge.

The seeds were put into kraft paper bags and stored in a controlled environment (14 °C and 63% RH) during the experiment to reduce deterioration.

The following evaluations were made:

Seed moisture content: determined by the oven method at 105 ± 3 ºC, for 24 hours, using two subsamples of 5.0 g of seeds for each repetition (Brasil, 2009). The results were expressed as a mean percentage for each lot on a wet weight basis.

Germination: four subsamples of 50 seeds were taken for each repetition (totaling 16 repetitions), distributed on a moistened roll paper towel with a amount of water equivalent to 2.5 times the substrate weight and kept in a germinator at

20 °C, under a constant light regime. A count of normal plants was made on the fifth day after sowing, based on the rules established in The Rules for Seed Analysis (Brasil, 2009). The results were expressed as a percentage.

Tetrazolium test (TZ): four replications of 50 seeds were used for each lot, totaling 200 seeds (Brasil, 2009). Ten per cent more seeds were used as a safety measure in the case of mistakes in preparation (sections), but only 50 seeds per replication were used during the evaluation. The following methodologies were tested:

1) Pre-conditioning between paper towels: seeds were wrapped between moistened paper towels for 18 h, at 20 °C (Figure 1A), with a quantity of water equivalent to 2.5 times the weight of the paper (Brasil, 2009), with the following combinations of preparation and coloration being tested: *1.1.)* seed with a longitudinal section along the embryo and endosperm (Figures 1B and 1C), with coloration of the two halves on a sheet of moistened filter paper with a tetrazolium solution equivalent to 2.5 times the weight of the paper, placed for coloration at 30 °C (Brasil, 2009; ISTA, 2008) and 40 ºC (Souza et al., 2010), for 2 hours, at concentrations of 0.1% (Dias and Barros, 1995), 0.5% (Brasil, 2009) and 1.0% (ISTA, 2008); *1.2.)* seed with a longitudinal section along the embryo and endosperm, with one of the halves discarded and immersion of the other half in 3 mL of tetrazolium solution, in a plastic cup (50 mL capacity), placed for coloration at 30 °C (ISTA, 2008; Brasil, 2009) and 40 ºC (Souza et al., 2010), for 2 and 3 hours, at concentrations of 0.1% (Dias and Barros, 1995) and 0.075% (França-Neto et al., 1998).

Figure 1. A - Forms of pre-conditioning of wheat seeds (between paper towels and immersion in water); B and C – details of longitudinal seed sectioning along the embryo and the endosperm.

2) Pre-conditioning by immersion: the seeds were directly immersed in 30 mL of water (Figure 1A), in a beaker (50 mL capacity) for 18 h, at 20 °C (ISTA, 2008), with the following combinations of preparation and coloration being tested: seed with a longitudinal section along the embryo and endosperm (Figures 1B and 1C), with coloration of both halves on a sheet of moistened filter paper with a tetrazolium solution equivalent to 2.5 times the weight of paper placed to color at 30 °C (ISTA, 2008; Brasil, 2009) and 40 ºC, for two hours, at concentrations of 0.1% (Dias and Barros, 1995), 0.5% (Brasil, 2009) and 1.0% (ISTA, 2008).

Seed moisture content was determined for both forms of pre-conditioning according to item 1.

After each period of coloration, the seeds were maintained on the filter paper (coloration on paper) or immersed in water (coloration by immersion) and kept at temperatures of 5 to 10 °C until evaluation, which was made on the same day as the coloration.

Figure 2 shows the details of the external and internal morphology of the wheat seed, indicating the vital areas (coleoptile, plumule, mesocotyl, radical, coleorhizae and the central region of the scutellum).

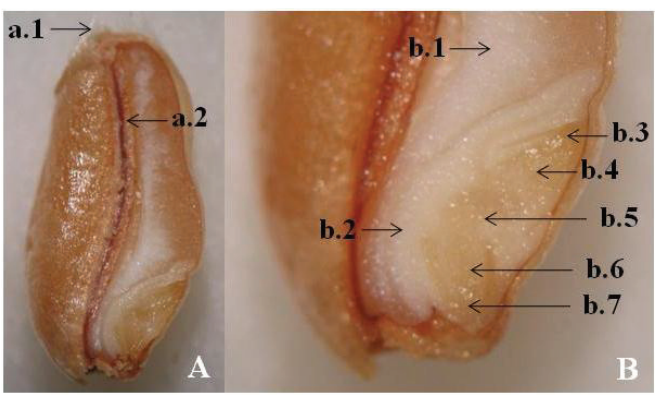

Figure 2. Wheat caryopsis, showing: the external morphology (A), represented by trichomes (a.1) and pericarp + tegument (a.2); and the internal morphology (B), composed of the endosperm (b.1), scutellum (b.2), coleoptile (b.3), plumule (b.4), mesocotyl (b.5), radicle (b.6) and coleorhiza (b.7).

The determination of wheat seed viability was based on the methodology used for corn seeds as described by Dias and Barros (1995), and adapted for wheat seeds in this study. The following criteria were used:

- *Viable seeds:* with brilliant rose coloration, superficial, uniform and without embryonic lesions (Figure 3A); seeds with small points of deterioration or dead tissues in non-vital regions (Figure 3B); seeds with a brilliant rose-colored embryo or with an intense and deep red coloration, which showed small areas or points more intensely colored or discolored at the extremities of the scutellum, without reaching the vital region (Figures 3B, 3C and 3D); seeds which were damaged in areas of the scutellum as long as the vital embryonic regions were

intact (Figures 3E and 3N); seeds whose damage reaches the radicle but with the mesocotyl (seminal roots) region intact (Figures 3G and 3J);

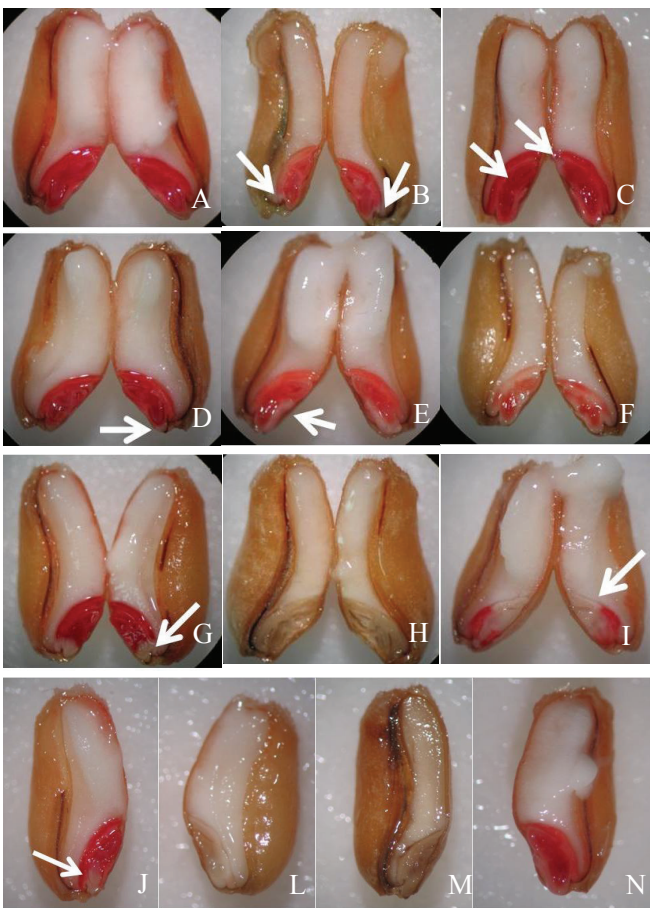

Figure 3. Viable wheat seeds: A, B, C, D, E, G, J and N; unviable wheat seeds: F, H, I, L and M.

- *Unviable seeds*: intense coloration of the embryo with discolored areas on the plumule and/ or the coleoptile and/ or the radicle, plus the mesocotyl region and/ or central part of the scutellum (Figure 3F); seeds with part or all of the scutellum discolored, and part or all of the central axis discolored (Figure 3I); embryo totally discolored (Figures 3H, 3L and 3M).

Except for the determination of moisture content, the data obtained from each test were analyzed according to an entirely random design with four repetitions. Pearson's Correlation Coefficient was also calculated between the germination test data and the most promising procedures from the tetrazolium test. The analysis was made by transforming data to arcsine $\sqrt{x}/100$. The means were compared using Tukey's test ($p \leq 0.05$).

Results and Discussion

The values for the moisture content of the five seed lots were similar (Table 1), which was an important condition for conducting the tests. The results of the germination test indicated that lot 1 had the highest viability and lots 4 and 5 had low viability, while lots 2 and 3 had intermediate results (Table 1). Therefore, lots with distinct physiological qualities were used, which was crucial for fulfilling the objectives of this study.

Table 2 shows the results of wheat seed viability from the tetrazolium test. It was observed that when the seeds were pre-conditioned between paper towels, with coloration of both halves for 2 h, at 30 °C, in a 1.0% tetrazolium solution (Figure 4A), the classification of viability was the same as that of the germination test (Table 1), with a highly significant correlation coefficient (0.97**). This type of seed preparation (longitudinal section along the embryo and the endosperm), using a 2 h coloration time, enabled a clear view of the embryonic tissues and was faster when compared to the methods recommended in the literature: incision and removal of the embryo or a longitudinal section along the embryo and ¾ of the endosperm, for coloration periods varying from 3 to 24 h (ISTA, 2008; Brasil, 2009).

According to Kruse (1996), an advantage of the preparation with incision of the embryo is that of enabling an evaluation of the viability of the scutellum and the radicle. On the other hand, the longitudinal section along the embryo and the endosperm allows a view of all the internal embryonic structures, and it is recommended for other cereals, as triticale (Souza et al., 2010).

With the coloration of both halves of the wheat seed, the whole seed can be observed internally; thus, a more reliable assessment can be made about its viability. However, preparing this seed before coloration is time-consuming because both halves have to be positioned on a sheet of moist paper.

By using the same method of pre-conditioning and preparation (imbibition between paper towels / coloration of both seed halves on paper), but with coloration at 40 °C (Table 2, upper part), it was not possible to observe the same ranking of the lots of the germination test (Table 1).

In the case of pre-conditioning by immersion (Table 2, lower part), a distinct classification from the one obtained in the germination test (mainly for showing the worst lots) was observed or there was even no separation of the viability between lots (combination 40 °C/ 1.0%). This conflicts with the recommendations of ISTA (2008) and Brasil (2009), which advise the use of a pre-conditioning by immersion, at 20 °C, for 4 to 18 hours for wheat. Souza et al. (2010), working with triticale seeds, also verified that the pre-conditioning process

by immersion was inefficient at separating lots in a similar manner to the germination test.

Table 2. Results of seed viability of five lots of wheat seeds, cultivar BRS 208, obtained from different procedures of the tetrazolium test (TZ).

Pre-conditioning between paper towels/ coloration of both halves of the seed on paper (2 h)						
Lots	30 °C			40 °C		
	Concentrations of TZ salt ..... %			Concentrations of TZ salt ..... %		
	0.1	0.5	1.0	0.1	0.5	1.0
	..... % of viable seeds.....					
1	84 abc*	86 a	93 a	64 ab	92 a	92 a
2	83 bc	76 b	89 ab	74 a	85 b	89 ab
3	86 ab	82 ab	88 ab	57 b	81 bc	89 ab
4	88 a	83 ab	85 b	57 b	82 bc	85 bc
5	80 c	81 ab	82 b	63 ab	76 c	82 c
C.V. (%)	3.2	4.3	4.5	7.9	3.8	3.7
Pearson's C.C.	-	-	0.97**	-	-	-
Hydration by immersion / coloration of both halves of the seed on paper (2 h)						
Lots	30 °C			40 °C		
	Concentrations of TZ salt ..... %			Concentrations of TZ salt ..... %		
	0.1	0.5	1.0	0.1	0.5	1.0
	..... % of viable seeds					
1	76 ab*	90 a	88 a	54 ab	90 ab	92 a
2	63 bc	89 a	84 ab	64 a	91 a	91 a
3	80 a	86 a	74 cd	50 bc	91 a	89 a
4	79 a	65 b	77 bc	53 ab	74 b	87 a
5	58 c	83 ab	64 d	38 c	85 b	83 a
C.V. (%)	7.9	8.9	4.8	7.9	8.2	5.4

*Means followed by the same letter in the column do not differ by Tukey's test ($p \leq 0.05$);
**significant at 1% of probability according to the "t" test.

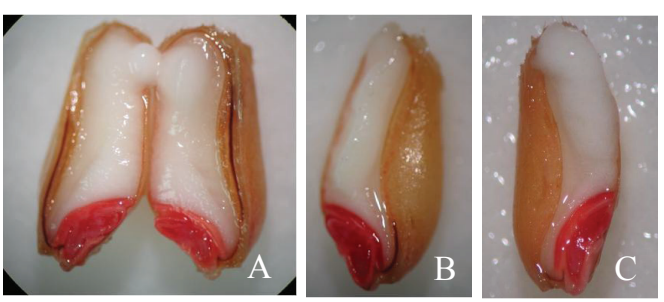

Figure 4. Pre-conditioning between paper towel (18 h / 20 °C), with: (A) coloration of both halves of the seed on paper (1.0% / 30 °C / 2 h); (B) coloration of one half of the seed by immersion (0.1% / 30 °C / 3 h); (C) coloration of one half of the seed by immersion (0.075% / 40 °C / 2 h).

After two forms of pre-conditioning, there were differences as for seed moisture content according to the procedure tested (Table 3). The imbibitions between paper towels resulted in lower moisture levels compared to imbibitions by immersion, considering the same time period (18 h) and temperature (20 °C). When the seeds were pre-conditioned by immersion, moisture levels between 37.0% and 38.4% were obtained, and this procedure did not result in a similar classification of lots as that of the germination test (Table 2, lower part). These results can be associated with the rapid entrance of water into the seed, causing alterations in cell shape and structure, which may be temporary or permanent, depending on intensity.

Table 4 shows the results of the tetrazolium test, using pre-conditioning between paper towels, with coloration by immersion of one seed half. The combination 0.1% / 30 °C / 3 h (Table 4, upper part) resulted in a reliable separation of the lots (Figure 4B), into similar classes of viability as those obtained in the germination test. Lot 1 had the highest quality; lots 2 and 3 had medium quality, and lots 4 and 5 had the worst performance, with a highly significant correlation coefficient (0.96**). This type of preparation (discarding one half of the seed and immersion of the other half in a tetrazolium solution) is very practical, as it reduces seed preparation time without

affecting tissue observation, which is another advantage of using a more dilute and economical tetrazolium solution. When a concentration of 0.075% tetrazolium was used, with coloration of one half of the seed by immersion, at 40 ºC, for 2 hours (Table 4, lower part), a reliable separation of those lots with the lowest viability (lots 4 and 5) was obtained, as classified in the germination test (Table 2), but it was impossible to identify those lots with an intermediate quality (lots 2 and 3). This procedure allowed a clear coloration of the embryonic tissues (Figure 4C), using a tetrazolium solution of lower concentration, resulting in more economy, and can be used to test incoming lots with the aim of a rapidly discarding lower–quality ones.

Table 3. Mean percentage of moisture content of seeds from five lots of wheat, cultivar BRS 208, after pre-conditioning of the seeds between paper towel and by immersion at 20 °C for 18 hours.

Lots	Moisture content after pre-conditioning of seeds (18h / 20ºC)	
	Between paper towels	By immersion
	..... %	
1	24.6	37.0
2	25.3	38.4
3	25.1	38.0
4	24.9	37.9
5	25.8	38.3

Table 4. Results of viability for seeds from five lots of wheat, cultivar BRS 208, obtained from different procedures of the tetrazolium test (TZ).

Pre-conditioning between paper towel/ Coloration of one half of the seed by immersion				
Concentrations of TZ salt at 0.1%				
Lots	30 °C		40 °C	
	Coloration time (h)		Coloration time (h)	
	2	3	2	3
	..... % of viable seeds.....			
1	82 a[1]	90 a	90 a	92 a
2	72 b	85 ab	86 b	85 b
3	62 c	83 ab	86 b	89 ab
4	71 bc	82 b	82 c	84 b
5	69 bc	81 b	78 d	76 c
C.V. (%)	4.7	4.4	2.0	3.0
Pearson's C.C.	-	0.96**	-	-
Concentrations of TZ salt at 0.075%				
Lots	30 °C		40 °C	
	Coloration time (h)		Coloration time (h)	
	2	3	2	3
	..... % of viable seeds.....			
1	82 a[1]	81 a	86 a	88 a
2	78 a	72 b	80 a	80 bc
3	65 b	70 b	82 a	84 ab
4	68 b	64 b	70 b	74 c
5	55 c	53 c	62 b	63 d
C.V. (%)	4.0	4.5	4.4	3.8
Pearson's C.C.	-	-	0.88*	-

[1]Means followed by the same letter in the column do not differ according to Tukey's test ($p \leq 0.05$);
**and * significant at 1% and at 5% of probability, respectively, according to the "t" test.

França-Neto et al. (1998) recommended the use of 0.075% tetrazolium solution at a temperature of 40 °C to evaluate the viability of soybean seeds, since this concentration allows a suitable view of the embryo and of any mechanical damage and damage by moisture, stinkbugs and temperature. The same concentration was recommended for corn seeds (Dias and Barros, 1995).

For the remaining procedures tested (Table 4), the ranking of the lots was inconsistent and it was impossible to obtain the same classification as from the germination test (Table 1).

Conclusions

The tetrazolium test is efficient at evaluating wheat seed viability by pre-conditioning seeds between paper towels (18 hours at 20 °C), and by adopting the following combinations of preparation and coloration:

Coloration of both halves of the seed on paper (2 h, at 30 °C), in a 1.0% tetrazolium solution;

Coloration of one half of the seed by immersion (3 h, at 30 °C), in a 0.1% tetrazolium solution;

Coloration of one half of the seed by immersion (2 h, at 40 °C), in a 0.075% tetrazolium solution. This procedure is recommended for identifying and discarding lots with lower viability.

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