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Determination of pre-wetting procedure of coriander seeds for tetrazolium test

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ABSTRACT

Searching to determine the quality of seeds using rapid and effective tests, the aim of this study was to evaluate the influence of pre-wetting procedures on determining the viability of coriander seeds by the tetrazolium test. Ten seed batches of coriander were used. The experiment was carried out in a completely randomized design, in a 10x2 factorial scheme (10 batches x moistening and soaking in water) with four replications. Seeds of each batch were previously evaluated through water content, germination with and without fungicide treatment, and the emergence of seedlings in the sand. The seeds were pre-wetted in two ways: rolls of filter paper and directly in water for 16 hours at 20ºC temperature. Despite the numerical difference between the pre-wetting forms, these differences were not significant. Thus, we considered that both ways of pre-wetting are appropriate. Coriander seeds were influenced by pre-wetting, resulting in a quick availability evaluation through the tetrazolium test. Thus, pre-wetting can be carried out by water immersion or paper through longitudinal cutting.

Keywords: *Coriandrum sativum*, viability, rapid test, pre-soaking.

RESUMO

Determinação de procedimento de pré-umedecimento em sementes de coentro para o teste de tetrazólio

Na busca em determinar a qualidade das sementes por meio de testes rápidos e eficazes, objetivou-se avaliar a influência de procedimentos de pré-umedecimento na determinação da viabilidade de sementes de coentro pelo teste de tetrazólio. Foram utilizados dez lotes de coentro. O experimento foi realizado em delineamento inteiramente casualizado, em esquema fatorial 10x2 (10 lotes x umedecimento e embebição em água) com quatro repetições. As sementes de cada lote foram previamente avaliadas por meio do teor de água, da germinação com e sem tratamento químico com fungicida e da emergência de plântulas em areia. O pré-umedecimento das sementes foi realizado entre rolo de papel e por imersão em água, ambos por 16 horas a 20ºC. Apesar de ter havido diferença entre as formas de pré-umedecimento, as diferenças nos percentuais de eficiências entre formas de pré-umedecimentos não foram significativas. Assim, pondera-se que ambas as formas de pré-umedecimentos são adequadas. As sementes de coentro foram influenciadas pelo pré-umedecimento resultando em uma rápida avaliação de sua viabilidade através do teste de tetrazólio, podendo ser realizado o pré-umedecimento por imersão em água ou papel, mediante corte longitudinal.

Palavras-chave: *Coriandrum sativum*, viabilidade, teste rápido, pré-embebição.

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Like many other vegetables, the coriander crop (*Coriandrum sativum*, Apiaceae) is propagated by seeds (the achenes), even though the most important structures of the plant in food are the leaves and stem, whose demand is more significant in the North and Northeast regions of Brazil (Oliveira *et al*., 2007). Morphologically, the whole coriander fruit is classified as a diaquenium; it consists of two achenes that are generally sold whole (Silva *et al*., 2012; Nascimento *et al*., 2014). Thus, it is possible that just one of the fruits (seed) is viable.

The evaluation of the seed's physiological quality, for purposes of commercialization, has been based on the germination test. It takes 21 days to obtain germination test results for coriander crop (Brasil, 2009; ISTA, 2012). Intermediate countings can be performed since some crops, such as the coriander, show extended germination test duration and, usually, indicates infected seeds, which can contaminate other seedlings in the substrate during the test (Brasil, 2009). Given the above, the tetrazolium test is an alternative for a rapid seed viability test of several crops. This test is widely used by seed producer companies in different production stages, streamlining decision-making (Walter *et al*., 2020). The tetrazolium test is based on the activity of dehydrogenase enzymes which participate in the breathing process, forming a red and insoluble substance in the living tissues of the seeds (Elias *et al*., 2012; França-Neto & Krzyzanowski, 2019).

Despite being considered a simple test, several factors can interfere in the conduction, such as the pre-wetting process (Souza *et al.*, 2014). In that regard, the seed hydration process, which is determined according to each species' morphological and physiological traits, is carried out to facilitate the seed cutting and the activation of the enzymatic metabolism (AOSA/SCST, 2010). In this process, the choice of substrate and wetting time can be decisive when cutting and evaluating the achene.

In studies on the adequacy of tetrazolium test methodologies, the correct choice of the substrate in combination with soaking temperature has been one of the factors analyzed in order to select more suitable procedures for crops such as melon (Lima *et al.*, 2007), cucumber (Lima *et al.*, 2010) and carrot (Lima *et al.*, 2018). For coriander, Working Sheets on Tetrazolium Testing (ISTA, 2003) suggests that seeds should be pre-moistened in water for 24 hours. The Association of Official Seed Analysts (AOSA) suggests about 16-hour pre-wetting (AOSA/SCST, 2010) but does not specify the type of substrate.

Considering the relevance of the tetrazolium test for a rapid estimate of vegetable species viability and little information on using this method in Brazil for the coriander crop, the authors highlight the importance of developing studies that address specific methodological factors for this species. Thus, the aim of this study was to evaluate the influence of the pre-wetting procedure to determine the viability of coriander seeds using the tetrazolium test.

MATERIAL AND METHODS

The experiment was carried out in Laboratório Didático de Análise de Sementes of Faculdade de Agronomia "Eliseu Maciel" (31°48'6"S, 52°24'55"W, 21 meters altitude), at Universidade Federal de Pelotas-RS. Ten batches of marketable coriander seeds cv. Verdão were used. These seeds were produced in the South Region of Rio Grande do Sul. Each batch consisted of 500 grams of seeds (achene).

The experiment was carried out in a 10x2 factorial scheme [10 batches X 2 ways of pre-wetting procedures (paper and water)], in a completely randomized design, with four replications of 100 seeds. The authors evaluated two seeds per fruit to decrease the possible risk of a false negative result. If one of the seeds is viable, it is counted in the percentage of viability, even if the other seed present in the fruit is non-viable, according to Figures 1B, C, and D. To evaluate the initial quality of the seeds, we performed the following tests:

Water content (TA)

In a stove at 105±3ºC for 24 h, using two subsamples per 4.5-gram-seed batch, according to the recommendations of Regras para Análise de Sementes (Rules for Seed Analysis, RAS) (Brasil, 2009).

Germination (G)

In gerbox-type plastic boxes, using 100 seeds per replication, distributed on two blotting paper sheets, moistened with distilled water equivalent to 2.5 times the weight of dry paper, and kept in the germination room at a constant temperature of 20±1ºC (Brasil, 2009). The evaluation was done 21 days after sowing, considering the percentage of normal seedlings, according to Brasil (2009).

Using the results, we decided to repeat the experiment with fungicide-treated seeds, though, as these seeds are frequently contaminated by fungi, which negatively influence the germination test results. We applied Metalaxil-m + Fludioxonil at a dose of 200 mL 100 kg^{-1} seeds to treat the seeds. We decided to analyze germination isolated for a better data interpretation, using a 2x10 factorial scheme (with and without treatment x seed batches).

Seedling emergence in the sand (EPA)

Carried out in a germination room at constant temperature of 20ºC. The authors used four replications of 50 seeds, sown on trays containing sterile sand (average particle size) with 1.5-cm sowing depth. The maintenance of moisture in the trays kept it close to the field capacity, based on daily observations. The final counting of emerged seedlings was carried out 21 days after sowing.

For conducting the tetrazolium test (TZ), two pre-wetting procedures were tested: 1) in the paper substrate, the seeds were stored between Germitest® paper sheets (Figure 2A), moistened equivalent to 2.5 times the weight of dry paper, for 16 h at a temperature of 20ºC. 2) in pre-wetting with water (Figure 2B), the seeds were immersed for 16 h at a temperature of 20±1ºC. During this period, the whole material was kept in the germination room with a constant temperature of 20±1ºC. After the pre-wetting time, we cut the seeds longitudinally (Figure 2C), keeping half of the two seeds in the fruit (Figure 2D). Then, the seeds were exposed to 0.5% tetrazolium salt solution for five hours, in the dark, at 40ºC, at germination chambers BOD type.

Seed cutting, temperature, concentration, and staining time were chosen based on the preliminary tests, aiming to find more rapid and easy-execution alternatives to conduct the trial. We highlight that only one cutting was used (longitudinal along the fruit), being performed before staining with tetrazolium salt solution, reducing the execution time from 48 to 24 hours, as well as facilitating the execution of the test and more accurately estimating the feasibility, speeding up the process. However, in this study, we pointed out only the moistening step and cutting execution.

For staining time, the seeds were evaluated, considering the color and integrity of the tissues, classifying them as viable and non-viable. The viable seeds were: a) bright red seeds with no discolored areas, showing firm tissues (endosperm and embryo) in the two halves of the fruit (Figure 1A); b) at least one of the fruit's seed with bright red color throughout its length (Figures 2B, C and D), 10x images are captured using articulated table magnifiers.

The non-viable seeds were: a) Seeds with no color in the embryo, even if the endosperm had color (Figures 1E and F); b) Seeds with very soft pink color, showing little activity in the seed tissues (Figure 1G); c) Seeds with intense carmine red in both halves and absence of color in more than 1/3 in the critical region of the endosperm (close to the embryo) and the embryo; d) Absence of full color in both seeds of the fruit (Figure 1H).

To calculate the viability results, the authors summed the percentual of viable seeds, comparing the results with the obtained in standard germination test, using efficiency analysis of the tetrazolium test: TZn efficiency = [1-(|G-TZn|)/G]100 (Equation 1)

Where: G= % of normal seedlings obtained using the germination test. TZn= % of viable seeds obtained using tetrazolium tests.

Except the results obtained in Equation 1, the obtained data were submitted to analysis of variance ($p < 0.05$) and, when significant by F test, the averages were compared using Scott-Knott test for the batches and Tukey test for the germination with and without chemical treatment, at the level of 5% probability. For statistical analysis, Sisvar software, version 5.3, was used.

RESULTS AND DISCUSSION

The initial water content of the seeds ranged from 9.1% to 10.5% (Table 1), with a maximum difference of 1.4%, showing uniformity in batches. This proximity between values is essential to avoid differences in metabolic activities and moisture speed during the experiment (Steiner *et al*., 2011).

After pre-wetting, the seeds showed 52% average water content. Several studies on tetrazolium test methodologies report water content between 25 and 30% as enough to activate the seed metabolism (Lima *et al*., 2010; Flores *et al*., 2015; Paraíso *et al*., 2019), which is necessary for such a study. The results of this study suggest that 16 hours are enough to reactivate seed enzymatic metabolism, a fundamental step for staining living tissues by the tetrazolium test. Working Sheets on Tetrazolium Testing (ISTA, 2003) suggests a 24-hour pre-wetting period for coriander seeds, making the test longer. Working Sheets on Tetrazolium Testing (ISTA, 2003) recommendations establish that seeds should be separated first from fruits and only one of the seeds should be used, being soaked in water for 24 hours. Afterward, a longitudinal cutting before staining by the tetrazolium salt solution for 24 hours. In the evaluation, the seed should be cut longitudinally for a second time to observe the structures. In this study, with only one cutting, the authors could obtain the same results.

A significant effect was observed in batches for germination, regardless of whether the seed was treated or not (Table 1). In order to evaluate the germination percentage of the no-chemical-treated seeds (Table 1), three different levels of quality were noticed. The batches 2, 4, 5, 6, and 7 showed lower performance than the others, showing values between 79 and 86%. Considering all the evaluated batches, only batch 4 showed germination inferior to 80%. However, we highlight that all batches showed germination above the minimum standard required for commercializing coriander seeds in Brazil, which is 65% (Brasil, 2019). This difference among the batches is interesting since it shows that pre-wetting is recommendable for any vigor level of a batch.

Concerning germination of

Table 1. Average values for water content (TA), seed germination with and without chemical treatment, and the seedling emergence in sand (EPA) in different coriander seed batches. Pelotas, UFPel, 2020.

Batches	TA (%)	Germination (%)		EPA
		No-treated	Treated**	(%)
1	10.5[ns]	89Ab	91Ab	90[ns]
2	9.4	81Bc	95Aa	86
3	9.1	95Aa	96Aa	94
4	10.1	79Ac	84Ad	87
5	9.8	81Bc	90Ac	84
6	9.9	84Bc	92Ab	85
7	9.1	86Ac	86Ad	92
8	9.6	97Aa	98Aa	96
9	9.7	97Aa	98Aa	96
10	9.8	90Ab	94Ab	86
Average	9.7	88	93	89
CV (%)	-	5.15	3.42	7.26

Averages followed by the same uppercase letters in the lines and lowercase letters in the columns do not differ from each other by the Scott-Knott test at 5% probability; ns = not significant; CV = coefficient of variation; **seeds treated with Metalaxil-m + Fludioxonil.

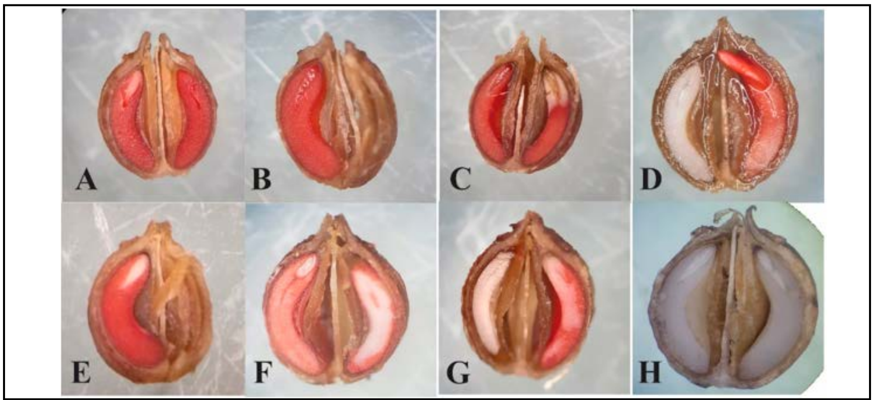

Figure 1. Viability of coriander seeds by the tetrazolium test: viable (A, B, C, D) and non-viable (E, F, G, H). Pelotas, UFPel, 2020

Table 2. Viability of coriander seed batches and efficiency of the tetrazolium test (TZ) using two forms of pre-wetting (paper and water). Pelotas, UFPel, 2020.

Batches	Pre-wetting (%)		Average	Efficiency of TZ test (%)			
	Paper	Soaking in water		Paper	Water	Paper[1]	Water[1]
1	92 Ba	95 Aa	94	96 Ba	93	100	94
2	93 Ba	95 Aa	94	86	83	97	93
3	90 Ba	95 Aa	93	95	99	95	92
4	90 Ba	93 Aa	92	86	82	93	97
5	92 Ba	97 Aa	95	87	80	98	97
6	90 Ba	96 Aa	93	93	85	97	99
7	88 Ba	95 Aa	92	98	90	98	96
8	90 Ba	96 Aa	93	93	98	92	100
9	94 Ba	97 Aa	96	97	100	96	100
10	93 Aa	89 Ba	91	97	99	99	98
Average	91	95	-	93	91	96	95

Averages followed by the same uppercase letters in the lines and lowercase letters in the columns do not differ from each other by the Scott-Knott test at 5% probability; [1]seeds treated with Metalaxil-m + Fludioxonil.

Figure 2. Pre-wetting of coriander seeds for the tetrazolium test between paper sheets (A) and soaking in water (B). Longitudinal cut of the seeds (C) and (D) and detail of the seed structure showing the diachenes (two embryos) and the endosperm (E). Pelotas, UFPel, 2020.

fungicide-treated seeds, we could notice separation in four levels of quality (Table 1). The lowest values found were 84 and 86% for batches 4 and 7, respectively. These values were 5% superior comparing with the general average of no-treated seeds. According to Pereira *et al.* (2005), the coriander crop shows some problems related to seed quality, which is one factor associated with pathogens that negatively influence the physiological quality and seedling development. According to Gadotti *et al.* (2020), seeds infected by pathogens can interfere with seedling development during germination test. In this sense, the treatment using fungicides possibly contributed to minimizing the deleterious effects of fungi on physiological performance. Thus, we ruled out the possibility that pre-wetting in treated seeds causes some deleterious effect on the batch.

For seedling emergence (Table 1), the authors observed a behavior different from the germination, showing no significant difference among the seed batches. The average percent for an emergency in the sand was 89%, similar to the value found in germination with and without fungicide treatment, which obtained 93 and 88%, respectively; besides the difference observed among the batches ($p<0.05$). The seedling emergency in the sand was performed under controlled conditions and used as an alternative to germination test, although it is not indicated for the germination test in coriander crop (Brasil, 2009; ISTA, 2012), this test was performed to minimize the interference of pathogens adhered to the achene surface.

We verified no interaction between batch and pre-wetting ways about the tetrazolium test for seed viability but only between the pre-wetting ways (Table 2). The seeds soaked in water showed higher viability values, a general average of 95%, compared with the seeds which were pre-conditioned between paper, which showed an average of 91%. We highlight that both substrates used in the experiment resulted in viability values close to those observed for the germination average of treated seeds, 93% (Table 1).

The tetrazolium test for viability in relation to treated seed germination was 96% for seeds that were pre-conditioned between paper sheets and 95% for those pre-conditioned in water (Table 2). The efficiency of seed viability to the germination test without fungicide treatment was 93% for pre-wetting between paper sheets and 91% for water. In studies on the efficiency of tetrazolium test methodology about germination, Lima *et al.* (2018) obtained higher efficiency in soaking carrot seeds using the paper substrate for two hours, moistened with distilled water, corroborating this study data.

Despite the difference between the pre-wetting ways for viability data

(Table 2), we concluded, using the analysis of the efficiency of tetrazolium test methodology, that the differences in the percentages of efficiencies between forms of pre-wetting were minimal. Thus, we considered that both forms of pre-wetting are appropriate.

Considering the dynamic, fast-paced seed industry, tetrazolium testing has become a popular technology for evaluating seed viability as it is a fast and alternative method. However, not yet officially recognized to determine seed viability (Soares *et al*., 2016), except for some Poaceae in Brazil, such as *Lollium multiflorium* (ryegrass) and species of *Brachiaria spp.* and *Panicum maximum* that can currently be marketed based on the percentage of viability by the tetrazolium test (Brasil, 2008, 2016).

Thus, the tetrazolium test is a good alternative for a fast quality evaluation of coriander seeds, favoring the vegetable seed industry, mainly when treated with fungicides. The coriander seeds were influenced by pre-wetting, resulting in a fast evaluation of viability using the tetrazolium test, possibly the pre-wetting in water or between paper sheets, with just one longitudinal cutting.

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