

The envelope method and substrate wetting in the germination test of onion seeds¹

Método do envelope e umedecimento do substrato no teste de germinação de sementes de cebola

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ABSTRACT - The Rules for Seed Analysis reports the use of a paper envelope in germination tests, but without any description of the exact method. The objective of this study was to evaluate the viability of the envelope method and the optimal level of substrate wetting when performing germination tests of onion seeds. The study was performed in two stages. In the first stage, a batch of seeds was evaluated using two seeding methods (on paper and in a paper envelope) and five levels of substrate wetting with differing amounts of water: 1.5, 2.0, 2.5, 3.0, and 3.5 times the dry mass of the paper. A completely randomized design was used in a 2×5 factorial scheme. In the second stage of the study, the two seeding methods mentioned in the prior were tested in eight seed batches using a completely randomized design and a 2×8 factorial scheme. The degree of moisture, germination, and the first count of germination were evaluated. The time spent for the installation, seeding, counting, and disassembling of the germination test were determined. It was concluded that the envelope method is fit for use in onion seed germination tests. The optimal amount of water to add to the substrate is 2.5 times the dry mass of the paper substrate. The envelope method is fast, practical, and more efficiently uses space within germinator chambers, making it easily incorporable into routine tests within seed analysis laboratories.

Key words: *Allium cepa* L.. Analysis of seeds. Quality control. Water levels. Methodology.

RESUMO - Nas Regras para Análise de Sementes há relato do uso de envelope de papel para o teste de germinação, porém sem qualquer descrição do método. Assim, objetivou-se avaliar a viabilidade do método do envelope e do nível de umedecimento do substrato no teste de germinação de sementes de cebola. A pesquisa foi executada em duas etapas. Na primeira, um lote de sementes foi avaliado por meio de dois métodos de sementeira (sobre papel e em envelope de papel) e cinco níveis de umedecimento do substrato em água: 1,5; 2,0; 2,5; 3,0 e 3,5 vezes a massa seca do papel. Foi utilizado o delineamento inteiramente casualizado em esquema fatorial de 2x5. Na segunda etapa da pesquisa, os dois métodos de sementeira citados na primeira etapa foram testados em oito lotes de sementes, utilizando o delineamento inteiramente casualizado e esquema fatorial de 2x8. Foi avaliado o grau de umidade, a germinação e a primeira contagem de germinação. Foi cronometrado o tempo despendido para a instalação, sementeira, contagem e desmonte do teste de germinação. Concluiu-se que o método do envelope é viável para o teste de germinação de sementes de cebola. O umedecimento do substrato em água mais favorável à germinação é de 2,5 vezes a massa seca do papel. O método do envelope é rápido, prático e ocupa menor espaço nos germinadores, adaptando-se à rotina de um laboratório de análise de sementes.

Palavras-chave: *Allium cepa* L.. Análise de sementes. Controle de qualidade. Níveis de água. Metodologia.

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INTRODUCTION

In Brazil, the annual average area cultivated with onions (*Allium cepa* L.) is 60 thousand hectares. Considering that the average seed consumption is 2.0 kg ha⁻¹, the demand for this input is 120 tons per year (OLIVEIRA *et al.*, 2014).

To safeguard the producer, federal seed standards require a minimum germination value of 80% for onion seeds before batches can be sold on the domestic market (BRASIL, 2009). The germination test is the main parameter used to evaluate the physiological quality of the seeds and allows for the calculation of the germination potential of a batch under favorable conditions (AZEREDO *et al.*, 2010; GENTIL; TORRES, 2001; MARTINS; MACHADO; CAVASINI, 2008).

The results of this test are used to determine the seeding rate, in assessing batch value, and in commercialization, as it allows the standardization of results from many laboratories (MARTINS; BOVI; SPIERING, 2009). Germination tests should follow the standard procedures recommended by the RSA - Rules for Seed Analysis (BRASIL, 2009), an official publication that standardizes seed analyses so that germination occurs under the optimal conditions for each individual species.

According to the Brazilian and international rules for analyzing seeds (BRASIL, 2009; INTERNATIONAL SEED TESTING ASSOCIATION, 2004), for onion seeds, the germination test should be conducted by sowing the seeds on or between paper or in sand before placing them inside transparent plastic boxes kept in a germination chamber at 20 or 15 °C for 12 days. In the laboratory the paper substrate is the most commonly used for ease of handling and disposal (BARROSO; FRANKE; BARROS, 2010).

In the RSA, there is a report on the use of a paper envelope as a substrate in germination tests, however the proper procedure for preparing and conducting a germination test in this way is not described (BRASIL, 2009). Therefore, it is necessary to study its applicability, feasibility, efficiency, and advantageousness to verify if this method could be used more comprehensively as an alternative substrate to the traditional paper method.

Due to the importance of using the right amount of water in germination tests, RSA (BRASIL, 2009) normalized the wetting of the substrate, recommending for the paper germination test that a water volume equivalent to 2.0 to 3.0 times the dry mass of the substrate be added. However, this information was only detailed for the traditional methods: sowing seeds on, between, or enclosed within paper. There were no specific recommendations found for the paper envelope method, and thus the optimal level of substrate wetting for this method is unknown.

In spite of this general recommendation, distinct requirements regarding the level of substrate wetting for the germination test are typically verified in the literature according to the species, including vegetable crops such as maroon cucumber - *Cucumis anguria* (GENTIL; TORRES, 2001), wild cabbage - *Brassica oleracea* (AZEREDO *et al.*, 2010) and garden peach tomato (cocona) - *Solanum sessiliflorum* (PEREIRA; SANTOS; MEDEIROS FILHO, 2011), in which maximum germination was achieved by wetting the substrate paper with water in the amounts of 1.0 to 2.5; 2.0 to 2.5, and 2.5 to 3.5 times the dry paper mass, respectively.

Thus, for routine testing by seed analysis laboratories, methods capable of providing the maximum percentage of germination are sought quickly and practically (GASPAR-OLIVEIRA *et al.*, 2007; MARTINS; MACHADO; CAVASINI, 2008).

In view of the above, the objective of this research was to evaluate the viability of the envelope method and to determine the most appropriate level of substrate wetting in the germination test of onion seeds.

MATERIAL AND METHODS

The research was conducted using eight batches of onion seeds (*Allium cepa* L.) of the cultivar Franciscana IPA 10 in the Seed Analysis Laboratory of the Department of Plant Production of the Faculty of Agrarian and Veterinary Sciences of UNESP, Jaboticabal Campus.

The moisture content of the seeds in all batches was determined by the greenhouse method, using subsamples of 2.0 g of seeds which were exposed to temperatures of 105 ± 3 °C for 24 hours (BRASIL, 2009).

The study was carried out in two stages: in the first stage, a seed batch was evaluated using two seeding methods (on paper and in a paper envelope) and five levels of substrate wetting with amounts of water equivalent to 1.5, 2.0, 2.5, 3.0, and 3.5 times the dry mass of the paper. The paper was autoclaved and moistened with distilled water prior to seeding.

Seeding on paper: representing the control treatment as it is the traditional method used in onion seed germination tests, two sheets of blotting paper were packed in a transparent box (11.0 × 11.0 × 3.5 cm) with a lid and eight subsamples of 50 seeds were sown on the moistened paper.

Seeding in a paper envelope: two rows of 25 seeds were sown on the upper half of a horizontally laid sheet of moistened germitest paper (Figure 1 and Figure 2A). The seeding was completed with the aid of a perforated

Figure 1 - Seeding of onion seeds with the aid of a perforated countersink ruler (A); seeds positioned on the upper third of the germitest paper sheet after removal of the ruler (B)

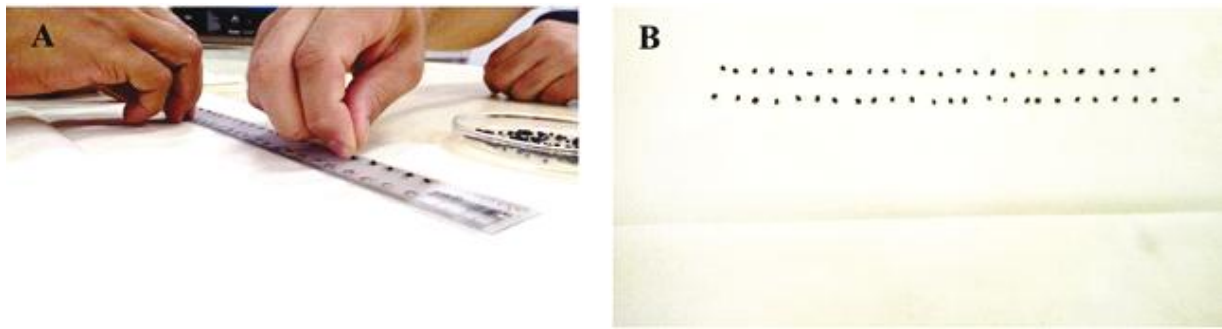
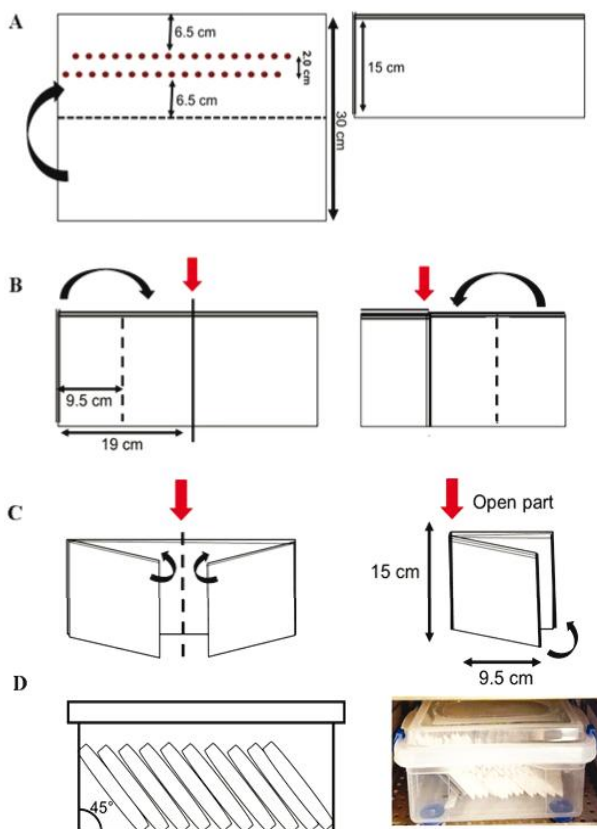


Figure 2 - Seeding on germitest paper and horizontal folding (A), vertical folds for envelope preparation (B and C), packaging and positioning of envelopes in the plastic box (D) for conducting the germination test of onion seeds 'Franciscana IPA 10'



countersink ruler with 25 perforations, each the size of a single seed. For the preparation of the envelope, the sheet was folded in half so that the seeds remained between the two halves of the paper.

Then, 1/4 of the left side of the sheet was folded in half and the same procedure was repeated on the right

side, so that the two edges of the envelope were then in the center (Figure 2B).

By folding vertically at the meeting point of the edges, the envelope was made, with the opening facing upwards. The final dimensions of these envelopes were 9.5×15.0 cm (Figure 2C). The envelopes were packed in transparent plastic boxes with a lid ($32.0 \times 20.0 \times 9.0$ cm) at an incline of 45° to the horizontal (Figure 2D).

Germination and the first count test: for both methods germination tests were conducted in a BOD germination chamber at 20°C with a 12-hour photoperiod (Figure 3). The first germination count on the sixth day after seeding was evaluated by calculating the percentage of normal seedlings. A final count of normal, abnormal and dead seedlings was performed on the 12th day after seeding and the data was expressed as a percentage (BRASIL, 2009).

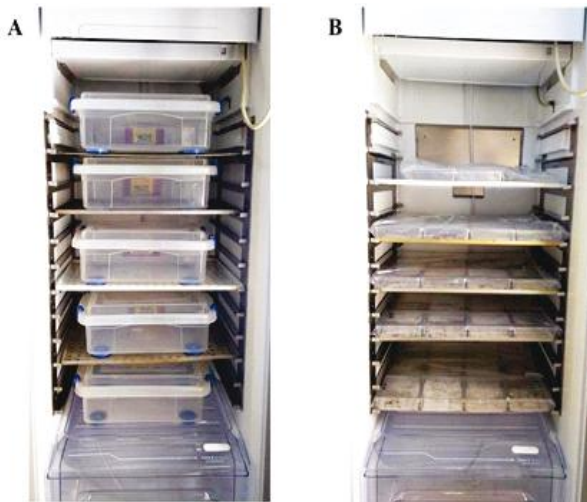
In the second stage of the study, the two seeding methods described above (on paper and in a paper envelope) were tested in eight seed batches. The optimal level of substrate wetting for germination was used, as identified in the previous step, which corresponded to adding an amount of water equivalent to 2.5 times the amount of the dry paper mass.

To evaluate the germination performance of the seeds, the germination test and first germination count test were performed as described in the first step. At this stage, the following procedures were timed to evaluate the total time required to complete the germination test using each seeding method.

Installation: the preparation of the materials (substrate and boxes), identification of the treatments, and wetting of the substrate were all timed.

Seeding: the timer was started when seeds were first seeded on the already moistened substrate and was stopped when the boxes were ready to be placed in the

Figure 3 - Plastic boxes wrapping the paper envelopes (A) and traditional boxes with filter paper (B) inside the BOD during the germination test



germinator. For the envelope method, the preparation of the envelopes was also timed.

Evaluation: the timer was started when the boxes were removed from the germination chamber and was stopped after the boxes had been opened, the envelopes unfolded (in tests using the envelope method) and the contents of the boxes had been observation and closing in the accounting of the normal seedlings in the test of the first count and the normal, abnormal seedlings and dead seeds in the final count of germination.

Disassembly: timing was done by using a digital timer during waste disposal and the cleaning of the materials used in the test (Figure 4).

The experiment utilized a completely randomized design. In the first stage, the data was analyzed using a

2×5 factorial scheme, with two seeding methods for the germination test (envelope and plastic box) and five levels of substrate wetting (1.5; 2.0; 2.5; 3.0 and 3.5), with eight repetitions. For the second stage, the data was analyzed using a 2×8 factorial scheme, with two seeding methods used for the germination test at 2.5 times the dry paper mass of water and eight seed batches, each with eight repetitions.

The data was analyzed using an F test and when results were significant the means of the treatments were compared using a Tukey test at 5% probability. Regression analyses were performed when effect treatments were quantitative. The germination data was then $\arcsin(x/100)^{0.5}$ transformed to meet the assumptions of normality and homogeneity and were analyzed using the Shapiro-Wilk test.

RESULTS AND DISCUSSION

The values for the initial seed moisture content were similar for the eight batches studied, ranging from 9.0 to 9.3%. Therefore, seed germination differences could be attributed to variation in the physiological quality of the batches (AZEREDO *et al.*, 2010; SILVA *et al.*, 2017; TOMAZ *et al.*, 2015, 2016).

In the first stage of the experiment, it was verified that the seeding methods and the substrate wetting levels had no influence on germination or the speed of this process, as evaluated by the first count test (Table 1).

The isolated influence of wetting on the percentage of abnormal seedlings and the interaction of seeding method and level of wetting was only observed in the percentage of dead seeds.

Higher levels of substrate wetting resulted in an increased proportion of abnormal onion seedlings, regardless of seeding method (Figure 5A).

Figure 4 - Cleaning the plastic boxes with cotton and alcohol (70%) to install the germination test (A and B)

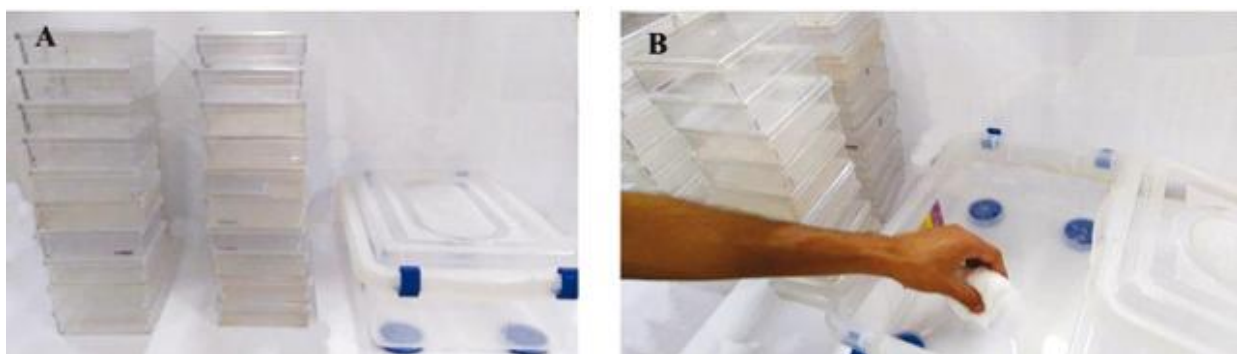


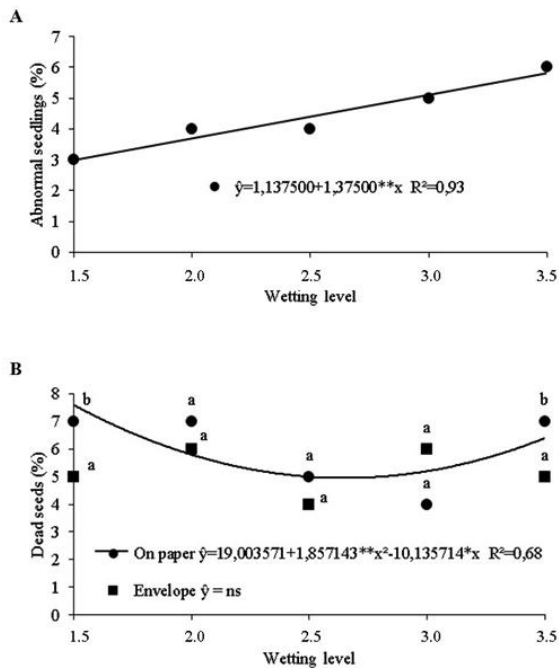
Table 1 - Variance analysis and mean values for the germination test, first count, abnormal seedlings and dead seeds according to seeding method (M), on paper or envelope, and five levels of substrate wetting (W) for one batch of onion seeds 'Franciscana IPA 10'

Evaluated parameters	Variation factors			C.V. (%)	Mean
	W	M	W x M		
Germination (%)	0,48 ^{ns}	0,002 ^{ns}	0,40 ^{ns}	5,85	88
First count (%)	0,70 ^{ns}	2,150 ^{ns}	0,27 ^{ns}	8,32	82
Abnormal seedlings (%)	6,27**	0,990 ^{ns}	2,17 ^{ns}	39,23	05
Dead seeds (%)	6,21**	10,490**	3,35**	27,55	06

** e^{ns}= significant at the 1% probability level and not significant according to the F test, respectively

The wetting of the substrate with the maximum level of 3.5 times the dry mass of the paper in water doubled the percentage of abnormal seedlings when compared to batches using the minimum wetting level of 1.5. These results may have been due to a deficiency in oxygen supply, an essential factor in the germination process. Issues with gas exchange during the germination process may have caused damage via very rapid imbibition, as excess water limits the absorption of oxygen thus inhibiting respiration and causing the delay or paralysis of the germination process, as well as causing abnormal seedling growth (CARVALHO; NAKAGAWA, 2012).

Figure 5 - Percentage of abnormal seedlings (A) and dead seed (B) as a function of the level of substrate wetting (1.5, 2.0, 2.5, 3.0 and 3.5 times the dry mass of the paper in water) and seeding method (on paper or in an envelope) during the germination test (B) using one batch of onion seeds 'Franciscana IPA 10'



In similar studies, substrate wetting levels in seed germination verified the deleterious effect caused by excess water in the substrate due to reduced aeration (AZEREDO *et al.*, 2010; GENTIL; TORRES, 2001; MARTINS; BOVI; SPIERING, 2009).

Wetting of the substrate with an amount of water equal to 2.7 times the dry mass of the paper resulted in the most favorable conditions for the germination test, because at this ratio of wetting there was the least mortality of onion seeds for both seeding methods (Figure 5B). Thus, the 2.5 times wetting level, commonly used in routine laboratory tests and in research with onion seeds (CASEIRO; MARCOS FILHO, 2005; DIAS *et al.*, 2006; GADOTTI; MENEGHELLO; TILLMANN, 2013; HÖLBIG; BAUDET; VILLELA, 2011; PINHEIRO *et al.*, 2014), is the closest to the ideal moisture level.

In the second stage of the study (Table 2), there was a significant interaction between the seeding method and the batch for all evaluated parameters, except those that were timed in the conduction stages.

The envelope method was equal to or superior to the on paper method (control) based on percentage germination, with a germination success rate of 88% (Table 3). The envelope method was also superior to or equal to the on paper method in 100% of the batches based on the first germination count. These results validate the envelope seeding method as an effective substrate for use in onion seed germination tests. Similarly, most batches utilizing the envelope method had fewer abnormal seedlings and dead seeds than batches sowed using the on paper method.

The RSA rules and procedures are periodically reviewed by a committee made up of researchers and practitioners familiar with the subject area. However, modifications can only be made based on research results (TOMAZ *et al.*, 2015, 2016). These studies should allow for germination to occur under the

Table 2 - Variance analysis and mean values for the germination test, first count, abnormal seedlings, dead seeds and the time spent (seconds/sample) for the execution of the installation, sowing, evaluation, disassembly and total time (M) on paper or envelope and seed batches (B) of onion 'Franciscana IPA 10'

Evaluated parameters	Fator de variação			C.V. (%)	Mean
	B	M	B x M		
Germination (%)	23,82**	1,75 ^{ns}	3,74**	2,45	93
First count (%)	160,39**	94,33**	17,30**	6,65	48
Abnormal seedlings (%)	9,99**	4,37*	2,64*	68,98	01
Dead seeds (%)	19,71**	0,94 ^{ns}	4,04**	36,07	06
Installation (s)	0,02 ^{ns}	1.059,20**	0,03 ^{ns}	3,08	172,8
Sowing (s)	0,89 ^{ns}	0,89 ^{ns}	0,90 ^{ns}	9,70	158,8
Evaluation (s)	0,30 ^{ns}	0,007 ^{ns}	0,16 ^{ns}	11,01	91,8
Disassembly (s)	0,58 ^{ns}	30.017,89**	0,17 ^{ns}	3,39	27,2
Total time (s)	1,35 ^{ns}	3.101,83**	0,80 ^{ns}	3,71	450,6

Table 3 - Percentage of germination, first count, abnormal seedlings, and dead seeds for both seeding methods, on paper (OP) and envelope (E) observed in batches of onion seeds 'Franciscana IPA 10'

Batches	Germination		First count		Abnormal seedlings		Dead seed	
	E	OP	E	OP	E	OP	E	OP
	----- (%) -----							
1	94 ABCa	96 Aa	41 Ca	25 Db	0 Aa	1 ABa	6 ABa	3 Aa
2	90 CDb	95 Aa	38 Ca	26 CDb	2 Ca	3 Ba	8 BCb	4 Aa
3	85 Da	83 Ba	36 Ca	28 CDb	3 Cb	1 ABa	12 Ca	15 Bb
4	95 ABa	95 Aa	55 Ba	33 Cb	1 ABa	1 ABa	4 ABa	5 Aa
5	92 BCa	95 Aa	66 Aa	69 Aa	1 ABa	1 ABa	7 ABa	5 Aa
6	96 ABa	97 Aa	66 Aa	54 Bb	0 Aa	0 Aa	4 ABa	3 Aa
7	98 Aa	93 Ab	58 Ba	59 Ba	0 Aa	1 ABa	2 Aa	6 Ab
8	90 Ca	94 Aa	54 Ba	58 Ba	2 BCb	1 ABa	8 BCa	6 Aa

Means followed by the same lowercase letter in the row and upper case in the column do not differ by Tukey test at the 5% probability level. *, ** e, n s = significant at the 5% level, 1% probability and not significant according to the F test, respectively

optimum conditions for each species (ALVES; SILVA; CÂNDIDO, 2015; BRASIL, 2009).

As for the time spent on the installation, seeding, and dismantling procedures of the germination test, it was found that the envelope method was faster than the traditional on paper method (Tables 2 and 4). The only procedure that demanded the same time between the two methods was the test count (evaluation).

This result may allow seed companies to save time and labor which are fundamental factors in internal quality control procedures.

In addition, the envelope method was more space-efficient, as 5 boxes each containing 40 envelopes fit within

a BOD germination chamber, totaling 200 subsamples. Using the traditional method, only 60 boxes containing blotting paper totaling 60 subsamples could fit within the chamber (Figure 3A and B).

The installation and disassembly of the test using the traditional method (on paper) was more time consuming than when the envelope method was used due to the greater number and smaller dimensions of the plastic boxes used in the test (Figure 4A and B). This made the internal cleaning using 70% alcohol more difficult during installation. In the traditional method the disassembly required more time during substrate removal, because the paper stuck to the bottom of each box and had to be removed manually with tweezers. For the envelope

Table 4 - Time spent (seconds/sample) for the execution of the installation stages, seeding, evaluation, disassembly, and total time for conducting the germination test according to the seeding method (M), on paper (OP) or envelope (E) in batches (B) of onion seeds 'Franciscana IPA 10'

Method	Installation		Sowing		Evaluation		Disassembly		Total time	
	E	OP	E	OP	E	OP	E	OP	E	OP
	----- (s) -----									
	104,5 a	241,0 b	130,9 a	186,6 b	91,6 a	91,9 a	7,2 a	47,2 b	334,3a	566,8 b

Means followed by the same lowercase letter in the row and upper case in the column do not differ by Tukey test at the 5% probability level. *, ** e, **s = significant at the 5% level, 1% probability and not significant according to the F test, respectively

method, the envelopes were easily removed from the box and unfolded for evaluation and then discarded. In addition, initial seeding using the envelope method was faster because a perforated countersink ruler facilitated the procedure (Figure 1).

This result leads to the assertion that in routine laboratory analyses, the envelope method is more cost and time efficient as less consumables and fewer researchers are required to deliver results.

CONCLUSIONS

1. The envelope method is an efficient way to complete germination tests of onion seeds;
2. The envelope method is faster and more practical, adapting better to the routine tests of a seed analysis laboratory;
3. The level of substrate wetting most favorable for germination using the envelope method is 2.5 times the dry mass of the paper substrate.

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