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Parameters of the Accelerated Aging Test for the Determination of Tobacco (*Nicotiana tabacum* L.) Seed Vigor

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Abstract

Vigor tests are very important to obtain additional information to the standard germination test and can assist in decision-making. The aim of this work was to evaluate accelerated aging test methodologies for the determination of vigor in tobacco seeds. For the study, ten seed lots of tobacco were used. The initial quality of the tobacco seeds was determined through the germination test, first count of germination, germination speed index, seedling emergence at 7, 14 and 21 days after sowing, emergence speed index and the accelerated aging with water performed as proposed by the Association of Official Seed Analysts. After the determination of seed initial quality, the accelerated aging test was studied in the following methods: accelerated aging with water, saturated saline solution (40g NaCl 100mL^{-1} water) and unsaturated saline solution (11g NaCl 100mL^{-1} water), submitted to the temperatures of 45 and 41 ℃, for the exposure periods of 24, 48 and 72 hours. According to the results obtained, the accelerated aging test with water conducted under a temperature of 45 ℃ combined with a 24-hour exposure period is shown as adequate for evaluating the vigor of tobacco seeds.

Keywords: physiological quality; seed analysis; physiological potential

1. Introduction

The tobacco crop has major socioeconomic relevance in Brazil, as one of the most significant agroindustry activities in the southern region, comprised by the states of Rio Grande do Sul, Santa Catarina and Paraná, where the production is concentrated. Tobacco cultivation involves about 150 thousand farm households and is present in nearly 566 municipalities, generating 40 thousand direct jobs in the industries (AFUBRA, 2018).

Crop establishment in tobacco is performed through seedling transplant, therefore, the use of good quality seedlings is essential to obtain uniform plant stands and to guarantee satisfactory yields. Both for crops that are sown directly in the field and species in which seedlings are produced on greenhouses and then transplanted to the field, the use of seeds with high physiological quality is fundamental to obtain adequate plant populations (Nascimento *et al.* 2011). For species where harvest is performed during the vegetative stage, the effects of low seed vigor on seedling emergence may be critical, leading to delays in stand establishment and affecting plant growth uniformity (Marcos Filho, 2015).

Seed quality is characterized by the sum of four attributes: genetic, physic, physiological and sanitary. Between these attributes, seed physiological quality, represented by germination and especially by vigor, is extremely important since this characteristic is directly related with an adequate establishment of seedlings in the field (Peske *et al.* 2012). Additionally, for tobacco seed commercialization a minimum value of 98% for purity and 80% for germination are mandatory, with an exception for foundation seeds, in which the minimum standards for germination are 60%, but may be marketed with up to 10 (ten) percentual points below the standard, provided the transaction is made directly by the producer and the user, with the formal consent of the latter (Brasil, 2013).

However, germination is defined as the emergence and development of the structures of the embryo, with the ability to originate a normal seedling under favorable environmental conditions, which does not precisely correspond to the performance of seed lots in the field. Seed vigor, on the other hand, comprises the seed proprieties which determine the potential for emergence and rapid and uniform development of normal seedlings under adverse environmental conditions (Peske *et al.* 2012). Thus, vigor tests are very important to provide additional information to the standard germination test and can assist decision-making. Between the tests available, the accelerated aging test is one of the most sensitive and efficient for the evaluation of seed vigor for several species (Marcos Filho, 2015). However, few studies that have tested methods of evaluating tobacco seed vigor can be found in the literature. Therefore, considering the major socioeconomic importance of this crop and the limited information for the evaluation of tobacco seed vigor, this study was performed with the aim of determining an adequate methodology of vigor testing for tobacco seeds using the accelerated aging test.

2. Material and Methods

2.1 Location

The present work was performed at the Didactic Laboratory of Seed Analysis "Flávio Farias Rocha" of the Federal University of Pelotas (UFPel), and in a farm located at the municipality of Venâncio Aires – RS. Ten seed lots of tobacco (*Nicotiana tabacum* L.) were used.

2.2 Seed Quality Characterization

Initial seed quality was evaluated using the following tests:

2.2.1 Water Content (W.C.)

Evaluated using the kiln method at 105±3 ℃ for 24 hours (Brasil, 2009). Two repetitions of 0.2 g for each seed lot were evaluated. The results were indicated in wet basis and expressed in percentage.

2.2.2 Germination (G)

Each repetition was composed of four subsamples of 50 seeds sown into acrylic germination boxes (11 x 11 x 3.5 cm) deposited over two sheets of blotting paper, previously moistened using distilled water by 2.5 times the weight of the dry paper. The test was carried out under alternated temperature 20 – 30 ℃ with an eight-hour daily photoperiod combined with the higher temperature. Evaluations were performed 16 days after sowing (Brasil, 2009). The results were expressed as the percentage of normal seedlings.

2.2.3 First Count of Germination (FCG)

Performed alongside the standard germination test, counting normal seedlings at the seventh day after sowing. The results were expressed as the percentage of normal seedlings.

2.2.4 Speed of Germination Index (SGI)

Performed alongside the standard germination test, data was obtained from daily evaluations carried out at the same day period until stabilization of germination, evaluations started when the first seedling was observed. The index was obtained through the equation proposed by Maguire (1962): SGI = (G1/N1) + (G2/N2) + (Gn/Nn), where, SGI = speed of germination index; G1, G2,…, Gn = Number of seedlings counted at each day; N1, N2,…, Nn = number of days between sowing and each evaluation.

2.2.5 Seedling Emergence (SE)

Performed in polystyrene seedling trays of 200 cells filled with coconut fiber as substrate (Amafibra, type 47), divided in 8 partitions (5 x 5 cells each). Each repetition contained four subsamples of 50 seeds which were uniformly distributed over each partition previously determined and identified in each tray. The trays were held in a low tunnel and a floating system was used for irrigation, with a water blade of 10 centimeters. The evaluations were performed at 7, 14 and 21 days after sowing. Results were expressed as the percentage of normal seedlings.

2.2.6 Speed of Emergence Index (SEI)

Performed alongside the seedling emergence test, daily evaluations and the calculation of the indexes were carried out as described for SGI.

2.2.7 Standard Accelerated Aging (SAA)

Performed according to the conditions described by AOSA (2009). Due to the reduced dimensions of tobacco seeds, adapted screens were developed. The test was performed on acrylic germination boxes, containing 40 mL of distilled water at the bottom. For each repetition, approximately 0.2g of seeds were distributed in a uniform layer over the adapted screen. Each box received four adapted screens, containing the seeds of each lot, which were deposited over a suspended metallic screen. The boxes were posteriorly closed with acrylic covers. Next, the boxes were held under incubation at 43 ℃, for 72 hours. After the aging period, 200 seeds (four subsamples of 50 seeds) for each seed lot were subjected to the germination test and evaluated at seven days after sowing. The remainder seeds were used to verify the water content reached using the kiln method described previously.

2.3 Accelerated Aging Methods

After seed quality characterization, alternative methodologies to the “standard” accelerated aging test, described by AOSA (2009), were studied using two temperatures (41 and 45 ℃) combined with three aging periods (24, 48 and 72 hours) and three different solutes (distilled water [AAW], unsaturated saline solution [AUSS] and saturated saline solution [ASSS]). The unsaturated saline solution was obtained using a 11g% (w/v) NaCl solution and the saturated saline solution was obtained using a 40g% (w/v) NaCl solution. These methodologies are described by Jianhua; McDonald (1996) and were determined accordingly to the Van’t Hoff equation described by Salisbury; Ross (1992). The other conditions of the test remained as previously described.

2.4 Experimental Design and Statistical Analysis

The experiment was carried out under randomized block design with three statistical repetitions. Data from the tests were subjected to the analysis of variance, wherein the effects of the treatments (seed lots) were evaluated by the F test and, when significant, the averages were compared by the Scott-Knott test at the 5% probability level of error. The results were also subjected to the Pearson correlation analysis and the significance of the coefficients was verified by the Student’s t-test, at the 5% probability level of error. Data from water content were not analyzed and were only used for the initial characterization of the seed lots and for monitoring during seed aging.

3. Results and Discussion

3.1 Initial Quality of Tobacco Seed Lots

Data from the initial quality evaluations of tobacco seed lots can be observed in Tables 1, 2 and 3. According to Table 1, there were no statistical differences between the lots for germination, demonstrating that the lots presented resembling viability. Similarly, seed initial water content were similar between the seed lots, where the average obtained for the lots was of 5.1%, with a maximum variation between lots of 1.2 percentual points, which is within the tolerated range, that is up to 2%. This homogeneity is highly important for the execution of the tests, since if the initial water content between samples is very distinct, there will be marked variation in the moistening speed during aging and, certainly, different deterioration intensities (Marcos Filho, 2015). The low initial water contents and the resemblance between the lots evaluated allows a greater reliability of the results obtained (Tunes *et al*. 2011). Coimbra *et al*. (2009) highlighted that seed initial water content is a primordial factor for standardizing quality assessment tests.

Table 1. Initial water content (W.C.), First count of germination (FCG), germination (G) and speed of germination index (SGI) of ten tobacco seed lots, Pelotas-RS, UFPel, 2018

Lot	W. C. (%)	FCG (%)	G (%)	SGI
L1	5,2	97 a	98	6.16 a
L2	4,9	97 a	97	6.02 a
L3	5,2	97 a	98	6.36 a
L4	5,1	95 b	96	6.03 a
L5	5,2	96 a	98	5.92 a
L6	5,1	96 a	97	6.47 a
L7	5,0	93 b	97	6.18 a
L8	4,4	94 b	97	5.71 b
L9	5,0	97 a	98	5.45 b
L10	5,6	94 b	96	5.34 b
Average	5,1	96	97^{ns}	5.96
C.V. (%)	-	1.31	0.98	4.03

*Averages followed by the same letter in the column do not differ between each other by the Scott-Knott test at the 5% probability level of error. ^{ns} – Not significant by the F test.

The first count of germination and speed of germination index (Table 1), as well as seedling emergence, evaluated at the seven days after sowing, the speed of emergence index (Table 2), and the standard accelerated aging (Table 3), were efficient for the stratification of seed lots according to vigor levels.

Table 2. Seedling emergence evaluated at seven (SE7), fourteen (SE14) and twenty-one (SE21) days after sowing and speed of emergence index (SEI) of ten tobacco seed lots, Pelotas-RS, UFPel, 2018

Lot	SE7 (%)	SE14 (%)	SE21 (%)	SEI
L1	72 a	85	87	6.46 a
L2	69 a	84	87	6.45 a
L3	65 a	78	80	5.98 a
L4	65 a	79	84	5.98 a
L5	54 b	77	83	5.53 b
L6	51 b	75	79	5.32 b
L7	63 a	79	83	5.91 a
L8	63 a	76	78	5.70 b
L9	58 b	78	84	5.68 b
L10	50 b	75	79	5.24 b
Average	61	79^{ns}	82^{ns}	5.82
C.V. (%)	16.64	10.05	8.29	10.02

*Averages followed by the same letter in the column do not differ between each other by the Scott-Knott test at the 5% probability level of error. ^{ns} – Not significant by the F test.

Table 3. Standard accelerated aging with water (SAA), as proposed by AOSA, and water content reached after the SAA (W.C. SAA) of ten tobacco seed lots, Pelotas-RS, UFPel, 2018

Lot	SAA (%)	W.C. SAA (%)
L1	93 a	18.7
L2	92 a	18.7
L3	86 b	22.0
L4	86 b	20.6
L5	85 b	19.6
L6	83 b	22.2
L7	83 b	22.0
L8	83 b	19.0
L9	81 b	22.5
L10	76 b	20.6
Average	85	22.6
C.V. (%)	4.91	-

*Averages followed by the same letter in the column do not differ between each other by the Scott-Knott test at the 5% probability level of error.

According to the results presented in Table 1, the first count of germination divided the lots into two levels of vigor, where L1, L2, L3, L5, L6 and L9 were classified as high vigor whereas seed lots L4, L7, L8 and L10 as of inferior vigor. However, the stratification of seed lots using this test was not very similar to the results observed for seedling emergence, at seven days after sowing, and the speed of emergence index (Table 2), and the standard accelerated aging (Table 3). Therefore, this variable did not present significant correlations with the seedling emergence test, evaluated at the seven days after sowing, and with the speed of emergence index, as demonstrated in Table 4. The seed lots did not differ statistically when seedling emergence was evaluated at 14 and 21 days after sowing, as can be observed in Table 2.

Table 4. Linear correlations [Pearson correlation coefficient (r)] between the physiological quality variables of ten tobacco seed lots: germination (G), first count of germination (FCG), speed of germination index (SGI), seedling emergence evaluated at seven (SE7), fourteen (SE14) and twenty one (SE21) days after sowing, speed of emergence index (SEI) and the standard accelerated aging (SAA AOSA) as proposed by AOSA, Pelotas-RS, UFPel, 2018

Variable	G	FCG	SGI	SE7	SE14	SE21	SEI	SAA AOSA
G	1.0							
FCG	0.64*	1.00						
SGI	0.23^{ns}	0.24^{ns}	1.00					
SE7	0.23^{ns}	0.28^{ns}	0.32^{ns}	1.00				
SE14	0.28^{ns}	0.46^{ns}	0.25^{ns}	0.85**	1.00			
SE21	0.28^{ns}	0.46^{ns}	0.15^{ns}	0.66*	0.92**	1.00		
SEI	0.26^{ns}	0.40^{ns}	0.34^{ns}	0.96**	0.95**	0.81**	1.00	
SAA	0.38^{ns}	0.57^{ns}	0.55^{ns}	0.82**	0.88**	0.74*	0.89**	1.00

******Significant by the t-test at the 1% probability level of error; *****Significant by the t-test at the 5% probability level of error. $^{\mathbf{ns}}$ – Not significant by the t-test.

The slower speed of emergence for seedlings derived from low vigor seeds is attributed to the necessity of restructuring cellular membranes, which were unstructured and/or damaged by oxidative processes that are common during deterioration, before the growth of the embryonic axis, which is inherent to the germinative process, can be initiated. Therefore, the time required by the repair mechanisms widen the period for emergence to occur (Villiers, 1973), such fact may explain the greater sensibility to distinguish vigor levels when seedling emergence is evaluated at seven days after sowing.

For seedling emergence at 14 and 21 days after sowing, the trend is that emergence tends to be similar between the seed lots evaluated. According to Larsen *et al.* 1998, the influence of seed vigor may exist even if there are no differences in the initial seedling population. These authors claim that low vigor seeds slowly emerge as seedlings and, even if differences in the initial development attenuate during the progress of plant cycle, plants derived from low vigor seeds usually have inferior growth rate and higher sensitivity to environmental adversities.

The seedling emergence test evaluated at the seven days after sowing (Table 2) allowed the separation of tobacco seed lots into two levels of vigor, where L1, L2, L3, L4, L7 and L8 were classified as high vigor and L5, L6, L9 and L10 as inferior vigor seed lots. Studies carried out by Crivellari (2016) demonstrated that the evaluation of emergence at seven days after sowing, for bare tobacco seeds, performed under controlled conditions and at 25 ℃, were efficient in stratifying seed lots according to vigor levels. Similarly, in this study, seedling emergence performed in a low tunnel and using the floating system for irrigation, in which the environmental conditions are not completely controlled, the evaluation of seedling emergence at the seven days after sowing and the speed of emergence index were efficient in stratifying seed lots according to vigor levels.

The speed of emergence index (Table 2) stratified the seed lots according to the physiological quality in two levels of vigor, L1, L2, L3, L4 and L7 were classified as high vigor and L5, L6, L8, L9 and L10 as low vigor seed lots.

According to data obtained through the standard accelerated aging (Table 3), the test stratified the lots into two vigor levels, wherein L1 and L2 were superior to the others. Furthermore, the test presented significant correlation with the seedling emergence test at seven days after sowing (r= 0.82) and with the speed of emergence index (r= 0.89), as demonstrated in Table 4. The average water content between lots, obtained after the standard accelerated aging (Table 3), was of 22.6% with a maximum variation of 3.9 percent. However, according to AOSA (2009), the water content reached for tobacco seeds after the accelerated aging test with water must be within 40 and 50%, depending of the initial water content, deterioration level and chamber type (B.O.D or Water-Jacketed CO_2 Incubators).

After the analysis of the results from Table 1, the correlation of each test with the main indicators of vigor, which are the seedling emergence at the seven days after sowing and the speed of emergence index, was evaluated (Table 4). The first count of germination promoted the stratification of lots, however not properly, not presenting significant correlations with the indicators mentioned. According to Medeiros (2008), Carvalho (2009) and Crivellari (2016), which studied the physiological quality of tobacco seeds, the first count of germination is not suitable for assessing tobacco seed vigor.

For the speed of germination index, seven lots presented superior vigor and three were categorized as of inferior vigor (Table 1). However, the results obtained did not present significant correlations with the speed of emergence index and with seedling emergence at seven days after sowing (Table 4), indicating that the test is not suitable for the evaluation of tobacco seed vigor, when the seedling emergence test is considered as the standard test.

3.2 Performance of the Accelerated Aging Methodologies

3.2.1 Accelerated Aging at 41 ℃

The average results for water content after each aging period using water (AAW), saturated saline solution (AUSS) and unsaturated saline solution (ASSS) at the temperature of 41 ℃ are presented in Table 5. These data were not evaluated statistically and were only used to characterize the seed lots after each aging period. The results presented in Table 5 for the

AAW and AUSS demonstrate a similar behavior to those observed in Table 8, which correspond to the seed water content reached after the accelerated aging at 45 ℃.

Table 5. Water content of ten tobacco seed lots, after three accelerated aging periods, using water (AAW), unsaturated saline solution (AUSS) and saturated saline solution (ASSS), at the temperature of 41 ℃, Pelotas-RS, UFPel, 2018

Lot	Water content (%)										
	AAW				AUSS				ASSS		
	24h	48h	72h		24h	48h	72h		24h	48h	72h
L1	18.5	20.6	20.6		11.4	10.7	11.4		5.7	6.6	6.3
L2	19.0	20.7	19.2		11.9	11.5	11.0		5.6	6.4	5.7
L3	17.1	18.1	17.8		9.0	10.6	10.0		4.1	4.0	5.9
L4	15.4	17.1	18.0		9.2	9.4	10.9		5.2	5.3	5.7
L5	18.4	20.5	18.2		10.6	11.6	12.7		6.7	5.9	8.2
L6	17.4	17.2	19.1		9.4	9.1	11.9		4.4	4.8	5.9
L7	17.8	19.0	20.7		9.8	10.6	12.0		4.0	3.6	5.6
L8	18.9	20.5	19.4		11.4	11.3	10.7		6.7	6.0	7.4
L9	19.3	20.8	19.9		10.9	11.5	11.2		5.6	4.8	6.2
L10	18.9	16.3	21.4		10.4	10.0	11.9		4.9	5.5	5.7
Average	18.1	19.1	19.4		10.4	10.6	11.4		5.3	5.3	6.3

The most expressive increase in seed water content reached at the end of the test was observed for AAW. AUSS promoted increases of 5.3% to 6.3% in the seed water content while ASSS resulted in a small addition to the water content. Additionally, in the AAW and AUSS methodologies the water content increased markedly at the first 24 hours of exposure, with minimal additional increases in the water content for exposure periods superior to 24 hours. ASSS promoted a small increase in the seed water content at the exposure period of 72 hours.

The distinguished increases for each solution used in the accelerated aging test is expected and was confirmed according to the results present in Tables 5 and 8. In the conditions of the accelerated aging test using 40 mL of distilled water at the bottom of each box, the air relative humidity is of approximately 100% (Marcos Filho, 2015). The use of the unsaturated saline solution promotes an air relative humidity in the interior of each box of 94%, reducing the rate of water absorption by seeds, methodology described by Jianhua; McDonald (1996) and determined accordingly to the Van't Hoff equation described by Salisbury; Ross (1992). Likewise, the use of the saturated saline solution reduces the air relative humidity inside the plastic boxes compared to the methodologies previously cited, which is around 76%, causing seed hydration to occur in even slower rates and reducing variation between samples (Jianhua; McDonald, 1996).

The results of the accelerated aging test performed at the temperature of 41 ℃ (Table 6) demonstrate that AAW and exposure periods of 24, 48 and 72 hours, as well as the AUSS with an exposure period of 24 hours and ASSS with an exposure period of 48 hours promoted the stratification of seed lots in vigor levels. The other methodologies performed at the temperature of 41 ℃ did not present statistical differences for the seed lots studied.

Table 6. Results (%) of ten tobacco seed lots in the accelerated aging tests using water (AAW), unsaturated saline solution (AUSS) and saturated saline solution (ASSS) after three exposure periods of 24, 48 and 72 hours at the temperature of 41 ℃, Pelotas-RS, UFPel, 2018

Lot	AAW (%)				AUSS (%)				ASSS (%)		
	24h	48h	72h		24h	48h	72h		24h	48h	72h
L1	97 a	96 a	93 a		96 a	96	94		95	97 a	95
L2	95 a	96 a	97 a		96 a	95	95		94	95 a	95
L3	96 a	94 a	95 a		95 a	94	94		96	94 b	93
L4	94 a	93 a	95 a		94 a	93	94		95	96 a	94
L5	93 a	93 a	92 a		93 b	93	90		95	95 a	94
L6	94 a	94 a	95 a		96 a	96	93		95	96 a	96
L7	93 a	92 a	94 a		92 b	90	88		93	93 b	92
L8	90 b	87 b	87 b		92 b	93	88		93	92 b	91
L9	92 a	92 a	93 a		92 b	95	88		94	93 b	93
L10	86 c	88 b	82 c		89 c	92	90		92	92 b	94
Average	93	93	92		94	94^{ns}	92^{ns}		94^{ns}	94	94^{ns}
C.V. (%)	2.32	3.07	2.14		1.74	2.36	3.72		1.63	1.93	1.65

*Averages followed by the same letter in the column do not differ between each other by the Scott-Knott test at the 5% probability level of error.

Deuner *et al.* (2017), while evaluating the most adequate methodology for the accelerated aging test in four sweet sorghum seed lots and the relation with field emergence, observed that tests with 24 hours exposure periods at the temperature of 41 ℃ for the accelerated aging with saturated saline solution and of 48 hours for the accelerated aging with water were efficient in the evaluation of sweet sorghum seed vigor, corroborating the results of this study. The authors also highlighted that a desirable vigor test, besides providing reliable results, must demonstrate the differences between seed lots and have fast execution.

According to the data presented in Table 6, the results obtained with AAW for the exposure periods of 24 and 72 hours stratified the seed lots in a similar manner, separating lots in three levels of vigor, wherein lots L1, L2, L3, L4, L5, L6, L7 and L9 were classified as high vigor, L8 as medium vigor and L10 as low vigor. However, AAW using the exposure period of 24 hours presented significant correlations with the speed of emergence index (r= 0.72), seedling emergence at seven days after sowing (r= 0.66), speed of germination index (r= 0.79) and with the standard accelerated aging (r= 0.85) (Table 7). On the other hand, for the 48 hours exposure period, the seed lots were ranked into two levels of vigor, wherein the lots L1, L2, L3, L4, L5, L6, L7 and L9 were classified as high vigor while L8 and L10 as low vigor. As demonstrated in Table 7, the results of this exposure period presented significant correlations with the first count of germination (r= 0.72), speed of germination index (r= 0.66), speed of emergence index (r= 0.64) and the standard accelerated aging (r= 0.79).

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Table 7. Linear correlations [Pearson correlation coefficient (r)] between the physiological quality variables of ten tobacco seed lots: germination (G), first count of germination (FCG), speed of germination index (SGI), seedling emergence evaluated at seven (SE7), fourteen (SE14) and twenty-one (SE21) days after sowing, speed of emergence index (SEI) and the standard accelerated aging proposed by AOSA (SAA AOSA) with alternative methodologies of accelerated aging performed at the temperature of 41 ℃: accelerated aging with water and exposure period of 24 (AAW24), 48 (AAW48) and 72 (AAW72) hours, accelerated aging with unsaturated saline solution and exposure period of 24 (AUSS24), 48 (AUSS48) and 72 (AUSS72) hours and accelerated aging with saturated saline solution and exposure period of 24 (ASSS24), 48 (ASSS48) and 72 (ASSS72) hours. Pelotas-RS, UFPel, 2018.

Variable	G	FCG	SGI	SE7	SE14	SE21	SEI	SAA AOSA	AAW24	AAW48	AAW72	AUSS24	AUSS48	AUSS72	ASSS24	ASSS48	ASSS72
G	1.0																
FCG	0.64*	1.00															
SGI	0.23^{ns}	0.24^{ns}	1.00														
SE7	0.23^{ns}	0.28^{ns}	0.32^{ns}	1.00													
SE14	0.28^{ns}	0.46^{ns}	0.25^{ns}	0.85**	1.00												
SE21	0.28^{ns}	0.46^{ns}	0.15^{ns}	0.66*	0.92**	1.00											
SEI	0.26^{ns}	0.40^{ns}	0.34^{ns}	0.96**	0.95**	0.81**	1.00										
SAA AOSA	0.38^{ns}	0.57^{ns}	0.55^{ns}	0.82**	0.88**	0.74*	0.89**	1.00									
AAW24	0.53^{ns}	0.63^{ns}	0.79**	0.66*	0.66*	0.60^{ns}	0.72*	0.85**	1.00								
AAW48	0.42^{ns}	0.72*	0.66*	0.47^{ns}	0.70*	0.74*	0.64*	0.79**	0.89**	1.00							
AAW72	0.36^{ns}	0.53^{ns}	0.72*	0.48^{ns}	0.51^{ns}	0.58^{ns}	0.57^{ns}	0.67*	0.88**	0.85**	1.00						
AUSS24	0.36^{ns}	0.67*	0.80**	0.52^{ns}	0.57^{ns}	0.46^{ns}	0.60^{ns}	0.84**	0.91**	0.86**	0.81**	1.00					
AUSS48	0.42^{ns}	0.85**	0.25^{ns}	0.17^{ns}	0.34^{ns}	0.27^{ns}	0.26^{ns}	0.49^{ns}	0.50^{ns}	0.56^{ns}	0.38^{ns}	0.70*	1.00				
AUSS72	0.04^{ns}	0.58^{ns}	0.58^{ns}	0.43^{ns}	0.52^{ns}	0.39^{ns}	0.53^{ns}	0.68*	0.65*	0.74*	0.53^{ns}	0.78**	0.56^{ns}	1.00			
ASSS24	0.53^{ns}	0.71*	0.69*	0.29^{ns}	0.26^{ns}	0.25^{ns}	0.33^{ns}	0.57^{ns}	0.83**	0.73*	0.71*	0.78**	0.56^{ns}	0.64*	1.00		
ASSS48	0.19^{ns}	0.55^{ns}	0.65*	0.34^{ns}	0.53^{ns}	0.54^{ns}	0.45^{ns}	0.73*	0.78**	0.83**	0.64*	0.83**	0.60^{ns}	0.76*	0.74*	1.00	
ASSS72	0.04^{ns}	0.53^{ns}	0.34^{ns}	0.12^{ns}	0.28^{ns}	0.34^{ns}	0.08^{ns}	0.35^{ns}	0.35^{ns}	0.66*	0.33^{ns}	0.56^{ns}	0.63*	0.70*	0.40^{ns}	0.75*	1.00

**Significant t-test at the 1% probability level of error; * Significant by the t-test at the 5% probability level of error. ^{ns} – Not significant.

The accelerated aging with unsaturated saline solution (AUSS) and a 24 hours exposure period (Table 6), stratified the lots into three levels of vigor, classifying lots L1, L2, L3, L4 and L6 as high vigor, L5, L7, L8 and L9 as medium vigor and L10 as low vigor, and obtained significant correlations (Table 7) with the first count of germination (r= 0.67), speed of germination index (r= 0.80) and the standard accelerated aging (0.84). When the saturated saline solution (ASSS) was used, with an exposure period of 48 hours, the seed lots were classified into two levels of vigor, grouping lots L1, L2, L4, L5 and L6 as high vigor seed lots and L3, L7, L8, L9 and L10 as of inferior vigor, presenting a significant correlation with the speed of germination index (r= 0.65) and the standard accelerated aging (r= 0.73).

Nevertheless, analyzing data from Table 6 combined with the correlations demonstrated in Table 7, the accelerated aging method which demonstrated more similar results with the vigor tests applied for the initial characterization of the lots was the accelerated aging with water

(AAW) at the temperature of 41 ℃ and an exposure period of 24 hours. However, despite presenting significant correlations with the seedling emergence test at seven days after sowing (r= 0.66) and the speed of emergence index (r= 0.72), these correlations were inferior to those observed for the standard accelerated aging method proposed by AOSA, which were of 0.82 and 0.89, respectively.

Crivellari (2016), studying the performance of tobacco seeds through the accelerated aging test with water at the temperature of 41 ℃, concluded that the artificial aging at 41 ℃ for 72 hours is efficient in stratifying tobacco seed lots from the cultivars CSC 447 and BAT 210 into different levels of vigor. On the other hand, Carvalho (2009) concluded, using five tobacco seed lots of the cultivar CSC 439, that the most adequate conditions for the accelerated aging test of tobacco seeds are 41 ℃ for 12 hours of exposure with the evaluation performed at seven days after sowing, using the method with water for bare seeds.

3.2.1 Accelerated Aging at 45 ℃

The average results of the seed water content after each aging period using AAW, AUSS and ASSS at the temperature of 45 ℃ are presented in Table 8. These data were not analyzed statistically and were only used to characterize lots after the aging period.

Table 8. Water content of ten tobacco seed lots, after three accelerated aging periods, using water (AAW), unsaturated saline solution (AUSS) and saturated saline solution (ASSS), at the temperature of 45 ℃, Pelotas-RS, UFPel, 2018

Lot	Water content (%)								
	AAW			AUSS			ASSS		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
L1	17.8	20.0	17.7	11.5	10.9	11.7	6.1	6.6	6.9
L2	17.9	20.2	18.8	11.7	11.6	12.9	6.3	6.2	7.4
L3	18.9	19.6	17.6	9.8	10.8	11.3	6.9	6.4	6.1
L4	19.4	19.7	18.7	9.7	10.9	10.5	4.8	5.8	6.8
L5	18.7	18.2	18.1	10.8	10.6	12.5	7.3	6.4	8.0
L6	18.3	19.5	19.2	10.4	9.6	10.6	6.6	6.8	6.4
L7	19.2	19.8	18.7	9.5	10.2	10.6	4.5	6.9	6.4
L8	18.1	18.5	19.4	11.7	10.8	12.2	6.9	5.5	6.9
L9	21.6	18.1	19.2	11.9	11.0	11.7	6.4	5.3	8.2
L10	21.6	22.1	19.6	11.6	12.3	11.8	6.0	7.4	6.9
Average	19.2	19.6	18.7	10.8	10.9	11.6	6.2	6.3	7.0

The tobacco seeds subjected to AAW, AUSS and ASSS presented increases in the water content after the exposure periods of 24, 48 and 72 hours at the temperature of 45 ℃ (Table 8), with a more expressive increase for AAW. The use of AUSS promoted an average increase of 5.7 to 6.5% in the seed water content while the use of ASSS resulted in a small increase in this characteristic. For the AAW and AUSS methodologies, the seeds obtained more markedly increases in the water content up to the 24 hours, followed by minimal increases after this period.

Still in Table 8, a greater uniformity was observed in the water content between seed lots subjected to ASSS and AUSS with different exposure periods. Depending of the solute used, an air relative humidity inferior to the observed for the accelerated aging with water can be obtained, causing seed water absorption to occur in a lower intensity and in a more uniform manner, resulting in a slower intensity of deterioration and lower variation between results (Jianhua; McDonald, 1996).

According to Tunes *et al*. 2012, the use of the unsaturated and saturated saline solutions reduces water absorption and deterioration rate in rice seeds during the accelerated aging test. Similar results were reported by Lemes *et al*. 2015, where the use of saline solutions in the aging chamber reduced water absorption in *Cynodon dactylon* seeds during the accelerated aging test when compared to the method with water. Likewise, Deuner *et al.* (2017) observed that the accelerated aging with saturated saline solution in sweet sorghum seeds causes a gradual increase in water content, allowing more uniform results.

The results from the accelerated aging test performed at the temperature of 45 ℃ (Table 9) demonstrate that AAW and an exposure period of 24 hours, AUSS with the exposure period of 48 hours as well as ASSS using the exposure periods of 24 and 48 hours promoted the stratification of the lots into two levels of vigor. The other methodologies tested at the temperature of 45 ℃ were not efficient.

The AAW method combined with the exposure period of 24 hours promoted the stratification of the lots in a similar manner to the vigor tests used in the initial characterization of seed quality, classifying the lots into two levels of vigor, L1, L2, L3, L4 were determined as of high vigor and L5, L6, L7, L8, L9 and L10 as of low vigor. Significant correlations were observed with the speed of emergence index (r= 0.83), seedling emergence at seven days after sowing (r= 0.80), speed of germination index (r= 0.68) and the standard accelerated aging (r= 0.85) (Table 10).

The AUSS method and an exposure period of 48 hours and the ASSS method and an exposure period of 24 hours presented similar results to the variable SGI, with significant correlations of r= 0.76 and r= 0.78, respectively. On the other hand, for ASSS and an exposure period of 48 hours, there were no significant correlations with the vigor tests applied for the characterization of seed lot initial quality.

According to the data presented in Table 9, combined with the correlation analysis demonstrated in Table 10, the methodology that obtained the most similar results compared to the variables tested in the initial characterization of seed lot quality was the AAW performed at the temperature of 45 ℃ combined with an exposure period of 24 hours, where the obtainment of results in a short period stands out, with similar results to those observed for the speed of emergence index, the seedling emergence at seven days and the standard accelerated aging, which is performed with an exposure period of 72 hours at the temperature of 43 ℃. Tunes *et al*. 2012 highlights that the obtainment of vigor tests results in the shorter period possible is desirable and reduces the costs associated with the electricity consumption of the equipment used. Besides other characteristics, an efficient vigor test, must provide rapid results (Marcos Filho, 2015).

Table 8. Results (%) of ten tobacco seed lots in the accelerated aging tests using water (AAW), unsaturated saline solution (AUSS) and saturated saline solution after three exposure periods (24, 48 and 72 hours) at the temperature of 45 ℃, Pelotas-RS, UFPel, 2018

Lot	AAW (%)				AUSS (%)				ASSS (%)		
	24h	48h	72h		24h	48h	72h		24h	48h	72h
L1	96 a	91	89		94	93 a	92		95 a	95 a	96
L2	93 a	90	86		95	92 a	94		95 a	96 a	95
L3	94 a	92	88		93	94 a	89		97 a	95 a	95
L4	92 a	92	87		91	91 a	92		94 a	95 a	93
L5	86 b	86	78		89	88 b	88		94 a	91 b	93
L6	90 b	85	82		93	93 a	82		96 a	96 a	96
L7	89 b	86	83		88	92 a	80		92 b	91 b	92
L8	87 b	87	82		90	91 a	87		93 b	93 b	91
L9	88 b	87	81		90	86 b	87		92 b	94 a	91
L10	84 b	89	80		90	90 a	85		92 b	94 a	91
Average	90	88^{ns}	83^{ns}		91^{ns}	91	87^{ns}		94	94	93^{ns}
C.V. (%)	3.17	2.96	6.06		3.18	1.96	6.8		1.79	1.63	2.67

*Averages followed by the same letter in the column do not differ between each other by the Scott-Knott test at the 5% probability level of error. ^{ns} – Not significant by the F test

Guiscem *et al.* (2001) also observed a significant and positive correlation between the accelerated aging test and field seedling emergence in sweet corn seeds BR 400. However, Bertolin *et al.* (2011) did not find a significant correlation between the accelerated aging and seedling emergence while evaluating bean seeds.

The AUSS method and an exposure period of 48 hours at the temperature of 45 ℃ presented significant results when comparing seed lots, stratifying lots into two levels of vigor, where L1, L2, L3, L4, L6, L7, L8 and L10 were classified as of high vigor and L5 and L9 as low vigor. This method did not present significant correlations with the seedling emergence at seven days after sowing and the speed of emergence index, however, a significant correlation (r= 0.76) was observed for the speed of germination index.

The accelerated aging test with the use of the saturated saline solution (ASSS) promoted the stratification of the seed lots for the exposure periods of 24 and 48 hours, however, no significant correlations with the seedling emergence at seven days after sowing and the speed of emergence index were observed. Nevertheless, the exposure period of 24 hours presented a significant correlation with the speed of germination index (r= 0.78), as can be seen in Table 10.

Table 9. Linear correlations [Pearson correlation coefficient (r)] between the physiological quality variables of ten tobacco seed lots: germination (G), first count of germination (FCG), speed of germination index (SGI), seedling emergence evaluated at seven (SE7), fourteen (SE14) and twenty one (SE21) days after sowing, speed of emergence index (SEI) and the standard accelerated aging proposed by AOSA (SAA AOSA) with alternative methodologies of accelerated aging performed at the temperature of 45 ℃: accelerated aging with water and exposure periods of 24 (AAW24), 48 (AAW48) and 72 (AAW72) hours, accelerated aging with unsaturated saline solution and exposure period of 24 (AUSS24), 48 (AUSS48) and 72 (AUSS72) hours and accelerated aging with saturated saline solution and exposure periods of 24 (ASSS24), 48 (ASSS48) and 72 (ASSS72) hours. Pelotas-RS, UFPel, 2018

Variable	G	FCG	SGI	SE7	SE14	SE21	SEI	SAA AOSA	AAW24	AAW48	AAW72	AUSS24	AUSS48	AUSS72	ASSS24	ASSS48	ASSS72
G	1.00																
FCG	0.64*	1.00															
SGI	0.23^{ns}	0.24^{ns}	1.00														
SE7	0.23^{ns}	0.28^{ns}	0.32^{ns}	1.00													
SE14	0.28^{ns}	0.46^{ns}	0.25^{ns}	0.85**	1.00												
SE21	0.28^{ns}	0.46^{ns}	0.15^{ns}	0.66*	0.92**	1.00											
SEI	0.26^{ns}	0.40^{ns}	0.34^{ns}	0.96**	0.95**	0.81**	1.00										
SAA AOSA	0.38^{ns}	0.57^{ns}	0.55^{ns}	0.82**	0.88**	0.74*	0.89**	1.00									
AAW24	0.30^{ns}	0.57^{ns}	0.68*	0.80**	0.76*	0.59^{ns}	0.83**	0.85**	1.00								
AAW48	0.11^{ns}	0.34^{ns}	0.09^{ns}	0.63^{ns}	0.52^{ns}	0.35^{ns}	0.61^{ns}	0.46^{ns}	0.64*	1.00							
AAW72	0.07^{ns}	0.37^{ns}	0.53^{ns}	0.84**	0.70*	0.47^{ns}	0.81**	0.71*	0.94**	0.80**	1.00						
AUSS24	0.15^{ns}	0.71*	0.45^{ns}	0.47^{ns}	0.58^{ns}	0.38^{ns}	0.56^{ns}	0.69*	0.76*	0.52^{ns}	0.68*	1.00					
AUSS48	0.12^{ns}	0.00^{ns}	0.76*	0.43^{ns}	0.28^{ns}	0.02^{ns}	0.39^{ns}	0.45^{ns}	0.64*	0.38^{ns}	0.69*	0.57^{ns}	1.00				
AUSS72	0.12^{ns}	0.60^{ns}	0.02^{ns}	0.63*	0.66*	0.57^{ns}	0.68*	0.70*	0.55^{ns}	0.75*	0.56^{ns}	0.61^{ns}	0.05^{ns}	1.00			
ASSS24	0.32^{ns}	0.63^{ns}	0.78**	0.28^{ns}	0.24^{ns}	0.06^{ns}	0.32^{ns}	0.58^{ns}	0.69*	0.38^{ns}	0.56^{ns}	0.76*	0.67*	0.37^{ns}	1.00		
ASSS48	0.15^{ns}	0.57^{ns}	0.25^{ns}	0.23^{ns}	0.28^{ns}	0.14^{ns}	0.28^{ns}	0.34^{ns}	0.57^{ns}	0.51^{ns}	0.58^{ns}	0.87**	0.42^{ns}	0.46^{ns}	0.59^{ns}	1.00	
ASSS72	0.30^{ns}	0.62^{ns}	0.82**	0.35^{ns}	0.49^{ns}	0.36^{ns}	0.46^{ns}	0.72*	0.78**	0.30^{ns}	0.60^{ns}	0.82**	0.68*	0.33^{ns}	0.89**	0.59^{ns}	1.00

******Significant by the t-test at the 1% probability level of error; ***** Significant by the t-test at the 5% probability level of error. ^{ns} – Not significant.

According to studies performed by Medeiros (2008), the seed vigor of tobacco hybrids CSC 405, K 326 and CSC 459 can be evaluated through the accelerated aging test with saturated saline solution at the temperature of 45 ℃ and an exposure period of 72 hours, however, the evaluation of this method is only performed at 16 days after sowing.

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