Comunicata Scientiae
Horticultural Journal

ORIGINAL ARTICLE
published: 22 May 2021
https://doi.org/10.14295/CS.v12.3252

Evaluation of pea seed vigor by the accelerated aging and controlled deterioration tests

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Abstract

Vigor tests were developed to detect differences in seed lots due to limitations of germination tests. The objective of this work was to investigate the efficiency of the accelerated aging and controlled deterioration tests to assess the vigor of pea seed lots. The water content and physiological quality of five pea lots cv. Aragon were determined using the germination and vigor tests (first count, electrical conductivity, seedling emergence and speed of emergence index, accelerated aging with the traditional system and with saturated NaCl solution at 41°C for 48, 72 and 96 hours; and controlled deterioration test with 15, 20, 25% water content for 48, 72 and 96 hours at 42°C). The accelerated aging test with saturated NaCl solution for 96 hours was efficient to separate pea seeds lots. Combinations of 20% water for 72 hours and 25% water for 48 and 72 hours were efficient to separate pea seed lots using the controlled deterioration test.

Keywords: *Pisum sativum*, quality control, vigor tests

Introduction

Pea is considered a vegetable with high nutritional value and its production in Brazil is allocated to human consumption. The use of seeds possessing high physiological potential is important for the establishment of plantations (Kavan et al., 2019) and enable higher yields (Catão et al., 2013).

Seed technology has pursued the improvement of tests used to evaluate the physiological potential aiming to obtain results which may express the real performance of seed lots (Rocha et al., 2018). The germination test overestimate real values for plant emergency in the field (Bertolin et al., 2011), once these tests are performed under optimal water, aeration and temperature conditions (Brasil, 2009). Therefore, result from this test is considered insufficient requiring also the results obtained from vigor tests (Ohlson et al., 2010).

Among vigor tests, the accelerated aging test is one of the most used in Brazil and worldwide for quality control programs (Guiscem et al., 2010). This test has as a principle the significant increase in seed deterioration rates occurring due to the exposure to high levels of temperature and relative humidity (Ohlson et al., 2010).

The accelerated aging test may be considered as one of the most sensitive to evaluate seed vigor (Marcos Filho, 2015). However, due to the high humid atmosphere during the test, different water absorption rates have been observed among seeds. This may result in different degrees of deterioration of seeds lots (Powell, 1995). For this reason, the use of saturated salt solutions has replaced distilled water in performing the test by adapting humid atmosphere, the rate of seed water absorption to the speed and intensity of deterioration (Lima et al., 2015).

Differences in vigor among seed lots have also been detected through the controlled deterioration test,

Received: 06 December 2019
Accepted: 12 December 2020

which has a principle equivalent to the accelerated aging. Controlled deterioration test is performed with the initial water content equalized for all seed lots (Powell, 1995), so that the same degree of aging or deterioration will be imposed in a uniform. This allows comparing and classifying seed lots, regarding their deterioration degree, in a more accurate way.

When evaluating vigor, the accelerated aging test is more drastic than the controlled deterioration test. In the controlled deterioration test the seed water content remains constant, while in the accelerated aging test the seed water content increases during the exposure period. This can cause damage to the seeds during imbibition and in this perspective, we hope to consolidate the controlled deterioration test to be a safe alternative in assessing the vigor of pea seeds.

For this reason, the controlled deterioration test has been used largely to evaluate seed vigor in various crops (Torres et al., 2012; Torres et al., 2013; Lopes et al., 2013; Medeiros et al., 2014). In face of these facts, the objective of this work was to investigate the efficiency of the accelerated aging and controlled deterioration tests to assess the vigor of pea seed lots.

Material and Methods

The experiment was performed at the Seed Laboratory from the Department of Plant Science at the Gammon Educational Foundation, located in Paraguaçu Paulista, São Paulo, Brazil, between August and December 2018. Five pea seed lots, cultivar Aragorn, were used in the experiment. Samples from each lot were analyzed to determine their water content and their physiological quality was evaluated using tests of germination and vigor. Water content was determined using an oven at 105±3°C for 24 hours, with two sub-samples with approximately 10g for each lot (Brasil, 2009).

Four replicates with 50 seeds for each lot were used in the germination test, with seeds being uniformly distributed on two sheets of germitest paper, dampened with distilled water at a proportion of 2.5 times the weight of the dry paper sheets and set to germinate at 20°C and photoperiod of 12 hours. The germination counts were performed after five and eight days of sowing for determining the percentage of normal seedlings (Brasil, 2009).

The first germination counting was achieved along with the germination test, determining the percentage of normal seedlings five days after the experiment started (Brasil, 2009). The results were expressed as germination percentage in the first and last counts.

The electrical conductivity test was performed with four sub-samples with 50 seeds, weighed with a precision of two decimal units and set in plastic cups (200mL) containing 75mL distilled water for 24 hours at 25°C. The electrical conductivity was accessed immediately after the imbibition period using a Tecnal Tec-4MP conductivity meter. Data were transformed to $\mu S\ cm^{-1}\ g^{-1}$.

To test the emergence seeds were sowed in the field with four sub-samples containing 50 seeds from each lot, distributed at 3.0 cm depth and 0.5 cm spacing in furrows with 1.0 m length. The medium textured soil was moistened at approximately 60% of its water retention capacity. Ten days after the stand was stabilized, the number of emerged plants was evaluated and the results were expressed as emergence percentage. The speed of emergence index was accessed simultaneously to the emergence test, calculating the number of normal plants emerged every day in the same schedule. The index was calculated as proposed by Maguire (1962).

The accelerated aging test was performed with 240 seeds from each lot, which were set in a single layer aluminum grids inside gerbox plastic boxes containing 40mL distilled water or saturated solution of NaCl (40g $100ml^{-1}$) (Marcos Filho, 1999). These boxes were kept in an incubator type BOD at 41°C for 48, 72 and 96 hours. After the exposure period the water content and germination was determined.

In the controlled deterioration test, seed samples from each lot were divided in three subsamples with three hundred seeds, aiming to adjust humidity to 15, 20 and 25%, through the humid substrate method (Rossetto et al., 2004). After reaching the desired humidity degree, seeds were conditioned in glass containers and preserved at 10°C for 12 hours. At the end of this period, seeds were conditioned in aluminum bags and sealed. Aluminum bags were preserved at 42°C in water bath for 48, 72 and 96 hours. After the exposure period, sealed bags were immersed in water for 30 minutes and then the water content of seeds was determined (Brasil, 2009). The germination test proceeded as previously described and the evaluation was performed five days after the test was installed.

The experiment was set in a complete random design, with four replicates per lot. In order to analyze data statistically, the F test and variance analysis at 5% probability were used, when significant effects occurred means were compared by the Tukey test at 5% probability, using the software Sisvar 5.0 (Ferreira, 2011). Pearson's linear correlation was also used for results of the vigor tests.

Results and Discussion

Results regarding seed's water content were similar between the seed lots studied (Table 1). This fact is important once the homogeneity of the initial water content in seeds contributes to obtain consistent test results (Marcos Filho, 2015). Guedes et al. (2011) emphasizes that differences of 1 to 2% in the water content between samples are not significant and the tests may be performed without problems.

Table 1. Water content (WC), first germination count (FGC), germination (G), electrical conductivity (EC), emergency (E) and emergency speed index (ESI) for the initial characterization of the physiological quality of lots of pea seeds.

Lots	WC (%)	FGC (%)	G (%)	EC (μS cm^{-1} g^{-1})	E (%)	ESI
1	10.3	76±3.1 ab	84±4.6 a	33.87±3.4 b	76±3.5 ab	12.5±0.7 bc
2	10.4	67±2.7 b	82±4.5 a	31.46±3.2ab	75±3.4 ab	8.4±0.5 d
3	10.0	79±3.2 a	87±4.8 a	40.18±4.1 b	79±3.6 ab	10.8±0.6 cd
4	10.4	79±3.2 a	86±4.7 a	25.73±2.6 a	85±3.9 a	14.7± 0.9 a
5	10.9	74±3.0 ab	81±4.5 a	36.52±3.7 b	73±3.3 b	12.1±0.7 ab
CV (%)		4.08	5.55	10.16	4.56	5.99

*Means followed by the same letter in column are not statistically different by Tukey test at 5% probability. Values represent Mean ± standard error.

There was no difference regarding germination between the five pea seed lots studied (Table 1). According Araujo et al. (2011), seed lots with similar germination rates are fundamental in studies aiming to determine methods for evaluation seed vigor once the objective is to separate lots of seeds with similar germination. If the seeds' germination potential shows accentuated differences, the germination test by itself can detect differences in the physiological potential of seeds (Marcos Filho & Novembre, 2009). The first germination counting test allowed to classify lots 3 and 4 as of superior quality and lot 2 as of inferior vigor (Table 1).

The test of emergence in the field showed a different behavior for the seed lots studied, with lot 4 being of superior quality and lot 5 of inferior quality, therefore diverging from the germination test results. Concerning to the speed of velocity index (ESI), lot 4 was classified as of a better quality and lot 2 as of a worst quality (Table 1). Emergence test is considered the best indicative to infer about seed vigor, once to execute it, similar conditions to those seeds will support during the seeding in the field, must be used (Guedes et al., 2011).

In the electrical conductivity test (Table 1), only lot 4 was superior, being lots 1, 3 and 5 considered as inferior and lot 2 as intermediary. This test is commonly used due to some advantages such as allowing the detection of the first symptoms of seed deterioration, that is, the loss of structural integrity of cellular membranes (Silva et al., 2014).

In the initial characterization of pea seed lots only the germination test did not allow to separate seed lots by their physiological quality levels, therefore, vigor test are fundamental to evidence differences existing between seed lots (Marcos Filho, 2015). Therefore, is important that tests used to evaluate the physiological quality must be efficient to track the evolution process of seed deterioration, especially when these seeds are submitted to adverse conditions (Carvalho et al., 2009).

Using the test of accelerated aging by the traditional method allowed to verify that in the 72 hours period there were no significant differences in seed vigor between all five pea lots studied. However, for the 96 hours period lots 1, 3 and 4 showed superior vigor than lots 2 and 5. Thus, it is possible to verify a lower efficiency to separate seed lots, once in the emergence test these lots were classified as of intermediary quality (Table 2).

However, when the accelerated aging test was applied using the saturated NaCl (40g) solution method (Table 2), it was notorious that in the 96 hours period lot 4 was classified as of superior vigor, lots 1, 2 and 3 as intermediary and lot 5 as of inferior vigor. These results confirm data verified in the emergence test classifying the vigor of seed lots (Table 1). Classification of seed lots according to the accelerated aging test was also achieved in wheat (Ohlson et al., 2010), carrot, pea, beans and soybean (ISTA, 2014).

Water contents achieved after the application of the accelerated aging treatments are showed in Table 2. Analyzing the results it was evidenced that with the traditional accelerated aging method (100% UR), the water content varied from 3.5% to 4% within the exposure periods tested. It is important to emphasize that water content at the end of the accelerated aging test is one of the indicators for uniformity performance of this test. Marcos Filho (2015) observed that variations from 4% to 5% between samples are considered as acceptable. Lower and more uniform values of water content were observed when using the saturated saline solution, thus promoting less drastic effects on seed aging. Therefore, the deterioration degree of seeds is reduced when compared to the common values observed when using the traditional method. Similar results were observed

when studying coriander (*Coriandrum sativum*) (Radke et al., 2016) and rice seeds (Monteiro et al., 2017).

According the controlled deterioration test results (Table 3), it is possible to verify the 72 hours exposure period with seed water content set at 20%, classified vigor for lot 4 as superior, lot 5 as inferior and lots 1, 2 and 3 as intermediary.

Table 2. Accelerated aging (%) by traditional systems and with saturated solution of NaCl, for periods of 48, 72 and 96 hours and water content (%), obtained from five lots of pea seeds.

	----------Traditional----------			----------Saturated Solution----------		
Lots	48	72	96	48	72	96
			Accelerated aging			
1	57±5.1ab	43±13.7a	35±6.4a	60±6.0ab	56±17.0a	48±11.3ab
2	53±4.8ab	47±14.9a	21±6.7b	69±6.9a	58±17.6a	52±12.2ab
3	46±4.1b	47±14.9a	33±10.5a	41±4.1c	50±15.1a	47±a11.0b
4	65±5.8a	46±14.6a	30±9.5a	69±6.9a	53±16.0a	66±15.5a
5	55±4.9ab	44±14.0a	15±4.8b	50±5.0b	57±17.3a	31±7.3b
CV (%)	8.97	31.76	18.42	10.07	30.27	23.45
			Water Content			
1	21.5±1.1a	26.0±1.7a	26.4±1.4a	12.6±0.2c	13.1±0.7b	13.2±0.7ab
2	22.0±1.1a	22.8±1.5ab	27.7±1.4a	12.7±0.2b	13.2±0.7ab	12.9±0.7b
3	20.3±1.0ab	25.3±1.6ab	25.9±1.3ab	13.1±0.3b	13.2±0.7ab	13.5±0.7ab
4	18.6±0,9b	21.9±1.4b	23.7±1.2b	12.9±0.2b	13.1±0.7b	13.1±0.7b
5	22.1±1.1a	24.2±1.6ab	26.5±1.4a	13.4±0.3a	13.9±0.7a	13.9±0.7a
CV (%)	4.98	6.49	5.21	1.93	5.11	5.05

*Means followed by the same letter in column are not statistically different by Tukey test at 5% probability. Values represent Mean ± standard error.

Table 3. Controlled deterioration in periods of 48, 72 and 96 hours, with water content adjusted to 15, 20 and 25% and water content (%), obtained from five lots of pea seeds.

	----------15%----------			----------20%----------			----------25%----------		
Lots	48	72	96	48	72	96	48	72	96
				Controlled Deterioration					
1	57±19.6a	69±6.2a	48±3.9a	46±11.0ab	64±5.3ab	55±6.6a	44±10.0b	57±6.7b	51±8.1a
2	59±20.3a	53±4.7bc	50±4.0a	50±12.0ab	61±5.0ab	48±5.8a	33±7.5bc	45±5.3bc	48±7.6a
3	51±17.6a	52±4.6cd	49±3.9a	30±7.2b	51±4.2bc	48±5.8a	21±4.8bc	36±4.2c	20±3.2b
4	50±17.2a	60±5.4ab	51±4.1a	59±14.1a	69±5.7a	45±5.4a	67±15.2a	65±7.6a	50±7.9a
5	49±16.9a	42±3.7d	33±2.7b	32±7.6b	45±3.7c	22±2.6b	14±3.2c	21±2.5d	11±1.7b
CV (%)	34.42	8.92	8.06	23.90	8.21	11.98	22.68	11.72	15.89
				Water Content					
1	14.9±0.4b	15.1±0.7a	15.3±0.7a	19.4±0.6a	20.1±0.8a	19.6±0.7a	25.2±0.5a	25.2±0.4ab	25.1±0.4ab
2	14.7±0.3b	14.8±0.7a	14.7±0.7a	20.8±0.6a	20.3±0.8a	19.6±0.7a	25.1±0.5a	24.8±0.4a	25.5±0.4a
3	15.2±0.4a	15.5±0.7a	14.8±0.7a	19.9±0.6a	19.4±0.7a	20.4±0.7a	24.8±0.5a	25.3±0.4b	24.3±0.4b
4	15.7±0.4a	14.3±0.7a	15.1±0.7a	19.7±0.6a	19.3±0.7a	20.8±0.7a	25.7±0.5a	24.7±0.4a	24.7±0.4ab
5	15.4±0.4b	15.7±0.8a	15.3±0.7a	20.5±0.6a	20.6±0.8a	20.4±0.7a	25.1±0.5a	24.9±0.4ab	25.6±0.4a
CV (%)	2.36	4.78	4.80	2.93	3.86	3.54	2.04	1.68	1.65

*Means followed by the same letter in column are not statistically different by Tukey test at 5% probability. Values represent Mean ± standard error.

For the exposure periods of 48 and 72 hours, with water content of 25%, lots were classified identically. Torres et al. (2012), while studying coriander seeds set at 18%, 21% and 24%, observed that 21% water content showed higher efficiency to separate seed lots in different vigor levels. The results of controlled deterioration test corroborate data observed in the emergence test (Table 1) and with the aging test using the saturated solution (Table 2).

Data regarding the water content of pea seed lots after the exposure period to controlled deterioration test are showed in Table 3. Water content in seeds after the three deterioration periods remained practically unaltered when compared to the beginning of the test, with values near to the established. This fact is important for the reliability of test results, once it ensures a similar deterioration process between seed lots (Dutra & Medeiros Filho, 2008). According TeKrony (2003), one percent differential point in the humidity degree between seed lots may cause great impact on germination after controlled deterioration, especially for lots with intermediate and low vigor.

Independently of water content setup (15, 20, 25%) the period of 96 hours exposure promoted slight differentiation of the seed lots. Prolonged exposure periods associated to high temperature and relative humidity may probably have caused alterations which influenced

protein and nucleic acid synthesis and DNA metabolism (Vázquez et al., 1991). According Basajavarajappa et al. (1991), changes in the respiratory process and membrane functioning may also occur, due principally to peroxidation of lipids, interfering on germination.

There was significant correlation between the tests of controlled deterioration, seedling emergence in the field and electrical conductivity. The accelerated aging with saline solution tests for 96 hours and controlled deterioration (20%/72 hours, 25%/48 hours and 25%/72 hours) also correlate (Tables 4 and 5). Rossetto et al. (2004) evaluated the controlled deterioration test in peanut seeds with water content at 15% and 20% and observed the 15% setting showed high correlation with other seed vigor tests.

Table 4. Pearson's linear correlation coefficient between the results of controlled deterioration (CD) in relation to germination (G), first germination count (FGC), electrical conductivity (EC), emergency (E) and emergency speed index (ESI).

	G	FGC	EC	E	ESI
CD-15%/48h	-0.035$^{n.s}$	0.059$^{n.s}$	-0.499*	-0.362$^{n.s}$	0.134$^{n.s}$
CD-15%/72h	0.338$^{n.s}$	0.237$^{n.s}$	-0.320$^{n.s}$	0.272$^{n.s}$	0.256$^{n.s}$
CD-15%/96h	0.341$^{n.s}$	0.075$^{n.s}$	-0.412*	0.569**	-0.128$^{n.s}$
CD-20%/48h	0.265$^{n.s}$	0.011$^{n.s}$	-0.759**	0.160$^{n.s}$	0.161$^{n.s}$
CD-20%/72h	0.273$^{n.s}$	0.047$^{n.s}$	-0.444*	0.480*	0.174$^{n.s}$
CD-20%/96h	0.324$^{n.s}$	0.088$^{n.s}$	-0.180$^{n.s}$	0.463*	-0.134$^{n.s}$
CD-25%/48h	0.310$^{n.s}$	0.183$^{n.s}$	-0.680**	0.597**	0.453*
CD-25%/72h	0.345$^{n.s}$	0.165$^{n.s}$	-0.597**	0.656**	0.371$^{n.s}$
CD-25%/96h	0.385$^{n.s}$	-0.029$^{n.s}$	-0.593**	0.334$^{n.s}$	-0.101$^{n.s}$
G	-	0.689**	-0.214$^{n.s}$	0.485*	0.026$^{n.s}$
FGC	-	-	-0.131$^{n.s}$	0.289$^{n.s}$	0.458*
EC	-	-	-	0.499*	-0.214$^{n.s}$
E	-	-	-	-	0.287$^{n.s}$
ESI	-	-	-	-	-

n.s non-significant, ** significant at 1%, and * significant at 5% probability.

Table 5. Pearson's linear correlation coefficient between the results of controlled deterioration (CD), germination (G), first germination counting (FGC), electrical conductivity (EC), field emergence (E) and (ESI), in relation to the accelerated aging test (AA).

	AA/48h H_2O	AA/72h H_2O	AA/96h H_2O	AA/48h NaCl	AA/72h NaCl	AA/96h NaCl
CD/15%/48h	0.286$^{n.s}$	0.215$^{n.s}$	-0.062$^{n.s}$	0.650**	0.321$^{n.s}$	0.311$^{n.s}$
CD/15%/72h	0.555**	0.248$^{n.s}$	0.622**	0.343$^{n.s}$	0.350$^{n.s}$	0.457*
CD/15%/96h	0.065$^{n.s}$	0.559*	0.453*	0.122$^{n.s}$	0.318$^{n.s}$	0.383*
CD/20%/48h	0.599**	0.130$^{n.s}$	-0.161$^{n.s}$	0.592**	0.283$^{n.s}$	0.374$^{n.s}$
CD/20%/72h	0.613*	0.487*	0.460*	0.705**	0.647**	0.736**
CD/20%/96h	-0.128$^{n.s}$	0.121$^{n.s}$	0.505*	0.254$^{n.s}$	0.345$^{n.s}$	0.272$^{n.s}$
CD/25%/48h	0.656*	0.239$^{n.s}$	0.426*	0.576*	0.397*	0.574**
CD/25%/72h	0.588**	0.459*	0.556**	0.605**	0.485*	0.623**
CD/25%/96h	0.494*	0.217$^{n.s}$	0.439*	0.638*	0.430*	0.459*
G	-0.032$^{n.s}$	-0.149$^{n.s}$	0.505*	-0.073$^{n.s}$	0.039$^{n.s}$	0.041$^{n.s}$
FGC	0.078$^{n.s}$	-0.198$^{n.s}$	0.389*	-0.144$^{n.s}$	0.025$^{n.s}$	0.025$^{n.s}$
EC	-0.576 **	-0.062$^{n.s}$	-0.114$^{n.s}$	-0.596 **	-0.156$^{n.s}$	-0.376$^{n.s}$
E	0.211$^{n.s}$	0.086$^{n.s}$	0.549 *	0.033$^{n.s}$	0.184$^{n.s}$	0.416*
ESI	0.527*	-0.183$^{n.s}$	0.162$^{n.s}$	0.278$^{n.s}$	-0.091$^{n.s}$	0.099$^{n.s}$

n.s não-significativo, **significativo a 1%, e * significativo a 5% de probabilidade.

Various authors also attained good correlation values between laboratory tests and emergence in the field (Araujo et al., 2011; Torres et al., 2012; Torres et al. 2013). However, those results many times may be incompatible due to the seedling emergence tests not always being adequate to detect differences between the physiological potential of seed lots, and also, due to the environmental conditions during seeding (Marcos Filho, 2015). In face of the results observed, the controlled deterioration test associated to the emergence test may be used to evaluate pea seed vigor, nevertheless, the quality of seed lots may vary in the case of environmental conditions being different from the ones studied in the present research.

Conclusions

The accelerated aging test with saturated saline solution of NaCl for 96 hours is efficient to separate

pea seed lots. The controlled deterioration test, with combinations of 20% of water content for 72 hours of exposure and 25% water for 48 and 72 hours of exposure allows to separate pea seed lots.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.