Vigor of lentil seeds evaluated by the tests of accelerated aging and controlled deterioration

Vigor de sementes de lentilha avaliadas pelos testes de envelhecimento acelerado e deterioração controlada

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Luana de Souza Marinke
Londrina State University, Department of Agronomy, CEP: 86057-970, Londrina, PR, Brazil
E-mail: luuanasm@hotmail.com

Hugo Cesar Rodrigues Moreira Catão
Federal University of Uberlândia, Institute of Agrarian Sciences, CEP: 38410-337, Uberlândia, MG, Brazil
E-mail: hugo.catao@ufu.br

Gabriel Zanardo Martins
North Paraná State University, Department of Plant Sciences, CEP 86360-000, Bandeirantes, PR, Brazil
E-mail: gabrielzm97@gmail.com

Ítala Menegon Castilho
Integrated Colleges of Ourinhos, Department of Plant Sciences, BR 153, Km 338, CEP: 19909-100, Ourinhos, SP, Brazil
E-mail: italamenegon97@gmail.com

Franciele Caixeta
General Mills Brasil Alimentos Ltda., R. Benjamin Constant, 17, CEP: 86390-000, Cambará, PR, Brazil.

ABSTRACT

The objective of this work was to investigate the efficiency of the accelerated aging and controlled deterioration tests in the evaluation of lentil seeds’ vigor. Water content was determined and the physiological quality was evaluated using germination and vigor tests (first count, seedling emergence, indices of germination speed and emergence, accelerated aging test with traditional method and with NaCl saturated solution at 41°C and 45°C for 24, 48, 72 hours and controlled deterioration with 20% and 24% water content for 24, 48 and 72 hours at 45°C) in seven lots of lentil seeds cv Silvina. The use of traditional accelerated aging test and the NaCl saturated solution method at 41°C for 48 hours were efficient to classify lentil seeds’ lots. The combination of 20% and 24% water for 48 hours exposure and of 20% water for 72 hours exposure were efficient to classify the lentil seed lots by the controlled deterioration test.

Keywords: Lens culinaris, seed analysis, vigor tests.
RESUMO

O objetivo do trabalho foi analisar a eficiência dos testes de envelhecimento acelerado e deterioração controlada na avaliação do vigor de lotes de lentilha. Sete lotes de sementes da cultivar Silvina foram submetidos à determinação do teor de água e à avaliação da qualidade fisiológica, empregando os testes de germinação e vigor (primeira contagem, emergência de plântulas, índices de velocidade de germinação e emergência, envelhecimento acelerado pelos sistemas tradicional e com solução saturada de NaCl a 41ºC e 45ºC por 24, 48, 72 horas e deterioração controlada com teor inicial de água de 20% e 24% por 24, 48 e 72 horas a 45ºC). O teste de envelhecimento acelerado tradicional e com solução saturada de NaCl, a 41ºC por 48 horas é eficiente para classificar os lotes de sementes de lentilha. O teste de deterioração controlada, nas combinações de 20% e 24% de água por 48 horas de exposição e 20% de água por 72 horas de exposição permitem classificar os lotes de sementes de lentilha.

Palavras-chave: Lens culinaris, análise de sementes, testes de vigor.

1. INTRODUCTION

Lentil is one of the ancients’ leguminous vegetables cultivated by man. Although Brazil has favorable climatic conditions to grown this crop its production is relatively small, thus forcing importation in order to supply the internal market (Freitas & Nascimento, 2006). With the intention of increasing the probabilities of success cultivating this crop, the utilization of seeds possessing high physiological potential is essential when planning to establish plantations (Kavan et al., 2019), allowing uniformity of seedlings and higher yields (Catão et al., 2018).

Seed technology has intended to improve tests used to evaluate physiological potential (germination and vigor), aiming the results may express the real performance potential of seed lots (Rocha et al., 2018), minimizing the risk of using low quality seeds. Results provided by germination tests overestimate the real values for plant emergence in the field (Kavan et al., 2019), once these tests are performed under optimal conditions of water availability, aeration and temperature (Brasil, 2009). Thus, the results from germination tests are considered insufficient to evaluate the physiological potential of seeds, requiring also the results obtained through vigor tests (Rocha et al., 2018).

Amongst vigor tests, accelerated aging is one of the most used tests in Brazil and worldwide in quality control programs from seed producing companies (Torres et al., 2014). This test has as a principle the significant increase in seed deterioration rates due to the exposure to high levels of temperature and relative humidity, considered as the main environmental factors for deterioration intensity and velocity (Marcos Filho, 2015).

Among the available vigor tests, the accelerated aging test may be considered as one of the most sensitive for evaluation of seed vigor (Marcos Filho, 2015). However, due to the
high humid atmosphere during the execution of the test, different rates of water absorption have been observed among seeds. This may result in different deterioration degrees in seed samples tested under the same aging period (Powell, 1995). Due to this reason, the use of saline saturated solutions, substituting distilled water during the tests, allows to adjust the humid atmosphere, the seed’s water absorption rate, and the velocity and intensity of seed deterioration (Lima et al., 2015).

Differences in vigor of seed lots have also been detected through the controlled deterioration test, which has a principle equivalent to the accelerated aging test. This test is performed with equalized water content in all seed lots, using pre-determined and constant water contents (Powell, 1995), so that the same aging or deterioration degree is uniformly imposed to all seed lots. This allows comparing and classifying seed lots regarding their deterioration level in a more accurate manner.

While evaluating vigor, the accelerated aging test is more drastic than the controlled deterioration test, once in this last the water content of seeds is maintained constant, while in the accelerated aging test the water content increases during the exposure period. Therefore, the controlled deterioration tests has been used when evaluating vigor in cambre seeds (Leão-Araújo et al., 2017), *Crotalaria juncea* (Costa e Silva et al., 2015); okra (Torres et al., 2013); eggplant (Lopes et al., 2013) and melon (Medeiros et al., 2014). Hence, before these facts, the objective of the present work was to study the accelerated aging and controlled deterioration methods to evaluate the vigor of lentil seeds.

2. 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, using seven lots of lentil seeds cultivar Silvina. Seed lots were evaluated by means of germination test, seedling emergence, speed of emergency and emergence indices, traditional accelerated aging and accelerated aging using sodium chloride saturated solution; and controlled deterioration. The water content of seeds from each lot was also determined.

Samples from each lot were analyzed using the germination and vigor tests and had their water content and physiological quality determined. The water content was determined using an oven at 105±3°C for 24 hours, using two sub-samples with approximately 10g for each lot (Brasil, 2009).
In the germination test, four replicates with 50 seeds for each lot were used, uniformly distributed on two germitest paper sheets moistened 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 12 hours photoperiod. Counting was performed five days after seeding the paper sheets (Brasil, 2009). The first germination count was performed in conjunction with the germination test, determining the percentage of normal seedlings five days after the experiment started (Brasil, 2009). The results were expressed as percentage of germination in the first and the final count.

In the seedlings’ emergence test, four replicates of 50 seeds for each lot were also used. Seeds were distributed in polystyrene trays containing 200 cells filled with commercial substrate. Trays remained in a protected environment at mean temperature of 25°C. The number of emerged seedlings was evaluated ten days later and the results were expressed as emergence percentage.

Evaluations of speed of germination and emergence were performed simultaneously to the germination and emergence tests, calculating the number of normal seedlings every day in the same schedule. The indices were calculated as proposed by Maguire (1962).

In the accelerated aging test, 240 seeds from each lot were set in a single layer over an aluminum grid inside plastic boxes containing 40ml of distilled water or saturated NaCl (40g 100ml⁻¹) solution. These boxes were preserved in B.O.D chamber at 41 and 45°C, for 24, 48 and 72 hours. After the exposure period the water content was determined and the germination test was set up as previously described.

In the controlled deterioration test, initially, seed samples from each lot were subdivided in two sub-samples, aiming to adjust humidity to 20 and 24%, by means of the humid substrate method. After attaining the desired humidity 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 containers previously sealed. These containers were preserved at 45°C, in water bath, for 24, 48 and 72 hours. After the exposure period these sealed containers were submerged in water for 30 minutes and then the water content was determined (Brasil, 2009) and the germination test was set up, as previously described. 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. The F test and variance analysis at 5% probability were used in order to statistically analyze data, when significant effects occurred means were compared by the Tukey test at 5% probability.
3. RESULTS AND DISCUSSION

Values for water content and the results from the germination and vigor tests are showed in Table 1. Data regarding seeds’ water content were similar for all the studied lots. This fact is important for the test execution, once the uniformity of seeds’ initial water content contributes to obtain consistent results. Differences from 1 to 2% in water content between samples are not significant and the tests may be executed efficiently (Marcos Filho, 2015).

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

| Lots | WC  | FGC | G   | GSI  | E   | ESI |
|------|-----|-----|-----|------|-----|-----|
| 1    | 7.2 | 22b | 93a | 32.3ab | 78ab  | 69.7a |
| 2    | 6.9 | 35b | 94a | 32.8ab | 76b  | 68.8a |
| 3    | 7.1 | 18c | 89a | 31.6b | 70c  | 55.9b |
| 4    | 7.2 | 52a | 96a | 33.1a | 80ab | 70.1a |
| 5    | 7.8 | 45a | 90a | 33.3a | 88a  | 70.7a |
| 6    | 7.6 | 47a | 91a | 33.1a | 89a  | 70.9a |
| CV(%)| 13.2| 4.9 | 2.0 | 10.4 | 2.0 |

* Means followed by the same letter in column are not statistically different by Tukey test at 5% probability.

There were no significant differences observed in viability between the six lots of lentil seeds by the germination test. Although high germination values were observed in seed lots, it does not necessarily means those seed lots have high vigor. One must consider germination tests are performed under favorable temperature and humidity conditions (Marcos Filho, 2015). According Castilho et al. (2019), lots with similar germination are fundamental in studies aiming to determine better methods to evaluate the vigor of seeds, once the objective is to separate seed lots with similar germination. If the germination potential of seeds displays accentuated differences, the germination test by itself detects differences in the physiological potential of seeds (Marcos Filho, 2015).

Through the first count of germination it was possible to classify lots 4, 5 and 6 as of high vigor and lot 3 as of inferior vigor. The first count germination test is a vigor predictor. The emergence test showed lots 5 and 6 as possessing high vigor, while lot 3 had the lowest vigor. The speed of germination and emergence indices also showed the same classification. These results are in agreement with those obtained in the first count of germination. According Marcos Filho (2015), the emergence of seedlings is an indicator of test efficiency to evaluate the physiological potential in seed lots.
In the initial characterization of lentil seed lots only the germination test was unable to separate seed lots in levels of physiological quality, thus, vigor tests are fundamental to expose differences between seed lots (Marcos Filho, 2015). Therefore, is of a fundamental importance that tests to evaluate the physiological quality are efficient to track the evolution process of seed deterioration, especially when these seeds are submitted to adverse conditions.

The traditional accelerated aging test allowed to verify the period of 48 hours at 41°C was efficient to separate lots by its physiological potential, once it was possible to observe that lots 5 and 6 were classified as of high vigor, lots 1, 2 and 4 as intermediary and lot 3 as of inferior quality (Table 2). Classification of seed lots according to the accelerated aging test was also achieved in carrot, pea, beans and soybean (ISTA 2014).

Table 2. Accelerated aging traditional (AAT) and accelerated aging with saturated NaCl solution (AASS) of lentil seed lots for periods of 48, 72 and 96 hours, at 41 and 45°C.

| Lots | 41°C   | AAT | AASS | 45°C   | AAT | AASS |
|------|--------|-----|------|--------|-----|------|
|      | 24 hours | 48 hours | 72 hours | 24 hours | 48 hours | 72 hours |
| 1    | 80ab    | 85ab | 74a  | 78b    | 77b | 74a  |
| 2    | 84ab    | 82ab | 71a  | 83ab   | 87ab | 78a  |
| 3    | 78b     | 70c  | 78a  | 75b    | 70c | 66b  |
| 4    | 84ab    | 80b  | 77a  | 81ab   | 80ab | 77a  |
| 5    | 93a     | 92a  | 78a  | 93a    | 94a | 84a  |
| 6    | 95a     | 92a  | 73a  | 87a    | 90a | 80a  |
| CV(%)| 8.0     | 5.2  | 7.6  | 10.0   | 6.8 | 12.7 |

* Means followed by the same letter in column are not statistically different by Tukey test at 5% probability.
Aging test with NaCl saturated solution also showed the period of 48 hours at 41°C being the most efficient to separate seed lost in different vigor levels, showing the same classification obtained with the traditional method (Table 2). These results support data verified with the emergence test classifying the vigor of seed lots (Table 1). Although all other procedures used showed significant results, these were not sufficiently efficient to stratify lentil seed lots in levels of different physiological potential.

Independently of the exposure period, both the traditional and the NaCl saturated solution aging methods at 45°C temperature were not able to differentiate vigor levels in the seed lots studied. There was a higher reduction in germination observed for the 72 hours period (Table 2). Rocha et al. (2018) verified the increment of temperature promotes more drastic effects in germination than the extension of the exposure period to the accelerated aging test, which is confirmed in our study at a temperature of 45°C.

Seed deterioration increased with the exposure period to the aging test, once within each temperature a reduction in germination was verified, as observed also by Kavan et al. (2019). The deterioration process is determined mainly by the interaction between genetic heritage, seed water content and temperature.

Water content values obtained after performing the accelerated aging treatments are exhibited in Table 3. It was verified that in the traditional accelerated aging method (100% relative humidity), the variation of water content in the exposure periods remained among 3.0% to 4.3%.

Table 3. Water content of lentil seed lots before and after accelerated aging traditional (AAT) and accelerated aging with saturated NaCl solution (AASS) for periods of 48, 72 and 96 hours at 41 and 45°C.

| Lots | Water content | Water content after accelerated aging at 41°C | Water content after accelerated aging at 45°C |
|------|---------------|---------------------------------------------|---------------------------------------------|
|      |               | 24 hours | 48 hours | 72 hours | 24 hours | 48 hours | 72 hours |
|      |               | AAT | AASS | AAT | AASS | AAT | AASS | AAT | AASS |
| ---  | --------------|-----|------|-----|------|-----|------|-----|------|
| 1    | 7.2           | 24.4| 8.8  | 26.6| 9.1  | 28.2| 9.1  |
| 2    | 6.9           | 26.4| 8.5  | 22.6| 9.0  | 25.9| 10.1 |
| 3    | 7.1           | 23.8| 8.9  | 22.9| 8.8  | 26.5| 9.4  |
| 4    | 7.2           | 25.6| 9.6  | 23.7| 9.8  | 28.2| 9.1  |
| 5    | 7.8           | 27.8| 9.8  | 26.2| 10.3 | 24.9| 9.7  |
| 6    | 7.6           | 25.0| 9.8  | 26.9| 9.8  | 23.9| 9.4  |
Water content at the end of the accelerated aging test is one of the indicators of performance uniformity in this test. Marcos Filho (2015) verified that variations from 4% to 5% in water content between samples are considered tolerable. Lower and more uniform values for water content were observed using the saturated saline solution, thus promoting less drastic effects in seed aging. Therefore, the deterioration degree of seeds is attenuated when compared to the ordinarily observed when using the traditional method. Similar results were obtained in studies with coriander (*Coriandrum sativum*) seeds (Radke et al.; 2016) and rice seeds (Monteiro et al., 2017).

According to the results obtained in the controlled deterioration test (Table 4) it was verified the period of 24 hour in water bath was not enough to separate lots in a consistent manner.

| Lots | 24 hours 20% | 24 hours 24% | 48 hours 20% | 48 hours 24% | 72 hours 20% | 72 hours 24% |
|------|--------------|--------------|--------------|--------------|--------------|--------------|
| 1    | 99a          | 97a          | 88b          | 70bc         | 36c          | 20b          |
| 2    | 99a          | 99a          | 90ab         | 74b          | 45bc         | 32a          |
| 3    | 99a          | 98a          | 74c          | 66c          | 21d          | 11b          |
| 4    | 96a          | 96a          | 95a          | 78ab         | 57b          | 50a          |
| 5    | 99a          | 99a          | 98a          | 89a          | 62a          | 51a          |
| 6    | 97a          | 98a          | 95a          | 87a          | 65a          | 48a          |
| CV (%) | 4.82     | 8.92         | 10.06        | 7.2          | 11.72        | 15.82        |

* Means followed by the same letter in column are not statistically different by Tukey test at 5% probability.

On the other hand, using a period of 48 hours it was possible to detect differences in vigor, both for the water content set at 20% as well as at 24%. The 72 hours exposure period with seed water content adjusted at 20% was also efficient to stratify seed lots. In a general
manner seed lots 5 and 6 were the most vigorous, while lot 3 was the less vigorous. Costa e Silva et al. (2012) while studying *Crotalaria juncea* seeds set at 18%, 21% and 24%, observed that water content of 24% was the most efficient to separate seed lots in different vigor levels. Results from the controlled deterioration test support data observed in the emergence test (Table 1) and the traditional as well as the saturated solution aging tests (Table 2).

Data regarding the water content of lentil seed lots after the exposure period to the controlled deterioration test are showed in Table 5.

Table 5. Water content of lentil seeds lots adjusted for 20 and 24%, before and after deterioration controlled by 24, 48 and 72 hours.

| Lots | Water content | 24 hours 20% | 24 hours 24% | 48 hours 20% | 48 hours 24% | 72 hours 20% | 72 hours 24% |
|------|---------------|--------------|--------------|--------------|--------------|--------------|--------------|
|      |               | 24 hours     | 48 hours     | 72 hours     |              |              |              |
| 1    | 6.8           | 20.8         | 23.6         | 19.7         | 23.7         | 20.1         | 24.2         |
| 2    | 6.7           | 20.7         | 24.5         | 19.9         | 23.7         | 20.3         | 24.6         |
| 3    | 6.5           | 20.0         | 23.9         | 20.0         | 23.9         | 19.4         | 24.3         |
| 4    | 6.9           | 20.8         | 24.0         | 19.5         | 24.3         | 19.3         | 24.7         |
| 5    | 7.1           | 20.6         | 23.6         | 19.7         | 24.5         | 20.6         | 24.1         |
| 6    | 7.0           | 19.8         | 23.8         | 19.9         | 23.9         | 20.4         | 23.8         |

Seeds’ water content after the three deterioration periods remained practically unaltered when compared to the beginning of tests, with values near to the established (20% and 24%). This fact is important for the reliability of the test results, once it guarantees a similar deterioration process between the seed lots (Torres et al., 2013). According TeKrony (2003), a difference of one percent in the humidity degree between seed lots may cause a great impact on germination after controlled deterioration, mainly for lots with intermediate and low vigor.

The 73 hours period set at 24% water propitiated slight differentiation of seed lots (Table 4). This is probably due to the fact that a long exposure period, associated to high temperature and relative humidity, may have caused cellular changes that influenced protein and nucleic acids synthesis, as well as affected DNA metabolism (Vásquez et al., 1991). According Basajavarajappa et al. (1991) modifications in the respiratory process and the function of membranes may also occur, caused mainly due to lipid peroxidation, interfering in germination.
The initial proposal for the controlled deterioration test suggests the water content adjust to be such that will allow to separate seed lots in a 24 hours exposure period. However, Torres et al. (2013) evaluated the use of water bath for 24 and 48 hours at 45ºC in okra seeds with water content adjusted at 20 and 24%, verifying higher efficiency to separate seed lots when using the combination of 24% water content for 48 hours in water bath.

An important point to be stressed while performing the controlled deterioration test is the precision required in the adjustment of the seeds’ water content (Powell, 1995). This stage is laborious and demands time to attain such precision. Lopes et al. (2013), while evaluating the physiological potential of melon seeds, commented the achievement of the controlled deterioration test was very laborious when compared to the accelerated aging test and considered that as a disadvantage. Powell (1995) also stated that controlled deterioration test requires a higher technical training than the accelerated aging test. In face of the results obtained, controlled deterioration and accelerated aging tests, with or without the use of saturated saline solution, provided consistent results and allowed the separation of lentil seed lots in different vigor levels, however, it is important to stress that the quality of lots may vary if the experimental conditions are different than in the present work.

4. CONCLUSIONS

Both the traditional accelerated aging test and the saturated NaCl solution test, at 41ºC for 48 hours are efficient to classify lentil seed lots.

The controlled deterioration test, at combinations of 20% and 24% water for 48 hours exposure and 20% water for 72 hours exposure, allowed the classification of lentil seed lots.

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