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Accelerated aging test in amaranth (*Amaranthus cruentus* L.) seeds

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Abstract

The use of vigor tests for assessing seed lot quality is an important tool for production and commercialization. However, research on evaluating the quality of amaranth seeds is still scarce. The objective of this work was to evaluate procedures for conducting the accelerated aging test, aiming at the identification of different levels of vigour among lots of amaranth (*Amaranthus cruentus* L.) seeds cultivar BRS Alegria. Five seed lots were used and submitted to tests of germination, first count of germination, seedling emergence and accelerated aging, using the aging periods of 24, 48 and 72 hours and adopting the traditional methodology with water and the use of saturated (40g NaCl in 100mL of water) and unsaturated (11g NaCl in 100mL of water) sodium chloride (NaCl) solution. The accelerated aging test at 41ºC and periods of 24, 48 and 72 hours of conditioning saturated NaCl solution allowed the stratification of the lots at vigour levels, similar to the results obtained by the seedling emergence test and the first count of germination. The accelerated aging test, using saturated saline solution (40g NaCl in 100mL of water) for 24 hours at 41°C, is therefore suitable for evaluating the physiological potential of amaranth seeds.

Keywords: *Amaranthus cruentus* L., pseudocereal, physiological potential, saline solution, vigour.

Introduction

Amaranth (*Amaranthus cruentus* L.) is a pseudocereal grown mainly in the south of America. Its seeds are used in human food due to the high content of proteins and carbohydrates and are therefore an important source for the production of flour and starch (Tapia-Blacido et al., 2010). The oil content of the seeds varies between 5.6 and 10.6% (Marcílio et al., 2005).

The quality of the seeds is directly related to their physiological potential, represented by germination and vigour, which express their ability to originate normal seedlings (Pereira et al., 2011). The germination test is used to determine the germination potential of a seed lot; however, this test alone may not efficiently determine seed quality. Therefore, vigour tests provide complementary information which allows the detection of physiological differences between lots with the same germination percentage (Radke et al., 2016).

Among the tests used, the accelerated aging test is one of the most sensitive test for the evaluation of seed vigour (Marcos Filho, 1999), since its results are related to the conservation potential of the seeds. One aspect of great relevance in the accelerated aging test is the seed size, being one of the quality components that most affects crop performance (Adebisi et al., 2013). Effects of seed size have been observed on seed germination, seedling emergence and related agronomic aspects in many crop species, including *Amaranthus cruentus* L. (Kaydan and Yagmur, 2008).

Research conducted on small seeds has shown poor results due to the marked variation of the moisture content of the samples after aging (Powell, 1995). In this sense, studies replacing water with saturated solutions of salts have shown greater efficiency in the detection of differences in the physiological quality between lots in relation to the accelerated aging test without the use of salt, agreeing with the results of Martins et al. (2006) on tomato (*Lycopersicon lycopersicum*), Torres and Marcos Filho (2005) on melon (*Cucumis melo*), Pereira et al. (2011), Tunes et al. (2011) and Radke et al. (2016) on coriander (*Coriandrum sativum*), Santos et al. (2011) on lettuce (*Lactuca sativa*) and chicory (*Cichorium intybus*), Kikuti and Marcos Filho (2012) on lettuce and Alves and Sá (2012) on arugula (*Eruca sativa*).

According to the solution used, specific relative humidity values are obtained, providing decreased intensity and rate of water uptake by the seeds, culminating in lower deterioration intensity and less variation between the results (Jianhua & Mcdonald, 1997; Tunes et al., 2012). The use of saturated or unsaturated saline solution limits the absorption of water by the seeds and the development of fungi, a fact that can be attributed to the formation of atmosphere superimposed on the saline solution, unpropitious for fungal growth (Ávila et al., 2006).

In this sense, the objective of this work was to evaluate procedures for conducting the accelerated aging test, aiming at the identification of different levels of vigour among lots of amaranth seeds.

Results and Discussion

Evaluation of the physiological potential of seeds

For the germination test (Table 1), there was no difference between the analyzed lots, whose germination was maintained between 89 and 91%, as it is important and coherent to compare the vigor of seed lots with similar germination (Marcos Filho, 1999).

The first count of germination showed to be more sensitive than the germination test, evidencing differences between the five lots of amaranth seeds (Table 1), with lots III and IV considered to be of superior vigor, lots I and II of intermediate vigor and lot V of lower vigor. The first count of germination to detect differences presented higher sensitivity of between seed lots was also confirmed by Bhering et al. (2006) and Torres et al. (2012), when evaluating different seed lots of cucumber (*Cucumis sativus*) and coriander (*Coriandrum sativum*), respectively. The first count of the germination test considered an indication of vigor, which, several times, better expresses germination rate differences between seed lots (Nakagawa, 1999). The seedling emergence test in the field composes a parameter that indicates the effectiveness of the tests in evaluating the physiological potential of seed lots (Marcos Filho, 1999).

In the seedling emergence test (Table 1), there were differences between the seed lots, and the results were similar to those indicated by the first count of germination. The seedling emergence and first count of germination tests allowed the classification of lots as regards the physiological potential, detecting differences not evidenced by the germination test.

Aging test

The initial seed water contents were similar for the five lots (Figure 1). This is desirable from the point of view of conducting the accelerated aging test. The uniformity of the water content of seeds is fundamental for the standardization of procedures and for obtaining consistent results (Marcos Filho, 2005). This relates to the fact that, within certain limits, wetter seeds affected more by the conditions of accelerated aging (Tunes et al., 2012).

The seeds submitted to the accelerated aging test (Figure 1) with the traditional procedure (water) reached a higher water content than those submitted to the modified procedures, unsaturated solution and saturated solution, this tendency observed in seeds of coriander (Radke et al., 2016). Studies with brassica seeds show that seeds tend to reach hygroscopic equilibrium at higher water contents, as the relative humidity of the air increases (Costa et al., 2008).
The water content corresponds to the equilibrium point, which increases with the increase of the relative humidity of the air and vice versa (Marcos Filho, 2005). Thus, seeds in contact with air with relative humidity of 100% will have a higher water content than seeds in contact with air presenting 94 and 76% relative humidity, respectively.
The results showed that the use of unsaturated and saturated solution of NaCl provided a lower absorption of water by the seeds. Similar results founded in radish seeds (Ávila et al., 2006); in kale, broccoli and cabbage seeds (Costa et al., 2008); in urucum seeds (Torres & Bezerra, 2009) and in parsley seeds (Tunes et al., 2013).

The use of NaCl solutions has the advantage of providing lower variation of water content between seeds and inhibition of fungal growth and development (Rodo et al., 2000). Facilitate the handling of seeds and eliminating this possible interference in the evaluation of the physiological potential of seeds during the tests, which was observed in previous works with seeds of other species, such as radish (Ávila et al., 2006) and brassica (Costa et al., 2008).

Data of the accelerated aging test, at 41 ºC and periods of 24, 48 and 72 hours of conditioning (Table 3), both using the traditional procedure (water) and using saturated and unsaturated NaCl solution, allowed the stratification of the lots at levels vigor, similarly to the results obtained in the seedling emergence and first count of germination (Table 3). Lots III and IV were indicated as being of greater vigor, lots I and II of intermediate vigor and lot V of lower vigor.

The accelerated aging test with the traditional methodology provided, in general, the stratification of the lots according to the levels of vigor determined by the other tests, and the germination percentage decreased as the seed exposure period increased. Similar results could observed in seeds of lettuce (Barbosa et al., 2011) and parsley (Tunes et al., 2013). Studies have shown that this effect is probably due to the high water content reached by the seeds after the aging period (Tunes et al., 2011).

In relation to the aging periods of 24 and 48 hours, using saturated or unsaturated saline solution, it was possible to rank the amaranth seed lots at three vigor levels, without causing drastic reductions in the percentage of normal seedlings at the end of the test.

Seed exposure to accelerated aging with saturated NaCl solution for 24 and 48 hours was efficient, with the first treatment being selected because, in the separation of amaranth seed lots at vigor levels, the shortest execution period is a characteristic desirable in this vigor test, providing results in the shortest possible time interval (Radke et al., 2016). A higher efficiency of the accelerated aging test with the use of saturated NaCl solution in the classification of seeds with different levels of vigor was also observed in seeds of bell pepper (Torres & Minami, 2000), carrot (Rodo et al., 2000), melon (Torres & Marcos Filho, 2003), chilli pepper (Torres, 2005) and lentil (Freitas & Nascimento, 2006).

Materials and Methods

Experimental site and Climatic conditions seeds cultivars, age of seeds and storage condition

The amaranth seeds were obtained in experiments developed in the Didactic and Experimental Field of the Phytotechnology Department of the Eliseu Maciel Agronomy School of the Federal University of Pelotas (FAEM/UFPel), in

Table 1. Germination, first count of germination and seedling emergence test of amaranth, cv. BRS Alegria. UFPel/RS, 2016/17.

Lot	Germination (%)		First count of (%)		Seedlingemergence test (%)	
I	91	a [1/]	58	b	64	b
II	89	a	58	b	62	b
III	90	a	64	a	68	a
IV	90	a	62	a	66	a
V	89	a	42	c	59	c
C.V. (%)	7,5		6,0		4,9	

[1/] Means followed by the same letter in the column do not differ by Tukey test (p≤0.05). C.V.: coefficient of variation.

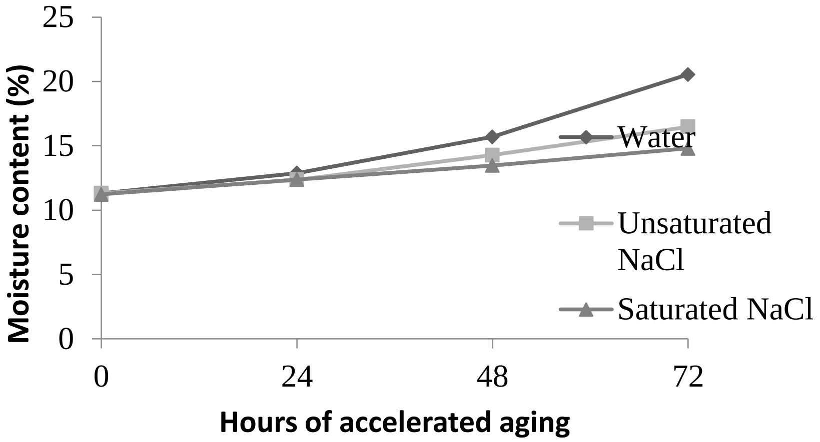

Fig 1. Average moisture content (%) of five lots of amaranth seeds, cv. BRS Alegria, after 0, 24, 48 and 72 hours of accelerated aging using different solutions. UFPel/RS, 2016/17.

Table 2. Accelerated aging (% of normal seedlings), at 24, 48 and 72 hours, of amaranth seeds, cv. BRS Alegria, as a function of different lots and solutions. UFPel/RS, 2016/17.

Lots	Type of solution					
	Water		Unsaturated NaCl		Saturated NaCl	
	Accelerated aging (% of normal seedlings)					
	24 hours					
I	34	b [1/]	48	b	60	b
II	33	b	46	b	62	b
III	41	a	52	a	68	a
IV	40	a	50	a	68	a
V	17	c	22	c	32	c
C.V. (%)	9,2					
	48 hours					
I	26	b	24	b	32	a
II	24	b	22	b	32	a
III	30	a	28	a	26	b
IV	29	a	26	a	30	b
V	11	c	08	c	14	c
C.V. (%)	9,9					
	72 hours					
I	10	b	18	b	24	a
II	12	a	20	b	22	a
III	16	a	26	a	20	b
IV	14	a	24	a	21	b
V	04	c	06	c	10	c
C.V. (%)	10,3					

[1/] Means followed by the same letter in the column do not differ by Tukey test (p≤0.05). C.V.: coefficient of variation.

the municipality of Capão do Leão (31°52'00'' S and 52°21'24'' W, altitude of 30 meters), in Rio Grande do Sul (RS), Brazil, in the 2012, 2013 and 2015 harvests. The region's climate, according to the classification of Köppen & Geiger (1928), is humid subtropical (Cfa). The seeds used were from the cultivar BRS Alegria, from Embrapa Products and Market. The seeds were harvested and stored in a cool chamber (Temperature 4^0C) at 35% relative humidity.

Conduction of experiment and experimental design

Prior to the experiment, the seeds were stored in the Laboratory of Seed Analysis of UFPel, Capão do Leão Campus - RS. The experimental was conducted in a factorial scheme (lot x types of solution) in a completely randomized design with four replicates. Different lots of amaranth seeds (I, II, III, IV and V) were used with similar germination and different levels of vigor determined previously with the seedling emergency test, a fundamental condition for conducting the experiment, since the accelerated aging test aims to classify vigor levels similar to the field emergency test. Three types of solutions (water, unsaturated and saturated) were evaluated for conducting the accelerated aging test. Regarding the types of solutions employed, water corresponded to the absence of NaCl; unsaturated solution corresponded to 11 g NaCl 100 mL^{-1} distilled water and saturated solution corresponded to 40 g NaCl 100 mL^{-1} distilled water (Jianhua & McDonald, 1997).

Traits measured

The seeds were submitted to the tests of germination, first count of germination, seedling emergence, accelerated aging and determination of the moisture content. The germination test was performed with four replicates of 50 seeds, using two sheets of blotting paper, moistened with distilled water in the ratio of 2.5 times the mass of dry paper. The seeds were exposed at alternating temperatures (20-30°C) and the evaluations were performed at 5 and 14 days after sowing, with the results being expressed as percentage of normal seedlings (Brasil, 2009).

The first count of germination was conducted along with the germination test, with the first count being performed five days after the test, according to the Rules for Seed Analysis (Brasil, 2009), with the results being expressed as percentage of normal seedlings. For seedling emergence, four replicates of 100 seeds per lot were used. Sowing was performed in beds with a spacing of 1.0 x 0.05 m and a depth of 0.02 m. The final count was performed at 21 days after sowing, computing the percentage of emerged seedlings (Nakagawa, 1994).

The moisture content was determined before and after the accelerated aging test. The greenhouse method (Brasil, 2009) was employed, using two replicates of approximately three grams of seeds, at 105 ± 3ºC for 24 hours, with the results being expressed as percentage (wet basis).

For the accelerated aging test, gerbox plastic boxes were used as an individual compartment (mini chamber), containing a tray with an aluminium screen in which the seeds, after weighing (0.5 mg), were distributed, forming a uniform layer. To each individual compartment, 40 mL of distilled water were added and the boxes were maintained in a BOD chamber at 41ºC for 24, 48 and 72 hours. Subsequently, the seeds were submitted to the germination test, being evaluated after seven days, and the results were expressed as percentage of normal seedlings.

Statistical analysis

The data obtained were analysed for normality by the Shapiro Wilk test, for homoscedasticity by the Hartley test and for independence of waste by graphic analysis. Subsequently, they were submitted to analysis of variance through the F test ($p \leq 0.05$). When statistically significant, the effects of lots and types of solutions were compared by Tukey´s test ($p \leq 0.05$).

Conclusion

The accelerated aging test, using saturated saline solution in the 24 hour combination at 41ºC, is adequate to evaluate the physiological potential of amaranth seeds. The 48-hour period, either for the accelerated aging method using saturated saline solution or unsaturated saline, is also effective in evaluating the expression of vigor in amaranth seeds. The traditional method is not suitable due to the provision of variation of the water content between the samples, exceeding what is tolerable.

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