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RESEARCH NOTE

Physiological quality of popcorn seeds assessed by the accelerated aging test[1]

Cristiane da Silva Rocha[2], Hugo César Rodrigues Moreira Catão[2*], Franciele Caixeta[3], Heloisa Karoline Kavan[2], Talis Melo Claudino[4], Diego Gonçalves Caixeta[5]

ABSTRACT - Despite the insufficient internal supply of popcorn kernel cultivars, the Brazilian production is continuously increasing. Therefore, searching for tests that offer fast results and reliable information on the physiological potential of the seeds is very important. The objective of this study was to assess the efficiency of the accelerated aging test, by using different times and exposure temperatures, for the evaluation of the physiological quality of popcorn seed lots. Popcorn seeds from three lots of the hybrid AP 8203 were used. For the initial characterization of the lots, the following tests were conducted: seed moisture content, first and final germination count, and field emergence. The accelerated aging test was performed in a 3x4x2 factorial scheme. After aging, the seeds were tested for moisture content, germination, and electrical conductivity. The experiment was carried out in a completely randomized design. The characteristic vigor was affected during aging, as evidenced by the increase in the amount of leachate detected by the electrical conductivity test. Seed lot 3 was most vigorous in the aging test conducted for 48 h at 45 °C. The accelerated aging test at 42 ºC for 48 h provides consistent information to differentiate seed lots of popcorn.

Index terms: vigor, germination, temperature.

Qualidade fisiológica de sementes de milho pipoca avaliadas pelo teste de envelhecimento acelerado

RESUMO - A oferta nacional de cultivares de milho pipoca é pequena, contudo a produção é crescente. Assim, a busca por testes que ofereçam rapidez e informações seguras sobre o potencial fisiológico das sementes torna-se de fundamental importância. Objetivou-se avaliar a eficiência do teste de envelhecimento acelerado na avaliação da qualidade fisiológica de lotes de sementes de milho pipoca, testando-se diferentes períodos e temperaturas de exposição. Foram utilizadas sementes de milho pipoca do híbrido AP 8203 provenientes de três lotes. Para a caracterização inicial dos lotes, determinou-se o teor de água, a primeira e a contagem final do teste de germinação e a emergência em canteiro. O teste de envelhecimento acelerado foi realizado em esquema fatorial 3x4x2. Após o envelhecimento, determinou-se o teor de água, e as sementes foram submetidas aos testes de germinação e condutividade elétrica. O delineamento experimental utilizado foi o inteiramente casualizado. O vigor foi afetado durante o envelhecimento devido ao aumento da quantidade de lixiviados detectado no teste de condutividade elétrica. O lote de sementes 3 foi o mais vigoroso pelo teste de envelhecimento conduzido por 48 h na temperatura de 45 ºC. O teste de envelhecimento acelerado utilizando a temperatura de 42 ºC, durante 48 h, fornece informações consistentes que permitem diferenciar lotes de sementes de milho pipoca.

Termos para indexação: vigor, germinação, temperatura.

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[2]Departamento de Fitotecnia, Faculdades Gammon Paraguaçu Paulista, 19700-000 – Paraguaçu Paulista, SP, Brasil.
[3]Generall Mills Alimentos Ltda, 86390-000 – Cambará, PR, Brasil.
[4] Faculdades Integradas de Ourinhos, 19909-100 – Ourinhos, SP, Brasil.
[5]Universidade Federal de Viçosa, 36570-900 – Viçosa, MG, Brasil.
*Corresponding author <hugocatao@yahoo.com.br>

Introduction

The supply of national popcorn cultivars is insufficient, mostly due to the inexistence of hybrids and varieties adapted to the soil and environment existent in Brazil, which augments the importation of seeds (Catão and Caixeta, 2017). However, popcorn kernel production is a growing business and an advantageous income source due to the value added to the product. This expansion is a consequence, at least partially, of the selection, development, and adaptation of foreign cultivars to the climate conditions of the country (Catão and Caixeta, 2017).

The use of seeds with certified quality is a preponderant factor for establishing crops, as they result in larger volumes of production (Catão et al., 2013), more uniformity, and higher vigor of seedling and plant populations (Bittencourt et al., 2012). In addition to that, they do not transmit pathogens to the planting areas (Dias et al., 2010). Thus, seeds are a crucial factor for the success or failure of production, once it holds all the productive potentialities of the plant.

According to Bittencourt et al. (2012), it is vital to assess the physiological potential of the seeds through tests that provide reliable information so that decisions can be taken during their production and commercialization. To consider only the results from the germination tests is not enough to evaluate the physiological potential of the seeds in the field (Ohlson et al., 2010), once it is performed in optimum conditions of water availability, aeration, and temperature (Brasil, 2009). On that account, vigor tests are used along with the germination one, to help in the decision-making process and to make the quality control faster and more efficient (Bittencourt et al., 2012).

The accelerated aging test gives excellent information on the physiological quality of seeds. In this evaluation, they are submitted to conditions of high temperature and relative humidity to estimate the relative storage potential of the seed lots (Delouche and Baskin, 1973; Santos et al., 2002). Several species can have their vigor appraised through accelerated aging, and many producer companies have included the test in their quality control programs, once it provides, within a few days, rather trustful data on the storage potential of the processed lots. Depending on the history of the lot, it is also possible to obtain information on the potential of seedling emergence in the field (Santos et al., 2002).

The induced aging process of corn seeds is carried out at 42 ºC for 96 h of exposure (Marcos-Filho, 1999). Nevertheless, some scientific reports encourage more studies on the accelerated aging of corn and wheat seeds, in order to provide better understanding and more accurate data, regarding the combinations of temperature and exposure time (Marcos-Filho, 1999; Santos et al., 2002).

For popcorn seeds, few references in scientific literature mention the use of the accelerated aging test to assess the physiological condition of the lots. In the face of that, the present work aimed at evaluating the physiological quality of popcorn seed lots through the accelerated aging test, by using different temperatures and exposure times.

Material and Methods

The research was carried out in the Laboratory of Seed Analysis of the Department of Plant Sciences at *Faculdades Gammon*, in the city of *Paraguaçu Paulista*, state of *São Paulo* (SP). Three lots of popcorn seeds of the hybrid AP 8203 were used. AG Alumni Seeds produced them in Indiana, USA, 2016 harvest, and they were donated by the company General Mills Brazil, based in *Cambará*, state of *Paraná* (PR).

The initial characterization of the lots comprised the following tests:

Moisture content: it was determined through the oven method at 105±3 °C, and using two subsamples with 25 g of seeds each, as stated in Brasil (2009).

Germination test: it was conducted in four replications of 50 seeds, which were sowed in a germitest paper that had been previously moistened with distilled water, in the proportion 2.5 mL. g^{-1} of paper. The seeds were placed inside germinators at the constant temperature of 25 ºC. Two counts of the normal seedlings were performed: on the fourth and seventh days after the test setting (Brasil, 2009). The results were expressed as the percentage of germination of each count.

Emergence test: the seeds were sowed in polyethylene trays containing sand as substrate, which had been moistened up to approximately 60% of its water retention capacity. The trays were kept under environment conditions (room temperature and natural light). Four subsamples of 50 seeds from each lot were used. After the stabilization of the stand, the emerged seedlings were counted, and the results expressed as percentage.

All tests previously described were executed in a completely randomized design.

The accelerated aging test was also performed following a completely randomized design, in a factorial scheme 3x4x2 (three lots: L1, L2, and L3; four aging times: zero, 48, 72, and 96 h; and two temperatures: 42 ºC and 45 ºC). The seeds from each lot were spread on aluminum screens fixed inside plastic gerboxes. After the accelerated aging, the seeds were submitted to the moisture content, germination, and electrical conductivity tests. The moisture content and germination were determined as previously described, however, in the latter, the normal seedlings were assessed on the fourth day after setting the test.

The electrical conductivity test was performed in four replications of 50 seeds. First, they were exposed to the temperatures (41 and 45 ºC) for each aging time (0, 24, 48, 72, and 96 h). Next, they had their weigh measured and were put inside disposable plastic cups (200 mL capacity) containing 75 mL of deionized water, at 25 ºC for 24 h. After this period of imbibition, a conductivity meter (Tecnal Tec-4MP), with constant electrode 1, was used to do the readings. The data were expressed in $\mu S.cm^{-1}.\ g^{-1}$.

The statistical study of the data was performed by applying the F test and the analysis of variance, at a 5% significance level. When significant effects were observed, the qualitative means were compared through the Scott-Knott's test, at a 5% probability level, with the software SISVAR 5.0 (Ferreira, 2011). The quantitative means were submitted to polynomial regression ($p < 0.05$) e plotted on graphs.

Results and Discussion

Table 1 shows the outcome of the germination and emergence tests, according to which no significant differences were noticed among the lots. The emergence test is considered the best indicator to infer about the vigor of seeds because its execution simulates conditions that seeds might be exposed to after being sown in the field (Guedes et al., 2011). One of the aims of the vigor test is to uncover physiological quality differences that are not detected by the germination test (Marcos-Filho, 2005).

The initial moisture content of the seeds was 12.9%, 12.2%, and 12.7%, in lots 1, 2, and 3, respectively (Table 1). This range is adequate, once variations of 1% to 2% in moisture content among samples do not compromise the execution of the tests (Guedes et al., 2011). The moisture content reached after the accelerated aging treatments are displayed in Table 2, and Figures 1A and 1B. By analyzing the results of time and temperature combinations, it was possible to verify a rise in the moisture content of the seeds, as the exposure time to accelerated aging increased. In general, 42 °C for 96 h caused a more significant reduction in moisture content than the conditions of 45 °C for 96 h did.

The moisture content by the end of the accelerated aging period is one of the uniformity indicators of the test, and Marcos-Filho (1999) reported that variations of 4% to 5% among samples could be considered acceptable. Seeds aging causes degenerative alterations in the membrane system, which compromises its integrity and lowers its selectivity. This fact may lead to free water and solute exchange between the cell and exterior medium, reducing seeds viability (Vieira et al., 1994; Binotti et al., 2008).

It is possible to assess the germination as an effect of the temperature in each lot, as well as the germination of the lots according to each temperature (Table 3). At the temperature of 42 ºC, lot 3 showed more viability than the others. After 48 h of aging, there was a decline in seed germination in all lots, but it was less pronounced in lot 3. A reduction in the germination of the lots was also noticed at 45 ºC. In this case, lot 3 presented the highest viability up to 48 h of aging. However, after 72 h, its germination sharply decreased, henceforth not differing from the other lots.

When the germination of the lots was analyzed by temperature, a reduction was noticed at 42 ºC after 48 h (Table 3). Lot 1 showed germination values of 22% and 7%, after 72 and 96 h of aging, respectively. On the other hand, seeds from lots 2 and 3 displayed higher viability for the same period. At 45 ºC, after 48 h submitted to the deteriorating conditions, seeds also had their germination diminished. Nonetheless, such decline was more marked after 72 h, regardless of the lot in study.

The data available on Table 3, and also Figures 2A and 2B, evidence that the exposure times of 48 and 72 h were the most efficient for differentiating the lots at 42 ºC. Seeds exposition for 48 h was effective to classify the lots into levels of vigor, with lot 3 standing out from the others.

Table 1. Mean values of moisture content (MC), first germination count (FGC), germination (GER), and seedling emergence (E) obtained from the initial characterization of the physiological quality of popcorn seed lots (hybrid AP820).

Lots	MC (%)	FGC (%)	GER (%)	E (%)
L1	12.9	89 a	95 a	90 a
L2	12.2	84 b	95 a	94 a
L3	12.7	94 a	99 a	90 a
CV (%)		4.73	3.14	4.65

Table 2. Mean values of moisture content (%) of popcorn seed lots (hybrid AP8203), before and after the accelerated aging periods at 42 ºC and 45 ºC.

Lots	Aging periods			
	0 h	48 h	72 h	96 h
	42 ºC			
L1	12.9	22.3	23.0	25.2
L2	12.2	22.4	23.1	24.9
L3	12.7	21.7	22.7	25.5
	0 h	48 h	72 h	96 h
	45 ºC			
L1	12.9	20.3	22.8	21.9
L2	12.2	21.9	22.1	22.0
L3	12.7	19.7	20.1	22.9

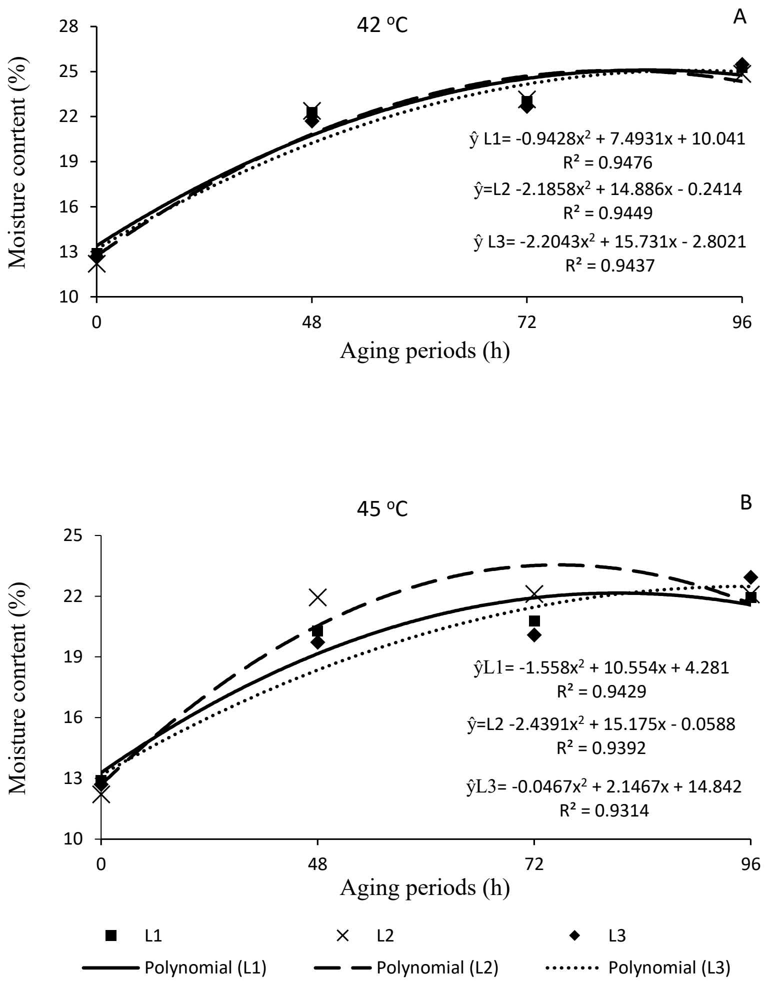

Figure 1. Moisture content (%) of popcorn seeds (AP8203) from different lots, before and after the accelerated aging at 42 ºC and 45 ºC.

The exposure time of 72 h at 42 ºC was also sufficient for lot classification. However, it should be taken into consideration that a desirable characteristic in a vigor test is the time taken to execute it and obtain the results. Consequently, such a longer exposition period is not advantageous, once 48 h are enough to provide the same outcome. Similar data were obtained after the accelerated aging of soybean (Silva et al., 2010), sorghum (Vazquez et al., 2011), rice (Tunes et al., 2012), and corn seeds (Bittencourt et al., 2012).

After aging for 72 and 96 h at 45 ºC, the seeds had their germination drastically reduced, in a way that it was impossible to assort the lots by levels of vigor. Only in the 48 h exposure time, it became possible to differ lot 3 from the others. Silva et al. (2010) found that a rise in temperature provokes more drastic effects on seed germination than an increase in the exposure time does. Such fact was confirmed in the present work at the temperature of 45 ºC.

Seed deterioration grew as the time of exposure to the accelerated aging test increased, once a reduction in germination could be observed within each temperature. This fact was also reported by Santos et al. (2004) and Binotti et al. (2008). Delouche (2002) affirmed that the deterioration process mostly derives from the interaction among genetic features, seed moisture content, and temperature.

As previously mentioned, the aging of seeds triggers degenerative alterations in the membrane system. Additionally, it modifies the respiratory metabolism, the protein synthesis and, by extension, the DNA metabolism (Basajavarajappa et al., 1991; Vázquez et al., 1991). Guedes et al. (2011) also realized that aging delays the germination process, causing the embryo to grow less, and making seeds more susceptible to environmental stress, thus compromising their viability.

Figure 3 portraits the reduction in seed vigor during the accelerated aging at both temperatures (42 ºC and 45ºC),

Table 3. Mean values of germination (%) of popcorn seeds (hybrid AP8203) submitted to different aging periods (h), according to temperature (42 ºC and 45 ºC) and seed lot.

Temperature (ºC)	Lots	Aging periods (h)			
		0	48	72	96
42	L1	90 Aa	62 Cb	22 Cb	7 Cc
	L2	91 Aa	74 Bb	46 Bc	17 Bd
	L3	92 Aa	87 Aa	78 Ab	30 Ac
45	L1	94 Aa	54 Bb	3 Ab	1 Ab
	L2	89 Aa	52 Bb	5 Ac	1 Ac
	L3	93 Aa	71 Ab	8 Ac	2 Ac
Lots	Temperature (ºC)	Aging periods (h)			
		0	48	72	96
L1	42	90 Aa	62 Ab	22 Ab	7 Ac
	45	94 Aa	54 Bb	3 Bb	1 Ab
L2	42	91 Aa	74 Bb	46 Ac	17 Ad
	45	89 Aa	52 Bb	5 Bc	1 Bc
L3	42	92 Aa	87 Aa	78 Ab	30 Ac
	45	93 Aa	71 Bb	8 Bc	2 Bc
CV (%)					12.76

Means followed by the same lowercase letter, in the column, and uppercase letter, in the row, do not differ statistically, according to the Scott-Knott test, at a 5% probability level.

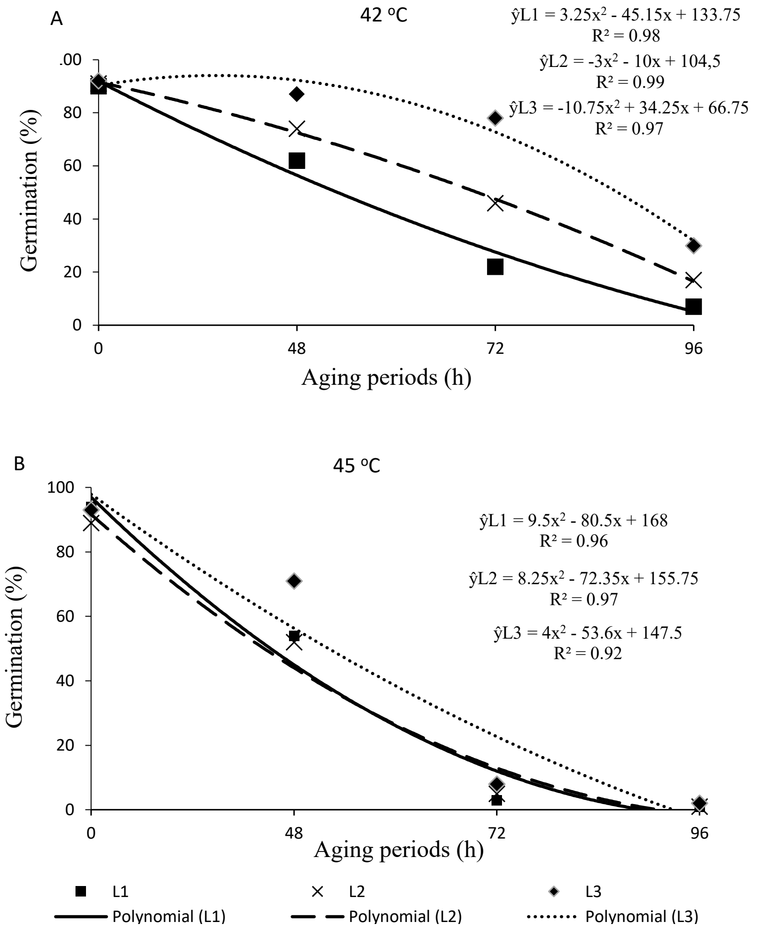

Figure 2. Germination (%) of popcorn seeds (AP8203) from different lots, before and after accelerated aging at 42 ºC and 45 ºC.

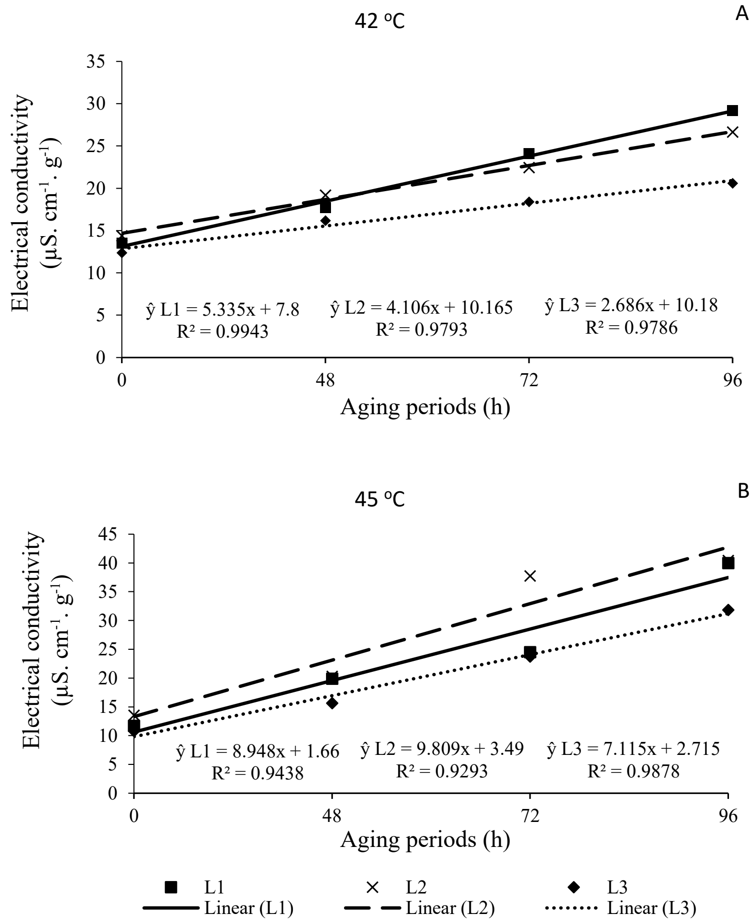

Figure 3. Electrical conductivity (µS. $cm^{-1}.g^{-1}$) of popcorn seeds (AP8203) from different lots, before and after accelerated aging at 42 ºC and 45 ºC.

measured through electrical conductivity. With the results of this test, it was possible to perceive an increase in the amount of leachate substances during the storage of the seeds, with the highest intensity being observed at 45 ºC. According to Bewley and Black (1994), this phenomenon occurs due to the destabilization of the cell membrane system, once seeds get more susceptible to deleterious effects of the oxygen gas. As a consequence, they become more propense to suffer enzymatic action and oxidation of compounds, therefore quickly consuming their reserves.

Based on the physiological results, the popcorn seeds had their viability and vigor reduced by the stress conditions imposed by the accelerated aging. This fact might have occurred due to the consumption of reserve substances, caused by the acceleration of metabolism in a situation of high temperature and relative humidity. On account of that, Guedes et al. (2011) affirmed that the viability loss in seeds is the outcome of critical metabolic events, and that the deterioration is manifested in different forms, which might even stop germination completely.

Conclusions

Vigor is affected by the deterioration conditions of temperature and relative humidity to which seeds are submitted to during aging. This effect is perceived by the increase in the amount of leachate substances detected by the electrical conductivity test. Seed lot 3 is the most vigorous one, according to the accelerated aging test carried out for 48 h at 45 ºC. Performing this test at 42 ºC for 48 h provides consistent information for differentiating popcorn seed lots.

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