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Adjustment of initial moisture content and accelerated aging test for supersweet corn (*sh2*) seeds

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Abstract: The accelerated aging test is an important method for assessing vigor in a seed quality program. However, the results are influenced by several factors (such as initial moisture content of the seeds) that are used as indicators of the uniformity of the performed tests. In this study, we compared the efficiency of the standardization of the initial moisture content of supersweet corn seeds in the accelerated aging test. Five lots of the hybrid Tropical Plus® supersweet corn (*sh2*) were used to evaluate germination, precocity of primary root emission, first count of germination, field seedling emergence, electrical conductivity, seed moisture content, cold test, and accelerated aging test without standardization (traditional) and with standardization of the initial moisture content to 14%, 12%, and 10% for 3 aging periods (48, 72, and 96 h). The results showed that the standardization improved the sensitivity of the accelerated aging test, allowing the seed lots to be classified into different vigor levels. Among the different combinations of initial moisture contents and aging periods, standardization of the initial moisture content to 14% or 12% and exposure period to 96 or 72 h allowed the best vigor classification of the supersweet corn (*sh2*) seeds.
Keywords: vigor, germination, seed quality.

Introduction

The supersweet corn seeds have a high sugar content and low starch content because of mutant alleles block the conversion of sugar into starch in the endosperm during seed development (Qi et al., 2009). For presenting higher contents of sugars, the genes shrunken-2 (*sh2*), brittle and brittle-2 are the most used for the production of seeds of commercial sweet corn (Okomura et al., 2013). Besides the change in sugar content, a feature desired by the food industry and consumers, these genes are responsible for the wrinkled appearance and lower pericarp thickness that cause the seeds to be more sensitive to mechanical damage, pathogen entry, thermal damage during drying, and deterioration (Zucareli et al., 2012; 2014). Because of these factors, supersweet corn seeds generally show lower germination, vigor, and seedling field emergence than corn seeds with a starchy endosperm (Kwiatkowski & Clemente, 2007). This low physiological quality of the seeds represents a barrier for supersweet corn seed production in Brazil, making it necessary to improve the vigor and germination of these seeds.

Deterioration has been described as a cumulative, irreversible, degenerative, and inexorable process (Carvalho & Nakagawa, 2012). Loss of germination is the final step of this process, and it involves physiological, biochemical, physical, and cytological changes in the seeds. Because viability tests are conducted under standardized and favorable conditions, small differences in physiological quality between lots are not detectable. Thus, the use of vigor tests allow us to measure the differences in physiological potential and provide a better raking of seed lots, including identification of lots with a higher probability of performing well after sowing (Ventura et al., 2012; Marcos Filho, 2015a).

Seed vigor can be assessed directly or indirectly with physiological tests, such as measurement of seedling length and radicle protrusion; biochemical tests, such as electrical conductivity and tetrazolium analyses; and tests that evaluate tolerance to stress conditions, such as cold and accelerated aging tests (Marcos Filho, 2015b). Among the vigor tests, the accelerated aging test has been acknowledged as one of the most relevant tests, as it provides information with a high degree of consistency (Marcos Filho, 2011). Basically, the test consists of exposing the seeds to high temperatures (40 °C to 45 °C) and humidity (around 100% relative humidity) for a specific period, increasing the deterioration rate. After this aging treatment, the seeds are analyzed using a germination test; high-

vigor seeds are more tolerant to the stress conditions and produce a higher percentage of normal seedlings (Marcos Filho, 1999). However, several factors, other than temperature and aging period, affect the seed behavior during the test, for example, genotype, sample size, and seed moisture content.

Because vigor is a more sensitive parameter of seed physiological quality, the test variables need to be controlled more rigorously (Marcos Filho, 2015b). The moisture content affects the deterioration rate in the accelerated aging test. Determination of seed water content before and after the aging period is recommended; variations up to 2% among lots acceptable to check for result consistency, indicating test uniformity (Marcos Filho, 2015a). Vieira et al. (2005) used corn seeds and Scappa Neto et al. (2000; 2001) used soybean and bean seeds to standardize the seed water content before the accelerated aging test. The possibility of adjustment of an indicative parameter for test conformity typifies a high control level, allowing test standardization (Marcos Filho, 2011). Improvement of the methods used to assess physiological quality is a necessity for internal seed quality control programs. On the basis of the above-mentioned considerations, the aim of this study was to evaluate the efficiency of standardization of the initial water content of supersweet corn seeds at different exposure periods during the accelerated ageing test.

Methods

Five lots of hybrid supersweet corn seeds, Tropical Plus® (*sh2*), produced in Ituiutaba – MG in the crop year 2013/14 by the company Syngenta Ltd were used. The experiment was performed at the Seeds Analysis Laboratory, Federal University of Mato Grosso. The following tests were performed: *Seed moisture content*, measured before and after the accelerated aging test by using the oven method at 105 °C (±3 °C) for 24 h (Brasil, 2009), with 2 replicates of 25 seeds. The results were expressed in mean percentage. *Precocity of primary root emission*, the seeds were distributed on moist paper-towel sheets with water equivalent to 2.5 times the weight of the dry paper and maintained in a germinator at 30 °C, with 4 replicates of 50 seeds. The number of seeds that emitted a primary root was counted after 32 h (Toledo et al., 1999). *Germination and first germination counts*, seeds on paper-towel rolls were moistened with water equivalent to 2.5 times the weight of dry paper at 30 °C, with 4 replicates of 50 seeds. The evaluations were performed at 4 (first germination count) and 7 days after seeding, according to the criteria established by the Seed Analysis Rules (Brasil, 2009). The results were expressed as the mean percentage of normal seedlings per lot. *Field seedling emergence*, for this field experiment, 4 replicates of 100 seeds were manually distributed in rows that were 0.40 m apart to a depth of 5 cm. Seedling emergence was evaluated 21 days after sowing, and the mean percentage of emerged normal seedlings for each lot was recorded (Vieira & Carvalho, 1994). *Electrical conductivity (EC)*, 4 replicates of 50 seeds were weighed to the nearest 0.01 g and immersed in 75 mL of deionized water in 200 mL plastic vials. Seed hydration occurred in a germinator at 30 °C for 24 h. After soaking, EC was determined in a conductivity meter (TK-W12D) with an electrode with constant 1 and the results were expressed as $\mu S \cdot cm^{-1} \cdot g^{-1}$ (Vieira & Carvalho, 1994). *Cold test*, 4 replicates of 50 seeds were distributed on moist paper-towel rolls with water equivalent to 2.5 times the weight of the dry paper and maintained for 7 days at 10 °C and then the rolls were placed 4 days at 30 °C. The number of normal seedlings was counted after this period (Vieira & Carvalho, 1994). *Accelerated aging without adjustment of the initial water content (traditional)*, the seed samples were set on a single layer of aluminum screen and placed in plastic boxes (11 cm × 11 cm × 3 cm) containing 40 mL of water at the bottom; the screen prevented the seeds from touching the water. The boxes were sealed in BOD chambers at 42 °C for 48 h, 72 h, and 96 h (Marcos Filho, 1999; Santos et al., 2002). Then, the germination test was performed and evaluated four days after sowing. The seed moisture content was also determined after aging to observe if the test conditions were uniform. *Accelerated aging with adjustment of the initial water content*, the adjustment of the initial water content was performed for 250 seeds arranged on an aluminum screen placed in plastic boxes of the gerbox type containing 40 mL of water, and wetting was performed using the wet atmosphere method (Rosseto et al., 1995). Then, the seeds were divided in 5 replicates of 50 seeds and dried using the oven method at 38 °C to adjust the water content to 14%, 12%, and 10% (Ducatti et al., 2016). The loss of water during the drying process on the basis of the weight of the seed was calculated using the following equation: Wx = Wo (100 - Uo/100 - Ux), where, Wx is the final weight of the sample, Wo is the initial weight of the seeds, Uo is the initial water content of the seeds, and Ux is the intended water content (Martins et al., 1999). After the adjustment, the samples were evaluated for accelerated aging for 48 h, 72 h, and 96 h, as described above.

The data were analyzed for variance by using the F test, arranged in a completely randomized design, and the means were compared using the Tukey test at 5% probability. For the accelerated aging methods, a 5 × 3 × 3 factorial design (lots × water content × periods) was used. The determination of simple correlation coefficients (r) between the accelerated aging tests with and without adjustment of the initial moisture content and physiological tests was also performed.

Results and discussion

The seed moisture content ranged among the lots from 11% to 12.5%, with differences lower than 2%, according to the recommended values for the accelerated aging test; this indicated test uniformity (Marcos Filho, 1999). The results of the

traditional accelerated aging tests were similar, with a variation limit of 4%; the seed moisture content ranged from 24.6% to 26.3% after 48 h, 24.4% to 26.6% after 72 h, and 26% to 26.8% after 96 h and gradually increased with the aging period, as reported by Coimbra et al. (2009) and Venancio et al. (2012). The data from seed moisture content after the accelerated aging with standardization of moisture content also remained similar, as shown in Figure 1. This uniformity is essential for providing the same imbibition and deterioration rates to the seeds (Marcos Filho, 1999).

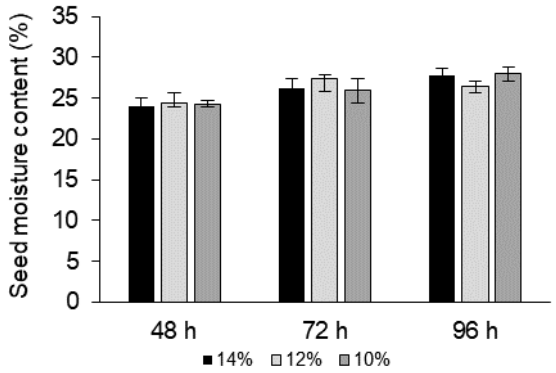

Figure 1. Average seed moisture content (%) after the accelerated aging test in 3 different periods of aging 48, 72, and 96 h) with adjustment of initial water content in 14%, 12%, and 10% of 5 lots of the supersweet corn seed Tropical Plus®.

Germination, first germination count, precocity of primary root emission, field seedling emergence, electrical conductivity, cold test, and traditional accelerated aging for 96 h and 72 h were not statistical different among the seed lots (Table 1). The germination of seed lots ranged from 70% to 85%. These values were higher than the minimum standards (60%) established for the commercialization of supersweet corn seeds (Brasil, 2013).

Among the tests, only the traditional accelerated aging test with an exposure time of 48 h was sensitive for identifying differences among the lots and classifying them into 3 vigor classes (Table 1): high vigor lot (L1), low vigor lot (L4), and intermediate vigor lots. Santos et al. (2002) verified the test efficiency in a study using the sweet corn seed BR 400; Alvarenga et al. (2012), hybrid supersweet corn (*sh2*); Alvarenga et al. (2013), hybrids SWB 551 and SWB 585; and Zucareli et al. (2014), cultivars BR 401 and 402. However, according to Coimbra et al. (2009), accelerated aging of the sweet corn seed DO-04 in distilled water (100% RH) and saturated NaCl solution (76% RH) was not efficient for detecting vigor differences.

The accelerated aging test with adjustment of the initial moisture content to 14% and 12% for 96 h (Table 2) provided a better distinction of physiological quality among the seed lots, allowing separation of the lots into 3 vigor levels. L2 was more vigorous; L1, L4, and L5 showed intermediate vigor; and L3 showed the lowest vigor. In general, the increase in the aging period increased the sensitivity for identifying the physiological quality of the seeds, especially for a period of 96 h and adjustment of 14%. Venancio et al. (2012) also observed higher sensitivity with longer periods of exposure in corn seeds. After an adjustment of 10% for the same period, the lots were divided into 3 vigor levels; however, L3 showed the highest vigor.

The adjustment to 14% and 12% in 72 h (Table 2) allowed the lots to be classified into only 2 vigor levels. Under these conditions, lots with intermediate physiological potential were not detected. Despite the lower sensitivity at 96 h, an improvement over the traditional accelerated aging test (without standardization of the seed moisture content) was observed.

Table 1. Germination (G), precocity of primary root emission (PRE), first germination count (FG), field seedling emergence (FSE), cold test (CT), traditional accelerated aging test (AA), and electrical conductivity (EC) of 5 lots of the hybrid supersweet corn seeds Tropical Plus®.

Lots	G	PRE	FG	FSE	CT	AA (%)			EC
			(%)			48 h	72 h	96 h	($\mu S \cdot cm^{-1} \cdot g^{-1}$)
L1	83	69	54	91	71	48 A*	39	29	35
L2	84	73	62	90	67	41 AB	36	30	36
L3	85	72	51	90	71	41 AB	41	23	36
L4	78	68	58	90	63	32 B	39	23	41
L5	70	62	56	90	63	37 AB	39	33	44
CV (%)	9.9	10.1	10.3	4.0	11.7	15.0	14.5	18.4	11.6

* Means followed by the same letter in a column or without letters do not differ significantly, according to the Tukey test ($p < 0.05$). CV = coefficient of variation.

Table 2. Percentage of normal seedlings after 96 h, 72 h and 48 h of accelerated aging tests with standardization of the initial moisture content to 14%, 12%, and 10% at 42 °C for 5 lots of the hybrid supersweet corn seed Tropical Plus®.

Lots	96 h			72 h			48 h		
	14%	12%	10%	14%	12%	10%	14%	12%	10%
L1	54 ABa	38 Ba	21 BCa	47 Aa	51 Aa	6 Cb	32 Ab	61 Aa	17 Bb
L2	58 Aa	56 Aa	24 Bb	50 Aa	45 Aa	47 Aa	35 Aa	41 Ba	43 Aa
L3	11 Cb	13 Cb	40 Aa	24 Bb	15 Bc	48 Aa	28 Ab	33 Bab	48 Aa
L4	47 ABa	42 Ba	9 Cb	43 Aa	44 Aa	28 Bb	35 Aa	36 Ba	46 Aa
L5	44 Ba	42 Ba	20 BCb	51 Aa	44 Aa	43 Aa	36 Aa	41 Ba	32 Aa
C.V (%)		15.0			12.9			17.8	

* Means followed by the same letter (uppercase in the column and lowercase in the row, for each group) do not differ significantly, according to the Tukey test ($p < 0.05$). CV = coefficient of variation.

Differences between vigor levels were not observed in the seeds with an initial moisture content of 14% that were aged for 48 h (Table 2). With the initial moisture content was adjusted to 12%, only 2 vigor levels were observed: L1 was the most vigorous, and the other lots, less vigorous and similar to each other. L3 showed the worst performance in the accelerated aging test in 96 h (14% and 12%), 72 h (14% and 12%), and 48 h (12%). When the initial moisture content was adjusted to 10%, L3 was the most vigorous in all the aging periods (96 h, 72 h, and 48 h).

Examining the results of accelerated aging tests, the aging period of 96 h and 72 h were the most efficient in separating lots in vigor levels. The period of 96 h separated lots in 4 vigor levels (14% and 10%) and 3 vigor levels (12%), while the aging period of 72 h in 3 levels (10%). The aging period of 72 h (14% and 12%) and 48 h were less efficient in showing the differences among the lots.

Overall, the accelerated aging test conducted with adjustment of the initial seed moisture content showed greater sensitivity for stratifying the lots at different levels of vigor than the traditional aging and other vigor tests. Adjustment of the initial water content had a significant effect on the results of the accelerated aging test. On the basis of the water content, variations in the hydration and deterioration rates of the seeds were observed; in general, the seeds with higher water content were more sensitive to the test conditions (Marcos Filho, 2015a).

In summary, different water contents of the seeds affect the hydration process and, thereby, deterioration rates (Marcos Filho, 2015a). Therefore, it is important to standardize the initial water content, allowing us to observe the germination results on the basis of only the physiological quality of the lots. Furthermore, through these tests, differences greater than 2% in the initial moisture content of samples were proven to compromise the results of the accelerated aging test (Marcos Filho, 1999).

The same effect was observed in a study by Vieira et al. (2005), in which the adjustment of the initial water content in maize seed at 8%, 10%, and 12% allowed the lots to be differentiated into different vigor levels and showed the importance of standardization of the initial seed moisture content. Scappa Neto et al. (2000), when studying the effect of initial moisture content (8%, 10%, and 12%) in AA using soybean seeds, concluded that the 12% adjustment is more appropriate to conduct the test. However, in the same conditions and with bean seeds, aging results have not changed due to the initial moisture content (Scappa Neto et al., 2001).

Because the aging tests allow us to select lots of different vigor classes, the results were evaluated using correlation, and a high positive correlation between the accelerated aging data 96 h/12% and first counting test (0.92) was detected (data not shown). The correlation analysis was also performed for combinations of periods of accelerated aging and initial moisture contents. A significant correlation was observed between the tests conducted using 96 h/14% and 96 h/12% (0.93) and 72 h/14% (0.92) and 72 h/12% (0.96). A significant positive correlation was detected for the test performed using 96 h/12% and 72 h/14% (0.92).

Significant associations indicate a similar pattern of behavior among the tests. In this study, significant correlations helped in identifying alternatives among the evaluated tests, such as reducing the aging period of 96 h to 72 h and recommended initial moisture content of 14% or 12%. Because the standardized moisture content for the accelerated aging test ranges from 11% to 13%, it is recommended to adjusting the initial moisture content to 12% in supersweet corn seeds.

In general, the results obtained in this study revealed that the standardization of the initial moisture content in the seeds enhances the efficiency of the accelerated aging test, making it possible to identify lots of intermediate vigor. Because water content is the parameter used to control test uniformity, its standardization is key for performing the accelerated aging test. The test was able to classify the lots with efficiency and sensitivity, which makes the method a good alternative for evaluation of seed vigor.

Conclusion

The standardization of the initial water content of supersweet corn seeds for the accelerated aging test increases the efficiency of the test in identifying differences in the physiological potential of the seed lots.

The adjustment of the initial water content of the supersweet corn seeds to 14% and 12% in combination with 72 h or 96 h of accelerated aging at 42 °C provides consistent identification of differences in the physiological potential of the seed lots of commercial value.

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