




Accelerated Aging as a Vigor Test in *Encholirium spectabile* Seeds

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ABSTRACT

The objective was to verify how differences in the accelerated aging test, traditional method (water) or saturated NaCl solution influence the differentiation of the physiological potential of *Encholirium spectabile* seeds from two geographic regions of Brazil (Graça-CE and Serra Talhada-PE). The experimental design was completely randomized in a $2 \times 2 \times 5$ factorial scheme, in which two methods (water and saturated NaCl solution), seeds from two geographic regions and five aging periods (0 h, 24 h, 48 h, 72 h and 96 h) were evaluated. Results indicated that seeds from Graça-CE were less vigorous than those from Serra Talhada-PE. However, both aging methods (water and saturated NaCl solution) increased the physiological potential of seeds harvested from Graça-CE. *E. spectabile* seeds presented different levels of physiological potential by the accelerated aging test.

Keywords: Bromeliaceae, physiological conditioning, seed deterioration.

1. INTRODUCTION AND OBJECTIVES

Studies on the applicability of methods for the evaluation of the physiological potential in bromeliaceous seeds deserve attention, due to the importance attributed to numerous species of this family. Among them, the *Encholirium spectabile* Mart. ex Schult. & Schult. f., a native species belonging to the Bromeliaceae family, subfamily Pitcairnioideae, is popularly known in Brazil as “macambira-de-flecha” (Oliveira et al., 2013).

This species is propagated by seeds, and knowledge about its physiological performance is valuable because the species *E. spectabile* presents potential to be explored commercially for ornamental purposes and its medicinal characteristics, associated with the presence of phenolic compounds that activate cytoprotective mechanisms (Carvalho et al., 2010). In addition, the species is used in ruminant feeding in the Brazilian semi-arid region, especially during the dry season (Bessa, 1982; Lima, 1996).

It is also worth noting the high potential of the species for soil conservation, considering that the species forms clumps and its roots can act by holding the soil and preventing erosion. Even if its sustainable exploration is not immediate, the lack of knowledge of its potential use, together with the rapid and disorderly occupation of the semi-arid region of the Brazilian Northeast, may accelerate the species depletion.

The accelerated aging test is one of the most sensitive and efficient evaluations of seed vigor for several species and has been improved by several researchers (Marcos Filho, 2015). This test is based on the fact that the seed deterioration rate is increased by its exposure to high temperature and relative humidity (RH). Seeds with low vigor present a greater reduction of their viability when submitted to such stressful situations, whereas more vigorous seeds are less affected and show a relatively higher germination, even when subjected to the aging test (Marcos Filho, 1999).

The interaction between temperature and seed exposure time during the aging test are crucial factors for the assay efficiency, but for many species, this interaction has yet to be established (Guedes et al., 2011). Another factor to consider is the difference in the amount of water absorbed by the seeds when exposed to high humidity, especially when evaluated seeds are small, since they absorb water rapidly, intensifying the deterioration process (Alves & Sá, 2012). For these reasons, the accelerated

aging test has been performed using saturated mineral solutions (NaCl, KCl and NaBr) in replacement of water to delay the effects caused by rapid absorption of water by small seeds.

In *Lactuca sativa* L. and *Cichorium intybus* L. seeds, accelerated aging with saline NaCl solution for 48 h and 72 h, respectively, was efficient in differentiating vigor levels (Santos et al., 2011). Alves et al. (2012) also demonstrated that the accelerated aging test with saturated NaCl solution at 41 °C for 48 h was a promising option for evaluating the physiological potential of *Solanum gilo* Raddi seeds.

The applicability of the accelerated aging test in assessing the physiological potential of native species seeds was verified in *Copaifera langsdorffii* Desf. (Ferreira et al., 2004), *Anadenanthera colubrina* (Vellozo) Brenan (Garcia et al., 2004), *Dictyoloma vandellianum* A.D. Juss. (Flavio & Paula, 2010), *Dalbergia nigra* (Vell.) Fr. All. (Guedes et al., 2011), *Myracrodruon urundeuva* Allemão (Araújo et al., 2013), *Chorisia glaziovii* (O. Kuntze) (Guedes et al., 2013) and *Poincianella pyramidalis* (Tul.) LP Queiroz (Lima et al., 2014), for example. However, there are still few studies that contemplate seeds of native species in the face of the existing diversity.

Thus, the objective of this study was to verify how the accelerated aging test, a traditional method with water or saturated NaCl solution, influences the differentiation of the physiological potential of *E. spectabile* seeds from two geographic regions (Graça-CE and Serra Talhada-PE).

2. MATERIALS AND METHODS

2.1. Collection and processing of seeds

E. spectabile dried fruit stems were collected from 10 matrices randomly selected in two geographic regions, one located in the city of Graça, Ceará state (4°02'48" S and 40°44'59" W) and another in the city of Serra de Talhada, Pernambuco state (7°59'9" S, 38°17'45" W), which were collected in September and October 2013.

After collection, the fruits were manually opening to obtain seeds. Any damaged and malformed seeds were discarded. They were then homogenized in terms of size, by using a mesh sieve with a diameter ≥ 2 mm and ≥ 4 mm, respectively, and placed in plastic containers in an air-conditioned room at 25.5 °C and 58% RH, for 12 months.

2.2. Accelerated aging (traditional method and saturated NaCl solution)

The assays were carried out in a completely randomized design in a 2 × 2 × 5 factorial scheme, for investigation of two methods (water and saturated NaCl solution), seeds from two geographic regions and five aging periods (0 h, 24 h, 48 h, 72 h and 96 h).

Seeds from both regions were submitted to the accelerated aging test (traditional method), in which Gerbox-type transparent plastic boxes were used as individual compartments (small chambers) that contained interior supports coupled to aluminum screens (Marcos Filho, 1999).

As the *E. spectabile* seeds are very small, a piece of tulle tissue was laid over each aluminum screen to prevent the seeds from falling into the water. Approximately 0.5 g of seed were distributed to form a uniform layer, which filled the entire screen. Forty milliliters of distilled water were previously added into the boxes, which were then capped and kept in a Biochemical Oxygen Demand (BOD) chamber at 41 °C for 24 h, 48 h, 72 h and 96 h.

The accelerated aging test with saturated NaCl solution was performed in a similar way to the traditional procedure, but instead of water, 40 mL saturated NaCl solution was added into each plastic box. The solution was prepared with 40 g NaCl in 100 mL distilled water, establishing an environment with 76% RH (Jianhua & McDonald, 1997).

To evaluate the physiological potential of seeds from each geographic region, before and after the periods of traditional accelerated aging and with a saturated NaCl solution, the following tests were conducted:

Determination of water content of seeds: Based on the gravimetric technique (Brasil, 2009), 0.4 g of seeds per

treatment was oven-dried at 105 °C ± 3 °C for 24 h, and the results were expressed as a percentage of the wet basis.

Germination test: Four replicates of 50 seeds were distributed in Gerbox plastic boxes, on two sheets of blotting paper properly sterilized in an oven at 105 °C for 2 h and moistened with distilled water equivalent to 2.5 times the dry paper mass (Brasil, 2009).

The boxes were kept inside plastic bags, under continuous lighting in an air-conditioned room at 25 °C and 45.37% RH, with daily monitoring by using a digital thermo-hygrometer. Normal seedlings were those that had developed primary root and total expansion of the cotyledonary sheath at the 15th day after the test installation. The results were expressed as a percentage of normal seedlings.

Index of germination speed: Daily counts of normal seedlings were recorded from the test installation until the 15th day, according to Maguire (1962).

Mean germination time: In conjunction with the germination test, the mean germination time was calculated according to Labouriau and Valadares (1976), and the results were expressed in days.

Data were submitted to analysis of variance by the F-test and means were compared by Tukey’s test at 5% probability level, using Sisvar software (Ferreira, 2011).

3. RESULTS AND DISCUSSION

E. spectabile seeds evaluated in this study showed no initial differences in the water content. However, there was a significant difference between the recorded water contents when considering the geographical region in which the seeds were collected, regardless of whether the seeds were aged with water or with saturated NaCl solution. However, the values were within the acceptable limits, according to Marcos Filho (1999) (Table 1).

Table 1. Water content of *Encholirium spectabile* Mart. ex Schult. & Schult. f. seeds from two geographic regions, before and after the traditional accelerated aging test with water (100% relative humidity) and saturated NaCl solution (76% relative humidity) at 41 °C.

| Aging period (h) | Geographic region | | | |
|------------------|-------------------|-----------|------------------|-----------|
| | Graça-CE | | Serra Talhada-PE | |
| | H ₂ O | NaCl | H ₂ O | NaCl |
| 0 | 9.61 Ad | 10.77 Ab | 8.78 Ac | 9.17 Ab |
| 24 | 32.19 Ac* | 11.08 Bb‡ | 25.36 Ab* | 19.31 Ba‡ |
| 48 | 32.52 Abc* | 21.89 Ba‡ | 29.24 Aa* | 19.28 Ba‡ |

Table 1. Continued...

| Aging period (h) | Geographic region | | | |
|------------------|-------------------|-----------|------------------|-----------|
| | Graça-CE | | Serra Talhada-PE | |
| | H ₂ O | NaCl | H ₂ O | NaCl |
| 72 | 35.38 Aa* | 21.63 Ba* | 30.10 Aa* | 18.99 Ba* |
| 96 | 34.84 Aab* | 12.42 Bb* | 29.59 Aa* | 18.86 Ba* |
| CV (%) | 5.78 | | | |

Means followed by the same lowercase letters in the column and uppercase letters in the row, by geographical region, do not differ by Tukey's test ($P \leq 0.05$). * in the line indicates the difference between geographical regions for the method with water, and ** for the method with saturated NaCl solution, in each aging period. CV: coefficient of variation.

When considering the type of aging technique used, seeds from both geographic regions obtained a higher water content when they were submitted to the traditional accelerated aging test with water. This result was already predictable since the NaCl solution reduces the intensity with which seeds absorb water, corroborating the findings of Tunes et al. (2012) and Radke et al. (2016), who observed the same trend with *Oryza sativa* L. and *Coriandrum sativa* L. seeds, respectively. There was also an increase in the water content as a function of the aging exposure time of the seeds from both regions' tests (Table 1).

Seeds from Graça-CE were less vigorous than those from Serra Talhada-PE before the aging test, as well as when aged for 96 h by both methods (water and saturated NaCl solution) (Table 2). This result was justified by the decrease in the mean value of germination after aging for 96 h in comparison to the other aging periods, possibly due to a more intense

deterioration of the seeds relative to those aged for shorter periods.

Regarding the aging method adopted (Table 2), the geographic region of the seeds led to a difference in the germination when aged for 72 h. In the accelerated aging test with water, the germination was higher for the seeds from Graça-CE, whereas in the aging test with NaCl solution, the highest percentage of germination was recorded in seeds from Serra Talhada-PE. Similar results were found in *Mabea fistulifera* Mart. seeds aged in saline solution at 41 °C for 72 h (Gomes & Lopes, 2017).

For the seeds harvested in the city of Graça-CE, the germination speed index differed significantly between the accelerated aging methods adopted when the seeds were aged for 24 h, 48 h and 72 h. Regarding the seeds harvested in Serra Talhada-PE, the type of aging procedure influenced the germination rate when the seeds were in conditions of high temperature and humidity for 24 h and 72 h (Table 3).

Table 2. Germination (%) of *Encholirium spectabile* Mart. ex Schult. & Schult. f. seeds from two geographic regions, before and after traditional accelerated aging methods with water (100% relative humidity) and saturated NaCl solution (76% relative humidity) at 41 °C.

| Aging period (h) | Geographic region | | | |
|------------------|-------------------|------------|------------------|-----------|
| | Graça-CE | | Serra Talhada-PE | |
| | H ₂ O | NaCl | H ₂ O | NaCl |
| 0 | 61.50 Ab* | 59.50 Ad* | 86.00 Aab* | 85.50 Aa* |
| 24 | 86.50 Aa | 90.50 Aa | 82.50 Bb | 93.50 Aa |
| 48 | 87.50 Aa | 81.50 Aab | 85.00 Aab | 88.00 Aa |
| 72 | 87.00 Aa* | 73.00 Bbc* | 75.00 Bb* | 92.00 Aa* |
| 96 | 51.00 Bb* | 66.00 Acd* | 95.00 Aa* | 90.50 Aa* |
| CV (%) | 7.63 | | | |

Means followed by the same lowercase letters in the column and uppercase letters in the row, by geographical region, do not differ by Tukey's test ($P \leq 0.05$). * in the line indicates the difference between geographical regions for the method with water, and ** for the method with saturated NaCl solution, in each aging period. CV: coefficient of variation.

Table 3. Germination speed index of *Encholirium spectabile* Mart. ex Schult. & Schult. f. seeds from two geographic regions, before and after traditional accelerated aging methods with water (100% relative humidity) and saturated NaCl solution (76% relative humidity) at 41 °C.

| Aging period (h) | Geographic region | | | |
|------------------|-------------------|-----------|------------------|----------|
| | Graça-CE | | Serra Talhada-PE | |
| | H ₂ O | NaCl | H ₂ O | NaCl |
| 0 | 3.19 Bb* | 4.21 Ab* | 6.87 Aab* | 6.97 Aa* |
| 24 | 6.98 Aa* | 5.38 Ba* | 6.11 Bb* | 6.95 Aa* |
| 48 | 6.97 Aa | 5.25 Ba* | 6.53 Aab | 7.11 Aa* |
| 72 | 7.12 Aa* | 5.31 Ba* | 6.10 Bab* | 7.07 Aa* |
| 96 | 3.96 Ab* | 4.42 Aab* | 7.14 Aa* | 6.97 Aa* |
| CV (%) | 8.17 | | | |

Means followed by the same lowercase letters in the column and uppercase letters in the row, by geographical region, do not differ by Tukey's test ($P \leq 0.05$). * in the line indicates the difference between geographical regions for the method with water, and ** for the method with saturated NaCl solution, in each aging period. CV: coefficient of variation.

There was no significant difference between aging periods for *E. spectabile* seeds from Serra Talhada-PE exposed to the saturated NaCl solution (Table 3). However, it is important to emphasize that these seeds provided higher germination rates than those observed in seeds from Graça-CE, under the same aging conditions.

Instead of intensifying the deteriorative process of seeds harvested in Graça-CE, accelerated aging with water favored the germination and seedlings uniformity during 24 h, 48 h and 72 h. Namely, during such periods, the conditions of high temperature

and RH provided physiological conditioning of the seeds (Tables 2 and 3). Seed reinvigoration due to physiological conditioning treatments, i.e., seed hydration without protrusion of the primary root, may reflect an increased rate and uniformity of seedling emergence (Khan, 1992).

Periods of accelerated aging, regardless of the technique employed, did not influence the germination time of *E. spectabile* seeds from both geographic regions. However, when not aged, the seeds harvested in Graça-CE took comparatively longer to germinate and to yield normal seedlings (Table 4).

Table 4. Average germination time of *Encholirium spectabile* Mart. ex Schult. & Schult. f. seeds from two geographic regions, before and after traditional accelerated aging methods with water (100% relative humidity) and saturated NaCl solution (76% relative humidity) at 41 °C.

| Aging period (h) | Geographic region | | | |
|------------------|-------------------|----------|------------------|----------|
| | Graça-CE | | Serra Talhada-PE | |
| | H ₂ O | NaCl | H ₂ O | NaCl |
| 0 | 2.62 Ab* | 1.93 Ba* | 1.59 Aa* | 1.55 Aa* |
| 24 | 1.56 Ba | 2.14 Aa* | 1.75 Aa | 1.74 Aa* |
| 48 | 1.60 Ba | 1.92 Aa* | 1.67 Aa | 1.56 Aa* |
| 72 | 1.65 Aa | 1.76 Aa | 1.55 Aa | 1.67 Aa |
| 96 | 1.53 Ba | 2.09 Aa* | 1.71 Aa | 1.66 Aa* |
| CV (%) | 11.61 | | | |

Means followed by the same lowercase letters in the column and uppercase letters in the row, by geographical region, do not differ by Tukey's test ($P \leq 0.05$). * in the line indicates the difference between geographical regions for the method with water, and ** for the method with saturated NaCl solution, in each aging period. CV: coefficient of variation.

In determining the influence of the origin of the seeds, overall, the average germination times indicated that the seeds harvested in Serra Talhada-PE were the most vigorous, regardless of the accelerated aging test (water versus saturated NaCl). However, the traditional assay (water) contributed to reducing the average germination time of seeds harvested in Graça-CE (Table 4), showing that there was a significant improvement in the physiological potential of *E. spectabile* seeds of this region.

These results indicate that the germinative behavior observed after exposure of *E. spectabile* seeds to high temperature and RH by the traditional accelerated aging test or with saturated NaCl solution may be related to the strategies used by species to survive potential environmental pressures, such as high temperatures and water deficit (Figueiredo et al., 2014). It is noteworthy that the species grows fully exposed to the sun on rock outcrops, and germination occurs most expressively after the first rains, that is, under high humidity (Ramalho et al., 2004).

Thus, it should be considered that a stress vigor test can provide deleterious conditions for a particular group of seeds (e.g., cultivated species). However, for seeds from species that have not been domesticated or that germinate in nature under similar conditions to those used in a given stress test, it can be seen that instead of being stressed, the seed may benefit from such conditions, resulting in enhanced germination and vigor.

4. CONCLUSIONS

Encholirium spectabile seeds presented different levels of physiological potential by the accelerated aging test, which in turn provided the reinvigoration of seeds harvested in the city of Graça-CE, when using the traditional method with water.

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