

NOTE

Accelerated aging for evaluation of vigor in *Brachiaria brizantha* 'Xaraés' seeds¹

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ABSTRACT: The aim of this study was to adjust the accelerated aging test to evaluate the physiological potential of seed lots of *Brachiaria brizantha* 'Xaraés', represented by four lots. Seeds were tested by traditional accelerated aging and with saturated NaCl solution in five aging periods: 0, 24, 48, 72 and 96 hours. The profile of the lots was determined by the following measures: moisture content, germination test, first germination count, germination speed index, initial stand, emergence, and emergence speed index. The accelerated aging test makes it possible to separate the lots by the method of saturated NaCl solution for 24 hours and it is appropriate for evaluation of seed physiological potential.

Index terms: physiological quality, accelerated aging, vigor.

Envelhecimento acelerado para avaliação do vigor de sementes de *Brachiaria brizantha* cv Xaraés

RESUMO: Objetivou-se, com esse estudo, adequar o teste de envelhecimento acelerado, para avaliar o potencial fisiológico de lotes de sementes de *Brachiaria brizantha* cv Xaraés representada por quatro lotes. As sementes foram submetidas ao envelhecimento acelerado tradicional e com solução saturada de NaCl, em cinco períodos de envelhecimento, 0, 24, 48, 72 e 96 horas. Para caracterização dos lotes, foram determinados o grau de umidade, o teste de germinação, primeira contagem da germinação, índice de velocidade de germinação, estande inicial, emergência e índice de velocidade de emergência. Conclui-se que o teste de envelhecimento acelerado possibilita a separação dos lotes pelo método com solução saturada de NaCl por 24 horas, sendo apropriado para a avaliação do potencial fisiológico das sementes.

Termos para indexação: qualidade fisiológica, envelhecimento acelerado, vigor.

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INTRODUCTION

There are approximately 47 million hectares of natural pasture and 112 million hectares of planted pasture in Brazil. This total area of pasture is nearly three times greater than that used for grain production, which currently exceeds 56 million hectares (IBGE, 2018). Establishment and growth of pastures can occur through vegetative processes or, in the case of large properties with a high level of technology, through seeds. Thus, seed quality is fundamental for obtaining an adequate and uniform stand (Costa et al., 2011).

For evaluation of this quality, the germination test is the official procedure that evaluates the capacity of seeds to produce normal seedlings under ideal conditions. Nevertheless, this test does not always reveal differences of performance between seed lots during storage or in the field (Carvalho and Nakagawa, 2012). Thus, it is important to evaluate seed vigor to complement the information provided by the germination test.

Among the vigor tests developed, accelerated aging is one of the most studied for various species grown, and it is used in diverse quality control programs adopted in seed companies by exhibiting efficiency in comparison of vigor and in estimation of the storage potential of the seed lots. The accelerated aging test has shown a good relationship to the seedling emergence test in the field (Marcos-Filho, 2015a).

Initially developed for the purpose of estimating the longevity of stored seeds (Delouche and Baskin, 1973), accelerated aging has been widely studied with the aim of standardizing it, for its use is quite promising. In this test, the seeds are subjected to simulated stress conditions through high temperatures and high relative humidity (100%), generating high respiration rates and consumption of seed reserves, causing degenerative changes in seed metabolism and changes in the antioxidant defense system and in the soluble sugar content in the embryo (Bijan-zadeh et al., 2016; Barreto and Garcia, 2017; Moncaleano-Escandon et al., 2013; Das and Roychoudhury, 2014; Lehner et al., 2008). Thus, more vigorous seeds better tolerate this aging condition than those of lower vigor and produce a higher percentage of normal seedlings, evaluated by the germination test after aging (Baalbaki et al., 2009; Marcos-Filho, 2015b; Pereira et al., 2012; Silva et al., 2010; Moraes et al., 2016).

Yet accelerated aging with water, which leads to relative humidity of 100%, can cause accentuated variations among the moisture contents of the samples, interfering in the results of the test. Thus, Jianhua and McDonald (1996) aimed to reduce the relative humidity during the test and proposed the use of a saturated NaCl saline solution, which leads to relative humidity of 76%, causing moderate aging, similar to the “natural aging” of the seeds, in contrast with the traditional procedure.

Although the accelerated aging test has been widely studied for different species, the information available from it, aiming at evaluation of the vigor of forage seeds, is old or quite scarce. Accelerated aging at 43 °C for 48 h was used by Dias et al. (2004) to monitor the physiological quality of *Brachiaria brizantha* cv Marandu seeds during storage. Cavalcante-Filho and Usberti (2008) used accelerated aging to break the dormancy of *B. brizantha* cv Marandu seeds and Mulato 1 and Mulato 2 hybrids. These authors concluded that the temperature of 43 °C for 72 h was effective in breaking dormancy. Usberti (1990) used accelerated aging to estimate storage of *B. decumbens* seeds and observed its effectiveness when conducted at 43 °C and 100% RH for 36 and sixty hours.

In this context, the aim of this study was to adjust the method of the accelerated aging test to evaluate the physiological quality of *Brachiaria brizantha* cv Xaraés seeds.

MATERIAL AND METHODS

The study was conducted in the Seed Analysis Laboratory of the *Universidade Federal dos Vales do Jequitinhonha e Mucuri* (UFVJM) in the city of Diamantina, MG. Four lots of *Brachiaria brizantha* cv Xaraés seeds were used, which were evaluated regarding physiological quality through the following determinations and tests:

Moisture content: this was determined by the laboratory oven method at 105 °C for 24 hours (Brasil, 2009).

Germination test: four replications of fifty seeds per lot, distributed on two sheets of blotting paper moistened with distilled

water in the amount of 2.5 times the weight of the substrate, were placed in a plastic box and then in a B.O.D. type germinator at a temperature alternating between 20 °C–35 °C with constant lighting. Evaluations began at seven days (first germination count) and ended at 21 days after setting up the trials, based on the criteria recommended in Brasil (2009). The germination speed index (GSI) was performed together with the germination test, counting the number of germinated seeds daily until stabilization; calculations were made according to the formula of Maguire (1962).

Seedling emergence test: four replications of fifty seeds were sown at 1 cm depth in a soil and sand substrate (1:2) in plastic trays. Moisture of the substrate was adjusted to 60% of water holding capacity. When emergence began, daily evaluations were made, computing the initial stand on the 7th day and the number of emerged seedlings until stabilization. The emergence speed index (ESI) was determined according to the formula of Maguire (1962).

Accelerated aging (traditional procedure): two hundred seeds were placed on a metal screen in a plastic box containing 40 mL of distilled water at the bottom. The plastic boxes were placed in a B.O.D. type germination chamber for 24, 48, 72 and 96 hours, at the temperature of 45 °C. After passing through each period of accelerated aging, the seeds were placed for germination. The percentage of normal seedlings was evaluated on the 21st day after sowing (Brasil, 2009). Moisture content was also determined after each period of accelerated aging (Brasil, 2009).

Accelerated aging (saturated saline solution): this was conducted as described for the traditional accelerated aging test; however, a saturated sodium chloride (NaCl) solution at the proportion of 40 g of NaCl to 100 mL of water was placed within each plastic box, which leads to relative humidity of 76% (Jianhua and McDonald, 1996).

A completely randomized experimental design (CRD) was used, with four replications. A 4 × 5 factorial arrangement (four seed lots and five accelerated aging periods) was adopted for each method, both the traditional method and the method with saturated NaCl solution. Analysis of variance was used on the data and the mean values were clustered by the Scott-Knott test at 5% probability. The result of the accelerated aging test was correlated with those previously described through simple correlation.

RESULTS AND DISCUSSION

The moisture content of the seed lots evaluated was similar, ranging from 8.34% to 9.95%, i.e., there was a variation of less than one percentage point (pp) among the lots (Table 1). This indicates that the test was in the recommended range because, according to Marcos-Filho (2015a), the test should be set up with samples whose moisture content does not vary more than two percentage points. Variations in percentage points greater than this impede standardization of any method, as well as impede obtaining uniform results among laboratories or even within the same laboratory.

The tests performed in the laboratory to obtain the initial quality of the seed lots (Table 1) did not stratify the lots, or when stratification occurred, it was only in two levels of quality, indicating similarity among the lots. This occurs because laboratory tests are performed under artificial ideal conditions, and the physiological potential of the seeds may be overestimated, which may limit ranking of the seed lots (Haesbaert et al., 2017).

Table 1. Results of normal seedlings in moisture content (MC), germination (G) (%), first germination count (FGC) (%), germination speed index (GSI), emergence (E) (%), initial stand (IS) (%) and emergence speed index (ESI), obtained from four lots of *Brachiaria brizantha* cv Xaraés seeds.

Lot	MC	G	FGC	GSI	E	IS	ESI
1	8.34	64 a	19 b	3.25 b	53 b	25 a	3.11 b
2	8.31	70 a	32 a	4.23 a	63 a	23 a	3.74 a
3	9.95	64 a	13 b	3.25 b	43 c	18 b	2.53 c
4	9.94	66 a	16 b	3.54 b	31 d	14 b	1.86 d
CV (%)	–	8.70	22.99	6.36	8.65	13.21	7.18

Mean values followed by the same lowercase letter in the column do not differ from each other by the Scott-Knott test at 5% probability.

In contrast, in the results of the tests related to seedling emergence, significant differences were observed among the lots; in the emergence test and the emergence speed index, the lots were ranked in four levels of quality.

In these results, different responses of the lots were found when they were subjected to the different specific conditions of each test. In this respect, Marcos-Filho (2015a) highlights the need to use various tests to evaluate seed vigor, due to variation in the efficiency of the procedures currently available.

Comparing exposure to traditional accelerated aging and the use of a saturated NaCl solution (Table 2), it was found that, regardless of the exposure period, the use of a saturated NaCl solution resulted in moisture contents that were considerably lower and more uniform than those observed for the seeds aged by the traditional procedure.

This occurs because, according to Jianhua and McDonald (1996), in using the saturated NaCl solution, there will be a reduction in relative humidity within the plastic box, and the seed will absorb water at a slower rate, resulting in lower and more uniform moisture contents compared to traditional accelerated aging. This was also observed by Radke et al. (2018) in chia seeds.

In addition, the use of saturated NaCl solution reduced fungal development during the test, due to restriction in relative humidity in the environment within the plastic boxes, which does not favor proliferation of microorganisms. In addition, it is probable that the salt solution releases chlorine and sodium ions to the medium and these chlorine ions have antifungal activity and contribute to a reduction in proliferation of fungi (Ávila et al., 2006). Various studies using salt solutions as an antifungal agent have proven to be effective in as in inhibition of conidia germination much as and inhibition of mycelium growth (Fallanaj et al., 2013; Youssef and Hussien, 2020). The absence of fungi during the accelerated aging test with saturated NaCl solution was also observed by Tunes et al. (2011) in ryegrass seeds.

The results of normal seedlings after the traditional accelerated aging test (Table 3) show that there was little sensitivity in classification of the lots up to 72 hours of aging, with separation into only two levels of quality.

Only after 96 hours of aging was it possible to observe differentiation of the lots into three levels of quality, such that lot 2 was of higher quality, lots 1 and 3 of intermediate quality, and lot 4 of lower quality.

Nevertheless, in spite of this differentiation, for these periods of accelerated aging of 72 and 96 hours, the decline in germination was drastic, as also observed by Oliveira et al. (2014) in physic nut and by Silva et al. (2017) in rattlepod (*Crotalaria*).

This may have occurred because, according to Marcos-Filho (2015a), long periods of traditional accelerated aging can bring about drastic conditions, making it difficult to detect significant differences in quality among seed lots. In addition, this reduction in germination may be related to the high moisture content of the seeds after aging, since seeds with a higher moisture content absorb water more rapidly, leading to deterioration, compared to seeds with lower moisture content (Morais and Rossetto, 2013; Tunes et al., 2012). Such a result can be attributed to the stress condition caused by accelerated aging, which triggers various metabolic reactions, producing what are known as reactive oxygen species (ROSs). An increase in production and accumulation of ROSs and their imbalance with antioxidant mechanisms characterize oxidative stress (Kibinza et al., 2011; Mittler, 2017). Various studies have shown the major damage that

Table 2. Moisture content of *Brachiaria brizantha* cv Xaraés seed lots under testing by traditional accelerated aging and accelerated aging with NaCl solution for 24, 48, 72 and 96 hours.

Lot	Treatments / Aging periods (hours)							
	Traditional				NaCl			
	24	48	72	96	24	48	72	96
1	14.42	15.57	15.89	20.13	8.29	9.14	10.30	10.31
2	11.90	11.35	12.22	16.74	7.95	7.05	7.95	7.77
3	17.73	19.15	19.47	24.01	10.08	11.48	11.74	11.17
4	18.87	22.38	22.58	27.71	10.24	9.88	7.74	9.63

deterioration can cause in seeds through oxidative stress, including peroxidation and degradation of membrane lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, and, at higher levels, cell death (Ahmad et al., 2012; Jeevan Kumar et al., 2015; Jyoti and Malik, 2013; Mishra et al., 2011).

Through the method that uses saturated NaCl solution (Table 3), it was possible to distinguish the lots in four levels of quality after only 24 hours – lot 2 had higher quality, followed by lots 1, 3 and 4, thus coinciding with the results of emergence and ESI testing (Table 2).

Use of the saturated NaCl solution led to lower seed deterioration, even after 96 hours of aging. This occurs because the use of this solution reduces the speed of water absorption by seeds, affecting seed vigor in a milder way, thus obtaining less drastic and more uniform results compared to the traditional method. Yet the effectiveness of the test is not diminished.

Consistent results using this procedure for seed vigor evaluation were obtained by Tunes et al. (2013) in parsley seeds, by Lemes et al. (2015) in Bermuda grass, and Radke et al. (2016) in coriander.

The effectiveness of the traditional accelerated aging test or the saturated saline solution test for seed vigor evaluation was also observed through the correlation between the results of the accelerated aging test and the results obtained in evaluation of seed lot quality, especially when correlated with the seedling emergence test (Figure 1). The correlation of data of a vigor test with seedling emergence in the field is crucial for the test to be considered effective, since a vigor test should not only classify lots into different vigor classes, but should also be as near as possible to results of seedling emergence in the field (Marcos-Filho, 2015a; Melo et al., 2017).

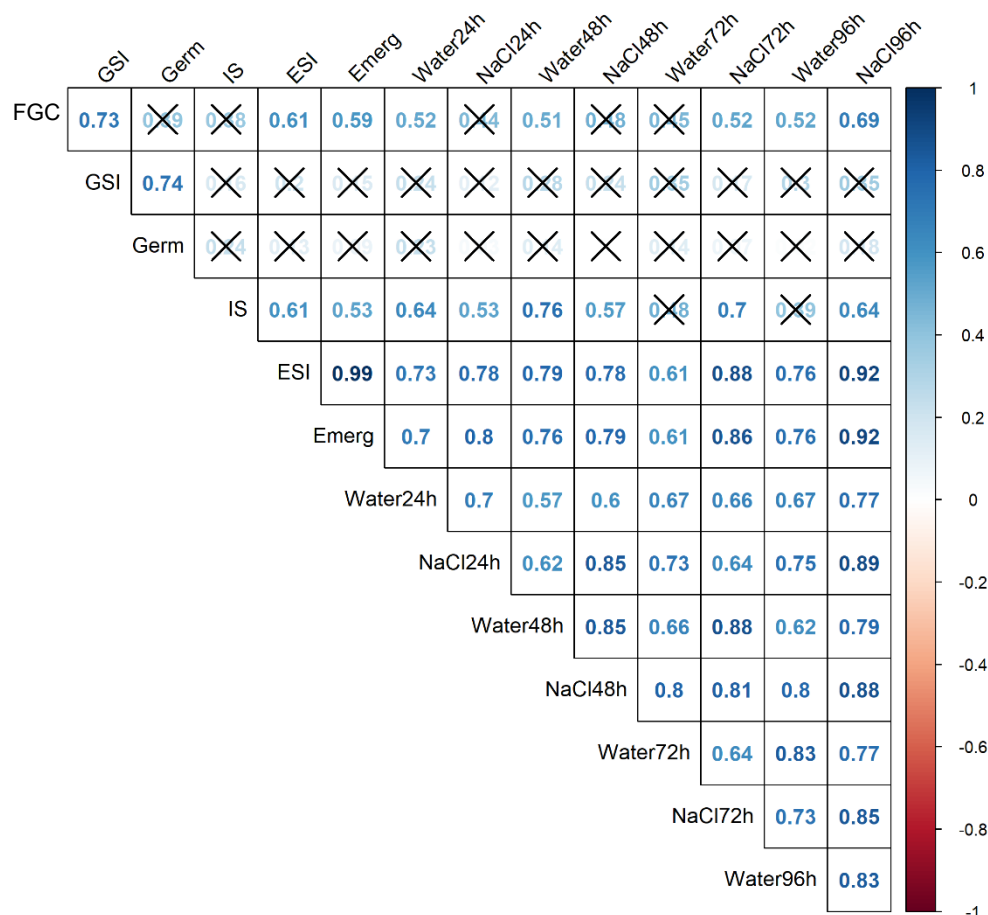
Nevertheless, due to wide variation in the moisture content of the samples when using the traditional accelerated aging test, the accuracy of the results was negatively affected, advising against the use of this method for evaluation of seed physiological quality. This same result was observed by Gordin et al. (2015), who evaluated the physiological quality of *Guizota abyssinica* seeds and did not recommend the use of traditional accelerated aging, even when it stratified the lots in vigor levels or correlated with emergence; the author suggested using the saturated saline solution.

In this respect, from the results observed in this study, it can be said that the method of accelerated aging with saturated NaCl solution was effective in detecting subtle differences among seed lots with similar germination. Thus, the period of 24 hours of aging with the use of a saturated NaCl solution proved to be more adequate since it can be performed in a shorter period, a desirable characteristic in a seed vigor test, as this allows energy savings in equipment and provides results in a faster way.

Table 3. Results of normal seedlings (%) obtained in the germination test by *Brachiaria brizantha* cv Xaraés seeds under different periods of traditional accelerated aging and accelerated aging with saturated NaCl solution.

Lot	Treatments / Aging periods (hours)							
	Traditional				NaCl			
	24	48	72	96	24	48	72	96
1	52 b	49 a	37 a	27 b	51 b	55 a	58 a	40 b
2	63 a	46 a	44 a	45 a	62 a	57 a	58 a	52 a
3	51 b	26 b	25 b	22 b	42 c	27 b	34 b	30 c
4	36 c	27 b	26 b	15 c	31 d	21 b	18 c	22 c
CV (%)	15.08				16.07			

Mean values followed by the same letter in the column do not differ from each other by the Scott-Knott test at 5% probability.



Values marked with an "X" do not have significant correlation at 5% probability by the t-test.

Figure 1. Simple Pearson correlation (r) between the variables of first germination count (FGC), germination speed index (GSI), germination (G), initial stand (IS), emergence speed index (ESI), emergence (E) and the different procedures of the accelerated aging test performed in *Brachiaria brizantha* cv Xaraés seeds.

CONCLUSIONS

The accelerated aging test allows separation of *B. brizantha* cv Xaraés seed lots by the method with saturated NaCl solution for 24 hours.

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