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RESEARCH ARTICLE

METHODOLOGICAL ADJUSTMENTS TO THE VIGOR TESTS IN *BRACHIARIABRIZANTHA* MARANDU CULTIVAR SEEDS

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ABSTRACT

The objective of this study was to adjust the accelerated aging and electrical conductivity tests, to evaluate the physiological potential of lots from *Brachiariabrizantha* Marandu cultivar seeds, represented by four lots. For the accelerated aging test, seeds were submitted to traditional accelerated aging and with saturated NaCl solution and five periods of aging, 0, 24, 48, 72 and 96 h. For the electrical conductivity test, seeds were submitted to 12 periods of imbibitions in water for 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 h, using samples of 25 seeds in 25 mL and 50 mL, 50 seeds in 50 mL and 75 mL of water. To characterize the profile of lots, were determined moisture content, germination tests, first count of germination, speed of germination, initial stand, emergence, and speed of emergence index. The accelerated aging test makes possible the separation of lots of *B. brinzatha*cv Marandu by the method with saturated NaCl solution for 72 h. For the electrical conductivity test, the imbibition period during 14 h using 50 seeds in 50 mL to cv Marandu is appropriate for evaluating the seed physiological potential.

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INTRODUCTION

Brazil has the fourth higher area with pasture in all world (Anualpec, 2011). There are in the country approximately 60 millions of hectares of natural pastures and 120 millions of cultivated pastures, of which 8 millions are renewed annually. This total area with pasture is more than three times the used for the production of grains that actually overcome 50 millions of hectares (Souza, 2012). The quality of seeds used to the implantation of pastures becomes of huge importance, once the technological level of properties it is increasing. The germination test is the official procedure to evaluate the capacity of seeds in produce normal seedlings in ideal conditions, but not always reveals the difference of development between lots of seeds during the storage or in field (Carvalho and Nakagawa, 2012). In this way, it is important to evaluate the vigor of seeds like complement to the information provided by the germination test. Among the tests already in use, the accelerated aging and the electrical conductivity tests are the most studied to various cultivated species, mainly cause they present efficiency in the comparison of vigor and in the estimation of storage potential of seeds lots, presenting good relation with the emergence of seedlings in field (Vieira and Krzyzanowski, 1999; Marcos Filho, 1999). The accelerated aging test is based on the fact that seeds with higher vigor are more tolerant to the relative humidity and high temperatures after a period of exposition, evaluating the answer of seeds through the germination test (Marcos Filho, 1999). The electrical conductivity test is based in lower speed of structuration of membranes in seeds less vigorous when soaked in water. Higher liberation of exudates to the exterior of cell happens and however higher conductivity electric (Marcos Filho, 2005). With this, is considered the vigor of seeds

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inversely proportional to the reading of electric conductivity (Vieira and Krzyzanowski, 1999). According with Rodrigues, *et al*. (2006) and Panobianco *et al.* (2007) many are the factors that can affect the results of the electrical conductivity test, between them, the quality and quantity of water used for immersion, the period of immersion, the moisture, mass and quantity of seeds, the age and the integrity of seeds membranes, the genotype and the temperature. The objective with this study is to adequate the methodology of the accelerated aging and the electrical conductivity tests to the evaluation of physiological quality of *Brachiariabrizantha* cv Marandu.

MATERIALS AND METHODS

Were used four lots of *Brachiariabrizantha* cv. Marandu seeds, which had their physiological quality, evaluated by the following determinations and tests:Moisture content: determined by the oven method at 105° C for 24 hours using five replicates of 50 seeds from each treatment (BRASIL, 2009). The results were expressed in percentage. In the germination test were used four replications of 50 seeds per lot. Seeds were distributed between blotter paper moistened with distilled water. Was used the amount equivalent to 2,5 times the weight of dry paper. Following, the gearboxes were kept in germinator type B.O.D regulated to the alternating temperature of20 ºC-35 ºC, with illumination present during the period of 8h associated with higher temperature. The evaluation was realized at twenty-first day after the installation of the test by determining the percentage of normal seedlings (BRASIL, 2009). The first count was realized together to the germination test, by determining the percentage of normal seedlings at seventh day after the test installation. At the end of the test, seeds that did not germinate were submitted to tetrazolium test (ISTA, 2008), and the viable seeds were added to the final count of the germination test. The germination speed index (GSI) was done at the same time of the germination test, computing the number of seeds that germinates daily, until the stabilization, according Maguire (1962). Seedling emergence test - four replicates of 50 seeds per lot were utilized, distributed in plastic trays containing the substrate soil and sand in the proportion of (1:2), with 1cm of depth. The moisture of substrate was adjusted to 60% of retention capacity. When the emergence started, were realized daily evaluations, computing the initial stand at seventh day and the number of emerged seedlings until the stabilization. The emergence speed index (ESI) was determined according Maguire (1962). Accelerated aging (traditional procedure): 200 seeds were distributed so as to form a simple layer over the surface of the metallic screen suspended inside each plastic box (internal compartment), containing 40 mL water. The boxes covered with lids, remained inside the chamber during five aging periods (24, 48, 72, 96h and without aging), using the temperature of 45°C. Seeds were then placed for germination according to methodology described for the germination test. The evaluation was performed twenty one days after sowing and the results were expressed as mean percentage of normal seedlings for each lot. In order to monitor the test, seed moisture content was measured before and after the aging periods (Brasil, 2009). Accelerated aging (saturated salt solution): was conducted in a way similar as described for the traditional test, except that the bottom of each plastic box (individual compartment) received 40 mL of a saturated NaCl solution, as a replacement for water. This solution had the proportion of 40 g NaCl 100 mL^{-1} water, thus establishing an environment of 76% relative humidity. Electrical conductivity: were evaluated the effect of imbibition periods (2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 e 24 h) and of the combination of seeds quantity in volume of deionized water (25 seeds in 25 mL; 25 seeds in 50 mL; 50 seeds in 50 mL; 50 seeds in 75 mL). The test was carried out with four replicates of 50 seeds for each lot. The periods of seeds imbibition were defined after pretesting and according with imbibition curve of seeds in water. Seeds were weighed in balance with 0,001g of precision, placed into plastic cups containing deionized water and kept in germination chamber for each imbibition period, at 25ºC. The reading of electric conductivity was realized in condutivimeter DIGIMED®, model DM-21 and the mean values, for each lot, were expressed in µS/cm/g of seeds. Was used the completely randomized design (DIC) with four replications. To the accelerated aging test, was adopted the factorial scheme 4 x 5 (four lots of seeds and five periods of aging) for each method, traditional or with saturated solution of NaCl. For the electrical conductivity test, was adopted the factorials scheme of 4 x 12 x 4 (four lots of seeds, 12 periods of imbibition and 4 treatments combining the number of seeds and the water volume). The data were submitted to analysis of variance and the means were compared between each other by Scott-Knott test at 5% probability. Statistical analyzes were performed with the aid of SISVAR® statistical program.

RESULTS AND DISCUSSION

The moisture content of seeds, presented similarities for the 4 studied lots, being equal to 10%. The moisture content of seeds is of extreme importance for the standardization of any method, as well as in the obtainment of uniform results between laboratories and inside the same laboratory. Seeds with higher moisture content have more chances to start the process of deterioration quickly when in conditions of accelerated aging, in comparison to seeds with lower moisture content (Marcos Filho, 2005). Was observed according the results of germination test, first count of germination and GSI (Table 1), that the lots 1 and 4 are of superior quality and the lot 2 and 3 of inferior quality. For the emergence test can be observed the superiority of lot 1, followed for the lots 4, 3 and 2 respectively, differentiating the lots in four level of quality. With the test of initial stand was possible to differentiate the lots in two levels of quality, confirming the inferiority of lot 2, and the superiority of lots 1, 3 and 4. Examining the results of ESI, was possible to distinguish the lots in three levels of quality, being the lot 1 of higher quality, the 4 and 3 intermediate and the lot 2, inferior. After the exposition to the traditional accelerated aging and with the use of saturated solution of NaCl (Table 2), was verified that, independently of the period of exposition, the use of saturated solution of NaCl result in lower and uniform contents of water in comparison to the observed for seeds aged by the traditional procedure. According Jianhua and McDonald (1996), with the use of saturated solution of NaCl will occur a reduction in relative humidity inside the gerbox, so that the seed will absorb water in lower speed, resulting in degrees of humidity inferiors and uniforms, when compared to the traditional accelerated aging, promoting lower effects in seeds and without reduce the efficiency of test.

Table 1.Germination (G), first count (FC), germination speed index (GSI), emergence (E), initial stand (IS), and emergence speed index (ESI) of 4 lots of *Brachiariabrizantha* cv Marandu seeds

Lots	G (%)	FC (%)	GSI	E (%)	IS (%)	ESI
1	81 a	58 a	5,07 a	80 a	33 a	10,07 a
2	55 b	33 b	3,53 b	16 d	3 b	1,25 c
3	58 b	34 b	3,56 b	45 c	22 a	6,17 b
4	77 a	52 a	5,39 a	65 b	18 a	5,94 b
CV (%)	14,20	19,37	12,99	9,50	24,85	15,42

Means followed by the same lower case letter in the column do not differ by the Scott-Knott test at the 5% level.

Table 2. Moisture content (%) in different periods of accelerated traditional aging and with saturated solution of NaCl of *Brachiariabrizantha*cv Marandu seeds

Periods of aging (hours)	Treatments	
	Traditional	NaCl
Time zero	10 A	10 A
24	14 A	10 A
48	15 B	10 A
72	16 B	10 A
96	17 B	12 A
CV (%)	9,71	

Means followed by the same capital letter in the column do not differ by the Scott-Knott test at 5%.

Table 3. Germination (%) of *Brachiariabrizantha*cv Marandu seeds, submitted to different periods of accelerated traditional aging and with saturated solution of NaCl

Lots	Treatments / Periods of aging (hours)									
	Traditional					NaCl				
	0	24	48	72	96	0	24	48	72	96
1	81 aA	76 aA	80 aA	76 aA	85 aA	81 aA	72 aA	75 aA	77 aA	77 aA
2	55 aB	60 aA	46 bB	39 bB	48 bB	55 aB	31 bB	25 bC	29 bD	18 bC
3	58 aB	68 aA	58 aB	44 bB	45 bB	58 aB	61 aA	46 aB	51 aC	54 aB
4	77 aA	72 aA	81 aA	69 aA	47 bB	77 aA	73 aA	71aA	66 bB	59 bB
CV (%)	12,38									

Means followed by the same lower case letter in the line and capital letter in the column do not differ by the Scott-Knott test at the 5% level.

Table 4.Electrical conductivity test (µS/cm/g) of *Brachiariabrizantha* cv Marandu seeds in function of the treatments: 25 seeds in 25 mL of deionized water, 25 seeds in 50 mL of deionized water, 50 seeds in 50 mL of deionized water and 50 seeds in 75 mL of deionized water and the periods of immersion

Trat	Lots	Periods of immersion (hours)											
		2	4	6	8	10	12	14	16	18	20	22	24
25/25	1	47 aA	61 aA	66 aA	72 aA	76 aA	77 aA	110 bA	121 bA	124 bA	125 bA	129 bA	130bA
	2	171 aC	188 aC	190 aC	193 aC	201 bC	208 bC	218 bC	228 bB	234 cC	241 cC	258 dC	265 dC
	3	126 aB	143 aB	153 bB	163 bB	170 bB	171 bB	199 cB	204 cB	206 cB	208 cB	209 cB	209 cB
	4	145 aB	162 aB	168 aB	169 aB	174 aB	175 aB	182 bB	197 bB	188 bB	189 bB	184 bB	199 bB
25/50	1	18 aA	23 aA	26 aA	29 aA	32 aA	33 aA	39 bA	44 bA	46 bA	47 bA	47 bA	49 bA
	2	82 aB	93aB	99 aC	101 aC	105 aC	106 aC	106 aC	106 aC	107 aC	108 aC	108 aC	110 aC
	3	61 aB	72aB	79 aB	85 bB	91 bC	91 bC	95 bC	98 bC	102 bC	106 bC	109 bC	110 bC
	4	68 aB	72 aB	70 aB	70 aB	73 aB	73 aB	74 aB	75 aB	78 aB	79 aB	81 aB	82 aB
50/50	1	22 aA	26 aA	29 aA	32 aA	35 aA	35 aA	37 aA	42 aA	44 aA	44 aA	44 aA	44 aA
	2	91 aC	100 aC	102 aC	104 aC	111 aC	121 aC	128 bD	137 bD	143 bD	148 bD	149 bD	156 bD
	3	85 aC	96 aC	100 aC	101 aC	102 aC	102 aC	102 aC	104 aC	105 aC	108 aC	110 aC	111 aC
	4	64 aB	69 aB	72 aB	72 aB	75 aB	76 aB	76 aB	77 aB	77 aB	78 aB	79 aB	86 aB
50/75	1	14 aA	17 aA	19 aA	21 aA	23 aA	24 aA	25 aA	28 aA	28 aA	29 aA	29 aA	27 aA
	2	55 aB	56 aB	56 aB	58 aB	58 aB	62 aB	73 bB	78 bB	86 bB	86 bB	89 bC	91 bC
	3	51 aB	58 aB	62 aB	66 aB	69 aB	69 aB	75 aB	79 aB	78 aB	77 aB	80 aC	83 aC
	4	39 aB	40 aB	42 aB	42 aA	42 aA	43 aA	44 aA	47 aA	49 aA	50 aA	52 aB	52 aB
CV (%)		16,22											

Means followed by the same lower case letter in the line and capital letter in the column do not differ by the Scott-Knott test at the 5% level

This fact was also observed by Silva, *et al*. (2010), in seeds of bermudagrass, Souza *et al.* (2009), with black oat, Tunes *et al.* (2011), in ryegrass and Torres *et al*. (2009) in melon. Besides this, the use of saturated solution of NaCl, reduced the development of fungi during the test, due to the restriction of relative humidity in the environment inside of the plastic boxes, what does not favor the proliferation of microorganisms. It is probable that the saline solution releases chlorine and sodium ions to the environment and these ions of chlorine by having antifungal action, contributes to the reduction of proliferation of fungi (Ávila *et al.,* 2006).The absence of fungi during the accelerated aging test with saturated solution of NaCl was also

observed by Tunes, *et al*. (2011), in seeds of ryegrass, Souza *et al.* (2009), in black oat and Silva *et al*. (2010), in seeds of Bermuda grass. Analyzing the results of traditional accelerated aging test (Table 3), it can be observed that, after 24 hours of aging there was no differentiation of lots, however, was observed an increase of percentage of seeds germination from lots 2 and 3, when compared to the time zero. This increase was not statistically significant, however was sufficient to match statistically the quality of lots after 24 hours of aging. According Meschede *et al*. (2004), the accelerated aging is a technology efficient to overcome the dormancy of *B. brizantha*cv Marandu seeds, however the period of exposition can varies with the initial quality of lot. Still on Table 3, after 48 and 72 hours of aging by the traditional method, was observed the superiority of lots 1 and 4 and the inferiority of lots 2 and 3, resembling to the results presented on germinations test, first count of germination and GSI (Table 1). After 96 hours of aging, it is confirmed the superiority of lot 1 in relation to the other lots. According Marcos Filho (1999), long periods of traditional accelerated aging can cause very drastic conditions, capable to difficult or impede the detection of significant differences of quality between the seeds lots. This was also observed by Silva *et al*. (2010), in seeds of Bermuda grass. In the accelerated aging test with saturated solution of NaCl (Table 3), was observed after 24 hours of aging, the inferiority of lot 2, how was observed by the tests of emergence, initial stand and ESI (Table 1). After 48 hours of aging was possible to differentiate the lots in three levels of quality, being the lots 1 and 4 of superior quality, the lot 3 of intermediate quality and the lot 2 like inferior quality. Examining the results after 72 hours, was possible to stratify the lots in four levels of quality, being the lot 1 of superior quality, followed by the lots 4, 3 and 2, respectively. These results coincide with the results obtained in the emergence test (Table 1). The period of 96 h of aging also classified the lots in three levels of quality, being the lot 1 of superior quality, followed by the lots 3 and 4 intermediates and the lot 2 as inferior quality. The superiority of lot 1 was detected in the emergence test and ESI, as well as the inferiority of lot 2 that beyond these tests was detected yet, by the initial emergence (Table 1).

The use of saturated solution of NaCl, when compared to the use of pure distillated water, decreased the absorption of water by the seeds (provides a lower relative humidity) and the process of deterioration, even in front of a methodology that causes a stress in seeds with the use of high temperature (Tunes *et al*., 2011). Lopes *et al.* (2009), Silva *et al*. (2010), and Fanan *et al*. (2006), working with ryegrass, Bermuda grass and wheat seeds, respectively, obtained the same conclusion. To the establishment of preconditioning periods for the realization of electrical conductivity test, was realized a pre-test with an imbibition curve to four lots of seeds, where it was observed the three-phase standard of imbibition for all lots, with fast imbibition in the beginning of the process and root protrusion with 24 hours. According the data can be conclude that the maximum time to be used on the electrical conductivity test, must be 24 hours, since this was the period necessary to occur the beginning of root protrusion of seeds. The electrical conductivity (Table 4) presented significant triple interaction ($p < 0,05$).

Observing the Table 4, it can be realized that during the periods of immersion, there is an increase of quantity of electrolytes released by seeds, demonstrating the influence of immersion period of seeds on the quantity of solutes leached into the solution. This was also reported by Paiva *et al*. (2008) for macrotyloma seeds, Machado *et al*. (2011), for ground pea seeds and Vazquez *et al.* (2011), for sorghum seeds. In the combination of 25 seeds in 25 mL of water, after two hours of immersion, was possible to observe that lot 1 was superior, lot 3 and 4 intermediate and the lot 2 inferior. During all the period of immersion the classification of lots in three levels of quality, being the lot 1 the superior quality, the 3 and 4 of intermediate quality and the lot 2 inferior, was maintained until the 24h, period final of testIn the combination of 25 seeds with 50 mL and 50 mL seeds with 75 mL, only after 6h and 22h of immersion respectively, it was possible the separation of lots in three levels of quality. This occurs due to the initial liberation of electrolytes be intense in the beginning of the immersion of seeds in water, becoming difficult the distinction of quality between the lots (Vieira and Kryzanowski, 1999). In the treatment involving 50 seeds in 50 mL of water after two hours of immersion, already was possible to observe that the lot 1, presented higher physiological potential. However, the period of 2 hours was not enough to point significant differences of vigor between the lots 2 and 3. After 14 hours of immersion, was possible to classify the lots in four levels of quality, being the lot 1 of higher quality, followed by the lots 4, 3 and 2 respectively. According Menezes *et al*. (2007), with the increase of the period of immersion of seeds, the quantity of leachate released by the seeds more vigorous will be stabilizing, between others facts, by the reorganization of membranes, favoring the classification of lots in level of quality. Lopes and Franke (2010), working with ryegrass seeds, also found better distinction of lots using 50 seeds in 50 mL.

Conclusion

The accelerated aging test allows the distinction of *B. brinzatha* cv Marandu lots by the method with saturated solution of NaCl for 72 hours. For the electrical conductivity test, the period of imbibition of 14h, using 50 seeds in 50 ml is the better to the evaluation of physiological potential of seeds.

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