

Development of methodology to test the electrical conductivity of Marandú grass seeds¹

Desenvolvimento de metodologia para o teste de condutividade elétrica para sementes de capim-marandú

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ABSTRACT - The electrical conductivity test enables the evaluation of seed vigor for a maximum period of 24 hours, but the need to count the seeds to obtain a sample for analysis makes it difficult to apply this test to small seeds, such as forage grasses. The objective of this study was to establish a practical methodology for testing the electrical conductivity of *Brachiaria brizantha* (Hochst. Ex A. Rich.) Stapf 'Marandú' seeds and to estimate its effect on seedling emergence in the field. The water content, germination, first germination count, first emergency count, percentage, and rate of seedling emergence speed in the laboratory, seedling emergence in the field, and electrical conductivity of eight batches of seeds was analyzed. In the last test, the periods of conditioning (2, 4, 6, 8, and 24 hours) and the volumes (50 and 75 mL) of distilled water used were evaluated and a new sampling methodology based on the seed volumes, obtained with the aid of a 1.5 ml plastic tube (Eppendorf), was developed. The experimental design was completely randomized, with four replications. The electrical conductivity test using volume sampling from seeds immersed in 75 mL of water and readings taken after 2 or 4 hours was the most efficient method for evaluating the vigor of marandú grass seed batches and provided information equivalent to seedling emergence in the field.

Key words: *Brachiaria brizantha*. Forage grasses. Physiological quality. Vigor tests.

RESUMO - O teste de condutividade elétrica permite a avaliação do vigor das sementes no prazo máximo de 24 horas, porém a necessidade de contar as sementes para obter a amostra para a análise dificulta a aplicação deste teste em sementes pequenas, como de gramíneas forrageiras. Objetivou-se neste trabalho estabelecer uma metodologia prática para o teste de condutividade elétrica de sementes de *Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf. cv. Marandú visando a estimativa do vigor para a emergência de plântulas em campo. As sementes de oito lotes foram submetidas às seguintes avaliações: teor de água, germinação, primeira contagem de germinação, primeira contagem de emergência, porcentagem e índice de velocidade de emergência de plântulas em areia no laboratório, emergência de plântulas em campo e condutividade elétrica. Neste último teste foram avaliados os períodos de condicionamento (2; 4; 6, 8 e 24 horas), o volume de água destilada (50 e 75 mL) e testou-se nova metodologia de amostragem baseada no volume de sementes, obtida com o auxílio de um tubo de plástico de 1,5 mL (Eppendorf). O delineamento experimental utilizado foi o inteiramente casualizado, com quatro repetições. O teste de condutividade elétrica utilizando o método de amostragem por volume, de sementes imersas em 75 mL de água e leituras após duas ou quatro horas é eficiente na avaliação do vigor de lotes de sementes de capim-marandú, fornecendo informações equivalentes à emergência de plântulas em campo.

Palavras-chave: *Brachiaria brizantha*. Gramíneas forrageiras. Qualidade fisiológica. Testes de vigor.

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INTRODUCTION

Brachiaria brizantha 'Marandú' is the most widely cultivated commercial forage in Brazil; more than 60% of the cultivated pastures grow this plant and it representing 90% of the volume of fodder seeds traded in the country (BISCOLA; PEREIRA; COSTA, 2013; VERZIGNASSI *et al.*, 2012).

The physiological quality of the commercial seed batches of forage grasses is characterized by the germination test (BRASIL, 2008). However, because it is conducted under ideal conditions in the laboratory, there are limitations to this test when the objective is to estimate the emergence potential of seedlings in the field, especially under unfavorable environmental conditions (SENA; ALVES; MEDEIROS, 2015).

Compared with the germination test, the vigor tests provide more sensitive indexes of the physiological potential. As the degradation of cell membranes constitute one of the initial events in the seed deterioration process, the electrical conductivity test would theoretically be the most sensitive to estimate vigor (MATTHEWS; POWELL, 2006). This test is based on the determination of the amount of leachate in the seed imbibition solution, with the highest values corresponding to higher release of exudates and lower vigor (ARAUJO *et al.*, 2011; STEINER *et al.*, 2011).

However, several factors such as water volume, time, and temperature of the soaking solution, initial seed water content, and the number of seeds in the sample can affect the results of the electrical conductivity test. (ARAUJO *et al.*, 2011; DUTRA; MEDEIROS FILHO; TEÓFILO, 2006; LOPES; FRANKE, 2010; MACHADO *et al.*, 2011; NOGUEIRA *et al.*, 2013). These factors and the characteristics of the seeds have led technologists and researchers to try to better customize their methodology for use on the seeds of different species.

In some studies, the effects of using different volumes of deionized water for seed imbibition were evaluated. The best results were obtained with 50 mL for ryegrass and white oats (LOPES; FRANKE, 2010; SPONCHIADO; SOUZA; COELHO 2014), 75 mL for popcorn kernels (RIBEIRO *et al.*, 2009) and 100 mL and 100 mL for millet and black oats (GASPAR; NAKAGAWA, 2002; NOGUEIRA *et al.*, 2013).

The conventional method for testing electrical conductivity recommends a period of 24 h for imbibition, as verified for triticale seeds, a winter forage grass (STEINER *et al.*, 2011). However, this period may be shorter for some species with smaller seeds, such as 16 h for cowpeas and black oats (DUTRA; MEDEIROS FILHO; TEÓFILO, 2006; NOGUEIRA *et al.*, 2013), 6 h

for radish (NERY; CARVALHO; GUIMARÃES, 2009), 3 h for mung-green beans (ARAUJO *et al.*, 2011), 2 h for white oats (SPONCHIADO; SOUZA; COELHO, 2014) and 1 h for ryegrass (LOPES; FRANKE, 2010).

Most studies have used four replicates of 25, 50, or 100 seed samples (ARAUJO *et al.*, 2011; DUTRA; MEDEIROS-FILHO; TEÓFILO, 2006; LOPES; FRANKE, 2010; NOGUEIRA *et al.*, 2013; STEINER *et al.*, 2011). Additionally, it may be difficult for the seed analysis laboratories to work with such small seeds, as those of forage grasses.

Thus, one option would be to use a standard measure based on volume and a sufficient number of seeds per sample, making the test faster and more practical.

Thus, the aim of this study was to establish a practical methodology to test the electrical conductivity of *Brachiaria brizantha* 'Marandú' seeds to estimate their vigor, which determines the emergence of seedlings in the field.

MATERIAL AND METHODS

In this study, eight batches of Marandú grass seeds, collected from six municipalities of the main producing states of the country, were evaluated and are described in Table 1.

The seeds were sent to the Laboratory of Seed Analysis, Faculty of Agrarian and Veterinary Sciences - UNESP, Campus Jaboticabal-SP, and subjected to the following tests and analyses:

Water content - this was determined using the oven drying method at 105 ± 3 °C, for 24 h (BRASIL, 2009).

Germination test - this was conducted with four samples of 100 seeds each, seeded on two sheets of filter paper moistened with water (an amount equivalent to 2.5 times the mass of the dry paper), in transparent acrylic boxes (11.0 × 11.0 × 3.5 cm), maintained at 20-35 °C. The seedlings were considered normal when their plumule had emerged from the coleoptile and they showed a primary root with a minimum length of 0.5 cm length. Seed counts of germination were performed weekly until the 21 st day after sowing (BRASIL, 2009).

For the detection of dormancy, the remaining seeds from the germination test were submitted to the tetrazolium test. For this, they were sectioned longitudinally and medially through the embryo and one of the seed halves was immersed in 0.075% tetrazolium solution and incubated for 2 h at 41 °C (± 3 °C) in the absence of light (DELOUCHE *et al.*, 1976). Subsequently,

Table 1 - Geographical information and description of the provenance sites of the eight batches of *Brachiaria brizantha* 'Marandú'

Batches	Provenance sites	Latitude (S)	Longitude (W)	Altitude (m)
1	Quirinópolis - GO	20°41'08"	50°33'17"	480
2	Chapada Gaúcha - MG	15°28'06"	45°25'06"	871
3	Tupaciguara - MG	18°35'32"	48°42'18"	896
4	Chapadão do Ceú - GO	18°23'34"	52°39'57"	831
5	Uruana de Minas - MG	16°03'51"	46°15'15"	550
6	Chapada Gaúcha - MG	15°28'06"	45°25'06"	871
7	Luís Eduardo Magalhães -BA	12°05'58"	45°47'54"	765
8	Monte Alegre de Minas - MG	18°52'13"	48°52'31"	732

the seeds were washed in distilled water and the reading was taken immediately, with the seeds being classified as viable (dormant) and non-viable (dead), and the data expressed as a percentage (BRASIL, 2009).

Vigor tests:

First germination count - this was carried out in conjunction with the germination test, with normal seedling counts obtained on the fourth day after the tests were initiated, and the data was expressed as a percentage (GASPAR-OLIVEIRA *et al.*, 2008).

Seedling Emergence in sand - this was conducted with four subsamples of 50 seeds each, which were sown 2 cm deep in moist sand in plastic boxes (26.0 × 17.0 × 5.0 cm) and maintained at 28±3 °C. The percentage of seedlings that emerged from the 4th to the 30th day after sowing was determined.

Primeira contagem de emergência de plântulas em areia - realizada conjuntamente com o teste de emergência de plântulas em areia, contabilizando-se a porcentagem de plântulas emersas no quarto dia após a semeadura (GASPAR-OLIVEIRA *et al.*, 2008).

Speed of seedling-emergence index in sand - this was conducted in conjunction with the test of seedling emergence in sand from the 4th to the 30th day after sowing; the number of emerged seedlings per day, applying the formula proposed by Maguire (1962), was determined.

Electrical conductivity test - Four subsamples of 1.5 mL of seeds per batch, were quantified with the help of a plastic tube (Eppendorf) and weighed in a scale with a precision of 0.0001 g (Figure 1).

The sampled seeds were counted to determine the average number of seeds per sample, soaked in plastic cups containing 50 and 75 mL of distilled water at 25 °C, and the readings taken after 2, 4, 6, 8, and 24 h. Electrical conductivity was calculated in $\mu\text{S cm}^{-1} \text{g}^{-1}$.

Figure 1 - Use of a 1.5 mL tube to quantify seed samples of *B. brizantha* 'Marandú'. View of the tube in process of obtaining the seed sample (A), tube full of Marandú grass seeds (B), removal of the seeds that extrapolate the edge of the sample tube border with a ruler (C), and sample wrapping inside the cup before adding water for the electrical conductivity test (D)



Seedling emergence in the field - four subsamples of 50 seeds in 1.5 m long furrows, spaced 0.2 m apart, at a depth of 2 cm, were used in this experiment. They were sown in the third week of June 2015, with counts performed daily from the 4th to the 21st day after sowing and the results were expressed as a percentage (OLIVEIRA *et al.*, 2014). During the test conduction period, the average

maximum and minimum daily ambient temperatures in the field were 31 ± 3 and 18 ± 3 °C, respectively.

The design was completely randomized, with the comparison of means done using the Scott-Knott test at a 5% probability level. A Pearson's correlation coefficient was also determined between the values obtained in the germination, vigor, and emergence tests in the field. For the interpretation of correlations, the following criterion was followed based on Figueiredo Filho and Silva Júnior (2009): $r = 0.10$ to 0.30 (weak correlation); $r = 0.40$ to 0.60 (moderate); $r = 0.70$ to 1 (strong).

RESULTS AND DISCUSSION

The initial water content of the Marandú grass seed batches was between 10 and 12% (Table 2), which

is considered uniform as the maximum variation was 2%. This similarity is essential for vigor tests so that the tests are not affected by differences in metabolic activity and physiological performance of the seeds caused by differences in hydration levels (COIMBRA *et al.*, 2009; OLIVEIRA *et al.*, 2014; SENA; ALVES; MEDEIROS, 2015).

The average number of seeds per sample obtained in the plastic tubes was between 112 and 117 seeds, depending on the batch evaluated (Table 2). The variation of only four seeds between the batch samples and a low coefficient of variation (1.41%) for this variable allowed us to infer that the adopted methodology allowed for an efficient standardization and reproducibility in terms of obtaining samples. The uniformity of seed size in the different batches allowed the use of volume of plastic tubes in the sampling methodology.

Table 2 - Water content (WC), seedling emergence in the field (EF), germination (G), first germination count (FC), dormant seeds (D), dead seeds (DS), seedling emergence in sand (ES), first count of seedling emergence in sand (FCS), speed of seedling emergence index (SEI), seed electrical conductivity in 50 (EC₅₀) and 75 mL of water (EC₇₅), and number of seeds contained in 1.5 mL plastic tubes (no. of seeds) used in the electrical conductivity test of eight batches of Marandú grass seeds

Tests	Batches*								CV (%)
	1	2	3	4	5	6	7	8	
WC (%)	12,0	10,6	10,0	10,5	11,3	11,3	12,0	11,1	-
EF (%)	68 a	74 a	56 b	59 b	55 b	48 c	46 c	39 d	10,41
G (%)	71 a	72 a	70 a	75 a	70 a	68 a	60 b	60 b	8,55
FC (%)	36 a	25 b	35 a	27 b	23 c	10 d	22 c	23 c	21,44
D (%)	21 b	12 a	20 b	12 a	11 a	11 a	21 b	19 b	9,62
DS (%)	8 a	16 b	10 a	13 a	19 b	21 b	19 b	21 b	20,15
ES (%)	64 a	70 a	69 a	70 a	59 a	62 a	43 b	43 b	13,74
FCS (%)	24 b	28 b	35 a	14 c	7 d	28 b	10 c	12 c	14,98
SEI	8,43 a	8,11 a	8,63 a	7,13 b	3,83 c	7,44 b	2,84 c	2,92 c	11,43
EC ₅₀ 2 h	13 a	18 b	21 c	16 b	31 d	23 c	52 f	48 e	8,65
4 h	18 a	22 b	24 b	22 b	41 d	27 c	61 e	57 e	7,94
6 h	22 a	24 a	27 a	26 a	45 c	33 b	66 d	67 d	6,85
8 h	24 a	26 a	30 b	29 b	50 d	34 c	72 e	73 e	5,78
24 h	30 a	32 a	39 b	38 b	97 c	41 b	125 d	133 e	6,75
EC ₇₅ 2 h	9 a	10 a	16 b	14 b	20 c	22 c	30 d	30 d	9,47
4 h	12 a	13 a	19 b	17 b	25 c	24 c	39 d	38 d	8,14
6 h	15 a	15 a	23 c	19 b	29 d	27 d	43 e	42 e	7,13
8 h	17 a	17 a	25 c	23 b	33 e	29 d	46 f	45 f	8,59
24 h	25 a	23 a	30 c	27 b	58 e	35 d	82 g	72 f	4,39
No seeds	115 a	115 a	117 a	112 b	115 a	114 a	116 a	112 b	1,41

Means followed by the same letter on the wire do not differ from each other by the Scott-Knott ($P < 0,05$); * Batches 1 (Quirinópolis - GO); Batches 2 (Chapada Gaúcha - MG); Batches 3 (Tupaciguara - MG); Batches 4 (Chapadão do Ceú - GO); Batches 5 (Uruana de Minas - MG); Batches 6 (Chapada Gaúcha - MG); Lot Batches e 7 (Luís Eduardo Magalhães - BA); Batches 8 (Monte Alegre de Minas - MG)

In the electrical conductivity test, the number of seeds in the sample and the size of the seeds are factors that can affect the efficiency of the test (SILVA; MARTINS, 2009). Comparing batches of the same cultivar, but with different seed sizes could compromise the results of the test, as was found with corn seeds (MARTINELLI-SENEME; ZANOTTO; NAKAGAWA, 2000). This could explain the reported inefficiency of the test when compared with its use with batches of Marandú grass seeds.

The results of the germination, first count, emergence, speed index, first count of seedling emergence in sand, and electrical conductivity tests of seeds immersed in 50 mL of water for 4 and 24 h were not found to be highly reliable for analyzing the vigor of the seed batches (Table 3), as the correlation with seedling emergence in the field was lower than 0.70 (ARAÚJO *et al.*, 2011; FIGUEIREDO FILHO; SILVA JÚNIOR, 2009).

In contrast, there was a high correlation between seedling emergence in the field and the values of the electrical conductivity tests conducted using seeds immersed for 2, 6, and 8 h in 50 mL of water, or 75 mL of water at all the immersion periods evaluated. This characteristic is of fundamental importance, since the vigor tests should classify the batches into different levels of vigor in a manner proportional to their emergence in

the field (ARAÚJO *et al.*, 2011; LOPES; FRANKE, 2010; MARCOS FILHO, 2015).

There was a negative correlation between electrical conductivity and seedling emergence in the field (Table 2). Therefore, the increases in electrical conductivity corresponded to decreases in seed vigor. This fact corroborates reports of increases in electrical conductivity owing to the higher leaching of solutes and a subsequent decline in the physiological quality of the seeds (LOPES; FRANKE, 2010; MARCOS FILHO, 2015; NOGUEIRA *et al.*, 2013).

Therefore, the most promising methodologies to test electrical conductivity (of seeds immersed in 50 mL of water for 2, 6, and 8 h or in 75 mL of water immersed for all periods evaluated) were also compared, in terms of the classification of vigor of the Marandú grass seed batches using the means test, with the seedling emergence in the field and germination test (Table 2). These last two tests can be considered as standards of reference and classified the performance of the batches in decreasing order of physiological potential from 1 to 8.

The results of the seedling emergence test in the field facilitated the separation of the batches into four vigor classes: high (batches 1 and 2), medium-high (batches 3, 4 and 5), medium-low (batches 6 and 7), and low vigor (batch 8). Similarly, results of the germination test also supported the descending order of batch classification based on physiological potential from 1 to 8. However, the germination test was less rigorous than the seedling emergence in the field test, as it separated the batches into high potential seeds, from batch 1 to 6, and low physiological potential seeds, i.e., batches 7 and 8.

The low performance of these last batches can be attributed to the high percentage of dead and dormant seeds, because these made up 40% of the seeds that did not germinate in batches 7 and 8.

The minimum percentage germination necessary to trade *Brachiaria brizantha* seeds in Brazil is 60% (BRASIL, 2008). Therefore, all seed batches of Marandú grass would be approved for the seed market.

It was also verified that, except for the seeds of batches 7 and 8, which expressed a lower germination percentage than the others, the germination rates of the seeds were statistically equivalent to each other. This fact is relevant because one of the objectives of the vigor tests is to identify significant differences in the physiological quality of commercial batches of seeds with similar germination rates (COIMBRA *et al.*, 2009).

During the evaluation of seed quality, the germination means of the batches were compared to improve the efficiency of the electrical conductivity tests

Table 3 - Pearson correlation coefficients (r) between the values of the tests conducted in the laboratory and seedling emergence in the field, for the seeds of eight batches of *Brachiaria brizantha* 'Marandú'

Tests conducted in the laboratory x seedling emergence in the field	r
Germination	0.60
First count of germination	0.25
Seedling emergence in sand	0.61
First count of seedling emergence in sand	0.39
Speed of seedling emergence index in sand	0.63
Electrical conductivity 50 mL: 2 h	-0.71
4 h	-0.68
6 h	-0.72
8 h	-0.71
24 h	-0.68
Electrical conductivity 75 mL: 2 h	-0.82
4 h	-0.80
6 h	-0.79
8 h	-0.78
24 h	-0.70

so that they had high correlation (>70%) with seedling emergence in the field (Tables 2 and 3). Thus, only the immersion of 1.5 mL of seeds in 75 mL of water for 2 and 4 h allowed for the classification of the performance of the batches in a decreasing order of vigor, from batch 1 to 8, which was comparable with the results of the seedling emergence in the field test and the germination test.

Additionally, for the electrical conductivity test carried out in 75 mL of water, with readings after 2 and 4 h, the highest correlation (between -0.82 and -0.80, respectively) was found with the results of seedling emergence in the field test (Table 3).

These two methodologies classified the batches into four vigor classes: high (batches 1 and 2), medium-high (batches 3 and 4), medium-low (batches 5 and 6), and low (batch 7 and 8) (Table 2). The other methods for testing electrical conductivity were not as effective owing to contradictory or inconsistent results during the classification of the vigor of the batches when compared with the results of the seedling emergence in the field test.

These results demonstrated that the imbibition time may be shorter than 24 h, as suggested for most of the seeds of this species by Marcos Filho (2015). Similarly, Nery, Carvalho and Guimarães (2009) and Sponchiado, Souza and Coelho (2014), working with radish and white oats seeds, respectively, found that the imbibition time could be reduced to 2 h without impairing the reliability of the results, which corroborates the findings of this study.

One of the main requirements of seed companies is the practical, efficient, and rapid evaluation of seed quality, to enable agility in decision making, especially regarding harvesting, processing, and marketing (STEINER *et al.*, 2011).

Thus, the alternative method for conducting electrical conductivity test developed in this study, based on sampling by volume rather than the number of seeds, provided results for the evaluation of the vigor of the seed batches in 2-4 h. Therefore, this methodology should make testing of small seeds of Marandú grass easier and more practical for the seed analysis laboratories to adapt in their routine.

CONCLUSION

The electrical conductivity test using the volume sampling method of seeds immersed in 75 mL of water and readings taken after 2 or 4 h is efficient in evaluating the vigor of seed batches of Marandú grass, providing

information equivalent to that obtained from the seedling emergence in the field test.

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