DOI: 10.5433/1679-0359.2017v38n6p3491

Vigor tests in assessing the quality of signal grass seeds

Testes de vigor na avaliação da qualidade de sementes de capim-xaraés

Paulo Alexandre Fernandes Rodrigues de Melo[1]; Tatiane Sanches Jeromini[2*]; Carlos Eduardo Affonso[3]; Edna Ursulino Alves[4]; Cibele Chalita Martins[5]

Abstract

The correct assessment of the physiological quality of seed lots is necessary for the quality control program of companies. For such purpose, tests that detect differences in the physiological potential of seed lots and that meet the minimum market requirements. Thus, the study was conducted towards assessing the efficiency of laboratory tests in differentiating the quality of *Brachiaria brizantha* cv. Xaraés seed lots. Seeds from nine lots were assessed regarding water content, germination, first germination count, electrical conductivity and seedling emergence in sand in the laboratory (normal seedlings, first count and germination rate index), and the results were compared with those from the seedling emergence test conducted in the field. The experimental design used was completely randomized, with four replicates, and the Pearson correlation coefficient between the values from the germination, vigor and field seedling emergence tests was determined. The germination and seedling emergence in sand tests and the seedling emergence rate index efficiently assess the physiological quality of *Brachiaria brizantha* cv. Xaraés seed lots, providing data similar to those from seedling emergence in the field.
Key words: *Brachiaria brizantha.* Germination. Forrage grass. Vigor tests.

Resumo

A avaliação correta da qualidade fisiológica dos lotes de sementes é necessária para o programa de controle de qualidade das empresas, para isso se utiliza testes que detectam diferenças no potencial fisiológico de lotes e que atendam às exigências mínimas para a comercialização. Assim, o trabalho foi conduzido com o objetivo de verificar a eficiência de testes de laboratório na diferenciação da qualidade dos lotes de sementes de *Brachiaria brizantha* cv. Xaraés. As sementes de nove lotes foram avaliadas quanto ao teor de água, germinação, primeira contagem de germinação, condutividade elétrica, emergência de plântulas em areia no laboratório (plântulas normais, primeira contagem e índice de velocidade de emergência) e os resultados comparados aos obtidos no teste de emergência de plântulas em campo. O delineamento experimental utilizado foi o inteiramente casualizado, com quatro repetições e determinado o coeficiente de correlação de Pearson entre os valores obtidos nos testes de germinação e de vigor e o teste de emergência em campo. O teste de germinação, emergência de plântulas em areia e índice de velocidade de emergência de plântulas em areia são eficientes na avaliação da qualidade fisiológica de lotes de sementes de *Brachiaria brizantha* cv. Xaraés, fornecendo informações equivalentes à emergência de plântulas em campo.
Palavras-chave: *Brachiaria brizantha.* Germinação. Gramíneas forrageiras. Testes de vigor.

[1] Prof. Dr. Visitante, Universidade Estadual do Maranhão, UEMA, Campus de São Luís, São Luís, MA, Brasil. E-mail: pauloalexandrefernandes@outlook.com

[2] Discente de Doutorado em Agronomia/Produção Vegetal, Universidade Estadual Paulista, UNESP, Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP, Brasil. E-mail: tatiane_jeromini@hotmail.com

[3] Discente de Graduação, Curso de Engenharia Agronômica, UNESP, Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP, Brasil. E-mail: ceaffonso@outlook.com

[4] Profª Drª Adjunta, Universidade Federal da Paraíba, UFPB, Centro de Ciências Agrárias, Areia, PB, Brasil. E-mail: ednaursulino@cca.ufpb.br

[5] Profª Drª, UNESP, Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP, Brasil. E-mail: cibele@fcav.unesp.br

[*] Author for correspondence

Received: Mar. 17, 2017 - Approved: Sept. 25, 2017

Introduction

Brazil is the largest producer, consumer and exporter of forage grass seeds worldwide (MELO et al., 2016). Approximately 60% seeds produced are *Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf. seeds, and the cultivar Xaraés accounts for 15% exports of these signal grass seeds (EUCLIDES et al., 2010). Due to the competition in this market, seed companies have invested in quality control and laboratory tests for this purpose are in high demand (MARCOS-FILHO, 2015; OHLSON et al., 2009).

Seed physiological quality has been characterized by germination and vigor, and the latter may be defined as the sum of attributes that give the seed the potential to germinate, emerge and rapidly develop into normal seedlings in a wide diversity of environmental conditions (MARCOS-FILHO, 2015; MARTINS et al., 2014).

Vigor tests should detect differences in the physiological potential of seed lots with similar germination and compatible with the minimum market requirements (MARTINS et al., 2014; SOARES et al., 2010). These tests are classified as direct and indirect methods; direct methods try to simulate the conditions that occur in the field, and the indirect methods aim to assess physical, biological and physiological seed attributes indirectly related to vigor (SENA et al., 2015).

Assessing seed resistance to adverse field conditions has been relevant because the results from germination tests overestimate the seed physiological potential, which has caused discrepancies between the results from seed germination in the laboratory and seedling emergence in the field (LOPES; FRANKE, 2010).

Some vigor tests may be performed together with the germination test, for example, first germination count, which is conducted to facilitate performing the germination test and may be considered a vigor test because germination rate is one of the first characteristics affected in the seed deterioration process (COIMBRA et al., 2009; MACHADO et al., 2012). Using the same principle, the vigor of a seed lot may be efficiently assessed using the germinate rate index, as observed, for example, in millet (MACHADO et al., 2012), maize (SENA et al., 2015) and brachiaria hybrid cv. Mulato (FERREIRA et al., 2015) seeds.

Another quick vigor test is the electrical conductivity test, which indirectly assesses seed quality and is based on the concentration of electrolytes leaked by seeds during imbibition, providing reliable results within 24 hours for seeds of some winter forage grasses, such as ryegrass (LOPES; FRANKE 2010), triticale (STEINER et al., 2011) and black oat (NOGUEIRA et al., 2013).

Therefore, the tests that efficiently assess the seed physiological potential should be identified for each species, thereby enabling obtaining consistent and reproducible results (LOPES; FRANKE, 2010; MARCOS-FILHO, 2015; STEINER et al., 2011). Quality control laboratories usually perform several vigor tests simultaneously, in addition to the germination test, to classify seed lots safely into different levels of physiological quality (SOARES et al., 2010).

Thus, the objective of the present study was to assess the efficiency of laboratory tests in differentiating the physiological quality of *B. brizantha* cv. Xaraés seed lots.

Material and Methods

In this research study, nine *B. brizantha* cv. Xaraés seed lots were assessed: from Unaí – Minas Gerais (MG; lots 1 and 8), Chapada Gaúcha – MG (lots 2 and 4), Paraíso das Águas – Mato Grosso do Sul (MS; lots 3 and 7), Primavera do Leste – MT (lot 9), Rosário do Oeste – Mato Grosso (MT; lot 5) and Santo Anastácio – São Paulo (SP; lot 6). The study seeds were sent to the Seed Analysis Laboratory, School of Agricultural and Veterinary Sciences – São Paulo State University (Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP), Campus Jaboticabal – SP, to conduct the following assessments and testes:

Water content – assessed using the oven method at 105 ± 3 °C, for 24 hours (BRASIL, 2009).

Germination test – conducted with four 100-seed subsamples, sown on two filter paper sheets moistened with distilled water, at a volume of 2.5 times the dry paper mass, inside transparent acrylic boxes (11.0 x 11.0 x 3.5 cm), kept at 20-35 °C. Seedlings were considered normal when the plumule had emerged from the coleoptile, and the primary root was at least 0.5 cm long, performing weekly counts until the 21st day (BRASIL, 2009).

To detect dormancy, the remaining seeds from the germination test were subjected to the tetrazolium test. For such purpose, these seeds were sectioned longitudinally and medially through the embryo, immersing one of the seed halves in 0.075% tetrazolium solution and incubating for two hours at 41°C (±3 °C) in the dark (DELOUCHE et al., 1976). Then, the seeds were washed in distilled water, and the reading was performed immediately, classifying the seeds into viable (dormant) and nonviable (dead) seeds and expressing the data as percentage (BRASIL, 2009).

First germination count – performed together with the germination test, counting normal seedlings on the fourth day after starting the test and expressing the data as percentage (GASPAR-OLIVEIRA et al., 2008).

Seedling emergence in sand – conducted with four 50-seed subsamples, which were sown two centimeters deep in moistened sand, in plastic boxes (26.0 x 17.0 x 5.0 cm) containing sand substrate, kept at 28±3 °C, counting the number of seedlings emerged from the fourth to the 30th day after sowing, when seedling emergence stabilized, and expressing the results as percentage.

First count of seedling emergence in sand- performed together with the seedling emergence in sand test, counting the number of seedlings emerged on the fourth day after sowing and expressing the results as percentage (GASPAR-OLIVEIRA et al., 2008).

Seedling emergence rate index – performed together with the seedling emergence in sand test, from the fourth to the 30th day after sowing, tallying the number of seedlings emerged per day and using the equation proposed by Maguire (1962).

Electrical conductivity test – four 1.5-ml subsamples of seeds per lot were used, quantified using a microcentrifuge tube and weighed in a scale accurate to 0.0001 g. Sampling per volume instead of seed number was adopted to facilitate and make the test more practical, because the seeds are small, to adapt it to the routine of a seed analysis laboratory. The seeds were soaked in plastic cups containing 50 and 75 ml of distilled water at 25°C. The readings were performed 2, 4, 6, 8 and 24 hours later in a conductivity meter, and the results were expressed as µS cm^{-1}. The seeds sampled using microcentrifuge tubes were counted before soaking to calculate the mean seed number per sample.

Seedling emergence in the field – assessed by sowing, in the last week of January 2015, four 50-seed subsamples in 1.5-m-long furrows, spaced 0.2 m from each other, at a depth of two centimeters, performing daily counts, from the fourth to the 21th day after sowing, and expressing the results as percentage (OLIVEIRA et al., 2014). During the test, the average daily maximum and minimum ambient temperatures in the field were 39±3°C and 22±3°C, respectively.

The experimental design used was completely randomized, comparing means using the Scott-Knott test at 5% probability and the software Assistat. The Pearson correlation coefficient between the values from the germination, vigor and seedling emergence in the field tests was also determined. Correlation data were interpreted according to the following criterion described by Figueiredo-Filho e Silva-Júnior (2009): r = 0.10 to 0.30 (weak), r = 0.40 to 0.6 (moderate) and r = 0.70 to 1 (strong) correlation.

Results and Discussion

The water content of *B. brizantha* cv. Xaraés seeds ranged from 9 to 10% (Table 1), which is considered uniform because the maximum variation was 1%. This similarity between values is paramount to prevent differences in seed metabolic activity from affecting the physiological quality tests (ARAÚJO et al., 2011; COIMBRA et al., 2009; OLIVEIRA et al., 2014; SENA et al., 2015).

The first germination count, first count of seedling emergence in sand and electrical conductivity tests were not considered reliable for the vigor analysis of the seed lots in all water volumes and periods tests (Table 2) because no correlation with seedling emergence in the field was found (ARAÚJO et al., 2011; DIAS et al., 2004), or the correlation values (from 0.1 to 0.6) were considered weak of moderate (FIGUEIREDO-FILHO; SILVA-JÚNIOR, 2009).

Furthermore, according to the data outlined in Table 1, a relatively high percentage of dormant seeds, higher than 10%, was found in three lots (6, 7 and 9), and, in another three lots (7, 8 and 9), the percentage of dead seeds was higher than 30%. Most likely, these high incidence rates of dead and dormant seeds, combined, compromised the seed performance of lots 6, 7, 8 and 9, regarding germination, seedling emergence in the field and the results from the vigor tests (first germination count, first count of seedling emergence in sand and electrical conductivity).

In the electrical conductivity test, the mean seed number per 1.5-ml sample ranged from 94 to 104, depending on the study lot (Table 1). This variation between samples may have been caused by differences in seed size or dormancy because the lots 6, 7 and 9 had the highest dormancy and the lowest electrical conductivity values. According to Fessel et al. (2006), the comparison between lots of the same cultivar, albeit with different seed sizes, may compromise the results from the electrical conductivity test, which could be an explanation for the inefficiency of this test in comparing *B. brizantha* cv. Xaraés seed lots.

Table 1. Water content (WC), seedling emergence in the field (SE), germination test (normal seedlings – G; first count – FC; dormancy seeds – DS; and dead – NS), seedling emergence in sand (normal seedlings – SS; first count – FCS; and seedling emergence rate index – SES), electrical conductivity test of seeds in 50 (EC_{50}) and 75 ml of water (EC_{75}) and average number of seeds in the electrical conductivity test of nine lots of *Brachiaria brizantha* cv. Xaraés.

Tests		Lots*									
		1	2	3	4	5	6	7	8	9	CV (%)
WC (%)		9,2	9,2	9,6	9,1	9,0	9,4	9,6	10,0	9,1	-
SE (%)		85 a	76 a	79 a	74 b	73 b	69 b	67 b	68 b	54 c	5,82
G (%)		83 a	80 a	80 a	73 b	71 b	69 c	60 d	63 d	60 d	5,46
FC (%)		26 b	26 b	16 c	40 a	26 b	19 c	32 a	35 a	43 a	20,19
DS (%)		3 a	4 a	2 a	7 b	4 a	11 c	10 c	7 b	10 c	19,80
NS (%)		14 a	16 a	18 a	20 a	25 a	20 a	30 b	30 b	30 b	21,88
SS (%)		73 a	68 a	68 a	53 b	59 b	50 b	59 b	50 b	51 b	12,75
FCS (%)		19 a	6 c	2 d	13 b	3 d	2 d	7 c	5 c	11 b	26,10
SES		6,62 a	5,50 b	4,94 c	4,28 c	4,63 c	4,09 c	4,53 c	3,3 d	2,95 d	13,77
	2 hs	46 b	27 a	44 b	45 b	52 c	43 b	40 b	33 a	31 a	10,34
	4 hs	53 c	29 a	47 c	52 c	62 d	49 c	48 c	39 b	37 b	10,35
EC_{50} (µS cm^{-1})	6 hs	57 c	33 a	52 c	58 c	66 d	53 c	54 c	42 b	41 b	10,49
	8 hs	60 c	35 a	54 c	55 c	70 d	56 c	49 c	45 b	44 b	10,24
	24 hs	96 d	61 a	83 b	90 c	119 e	87 c	99 d	68 a	76 b	5,88

continue

continuation

EC_{75} (µS cm^{-1})	2 hs	30 b	23 a	30 b	33 b	27 b	31 b	26 b	22 a	22 a	10,46
	4 hs	34 c	27 a	34 c	39 c	32 b	38 c	31 b	26 a	26 a	10,80
	6 hs	38 b	29 a	36 b	42 b	34 a	42 b	34 a	28 a	31 a	12,37
	8 hs	39 b	30 a	38 b	43 b	37 b	44 b	35 a	29 a	34 a	12,51
	24 hs	61 d	54 b	51 a	66 d	57 c	69 d	49 a	44 a	55 c	7,42
Nº seeds		104 a	101 a	97 b	94 b	102 a	103 a	99 b	102 a	102 a	2,29

Means in the same line, followed by the same letter, are not significantly different according to de Scott-Knott (p<0,05) test.

The most reliable results to assess the quality of seed lots were obtained when using the standard tests (germination, seedling emergence in sand and germination rate index of seedlings in sand) because they were highly correlated with seedling emergence in the field, with values of 0.81, 0.67 and 0.69%, respectively (Table 2).

The correlation of data from a vigor test with seedling emergence in the field is crucial to consider the test successful because it should classify the lots into different levels of vigor as close as possible to seedling emergence in the field (ARAÚJO et al., 2011; LOPES; FRANKE, 2010; MARCOS-FILHO, 2015).

In the seed quality assessment, the means of lots were compared for all variables with significant correlation (Table 1) to identify more precisely the most efficient vigor tests, thus enabling classifying the performance of lots in decreasing order of vigor, from 1 to 9.

Table 2. Pearson correlation coefficient (r) between the results of laboratory tests with the seedlings emergence in the field for nine seed lots of *Brachiaria brizantha* cv. Xaraes.

Laboratory tests x seedlings emergence in the field		r
Germination		0,81
First count of seedlings emergence in the field		-0,50
Seedling emergence in sand		0,67
First count of seedling emergence in sand		0,17
Seedling emergence rate index in sand		0,69
Electrical conductivity 50 ml	2 hours	0,43
	4 hours	0,31
	6 hours	0,32
	8 hours	0,30
	24 hours	0,15
Electrical conductivity 75 ml	2 hours	0,47
	4 hours	0,41
	6 hours	0,32
	8 hours	0,27
	24 hours	0,33

Thus, the results from the seedling emergence in the field test enabled separating the lots into three classes of vigor: high (lots 1, 2 and 3), medium (lots 4, 5, 6, 7 and 8) and low (lot 9) vigor. Similarly, the germination test, even though it is not a seed vigor test, maintained the decreasing order of classification of lots in terms of physiological potential, from lot 1 to 9; nevertheless, the germination test was more accurate than the previous test because it separated the seed lots into four classes of: high (1, 2 and 3), medium-high (4 and 5), medium-low (6) and low (7, 8 and 9) physiological potential. Other studies reported results similar to the findings of the present study, regarding the efficiency of the germination test in differentiating commercial lots of *B. brizantha* seeds (LAURA et al., 2009; OHLSON et al., 2011).

The germination of seeds from all lots used in the research study was higher than 60%, and these seeds could be sold commercially because they meet the official standards of forage grass seeds (BRASIL, 2008).

Although studies on vigor tests for grass seeds, including sweet corn (COIMBRA et al., 2009), maize (GRZYBOWSKI et al., 2015) and millet (MACHADO et al., 2012) seeds, advocate that these tests allow differentiating seed lots with similar germination percentages, this premise is difficult to confirm in forage grasses because seed lots of these species have differences in germination larger than those assessed in large-crop seeds (LAURA et al., 2009; OHLSON et al., 2011).

Market standards for signal grass seeds recommend values higher than 60% germination (BRASIL, 2008), whereas this value should be higher than 80 or 85% for maize, soybean and rice, comprising lots with physiological quality more similar to each other (BRASIL, 2013). Therefore, due to these characteristics specific to the forage seed market and to the efficiency of the seed germination test in differentiating the physiological potential of seed lots (Tables 1 and 2), this test could be used in *B. brizantha* cv. Xaraés seed quality control to separate lots based on physiological potential.

The seedling emergence in sand test also showed high correlation with seedling emergence in the field (0.67%), separating the lots into only two classes of vigor: high (lots 1, 2 and 3) and low (lots 4, 5, 6, 7, 8 and 9) (Tables 1 and 2). Conversely, the rate of seedling emergence in sand, assessed using the germination rate index (GRI), was more sensitive because this rate separated the lots into four classes of vigor: high (lot 1), medium-high (lot 2), medium-low (lots 3, 4, 5, 6 and 7) and low (lots 8 and 9) vigor. This test also showed high correlation with seedling emergence in the field (0.69%), albeit more rigorously than the latter, identifying an additional class of vigor, and may be indicated as a promising vigor test for *B. brizantha* cv. Xaraés seeds.

Similarly, to this study, the seedling emergence in sand test efficiently assessed signal grass seed vigor in studies on seed scarification, fertilizer use, sowing depth (FOLONI et al., 2009) and harvesting methods (QUADROS et al., 2012).

Tests assessing the seedling emergence rate in the laboratory may be used to identify lots with faster seedling emergence in the field (MACHADO et al., 2012; SENA et al., 2015). The decreased seedling emergence results from the fact that seeds with lower vigor, at the beginning of the germination process, restore damaged organelles and tissues, and the time spent in that process lengthens the total period of seed germination and seedling emergence (SENA et al, 2015).

Conclusion

The germination and seedling emergence in sand tests and the seedling emergence germination rate efficiently assess the physiological quality of *B. brizantha* cv. Xaraés seed lots.

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