DOI: 10.5433/1679-0359.2020v41n3p1047

Use of SPAD index in elephant grass pre-breeding

Uso do índice SPAD em pré-melhoramento de capim elefante

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Highlights:

The use of morphoagronomic descriptors in pre-breeding is costly and time consuming.
We proposed screening elephant grass genotypes using SPAD index.
We found SPAD index was effective in separate Cameroon from Napier genotypes.

Abstract

The SPAD-502 (Soil Plant Analysis Development) chlorophyll meter is a simple diagnostic tool that instantly measures the chlorophyll content of plant leaves without damaging the leaf blade. This study aimed to evaluate the efficacy of the SPAD index as a tool for discriminating elephant grass acessions from different morphological groups. One hundred genotypes of the elephant grass *Pennisetum purpureum* from the Active Germplasm Bank at Embrapa Dairy Cattle, Minas Gerais, Brazil were evaluated. Following uniform cutting, six SPAD readings were taken from each accession every 60 days. Measurements were taken for two years. The experimental design was a 10 × 10 simple lattice with two replications. Joint deviance analysis for six SPAD measurements revealed great genetic variability between genotypes. SPAD values of Cameroon and Napier accessions were, on average, significantly different. The SPAD index was effective in detecting genetic variability between elephant grass acessions. However, it should not be used alone as a morphological descriptor, but in combination with other descriptors.

Key words: Chlorophyll meter. Crop breeding. *Pennisetum purpureum*.

Resumo

O clorofilômetro SPAD-502 (Soil Plant Analysis Development) é usado na medição indireta do conteúdo de clorofila, e é caracterizado pela rapidez e simplicidade, especialmente por ser uma medição não destrutiva do limbo foliar. O objetivo desse trabalho foi avaliar o índice SPAD como ferramenta para identificar acessos de diferentes grupos morfológicos de capim-elefante. Foram avaliados 100 genótipos do Banco Ativo de Germoplasma de Capim-elefante da Embrapa Gado de Leite, Minas Gerais, Brasil. Após corte de uniformização, foram realizadas seis leituras por acesso, a cada 60 dias. As medidas foram realizadas durante dois anos. O delineamento experimental foi o látice simples (10 x 10) com

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Received: Apr. 13, 2018 - Approved: Feb. 21, 2020

duas repetições. Foi observado efeito de acessos pela análise conjunta de *deviance* das seis medidas do índice SPAD mostrando alta variabilidade genética entre os materiais. Os grupos 'Cameroon' e 'Napier' foram diferentes entre si para o índice SPAD, que se mostrou eficaz na detecção de variabilidade genética entre cultivares; contudo, deve ser usado juntamente com outros descritores e não exclusivamente como descritor morfológico.
Palavras-chave: Clorofilômetro. Melhoramento vegetal. *Pennisetum purpureum*.

Elephant grass [*Pennisetum purpureum* Schum.; synonym *Cenchrus purpureus* (Schumach.) Morrone], native to Africa and introduced in Brazil as forage, is a promising source of renewable energy (J. R. A. S. C. Rocha et al., 2017). Elephant grass is a tall, erect, perennial plant with cespitose habit, cylindrical and full stems, broad and long leaves, and high biomass yield. For breeding purposes, the phenotypic description of elephant grass accessions requires the analysis of a set of morphoagronomic descriptors, including morphological, reproductive, agronomic, and biochemical traits (Pereira, 1999). However, using these descriptors is costly and time consuming, and tools are required to facilitate the work.

The SPAD-502 (Soil Plant Analysis Development) chlorophyll meter is a simple diagnostic tool that instantly measures the relative chlorophyll content of leaves without damaging the leaf blade that has been increasingly used to improve crop and pasture quality. The 'greenness' of the leaves is detected by measuring the amount of wavelength light absorbed by the leaf in the red and infrared regions. The absorbance in the red region indicates the amount of chlorophyll present, whereas the absorbance in the near-infrared region compensates for the thickness and water content of the leaf (R. N. C. Rocha et al., 2005; Silva, Jifon, Silva, & Sharma, 2007; Amarante et al., 2010; Araújo et al., 2013; Souza, Modesto, Pompeu, & Natale, 2014).

The efficiency of a dimensionless SPAD index as an indirect measure of chlorophyll content has been demonstrated in studies with various crops, including brachiaria grass (Maranhão, Silva, Bonono, & Pires, 2009), dwarf elephant grass (Araújo et al., 2013), sugarcane (Silva et al., 2007), and maize (R. N. C. Rocha et al., 2005; Amarante et al., 2010; Hurtado, Resende, Silva, Corazza, & Shiratsuchi, 2011). In addition, the SPAD index has been shown to correlate with dry matter yield (Souza et al., 2014). In maize, the SPAD index correlated with grain yield when the plant was at the four and eight fully expanded leaves stages (R. N. C. Rocha et al., 2005). Moreover, chlorophyll meter readings (SPAD) can be used to select stress-adaptive maize genotypes (Gekas et al., 2013). In sugarcane, the SPAD index correlated with drought tolerance or susceptibility (Silva, Santos, Vitorino, & Rhein, 2014). This study aimed to evaluate the efficacy of the SPAD index as a morphological descriptor of elephant grass genotypes from different morphological groups in pre-breeding programs.

The trial was conducted in the Active Germplasm Bank at Embrapa Dairy Cattle (BAGCE) in Coronel Pacheco, Zona da Mata region, southeastern Minas Gerais, Brazil (21°55'S 43°16'W, 426 m asl). The climate is Cwa according to the Köppen classification with hot and rainy summers and dry winters.

The experiment was conducted on 4-m single row plots spaced 1.5 m apart arranged in a 10×10 simple lattice design with two replications, totaling 100 genotypes: Cameroon (18), Napier (44), Mercker (5), and Dwarf (1), in addition to 32 genotypes that could not be assigned to specific varieties because of their unique morphological traits. Nitrogen-phosphorus-potash solid fertilizer (05-20-20) was applied annually at a rate of 250 kg ha^{-1}. SPAD readings were taken at the middle of the leaf lamina away from the primary vein using a SPAD-502Plus chlorophyll meter (Konica Minolta Sensing Inc, Osaka, Japan) every 60 days following uniform cutting. Measurements were taken for two years.

Statistical analyses were performed using the REML/BLUP (restricted maximum likelihood/best linear unbiased prediction) methodology according to Patterson and Thompson (1971) and Henderson (1975). In the random effects models, the level of significance for the likelihood-ratio test (LRT) was assessed using the chi-square test with one degree of freedom. Mean SPAD values were compared between the groups by the F-test using the Selegen-REML/BLUP software (Resende, 2007).

Joint deviance analysis of six SPAD measurements revealed a significant effect of genotype on SPAD values (Table 1), revealing a great genetic variability among the 100 BAGCE elephant grass genotypes investigated. In addition, there was a significant effect ($p < 0.05$) of the genotype × measurement time interaction on SPAD values (Table 1), indicating that the time of year of SPAD readings affects the accession ranking, based on this parameter. Also, more than one SPAD measurement is needed to predict genetic value with high accuracy. The accuracy of the SPAD index depends on several factors, including leaf maturity and phenological stage, and may be affected by seasonality (Amarante et al., 2010; Silva et al., 2014).

Table 1
Joint deviance analysis for six SPAD measurements in 100 elephant grass (*Pennisetum purpureum*) genotypes

Variable		Effect				
		Accession+	Block+	Accession × measurements+	Permanent environment+	Complete model
SPAD index	Deviance	4616.68	4612.51	4612.98	4654.29	4606.78
	LRT (X^2)	9.9**	5.73*	6.2*	47.51**	–

Chi-square: 3.84 and 6.63 for 5% and 1% significance levels, respectively.
**: significant at 1% by the chi-square test; *: significant at 5% by the chi-square test. +: Deviance of the model adjusted without these effects. LRT (X^2): likelihood ratio test.

The F-test for the effect of genotype showed a significant difference ($p < 0.05$) in SPAD values between the Cameroon and Napier groups (Table 2). Thus, SPAD values of Cameroon and Napier groups are, on average, significantly different. However, no significant differences in SPAD values were detected between the other groups.

Table 2
Deviance analysis for elephant grass *(Pennisetum purpureum)* genotypes Cameroon and Napier and F-test for the effect of Cameroon vs. Napier genotypes on the SPAD index

Effect	Deviance	LRT (X^2)		
Cameroon	72.24+	69.63**		
Complete model	2.61	–		
Napier	438.15+	301.73**		
Complete model	136.42	–		
Cameroon vs. Napier	DF = 1	SQ = 4.05	MS = 4.05	P-value = 0.02
Overall mean	29.96	–		
Cameroon mean	29.8	–		
Napier mean	30.03	–		
CV (%)	1.81			

Chi-square: 3.84 and 6.63 for 5% and 1% significance levels, respectively.
**: significant at 1% by the chi-square test; *: significant at 5% by the chi-square test.
DF: degree of freedom. CV: coefficient of variation
+: Deviance of the model adjusted without these effects. LRT (X^2): likelihood ratio test.

The six SPAD measurements taken from the BAGCE genotypes had a coefficient of repeatability (CoR) of 0.32. According to Resende (2007), CoR values greater than 0.6 indicate high repeatability, CoR values between 0.3 and 0.6 indicate intermediate repeatability, and CoR values smaller than 0.3 indicate low repeatability. The CoR value computed in this study (0.32) indicates that six SPAD measurements were sufficient to predict the genotypic value of elephant grass groups with 86% accuracy, which refers to the correlation between predicted and true genetic values of individuals (Pimentel et al., 2014).

In Cameroon elephant grass, eight readings were sufficient to estimate the chlorophyll content of leaves by the SPAD index with an acceptable error of 10%, whereas 16 and 80 SPAD measurements were required to determine the macronutrient and micronutrient content of plants, respectively (Souza et al., 2014). In sugarcane, five measurements from different plants within the same plot were sufficient to demonstrate a correlation between the SPAD index and drought tolerance (Silva et al., 2007). In maize, five readings from different plants were sufficient to satisfactorily demonstrate a correlation between the SPAD index and plant nutritional status (Hurtado et al., 2011).

The SPAD index accurately discriminated between Cameroon and Napier groups. There was great variability between genotypes within each elephant grass group. In addition, there was a significant interaction between SPAD values and environmental factors. Finally, the SPAD index was effective in detecting genetic variability between elephant grass accessions; however, it should not be used alone as a morphological descriptor, but in combination with other descriptors (dry matter, crude protein, plant height).

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