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Indirect method for quantifying the content of photosynthetic pigments in genotypes of dwarf elephant grass

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ABSTRACT. Equations that indirectly associate the rates obtained by chlorophyll meter SPAD-502 to chlorophyll and carotenoids rates of dwarf elephant grass genotype are provided. Leaves of the genotype of dwarf elephant grass were employed. After being collected, they were transported in light-protected styrofoam boxes to the Plant Physiology Laboratory of UENF. Leaf discs of the known area were then extracted. An average of 5 readings/leaf disc were undertaken by portable chlorophyll meter SPAD-502, with 6 discs for each range and distributed according to the following scale: 0-10; 10-20; 20-30; 30-40; 40-50; 50-60. Chlorophyll meter SPAD-502 provides a quick and efficient estimate of total chlorophyll, chlorophyll-*a* and chlorophyll-*b* in genotypes of dwarf elephant grass.

Keywords: carotenoids, chlorophyll-*a,* chlorophyll-*b*, chlorophyllometer, *Pennisetum purpureum.*

Método indireto de quantificação do conteúdo de pigmentos fotossintéticos em genótipos de capim-elefante anão

RESUMO. O objetivo deste ensaio foi determinar equações que indiretamente associem os valores obtidos no medidor de clorofila SPAD-502 aos teores de clorofila e carotenóides de genótipos de capim-elefante anão. Folhas de genótipos de capim-elefante anão foram utilizadas e, depois de coletadas, foram transportadas em caixas de isopor, protegidas da luz, até o laboratório de Fisiologia Vegetal da UENF. Posteriormente, os discos foliares de área conhecida foram extraídos. Com o auxílio do medidor portátil de clorofila (MPC) SPAD-502, foi obtida a média de 5 leituras/disco foliar e utilizaram-se 6 discos para cada intervalo, distribuídos de acordo com a seguinte escala de valores do MPC: 0-10; 10-20; 20-30; 30-40; 40-50; 50-60. O medidor de clorofila SPAD-502 possibilitou uma rápida e eficaz estimativa do conteúdo de clorofila total, clorofila-*a* e clorofila-*b* em genótipos de capim-elefante anão.

Palavras-chave: carotenóides, clorofila-*a*, clorofila-*b*, clorofilômetro, *Pennisetum purpureum.*

Introduction

The most important chemical compounds for converting light energy into chemical energy are the pigments found in chloroplasts. The two classes of photosynthetic pigments found in plants are chlorophylls and carotenoids, which are insoluble in water but soluble in organic solvents (MARENCO; LOPES, 2005). Chlorophyll-*a* is widespread in all photosynthetic cells and plays an important role in the bioconversion process of energy, whereas the other pigments are called accessory. Although the structure of chlorophyll-*a* and chlorophyll-*b* is basically the same, the leaf content of chlorophyll-*b* is about one third of the chlorophyll-*a* content. Carotenoids are yellow or orange pigments and are represented by carotenes and xanthophylls found in all photosynthetic cells. Their coloring in leaves is masked by the green color of chlorophyll (TAIZ; ZEIGER, 2004).

Integrating precision agriculture, there is currently is a need for real-time sensing of the nutritional status of plants (ZOTARELLI et al., 2002). Among the latest techniques to evaluate the plants' nutritional status in real time is the highlighting of the leaves' green color intensity analysis, since there is a significant correlation between the intensity of the green color and chlorophyll contents with N concentration in the leaf (GIL et al., 2002). The fast and low cost method to evaluate the green color in the leaf (TORRES

NETTO et al., 2002, 2005) has become easier due to recent technological advances and refinement of portable meters. The chlorophyll meter SPAD-502 is used in indirect quantification of chlorophyll, characterized by speed and simplicity, and mainly by providing a nondestructive evaluation of the leaf tissue (GUIMARÃES et al., 1999; TORRES NETTO et al., 2002, 2005). The intensity of the green color in leaves is detected by the device's measurement of the amount of light of wavelengths absorbed by the leaf in the red and infra-red region. The amount of absorption close to the red region indicates chlorophyll amount, while the amount of light absorbed near the infra-red region serves as an internal reference for the compensation of leaf thickness and water content (SWIADER; MOORE, 2002).

Current research determines the equations which indirectly associate the values of SPAD-502 to the chlorophyll contents and to the carotenoids of dwarf elephant grass genotypes.

Material and methods

The experiment was carried out at the Research Support Unit (UAP) of the Science and Agricultural Technologies Center (CCTA) of *Universidade Estadual Norte Fluminense* (UENF) in Campos dos Goytacazes (21º27'S, 41º15'W), Rio de Janeiro State, Brazil.

Plinthic Hapludox soil was used (EMBRAPA, 2006) and its chemistry was characterized. The soil featured 5.5 pH; 4 mg dm^{-3} P; 36 mg dm^{-3} K; 2.2 $cmol_c$ dm^{-3} Ca + Mg and 0.2 $cmol_c$ dm^{-3} Al. Dolomite lime was applied at 1.0 ton. ha^{-1} to raise base saturation to 60%.

Genotypes of dwarf elephant grass (*Pennisetum purpureum* Schum) (CNPGL 94-34-3, CNPGL 92-198-7, cultivar Mott) were retrieved from the breeding program of elephant grass of *Embrapa-Gado de Leite*.

Cuttings consisted of 4 lines (3-m length, each row 1 m spaced apart). A one square meter plot was used in the central area (one meter at each end and a row at each side were discarded) for sampling.

Uniform cuttings were made 30 cm above ground level, 60 days after cutting and fertilization was undertaken according to soil analysis, following Embrapa-Gado de Leite, *i.e* 100 kg ha^{-1} P_2O_5 (superphosphate) at planting. Nitrogen and potassium fertilization was carried out in three equal applications in November 2005, January and March 2006 (200 kg ha^{-1} N as urea and 200 kg ha^{-1} K_2O as potassium chloride). Samples were collected on the 35th day of growth at ground level for the subsequent division of leaf and stem + sheath.

Chlorophyll concentration was estimated with a portable chlorophyll meter (PCM) (SPAD-502, Minolta, Japan). Leaves of the genotypes were collected and transported in styrofoam boxes, protected from light, to the Plant Physiology Laboratory, after which the leaf discs of a known area (176.62 mm^2) were extracted. An average of 5 readings/leaf disc was obtained with PCM and six discs were used for each range, distributed according to the following scale: 0-10, 10-20, 20-30, 30-40, 40-50, 50-60.

Photosynthetic pigments (chlorophylls and carotenoids) were extracted, following Hendry and Price (1993). For the extraction of pigments, leaf discs were cut into small pieces and macerated in a porcelain mortar, with the addition of N_2 liquid. Further, 1.5 mL of extracting solution of ethanol/HCl 1.5N (85v:15v) were added and again macerated. The mortar was washed using a further 1.5 mL of the extracting solution. All macerated tissues were placed in a test tube wrapped in aluminum foil and refrigerated at 4ºC for 24 hours.

The supernatant was removed, placed in an Eppendorf tube and centrifuged at 10,000 rpm for 2 minutes, after which 500 μL were removed for later reading of wavelength with a spectrophotometer. The spectrophotometer (Biotech) gave simultaneous reading of wavelengths 490, 665 and 649 nm and measured the absorbance for carotenoids, chlorophyll-*a* and chlorophyll-*b*.

The concentrations of photosynthetic pigments were calculated according to equations below:

Chlorophyll concentration (μmol m^{-2}):

$$Clha = \left[(12.19 \times A665) - (3.45 \times A649)\mu g \cdot mL^{-1} \times 1.119\,\mu g \cdot mL^{-1} \right] \times (v)/s$$

$$Clhb = \left[(21.99 \times A649) - (5.32 \times A665)\mu g \cdot mL^{-1} \times 1.102\,\mu g \cdot mL^{-1} \right] \times (v)/s$$

$$Clhtotal = clha + clhb$$

Carotenoids concentration (μmol m^{-2}):

$$C = \left\{ \left[(1000 \times A490) - (2.14 \times Clha) - (70.16 \times Clhb) \right] / 220 \right\} \times (v)/s$$

where:

v = volume of solution in liters, s is the leaf disc area in $m^{2.}$

The CurvExpert® program was employed to fit the suitable mathematical model to the data set (Photosynthetic pigments versus SPAD-502 readings). The genotypes and cv. Mott were grouped because they had very similar behavior for the relationships under analysis and thus determined a single regression equation for all.

Results and discussion

SPAD-502 readings showed that the concentrations of chlorophyll-*a* and -*b* had a quadratic behavior. In fact, chlorophyll-*a* reached its maximum concentration at 35 by SPAD reading, decreasing as from this rate. Contrastingly, chlorophyll-*b* had more pronounced increments as from this rate. Thus, according to SPAD-502 readings, there was a complementariness between chlorophyll-*a* and -*b* (Figure 1 and 2).

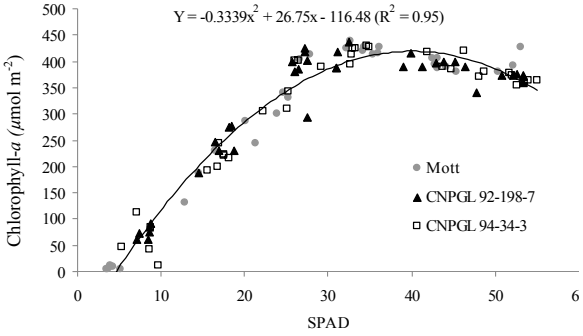

Figure 1. Relationship between the chlorophyll-*a* concentration and SPAD-502 readings in genotypes of dwarf elephant grass.

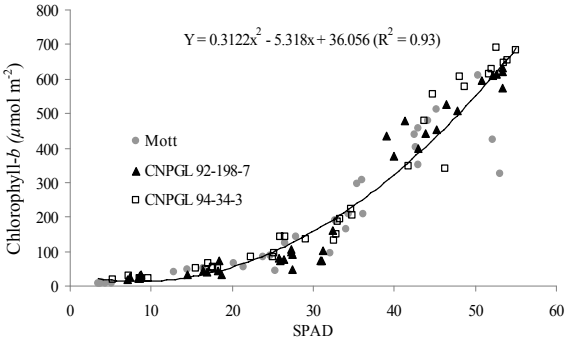

Figure 2. Relationship between chlorophyll-*b* concentration and SPAD-502 readings in genotypes of dwarf elephant grass.

Figure 3 shows a positive correlation between total chlorophyll concentration and SPAD-502 readings for cv. Mott and genotypes CNPGL 92-198-7 and CNPGL 94-34-3.

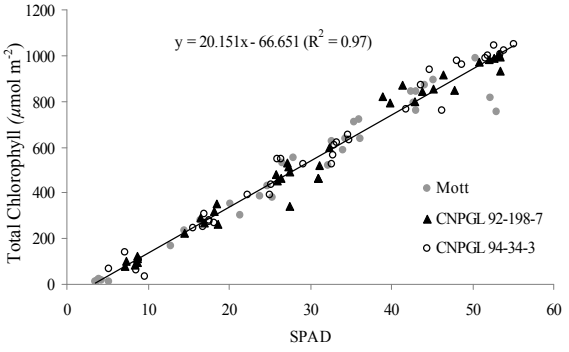

Figure 3. Relationship between total chlorophyll concentration and SPAD-502 readings in genotypes of dwarf elephant grass.

Thus, high rate of anthocyanin in CNPGL 94-34-3 genotype did not influence indirect estimation of total chlorophyll concentration. The linear behavior between the relationship of SPAD-502 readings and total concentration of chlorophyll is shown in Figure 3.

Although genotype CNPGL 94-34-3 provided a purple color owing to a higher concentration of anthocyanin, its total chlorophyll rate was similar to that of the other genotypes. Rates in this study corroborated those reported by Schaper and Chacko (1991) who obtained linear behavior between SPAD-502 and total chlorophyll rates. In experiments with two papaya cultivars, Torres Netto et al. (2002) also found a high relationship between the SPAD-502 readings and total chlorophyll rates, although these authors obtained a non-linear fit.

Salla et al. (2007) evaluated the correlation between chlorophyll concentration determined by the spectrophotometer and by SPAD-502 and reported that, since the use of SPAD-502 was not effective for all species under analysis, it must be supported by a previous study for each species.

The ratio between the concentration of chlorophyll-*a* and chlorophyll-*b* in plants (Figure 4) has been used as an indicator of responses to shade and early senescence stages (BROWN et al., 1991). Results indicated that rate of chlorophyll-*b* was generally 1/2 to 1/5 that of chlorophyll-*a*, and varied according to the intensity of the leaf's green color. Data were similar to those determined by Hall and Rao (1994) and by Monteith (1978).

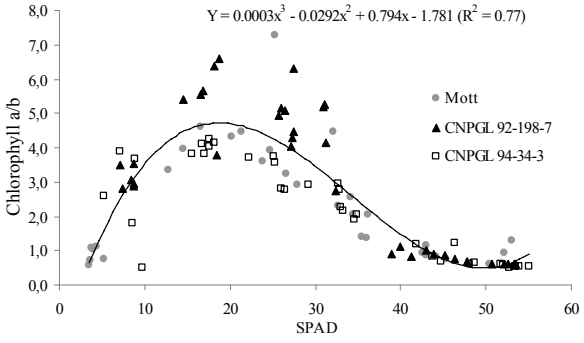

Figure 4. Relationship between chlorophyll-*a and -b* concentrations and SPAD-502 readings in genotypes of dwarf elephant grass.

The relationship between carotenoid rates and SPAD-502 readings is shown in Figure 5. It may be observed that the genotype CNPGL 92-198-7 showed higher contents of carotenoids, corresponding to the range of readings of SPAD-502 from 20 to 40. Silva et al. (2001) studied the photosynthetic characteristics of eight genotypes of

elephant grass and verified that increase in carotenoid concentration is in general associated to an increase in total chlorophyll concentration.

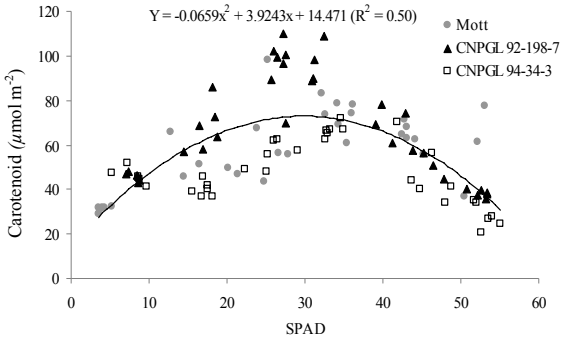

Figure 5. Relationship between carotenoid concentration and SPAD-502 reading in genotypes of dwarf elephant grass.

Current experiment reported a quadratic behavior for the relationship between total chlorophyll and carotenoids (Figure 6). The relationship between these pigments reflects the functions of carotenoids which, besides light collectors, are sunscreens since they preserve the chlorophylls from destruction by oxidation when excess of captured energy occurs (TAIZ; ZEIGER, 2004). The ratio between total chlorophyll and carotenoid concentrations has also been used as an indicator of senescence (HENDRY; PRICE, 1993). Changes in the chlorophyll/carotenoids relationship indicate plant disturbances caused by environmental factors, such as high water deficit, low temperature and high luminosity.

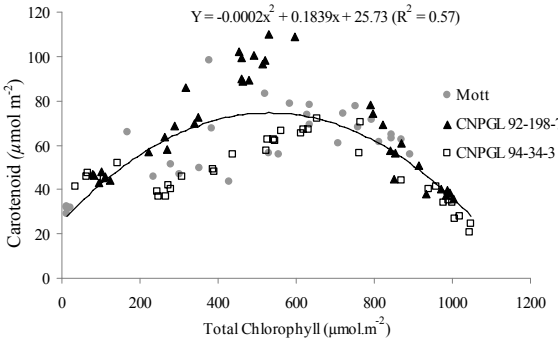

Figure 6. Relationship between carotenoid concentration and total chlorophyll in genotypes of dwarf elephant grass

Conclusion

PCM SPAD-502 provides a quick and efficient estimate of total chlorophyll concentration, chlorophyll-*a* and chlorophyll-*b* concentration in genotypes of elephant grass. The study of the relationship between SPAD-502 readings and carotenoid rates in these forages has low coefficient of determination. Therefore, the use of SPAD-502 readings for the estimate of this pigment is not recommended.

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