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Non-destructive determination of photosynthetic pigments in the leaves of castor oil plants

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ABSTRACT. The aim of the present work was to determine the relationship between photosynthetic pigments extracted in dimethyl sulfoxide (DMSO) and readings obtained by the portable chlorophyll meter, ClorofiLOG® 1030, to generate mathematical models that are able to report the contents of chlorophyll and carotenoids in the leaves of the castor oil plant. This work was conducted at Embrapa Cotton, located in the city of Campina Grande, state of Paraíba, Brazil, in October 2010. For the indirect analysis, portable equipment was used to read leaf discs of different shades of green. The chlorophyll in these discs was then determined using a classical method: 5 mL of DMSO was added, the samples were incubated in a water bath at 70ºC for 30 minutes, and a 3 mL aliquot was removed for spectrophotometric measurements at 470, 646 and 663 nm. The data were subjected to analysis of variance and regression analyses in which the readings obtained using the portable chlorophyll meter were the dependent variable and the photosynthetic pigments that were determined by the classical method were the independent variable. The results indicated that, with the exception of chlorophyll *b*, the ClorofiLOG® 1030 portable chlorophyll meter estimated the concentration of photosynthetic pigments with high precision, thus saving time and the chemical reagents that are typically used in conventional procedures.

Keywords: chlorophyll meter, calibration, chlorophyll.

Determinação não destrutiva de pigmentos da fotossíntese em folhas de mamoneira

RESUMO. Objetivou-se com o presente trabalho, estabelecer a relação entre os pigmentos fotossintéticos extraídos em DMSO e as leituras obtidas no clorofilômetro portátil ClorofiLOG® 1030, gerando modelos matemáticos capazes de predizer os teores de clorofila e de carotenóides em folhas de mamoneira. O trabalho foi conduzido na Embrapa Algodão, situada em Campina Grande, Estado da Paraíba, em outubro de 2010. Para a análise indireta, foi utilizado um equipamento portátil, sendo realizada a leitura em discos foliares com diferentes tonalidades de verde, sendo feita, nesses mesmos discos, a determinação da clorofila pelo método clássico. Para a extração da clorofila, utilizaram-se 5 mL de dimetilsulfóxido (DMSO), a qual foi mantida em banho-maria a 70ºC, por 30 minutos, e retirou-se 3 mL da alíquota para leitura em espectrofotômetro nos comprimentos de onda de 470, 646 e 663 nm. Os dados foram submetidos à análise da variância e regressão polinomial. A leitura obtida no clorofilômetro portátil foi a variável dependente, e os pigmentos fotossintéticos determinados pelo método clássico foi a variável independente. Os resultados indicaram que o clorofilômetro portátil ClorofiLOG® 1030, associado a modelos matemáticos, permitiu estimar a concentração dos pigmentos fotossintéticos, exceto a clorofila b, com alta precisão, com economia de tempo e com reagentes normalmente utilizados nos procedimentos convencionais.

Palavras-chave: clorofilômetro, clorofila, calibração.

Introduction

For many plants, the chlorophyll content in the leaves is highly correlated to the nitrogen concentration and yield (SMEAL; ZHANG, 1994). In addition to being a constituent of the proteins that participate in carbohydrate metabolism, almost 70% of the nitrogen in plants is incorporated in the chloroplast-related enzymes that carry out photosynthesis. Thus, there is an interdependence between nitrogen compounds and chlorophyll (LARCHER, 2000).

Such portable devices as the SPAD-502® (MINOLTA CAMERA COMPANY, 1989) and the ClorofiLOG® (FALKER AUTOMAÇÃO AGRÍCOLA, 2008) meters indirectly allow the relative determination of the amount of chlorophyll that is present in leaves through the amount of light that is transmitted by the leaf in two or three wavelengths. The ClorofiLOG® meter operates through two diodes that emit wavelengths that are located next to the peaks of each type of chlorophyll (635 and 660 nm); a third diode emits wavelengths for low absorbances (880 nm) and functions as an internal reference to compensate for the thickness of the leaves. During the measurement, the light that is transmitted through the sample is converted by a receiver into analog electrical signals (WASKOM et al., 1996). Regardless of the instrument used, when the sensors receive the signal, they provide a single reading that is proportional to chlorophylls *a* and *b* and to the total in a dimensionless unit (FALKER AUTOMAÇÃO AGRÍCOLA, 2008; MINOLTA CAMERA COMPANY, 1989). However, according to Blackmer and Schepers (1995), the nitrogen that is absorbed in excess by the plant is not accounted for in the reading of the device because photosynthetic pigments do not assimilate nitrate.

In classical methods, the determination of photosynthetic pigments is performed by spectrophotometry using organic solvents that have a chemical nature and polarity that are relevant for the quantification. Among these solvents, dimethyl sulfoxide (DMSO) has the advantage of having a high membrane permeability in addition to the efficient solubilization of proteins that are miscible in water, thus facilitating the extraction process (RONEN; GALUN, 1984). Chlorophyll *a* has a cyan color in solution, whereas chlorophyll *b* has a yellow-green color, and they are, therefore, quantifiable through colorimetry (RAJCAN et al., 1999; SENGE et al., 2006). This quantification follows the *Lambert-Beer Law*, which corresponds to the ratio of the incident intensity and the intensity that is transmitted by a body, called the transmission spectrum or absorbance (PERKAMPUS, 1992).

Studies on *Ricinus communis* have been intensified because of the distinction between this plant and other oilseed plants due to the high stability and reactivity of its oil (BELTRÃO, 2004). Because the photosynthetic efficiency may be estimated by the concentration of photosynthetic pigments, for experimentation with this plant, it is important that the efficiency of the photosynthetic pigments be determined using a rapid and inexpensive technique. The aim of this study was to determine the relationship between the photosynthetic pigments extracted in DMSO and the readings obtained from the portable chlorophyll meter, ClorofiLOG® 1030, to generate mathematical models that are able to report the contents of chlorophyll and carotenoids in the leaves of castor oil.

Material and methods

Leaves of castor oil plants (cultivars BRS Energia and BRS Paraguaçu) were collected and subjected to different nitrogen doses at a stage of full development before flowering to measure the photosynthetic pigments with portable equipment and to extract them using a classical model. The experiment was conducted at Embrapa Algodão, a research unit of Embrapa (Brazilian Agricultural Research Corporation), which is located in the city of Campina Grande, state of Paraíba, Brazil (7°13'32"S, 35°54'22"W). The predominant weather is AS', according to Koppen (1948), which is semiarid, hot and humid, with a maximum annual temperature of 28.6°C and a minimum of 19.5°C.

To determine the chlorophyll content, leaf discs with an area of 113 mm^2 were removed and individually measured using the ClorofiLOG 1030® portable chlorophyll meter (FALKER AUTOMAÇÃO AGRÍCOLA, 2008). The samples were then placed in a test tube and wrapped in aluminum foil for protection against light. DMSO (5 mL) was added, and the tubes were incubated at 70°C for 30 minutes for solubilization, according to the methodologies described by Arnon (1949) and Hiscox and Israelstam (1979). An 3 mL aliquot was transferred to a quartz container with a 3 cm^3 volume to determine the spectrophotometric absorbance at wavelengths of 470, 656 nm and 663 nm. The equations described by Wellburn (1994) were used for the quantification of chlorophylls *a* and *b*, the total chlorophyll content and carotenoids.

The data were subjected to analysis of variance, and regression analyses for significant data were applied. The corresponding coefficients of regression were obtained using the program, *Genes* (CRUZ, 2006). To generate the mathematical models, the readings of the index obtained from the portable chlorophyll meter were used as the dependent variable, and the concentration of pigments that were extracted by the classical method was used as the independent variable.

Results and discussion

As determined by the classical method, a close relationship was found between the measurement using the portable chlorophyll meter and the chlorophyll *a* content (μmol·m^2) (Figure 1). The relationship between the methods was best expressed by a linear model, and the adjusted coefficient of determination of the model was 0.93.

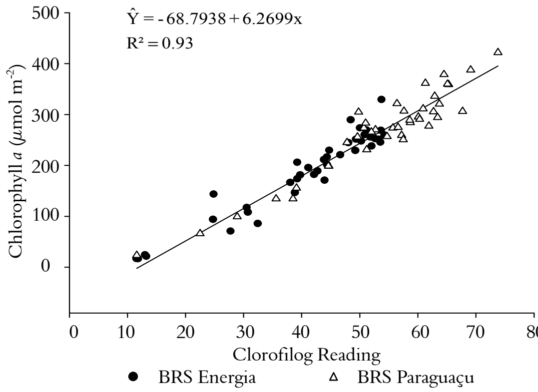

Figure 1. Relationship between the readings of the ClorofiLOG® portable chlorophyll meter and the contents of chlorophyll *a* in the leaves of the BRS Energia and BRS Paraguaçu castor oil cultivars.

The use of the leaf discs from the BRS Energia and BRS Paraguaçu cultivars indicated that there was a similar relationship between the degree of leaf greenness, as determined by the portable chlorophyll meter, and the chlorophyll *a* content, which was extracted by the classical method. Similarly, using corn and soybean plants, Markwell et al. (1995) found that there is a close relationship between the index that was obtained from the readings performed using the SPAD-502 portable chlorophyll meter and the chlorophyll that was extracted by the classical method. The similar results for chlorophyll *a* indicate that the index that was obtained by the portable chlorophyll meter is closely related to the amount of chlorophyll that is present in the leaves of the castor oil plant, regardless of the genotype evaluated. Thus, the model generated in Figure 1 may be used to estimate the content of chlorophyll *a* in the leaves of castor oil plants with great precision in a fast and efficient way and without the added cost of reagents.

For chlorophyll *b*, the relationship between the readings of the portable chlorophyll meter and the reading obtained by the classical method did not show good agreement, with a coefficient of determination of 53% (Figure 2). Thus, the use of the portable chlorophyll meter to determine the chlorophyll *b* content in the leaves of castor oil plants is not precise enough to allow its adoption in research activities.

Previous studies using portable equipment with such plants as cotton and coffee demonstrated the possibility of indirectly determining the chlorophyll b content (NEVES et al., 2005; TORRES NETTO et al., 2005); however, for cotton, the chlorophyll b content showed a lower relationship with the readings of the portable chlorophyll meter when compared with the other photosynthetic pigments. Furthermore, both Amarante et al. (2008), using apple, and Richardson et al. (2002), using *Betula papyrifera*, reported that the SPAD-502 estimate of chlorophyll b was less reliable than the estimates of chlorophyll a and the total chlorophyll content. According to Neves et al. (2005), these results may be explained by the fact that the absorption peak of chlorophyll a is similar to the wavelength emitted by the device and, thus, more difficult to separate.

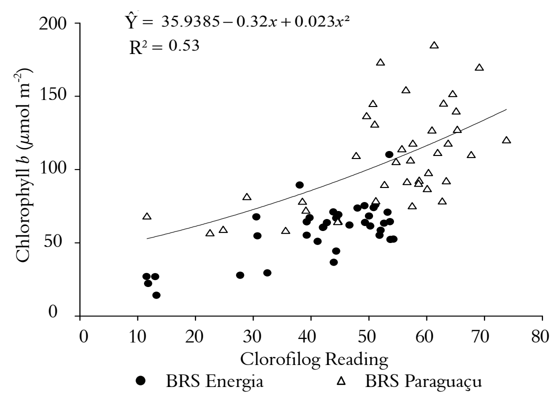

Figure 2. Relationship between the readings of the ClorofiLOG® portable chlorophyll meter and the contents of chlorophyll *b* in the leaves of the BRS Energia and BRS Paraguaçu castor oil cultivars.

The relationship between the carotenoids and the values of the ClorofiLOG® index in castor oil plant leaves is shown in Figure 3. The model that best expressed the relationship between the reading of the ClorofiLOG® device and the concentration of carotenoids in the leaf tissue was a quadratic model, with coefficients of determination of 80%. According to Taiz and Zeiger (2004), carotenoids play a key role in the protection of the photosystems because they dissipate the excess energy, preventing photo-oxidation; thus, their indirect quantification is relevant to the photosynthetic efficiency.

For the total chlorophyll, there was a significant relationship between the portable chlorophyll meter measurements and the total chlorophyll that was determined in the laboratory (the classical method), and the model that best expressed this relationship was a linear model, with a coefficient of determination of 0.84 (Figure 4). These data corroborate those obtained by Barbieri Junior et al. (2010) in a study that analyzed *tifton-85* using three different nitrogen doses in the topdressing fertilization, with coefficients of determination that ranged from 0.88 to 0.92. The strong relationship between the readings performed with the portable chlorophyll meter and the total chlorophyll that was extracted from the leaves was also found in other species, including corn

(ARGENTA et al., 2001), apple (AMARANTE et al., 2008), and tomato (SCHUELTER et al., 2003).

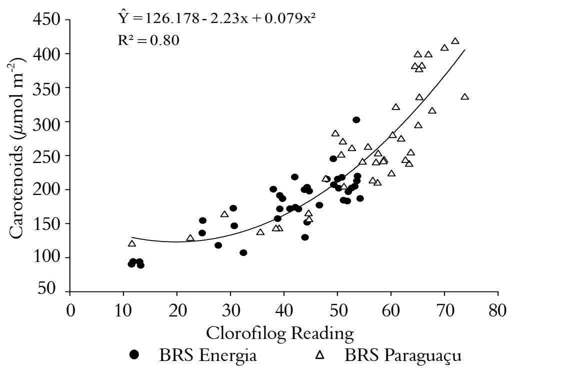

Figure 3. Relationship between the readings of the ClorofiLOG® portable chlorophyll meter and the contents of carotenoids in the leaves of the BRS Energia and BRS Paraguaçu castor oil cultivars.

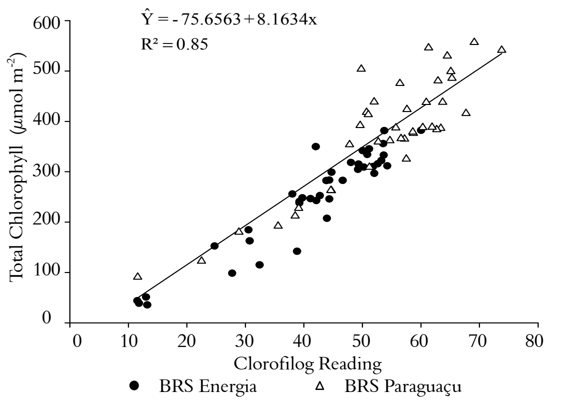

Figure 4. Relationship between the readings of the ClorofiLOG® portable chlorophyll meter and the total chlorophyll content in the leaves of the BRS Energia and BRS Paraguaçu castor oil cultivars.

The relationship between the chlorophyll *a* and the chlorophyll *b* is described in Figure 5. This relationship expresses the ability to capture light under shaded conditions, similar to the reports of Nakazono et al. (2001) and Larcher (2000). Although the chlorophyll *b* measurements by both methods did not agree well (Figure 2), the relationship between chlorophylls *a* and *b* had a high coefficient of determination. Similar results were described by Torres Netto et al. (2005) using the SPAD-502 portable chlorophyll meter in cotton and the coffee, with coefficients of determination of 0.73 and 0.92, respectively.

The relationship between the concentration of total chlorophyll and of carotenoids (Figure 6) and the readings performed by the ClorofiLOG® portable chlorophyll meter were adjusted to a quadratic model. These results are similar to those found by Brito et al. (2011) for cotton. This relationship between the concentration of total chlorophyll and the concentration of carotenoids is important for the protection against photo-oxidative, mainly in leaf senescence, as reported by Hendry and Price (1993).

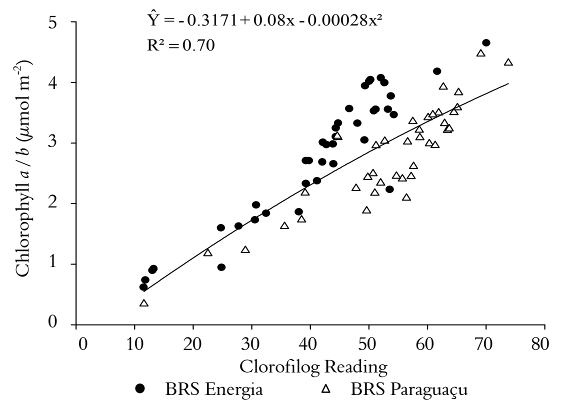

Figure 5. Relationship between the readings of the ClorofiLOG® portable chlorophyll meter and the contents of chlorophyll *a* and *b* in the leaves of the BRS Energia and BRS Paraguaçu castor oil cultivars.

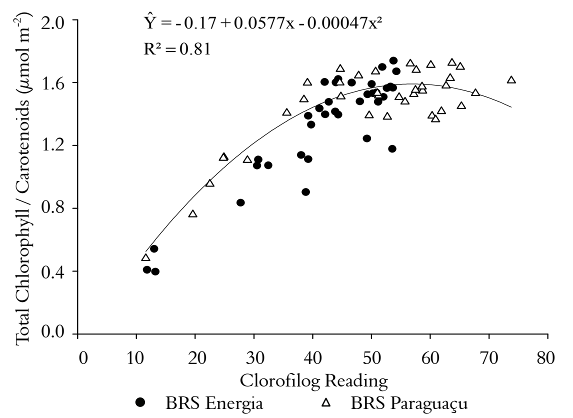

Figure 6. Relationship between the readings of the ClorofiLOG® portable chlorophyll meter and the contents of total chlorophyll/carotenoids in the leaves of the BRS Energia and BRS Paraguaçu castor oil cultivars.

Conclusion

Based on our mathematical models, with the exception of chlorophyll *b*, the ClorofiLOG® 1030 portable chlorophyll meter estimated the concentration of photosynthetic pigments with high precision, thus saving time and the chemical reagents that are typically used in conventional procedures.

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