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Indirect measurement of photosynthetic pigments in the leaves of *Jatropha curcas*

Quantificação indireta de pigmentos fotossintetizantes em folhas de *Jatropha curcas*

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Abstract

The aim of this work was to generate mathematical models capable of identifying photosynthetic pigments and soluble proteins from the leaves of *Jatropha curcas* using the relationship between classical readings performed by spectrophotometry and the chlorophyll meter, ClorofiLOG ® 1030. The work was conducted at Embrapa Cotton, in the city of Campina Grande, state of Paraíba, Brazil. For indirect analysis, portable equipment was used to read leaf discs at different stages of development. The chlorophyll in these discs was then determined using a classical method, while the Bradford method was used to determine soluble proteins. The data were subjected to analysis of variance and regression analyses, in which the readings obtained using the portable chlorophyll meter were the dependent variables and the photosynthetic pigments and soluble protein determined by the classical method the independents variables. The results indicated that with the exception of chlorophyll b and soluble protein, the mathematical models obtained with the portable chlorophyll ClorofiLOG ® 1030 can be used to estimate the concentration of photosynthetic pigments with high precision, thus saving time and the chemical reagents required for conventional procedures.
Key words: Chlorophyll meter, calibration, chlorophyll, soluble protein

Resumo

O objetivo do presente trabalho foi gerar modelos matemáticos capazes de reportar os pigmentos fotossintéticos e proteína solúvel nas folhas de *Jatropha curcas* por meio da relação entre leituras realizadas por espectrofotometria clássica e por clorofilômetro, ClorofiLOG® 1030. O trabalho foi realizado na Embrapa Algodão, na cidade de Campina Grande (PB). Para a análise indireta, foi usado o equipamento portátil para leituras em discos foliares com diferentes estádios de desenvolvimento. A clorofila nestes discos foi então determinada usando o método clássico, enquanto que para a determinação da proteína solúvel utilizou-se a metodologia de Bradford. Os dados foram submetidos a análise de variância e de regressão em que as leituras obtidas com o medidor portátil de clorofila foram as variáveis dependentes e os pigmentos fotossintéticos e de proteínas solúveis, determinadas pelo método clássico foram as variáveis independentes. Os resultados indicaram que, com exceção da clorofila b, e proteína solúvel, os modelos matemáticos obtidos por meio do clorofilômetro portátil ClorofiLOG® 1030 podem ser utilizados para estimar a concentração dos pigmentos fotossintéticos com alta precisão, poupando tempo e reagentes químicos normalmente utilizados em procedimentos convencionais.
Palavras-chave: Clorofilômetro, calibração, clorofila, proteína solúvel

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The technique of spectrophotometry is widely used to directly quantify organic molecules, such as proteins, nucleic acids and chlorophylls, as well as inorganic compounds. This technique is a common procedure for measuring leaf chlorophyll contents in plants, but is time-consuming and leads to the destruction of the samples. By contrast, the portable chlorophyll meter is based on non-destructive, simple and instantaneous principles, which allows leaf ontogenetic studies of crops (TORRES NETTO et al., 2005; BRITO et al., 2011).

In the classical detection of photosynthetic pigments, organic solvents are used, such as dimethyl sulfoxide (DMSO). Their advantage is their high diffusing capacity through leaf membranes, as well as being efficient in dissolving carrier proteins, thus facilitating the process of detection (HISCOX; ISRAELSTAM, 1979).

To obtain quick results regarding chlorophyll contents in order to help making decisions on fertilization and without added costs of reagents, handheld devices such as the chlorophyll meter are very useful. These devices have two or three diodes that emit different wavelengths through the leaf sample, such as the SPAD-502 and ClorofiLOG 1030, respectively. In the latter, two diodes emit wavelengths that lie in reflectance near the peak of each type of chlorophyll (635 and 660 nm), while the third diode is used for low absorbance, serving as an internal reference to compensate for the thickness of the leaves.

The light transmitted through the sample, inversely proportional to the amount of light used by chlorophyll, reaches a receiver and converts it into electric analog signals. Regardless of the instrument used, the sensors receive radiation to provide a single reading proportional to chlorophyll a, b carotenoid levels in dimensionless units. Due to soluble protein levels correlating with the nitrogen content through of chlorophyll there is a possibility of indirectly quantifying soluble protein concentrations using this principle.

Despite the differences between the equipments, the spectrophotometer uses basically the same principle, where light beams are emitted at wavelengths and absorbances are recorded by the spectrophotometer, which are later transformed by mathematical calculations to determine photosynthetic pigment concentrations. Furthermore, it is possible to indirectly estimate photosynthetic pigment levels through chlorophyll meters (RIGON et al., 2012b). This is because the chlorophyll content of leaves strongly correlates with the nitrogen concentration and yields. However, it should be noted that Wolff and Floss (2008) found no correlation between photosynthetic pigment levels and nitrogen and grain yield.

The models that describe the relationship between the readings of the portable equipments vary for each crop, according to the characteristics that are intrinsic to each species, and require independent calibration (UDDLING et al., 2007). This paper proposes an indirect determination of chlorophyll content and soluble protein using portable equipment and mathematical models, obtained by the relationship between the conventional determination of leaf samples from *Jatropha curcas*.

Jatropha curcas plants were derived from a breeding program and were subjected to different levels of nitrogen treatment. The leaves were collected randomly from different stages of development, which resulted in different levels of chlorophyll being obtained by the portable chlorophyll meter. The experiment was conducted at Embrapa Cotton, a research unit of Embrapa (Brazilian Agricultural Research Corporation) that is located in the city of Campina Grande, state of Paraíba, Brazil (7°13'32"S, 35°54'22"W), whose predominant weather is the type AS', that is, semiarid, hot and humid, with a maximum annual temperature of 28.6 ºC and a minimum of 19.5 ºC.

For analysis with the portable equipment for determination of chlorophyll, leaf discs with an area of 113 mm² were removed from different

stages of development of *Jatropha curcas*, using a perforated metallic cylinder. Subsequently, each leaf disc was measured on the portable chlorophyll meter ClorofiLOG® 1030. For the determination of leaf protein contents, three readings were taken by the same equipment, while new leaf samples with a mass of 0.2 g were analyzed by spectrophotometry.

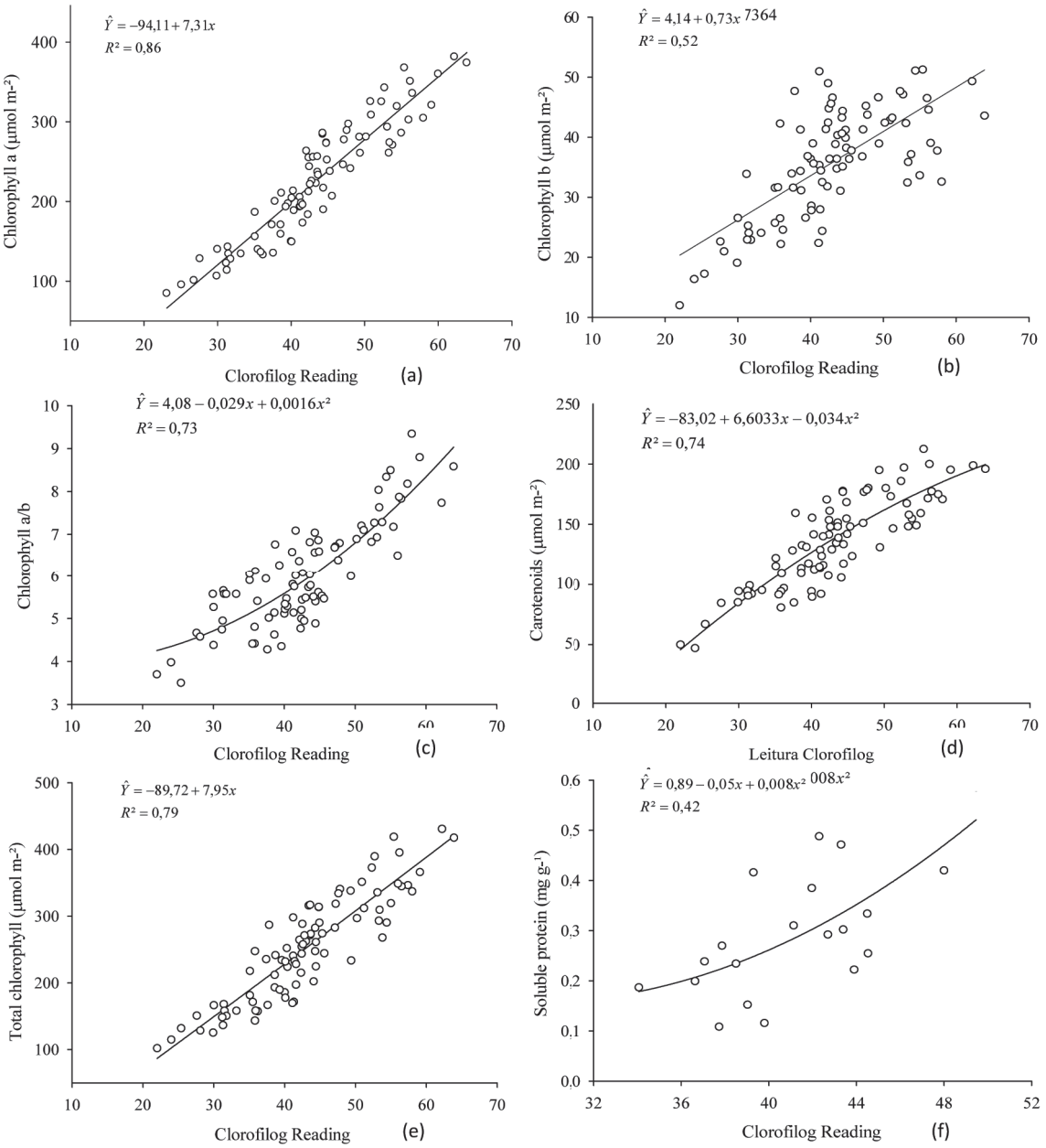
For the classical analysis by spectrophotometry, the leaf discs were placed in test tubes under refrigeration to prevent the denaturation of enzymes and proteins, and were previously wrapped in aluminum foil to protect against solar radiation. The methodology adapted by Hiscox and Israelstam (1979) was followed by dissolving the samples. In each test tube, 5 mL of DMSO were added, incubated at 70° C for 30 minutes, and then shaken every 10 minutes. As a result, the pigments were solubilized and 3 mL were transferred to an automatic pipettor quartz cuvette with a volume of 3 cm^{-3}. Absorbance was detected at wavelengths 480, 649 and 665 nm. The pigments were then quantified using equations.

To obtain soluble protein from leaf samples, 0.2 g of leaves were macerated with liquid nitrogen and maintained in 2 ml of potassium phosphate buffer (0.1 mol L; pH 6.5), using monobasic potassium phosphate (KH_2PO_4) and dibasic sodium phosphate (Na_2HPO_4) prepared in Milli-Q water. Later, they were centrifuged at 80,000 rpm for 20 minutes at 4 º C. Then, 50 µL of sample were added to a 1.5-mL quartz cuvette containing Bradford reagent (PA) to determine total protein levels at the wavelength 595 nm (BRADFORD, 1976). The technique used the dye Coomassie brilliant blue BG-250. The dye interacts with the protein macromolecules containing basic amino acid side chains or aromatic groups. At the pH of the reaction, interaction with the proteins causes a shift in the balance of the dye to the anionic form, which absorbs strongly at 595 nm.

Development of models of relationship the values for each wavelength were used in the equations reported by Wellburn et al. (1994) in µg/mL extracts, considering the volume of the extractor, the total leaf area and the extracted molar mass of chlorophyll b and carotenoids. The levels were expressed in µmol m^2 leaf blade. The transformed data were subjected to analysis of variance and polynomial regression for significant data, obtaining the degree of determination for each relationship, with relative chlorophyll content being the dependent variable, and photosynthetic pigments and soluble proteins independent variables.

The results showed that the portable chlorophyll meter readings displayed good accuracy and were comparable to those obtained with the classical method of chlorophyll a detection (µmol m^2) (Figure 1-A), with a determination coefficient of 0.86. Thus, the mathematical models generated can be used to estimate the level of this pigment with high accuracy, speed and efficiency, without the expense of reagents. Rigon et al. (2012b) also noticed a strong relationship between chlorophyll meter readings and those obtained conventionally in the laboratory for both sesame and castor oil. Similar observations were made by Torres Netto et al. (2005) for coffee plants and Brito et al. (2011) for cotton leaves. Therefore, the portable device and mathematical models can be used to estimate the content of chlorophyll a in the leaves of *Jatropha curcas* plants with great precision in a fast and efficient way and without the added cost of reagents.

Figure 1. Relationships between chlorophyll meter readings using the portable Clorofilog and levels of chlorophyll a (A), chlorophyll b (B), chlorophyll a/b (C), carotenoids (D), total chlorophyll (E) and soluble protein (F) in the leaves of *Jatropha curcas*.



Source: Elaboration of the authors.

However, for chlorophyll b, the meter readings gave a very low coefficient of determination and did not yield measurements similar to those of the conventional laboratory method (Figure 1 – B). This is due to the fact that the wavelength emitted by the device was closer to the absorption peak of chlorophyll a than b (NEVES et al., 2005). The measurement of chlorophyll b content through portable equipment is harder, as reported by Richardson, Duigan and Berlyn (2002), and Neves

et al. (2005). Most red light emitted by the device is absorbed by chlorophyll a. When Costa (2009) used the SPAD-502 portable chlorophyll meter to evaluate different cultivars of sugarcane, he also obtained low coefficients of correlation for chlorophyll b, which ranged from 0.46 to 0.78. Furthermore, both Amarante et al. (2008), in apple plants, and Richardson, Duigan and Berlyn (2002), in *Betula papyrifera*, reported that the SPAD-502 estimate of chlorophyll b was less reliable than that of chlorophyll a and the total chlorophyll content. However, this relation changes in accordance with the intrinsic characteristics of each species, a high coefficient of determination of 0.98 was obtained in sesame for chlorophyll b using portable equipment (RIGON et al., 2012a).

Regarding the contents of chlorophyll a/b, we observed that although chlorophyll b was not characterized with high efficiency, the ratio of chlorophyll a to b was determined with a coefficient of 0.73 (Figure 1-C). This relationship was also observed recently in cotton, coffee and sesame (BRITO et al., 2011; TORRES NETTO et al., 2005; RIGON et al., 2012a). This relationship is used as an indication of premature senescence and plant responses to shading when subjected to high rates of photosynthetic radiation (NAKAZONO et al., 2001). This can also be used as an indicator of leaf N partitioning, based on the positive relationship between chlorophyll *a*/b and the rate of light collected by the chlorophyll-protein complex of Photosystem II (KITAJIMA; HOGAN, 2003).

Efficacy was observed again between the classical and indirect measurements for the levels of carotenoids, with a determination coefficient of 0.74 (Figure 1-D). These pigments are responsible for protecting plant leaf against excess light and photo-oxidation. The quantification of these proteins indicates whether the plants have been subjected to stress that increases the levels of these pigments. In addition, carotenoids are usually associated with many proteins that constitute the photosynthetic apparatus (SIKUKU et al., 2010). Consequently, indirect quantification of carotenoids is an important marker of the overall state of the plant.

For total chlorophyll measurements, there was a significant relationship between the portable chlorophyll meter recordings and those procured in the laboratory (the classical method). The model that best expressed this relationship was a linear one, with a coefficient of determination of 0.79 (Figure 1-E). These results corroborate those of Britto et al. (2011) in cotton plants, who demonstrated that this index could be used to diagnose photooxidative protection during leaf senescence. Moreover, our data confirmed 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.

Although soluble proteins correspond to amino acid intermediates formed in the production of ammonia, the ability of the chlorophyll meter to characterize soluble protein levels was not satisfactory, yielding a determination coefficient of 0.42 (Figure 1-F).

Therefore, it was possible to obtain chlorophyll meter readings of photosynthetic pigment levels that were comparable to those provided by spectrophotometry. With the exception of chlorophyll *b* and soluble protein, the mathematical models obtained by the chlorophyll meter can be used to estimate the concentration of photosynthetic pigments with high precision, thus saving time and the costs of chemical reagents that are typically used in conventional procedures.

References

AMARANTE, C. V. T. do; STEFFENS, C. A.; ZANARDI, O. Z.; ALVES, E. de O. Quantificação de clorofilas em folhas de macieiras 'Royal Gala' e 'Fugi' com métodos ópticos não-destrutivos. *Revista Brasileira de Fruticultura*, Cruz das Almas, v. 30, n. 3, p. 590-595, 2008.

BARBIERI JUNIOR, É.; ROSSIELLO, R. O. P.;

MORENZ, M. J. F.; RIBEIRO, R. C. Comparação de métodos diretos de extração e quantificação dos teores de clorofilas em folhas do capim-Tifton 85. *Ciência Rural,* Santa Maria, v. 40, n. 3, p. 633-636, 2010.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, Bethesda, v. 7, n. 72, p. 248-54, 1976.

BRITO, G. G.; BRANDÃO, Z. N.; SILVA, V. B.; FRANKLIN, M. S.; SILVA, D. A. Non-destructive analysis of photosynthetic pigments in cotton plants. *Acta Scientiarum Agronomy*, Maringá, v. 33, n. 4, p. 671-678, 2011.

COSTA, C. T. S. *Crescimento, pigmentos fotossintéticos e produtividade de cana de açúcar, no quarto ciclo de cultivo.* 2009. Dissertação (Mestrado em Agronomia) – Universidade Federal de Alagoas, Rio Largo.

HISCOX, J. D.; ISRAELSTAM, G. F. A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany*, Guelph, v. 57, n. 12, p. 1332-1334, 1979.

KITAJIMA, K.; HOGAN, K. P. Increases of chlorophyll *a*/b ratios during acclimation of tropical woody seedlings to nitrogen limitation and high light. *Plant Cell and Environment*, Malden, v. 26, n. 6, p. 857-865, 2003.

NAKAZONO, E. M.; COSTA, M. C.; FUTATSIGI, K. E.; PAULILO, M. T. S. Crescimento inicial de *Euterpe edulis* Mart. em diferentes regimes de luz. *Revista Brasileira de Botânica*, São Paulo, v. 24, n. 2, p. 173-179, 2001.

NEVES, O. S. C.; CARVALHO, J. G.; MARTINS, F. A. D.; PÁDUA, T. R. P; PINHO, P. J. Uso do SPAD-502 na avaliação dos teores foliares de clorofila, nitrogênio, enxofre, ferro e manganês do algodoeiro herbáceo. *Pesquisa Agropecuária. Brasileira*, Brasília, v. 40, n. 5, p. 517-521, 2005.

RICHARDSON, A. D.; DUIGAN, S. P.; BERLYN, G. P. An evaluation of noninvasive methods to estimate foliar chlorophyll content. *New Phytologist*, Lancaster, v. 153, n. 1, p. 185-194, 2002.

RIGON, J. P. G.; BELTRÃO, N. E. de M.; CAPUANI, C.; BRITO NETO, J. F. de; SILVA, F. V. de F. Análise não destrutiva de pigmentos fotossintéticos em folhas de gergelim. *Agriambi*, Campina Grande, v. 16, n. 3, p. 258-261, 2012a.

RIGON, J. P. G.; CAPUANI, C.; BELTRÃO, N. E. de M.; BRITO NETO, J. F. de; SOFIATTI, V.; SILVA, F. V. DE F. Non-destructive determination of photosynthetic pigments in the leaves of castor oil plants. *Acta Scientiarum. Agronomy* Maringá, v. 34, n. 3, p. 325-329, 2012b.

SIKUKU, P. A.; NETONDO, G. W.; ONYANGO, J. C.; MUSYIMI, D. M. Chlorophyll fluorescence, protein and chlorophyll content of three nerica rainfed rice varieties under varying irrigation regimes. *ARPN Journal of Agricultural and Biological Science*, New City, v. 5, n. 2, p. 19-25, 2010.

TORRES NETTO, A.; CAMPOSTRINI, E.; OLIVEIRA, J. G.; SMITH, R. E. B. Photosynthetic pigments, nitrogen, chlorophyll *a* fluorescence and SPAD-502 readings in coffee leaves. *Scientia Horticulturae*, Amsterdam, v. 104, n. 2, p. 199-209, 2005.

UDDLING, J.; GELANG-ALFREDSSON, J.; PIIKKI, K.; PLEUEL, H. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. *Journal Photosynthesis Research,* Netherlands, v. 91, n. 1, p. 37-46, 2007.

WELLBURN, A. R. The spectral determination of chlorophylls a and b, as well as total Carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology*, v. 144, n. 3, p. 307-313, 1994.

WOLFF, W. M.; FLOSS, E. L. Correlação entre teores de nitrogênio e de clorofila na folha com o rendimento de grãos de aveia branca. *Ciência Rural*, Santa Maria, v. 38, n. 6, p. 1510-1515, 2008.