

SPAD index as an indirect estimate of chlorophyll content in okra plants

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ABSTRACT

Chlorophyll, the molecule responsible for the photosynthetic activity of plants, can be quantified by direct and indirect methods. The study aimed to relate the results of chlorophyll content obtained by different determination methods in okra leaves. Chlorophyll content was estimated using the SPAD index (non-destructive) and leaf pigments extraction with acetone (destructive). Analyzes were carried out on okra leaves (cultivar Santa Cruz 47) from the middle third of plants during the reproductive stage, varying colors from yellow to dark green under rainfed conditions. Determinations by the non-destructive method were conducted with SPAD-502 equipment, and determinations by the destructive way were carried out by spectrometry. Descriptive analysis, correlation, and regression were performed on the data. SPAD index ranged from 20.93 to 49.03 and showed a linear correlation with leaf pigments content. SPAD index showed a positive correlation with chlorophyll b and total chlorophyll contents. However, a negative relationship was observed for chlorophyll a content. Variation in carotenoid content was not directly related to the SPAD index. Estimating chlorophyll content by the non-destructive method directly correlates with the absolute content in okra leaves.

Keywords: *Abelmoschus esculentus*, Analysis methods, Leaf pigments.

Índice SPAD como estimativa indireta do conteúdo de clorofila em plantas de quiabo

RESUMO

A clorofila, molécula responsável pela atividade fotossintética das plantas, pode ser quantificada por métodos diretos e indiretos. O objetivo do estudo foi relacionar os resultados do conteúdo de clorofila obtidos por diferentes métodos de determinação em folhas de quiabo. Foi estimado o conteúdo de clorofila através do índice SPAD (não-destrutivo) e da extração dos pigmentos foliares com acetona (destrutivo). As análises foram realizadas em folhas de quiabo (cultivar Santa Cruz 47) do terço médio de plantas no estágio reprodutivo com coloração variando de amarela a verde escuro, em condição de sequeiro. Determinações pelo método não destrutivo foram realizadas com equipamento SPAD-502 e as determinações por método destrutivo foram realizadas por espectrometria, utilizando acetona como extrator. Foi realizada análise descritiva, correlação e regressão nos dados. O índice SPAD variou de 20,93 a 49,03, e apresentou correlação linear com o conteúdo de pigmentos foliares. O índice SPAD apresentou correlação positiva com os teores de clorofila b e clorofila total, entretanto para o conteúdo de clorofila a foi observado relação negativa. A variação do conteúdo de carotenoides não apresentou relação direta com o índice SPAD. A estimativa do conteúdo de clorofila por método não destrutivo apresenta relação direta com o conteúdo absoluto em folhas de quiabo.

Palavras-chave: *Abelmoschus esculentus*, Métodos de análises, Pigmentos foliares.

Among fruit-vegetables, okra (*Abelmoschus esculentus*) has great economic importance worldwide, with yield related to environmental conditions, nutrition, and plant physiological activity such as photosynthesis (Taiz et al., 2017; Schafleitner et al., 2021). The chlorophyll molecule is responsible for photosynthesis in plants, characterized by two forms (*a* and *b*); chlorophyll *b* expands the range of light used by photosynthesis and transfers energy to chlorophyll *a* molecule that uses it in photosynthesis (Taiz et al., 2017). Chlorophyll quantification during plant development helps in crop management, aiming to maximize yield (Jiang et al., 2017; Nogueira et al., 2018). It is an essential parameter in nutritional management, such as estimating the nitrogen status of plants (Li et al., 2019).

Quantification of chlorophyll content can be performed using direct (destructive) methods involving foliar pigments extraction (Lichtenthaler, 1987) and indirect (non-destructive) methods, such as estimation by SPAD index - Soil Plant Analysis Development (Wenneck et al., 2021a). In addition to being a non-destructive measure, it is fast and presents a low cost (Jiang et al., 2017). SPAD has been widely used as a management parameter in several crops, such as tomato (Nogueira et al., 2018), broccoli (Vidigal et al., 2021), lettuce, arugula, chives (Dias et al., 2020), cauliflower (Wenneck et al., 2021b), peas (Wenneck et al., 2021a), and potato (Li et al., 2019). The method can be used to monitor abiotic stress in plants, such as water deficit (Wenneck et al., 2021b), assist in plant nutrition (Vidigal et al., 2021), and infer the post-harvest quality of products (Dias et al., 2020).

Considering that the values obtained by SPAD are dimensionless, the correlation between the absolute chlorophyll content and the SPAD index is important to advance studies on the management of crops. Therefore, the study aimed to relate the values of different methods in determining chlorophyll content in okra leaves. The study was conducted at the Irrigation Technical Center (23°23'57"S, 51°57'07"W, and altitude of 542m) of the State University of Maringá, in Maringá-PR. The region has average annual precipitation between 1400 and 1600 mm, temperature between 21.1 and 22°C, solar radiation of 14.5 to 15 MJ m⁻² day⁻¹ (IAPAR, 2019).

2019). The experiment was carried out in a completely randomized design under rainfed conditions, determining the SPAD index and the chlorophyll content in 40 leaf samples.

The okra sowing (cultivar Santa Cruz 47) was conducted on September 8, 2021, with 0.30 m between plants and 1.00 m between rows. The collection of leaves took place 70 days after sowing (DAS), between 8 and 9 am, in the reproductive stage (at the beginning of the first fruits harvest). Leaves were collected from the middle third of plants with the color ranging from yellowish to dark green.

The soil in the area is classified as NITOSSOLO VERMELHO distroférrico, corresponding to a Ultisol in the soil taxonomy classification (EMBRAPA, 2018), with a clayey texture (80% clay) and presenting chemical parameters of pH CaCl₂ 5.3; aluminum: 0.0 cmol_c dm⁻³; calcium: 3.61 cmol_c dm⁻³; magnesium: 2.10 cmol_c dm⁻³; potassium: 0.87 cmol_c dm⁻³; phosphor (Mehlich I): 12.49 mg dm⁻³; organic matter: 2.02%, and cation exchange capacity: 8.95 cmol_c dm⁻³.

Indirect determination of chlorophyll content was performed with SPAD-502 equipment (Minolta®) with three readings per leaf. After non-destructive determination, leaves were collected for direct determination of chlorophyll content. Leaf pigments, chlorophyll (*a* and *b*), total chlorophyll (*a+b*), and carotenoids were determined by spectrometry using acetone as the extractor. To extract the pigments, the leaf tissue sample (150 mg) was submerged in 2 mL of pure acetone and kept at a dark and low temperature until the tissue was depigmented. The extract was placed in quartz cuvettes (2 mL) for absorbance determination in a spectrophotometer (AJX-6100PC) at wavelengths of 661.6, 644.8, and 470 nm. The pigment content was determined according to Lichtenthaler (1987).

Descriptive data analysis, linear correlation between variables, and regression analysis between leaf pigments and SPAD index were performed. SISVAR (Ferreira, 2019) and Microsoft Excell® software were used for data analysis. SPAD index values ranged from 20.93 to 49.03, with a mean of 38.37 (Table 1), total chlorophyll values ranged from 25.60 to 53.07 µg g⁻¹, while carotenoids had little expressiveness, ranging from 9.79 to 11.07 (Table 1).

Table 1. Descriptive analysis of SPAD, chlorophyll, and carotenoid values in medium leaves of okra (Santa Cruz 47 cultivar) at 70 DAS (reproductive stage) under rainfed conditions.

Parameters	SPAD ⁽¹⁾	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Total chlorophyll	Carotenoids
Average	38.37	19.32	25.83	45.58	10.02
Median	40.82	19.11	28.09	49.21	9.91
Standard deviation	7.22	0.55	8.48	7.95	0.59
Variance	52.18	0.30	71.84	63.20	0.35
Minimum	20.93	18.69	6.53	25.60	8.79
Maximum	49.03	20.27	35.14	53.07	11.07

⁽¹⁾ SPAD Index.

From parameters determined by destructive and non-destructive methods, mathematical models were obtained for prediction based on the SPAD index and chlorophyll content. Linear correlation was obtained between SPAD index values and chlorophyll content (Figure 1). Chlorophyll *a* reduced ($R^2 = 0.67$) according to the increase in SPAD index (Figure 1A) while chlorophyll *b* increased (Figure 1B), with a high determination coefficient ($R^2 = 0.90$).

Total chlorophyll had a determination coefficient of 0.81 (Figure 1C), reflecting the reduction in chlorophyll *a* and increased chlorophyll *b*. These results are similar to those obtained by Wenneck et al. (2021a) in *Pisum sativum* when analyzing the relationship between SPAD index and leaf pigments, getting a high correlation of 0.89.

SPAD index of cauliflower leaves subjected to a 30% water deficit during the cycle showed a high correlation with chlorophyll *b* content (Wenneck et al., 2021b). The rainfed condition of okra cultivation may have influenced to obtain similarity in this relationship (Table 2). Environmental conditions strongly affect chlorophyll *b* values and have less influence on chlorophyll *a* (Moura Neto et al., 2021). Under cultivation without water deficit, the SPAD index is related to the absolute content of chlorophyll *a* compared to chlorophyll *b* (Wenneck et al., 2021b).

From the analysis of Pearson linear correlation (Table 2), it was observed that chlorophyll *b* presents a

positive correlation with total chlorophyll (0.98) and a negative correlation (-0.90) with chlorophyll *a*.

It is inferred that the SPAD index presents a weak correlation with carotenoids and strong with green pigments of okra leaves, evidenced by low correlation values of chlorophyll with carotenoids (Table 2).

Studies show a high correlation between values obtained by SPAD-502 and chlorophyll content (Jiang et al., 2017; Shibaeva et al., 2020). Vidigal et al. (2021) demonstrate that the SPAD index also correlates highly with leaf nitrogen content. Furthermore, it has high accuracy even in leaves with interveinal chlorosis (Shibaeva et al., 2020). Other nutrients such as zinc and boron in okra plants can influence SPAD index values (Jahan et al., 2020).

Indirect determination (SPAD index) is sensitive to differentiate cultivars of the same plant species at different stages of phenological development and to differentiate cultivars that were grown in different growing regions, that is, under different environmental conditions. (Li et al., 2019; Souza et al., 2020).

Under water stress, the SPAD index tends to decrease in okra leaves. Still, applying biochar and arbuscular mycorrhizal fungi in the cultivation soil proved to be effective, increasing the index depending on the application of these inputs (Jaborova et al., 2021). Photosynthetic rate is influenced by chlorophyll content, closely related to SPAD index values according to the plant growth (Ferhi et al., 2017).

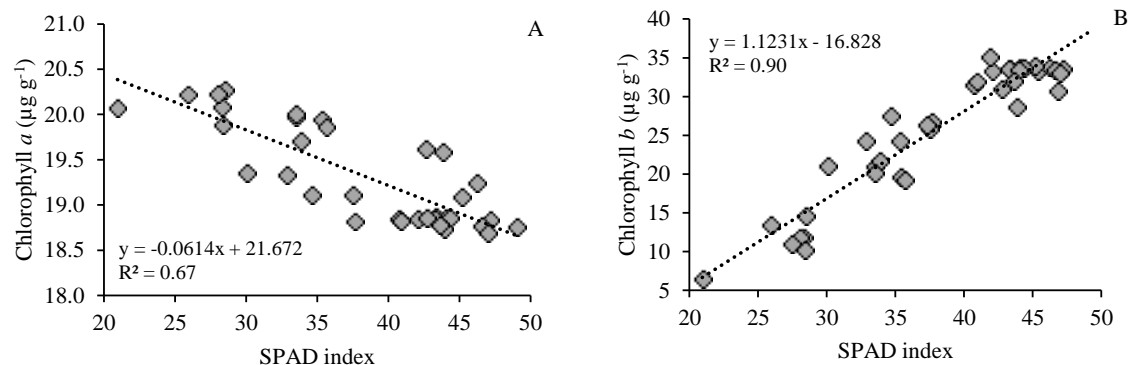


Figure 1. Leaf pigments content according to the SPAD index in medium leaves of okra (Santa Cruz 47 cultivar) at 70 DAS (reproductive stage) under rainfed conditions. A) Chlorophyll *a*; B) Chlorophyll *b*; C) Total chlorophyll

Table 2. Linear correlation between leaf pigments in medium leaves of okra (cultivar Santa Cruz 47) at 70 DAS (reproductive stage) under rainfed conditions.

	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Total chlorophyll	Carotenoids
Chlorophyll <i>a</i>	1.00	-	-	-
Chlorophyll <i>b</i>	-0.91	1.00	-	-
Total chlorophyll	-0.90	0.98	1.00	-
Carotenoids	0.00	0.19	0.15	1.00

Monitoring chlorophyll content in plants is a critical management practice, which, incorporated in agricultural production, helps in the sustainable development of agriculture, being part of the technologies that provide information for decision making (Nguyen et al., 2019). In order to use this tool properly and effectively in okra and other species, it is interesting to expand studies that aim to correlate the SPAD index with the absolute chlorophyll content, including analysis in different cultivars, growing seasons, soil, and climate conditions.

SPAD index shows a strong correlation with chlorophyll b and total chlorophyll in medium leaves of okra (cultivar Santa Cruz 47) at 70 DAS (reproductive stage) under rainfed conditions, representing this relationship by linear models. Therefore, chlorophyll content in okra leaves can be representatively determined using SPAD.

Authors' Contribution

Vinícius Villa e Vila contributed to the experimental set up, data collection, writing and revision. Gustavo Soares Wenneck contributed to the experimental set up, data collection, statistical analysis and revision. Daniele de Souza Terassi contributed to the data collection, experimental set up and revision. Roberto Rezende contributed to the experimental set up, revision and technical supervision. Reni Saath contributed to the experimental set up and revision.

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