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## Efficiency of portable chlorophyll meters in assessing the nutritional status of wheat plants

Alessana F. Schlichting<sup>1</sup>, Edna M. Bonfim-Silva<sup>1</sup>, Matheus de C. Silva<sup>1</sup>,  
William Pietro-Souza<sup>1</sup>, Tonny J. A. da Silva<sup>1</sup> & Lorraine do N. Farias<sup>1</sup>

<sup>1</sup> Instituto de Ciências Agrárias e Tecnológicas/Universidade Federal de Mato Grosso. Rondonópolis, MT. E-mail: [alessanamt@gmail.com](mailto:alessanamt@gmail.com); [embonfim@hotmail.com](mailto:embonfim@hotmail.com) (Corresponding author); [matheus\\_gbi@hotmail.com](mailto:matheus_gbi@hotmail.com); [william\\_pietro@hotmail.com](mailto:william_pietro@hotmail.com); [tonnyjasilva@hotmail.com](mailto:tonnyjasilva@hotmail.com); [nascimentoofarias@hotmail.com](mailto:nascimentoofarias@hotmail.com)

### Key words:

SPAD

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*Triticum aestivum*

### ABSTRACT

The objective of this study was to verify the efficiency of two portable chlorophyll meters (Minolta SPAD<sup>®</sup> 502 and Falker ClorofiLOG<sup>®</sup> 1030) in assessing the nutritional status of wheat plants, correlating the indices from the devices and the direct determination of chlorophyll content with the concentration of nitrogen (N) in the plant. The experiment was conducted in a greenhouse, in pots with 5 dm<sup>3</sup> of Oxisol, in a completely randomized design, with six N doses (0, 80, 160, 240, 320 and 400 mg dm<sup>-3</sup>) and five replicates. At 47 days after emergence, the readings of SPAD and Falker indices and the quantification of chlorophyll content and N concentration in wheat plants were performed, as well as analysis of variance and correlation test, both at 0.05 probability level. The chlorophyll meters Minolta SPAD<sup>®</sup> 502 and Falker ClorofiLOG<sup>®</sup> 1030 do not differ with respect to the indirect determination of chlorophyll in wheat plants. The Falker chlorophyll index was statistically equal to the chlorophyll content. Indirect chlorophyll indices and chlorophyll content showed a high correlation with the N concentration in the plant.

### Palavras-chave:

SPAD

Falker

*Triticum aestivum*

## Eficiência de medidores portáteis de clorofila na avaliação do estado nutricional de plantas de trigo

### RESUMO

Objetivou-se, neste trabalho, verificar a eficiência de dois medidores portáteis de clorofila (Minolta SPAD<sup>®</sup> 502 e Falker ClorofiLOG<sup>®</sup> 1030) na avaliação do estado nutricional de plantas de trigo correlacionando-se os índices dos aparelhos e a determinação direta do teor de clorofila com a concentração de nitrogênio na planta. O experimento foi realizado em casa de vegetação e conduzido em vasos com 5 dm<sup>3</sup> de solo - Latossolo Vermelho distrófico. O delineamento foi inteiramente casualizado, com seis doses de nitrogênio (0, 80, 160, 240, 320 e 400 mg dm<sup>-3</sup>) e cinco repetições. Aos 47 dias após a emergência foram realizadas as leituras dos índices SPAD e Falker, a quantificação do teor de clorofila e a concentração de nitrogênio nas plantas de trigo, além da análise de variância e do teste de correlação, ambos até 5% de probabilidade. Os clorofilômetros Minolta SPAD<sup>®</sup> 502 e Falker ClorofiLOG<sup>®</sup> 1030 não apresentam diferença entre si na determinação indireta de clorofila nas plantas de trigo. O índice Falker realizou leituras iguais, estatisticamente, do teor de clorofila. Os índices indiretos de clorofila e o teor de clorofila obtiveram alta correlação com a concentração de nitrogênio na planta.



## INTRODUCTION

Nitrogen (N) is considered as one of the most important nutrients in plant nutrition, since it is used in the synthesis of cell compounds, such as chlorophyll (Lima et al., 2001), thus influencing photosynthesis, production and transport of photoassimilates, growth rate between leaves and roots and the formation of roots (Taiz & Zieger, 2004). Thus, the concern about the adequate management of N fertilization is justified by the important physiological role played by the nutrient, aiming at maximizing the economic performance of crops.

For its diverse uses, wheat (*Triticum aestivum*) is among the most important plant species for human consumption. The state of Mato Grosso, in order to increase its agricultural diversity, has expanded wheat-planted areas every year and increased its production in the Cerrado (CONAB, 2013). Pietro-Souza et al. (2013) observed that N fertilization positively influences the initial development of wheat plants cultivated in a Red Latosol in the Cerrado region.

Therefore, practical and fast evaluation methods that allow assessing plant nutritional status become essential to facilitate crop management. Thus, portable leaf chlorophyll meters, or chlorophyll meters, are widely used, since they estimate chlorophyll contents in an instantaneous, indirect and nondestructive way, based on the optical properties of the leaves, constituting an alternative for the evaluation of N concentration in plants (Argenta et al., 2001).

In Brazil, two models of chlorophyll meters have been widely used: Minolta SPAD<sup>®</sup> 502 and Falker ClorofiLOG<sup>®</sup> 1030, in which the quantification of the chlorophyll index is determined by the light flux transmitted by the leaf, through wavelengths of different absorbances. The portable meter Minolta SPAD<sup>®</sup> 502 (Soil Plant Analysis Development) emits light at two wavelengths ( $\lambda$ ), 650 nm (red) and 940 nm (infrared), and indicates the SPAD chlorophyll index. The chlorophyll meter Falker ClorofiLOG<sup>®</sup> 1030 emits light at three wavelengths ( $\lambda$ ), 635 and 660 nm (red) and 880 nm (infrared), and indicates the Falker chlorophyll index. Both devices provide instantaneous readings proportional to the leaf contents of chlorophylls and carotenoids (Minolta, 1989; Falker, 2008), while the Falker device provides the results of chlorophyll a and b.

The chlorophyll index from the device Minolta SPAD<sup>®</sup> 502 showed positive correlation with leaf N concentrations in wheat plants (Fioreze & Rodrigues, 2012), although studies evaluating the possible differences in efficiency between different models of chlorophyll meters and their relationship with plant N concentrations are still incipient.

In this context, this study aimed to verify the efficiency of two portable chlorophyll meters (Minolta SPAD<sup>®</sup> 502 and Falker ClorofiLOG<sup>®</sup> 1030) in assessing the nutritional status of wheat plants, correlating the respective indices of the devices and the direct determination of chlorophyll content with plant N concentration.

## MATERIAL AND METHODS

The experiment was carried out in a greenhouse, at the Federal University of Mato Grosso, Campus of

Rondonópolis, in Rondonópolis-MT, Brazil (16° 28' S; 50°34' W; 284 m).

The experimental units were represented by plastic pots with capacity for 5 dm<sup>3</sup> of soil. The experiment was set in a completely randomized design with six N doses (0, 80, 160, 240, 320 and 400 mg dm<sup>-3</sup>) and five replicates. An amplitude of six doses was used for the validation of readings of the devices at various plant N concentrations.

The soil used in the experiment was from an area under Cerrado vegetation, in the region of Rondonópolis - MT, and was classified as dystrophic Red Latosol (EMBRAPA, 2013). Analyses performed in the layer of 0-0.2 m showed the following values of chemical and granulometric attributes: pH (CaCl<sub>2</sub>) - 4.1; P - 2.4 mg dm<sup>-3</sup>; K - 28 mg dm<sup>-3</sup>; Ca - 0.3 cmol<sub>c</sub> dm<sup>-3</sup>; Mg - 0.2 cmol<sub>c</sub> dm<sup>-3</sup>; Al - 1.1 cmol<sub>c</sub> dm<sup>-3</sup>; Cation exchange capacity - 5.9 cmol<sub>c</sub> dm<sup>-3</sup>; Base saturation - 6.5%; Organic matter - 22.7 g dm<sup>-3</sup>; Sand - 549 g kg<sup>-1</sup>; Silt - 84 g kg<sup>-1</sup>; Clay - 367 g kg<sup>-1</sup>, with clay loam texture.

Soil acidity was corrected using dolomitic limestone through the increasing base saturation method, raising it to 70%. Soil water content in the pots was maintained through the gravimetric method at 70% of the maximum soil water holding capacity.

Ten seeds of the wheat cultivar Guamirim were planted in each pot, which received fertilization with phosphorus (200 mg dm<sup>-3</sup> of P<sub>2</sub>O<sub>5</sub>) and potassium (150 mg dm<sup>-3</sup> of K<sub>2</sub>O), using single superphosphate and potassium chloride, respectively, at sowing. For N fertilization of the treatments, urea was used as the N source, divided into two applications, at 7 and 15 days after plant emergence. Thinning was performed 8 days after emergence, leaving five plants in each pot.

Data collection and plant cutting occurred at the beginning of flag leaf emergence, approximately 47 days after plant emergence. Indirect readings of leaf chlorophyll (chlorophyll index) were performed with two portable meters (Minolta SPAD<sup>®</sup> 502 and Falker ClorofiLOG<sup>®</sup> 1030) in the leaves +1 and +2, below the flag leaf, of the 5 plants of each experimental plot. The mean of the 10 readings was considered, in each experimental unit, as the index for each plot. All readings were performed in the mid-section of leaf blades (Matsunaka et al., 1997), from 9:00 to 10:00 a.m.

These same leaves used for the readings with chlorophyll meters were collected for chlorophyll quantification, wrapped in aluminum foil and taken to the laboratory in a thermal box with ice. Total chlorophyll was quantified according to the methodology proposed by Arnon (1949).

After removing the diagnostic leaves, the plants were cut at the base, placed in paper bags and dried in a forced-air oven at  $\pm$  65 °C, until constant weight. After drying, they were ground in a Willey-type mill and sieved through a 2-mm grid for N determinations in the leaves, according to the methodology of Malavolta et al. (1997).

In the statistical analyses, the efficiency of the devices (Minolta SPAD<sup>®</sup> 502 and Falker ClorofiLOG<sup>®</sup> 1030) was verified through analysis of variance and Tukey test at 0.05 probability level, with the statistical program Sisvar (Ferreira, 2008), considering the data chlorophyll contents as the efficiency reference. Correlation analysis was performed in order to

verify the relationship of the chlorophyll indices from the devices and chlorophyll contents with the N concentration in the plants, using the statistical program Assistat 7.6 (Silva & Azevedo, 2013).

### RESULTS AND DISCUSSION

The evaluated methods of chlorophyll measurement using the portable devices SPAD<sup>®</sup> 502 and Falker ClorofiLOG<sup>®</sup> 1030 in wheat plants showed the same efficiency ( $p \geq 0.01$ ) with respect to the reading of chlorophyll index. However, when these methods were compared with the laboratory quantification of chlorophyll contents (reference method), only the Falker index was significantly efficient ( $p \leq 0.01$ ) (Table 1).

Chlorophyll evaluation does not depend on the method used, whether direct or indirect. The response is the same when the Falker chlorophyll meter was used, because this device showed indices statistically equal to the chlorophyll contents measured at the laboratory, which shows the capacity of variation of chlorophyll indices, according to the nutritional status of wheat plants.

This efficiency of the variation of Falker meters, according to the nutritional status (N doses), was observed by Barbieri Júnior et al. (2012) and Mattje et al. (2013). The same efficiency occurred for SPAD readings in the study of Viana & Kiehl (2010). The efficiency of evaluation of the nutritional status, with respect to plant N, using the different devices is discussed in the literature, but not regarding the comparison between the efficiency of both meters and the method of chlorophyll extraction at the laboratory.

The increase in the values of chlorophyll indices in portable devices is a reflex of the N doses, due to the increase in chlorophyll content promoted by the higher total N concentration in leaf tissues, since 50-70% of the total N in the leaf constitute enzymes associated with the chloroplasts (Chapman & Barreto, 1997).

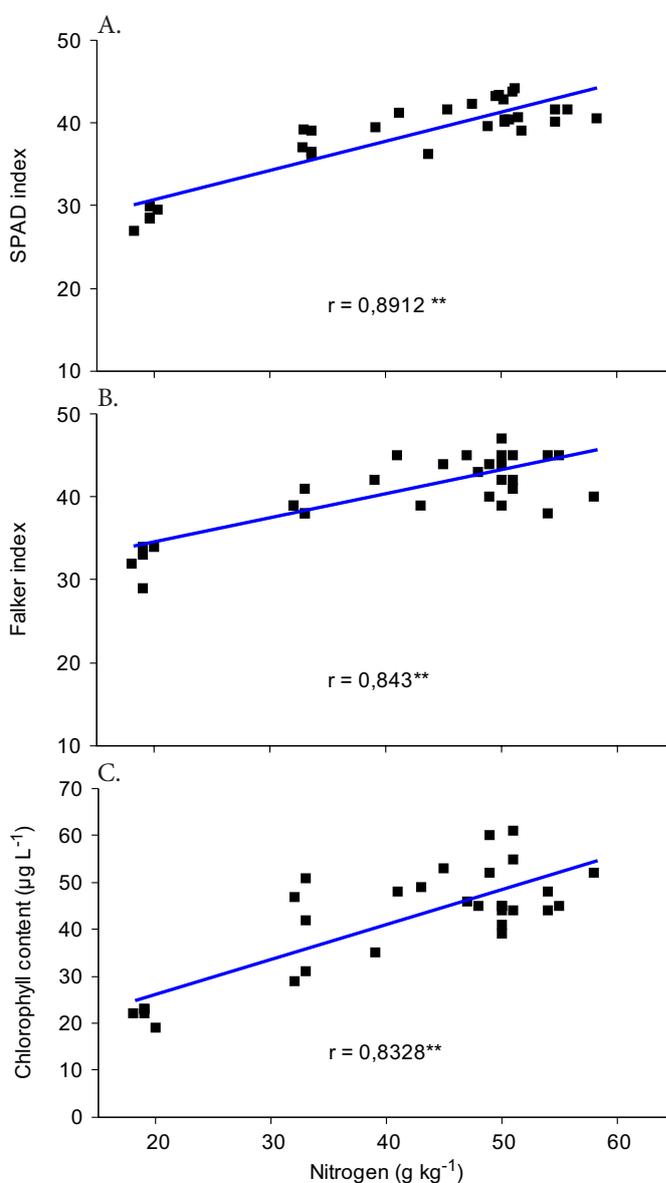
Monitoring plant nutrition with respect to N is essential. According to Jesus & Marengo (2008) and Singh et al. (2010), a portable device provides readings or indices that can be related to leaf chlorophyll contents. In addition, the measurements are instantaneous, practical, low-cost and can be performed at the field, which allows the correction of N deficiency in the same crop year. N fertilization contributes to the increase of crop yield, since more than 90% of plant biomass derives from the photosynthesis, which directly induces plant development (Makino, 2011).

According to Schadchina & Dmitrieva (1995), the determination of chlorophyll contents in the leaves is a precise variable in the estimation of N absorption by wheat

plants, since it is not influenced by the luxury consumption of N (Blackmer & Schepers, 1995). Schepers et al. (1992) highlights that the method of determination of chlorophyll contents (green leaves) is faster than the method of N detection in the plant, which is determined in the dry matter of the leaves.

In order to evidence the relationship between the chlorophyll indices from the devices and chlorophyll contents in wheat plants, the plant N concentration was also measured. All these factors were correlated ( $p \leq 0.01$ ) with plant N concentration (Figure 1), highlighting the efficiency of the portable meters of chlorophyll indices in the prediction of the nutritional status of wheat plants, cv. Guamirim.

According to Abreu & Monteiro (1999) and Schadchina & Dmitrieva (1995), chlorophyll content is directly correlated with N concentration in the leaves and, consequently, with



\*\*Significant at 0.01 probability level

Figure 1. Pearson's correlation in wheat plants 47 days after emergence. Between nitrogen ( $g\ kg^{-1}$ ) the SPAD index (A); between nitrogen ( $g\ kg^{-1}$ ) and the FALKER index (B) and between nitrogen ( $g\ kg^{-1}$ ) and the chlorophyll content ( $\mu g\ mL^{-1}$ ) (C)

Table 1. Chlorophyll content and Falker and SPAD indices in wheat plants at 47 days after emergence

Methodology of chlorophyll determination		
Destructive	Nondestructive	
Chlorophyll content ( $\mu g\ mL^{-1}$ )	Falker clorofiLOG <sup>®</sup> 1030	Minolta SPAD <sup>®</sup> 502
42.49 a	40.94 ab	38.48 b
CV: 10.41%		

Means followed by the same letter in the row do not differ statistically at 0.01 probability level; Least significant difference = 2.61; CV – coefficient of variation

plant nutrition and production, which are associated with the chloroplasts present in the leaves (Stocking & Ongun, 1962). Thus, since chlorophyll indices and total chlorophyll content in the leaves were determined in the same leaves (+1 and +2), there was a gradual increase in the degree of reliability of the results obtained in the present study, evidencing the positive correlations of the studied variables.

### CONCLUSIONS

1. The chlorophyll meters Minolta SPAD<sup>®</sup> 502 and Falker ClorofiLOG<sup>®</sup> 1030 do not differ with respect to the indirect determination of chlorophyll in wheat plants.

2. The FALKER chlorophyll index was statistically equal to the chlorophyll content determined in the laboratory.

3. Indirect chlorophyll indices and chlorophyll contents have a high correlation with the nitrogen concentration in the plant.

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