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Correlations between spad readings, chlorophyll and leaf nitrogen in coffee cultivars during fruiting stage

Correlações entre leituras spad, clorofila e nitrogênio foliar em cultivares de café durante estádio de frutificação

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Abstract: The methods currently used to determine the chlorophyll content and the amount of nitrogen present in leaves takes a long time to work, as well as the collection and destruction of the leaf tissues. Therefore, we have been studying the use of recent portable meters of chlorophyll, which enables a quick reading without requiring the destruction of leaves. The objective was to correlate the concentration of chlorophyll (*a*, *b* and total) as well as the concentrations of Nitrogen in the leaf tissue with indexes of SPAD-502 in four cultivars of arabica coffee. The experiment was conducted in the field during the agricultural year of 2006/2007. The experimental design was completely randomized, and the material was collected from the fourth pair of leaves below the apex of the plagiotrophycal, at the half height of the plants and on the two exposed sides between the lines of the coffee plantation. 20 leaves from four cultivars of arabica coffee, Topázio MG-1190, MG-1192 Rubi, Catuaí IAC-99 and Catucaí 2 SL, were used during four stages of leaf development, varying the intensity of green color of the leaves, consistent in the treatments. The estimations with SPAD - 502 showed high accuracy levels of chlorophyll (*a*, *b* and total) in the leaves, also there was a high correlation with the amounts of N extracted by the traditional method. Thus, the use of SPAD - 502 to evaluate the nutritional diagnosis of coffee leaves provides correlation, as well as being able to be repeated quickly, accurately and non-destructively.

Keywords: Arabic coffee, chlorophyllometer, Topázio, Rubi, Catuaí, Catucaí

Resumo: Os métodos atualmente usados para determinar o conteúdo de clororfila e a quantidade de nitrogênio nas folhas demandam longo tempo de trabalho, assim como a coleta e destruição dos tecidos vegetais. Entretanto, tem se estudado o uso de medidores portáteis de clorofila, o que permite uma rápida leitura e sem a necessidade de destruição das folhas. O objetivo foi correlacionar o concentração de clorofila (a, b e total assim o concentração de nitrogênio foliar nos tecidos foliares com o índice do SPAD-502 em quatro cultivares de café arábica. O experimento foi conduzido em campo durante a safra de 2007. O delineamento experimental foi o inteiramente casualizado, e os materiais foram coletados no quarto par de folhas a partir da parte distal do ramo plagiotrópico, terço médio das plantas e nos dois lados da linha de plantio. 20 folhas de cada cultivar de café arábica, Topázio MG-1190, MG-1192 Rubi, Catuaí IAC-99 e Catucaí 2 SL, foram utilizadas durante quatro estádios de desenvolvimento foliar que variaram de intensidade de coloração verde, tais fatores corresponderam nos tratamentos. A estimação com SPAD-502 mostrou altos níveis precisão de clorofila (*a*, *b* e total) nas folhas, também obteve uma alta correlação com os teores de nitrogênio extraído pelo método tradicional. Assim, o uso de SPAD-502 para avaliar o estado nutricional de folhas de cafeeiro promoveu correlação, assim como sendo capaz de ser repetido rapidamente, de precisão e não destrutivo.

Palavras-chave: Café arábico, clorofilômetro, Topázio, Rubi, Catuaí, Catucaí

Introduction

The coffee crops of Brazil were developed mainly in areas under water restrictions. Currently, these areas are developed for coffee because of the irrigation systems to encourage and make Brazil the world's biggest coffee producer and exporter.

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The Brazilian production 45 and 42 million bags in 2014 and 2015, respectively (CONAB, 2015). Due to the coffee crops being irrigated, fertigation can be used. This is a better method of distributing fertilizer, and it allows the fertilizer supply to be closer to the crops needs, minimizing the waste and maintaining a higher yield.

Nitrogen is an essential element used by plants, mainly in the synthesis of cellular components, such as chlorophyll. These pigments capture light for photosynthesis, turn light radiation into the chemical energy transients, ATP and NADPH. These are used in many plant reactions, such as the chlorophyll correspondence to photosynthetic efficiency and consequently plant growth and it's adaptability to different environments (Taiz e Zeiger, 2013).

The coffee plants used had symptoms of nitrogen deficiency, such as: yellowing of older leaves and the reduction of new sprouts. The visual diagnosis is subjective and it is not an efficient method to optimize the use on fertigation, other methods can be more effective. However, the SPAD is a chlorophyllometer, which can determine an index corresponding to chlorophyll content and leaf nitrogen.

Portable readers of chlorophyll (chlorophyllometer) have been used on the evaluation of the amount of chlorophyll in the leaf and measuring the intensity of green color in the leaves. These readers have the advantages of a fast and non-destructive evaluation, so it can easily be repeated and used in field conditions. Therefore, we can quickly evaluate the leaf nitrogen and nutritional status for further fertigation.

The chlorophyllometer readings can be influenced by specific factors such as leaf thickness, phonological stage, genotype and shading, and also the reading methodology. However, it should be considered that the cultivar readings on coffee plants are to be correlated to leaf nitrogen status and thus enabling it to be used in field conditions.

The objective of this study was to correlate the chlorophyll (*a*, *b* and total) contents and leaf nitrogen to SPAD-502 readings, on four coffee arabica cultivars.

Material and methods

This study was conducted under field conditions at the Center of Education, Research and Extension of the coffee business (CEPEA-Café), Federal University of Lavras (UFLA), 910 m of altitude, 21°14' of latitude south and 45°00' of longitude west.

The treatments were arranged in a completely randomized design. The fourth leaf pair at the plagiotrophycal branch was used at the median height of the two sides of the crop row. 20 leaves of four coffee arabica cultivars (Topázio MG-1190, Rubi MG-1192, Catuaí IAC-99 and Catucaí 2 SL) were collected, at four different leaf stages and intensities of green color (nearly completely yellow leaf to an intensely dark green leaf). At sampling time the plants were in the fruiting stage and the fruit were at grain expansion stage.

The plants sampled were selected by dividing the area into standardized locations, which had similar soil, vigor, salinity and management (fertility and cultural practices). Afterwards, the leaves were individually wrapped in aluminum foil and put in a polystyrene box and carried to the laboratory.

In a closed room with green light, four discs were collected on the middle third of each leaf, using a 346 mm^2 circle. The SPAD-502 chlorophyllometer was used to read the leaf circles. After the chlorophyll content was measured the four circles were soaked in a porcelain container of 20 mL of acetone (80%).

The material was filtrated with Watman n° 2 papers and the extract was read in the spectrometer (Beckman, DU-600B model) on 663 nm and 645 of absorbance, using quartz cuvette. The chlorophyll (*a*, *b* and total) amounts (Ca, Cb and Ct, respectively) were determined using the equations below, $Ca= (12.7 \times A_{663}) - (2.7 \times A_{645})$; $Cb= (-4.7 \times A_{663}) + (22.9 \times A_{645})$ and $Ct= (8.02 \times A_{663}) + (20.2 \times A_{645})$, described by Porra et al. (1989).

Samples of the same material were placed in forced-air drying ovens (60%) up to constant weight. The dry samples were grinded in a Wiley mill and the total nitrogen (N-total) was analyzed by the Kjeldahl method.

The data were submitted to simple linear correlation analysis. Regression analysis was carried out among SPAD index readings, extractable chlorophyll (*a*, *b* and total) and the leaf nitrogen content of each coffee cultivar.

Results and discussion

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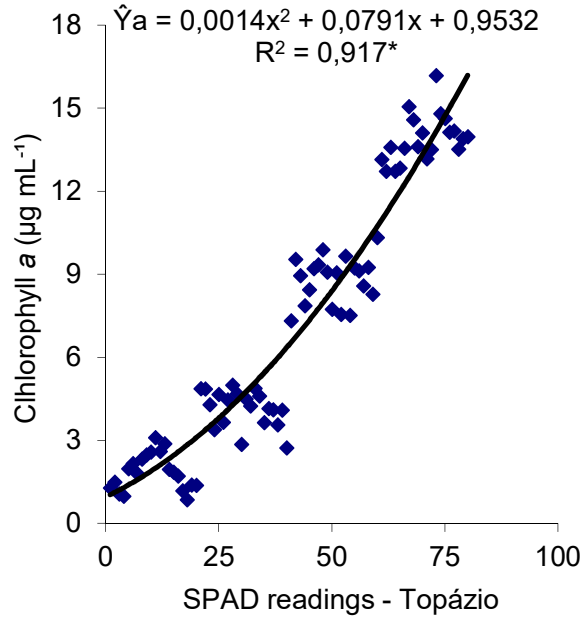
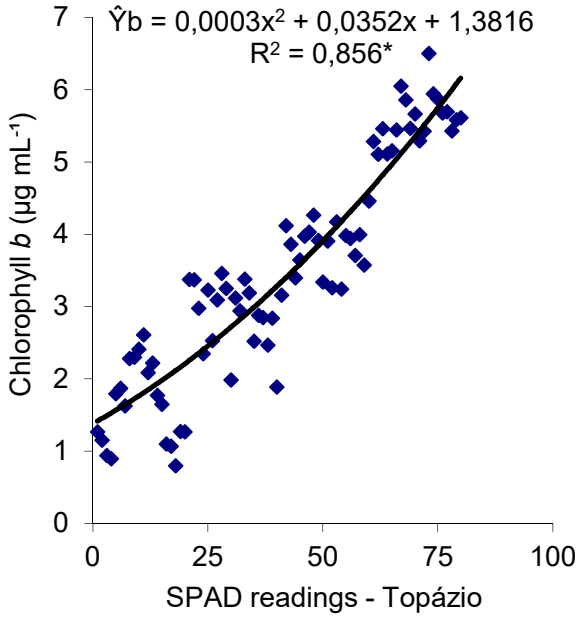
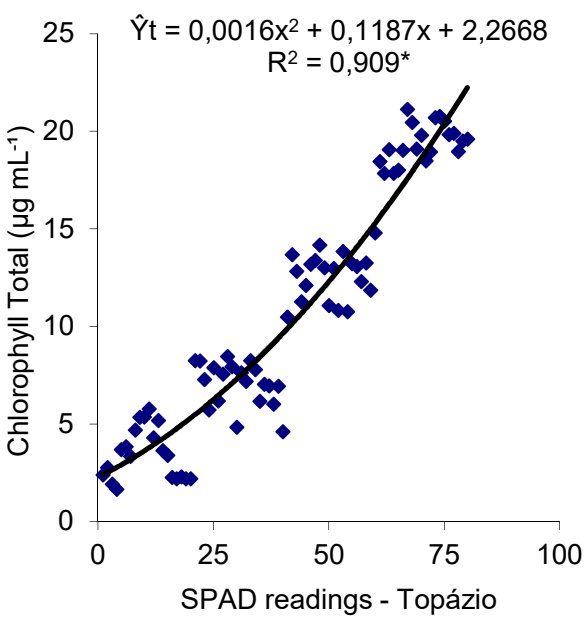
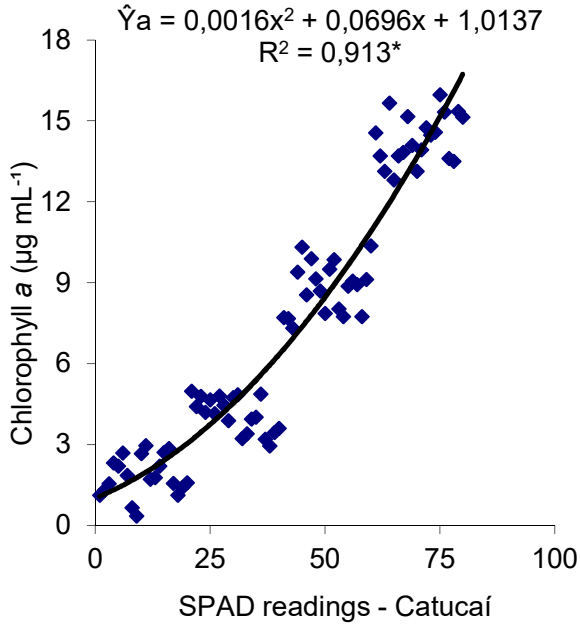
Simples linear correlation analysis showed a positive correlation among chlorophyllometer readings and extractable chlorophyll (*a*, *b* and total) of all coffee cultivars (Topázio, Catucaí, Catuaí and Rubi), and there were satisfactory coefficients to all variables (Figure 1).









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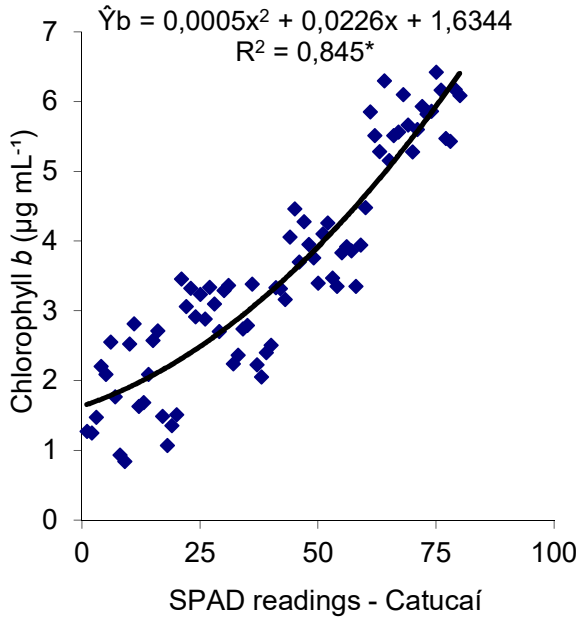

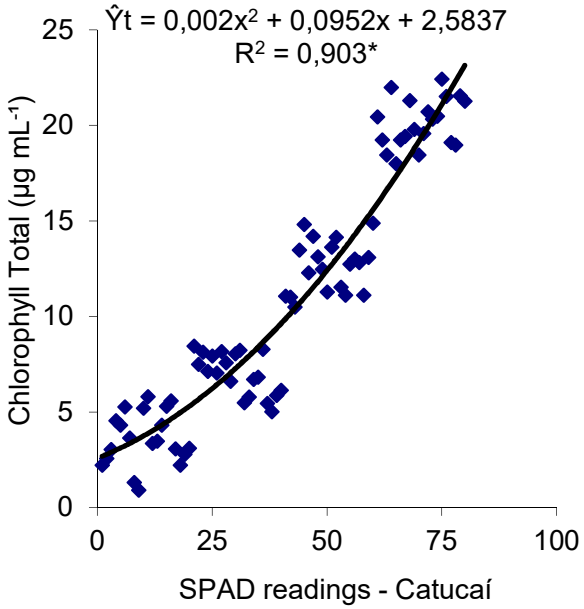

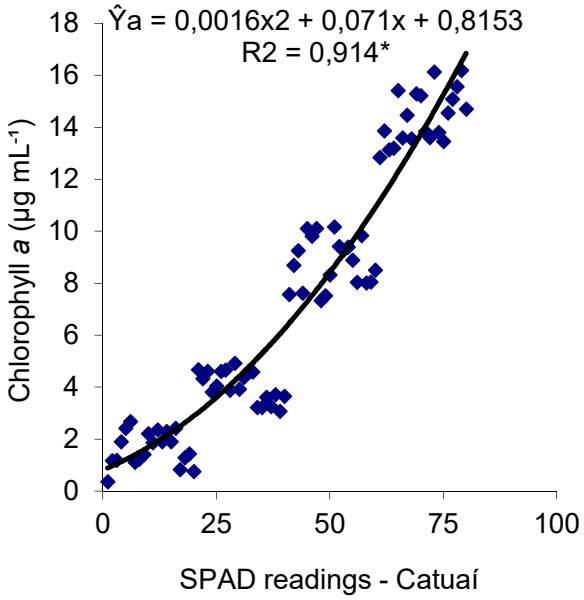

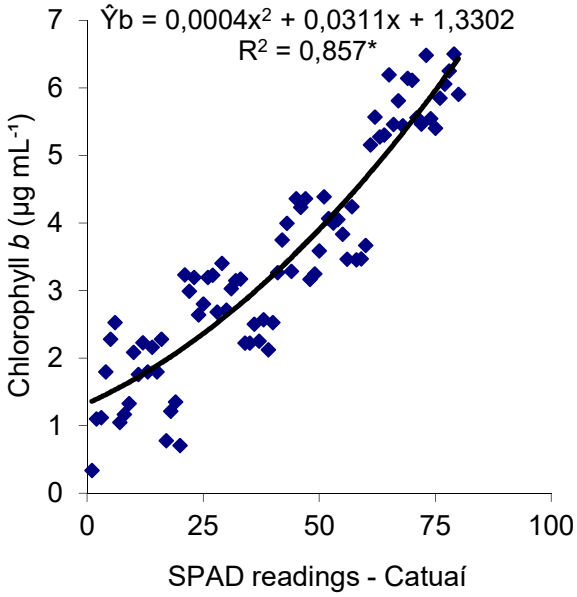

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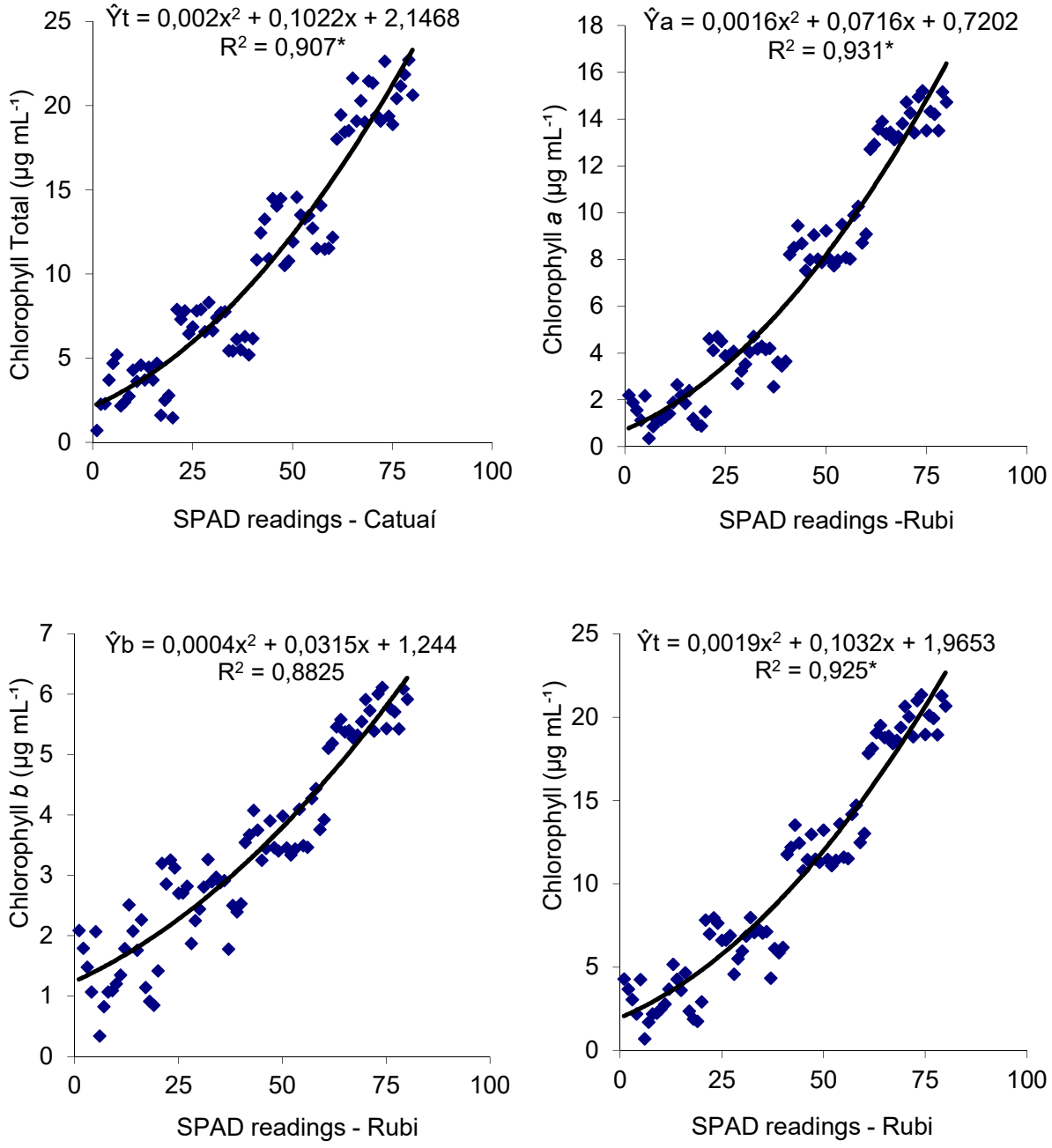

FIGURE 1 - Chlorophyll *a*, *b*, total and SPAD readings in leaves of *Coffea arabica* cultivars. Lavras – MG, 2006. (* significant at 5% of probability by test t).

All quadratic equations shown can be used to transform chlorophyllometer readings on the chlorophyll content of coffee plants, during fruiting stage including Topázio, Catucaí, Catuaí and Rubi cultivars. Lengthy and destructive evaluations can be changed to evaluations by SPAD to determine chlorophyll content.

Already known by chlorophyllometer readings we can estimate chlorophyll content and leaf nitrogen, due to the correlation among these variables (Torres Netto et al., 2005). However, there are few works which involve the utilization of the chlorophyllometer in coffee producing, and the evaluation and correlation of readings and leaf

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nitrogen in different stages. Research of this area should be highlighted, to provide data to field utilization of chlorophyllometer and better leaf nitrogen evaluation.

Brito et al. (2011) verify the correlation of extractable chlorophyll and chlorophyllometer readings on cotton plants. These authors concluded a possibility to evaluate photosynthetic pigments by chlorophyllometer with high precision, quickly and with no chemicals and laboratory use.

The relation analysis of SPAD readings and leaf nitrogen content in coffee estimated by the Kjedahl method showed positive relation of variables in both cultivars. The leaf nitrogen increased in the order that the SPAD readings were increasing (Figure 2).

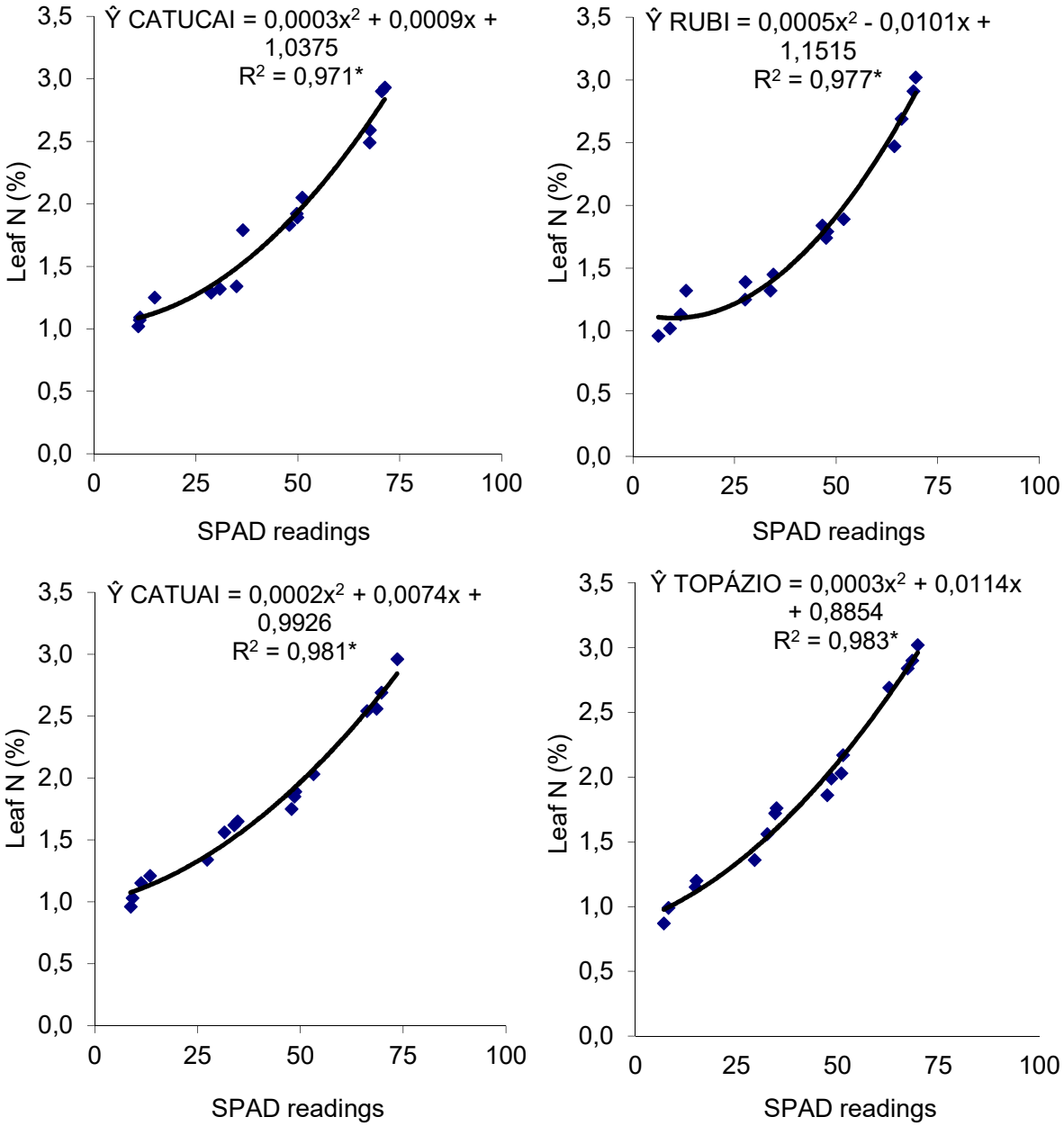

FIGURE 2 – Correlation among chlorophyllometer (SPAD) readings and leaf nitrogen of *Coffea arabica* cultivars. Lavras – MG, 2006. (*significant at 5% of probability by test t).

Torres Netto et al. (2005) noted increase according to the improvement of nitrogen status on *Coffea canephora* in different ages. Rigon et al. (2013) showed mathematical models which can be used to estimate photosynthetic pigments by

chlorophyllometer readings with high precision, saving time and cost of chemical analysis.

Araújo et al. (2013) evaluated the correlation between chlorophyll concentrations by SPAD in genotypes of dwarf elephant grass and reported a linear behavior between the relationship of SPAD readings and total concentration of chlorophyll, independently with higher rates of anthicyanin.

Coffea arabica showed correlations between SPAD readings, leaf N content and soil applied N dose, allowing a use to indicate crop fertilization, in order to obtain a higher yield (Reis et al., 2006). These authors recommended others specific studies of chlorophyllometer use by farmers.

Conclusions

SPAD-502 readings provide a quick and efficient estimation of chlorophyll concentration and nitrogen content of coffee leaves, independently of cultivars. Therefore, the use of SPAD-502 readings for the estimation of these variables and coffee nitrogen necessity is recommended due to higher correlation on all analyzed cultivars.

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