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Photosynthetic pigments, nitrogen, chlorophyll *a* fluorescence and SPAD-502 readings in coffee leaves

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Abstract

The chlorophyll meter (SPAD-502) is a simple, portable diagnostic tool that measures the greenness or relative content of leaves. Compared to the traditional destructive methods, the use of this equipment saves time, space and resources. The objective of this study was to establish a correlation between the photosynthetic pigments content extracted in DMSO, the total nitrogen content and the chlorophyll *a* fluorescence variables with the SPAD-502 readings in *Coffea canephora* Pierre leaves. The SPAD-502 has been shown to be a good tool to diagnose the integrity of the photosynthetic system in coffee leaves, and can thus help in the advanced interpretations of the photochemical process of these plants. The SPAD readings lower 40 show impairment in photosynthetic process. Thus, the portable chlorophyll SPAD-502 can be used to analyze the photosyn-

Abbreviations: F_0, minimal fluorescence; F_m, maximal fluorescence; F_v/F_m, ratio of variable to maximal fluorescence-maximum quantum efficiency of open photosystem II centres-quantum yield; q_p, photochemical quenching; q_N, non-photochemical quenching; Q_a, primary quinone acceptor of photosytem II; PSII, photosystem II; DMSO, dimethylsulphoxide; Chl, chlorophyll; Car, carotenoids

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thetic pigments, and total nitrogen can also help in interpretation of the photochemical process in coffee plants.

Keywords: *Coffea canephora* Pierre; Portable chlorophyll meter; Chlorophyll; Carotenoids; Fluorescence

1. Introduction

The methodologies used for chlorophyll extraction in plant materials are almost always based on methods that destructively extract leaf tissue using organic solvents that include acetone (McKinney, 1941; Bruisna, 1961), dimethylsulfoxide (DMSO) (Hiscox and Israelstam, 1979) methanol, *N*,*N*-dimethyl formamide and petroleum ether (Moran and Porath, 1980; Moran, 1982; Lichtenthaler and Wellburn, 1983; Inskeep and Bloom, 1985). During the extraction and dilution, significant pigment losses may occur thus leading to a high variability in the results. Shoaf and Lium (1976) used DMSO to modify the extraction methodology to eliminate the squashing and centrifuging stage. This method allowed longer storage periods for the extracted pigment, so that the spectrophotometer analyses need not to be performed immediately after extraction.

Although a high correlation between the chlorophyll content and photosynthesis rate was not obtained (Marini, 1986), the assessment of photosynthetic pigments, and consequently their relationships, is an important indicator of senescence (Brown et al., 1991). Chlorophyll loss is associated to environmental stress and the variation in total chlorophyll/carotenoids ratio may be a good indicator of stress in plants (Hendry and Price, 1993). In addition, measuring gas exchange and chlorophyll content repeatedly on the same leaves in field may provide useful information on the relationship between these parameters (Schaper and Chacko, 1991).

The chlorophyll meter (or SPAD meter) is a simple, portable diagnostic tool that measures the greenness or the relative chlorophyll concentration of leaves (Kariya et al., 1982). The meter makes instantaneous and non-destructive readings on a plant based on the quantification of light intensity (peak wavelength: approximately 650 nm: red LED) absorbed by the tissue sample. A second peak (peak wavelength: approximately 940 nm: infrared LED) is emitted simultaneous with red LED for to compensate the thickness leaf (Minolta Camera Co. Ltd., 1989). Compared with the traditional destructive methods, this equipment might provide a substantial saving in time, space and resources.

However, to determine the chlorophyll concentration in a sample, calibration curves between meter readings and the chlorophyll concentration in the tissue sample must be made. Recent research indicates a close link between leaf chlorophyll concentration and leaf N content, which makes sense because the majority of leaf N is contained in chlorophyll molecules (Peterson et al., 1993). Chlorophyll concentration or leaf greenness is affected by a number of factors, one being N status of the plant. Since the chlorophyll meter has the potential to detect N deficiencies, it also shows promise as a tool for improving N management (Peterson et al., 1993; Smeal and Zhang, 1994; Balasubramanian et al., 2000).

Carotenoids play an important role in the light harvesting complex and in the photoprotection of the photosystems. Several studies have shown that these compounds are very important in protecting the photosynthesis apparatus against photodamage, by interconversions among the xanthophyll molecules (Young et al., 1997; Ort, 2001). In the xanthophyll cycle, violaxanthin goes through de-epoxidation to give rise to anteroxanthin and finally zeaxanthin (Havaux, 1988). Zeaxanthin participates intensely in the regulation of heat dissipation of PSII energy, when this has an energetic overload (Ramalho et al., 2000; Ort, 2001). Therefore, an indirect, non-destructive quantification of the total carotenoids is of great importance for related studies.

Measurement of the chlorophyll *a* fluorescence is a quick, precise and non-destructive technique, widely used in investigating damage/repair caused in the photosynthesis plant system by various types of stresses (Smille and Nott, 1982; Havaux et al., 1988; Schreiber et al., 1988; Strand and Öquist, 1988; Ögren, 1994; Govindjee, 1995). In spite of the many studies related to chlorophyll *a* fluorescence in coffee, under various conditions (Da Matta et al., 1997; Ramalho et al., 2000), there is little information associating the fluorescence with the SPAD-502 readings. Association between SPAD readings and fluorescence measures can be important to optimize the advanced interpretations of data from the chlorophyll meter.

This study was carried out to determine if there was a correlation between photosynthetic pigments extracted in DMSO, total nitrogen content, chlorophyll *a* fluorescence variables and the SPAD-502 readings on *Coffea canephora* Pierre leaves.

2. Material and methods

2.1. Plant material and growth conditions

Coffee leaves (*C. canephora* Pierre) of different ages (second to fourth leaf pair on a plagiotropic branch counting from the apex) collected in commercial plantations from Campos dos Goytacazes, Rio de Janeiro State (21°27′S; 41°15′W) were used. The sampled leaves were transported in insulated boxes sheltered from light and brought to the Plant Physiology Sector at the Agricultural Science and Technology Center at the North Fluminense State University, Campos dos Goytacazes, RJ, Brazil.

2.2. SPAD readings

The mean of five readings from the portable chlorophyll meter (SPAD-502, Minolta, Japan) was obtained for each leaf disc of 169.72 mm^2 from individual leaves. SPAD-502 readings varying from 0 to 80 were obtained from 110 leaf discs. After readings, each disc was cut in fine strips and placed in a test tube containing 5 mL DMSO. The test tubes were then incubated at 70 °C for 30 min (Hiscox and Israelstam, 1979). After cooling the extract in the dark, a 3 mL aliquot was analysed spectrophotometrically at 480, 649 and 665 nm (SPEKOL, Zeiss, Germany). The chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), carotenoid (Car) concentrations were determined according to the equation proposed by Wellburn (1994).

2.3. Nitrogen content assessment

The mean of five readings from the SPAD-502 was obtained in laboratory for each leaf disc of 535.02 mm^2 from individual leaves. The discs were placed in six groups, each containing 15 discs, according to the SPAD-502 reading ranges (0–10, 10–20, 20–30, 30–40, 40–50, 50–60, 60–70, 70–80). After drying in an oven with forced air circulation at 70 °C for 48 h, the 20 mesh ground material was stored in hermetically sealed flasks for later analysis. The total nitrogen (TN) was determined by the micro-Kjeldahl method (Malavolta et al., 1989) after submitting the plant material to oxidation by sulphuric digestion (H_2SO_4 and H_2O_2).

2.4. Chlorophyll a fluorescence measurements

After measuring with the SPAD-502 the chlorophyll *a* fluorescence was determined on the leaf discs using a modulated light MINI-PAM portable fluorometer (Walz, Germany). The initial fluorescence (F_0), maximum fluorescence (F_m) were analyzed and quantum efficiency of open photosystem II centres-quantum yield (F_v/F_m) calculated. The leaf discs were previously adapted to the dark for 30 min so that all the centers of photosystem II (PSII) were at an open stage (all the primary acceptors oxidized) and dissipation through heat was minimum. The F_0 was obtained with low intensity modulated light (<0.1 μmol m^{-2} s^{-1}) not to induce any effect in the fluorescence variable. The F_m was obtained by 0.3 s pulses of saturating light of 20,000 Hz. The fluorescence variable (F_v) was calculated from the difference between F_m and F_0. The F_v and F_m values were used to obtain the F_v/F_m ratio.

3. Results and discussion

Fig. 1 shows the relationships between the SPAD-502 readings and chlorophyll and carotenoid concentrations. A polynomial quadratic mathematical model best fitted the relationship between the SPAD-502 readings and photosynthetic pigments. Relationships among the total chlorophyll concentration and SPAD-502 readings have been established for several plant species (Yadava, 1986; Marquard and Tipton, 1987; Schaper and Chacko, 1991). The simple linear mathematical model was fitted in the reported studies. These results are different to ours, which suggests that coffee has a different behaviour from other species regarding the mathematical fit of the studied relationships. In papaya leaves, relationships between SPAD readings and photosynthetic pigments concentrations was exponential (total chlorophyll and chlorophyll *a*), polynomial quadratic (carotenoids) and cubic (chlorophyll *b*) (Torres Netto et al., 2002).

The indirect carotenoids quantification can be obtained for values up to 30 using the SPAD-502 (Fig. 1d), in spite of the 650 nm quantifying system that is the wavelength relevant to chlorophyll absorption. These inferences can be obtained due to the direct linear relationship between the total chlorophyll and carotenoids concentration (Fig. 2a). However, the SPAD-502 readings lower than 40 not show a good relationship for carotenoids. Possibly, this fact is due to a poor relationship between carotenoids and total

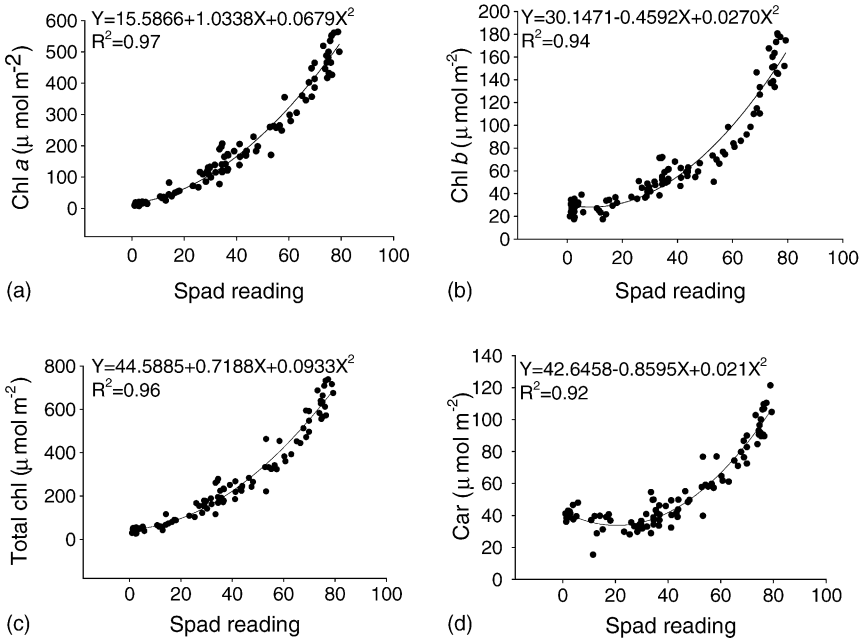

Fig. 1. Relationships between the SPAD-502 readings and chlorophyll *a* (Chl *a*) (a), chlorophyll *b* (Chl *b*) (b), total chlorophyll (total Chl) (c) and carotenoids concentration (Car) (d) in *C. canephora* Pierre leaves.

chlorophyll concentrations for carotenoids concentration lower than 40 μmol m^{-2} (Fig. 2a).

The ratio between chlorophyll and carotenoids has been much less widely used diagnostically, although this ratio is said to be a sensitive marker distinguishing natural full-term senescence and senescence due to environmental stresses such as desiccation in mosses (Buckland et al., 1991) and drought in flowering plants (Seel et al., 1992). Fig. 2b shows the relationship between the SPAD-502 readings and the total chlorophyll/carotenoid. The figure shows that for coffee, SPAD-502 readings lower than 40 indicate the beginning of possible impairment to the photosynthesis process, as this relationship is a very good indicator of disturbances caused in the plants by environmental factors (Hendry and Price, 1993). This figure also shows that the strength of the relationship decreases for SPAD-502 readings lower than 40. Thus, we propose the possible use of the chlorophyll meter as an indicator of stress in coffee leaves.

Chlorophyll molecules are components of the chloroplast membranes and occur at the ratio (*a*/*b*) of approximately 3.1 (Lichtenthaler et al., 1981). Plants exposed to high photosynthetic photon flux (PPF) present Chl *a*/*b* ratios around 3.2–4.0 and plants growing in environments with reduced PPF have a ratio around 2.5–2.9 (Lichtenthaler and Wellburn, 1983). From results of coffee leaves sampled from the second to fourth leaf pair on a plagiotropic branch counting from the apex, we hypothised that the variations in Chl *a*/*b* ratio were not due the shading. Wolf (1956) reported that chlorophyll *a* is more intensely

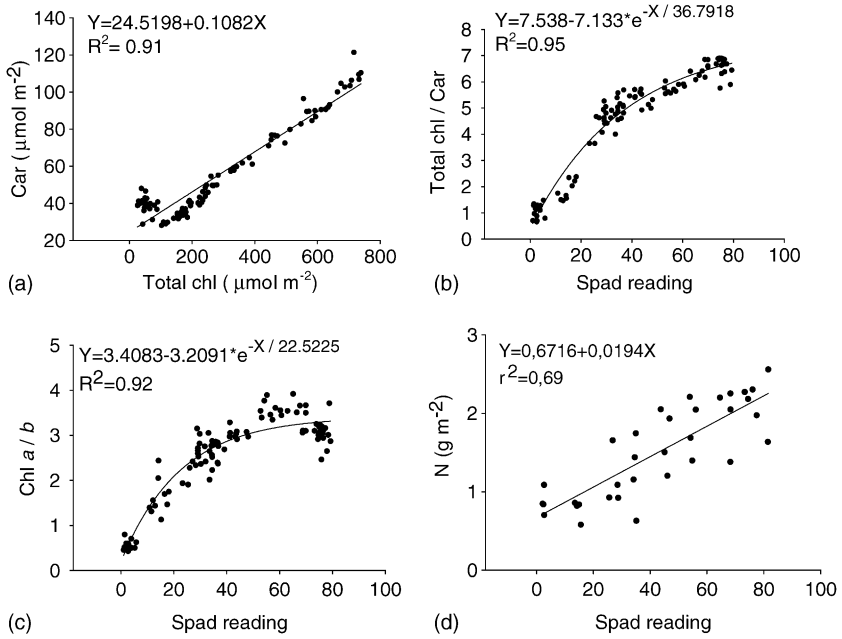

Fig. 2. Relationships between total chlorophyll concentration and carotenoids (a), SPAD-502 readings and the total Chl/Car ratios (b), Chl *a*/*b* ratios (c) and N content (d) in *C. canephora* Pierre leaves.

degraded than chlorophyll *b*. This information may explain the marked decrease in the *a*/*b* chlorophyll ratio when the SPAD-502 readings were below 40 (Fig. 2c).

Total N concentration increased linearly with SPAD-502 readings (Fig. 2d). In rice leaves, Takebe and Yoneyama (1989) shows a strong linear relationship between SPAD readings and weight-based leaf N concentration and this relationship varies with crop growth stage and/or variety, mostly because of thickness or specific leaf weight (Peng et al., 1995). The confounding effect of leaf thickness can be eliminated if foliar N concentration is expressed on a leaf area basis (Balasubramanian et al., 2000). Leaf area-based N concentration has a unique linear relationship with SPAD values of rice plants at all growth stages (Peng et al., 1995).

The relationship between the values obtained by the SPAD-502 and the chlorophyll fluorescence variables (F_0, F_m and F_v/F_m) are shown (Fig. 3a–c, respectively). The values of the F_0 (Fig. 3a) increased up to readings of 40. After this value, a stable behavior was found in the fitted curve. This fact was also observed for the F_m variables (Fig. 3b) and F_v/F_m (Fig. 3c). When all reaction centers are open (Q_a fully oxidized), the minimal fluorescence yield, F_0, is observed, whereas the maximal fluorescence yield, F_m, is found when all centers are closed (Q_a fully reduced) (Schreiber et al., 1994). According to the fitted model, the maximum quantum efficiency of the photosystem II, indicated by the F_v/F_m ratio, started to fall at around 40 (Fig. 3c). This ratio was positively correlated to the PSII quantum yield (Kitajima and Butler, 1975) for which values of 0.800 ± 0.050 correspond to highly efficient use of the excitation energy in photochemical processes

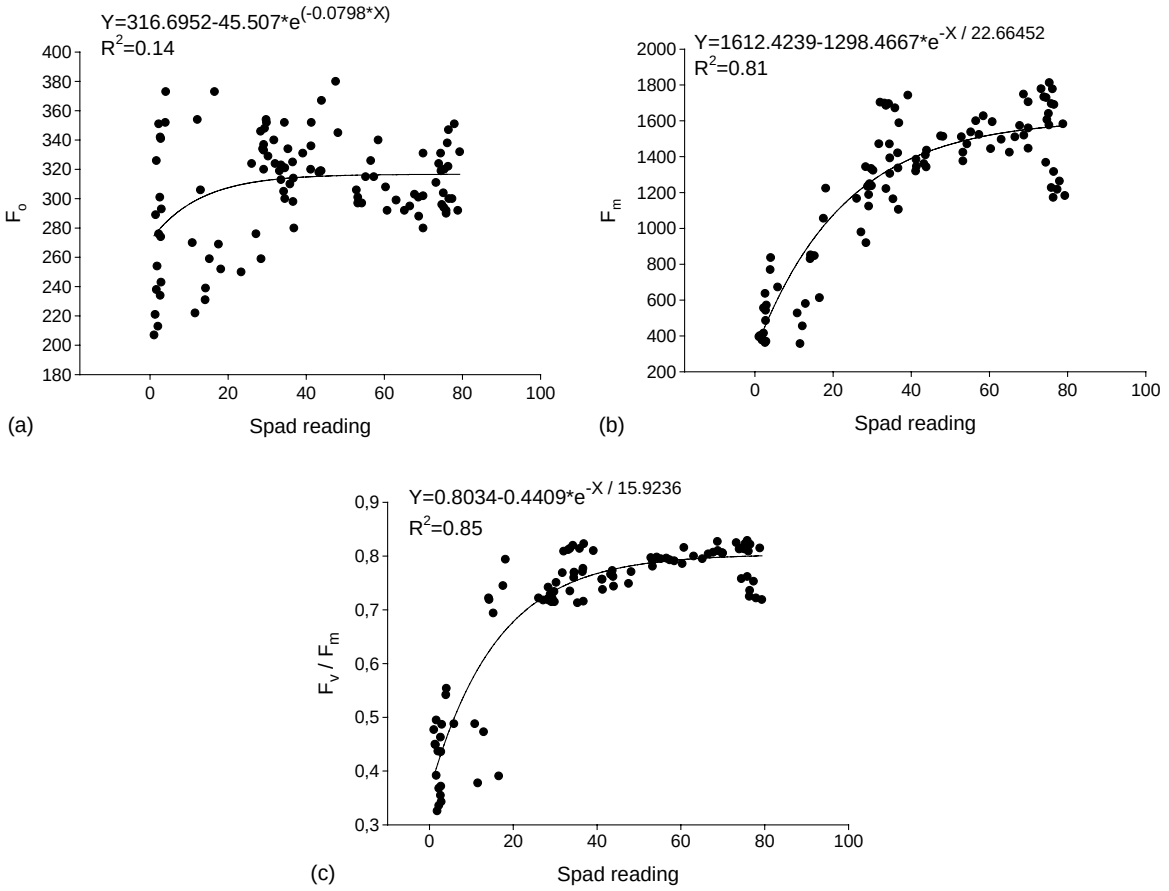

Fig. 3. Relationships between the SPAD-502 readings and the initial fluorescence (F_0) (a), the maximum fluorescence (F_m) (b) and maximum quantum efficiency of open photosystem II centres (F_v/F_m) (c) in *C. canephora* Pierre leaves.

(Björkman and Demmig, 1987; Bolhàr-Nordenkampf et al., 1989; Mohammed et al., 1995). This fact is in agreement with Figs. 2b, c and 3c. Thus, we propose that SPAD-502 readings around 40 showed to be the start of PSII impairment.

The F_m variable corresponds to the state of complete Q_a reduction (q_p = 0) and is considered proportional to the total chlorophyll content of a sample (Miranda et al., 1981). In our data, a positive correlation between the F_m values and the total chlorophyll concentrations (Fig. 4a) was observed for total Chl values up to 300 μmol m^{-2}. However, for larger values, a stable behavior in the F_m values was observed with the increase in total Chl. A positive linear relationship was obtained between the F_m values and the *a*/*b* chlorophyll ratio values (Fig. 4b). This relationship showed that high values of the Chl *a*/*b* ratio corresponded to high maximum fluorescence values. Lower values of F_m may be associated with increased non-photochemical quenching (q_N) (Araus et al., 1998). Additionally, a diminished electron flow from the water-splitting complex could cause impairment on the donor side, probably lowering F_m (Demmig-Adams et al., 1989). This fact shows that SPAD readings lower than 40 are related to impairment of the photochemical process (Figs. 3b and 4a and b).

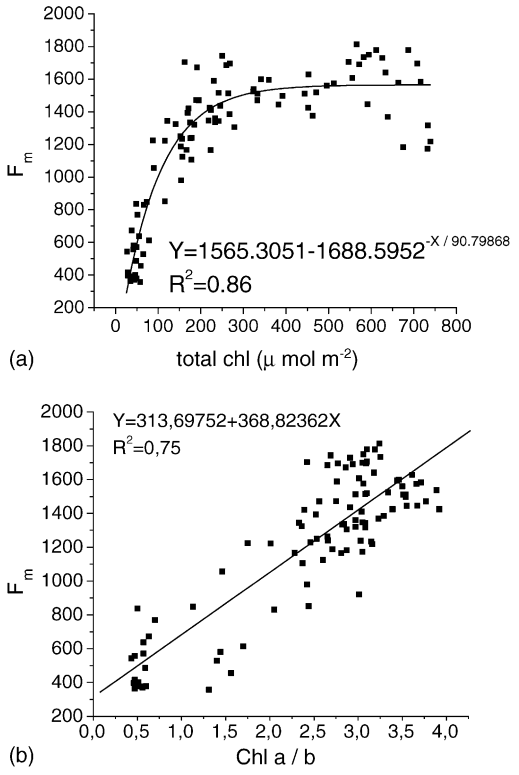

Fig. 4. Relationships between the maximum fluorescence and total chlorophyll concentration (a) and Chl *a*/*b* ratio (b) in *C. canephora* Pierre leaves.

In conclusion, the SPAD-502 can be a good tool to diagnose the integrity of the photosynthetic system in coffee leaves. This equipment can non-destructively and indirect efficiently assess the photosynthetic process in *C. canephora* Pierre plants. Thus the portable chlorophyll meter – SPAD-502 – can help in the advanced interpretations of the photochemical process in plants of the species under study. The SPAD readings lower 40 showed impairment in photosynthetic process.

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