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BRIEF COMMUNICATION

Nondestructive determination of nitrogen and chlorophyll content in olive tree leaves and the relation with photosynthesis and fluorescence parameters

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Abstract

For Tunisian olive tree orchards, nitrogen deficiency is an important nutritional problem, in addition to the availability of water. Establishment of relationships between nutrients such as nitrogen and ecophysiological parameters is a promising method to manage fertilisation at orchard level. Therefore, a nitrogen stress experiment with one-year-old olive trees (*Olea europaea* L. 'Koroneiki' and 'Meski') was conducted with trees respectively subjected to four nitrogen supply regimes (23.96 meq l^{-1}, 9.58 meq l^{-1}, 4.79 meq $l^{-1}$and 0 meq l^{-1} NO_3^-).

The current paper focuses on the use of the *SPAD-502* portable chlorophyll (Chl) meter, a nondestructive method for fertilisation management under nitrogen stress conditions of olive trees. Maximum net photosynthetic assimilation rates, chlorophyll fluorescence parameters and the SPAD Chl index were therefore measured simultaneously and the Chl and nitrogen content of the leaves were analysed. Significant correlations were established in the olive tree leaves between *SPAD-502* readings on the one hand and Chl content, nitrogen content, photosynthetic assimilation rate, and Chl fluorescence parameters (Φ_{PSII} and ETR) on the other hand.

Additional key words: electron flow; nitrogen deficiency; olive tree; photosynthesis; pigments; *SPAD-502*; quantum yield.

Introduction

For Tunisian olive tree orchards, nitrogen (N) deficiency is an important nutritional problem, in addition to the availability of water. The *SPAD-502* portable Chl meter allows rapid, nondestructive measurements of the N status and the Chl content of leaves and has been tested in some major crops (Smeal and Zhang 1994, Neilsen *et al.* 1995, Balasubra-Manian *et al.* 2000, Porro *et al.* 2001). For certain crops and terrestrial ecosystems, good correlations between Chl content and photosynthesis have been established (Brougham 1960, Dawson *et al.* 2003). In addition, significant correlations between leaf N and photosynthesis have been reported (Field and Mooney 1986). The *SPAD-502* Chl meter has therefore not only the potential to detect N deficiency, but might also

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Abbreviations: Chl – chlorophyll; F_t – steady-state fluorescence in the light-adapted leaves; F_m' – maximum fluorescence in the light; ETR – total electron flow, N – nitrogen; P_N – maximum net photosynthetic assimilation rate; Φ_{PSII} – quantum yield of PSII electron transport.
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provide additional information about the photosynthesis process. In this study, we investigated olive trees growing under different N-deficiency levels, searching for correlations among *SPAD-502* readings and destructively determined Chl *a* and leaf N content, maximum photosynthetic rates, and Chl fluorescence parameters.

Materials and methods

One-year-old olive trees (*Olea europaea* L. 'Koroneiki' and 'Meski') were grown in vermiculite (2-L containers) under greenhouse conditions from 22 January till 21 April 2008. The air temperature fluctuated between 20 and 32°C and the relative humidity of the air ranged between 60 and 70%. Plants were fertigated with a full-strength modified Hoagland's solution (EC = 2.5, pH = 6.5). After 34 days of culture, plants were randomly allocated to four groups and each group consisted of 16 plants (8 plants for 'Koroneiki' and 8 plants for 'Meski') Four N levels were provided for 58 days: (1) 100N received a full-strength nutrient solution throughout the entire experiment (NO_3^- = 23.96 meq l^{-1}); (2) 40N reduced N to 40% N (mild N stress with NO_3^- = 9.58 meq l^{-1}); (3) 20N reduced N to 20% N (moderate N stress with NO_3^-= 4.79 meq l^{-1}) and (4) 0N reduced N to 0% N (severe N stress with NO_3^- = 0 meq l^{-1}). The four treatments aimed to induce different leaf N levels.

After 58 days of N stress, maximum net photosynthetic assimilation rate (P_N) was measured simultaneously with several Chl fluorescence parameters using an open gas exchange system (*LI-6400; LI-COR*, Lincoln, NE, USA) with an integrated fluorescence chamber head (*LI-6400-40* leaf chamber fluorometer). Photosynthetically active radiation, air temperature and CO_2 concentration inside the sensor head were set at 1,500 μmol m^{-2} s^{-1}, 25°C and 450 μmol mol^{-1}, respectively, when measuring P_N, quantum yield of PSII electron transport (Φ_{PSII}) and total electron flow (ETR).

The steady-state (F_t) and the maximal (F_m') values of fluorescence are used to determine the Φ_{PSII} [(F_m' – F_t)/F_m'].

According to Krall and Edwards (1992) the total electron flow (ETR) can be derived from Φ_{PSII}, the light intensity incident on the leaf (PAR), the fractional absorption of light by the leaf (a) and the absorptance of PSI and PSII (f), ETR = Φ_{PSII} × PAR × a × f, where 'a' equals 0.84 and 'f' equals 0.5 (Schreiber 1997).

Measurements of P_N, Φ_{PSII}, and ETR were made on the 8th fully expanded leaf counted from the top of the olive tree and for three plants per cultivar per treatment. Measurements took place between 09:00 and 17:00 h and were done in 3 replications. The replications were measured respectively at 09:00, 12:00 and 15:00 for each treatment and each cultivar in order to cover as such the existing daily variability. The *SPAD-502* readings (*Konica Minolta Sensing, Inc*., Osaka, Japan) were determined for the 3rd and the 8th fully expanded leaf (two leaf-age classes) counted from the top of the tree (three measurements per leaf). Each measurement was done in seven replications. After the SPAD readings, Chl *a* for both leaf-age classes was extracted according to Moran (1982). Leaf discs (7 × 19.6 mm^2 $plant^{-1}$) were extracted with N, N dimethylformamide (DMF) and absorbance was measured at 664 and 647 nm (*UV-VIS, Biotek Uvikon XL*, USA).

Leaf N content was determined on the 8th fully expanded leaf counted from the top. Leaves from eight plants in each treatment were combined and analysed using the Kjeldahl method.

Data were analysed by polynomial regression analysis using statistical software *Version 16.0* (*SPSS,* Chicago, IL, USA).

Results and discussion

Recent research indicates a close link between leaf Chl concentration and leaf N content, which makes sense because the majority of leaf N is contained in Chl molecules (Peterson *et al*. 1993). Chl concentration or leaf greenness is affected by a number of factors, one being N status of the plant. Since the Chl 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).

SPAD values ranged from 45 to 80 for the 3rd leaf from the top compared to 70 to 95 for the 8th leaf (Fig. 1). A positive curvilinear relationship between the SPAD readings and the Chl a content was found ('Meski' R^2 = 0.72 and 0.90, p<0.001; 'Koroneiki' R^2 = 0.96 and 0.92, p<0.001 for 3rd and 8th leaf from the top, respectively) (Fig. 1) and among the SPAD readings and the N content ('Meski' R^2 = 0.84, p<0.001; 'Koroneiki' R^2 = 0.93 p<0.001 for the 8th leaf from the top) (Fig. 1). Cultivar differences were observed: lower SPAD values were found for 'Koroneiki' for a given N content compared to 'Meski' (Fig. 1). The Chl index (using the *SPAD-502* meter) has been successfully used to assess the N status in apple trees (Neilsen *et al*. 1995), grapevines (Porro *et al*. 2001), hardwood tree species (Chang and Robinson 2003) and woody ornamental plants (Demotes-Mainard *et al*. 2008). However, in most of such studies a simple linear regression was fitted to the data, which is different from our results. In coffee leaves, a polynomial quadratic fitting between SPAD readings and photosynthetic pigments was found (Netto *et al*. 2005). polynomial relationship gives good correlations for leaves of evergreen trees which are more leatherly and

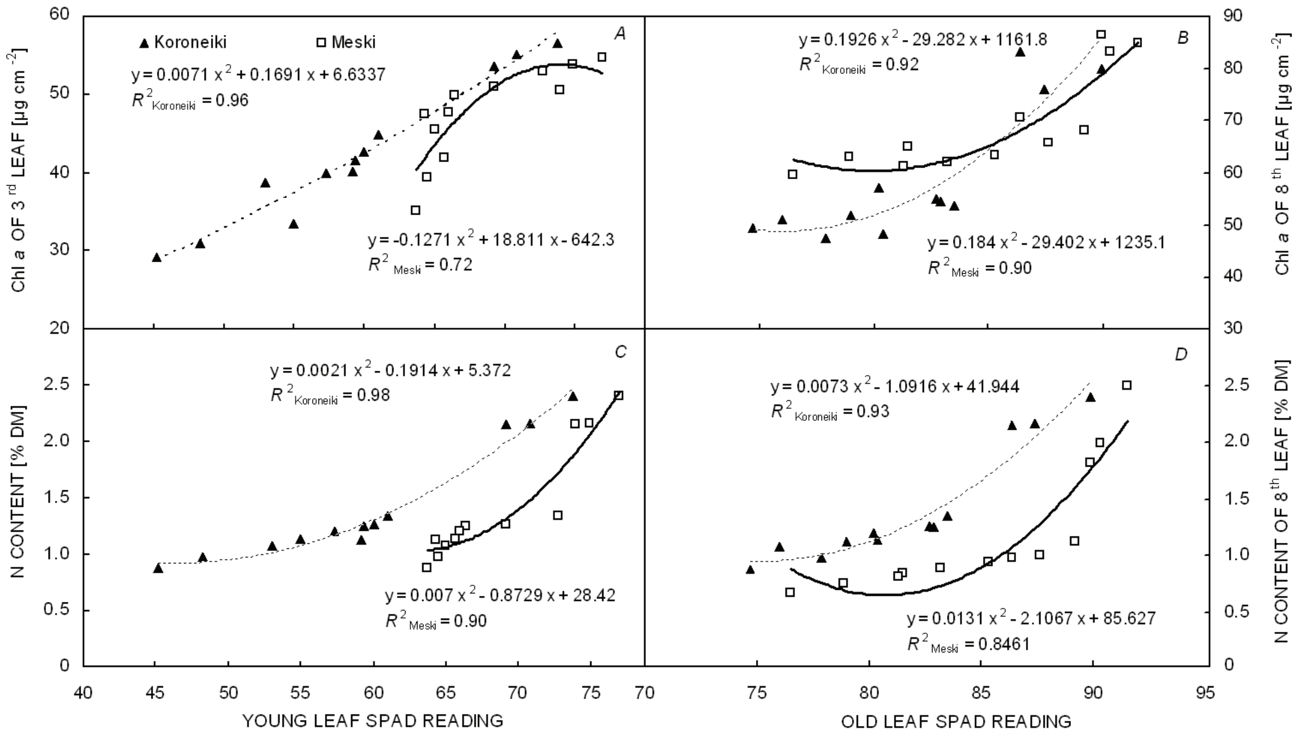

Fig. 1. Relationship between chlorophyll *a* (Chl *a*) concentration and *SPAD-502* readings (*A*: 3rd fully developed leaf from the top; *B*: 8th fully developed leaf from the top) and between nitrogen (N) content and *SPAD-502* readings (*C*: 3rd fully developed leaf from the top; *D*: 8th fully developed leaf from the top); this for two olive tree cultivars under nitrogen deficiency. (▲ 'Koroneiki', □ 'Meski', means $n = 12$). *Dotted* and *solid lines* indicate significant polynomial regressions for 'Koroneiki' and 'Meski', respectively. The coefficient of determination (R^2) is also shown.

often coated with wax.

In the present study, the Chl index was strongly correlated with Chl *a* (Fig. 1). Also a strong correlation between the SPAD readings and the N content was found, which supports the potential of the *SPAD-502* Chl meter to diagnose the N status in olive trees (Fig. 1). Leaf Chl meters (*SPAD-502*) have been used with various crops as an indirect indicator of plant N status (Turner and Jund 1991, Schepers *et al*. 1992, Chapman and Barreto 1997, Denuit *et al*. 2002, Gianquinto *et al*. 2003).

A wide SPAD-reading range was found for the 3rd leaf from the top, a correlation was made with the N content of the 8th leaf ('Meski' $R^2 = 0.90$, $p<0.001$; 'Koroneiki' $R^2 = 0.98$, $p<0.001$) (Fig. 1). The high correlations supported the idea that the higher colour sensitivity of younger leaves might be successfully used to judge changes in the whole-tree N status.

More than half of the N in leaves is used in photosynthetic proteins (Evans 1989), so it is also interesting to investigate the correlation between the destructively determined Chl *a* content and P_N and between SPAD readings and P_N (Fig. 2). Indeed, positive though nonlinear relations between Chl *a* content and P_N ('Koroneiki': $R^2 = 0.84$ $p<0.001$; 'Meski': $R^2 = 0.91$, $p<0.004$) and between SPAD readings and P_N ('Koroneiki': $R^2 = 0.85$, $p<0.001$; 'Meski': $R^2 = 0.87$, $p<0.004$) were found. These results confirm that Chl *a* content is strongly related to the photosynthetic process in olive trees as was also shown for other crops (Brougham 1960, Dawson *et a*l. 2003). In both cultivars, a Chl *a* content of at least 80 µg cm^{-2} seemed necessary for optimal CO_2 gas exchange. When N deficiency is imposed, gas exchange will be affected (Field and Mooney 1986). In this experiment, the significant correlation between SPAD readings, Chl *a* content and P_N showed that SPAD measurements can predict P_N in olive trees. The relationship between Φ_{PSII} and SPAD readings (Fig. 2) showed that leaf Φ_{PSII} reached its maximum SPAD values above 83 for both cultivars. Lower values indicate a decrease in the quantum yield of PSII and, hence, an induced disturbance in the electron transport or even possible damage to PSII causing reducing power of the biochemical processes. The relationship between ETR and SPAD readings was non-linear for 'Meski' and showed the same trend as was observed among SPAD and Φ_{PSII}. 'Koroneiki', however, showed a more linear relation among SPAD values ranging from 75 to 90 and ETR, which made it difficult to propose a threshold SPAD reading for this parameter. In the same context, Netto *et al*. (2005) showed for coffee leaves an exponential relation between the SPAD readings and the initial fluorescence, maximum fluorescence and maximum quantum efficiency of PSII centres (F_v/F_m). Our results showed a polynomial relationship

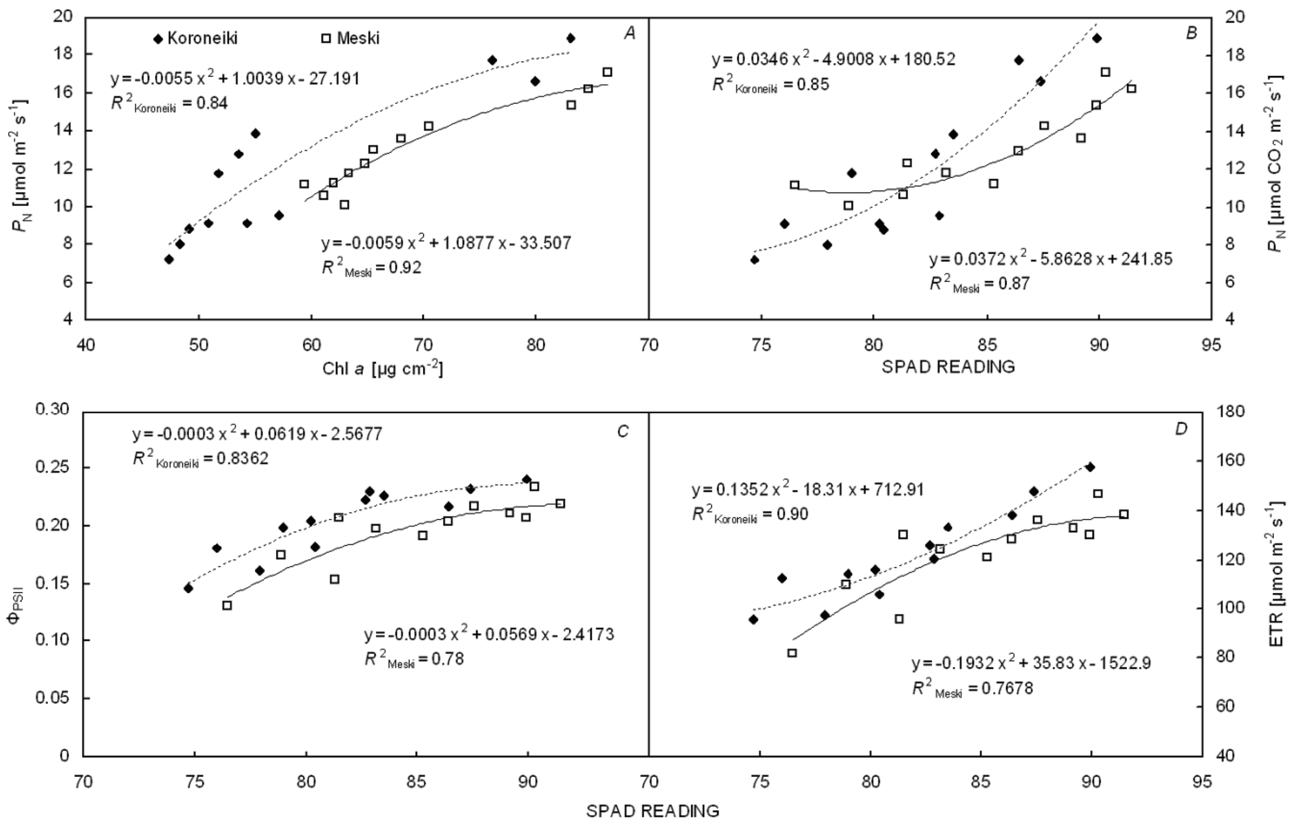

Fig. 2. Relationship between chlorophyll *a* (Chl *a*) concentration and maximum net assimilation rate (P_N) (*A*) and between *SPAD-502* readings and P_N (*B*), quantum yield of PSII (Φ_{PSII}) (*C*) and total electron flow (ETR) (*D*) in the 8th fully expanded leaves of the olive tree cultivars 'Koroneiki' and 'Meski'(▲'Koroneiki', □ 'Meski'). *Dotted* and *solid lines* indicate significant polynomial regressions for 'Koroneiki' and 'Meski', respectively. The coefficient of determination (R^2) is also shown.

between SPAD readings and Φ_{PSII} and ETR, but low correlations were found with F_v/F_m (data not shown).

In conclusion, *SPAD-502* measurements were useful to determine N deficiency and Chl *a* content in olive trees. Differences in cultivar responses and leaf age classes suggest that calibration for each cultivar is required. The *SPAD-502* Chl meter has a potential to be used as a rapid tool to support site-specific N management in olive orchards. Furthermore, this study indicates that SPAD readings can be used to assess certain parameters describing the photosynthetic capacity of the olive leaves under N stress.

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