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Spectral methods for nitrogen deficiency evaluation in maize plants

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In this study the potential of spectral methods (absorption and emission in visible range) for discriminating a nitrogen deficiency in maize plants was investigated. Nitrogen (N) is an essential nutrient for crop production but also contributes to eutrophication of surface water and degradation of drinking water quality Modern corn production requires the inputs of large quantities of N since economic losses consecutive to reduced yields under low N status are substantial. Synchronizing N availability with crop N need offers the potential to protect the environment without sacrificing production. Plants were cultivated in controlled hydroponic solutions under four level of nitrogen. Nitrogen deficient plants are characterized by lower chlorophyll content and reduced biomass. Variable chlorophyll fluorescence as well as transmission measurements were used in order to compare the different plant treatments. The results show that both methods were able to sense the nitrogen deficient plants, therefore presenting a potential for further use in assigning crop nutrient need, in a modern agriculture.

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1. Introduction

The photochemical conversion of solar energy into chemical energy, in the photosynthesis process, is associated with the photo-physical properties of the green pigment, the chlorophyll (Chl). Optical and spectroscopic properties of Chl were widely studied and well known from the literature [1-3]. In solution, Chl *a* emits around 30 % of the absorbed light, as fluorescence. The association of Chl with proteins in leaves, *in vivo,* is influencing the spectroscopic characteristics of the pigment by a complex overlapping. Though, the analysis of spectral properties of leaves allow to extract valuable parameters that may be used to assign physiological state of vegetation and to discriminate different stress situations. Previous studies [4] showed that the analysis of chlorophyll fluorescence from leaves may be used in monitoring vegetation under water or nitrogen stress, by observing different parameters computed from the excitation/emission spectra or using the variable Chl fluorescence yield.

In vivo, a red and far-red fluorescence from chlorophyll is a very sensitive indicator of the energetic status of the photosynthetic system [5], indicating how the multiple processes of energy absorption and utilization interact. At room temperature, most part of the leaf emission is due to Chl *a* from photosystem II (PSII). Another important characteristic of Chl fluorescence emission *in vivo* is derived from the quantum yield variability. The fluorescence emission ranges from a maximum of about 3 % to a minimum of about 0.5 %, from the incident light, depending on the status of the first electron acceptor molecule of PSII, if the electron transport is blocked or optimal [6].

Variable chlorophyll fluorescence is known from more than half a century [7] and it was extensively used in the last ten years by physiologists to assign the vegetation status (for a review see [8-9]). Development of PAM (pulse amplitude modulation) fluorimeters [10] and estimations of the photosynthetic linear electron flow from the variable Chl fluorescence measurements, by measuring PSII photochemistry yield through the $\Delta F / F_{\mathrm{m}}^{'}$ parameter [11], make from Chl fluorescence a very popular tool in plant physiology.

Chlorophyll fluorescence (ChlF), already shown to give good results in detecting nutrient stress on vegetation, is a non-destructive and non-intrusive probe of plant status (for a review see [9]). Remote sensing system based on laser-induced fluorescence (LIF) has great potential in terrestrial vegetation mapping for detecting vegetation stress. As an alternative, there are passive methods that are not using auxiliary excitation sources: passive fluorescence, excited by sunlight or reflectance. The ratio of the response from different spectral bands could be used for the assignment of vegetation status under various conditions.

Long-term stress events and mineral deficiencies may eventually reduce the chlorophyll and carotenoid content of leaves, and such parameters could be monitored by

reflectance measurements [12-14]. Chl fluorescence may bring additional information about the vegetation status by taking into account two of its characteristics: variable yield and spectral composition. Different stress factors affect the photosynthetic capacity and were essentially monitored by the decrease of the PSII quantum yield. Spectral changes in the fluorescence emission spectra have also been related with stress signatures. Nitrogen, as elemental constituent of chlorophyll, is essential in plant development and dramatically influences some crop growth.

In this paper we used only the variable chlorophyll fluorescence and leaf transmittance measurements, in detecting nitrogen deficiency in maize plants.

2. Experimental

2.1 Materials

Maize (*Zea mays* L. hybrid Norma) plants were grown from seeds in a modified Hoagland solution at 22/20 °C with a 16/8-h light/dark periodicity in a Conviron PGV-36 plant growth chamber, with a relative humidity of 75 % under white light illumination (300 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD)).

Four nitrogen treatments were applied by modifying Hoagland solution in order to obtain a final concentration of 1.5, 1.2, 0.75 and 0.3 mM NO_3 corresponding to four N-treatments from saturating to deficient in nitrogen, further named 100 %, 80 %, 50% and 20 %. For each treatment there were cultivated 15 plants (3 backers containing 5 seeds).

2.2 Methods

2.2.1 Spectral measurements

Spectral measurements were performed on small leaf pieces, cut from the middle of last full developed leaf of 4 weeks old maize plant.

Transmission spectra, in the range 400 to 800 nm, were fully recorded using the UV-VIS spectrometer UV-160A from Shimadzu.

Variable Chl fluorescence measurements were performed on the same leaf used for transmittance measurements, using a modulated chlorophyll fluorimeter PAM 2000 (Walz, Germany). The saturating pulse method as described by [10;15] was used for measuring various fluorescence levels for light or dark adapted leaves, from which the photochemical yield and electron transport flow have been computed as described bellow.

Variable Chl fluorescence measurements were performed under different light irradiance, after a minimum 30 min adaptation to darkness or 10 min adaptation to light. Minimal or stationary fluorescence (called F_o and F_s for dark and light adapted leaves, respectively) was measured previous to the application of 1 s saturating pulse, used to induce the maximal fluorescence (called F_m and F_m' for dark and light adapted leaves, respectively).

Optimum quantum yield of PSII photochemistry was calculated from F_o and F_m, with the equation 1:

$$\Phi_{PSII} = F_v/F_m = (F_m\text{-}F_o)/F_m \quad (1)$$

where F_v is the variable fluorescence, measured as $F_v = F_m - F_o$, for dark adapted leaves. Values around 0.8 are typical for an optimum Φ_{PSII}, for plants under physiological state, and are measuring the capacity of PSII to produce the photochemical act. Lower values are indicative for some PSII photosystem impairments.

The quantum yield of the linear photosynthetic electron flow, Φ'_{PSII}, when photosynthesis takes place, may be also computed from the variable fluorescence - as proposed by Genty et al. [11]:

$$\Phi'_{PSII} = \Delta F/F_m' = (F_m'\text{-}F_s)/F_m' \quad (2)$$

where $\Delta F = (F_m'\text{-}F_s)$ is the variable fluorescence in the presence of light background driving photosynthesis in plants. The electron transport rate (ETR) was estimated from the relationship:

$$ETR = \Delta F/F_m' \times PAR \times f_a \times ½, \quad (3)$$

where PAR is the photosynthetic active radiation photon flux density, f_a = 0.84 is the fraction of PAR that is absorbed by the pea leaf; ½ factor takes into account light absorption by the two photosystems, PSI and PSII. [11].

2.2.2 Leaf Chlorophyll content

The leaf chlorophyll concentration (Chl *a* and *b*) was determined by spectrophotometry, using the leaf acetone extract, according to [16]. Leaf samples of 1 cm^2 were collected and stored in liquid nitrogen until measurements were done. Samples were grinded using a mortar and pestle; 0.5 ml acetone 80% were added, followed by 5 min centrifugation at 4,000 g. Supernatant was collected and diluted with acetone to a final volume of 1 ml. Solution was transferred into a 1 cm optical path cell and absorption (*A*) at 663.2 nm and 646.8 nm was measured. Chlorophyll *a* and *b* concentrations in acetone extract are computed using equation (4) based on characteristic extinction coefficients at those wavelengths as proposed by [16]:

$$Chl_{a+b} = 7.15*A_{663.2} + 18.71*A_{646.8} \quad (4)$$

Dry matter was measured by weighting the shoots after drying them at 150 °C for 24 h.

3. Results and discussions

The different N treatments of plants conduct to significant differences in the maize plant developments, as seen in Fig. 1.

Fig. 1. The development of maize plants under different N-treatments.

Those variations in the plant development are accompanied by statistically significant differences in the leaf Chl content and biomass production, observed between deficient and saturated N-plants, as shown in Figs. 2 and 3.

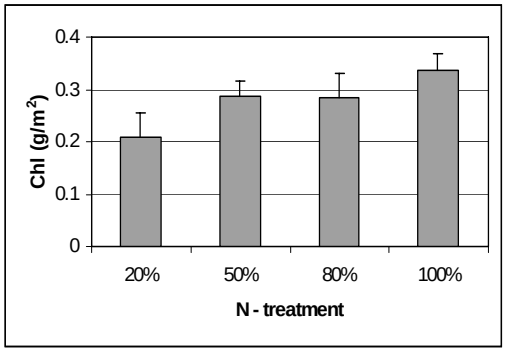

Fig. 2. Chlorophyll a + b content of maize leaves from different treated plants (mean values and standard deviations for 15 samples)

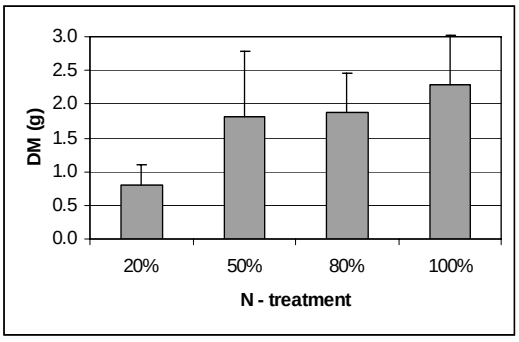

Fig. 3. Dry matter (DM) of maize leaves from different treated plants (mean values and standard deviations for 15 samples).

Chlorophyll fluorescence measurements were performed on last full developed leaf. The leaf was clamped and dark adapted for 30 min previous to measuring minimal F_o and maximal F_m fluorescence levels, from which optimum quantum yield was computed as F_v/F_m using relation (2). Under physiological conditions, maize plants have mean F_v/F_m of about 0.8, as obtained for control (100 % N) plants. Under nitrogen deficiency, this optimal PSII quantum yield is decreased to a mean value of about 0.72 in severe nitrogen deficient plants (20 % N). Intermediate treatments (50 %N and 80 %N) appear to be not significantly different from control. Another fluorescence parameter gives better results in discriminating between those intermediate treatments. It is the effective PSII quantum yield computed as $\Delta F/F_m'$, using the stationary F_s and maximal F_m' fluorescence levels for leaves that were light adapted to 150 μmol m^{-2} s^{-1} PPFD previously to measure fluorescence, using the actinic source of the PAM fluorimeter.

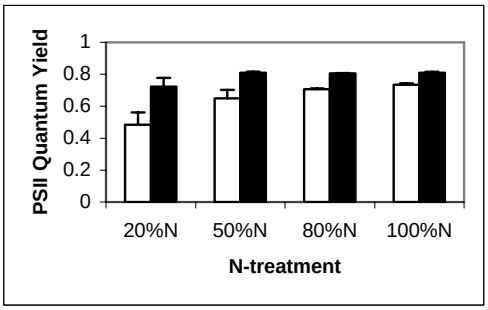

Fig. 4. PSII quantum yield, estimated by fluorescence, for different N-treated plants. Optimal quantum yield for dark-adapted plants (dark columns) and light adapted plants to 150 μmol m^{-2} s^{-1} PPFD (white columns).

For the measurements of leaf transmittance, full transmission spectra were recorded between 400 and 800 nm. The transmission spectra of leaf show the characteristic relative maximum in the green region (max. 545 nm) where leaf pigments are absorbing less and a rapid increase in transmission, starting from 680, nm and reaching a plateau above 700 nm (not shown).

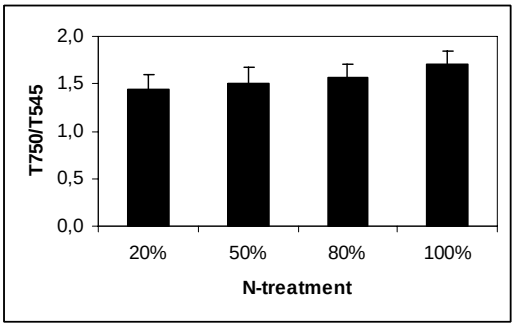

Fig. 5. Transmittance ratio T750/T545 for different N-treatments.

The ratio of transmissions corresponding to the far-red plateau and the relative maximum into the green spectral region was calculated from the transmission spectra, similarly with the reflectance parameter used by [12] and it was found to be related to leaf Chl concentration. The computed ratio T750/T545 was used as a spectral parameter and the results are presented in Figure

5. The mean values of the T750/T545 parameter show a decrease in deficient nitrogen plants as compared to the saturating ones, but do not allow discriminating between the different levels of nitrogen treated plants. The accuracy of our results could be affected by the low sensitivity of recording transmission signals (transmission values around 2 %, for leaf samples) in a spectrometer, not very suitable for this kind of samples. Transmittance measurements on leaf samples may be improved further by measuring leaf transmission, in this characteristic spectral region, in a narrow window, using special adapted experimental set-up instead of a spectrophotometer.

4. Conclusions

In our experiment, transmittance measurements give less satisfactory results in discriminating N-deficiency plant treatments by the decrease in their Chl content, as compared with fluorescence measurements. A decrease of the mean values of T750/T545 parameter in N-deficient plants was observed, but the method is still lacking in sensitivity and specificity. Chl fluorescence analysis brings more information on the vegetation status, by using the $\Delta F/F_m$' ratio in addition to the dark adapted F_v/F_m, in agreement with our previous observation in water stressed pea plants [17]. Diurnal variations of fluorescence must be taken into account, allowing a more precise discrimination of intermediary nitrogen deficiency. Taken together, those results conduct to the conclusion that an analysis of chlorophyll fluorescence quenching as function of irradiance may bring more detailed information about the physiological status of vegetation, as related to changes in the photosynthetic activity of the leaf during a diurnal cycle.

Generally, both methods: fluorimetry and transmission spectroscopy, show a potential in detecting spectral signatures that may allow the stress detection, taking the advantage of being rapid, simple, non-invasive and non-destructive techniques.

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References

[1] R. M. Ion, S. Apostol, Revista de Chimie **56** (6), 607 (2005)
[2] M. L. Pascu, L. Danaila, R. M. Ion, A. Popescu, M. Pascu, Proc. SPIE, **4166**, 140 (2000).
[3] L. Tugulea, Romanian Reports in Physics, **52**(5-6-7), 541 (2000).
[4] S. Apostol, A.Viau, N. Tremblay, J.-M.Briantais, S. Prasher, L.-E. Parent, I. Moya, Can. J. of Remote Sensing, **29**(1), 57 (2003).
[5] S. Apostol, Utilisation de la fluorescence chlorophyllienne pour le suivi à distance de la photosynthèse au niveau foliaire, Thèse de doctorat no. 6233, Université Paris Sud (2000)
[6] A. Rosema, W. Verhoef, J. Schroote, J. F. H. Snel, Remote Sens. Environ. **37**, 117 (1991)
[7] H. Kautsky, A. Hirsch, Naturwissenschaften **19,** 964 (1931).
[8] G. H. Krause, E. Weis, Annu. Rev. Plant. Physiol Plant. Mol. Biol. **42**, 313 (1991).
[9] Z. Cerovic, G. Samson, F. Morales, N. Tremblay, I. Moya Agronomie**. 19**, 543 (1999).
[10] U. Schreiber, U. Schliwa, W. Bilger, Photosynth. Res**. 10,** 51 (1986).
[11] B. Genty, J. Briantais, N. Baker, Biochim Biophys Acta, **990,** 87 (1989).
[12] C. Daughtry, C. Walthall, M. Kim, Brown de Colstoun, E., McMUrtrey, J., Remote Sens. Environ. **74**, 229 (2000).
[13] A. Gitelson, Y. Kaufman, M. N. Merzlyak, Remote Sens. Environ. **58**, 289 (1996).
[14] H. K. Lichtenthaler, A. A. Gitelson, M. Lang, J. Plant Physiol. **148**, 483 (1996).
[15] M. Bradbury, N. R. Baker, Biochim Biophys Acta, **765,** 275 (1984).
[16] R. J. Porra, W. A. Thomson, P. E. Kriedeman, Biochim. Biophys. Acta, **975**, 384 (1989).
[17] S. L. Apostol, C. Stihi, C. Oros, J. Optoelectron. Adv. Mater**. 9,** 2921 (2007).

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