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Laser-induced chlorophyll fluorescence to determine the nitrogen status of plants

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Abstract

The primary roles played by nitrogen in chlorophyll synthesis and photosynthesis suggest that deficiencies in this nutrient in the plant could be detected on the basis of changes in the plant's fluorescence spectra. The relationship between laser-induced fluorescence intensity at 680 and 740 nm, fluorescence ratio F680/F740 and the nitrogen nutritional status of wheat plants was characterized in two growth chamber experiments. The results showed that fluorescence intensity is related to the leaf chlorophyll content, especially to the intensity of the 680 nm fluorescence band. The fluorescence ratio F680/F740 is negatively correlated with the chlorophyll meter reading ($r^2=0.62$; $p<0.01$), as well as with the chlorophyll a content ($r^2=0.62$; $p<0.01$). The results showed that N-fertilization levels can be differentiated by means of fluorescence ratio F680/F740 measurements. Furthermore, the fluorescence ratio appears to be insensitive to light and air temperature conditions.

Introduction

The role played by N in chlorophyll synthesis suggests that a deficiency in this nutrient in the plant could be detected on the basis of changes in the plant's fluorescence spectra. Nitrogen is the major constituent of the tetrapyrole nucleus of chlorophyll. In consequence, the chlorophyll content of the leaf is positively correlated with the N concentration.

The main advantage of fluorescence detection compared to reflection measurements is the greater sensitivity achievable because the fluorescence signal has a very low background, since the signal comes only from the green plant parts (Lichtenthaler & Rinderle, 1988).

The chlorophyll fluorescence spectra of the upper side of the leaf exhibit two fluorescence maxima: one near 680 nm and a second one around 735 nm (Lichtenthaler & Rinderle, 1988). With increasing chlorophyll content, the 690 nm fluorescence band is decreased by a preferential reabsorption of the emitted fluorescence at 690 nm by chlorophylls. It occurs due to the partial overlapping of the absorption spectrum of the chlorophylls with the fluorescence emission spectrum between 640 nm to around 710 nm (Lichtenthaler & Rinderle, 1988).

The experiments had the objective to determine the relationship between the fluorescence intensity at 680 nm and 740 nm and the fluorescence ratio F680/F740 and the chlorophyll content of wheat leaves, as well as to investigate the influence of nitrogen supply and environmental conditions on the fluorescence yield and on the fluorescence ratio F680/F740.

Materials and methods

Two experiments were carried out under controlled environmental conditions. Wheat plants (*Triticum aestivum* cv. Thassos) were grown at a density of 12 plants per pot in 8 L pots (20 cm diameter) filled with a loamy soil. The average air temperature for day/night was 18/14°C, the relative air humidity was 60/75% day/night, and the maximum photosynthetically active radiation was 400 µmol m^{-2} s^{-1} during the day. The light period was 12 h. Every afternoon, the plants received an adequate amount of water.

The one-week-old wheat plants were divided in five groups, which received different amounts of N-fertilizer (NH_4NO_3 form) once a week. The final amount of N applied varied from 0.15 g N to 1.25 g N per pot.

The chlorophyll fluorescence intensity was measured at 18°C air temperature (if not otherwise specified) under steady-state conditions of fluorescence, i.e., in light-adapted plants. The excitation and the sensing of the fluorescence was performed at the adaxial leaf surface of the youngest fully expanded leaves. The leaves were cut from the plants and the fluorescence yield was quickly measured. The leaf area illuminated by the laser beam is about 7 mm^2 at about 14 cm distance. Before the measurements were done, the chlorophyll content of the leaf was estimated by using the chlorophyll meter (Minolta SPAD-502®).

The excitation wavelength of the laser beam was 640 nm, with an energy of 15 mW. Chlorophyll fluorescence was detected at two wavelengths (680 nm and 740 nm).

The photosynthetic pigments (chlorophylls) were extracted with acetone 100% and determined spectrophotometrically using the extinction coefficients and equations described by Schopfer (1989).

Results and discussion

The results of the experiments showed that the fluorescence intensity at 680 and 740 nm is affected by the chlorophyll content of wheat leaves, as estimated by the chlorophyll meter (Fig. 1). The 680 nm band decreased as the chlorophyll meter reading increased ($r^2=0.20$, $p<0.05$), while the fluorescence band at 740 nm slightly increased as the reading of the chlorophyll meter increased (r^2=0,05 not significant). With increasing chlorophyll content, the 680 nm fluorescence band is decreased by a reabsorption of the 680 nm band by the photosynthetic pigments. It occurs due to the partial overlapping of the absorption spectrum of the chlorophylls with the fluorescence emission spectrum. However, the correlation between the fluorescence intensity of single bands and the chlorophyll meter readings is low.

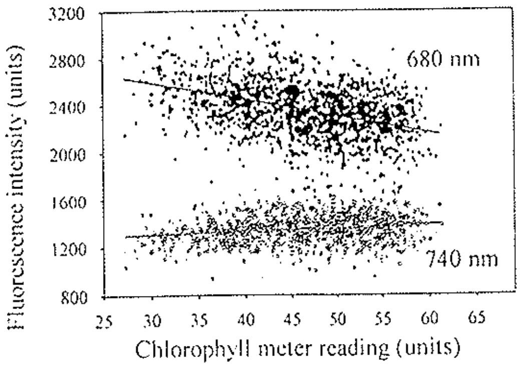

Figure 1. Fluorescence intensity at 680 and 740 nm in wheat leaves as related to the chlorophyll meter reading.

However, the correlation between fluorescence measurements and the chlorophyll meter readings was improved by using the ratio F680/F740 (Fig. 2). The ratio F680/F740 was better correlated with the chlorophyll meter readings (r^2=0.62, $p<0.01$) than were the values of the single fluorescence bands (Fig. 1 and 2).

The fluorescence ratio varies much less from leaf to leaf than the absolute fluorescence yield. For this reason, the ratio F680/F740 is more sensitive to estimate the chlorophyll content than are fluorescence values alone.

The fluorescence ratio F680/F740 was also negatively correlated with the chlorophyll *a* content (r^2=0.62, $p<0.01$) and with the total chlorophyll (*a*+*b*) content (r^2=0.60, $p<0.01$). Other authors also have shown that there is a negative correlation between the ratio F680/F740 and the chlorophyll content of leaves. Günther *et al.* (1991) showed that the fluorescence ratio of maize leaves decreased from 0.91 to 0.7 with increasing chlorophyll content from 24 to 36 $\mu g\ cm^{-2}$. In our work, we found a decrease in the fluorescence ratio from about 1.9 to 1.55 with increasing total chlorophyll content from about 25 to 65 $\mu g\ cm^{-2}$.

The results showed that N-fertilization levels can be differentiated by means of fluorescence measurements, as shown in Table 1. The mean fluorescence ratio in wheat leaves varied from 1.88 (plants without N-fertilization) to 1.55 (plants receiving 1.25 g N/pot up to this moment). The results also showed that 40 measurements per treatment were enough to get a reasonably reliable estimate of the chlorophyll status of the plant.

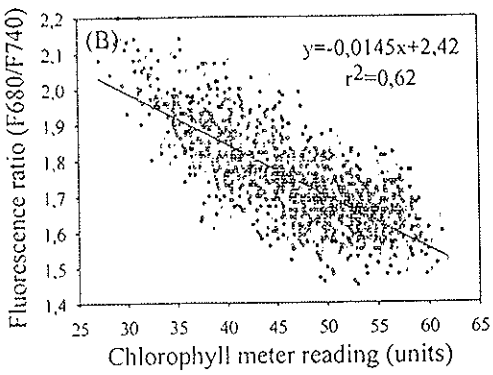

Figure 2. Fluorescence ratio F680/F740 in wheat leaves as related to the chlorophyll meter reading.

Table 1. Chlorophyll meter reading and the fluorescence ratio F680/F740 at the 8-leaf-stage as affected by the nitrogen supply.

Nitrogen applied	Chlorophyll meter Reading	Fluorescence Ratio[2]
g N/pot	units	F680/F740
without N	36.9 c[1]	1,88 c
0.15	39.4 c	1,83 c
0.40	45.8 b	1,66 b
0.85	52.5 a	1,58 a
1.25	54.5 a	1,55 a

[1] In a column, means followed by a common letter are not significantly different by Tukey's Multiple Range Test (α=0.01).
[2] Mean of 40 measurements.

Conclusions

The ratio F680/F740 is inversely related to the chlorophyll content. Taking into account that a positive correlation between leaf chlorophyll and N content exists, the use of this fluorescence method in remote sensing systems will permit monitoring on-line the spatial variation of the N-status of plants. The results of this project may lead to the development of a laser-induced fluorescence sensor, which allows farmers site-specific N fertilization.

References

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