LASER-INDUCED CHLOROPHYLL FLUORESCENCE AS A TOOL TO DETERMINE THE NITROGEN STATUS OF WHEAT

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ABSTRACT

The in vivo chlorophyll fluorescence emitted from green plant tissues can be taken as a very powerful instrument to obtain insight into the physiological status of plants. The relationship between the laser-induced fluorescence intensity at 680 and 740 nm and the fluorescence ratio F680/F740 and the nitrogen nutritional status of wheat plants was characterized in two growth chamber experiments. The results showed that the fluorescence intensity is related to the leaf chlorophyll content, especially to the intensity of the 680 nm band. The ratio F680/F740 is negatively correlated with the chlorophyll *a* content (r^2=0,62; p=0,01). The fluorescence ratio appears to be little sensitive to light and air temperature conditions.

INTRODUCTION

Laser-induced chlorophyll fluorescence is the optical emission from chlorophyll molecules that have been excited to a higher energy level by absorption of electromagnetic radiation.

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. In this regard, the fluorescence spectra can be used as an indicator of the relative concentration of N.

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 leaf side exhibit two fluorescence maxima: one near 690 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 the 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 at the Department of Plant Sciences, Chair of Plant Nutrition of the Technical University of Munich (Freising, Germany). Wheat plants (cv. Thassos) were grown at a density of 12 plants per pot in eight L pots (20 cm diameter) filled with a loamy soil. After ten days under natural conditions (greenhouse), the plants were transported into a controlled environment growth chamber. 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 control plants did not receive N fertilization.

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 upper leaf surface of the youngest fully expanded leaves in 50 leaves per treatment. The leaves were cut from the plants and the fluorescence was quickly measured. The leaf was kept at an angle of 90° with respect to the excitation beam. The leaf area illuminated by the laser beam is about 8 mm^2. Before the measurements were done, the chlorophyll content of the leaf was estimated by using the chlorophyll meter (Minolta SPAD-502®). This meter measures the greenness of the leaf, which is directly related to the chlorophyll content.

The measurements were done weekly in each treatment from the 5-leaf-stage (EC 21) until the 9-leaf-stage (EC 29). The first measurement was performed 35 days after plant emergence.

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) through interference filters and photomultiplier.

The photosynthetic pigments (chlorophylls) were extracted with acetone 100% and determined spectrophotometrically (wavelengths 645, 647, 652, 663 and 664 nm) using the extinction coefficients and equations described by Schopfer (1989).

The effect of light intensity and air temperature on the fluorescence intensity at 680 and 740 nm and on the fluorescence ratio F680/F740 was also studied. The fluorescence intensity was measured in different light intensities (30 and 350 µmol m^{-2} s^{-1}) and air temperatures (15 and 25°C).

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 SPAD-502® (Figure 1A). The 680 nm fluorescence band decreased as the chlorophyll meter reading (greenness) 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) (Figure 1A). This result is in agreement with the results reported by Subhash & Mohanan (1994) in rice, that showed an increase in the fluorescence intensity at 680 nm in nitrogen-deficient plants.

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, since the fluorescence intensity is strongly dependent on other parameters like geometry of the leaf subjected to the excitation beam, the roughness of the leaf surface (Schweiger et al., 1996) and the scattering properties of the leaf tissue (Briantais et al., 1986).

However, the correlation between fluorescence measurements and the chlorophyll meter readings was improved by the use of the fluorescence ratio F680/F740, instead of single-band fluorescence measurements (Figure 1B). The fluorescence 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 (Figures 1A and 1B).

According to Schweiger et al. (1996) fluorescence ratios (e.g. F680/F740) vary very little and 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.

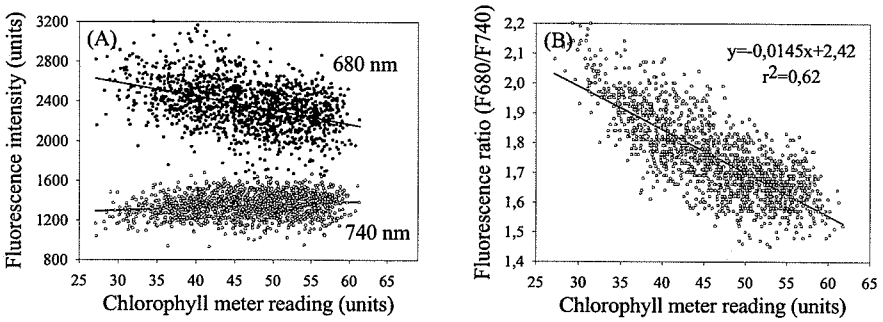

FIGURE 1. Fluorescence intensity of the 680 nm and 740 nm bands (A) and fluorescence ratio F680/F740 (B) as affected by the chlorophyll meter reading (SPAD-502).

The fluorescence ratio F680/F740 was 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) (Figures 2A and 2B). 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 μg 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 μg cm^{-2} (Figure 2B).

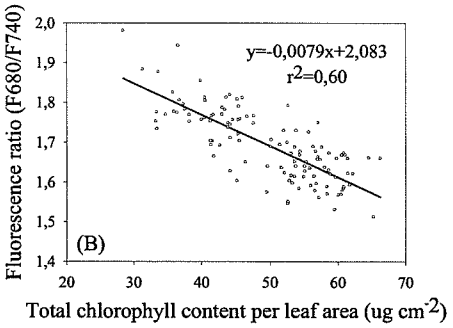

FIGURE 2. Correlation between the fluorescence ratio F680/F740 and the chlorophyll *a* content (A) and the total chlorophyll content (B) at the 7-leaf-stage.

The chlorophyll meter readings were well correlated with the leaf chlorophyll *a* content (r^2=0,84; p=0,01) (Figure 3A) and with the leaf N concentration (r^2=0,79, p=0,01) (Figure 3B), confirming that the chlorophyll meter can be used to estimate the chlorophyll content and the N nutritional status of wheat plants.

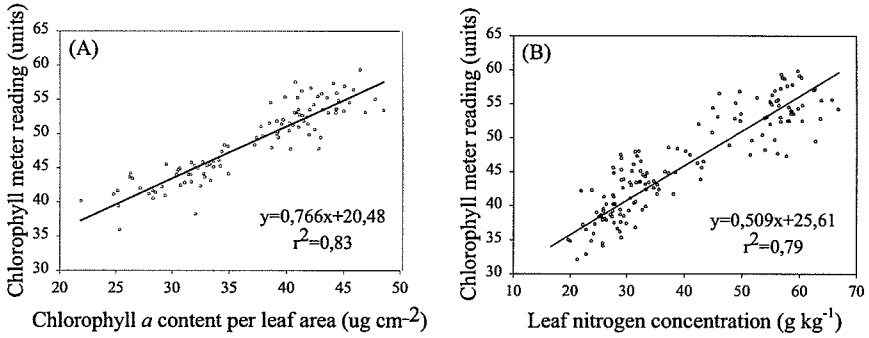

FIGURE 3. Correlation between the chlorophyll *a* content (A) and the leaf N concentration (B) and the chlorophyll meter reading at the 7-leaf-stage and at the 6-leaf-stage, respectively.

The results showed that N-fertilization levels can be differentiated by means of fluorescence ratio F680/F740 measurements (Table 1). In this sense, the mean fluorescence ratio in wheat leaves (measured at the 8-leaf-stage, 49 days after emergence) varied from 1,87 (plants without N-fertilization) to 1,58 (plants receiving 1,25 g N/pot up to this moment). The mean chlorophyll meter reading varied from 36,9 (plants without N-fertilization) to 54,5 (plants receiving 1,25 g N/pot) (Table 1).

TABLE 1. Chlorophyll meter reading and fluorescence ratio F680/F740 at the 8-leaf-stage (49 days after emergence) as affected by the nitrogen supply.

N applied up to the 8-leaf-stage	Chlorophyll meter reading	Fluorescence ratio (F680/F740)
g N/pot	units	
without N	36,9 c[(1)]	1,87 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	1,56 a

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

The fluorescence ratio F680/F740 was little affected by the light and air temperature conditions as shown in Figure 4. The results showed that the fluorescence ratio of wheat leaves was almost insensitive to the light intensity between 30 and 350 µmol m^{-2} s^{-1}, in spite of the great differences in the fluorescence yield between plants grown under different light intensities.

The fluorescence yield at 680 nm was about 30% higher and at 740 nm was about 38% higher at 30 µmol m^{-2} s^{-1} than at 350 µmol m^{-2} s^{-1} (data not shown). However, the fluorescence ratio was little affected by the light intensity (Figure 4A). This result agrees with the work of Günther et al. (1991) that did not found a significant dependence of the ratio F680/F740 and global irradiation under day light conditions. Also Stober & Lichtenthaler (1993) reported that the fluorescence ratio of wheat plants was little changed between field (1600 µmol m^{-2} s^{-1} in sunny days) and phytochamber plants (250 µmol m^{-2} s^{-1}), even though the total chlorophyll fluorescence intensity was very different between the two groups of plants. This behavior will permit that the laser-induced chlorophyll fluorescence system can operate under different weather conditions with the same accuracy, even at low light intensities, like at dawn and dusk.

The fluorescence intensity at 680 nm and 740 nm was also affected by the air temperature. The fluorescence intensity of both bands increased as the air temperature decreased (data not shown). The fluorescence intensity at 680 nm was about 38% higher and at 740 nm about 41% higher at 15°C than at 25°C (data not shown). According to Agati et al. (1997), the increase in total fluorescence intensity with a decreasing leaf temperature can be due to a lowering of the fluidity of the thylakoid membranes, which inhibits the reoxidation of plastoquinones, thus leading to a reduction in the electron transport rate and to an increase in the total fluorescence yield.

The fluorescence ratio F680/F740, on the other hand, was less affected by the air temperature than was the fluorescence intensity (Figure 4B). The increase in fluorescence yield with decreasing the air temperature was larger in the 730 nm fluorescence band than in the 680 nm one, leading to a decrease in the ratio F680/F730 from 1,8 to 1,7 (mean values) with a decrease in the air temperature from 25°C to 15°C. Also Lichtenthaler & Rinderle (1988) reported that the height of the ratio F680/F740 was little changed when the measurements were carried out at an air temperature of 20 to 25°C and at a lower temperature (5 to 10°C).

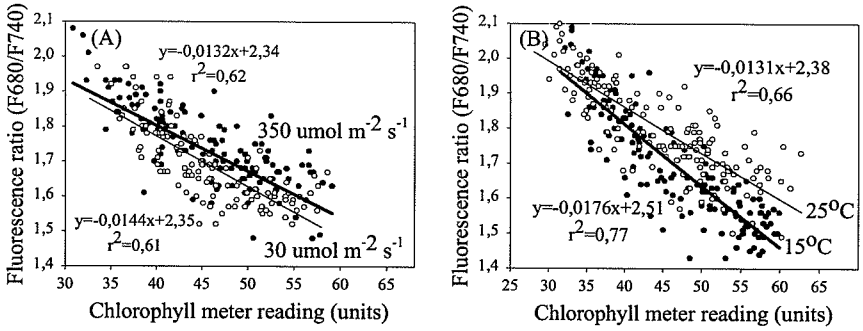

FIGURE 4. Fluorescence ratio F680/F740 as affected by the light intensity (A) and by the air temperature (B) at the 9-leaf-stage and at the 8-leaf-stage, respectively.

CONCLUSIONS AND FUTURE PERSPECTIVES

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 to monitor on-line the spatial variation of the N-status of plants and to control the amount of N fertilizer being applied. So far, the results are showing that the fluorescence ratio is almost insensitive to air temperature and light intensity. Thus, it is expected that this system can be operated under nearly all weather situations with the same accuracy. The results of this project may lead to the development of a laser-induced fluorescence sensor, which allows farmers site-specific N fertilization.

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