Photosynthesis Research **25**: 295–298, 1990.

Regular paper

The chlorophyll fluorescence ratio F690/F730 in leaves of different chlorophyll content

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Received 16 August 1989; accepted in revised form 16 May 1990

Key words: Fluorescence induction kinetics, maple leaves, spruce needles, two wavelength chlorophyll fluorometer

Abstract

The red laser-induced chlorophyll-fluorescence induction kinetics of predarkened leaf samples were registered simultaneously in the 690 and 730 nm regions i.e., in the region of the two chlorophyll fluorescence emission maxima. From the induction kinetics the chlorophyll fluorescence ratio F690/F730 was calculated. The ratio F690/F730 shows to be dependent on the chlorophyll content of leaves. It is significantly higher in needles of damaged spruces (values of 0.45–0.9) than in normal green needles of healthy trees (values of 0.35–0.5). During development and greening of maple leaves the ratio F690/F730 decreases with increasing chlorophyll content. Determination of the ratio F690/F730 can be a suitable method of monitoring changes in chlorophyll content in a non-destructive way in the same leaves during development or the yellowish-green discolouration of needles of damaged spruces in the Black Forest with the typical tree decline symptoms.

Abbreviations: F690/F730 – ratio of the fluorescence yield at the two fluorescence-emission maxima in the 690 and 730 nm regions; F_m – maximum fluorescence; F_s – steady-state fluorescence

Introduction

The red in vivo chlorophyll fluorescence emitted from divers green plant tissues can be taken as a very powerful instrument to obtain insight into the process of photosynthesis and into the physiological state and vitality of plants (Papageorgiou 1975, Krause and Weis 1983, Lichtenthaler 1987a, Walker 1988). The inverse relationship between in vivo chlorophyll fluorescence and photosynthetic activity first proposed by Kautsky and Frank (1943) can be applied to study the potential photosynthetic capacity of plants as well as to detect damage to the photosynthetic apparatus.

The chlorophyll fluorescence-emission spectra of green leaves (measured at room temperature) exhibit two maxima (or one maximum and a shoulder) in the 690 and 730 nm regions (French 1960, Lichtenthaler and Rinderle 1988). The registration of the complete fluorescence spectra allows the determination of the fluorescence intensity at the two maxima i.e., the ratio F690/F730. The latter may also be calculated from simultaneous measurements of the fluorescence kinetics in the 690 and 730 nm regions. The values of the ratio F690/F730 are considerably increased with decreasing chlorophyll content as is shown in this investigation with discoloured needles of damaged spruces and greening maple leaves.

Material and methods

Induction kinetics of the chlorophyll fluorescence were measured at room temperature simulta-

neously in the 690 and 730 nm regions by means of a laser-equipped portable two-wavelength chlorophyll fluorometer (Lichtenthaler and Rinderle 1988). Excitation was performed with a He/Ne-laser (Spectra physics, 8 mW; λ = 632.8 mm). The predarkened leaf sample (20 min) was illuminated by using a 3-arm glass-fiber system. The laser-induced chlorophyll fluorescence was detected by photodiodes. Red cut-off filters (Schott RG 665) were applied (to exclude excitation and stray light) in combination with interference filters in the 690 and 730 nm regions. The quantum irradiance of the red excitation light (632.8 nm) on the leaf surface amounted to 500 μmol m^{-2} s^{-1}. This intensity induced maximum fluorescence F_m and steady-state fluorescence F_s as was controlled using the PAM-fluorometer with saturating white light (Haitz and Lichtenthaler 1988).

The ratio F690/F730 was determined from the fluorescence induction kinetics registered with the two-wavelength chlorophyll fluorometer at room temperature. The ratio F690/F730 was calculated for the points of maximum fluorescence F_m and also for the steady-state fluorescence F_s 5 min after onset of illumination.

Measured were green needles of two healthy and yellowish-green needles of two damaged spruces (*Picea abies* (L.) Karst.) from the Mauzenberg forest area (Black Forest, 730 m above sea level). The damaged spruces were classified by forestry research scientists in damage class 3 (average needle loss 61–90%), for more details of forest tree damage classification confer Nagel et al. (1987). Fluorescence induction kinetics were also recorded in greening maple leaves (*Acer platanoides* L.) from two trees growing in the University Campus Park.

The chlorophylls were extracted with 100% acetone and the pigments quantitatively determined using the new extinction coefficients of Lichtenthaler (1987b). The needle and leaf area (projected area) was determined using a video camera (image processing system of SIS, Münster, Germany).

Results

In green needles the red laser-induced chlorophyll-fluorescence yield at 730 nm is much higher

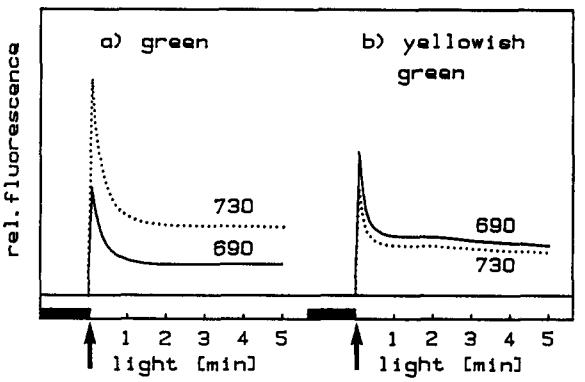

Fig. 1. Chlorophyll-fluorescence induction kinetics (Kautsky effect) of differently coloured spruce needles (*Picea abies* (L.) Karst.) measured with the two-wavelength fluorometer at 690 and 730 nm. The chlorophyll content for the green needles was 50 μg per cm^2 and for the yellowish-green needles 15 μg per cm^2 needle area. The ratios of the fluorescence emission in the two fluorescence maxima (ratio F690/F730) at the maximum fluorescence (F_m) and the steady state (F_s) are for the yellowish-green needles 1.3 at F_m and 1.2 at F_s and for the green needles 0.5 at F_m and 0.45 at F_s.

than that in the 690 nm region. This is inverse to the yellowish-green needles of damaged spruces (Fig. 1). From the fluorescence intensities in the 690 and 730 nm regions we determined the ratio F690/F730, which is much lower for green needles (0.45 at F_s) than for the yellowish-green needles (1.2 at F_s) of Fig. 1. This observation indicates that the ratio F690/F730 is dependent on the chlorophyll content of leaves. With this ratio we could demonstrate the increasing chlorophyll loss in the needles of damaged spruce trees with increasing age and needle year of the needles (Fig. 2). These spruces showed the typical yellowing effect of the older needle years, which is known as the mountain spruce disease (forest decline), which is thought to be caused by combined action of air pollution, acid rain and mineral deficiency. In the green needles of the healthy trees the ratio F690/F730 slightly decreases with increasing age and chlorophyll content from the 1988 to the 1986 needles. In contrast, the ratio F690/F730 in the needles of the damaged spruces increases considerably with increasing age from the 1988 to the 1984 needles. Figure 2 also demonstrates that the ratio F690/F730 is higher at maximum fluorescence F_m than at steady-state fluorescence F_s.

The inverse correlation between the height of the fluorescence ratio F690/F730 and the chloro-

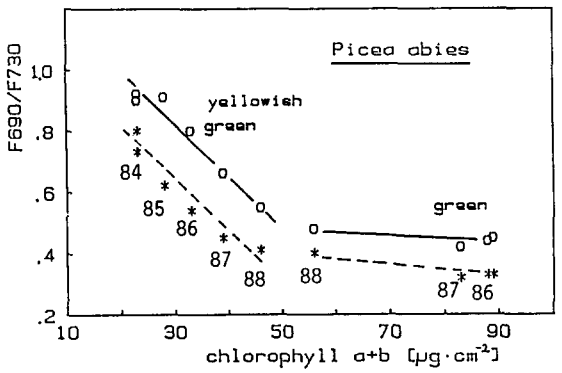

Fig. 2. Increase of the chlorophyll-fluorescence ratio F690/F730 with decreasing chlorophyll content in normal green needles of healthy spruces and yellowish-green needles of damaged spruces with lower chlorophyll content in the older needle years. The ratio F690/F730 was determined from the fluorescence induction kinetics measured with the two-wavelength fluorometer. It is given for the state of maximum fluorescence F_m (solid lines) and the steady-state fluorescence F_s (broken lines). The numbers 88 to 84 represent the needle years 1988 to 1984. Each point represents the mean of 4 determinations (SD = 5% or less).

phyll content was further demonstrated by registration of the fluorescence signatures during the development and greening of maple leaves (*Acer platanoides* L.) as shown in Fig. 3. The ratio F690/F730 is lower at steady-state than at maximum fluorescence. There is a good correlation

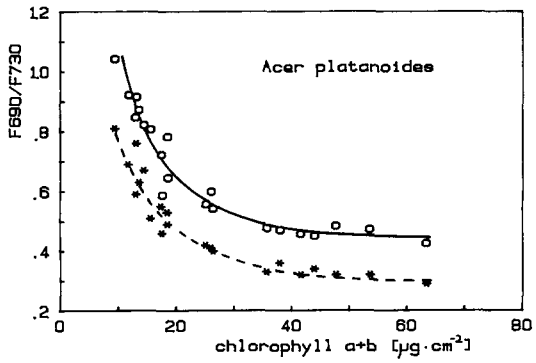

Fig. 3. Decrease of the chlorophyll fluorescence ratio F690/F730 with increasing chlorophyll content of leaves of *Acer platanoides* L. during the greening and leaf development period from April 18th to June 26th, 1989. The ratio F690/F730 was determined from the fluorescence-induction kinetics measured with the two-wavelength fluorometer. It is given for the state of maximum fluorescence F_m (solid line) and the steady-state fluorescence F_s (broken line). Each point represents the mean of 4 separate leaf measurements (SD < 7%).

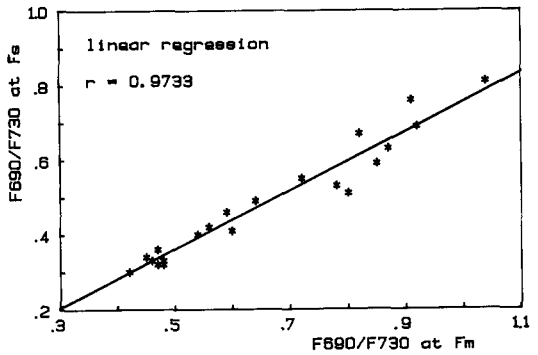

Fig. 4. Correlation (linear regression analysis) between the fluorescence ratios F690/F730 at F_m (maximum fluorescence) and at F_s (steady-state fluorescence) of Fig. 3.

between the ratio F690/F730 at F_m and F_s during the greening period of maple leaves as shown by a coefficient *r* of close to 1 (Fig. 4).

Discussion

The results obtained with maple leaves and those with the green needles of healthy and the yellowish discoloured needles of damaged spruces demonstrate that the ratio F690/F730 can be taken as an indicator of changes of the chlorophyll-content of leaves. Since chlorophyll fluorescence can be measured in a non-destructive way, one can follow via this ratio e.g., the greening of plants *in situ*. It is also possible to study the loss of chlorophyll in the needles of damaged spruces, which show the general forest tree decline symptoms i.e., needle loss and yellowish discolouration of the older needle years. Such spruces are found in many stands of the upper Black Forest regions and are characterized by lower chlorophyll content and a partially disturbed photosynthesis (Nagel et al. 1987).

In dark green leaves or needles the short wavelength chlorophyll fluorescence near 690 nm is partially suppressed due to reabsorption of the emitted fluorescence by chlorophyll (Lichtenthaler and Rinderle 1988). At lower chlorophyll levels the reabsorption process of the emitted 690 nm fluorescence decreases considerably, which appears to be the main reason for the increase of the ratio F690/F730. The slight decrease of the ratio F690/F730 during the induction kinetics from F_m to F_s is not yet well under-

stood. It may indicate that the relative amounts of reabsorption of the 690 nm fluorescence at F_m, where more fluorescence is emitted, is lower than at F_s. To which degree photosystem I may contribute at room temperature to the chlorophyll fluorescence emission is not known. This and the question whether state transitions, which change the distribution of light between the two photosystems, play a role in the decrease of the ratio F690/F730 from F_m to F_s is matter of further investigations.

During spring-time partial regreening of yellowish-green needles of damaged spruces can occur (Lichtenthaler et al. 1989). Such reaccumulation of chlorophyll should also be detectable via the ratio F690/730. Long-term stress conditions in plants appear to be always associated with a lower chlorophyll content and these may be monitored via the ratio F690/F730. From the induction kinetics with the chlorophyll fluorometer one can also determine the variable fluorescence and the fluorescence decrease ratio (Rfd-values defined as ratio of $(F_m-F_s)/F_s$ see Lichtenthaler 1987, Lichtenthaler and Rinderle 1988), which provide additional information on the functioning of the photosynthetic apparatus. Measurement of fluorescence induction kinetics with determination of the ratio F690/F730 may thus develop to a practical method in ecophysiology.

Acknowledgements

This work was supported in part by a grant from the PEF Project European Research Centre Karlsruhe, which is gratefully acknowledged. We wish to thank Annette Fahl and Bernhard Volk for technical assistance.

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