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Chlorophyll Fluorescence and Photon Yield of Oxygen Evolution in Iron-Deficient Sugar Beet (*Beta vulgaris* L.) Leaves[1,2]

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ABSTRACT

The response of sugar beet (*Beta vulgaris* L.) leaves to iron deficiency can be described as consisting of two phases. In the first phase, leaves may lose a large part of their chlorophyll while maintaining a roughly constant efficiency of photosystem II photochemistry; ratios of variable to maximum fluorescence decreased by only 6%, and photon yields of oxygen evolution decreased by 30% when chlorophyll decreased by 70%. In the second phase, when chlorophyll decreased below a threshold level, iron deficiency caused major decreases in the efficiency of photosystem II photochemistry and in the photon yield of oxygen evolution. These decreases in photosystem II photochemical efficiency were found both in plants dark-adapted for 30 minutes and in plants dark-adapted overnight, indicating that photochemical efficiency cannot be repaired in that time scale. Decreases in photosystem II photochemical efficiency and in the photon yield of oxygen evolution were similar when measurements were made (a) with light absorbed by carotenoids and chlorophylls and (b) with light absorbed only by chlorophylls. Leaves of iron-deficient plants exhibited a room temperature fluorescence induction curve with a characteristic intermediate peak I that increases with deficiency symptoms.

Leaves from Fe-deficient plants have reduced amounts of pigments and other chloroplast membrane components per unit area when compared with control plants (24). Previous studies have indicated that photosynthetic pigments are not uniformly decreased by Fe deficiency, xanthophylls being less affected than Chl and β-carotene (24); this relative enrichment in xanthophylls has been ascribed to a relative increase in lutein and in pigments within the violaxanthin cycle (also called VAZ pigments) (16). A detailed characterization of the changes in Chl and carotenoids induced by Fe deficiency in sugar beet has been described elsewhere (16).

The efficiency of photosynthetic energy conversion has been found to decrease in some carotenoid-enriched plant materials. In etiolated plants that contain large amounts of carotenoids but not Chl, excitation energy collected by carotenoids is not transferred to newly formed Chl *a* (8, 12); in these plants, some of the carotenoids are located in the prolamellar body, away from photosynthetically active structures (22). In *Poplar* leaves, Adams *et al.* (3) found that senescence induced decreases in the photon yield of oxygen evolution when white light was used for the measurements; however, when red light was used the photon yield of oxygen evolution remained fairly constant. This suggests that senescence induced a specific decrease in the efficiency of energy transfer from carotenoids to Chl; in these senescent leaves, a significant part of carotenoids may be located at the dense lipidic globules filled with products of chloroplast degradation, also disconnected from photosynthetically active structures (4).

It is unclear whether the efficiency of photosynthetic energy conversion is also affected in the carotenoid-enriched, Fe-deficient leaves. The photon yield of CO_2 fixation, measured in red light, has been shown to be unaffected by Fe deficiency in sugar beet, down to Chl contents of 10 $nmol \cdot cm^{-2}$ (23). However, other groups have detected decreases in the ratio of variable to maximum fluorescence arising from PSII both in cyanobacteria (13, 14, 19) and in sugar beet (16), suggesting that the efficiency of photosynthetic energy conversion can indeed be decreased by Fe deficiency. One possible explanation for these conflicting data is that Fe deficiency may affect the efficiency of photosynthetic energy conversion only below a Chl threshold value. Alternatively, the efficiency of photosynthetic energy conversion may be affected only when the light used for the measurements (*i.e.* blue light in fluorescence measurements) can be absorbed by carotenoids, but not when measurements are made with red light, absorbed only by Chl.

The aim of this work was to characterize the changes in the efficiency of photosynthetic energy conversion occurring in Fe-deficient plants. Intact leaf tissue has been used in this study, because chloroplasts and/or thylakoids isolated from Fe-deficient plants may not be fully representative of the starting leaf material. Using Fe-stressed sugar beet (*Beta vulgaris* L.) plants, we describe in this work the modifications in the photon yield of oxygen evolution measured in white and in red light, the characteristics of the Chl fluorescence kinetics from PSII measured at room temperature under several light

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[2] This paper is dedicated to the memory of Professor Cruz Rodríguez Muñoz, a pioneer in the field of plant physiology in Spain.

conditions, and the Chl fluorescence kinetics from PSII and PSI measured at 77 K.

MATERIALS AND METHODS

Plant Culture

Sugar beet (*Beta vulgaris* cv Monohill) was grown in a growth chamber in half-Hoagland nutrient solution, with or without Fe. Plants were grown with a PFD of 400 μmol photons$\cdot m^{-2}\cdot s^{-1}$ PAR at a temperature of 25°C, 80% RH, and a photoperiod of 16 h light/8 h dark. Young, rapidly expanding leaves were used for all measurements. All chlorotic leaves sampled showed no interveinal chlorosis, and exhibited a homogeneous color throughout the leaf.

Pigment Analysis

Pigments were extracted with acetone from liquid-nitrogen frozen leaf discs and stored at −30°C. Pigments were analyzed by the HPLC method described previously (20).

Absorptance Measurements

Absorptance was measured with a Shimadzu UV-3000 spectrophotometer equipped with an integration sphere accessory. This device has two sample holders at the entrance of the light into the sphere, one for the reference and the other for the sample beam. At the place where the sample light beam intersects with the other side of the sphere (R), there is a third holder, where a plate (white or black) can be inserted. All measurements were measured at 25 nm intervals from 750 to 400 nm, with the reference holder empty (air). First, a baseline was measured with the sample holder empty (air), and a white plate placed in R; baseline values were substracted from any further measurements. The transmittance of a leaf piece (I_T/I_O) was measured by placing a leaf piece in the sample holder. The reflectance of the same leaf piece (I_R/I_O) was then measured, by leaving empty the sample holder and placing the leaf piece in R, with a black plate underneath it. Leaf reflectance values were corrected for any significant reflectance of the black plate. The fraction of incident light absorbed—spectral absorptance—at each wavelength was calculated as $a_\lambda = I_A/I_O = (I_O - I_T - I_R)/I_O = 1 - (I_T/I_O) - (I_R/I_O)$. Integrated absorptance of the photons incident to the leaf in the photon yield determinations was calculated for the waveband 400 to 700 nm as

$$\int_{400}^{700} a_\lambda - d_\lambda$$

where a_λ is the spectral absorptance of the leaf and d_λ the relative spectral photon emittance of the light source at wavelength λ in nm.

Photon Yield

QY[3] were measured with a leaf-disc apparatus (LD2, Hansatech, Kings Lynn, UK). White light was obtained from a 100 W LS2 (Hansatech) halogen lamp. Red light was obtained with the same lamp fitted with a band-pass Corning 2–60 filter ($\lambda > 610$ nm). PFD (PAR, from 400–700 nm) was measured at 21 points homogeneously distributed throughout the illuminated area with a quantum meter (LI-1776, Li-Cor), and the mean used for calculations; mean coefficients of variation ranged from 3 to 7.4% depending on filter combinations. The temperature of the chamber was maintained at 25°C. All gasses were humidified by passing the gas flow through water maintained at 25°C. Calibration of the chamber was made as described in ref. 26. Light-response curves were obtained with CO_2-enriched air (5% CO_2, 21% O_2, balance N_2) (6) in the closed system mode. Six different PFD were used, adjusted with neutral density filters, between 20 and 120 μmol photons$\cdot m^{-2}\cdot s^{-1}$ for white light and between 30 and 100 μmol photons$\cdot m^{-2}\cdot s^{-1}$ for red light. Measurements were made from high to low PFD, and alternatively with white and red light. Photon yields were calculated by linear regression, from the slope of the relationship between net photosynthesis and PFD; only the linear part of the curve was used for calculations. Photon yields were corrected for the light absorptance of leaves with the same Chl content.

Chl Fluorescence

Continuous Chl fluorescence measurements were made on intact plants in the growth chamber (at 25°C). Experimental set-up was as described in ref. 16, except that a 620 nm short-pass filter (Ealing) was used. In some experiments, red light (610–720 nm) was used instead of blue-green light; in those experiments, the 620 nm filter was substituted by a Corning CS 2–60 band-pass filter and a 700 nm short-pass filter (Ealing). A leaf surface of about 10 cm^2 (defined with a black plastic mask) was illuminated through fiber optics. Light intensity was 150 μmol photons$\cdot m^{-2}\cdot s^{-1}$ at the leaf level. Fluorescence from PSII was detected as described in ref. 16. In some experiments, in which red light was used for illumination, fluorescence was detected through a 6 mm band-pass filter (Schott RG 665) and a 740 nm interference filter (Ealing). Measurements were made at the end of the 8 h period of darkness and 2 h after switching on the growth chamber lights. Preilluminated plants were kept in the dark for 30 min. In previous experiments, preilluminated plants were kept in the dark for different times; F_o and F_p were found not to change after 20 min of dark adaptation, indicating that the short-term fluorescence quenching mechanisms disappeared by that time (not shown).

Modulated Chl fluorescence measurements were made on intact plants in the growth chamber (at 25°C) with a PAM fluorometer (H. Walz, Effeltrich, FRG). F_o was measured by switching on the modulated light at 1.6 kHz; PFD was less than 0.1 μmol photons$\cdot m^{-2}\cdot s^{-1}$ at the leaf surface. F_m was measured at 100 kHz (modulated light of 3 μmol photons$\cdot m^{-2}\cdot s^{-1}$ at the leaf surface) with a 1 s pulse of 9000 μmol photons$\cdot m^{-2}\cdot s^{-1}$ of white light; F_m was already reached with pulses of 0.5 s. Signals were fed to a digital storage oscilloscope for the determination of F_o and F_m.

For measurements of PSII fluorescence at 77 K, discs were sampled from leaves in dark or light in the growth chamber and placed on a wet matting tissue in a light-tight metal

[3] Abbreviations: QY, photon yield of oxygen evolution; F_o, Chl fluorescence with all PSII reaction centers open; F_p, Chl fluorescence intensity at the peak of the continuous fluorescence induction curve; F_m, maximum Chl fluorescence emission at 77 K or at room temperature when using modulated fluorescence; F_v, variable part of Chl fluorescence ($F_p - F_o$) or ($F_m - F_o$); F_i, intermediate Chl fluorescence level; Q_B, secondary quinone acceptor in PSII.

holder. Leaf disks were dark-adapted in the holder for at least 30 min. Measurements were initiated 5 min after immersing the light-tight holder in liquid N_2. Light intensity (see above) was 6 μmol photons·m^{-2}·s^{-1} at the leaf level. The leaf disc was illuminated and fluorescence was collected through bifurcated fiber optics. Fluorescence was detected through a 3 mm red band-pass filter (Schott RG 665) supplemented with interference filters (Ealing) of 690 (PSII) or 740 nm (PSI). Fluorescence was measured with an end-on photomultiplier (Oriel), and the signal fed to a digital storage oscilloscope for the determination of F_o and F_m. F_m was measured 4 to 5 min after F_o, in order to ascertain that F_m had been reached.

RESULTS

Absorptance

Spectral absorptance curves of control, moderately Fe-deficient, and strongly Fe-deficient sugar beet leaves are shown in Figure 1. In moderately deficient leaves, Chl decreased by 80% from control values, whereas absorptance at the absorption maxima of Chl and carotenoids diminished by 10 and 5%, respectively. In strongly deficient leaves, Chl decreased by 95% from control values, and decreases in absorptance at the absorption maxima of Chl and carotenoids were 45 and 20%, respectively. The data show that, as Fe deficiency developed, massive losses in Chl produced little decreases in the absorption of carotenoids and only moderate decreases in the absorption of Chl. Iron deficiency, however, caused major decreases in the absorptance of green light. These phenomena may be ascribed to the efficient scattering carried out by the leaf tissue.

Integrated reflectance, transmittance, and absorptance were calculated for the white light used in quantum yield measurements as described in "Materials and Methods." Iron deficiency increased the integrated reflectance and transmittance of the leaf tissue (Fig. 2). Reflectance and transmittance increased from values of 8 to 10% in control leaves up to 23 and 50%, respectively, in extremely chlorotic leaves. Iron-deficient sugar beet leaves exhibited similar integrated reflectance but considerably higher integrated transmittance than *Poplar* senescent leaves at a comparable Chl content (3). The integrated absorptance of sugar beet leaves decreased from 0.80 to 0.63 when Chl decreased from 35 to 10 nmol·cm^{-2} (Fig. 2); an absorptance value of around 0.63 for sugar beet leaves with a Chl content of 10 nmol·cm^{-2} is in good agreement with values previously found by Terry (23). When Chl decreased below 10 nmol·cm^{-2}, however, absorptance decreased further, reaching values of around 0.2 for the lowest Chl sampled (less than 1 nmol·cm^{-2}).

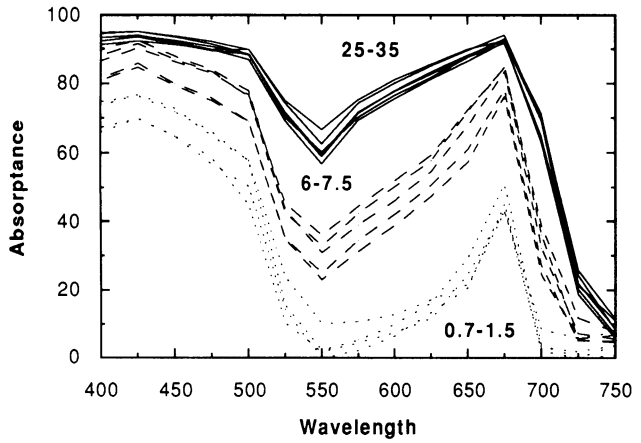

Figure 1. Spectral absorptance curves (% of absorbed photons at specific λ) from Fe-sufficient (25–35 nmol Chl·cm^{-2}), moderately Fe-deficient (6–7.5 nmol Chl·cm^{-2}), and strongly Fe-deficient (0.7–1.5 nmol Chl·cm^{-2}) sugar beet leaves.

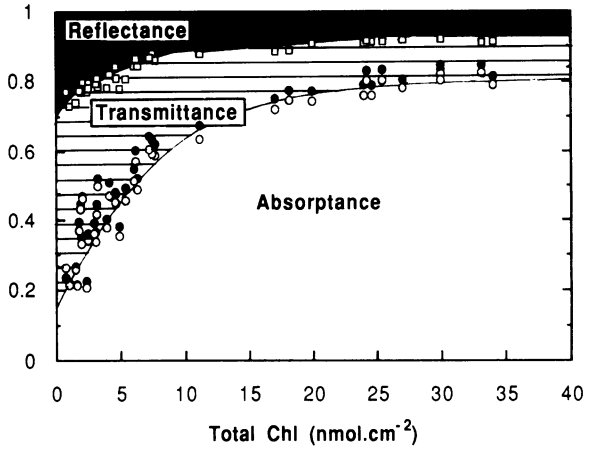

Figure 2. Fraction of total incident light that is absorbed (white area), transmitted (dashed area), and reflected (dark area) *versus* Chl content in sugar beet leaves affected by Fe deficiency. The absorptance is plotted for white (quartz-halogen lamp used for photon yield measurements, open circles) and red (white light plus a red filter, solid circles) light. See "Materials and Methods" for experimental details.

Absorptance of sugar beet leaves in red light was slightly higher than absorptance in white light for any Chl content (Fig. 2). This contrasts with studies made on senescent *Poplar* leaves, which exhibited lower absorptance in red light than in white light at low Chl contents (3). A possible cause for this discrepancy is that the relative contribution of carotenoids to integrated absorptance is much larger in senescent *Poplar* leaves than in Fe-deficient sugar beet leaves of similar Chl content. Leaves having 1.5 nmol Chl·cm^{-2} had ratios of absorptance in the blue region (due to carotenoids and Chl) to absorptance in the red region (due only to Chl) of 3.1 and 1.6 in a senescent *Poplar* leaf (3) and an Fe-deficient sugar beet leaf (Fig. 1), respectively.

Photon Yield of Oxygen Evolution

The QYs of leaf disks from Fe-sufficient and Fe-deficient plants of different Chl contents are shown in Figure 3. The QY of control plants was around 0.12. QY values decreased with decreasing Chl contents; samples with a Chl content of around 10 nmol·cm^{-2} had QY values around 0.09. For the lowest Chl contents, QY values ranged from 0.02 to 0.04. QY values obtained in red light were similar to those obtained in white light for sugar beet leaf disks at any total Chl content. This contrasts with data obtained for senescent *Poplar* leaves

(3), which indicated that QYs measured in red light, absorbed only by Chl, remained fairly constant throughout senescence. In Fe-deficient leaf tissue, QYs measured in red light are decreased considerably by Fe deficiency (Fig. 3).

Room Temperature Continuous PSII Chl Fluorescence

The absolute levels of F_o and F_p measured with continuous blue-green excitation light at room temperature exhibited an increasing trend when Chl decreased from 35 to 5 nmol·cm^{-2} (Fig. 4A). Further decreases in Chl led to decreases in the absolute values of F_o and F_p. The F_v/F_p ratio measured at room temperature remained fairly constant from 35 to around 7 nmol Chl·cm^{-2}; leaves that exhibited 7 nmol Chl·cm^{-2} still had F_v/F_p ratios of around 0.75 to 0.80 (Fig. 4B). Further decreases in Chl, however, led to major decreases in F_v/F_p ratios; the most chlorotic leaves sampled had Chl contents of around 1 nmol·cm^{-2} and F_v/F_p ratios of 0.2. Decreases in F_v/F_p ratios induced by Fe deficiency appeared both in overnight dark-adapted and in 30 min dark-adapted intact sugar beet leaves. The extent of the decrease was similar for both treatments at any given Chl content (Fig. 4B).

Low F_v/F_p ratios in Fe-deficient leaves, found when measuring Chl fluorescence with blue light, may have been an artifact caused by a carotenoid pool absorbing a significant fraction of incident light, but having a poor efficiency of energy transfer to Chl. The decrease in effective light intensity exciting Chl fluorescence may have led to an incomplete reduction of Q_B in fluorescence measurements, and in turn to low F_p and F_v/F_p ratios. We tested this possibility by measuring Chl fluorescence with red actinic light, and found that F_v/F_p ratios in Fe-deficient leaves were similar in blue (absorbed by carotenoids and Chl) and in red light (absorbed only by Chl) (not shown). Furthermore, the saturation curves F_v/F_p *versus* light intensity were found to be similar in red and blue light (not shown), indicating that both lights are equally efficient in exciting Chls.

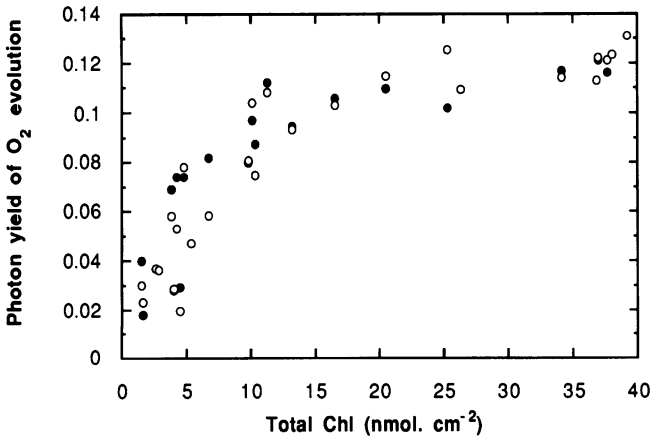

Figure 3. Photon yield of O_2 evolution (on an absorbed light basis) measured in white (400–700 nm, open circles) and red (610–700 nm, solid circles) light *versus* total Chl in sugar beet leaves affected by Fe deficiency.

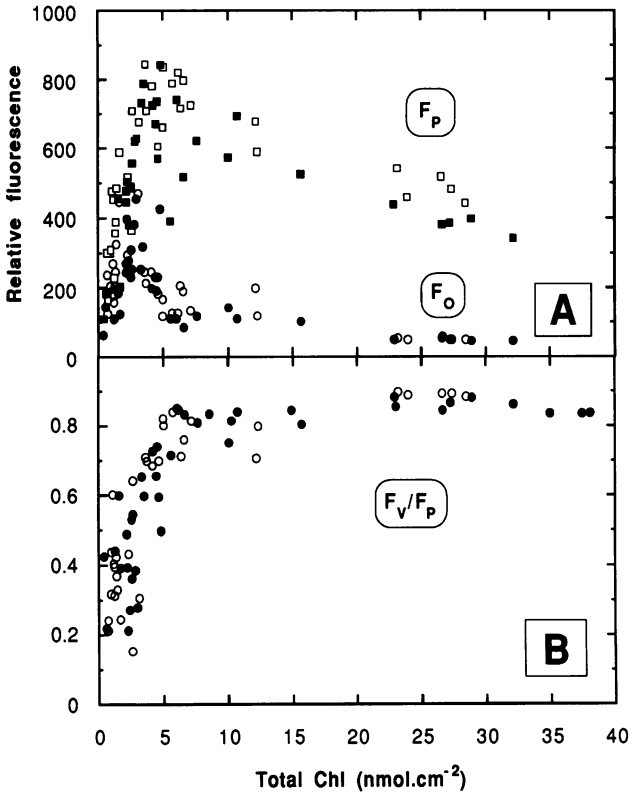

Figure 4. A, Relative fluorescence at F_o (circles) and F_p (squares) *versus* total Chl. B, Ratio of variable to maximum fluorescence (F_v/F_p) *versus* total Chl. All measurements were made at room temperature, in intact sugar beet leaves affected by Fe deficiency, with continuous fluorescence (620 nm short pass filter). Leaves were dark-adapted overnight (solid symbols) or dark-adapted for 30 min after being illuminated for 2 h (open symbols), as explained in the text.

Along with the decrease in the efficiency of PSII photochemistry, Fe-deficient plants exhibited modifications in the shape of the fluorescence induction curve (Fig. 5A). Iron-deficient plants exhibited marked increases in the relative magnitude of the F_o to F_i (also called F_{pl}) part of variable fluorescence, which is reached in less than 50 ms after the start of the illumination. The relative magnitude of the increase from F_o to F_i within the variable fluorescence—the ratio $(F_i - F_o)/F_v$—did not change significantly from controls down to Chl values of 20 nmol·cm^{-2}, the F_o to F_i rise accounting for 15% of total F_v in these samples (Fig. 5B). However, when Chl decreased further, the ratio $(F_i - F_o)/F_v$ increased rapidly to reach values of 1. The increases in F_i did not match closely the increases in F_o; the ratio F_i/F_o increased from values of around 2 in control leaves to values of 3 in deficient leaves with a Chl content of 5 nmol·cm^{-2} (Fig. 5C). In very chlorotic leaves, the F_i/F_o ratio decreased to values of around 1.2. In some of these extremely chlorotic leaves, the F_i peak was the maximum fluorescence in the induction curve. The time necessary to reach F_i decreased from 40 ms for controls to less than 10 ms for extremely Fe-deficient leaves (data not shown).

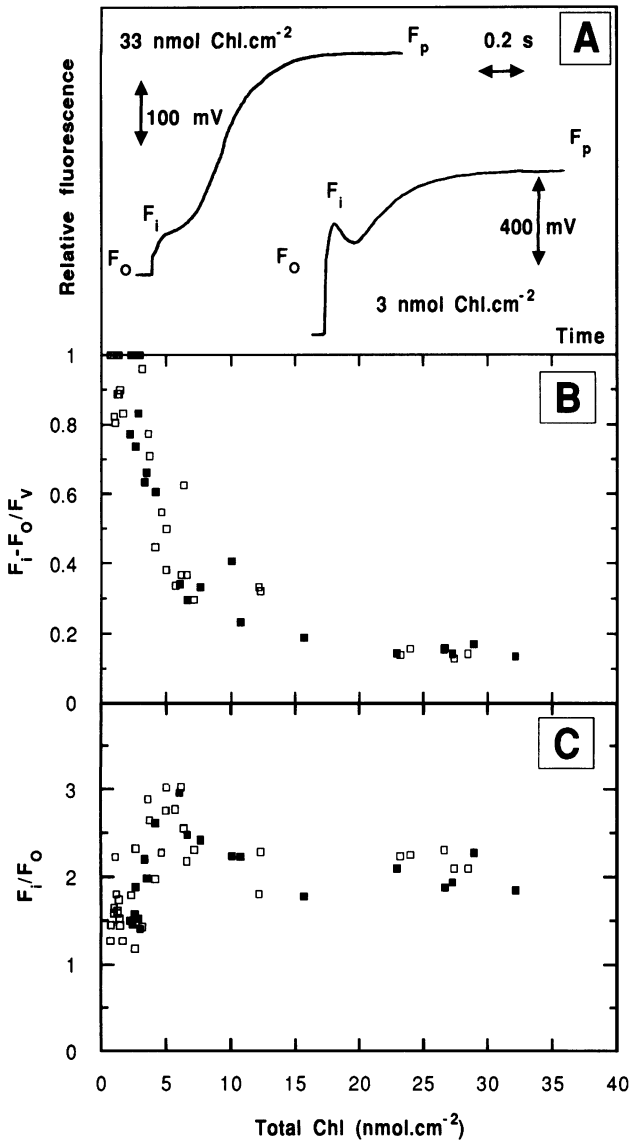

Figure 5. A, Fluorescence induction curves from control and strongly Fe-deficient leaves. B, The relative magnitude of the F_o to F_i part of variable fluorescence ($F_i - F_o/F_v$) *versus* total Chl. C, F_i/F_o ratio *versus* total Chl. All measurements were made at room temperature in intact sugar beet leaves. Leaves were dark-adapted overnight (solid squares) or for 30 min after 2 h light (open squares).

Room Temperature-Modulated PSII Chl Fluorescence

The absolute levels of F_o and F_m measured with modulated red excitation light at room temperature increased only slightly when Chl decreased from 35 to 15 nmol·cm^{-2} (Fig. 6A). When Chl decreased below these levels, F_o kept increasing, whereas F_m exhibited major decreases. As a result of these changes, the photochemical efficiency of PSII measured with modulated light at room temperature (F_v/F_m) decreased little when Chl decreased from 35 to around 10 nmol·cm^{-2}; leaves exhibiting 10 nmol Chl·cm^{-2} had F_v/F_m ratios of around 0.75 to 0.80 (Fig. 6B). Further decreases in Chl led to major decreases in F_v/F_m ratios; the most chlorotic leaves sampled had Chl contents of around 1 nmol·cm^{-2} and F_v/F_m ratios of less than 0.1. Decreases in F_v/F_m ratios induced by Fe deficiency appeared both in overnight dark-adapted and in 30 min dark-adapted intact sugar beet leaves. The extent of the decrease was similar for both treatments at any given Chl content (Fig. 6B).

77 K PSII Chl Fluorescence

The absolute levels of F_o and F_m from PSII measured at 77 K tended to increase slightly with Chl decreased from 35 to 5 nmol·cm^{-2} (Fig. 7A). Further decreases in Chl led to major decreases in the absolute values of F_m, whereas F_o kept increasing. The photochemical efficiency of PSII measured at 77 K (F_v/F_m) decreased little when Chl decreased from 35 to around 10 nmol·cm^{-2}; leaves exhibiting 10 nmol Chl·cm^{-2} had F_v/F_m ratios of around 0.75 to 0.80 (Fig. 7B). Further decreases in Chl led to major decreases in F_v/F_m ratios; the most chlorotic leaves sampled had Chl contents of around 1 nmol·cm^{-2} and F_v/F_m ratios of less than 0.2. Decreases in F_v/F_m ratios induced by Fe deficiency appeared irrespective of the light treatment (*i.e.* in discs from overnight dark-adapted plants and in disks from illuminated plants dark-adapted for 30 min). The extent of the decrease induced by Fe deficiency

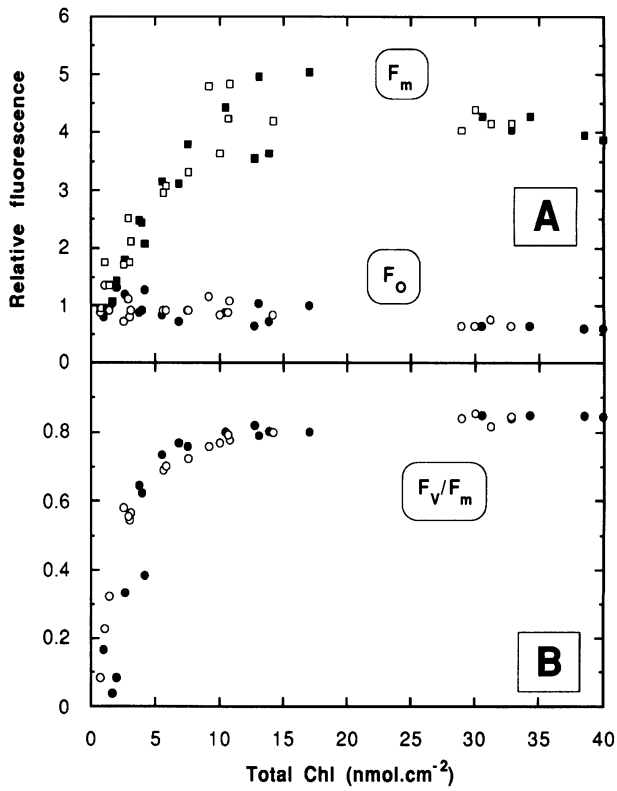

Figure 6. A, Relative fluorescence at F_o (circles) and F_m (squares) *versus* total Chl. B, Ratio of variable to maximum fluorescence (F_v/F_m) *versus* total Chl. All measurements were made at room temperature from intact sugar beet leaves, using modulated fluorescence. Plants were dark-adapted overnight (solid symbols) or dark-adapted for 30 min after being illuminated for 2 h (open symbols).

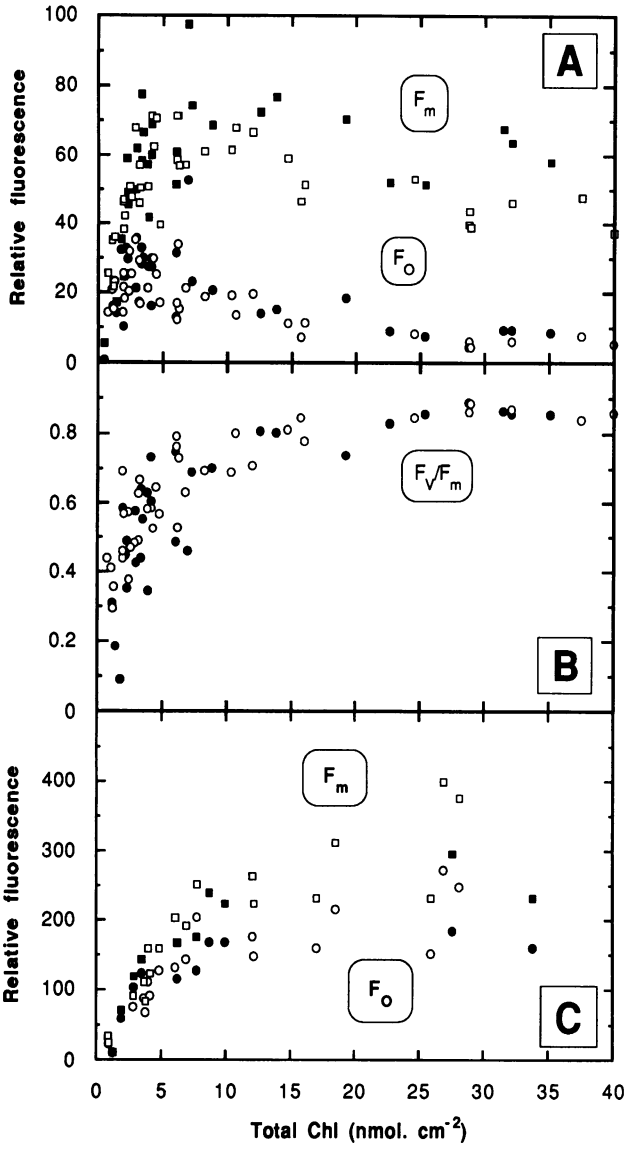

Figure 7. A, Relative PSII fluorescence at F_o (circles) and F_m (squares) *versus* total Chl. B, Ratio of variable to maximum PSII fluorescence (F_v/F_m) *versus* total Chl. C, Relative PSI fluorescence at F_o (circles) and F_m (squares) *versus* total Chl. Fluorescence was measured at 77 K from PSII (excitation 620 nm short pass filter, emission at 690 nm) and from PSI (excitation 620 nm short pass filter, emission at 740 nm) in discs of sugar beet leaves affected by Fe deficiency dark-adapted overnight (solid symbols) or dark-adapted 30 min after being illuminated for 2 h (open symbols).

was quite similar for both treatments at any given Chl content (Fig. 7B).

77 K PSI Chl Fluorescence

The absolute levels of F_o and F_m from PSI measured at 77 K decreased when Chl decreased from 35 to 1 nmol·cm^{-2} (Fig. 7C). The F_v/F_m ratios decreased only when Chl decreased below 10 nmol·cm^{-2} (not shown). Control leaves exhibiting 30 nmol Chl·cm^{-2} had F_v/F_m ratios of around 0.35, whereas the most chlorotic leaves sampled (around 1 nmol Chl·cm^{-2}) had F_v/F_m ratios of 0.05 (not shown).

DISCUSSION

The effect of Fe deficiency on the photochemical efficiency of PSII and on the photon yield of O_2 evolution in sugar beet can be described as consisting of two different phases. In the first phase, as Fe deficiency developed and sugar beet leaves lost Chl progressively, there was no parallel loss in the photochemical efficiency of PSII. Large decreases in Chl, down to 25% of control values, resulted in very small decreases (about 6%) in the photochemical efficiency of PSII. This finding is in agreement with previous data obtained by Terry (23), indicating that the quantum yield of CO_2 fixation was unaffected by Fe deficiency in this Chl range. The photon yield of O_2 evolution, however, was more affected than the photochemical efficiency of PSII (see discussion below).

In the second phase, the decreases in PSII photochemical efficiency and in photon yields of oxygen evolution became progressively larger, to reach values of around 0.2 and 0.02, respectively, in plants that had lost over 95% of their Chl. Decreases in the F_v/F_m and F_v/F_p ratios of fluorescence arising from PSII had been previously reported in cyanobacteria affected by Fe deficiency (13, 14, 19) and attributed to a decrease in the number of trapping centers relative to the Chl pool feeding into them (19). Results shown here confirm and extend preliminary results obtained for sugar beet (16). Other higher plant species (*i.e.* pear) grown in different environments exhibited a similar behavior (our unpublished data).

The decrease in the photochemical efficiency of PSII induced by Fe deficiency was irreversible even after a prolonged dark period. Both room temperature and 77 K fluorescence—measured from plants preilluminated for a few hours and then dark-adapted from 30 min and from plants dark-adapted overnight (8 h)—indicate that F_v/F_m and F_v/F_p ratios were little affected by long-term dark adaptation. All these data indicate that Fe deficiency induced a permanent or irreversible decrease in the photochemical efficiency of PSII. It should be noted that phenomena studied throughout this paper deal only with those light-dark conditions prevailing in the growth chamber during normal plant growth. There is little doubt that exposure of Fe-deficient plants to light intensities several times higher than the light used for growing the plants would result in other effects, including dark-reversible quenching of F_v/F_m, occurring long after the 30 min dark adaptation period used in our experiments; such effects, which may suggest that Fe-deficient plants are susceptible to photoinhibition, have been demonstrated in other studies (25). In our system, we did not detect any changes in F_v/F_m (or F_v/F_p) ratios occurring after the 30 min dark adaptation period. Fluorescence quenching mechanisms occurring in a shorter time scale will be dealt with in a separate report.

Our data also show that the light-induced full displacement of the xanthophyll cycle toward de-epoxidation that occurs in Fe-deficient plants (16) does not produce changes in the F_v/F_p and F_v/F_m ratios measured in the absence of an intrathylakoid ΔpH. This suggests that zeaxanthin, in Fe-deficient plants and in the dark, is not in close association to Chl. The possible presence in Fe-deficient plants of a zeaxanthin-asso-

ciated quenching of fluorescence, in the presence of ΔpH, is currently being studied and will be presented elsewhere.

The decreases in the photochemical efficiency of PSII and in the photon yield of oxygen evolution induced by Fe deficiency appeared when the light used for measurements excited carotenoids and Chl, but also when the light excited only Chl. Decreases in the photochemical efficiency of PSII and in the photon yield of oxygen evolution, measured with light containing the blue part of the spectrum that excites both carotenoids and Chl, may have arisen from a poor efficiency of energy transfer between carotenoids and Chl. The possibility that low F_v/F_p ratios may have been caused by measuring fluorescence with blue light was ruled out by measuring room temperature PSII fluorescence emission with red actinic light that excites only Chls; fluorescence measured with red actinic light exhibited decreases in the F_v/F_p ratio similar to that found with blue-green actinic light. Furthermore, decreases in the photon yields of oxygen evolution measured in white and in red light were also found to be similar. These data indicate that the loss of PSII photochemical efficiency did not arise specifically from a loss of energy transfer from carotenoids to Chl induced by Fe deficiency, but rather from a decreased photochemical efficiency of the whole of PSII pigment pool.

Chl fluorescence induction curves from Fe-deficient plants exhibited a characteristic shape, the fluorescence at point I approaching that at P in strongly deficient leaves. The F_o to F_i rise has been previously attributed to PSII units with a reduced antenna size ($PSII_\beta$ centers) (15), and more recently to PSII units lacking the ability to reduce Q_B (non-Q_B reducing PSII) (9, 10, 17). Preliminary results (not shown) indicate that the F_i to F_p rise in the fluorescence induction curve from thylakoids isolated from Fe-deficient plants was practically suppressed by dimethyl-*p*-benzoquinone, and the F_o to F_i rise was practically eliminated by dichloro-*p*-benzoquinone. These same characteristics have been described previously for OIDP curves from control and heat-treated thylakoids (9). The relative increase in the F_o to F_i rise in Fe-deficient plants may suggest that an increased proportion of PSII units are of the non-Q_B reducing type. The increase in the F_o-F_i rise may not necessarily be independent of the observed decreases in photochemical efficiency of PSII. For instance, in heat-treated thylakoids the observed increase in F_i was accompanied by a concomitant decrease in F_v/F_m ratios (9). The increase in the F_o-F_i rise and the decreases in photochemical efficiency of PSII observed may be two different consequences of the same process occurring in the thylakoid membrane of Fe-deficient plants.

The changes in F_o, F_m, and F_p absolute values exhibited patterns affected by reabsorption of fluorescence, similar to that found in other Chl-depleted plant tissues (3). When Chl fluorescence was measured at short wavelengths (680–690 nm), such as in continuous room temperature or 77 K PSII fluorescence measurements, the absolute values of F_o and F_m (or F_p) did not decrease even when Chl decreased by 80% from control values. When longer wavelengths were used (740 nm in 77 K PSI fluorescence), the absolute values of F_o and F_m exhibited decreases for similar losses in Chl. These changes were expected, because short wavelength fluorescence can be reabsorbed by the pigment beds in control, high Chl content leaves, whereas long wavelength fluorescence cannot.

The photon yield of oxygen evolution appears to be more sensitive than the photochemical efficiency of PSII in Fe-deficient plants; this contrasts with data obtained from healthy plants subjected to high-light stress, in which a close correlation between QY and photochemical efficiency of PSII has often been found (6, 11, 18). The response of QY and photochemical efficiency of PSII to Fe stress is similar to responses induced by water stress (5), SO_2 fumigation (1), and cold stress (2, 7), which have been suggested to arise from an impaired electron transport beyond the PSII-reaction center complex (2). This response is unlikely to be related to a specific effect of Fe deficiency on the oxygen-evolving system, because PSII fluorescence was affected to similar extents at room temperature and 77 K. A possible explanation for the different response of QY and photochemical efficiency of PSII is the occurrence of light-harvesting antenna state transitions in Fe-deficient plants (our unpublished data). State transitions may occur in deficient plants even at the low light levels used for photon yield measurements, thus diverting excitation away from PSII, and consequently decreasing oxygen evolution. This possibility, however, would be in conflict with the proposed role for state transitions in optimizing photosynthetic performance. A second process that may have contributed to the lack of correlation between QY and photochemical efficiency of PSII is the presence of a significant fraction of electron transport to molecular oxygen in Fe-deficient plants, even at 5% CO_2. Oxygen may act as an electron acceptor in several different ways (21) whose possible importance remain unexplored so far in Fe-deficient plants.

In summary, we have shown that Fe-deficient plants exhibit marked decreases in the photochemical efficiency of PSII and in the photon yield of oxygen evolution below a threshold Chl level. Decreases in the photochemical efficiency of PSII appear to be irreversible even after prolonged darkness. Decreases in the F_v/F_p ratios and in the photon yield of oxygen evolution appear also when actinic red light, specifically absorbed by Chl, was used for illumination. This indicates that decreases in the photochemical efficiency of PSII did not arise specifically from a poor efficiency of energy transfer from carotenoids to Chl, and suggests that the whole PSII was affected in Fe-deficient plants. We have also shown that Fe-deficient plants exhibit a characteristic increase of the I point in the room temperature fluorescence induction curve. The possibility that the increase in the F_o to F_i rise and the decreases in photochemical efficiency may have a common origin deserves further investigation.

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