

Down co-regulation of light absorption, photochemistry, and carboxylation in Fe-deficient plants growing in different environments

Ajmi Larbi · Anunciación Abadía · Javier Abadía · Fermín Morales

Received: 6 April 2006 / Accepted: 26 July 2006
© Springer Science+Business Media B.V. 2006

Abstract The regulation of photosynthesis through changes in light absorption, photochemistry, and carboxylation efficiency has been studied in plants grown in different environments. Iron deficiency was induced in sugar beet (*Beta vulgaris* L.) by growing plants hydroponically in controlled growth chambers in the absence of Fe in the nutrient solution. Pear (*Pyrus communis* L.) and peach (*Prunus persica* L. Batsch) trees were grown in field conditions on calcareous soils, in orchards with Fe deficiency-chlorosis. Gas exchange parameters were measured *in situ* with actual ambient conditions. Iron deficiency decreased photosynthetic and transpiration rates, instantaneous transpiration efficiencies and stomatal conductances, and increased sub-stomatal CO₂ concentrations in the three species investigated. Photosynthesis *versus* CO₂ sub-stomatal concentration response curves and chlorophyll fluorescence quenching analysis revealed a non-stomatal limitation of photosynthetic rates under Fe deficiency in the three species investigated. Light absorption, photosystem II, and Rubisco carboxylation efficiencies were down-regulated in response to Fe deficiency in a coordinated manner, optimizing the use of the remaining photosynthetic pigments, electron transport carriers, and Rubisco.

Keywords Apparent carboxylation efficiency · Fe deficiency · Light absorption · Peach · Pear · Photosynthesis down co-regulation · Photosystem II efficiency · Sugar beet · Xanthophyll cycle

Abbreviations

A	net CO ₂ uptake rate per unit leaf area
α	leaf absorptance
C_a	CO ₂ ambient concentration
ϵ	apparent carboxylation efficiency
Chl	chlorophyll
C_i	CO ₂ sub-stomatal concentration
E	transpiration rate
Φ_{PSII} and Φ_{exc}	actual and intrinsic photosystem II efficiencies, respectively
F_O and F_O'	minimal Chl fluorescence yield in the dark or during energization, respectively
F_M and F_M'	maximal Chl fluorescence yield in the dark or during energization, respectively
FR	far-red
F_S	Chl fluorescence at steady-state photosynthesis
F_V and F_V'	$F_M - F_O$ and $F_M' - F_O'$, respectively
g_s	stomatal conductance
Γ	CO ₂ compensation pressure
J_{max}	<i>in vivo</i> maximum rate of electron transport driving regeneration of RuBP
NPQ	non-photochemical quenching
PAR	photosynthetic active radiation
PCA	principal component analysis

A. Larbi · A. Abadía · J. Abadía · F. Morales (✉)
Department of Plant Nutrition, Aula Dei Experimental Station, Spanish Council for Scientific Research (CSIC), Apdo. 202, Zaragoza E-50080, Spain
e-mail: fmorales@eead.csic.es

Present Address:

A. Larbi
Institut de l'Olivier, Route de l'Aéroport, Km 1.5., B.P. 1087, 3000 Sfax, Tunisia

PPFD	photosynthetic photon flux density
PSI and PSII	photosystems I and II, respectively
qP	photochemical quenching
ROS	reactive oxygen species
Rubisco	ribulose-1,5- <i>bis</i> phosphate carboxylase
RuBP	ribulose <i>bis</i> phosphate
V + A + Z	violaxanthin + antheraxanthin + zeaxanthin
$V_{c,max}$	<i>in vivo</i> maximum rate of Rubisco carboxylation

Introduction

Plants growing in the field are often exposed to various types of adverse environmental conditions, which affect plant metabolism and growth and lead to reductions in crop yield (Dubey 1997). In the Mediterranean agricultural areas, one of the most important abiotic stresses leading to fruit crops yield reduction is Fe deficiency (Sanz et al. 1992; Tagliavini and Rombolà 2001). The major symptom of Fe deficiency is a decrease in leaf chlorophyll (chlorosis), which appears when plants are unable to take up enough Fe from the soil, where it is usually in large amounts but in unavailable chemical forms.

Iron deficiency-mediated reductions in photosynthesis have been reported for many plant species (Davis et al. 1986; Hurley et al. 1986a, b; Terry and Abadía 1986; Abadía 1992; Morales et al. 1998a, 2006) although many of these studies have been carried out with the model species sugar beet and in controlled growth environments. In this species, Fe deficiency was found to decrease photosynthesis by reducing the number of photosynthetic units per unit leaf area (Spiller and Terry 1980) and decreasing the energy conversion efficiency of the remaining units (Morales et al. 1998a). Terry (1983) compared the gas exchange characteristics of Fe-sufficient and Fe-deficient sugar beet leaves, and found that photosynthesis may be co-limited by light harvesting and electron transport capacity in addition to other factors (e.g., CO₂ supply) over a wide range of light/CO₂ environments. These changes were accompanied by a substantial reduction in the number of grana per chloroplast and thylakoid per granum (Platt-Aloia et al. 1983). Also, Fe deficiency in sugar beet decreased the concentration of light harvesting and electron transport components (Terry and Abadía 1986, and references therein), and also de-

creased the RuBP carboxylation capacity through diminished Rubisco enzyme activation (Taylor and Terry 1986; Winder and Nishio 1995) and down-regulation of gene expression (Winder and Nishio 1995). Other enzymes associated with photosynthetic carbon metabolism were not affected by Fe deficiency in sugar beet (Taylor et al. 1982; Taylor and Terry 1984).

Much less information is available, however, on the extent to which Fe deficiency-chlorosis reduces photosynthesis under field conditions. In the field, photosynthetic photon flux density (PPFD) fluctuations are the main cause of the variation in leaf photosynthetic rates, although air temperature and humidity, soil moisture content and leaf-to-air vapor pressure difference also modify leaf gas exchange properties (Tenhunen et al. 1980; Pettigrew et al. 1990; Thomas et al. 1999). Stomata behave optimally over a very small range of leaf-to-air vapor pressure difference (Thomas et al. 1999). In peach leaves, photosynthetic rates also depend on the leaf region, distance of the leaf from the fruit, crop load, and development time after bud break (Chalmers et al. 1975; Crews et al. 1975), but the possible influence of these factors on the development of leaf Fe chlorosis are still poorly known (Belkhodja et al. 1998b). Light effects are particularly important for species with continuously growing tree canopies, such as peach and pear, where leaves experience a changing light regime with time within the canopy (Andersen 1991; Le Roux et al. 2001).

One of the analytical techniques used to assess the degree of Fe deficiency-chlorosis is the measurement of the total leaf Fe concentration. This approach, however, presents major problems. Although there are linear relationships between leaf Fe and Chl concentrations in plants grown in hydroponics without Fe (Terry 1980; Terry and Low 1982), this is not the case with Fe-chlorotic field-grown plants. When leaves from Fe-deficient plants grown in the field were analyzed, the leaf Fe concentrations were reported to be relatively high in many cases (>80–100 µg Fe g⁻¹ dry weight), and were not well correlated with the leaf Chl concentration (Morales et al. 1998b, and references therein). This has been termed the “Fe-chlorosis paradox”, and has been traced to the effect of Fe chlorosis on leaf growth (Römheld 1997). Reductions in leaf growth would produce apparently high Fe concentrations, when expressed on a dry matter basis. Also, there are biochemical/physiological reasons by which the entrance of Fe in leaf cells may be hampered. Iron must be reduced from Fe(III) to Fe(II) by a Fe(III)-chelate reductase enzyme before entering into the

cells. An intrinsic decrease in Fe(III)-chelate reductase enzyme activity (González-Vallejo et al. 2000; Larbi et al. 2001), a possible shift in the apoplastic pH (González-Vallejo et al. 2000; López-Millán et al. 2001a) and accumulation of organic acids in the apoplastic space (López-Millán et al. 2000, and references therein) could lead to the accumulation of physiologically inactive Fe pools in some still unknown leaf compartment in chlorotic leaves (Morales et al. 1998b).

There are several processes of the leaf physiology that may interact with the development of Fe chlorosis. First, a Fe-deficient leaf not only has a reduced photosynthetic capacity but also absorbs more light per Chl (Abadía et al. 1999). The light absorbed not used in photosynthesis, especially under high light intensities in field conditions, could lead potentially to photoinhibitory and photo-oxidative processes. Heras (1960) reported that Fe-deficient leaves could re-green when exposed to PPFD levels lower than those used for plant growth. However, Fe-deficient leaves could remain without apparent damage in the field for months, which indicates that they have very efficient protective mechanisms. A second possibility for a role of photosynthetic processes in the development of chlorosis is the occurrence of Fe inactivation indicated above. The possible role of photosynthetic processes in the mechanisms of Fe inactivation remains unexplored.

The aim of this work was to investigate and compare the effects of Fe deficiency on the gas exchange characteristics in attached leaves of hydroponically grown sugar beet and field-grown pear and peach, under the environmental conditions occurring during growth. Measurements included gas exchange, modulated chlorophyll fluorescence and analyses of photosynthetic pigment composition by HPLC.

Materials and methods

Plant material

Sugar beet (*Beta vulgaris* L. Monohil hybrid from Hilleshög, Landskrona, Sweden) was grown in growth chamber with a PPFD of $350 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR (between 130 and $180 \mu\text{mol m}^{-2} \text{s}^{-1}$ placing the sensor at the growth angle of the leaves used for measurements), a temperature of 22°C , 80% relative humidity and a photoperiod of 16 h light/8 h dark. Seeds were germinated and grown in vermiculite for 2 weeks. Seedlings were grown for two more weeks in half-strength Hoagland nutrient solution with $45 \mu\text{M}$

Fe(III)-EDTA and then transplanted to 20 l plastic buckets (four plants per bucket) containing half-strength Hoagland nutrient solution (Terry 1980) with either 0 to initiate chlorosis (Fe-deficient plants) or $45 \mu\text{M}$ Fe(III)-EDTA (control plants). Thus, when Fe deficiency treatment was started, plants of the same age and size were also transferred into fresh complete media for use as controls. The pH of the Fe-free nutrient solutions was buffered at 7.7 by adding 1 mM NaOH and 1 g l^{-1} of CaCO_3 . This treatment simulates conditions usually found in the field leading to Fe deficiency (Susín et al. 1994). Young and recently expanded leaves were sampled with different degrees of Fe deficiency, covering the whole range of Chl concentrations. In some experiments, only control, Fe-sufficient ($390 \mu\text{mol Chl m}^{-2}$), severely ($80 \mu\text{mol Chl m}^{-2}$) and extremely ($30 \mu\text{mol Chl m}^{-2}$) Fe-deficient sugar beet leaves were used. Leaf Fe concentrations were approximately 150 and $45 \mu\text{g Fe g}^{-1}$ dry weight in Fe-sufficient and Fe-deficient leaves respectively (López-Millán et al. 2001b).

Leaves from pear (*Pyrus communis* L.) and peach (*Prunus persica* L. Batsch) were sampled from trees growing in calcareous soils (Belkhodja et al. 1998b; Larbi et al. 2003). The pear orchard is located in San Bruno (Centro de Investigación y Tecnologías Agroalimentarias, Diputación General de Aragón) in the Aula Dei Campus (Zaragoza, Spain). The soil of this site has clay-loamy texture, with 31% total calcium carbonate, 9.9% active lime, 2.86% organic matter and pH in water 8.0. Twenty-four years old trees of the cultivar Blanquilla (Agua de Aranjuez), grafted on quince A EM and with a frame of $5 \times 4 \text{ m}$, were used. The peach orchard (cv. “Babygold 7” grafted on seedling, 22-years-old, with a frame of $4 \times 5 \text{ m}$) was located in El Temple, Huesca, Spain. The soil has a clay-loamy texture, with 32% total calcium carbonate, 12.6% active lime, 1.89% organic matter, pH in water 8.4 and high permeability. Leaves with different degrees of Fe chlorosis were used, from fully green to severely Fe-chlorotic. In some experiments, only control, Fe-sufficient (250 or $200 \mu\text{mol Chl m}^{-2}$), severely (80 or $70 \mu\text{mol Chl m}^{-2}$) and extremely (25 or $35 \mu\text{mol Chl m}^{-2}$) Fe-deficient pear or peach leaves were used. Control pear leaves may have between 200 and $400 \mu\text{mol Chl m}^{-2}$. Leaf Fe concentrations were (Fe-sufficient/Fe-deficient leaves) approximately 120/100 $\mu\text{g Fe g}^{-1}$ dry weight in peach (Belkhodja et al. 1998b) and 130/100 in pear (Larbi 2003), respectively.

Experiments with pear and peach were carried out on cloudless days. Measurements were taken in the morning (between 7 and 10 h solar time; PPFD between 600 and $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$) at ambient relative humidity and

temperature. Day-to-day changes in water status cannot be eliminated from these experiments under natural conditions.

The leaf Fe concentration of young, rapidly expanding sugar beet leaves from plants grown hydroponically without Fe decreases concomitantly with the leaf Chl concentration (Terry and Low 1982). However, Fe chlorotic pear and peach leaves growing in the field often have large amounts of Fe that is immobilized somewhere in the chlorotic leaf in an unavailable form (Morales et al. 1998b). Therefore, we monitored the degree of Fe deficiency by measuring the leaf Chl concentration, which can be done non-destructively (see below) and is much simpler and accurate than measuring leaf Fe.

Photosynthetic pigment composition determinations and leaf absorptance

Leaf chlorophyll concentrations were estimated non-destructively with a SPAD-502 device (Minolta, Osaka, Japan). The SPAD-502 device uses two light-emitting diodes (650 and 940 nm) and a photodiode detector to measure sequentially transmission through leaves of red and infrared light. For calibration, leaf disks with different degrees of Fe deficiency were first measured with the SPAD, then extracted with 100% acetone in presence of Na ascorbate and finally Chl was measured spectrophotometrically (Abadía and Abadía 1993).

Leaf disks were taken from the same area of the leaves in which gas exchange and modulated Chl fluorescence were measured. Disks were cut with a calibrated cork borer, wrapped in aluminum foil, dropped in liquid-N₂ and stored (still wrapped in foil) at -20°C. Leaf extracts were prepared and stored as described previously (Abadía and Abadía 1993). Pigment extracts were thawed on ice, filtered through a 0.45 µm filter and analyzed by an isocratic HPLC method (Larbi et al. 2004).

Leaf absorptance values were measured as indicated elsewhere (Morales et al. 1991; Abadía et al. 1999).

Gas exchange measurements

Measurements were made on attached, recently fully expanded leaves of hydroponically grown sugar beet plants and on leaves of actively growing vegetative shoots in the field with a portable gas exchange system (CIRAS-1, PP Systems, Herts, U.K.), using a PLC broad leaf cuvette in closed circuit mode. Transpiration rate (E), stomatal conductance (g_s), net photosynthetic rate (A), and CO₂ sub-stomatal concentration (C_i)

were recorded during the measurements. The instantaneous transpiration efficiency (A/E) was also calculated. Except when responses to C_i were being examined, the ambient CO₂ concentration (C_a) was maintained at 350 ppm. Pear and peach leaves were chosen from the external part of the tree to avoid possible effects of prolonged shading, that diminishes the photosynthetic rate of pear and peach leaves (Andersen 1991). Pear and peach leaves were oriented and held perpendicularly to sunlight during the measurements.

In some experiments, gas exchange parameters were measured at different C_a values, ranging from approximately 6–27 to 1200–1900 ppm (11–15 C_a values per curve). Measurements were made first at a C_a of 350 ppm, and then C_a was subsequently lowered in a stepwise manner, set again at 350 ppm (used as reference) and finally increased stepwise. All measurements were carried out placing the leaf in the cuvette, when CO₂ concentration in the leaf cuvette had equilibrated and when the system had stabilized (3–5 min after changing the CO₂ concentration). These measurements gave a series of C_i values, ranging from 0 (or close to 0) to approximately 1100–1400 ppm. C_i data were grouped, each group having similar C_i values (from 3 to 25 values each group), and then the C_i mean values were calculated together with their corresponding A mean values. This way of doing resulted in a single curve per species and condition. Curves were fit using the Farquhar et al. (1980) model, following calculations described elsewhere (Long and Bernacchi 2003).

Incident light intensity

Incident photosynthetic photon flux densities (PPFD) were monitored with a portable CIRAS-1 system (PP Systems, Herts, U.K.) during the gas exchange measurements. PPFD measurements made with a quantum meter (Skye Instruments, Powys, U.K.) gave similar results (not shown).

Modulated chlorophyll fluorescence analyses

Modulated Chl fluorescence measurements were made on attached, recently fully expanded leaves of hydroponically grown sugar beet plants and on leaves of actively growing vegetative shoots in the field with a PAM 2000 fluorometer (Walz, Effeltrich, Germany). F_O was measured by switching on the modulated light at 0.6 kHz; PPFD was less than 0.1 µmol m⁻² s⁻¹ at the leaf surface. F_M and F_M' were measured at 20 kHz with a 1 s pulse of 6000 µmol photons m⁻² s⁻¹ of white light.

Fig. 1 Net photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; **a**), transpiration rate ($\text{mmol m}^{-2} \text{ s}^{-1}$; **b**), instantaneous transpiration efficiency (**c**), stomatal conductance ($\text{mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; **d**) and CO_2 sub-stomatal concentration (ppm; **e**) in hydroponically grown sugar beet affected by Fe deficiency. Measurements were made at 350 ppm CO_2 external concentration. Data are mean \pm SE ($n = 5-9$)

F_M was measured after 30–60 min of dark adaptation. The experimental protocol for the analysis of the Chl fluorescence quenching was essentially as described by Morales et al. (2000), and references therein. F_O and F_O' were measured in presence of far-red light ($7 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) in order to fully oxidize the PSII acceptor side (Belkhdja et al. 1998a). Dark-adapted, maximum PSII efficiency was calculated as F_V/F_M , where F_V is $F_M - F_O$. Actual (Φ_{PSII}) and intrinsic ($\Phi_{\text{exc.}}$) PSII efficiency were calculated as $(F_M' - F_S)/F_M'$ and F_V'/F_M' , respectively. Photochemical quenching (qP) was calculated as $(F_M' - F_S)/F_V'$. Non-photochemical quenching (NPQ) was calculated as $(F_M/F_M') - 1$. Pear and peach leaves were oriented and held perpendicularly to sunlight during the measurements.

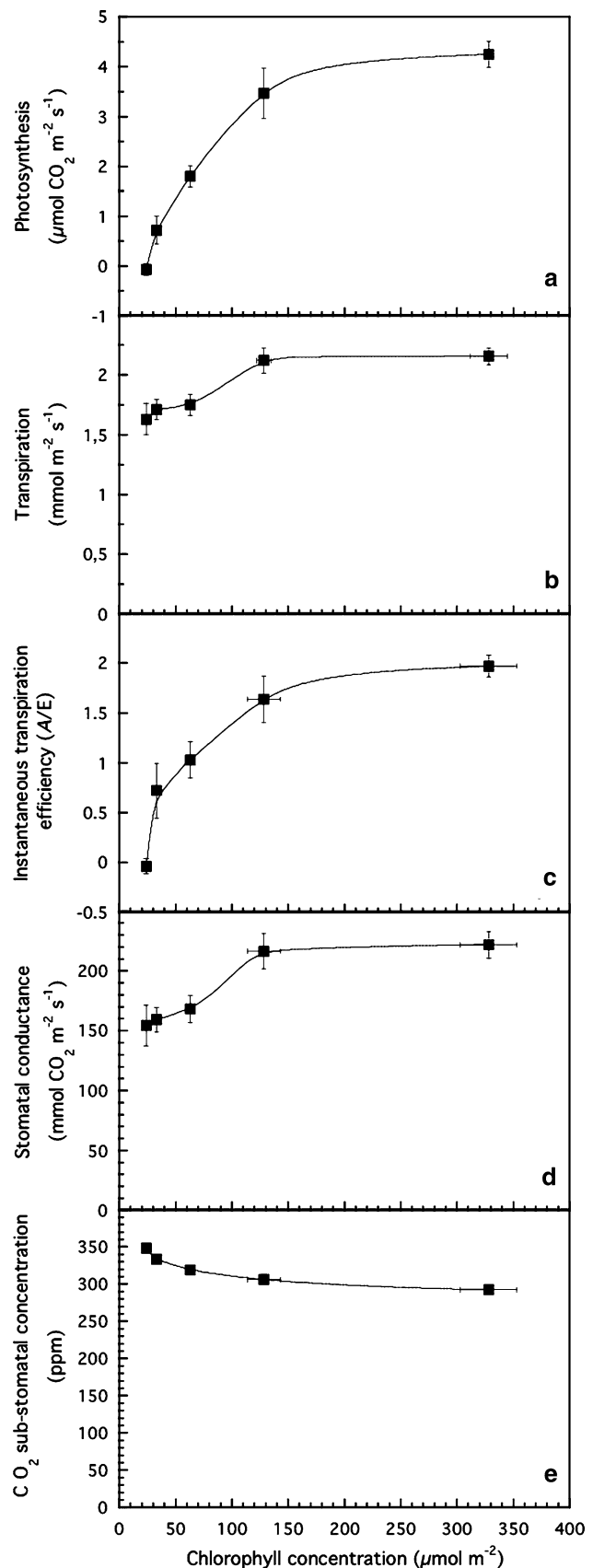
Statistical analyses

We used principal component analysis (PCA) to summarize the information of our multivariate data. PCA is a linear ordination method (ter Braak 1994). We selected those variables showing significant differences between treatments (Fe sufficiency, severe Fe deficiency and extreme Fe deficiency), and that were expected to have some relationship between them (Larbi 2003). These variables were Φ_{PSII} , $\Phi_{\text{exc.}}$, qP, ϵ , α , lutein/Chl, and $V + A + Z/\text{Chl}$. The analyzed covariance matrix was formed by these seven variables and the individual values ($n = 63$). The data were standardized to zero mean and unit variance. Ordination analyses were done using CANOCO ver. 4.5 (ter Braak and Šmilauer 1998).

Results

Effects of Fe deficiency on gas exchange characteristics

The photosynthetic rates of sugar beet, pear, and peach leaves decreased when the leaf Chl concentration decreased (Figs. 1a, 2a, 3a). A 50% Chl decrease from the control values caused major decreases in photosynthetic rates in peach (38%), whereas it caused relatively small



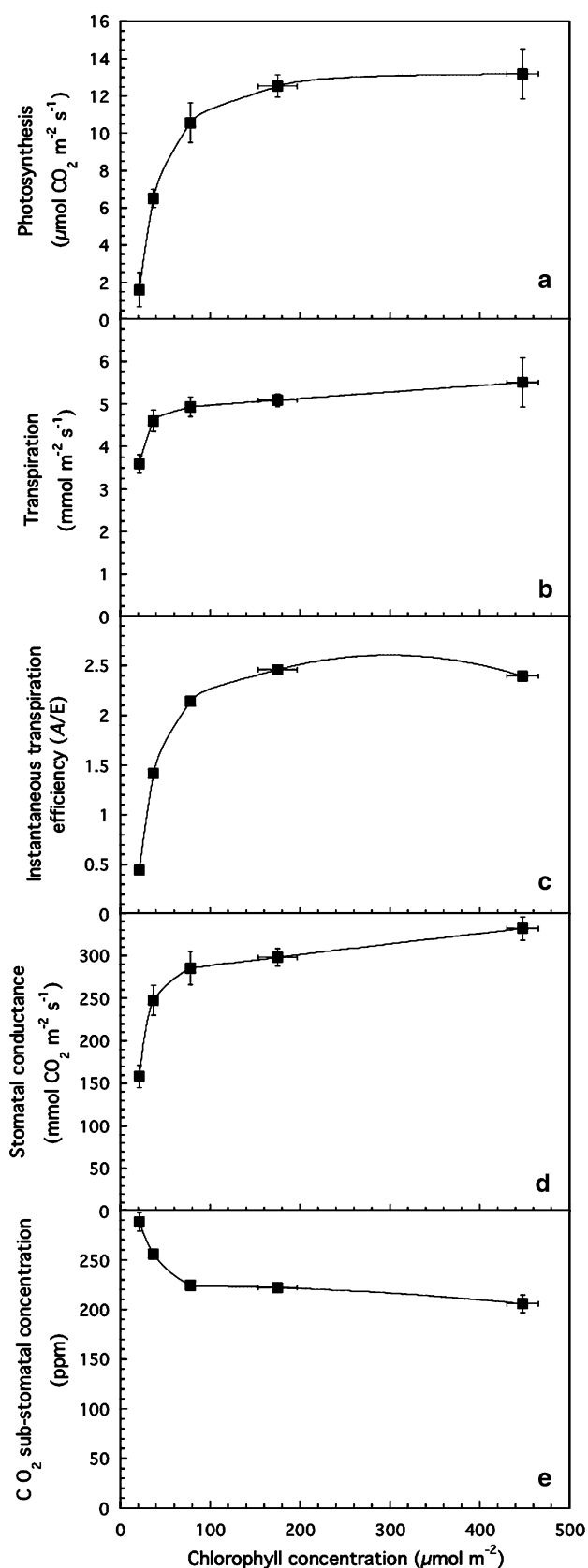


Fig. 2 Net photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; **a**), transpiration rate ($\text{mmol m}^{-2} \text{ s}^{-1}$; **b**), instantaneous transpiration efficiency (**c**), stomatal conductance ($\text{mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; **d**) and CO_2 sub-stomatal concentration (ppm; **e**) in leaves of field-grown pear trees affected by Fe deficiency. Measurements were made at 350 ppm CO_2 external concentration. Data are mean \pm SE ($n = 5$)

decreases in sugar beet (7%) and did not decrease photosynthetic rates in pear. Further decreases in Chl caused much more marked decreases in photosynthesis. Extremely Fe-deficient ($20\text{--}25 \mu\text{mol Chl m}^{-2}$) leaves had photosynthetic rates of approximately 0, 1.6–0.2, and $2.5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in sugar beet, pear, and peach, respectively.

Iron chlorosis did not affect transpiration down to Chl concentrations of 130 and $80 \mu\text{mol m}^{-2}$ in sugar beet (Fig. 1b) and pear (Fig. 2b) leaves, respectively. Peach leaf transpiration was already affected at $90 \mu\text{mol Chl m}^{-2}$ (Fig. 3b). When Chl decreased further, transpiration rates decreased markedly. The lowest transpiration rates found were approximately $1.5\text{--}4 \text{ mmol m}^{-2} \text{ s}^{-1}$ in extremely Fe-chlorotic leaves of the three species. In all cases, decreases in stomatal conductance in extremely deficient leaves were very large (Figs. 1d, 2d, 3d).

The instantaneous transpiration efficiency (A/E), calculated as the photosynthesis/transpiration ratio (Figs. 1c, 2c, 3c), followed in most cases trends similar to those of photosynthetic rates, since Fe chlorosis affected more photosynthesis than transpiration. Only in peach leaves photosynthetic rates (Fig. 3a) were affected before than the A/E values (Fig. 3c).

In sugar beet, sub-stomatal CO_2 concentrations (C_i) increased with Fe deficiency, from control values of 300 to 350 ppm in extremely Fe-deficient leaves (Fig. 1e). In pear leaves, C_i increased slightly from control values of approximately 205 to 225 ppm in leaves with $80 \mu\text{mol Chl m}^{-2}$, and extremely Fe-chlorotic leaves had the highest C_i values, reaching 290 ppm (Fig. 2e). Peach leaves showed a progressive increase in C_i with Fe chlorosis, from 150 ppm in control leaves to approximately 290 ppm in extremely Fe-deficient ones (Fig. 3e).

Effects of Fe deficiency on A/ C_i response curves

As the CO_2 sub-stomatal concentration (C_i) increased, the net CO_2 uptake (A) increased linearly first, then reached a plateau (Fig. 4). The response of A to C_i , the

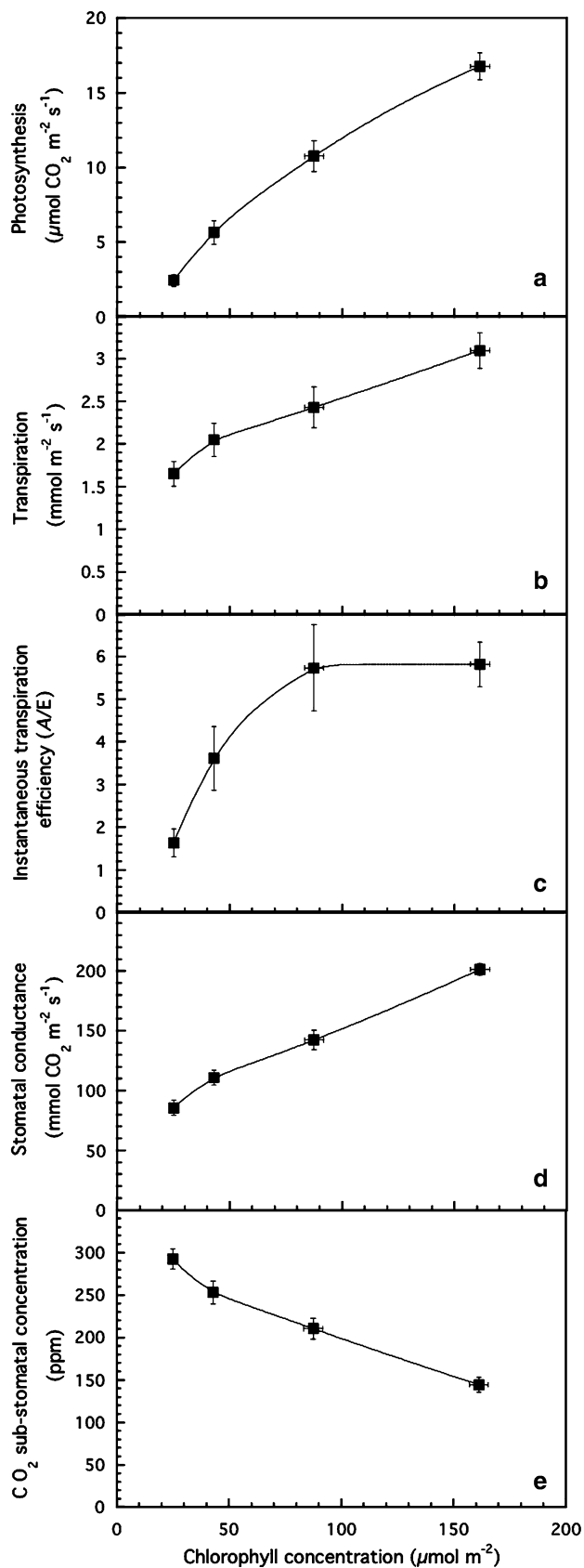


Fig. 3 Net photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; **a**), transpiration rate ($\text{mmol m}^{-2} \text{ s}^{-1}$; **b**), instantaneous transpiration efficiency (**c**), stomatal conductance ($\text{mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; **d**) and CO_2 sub-stomatal concentration (ppm; **e**) in leaves of field-grown peach trees affected by Fe deficiency. Measurements were made at 350 ppm CO_2 external concentration. Data are mean \pm SE ($n = 14\text{--}20$)

A/C_i response curve, showed clear differences between Fe-sufficient, severely and extremely Fe-deficient leaves of sugar beet (Fig. 4a), pear (Fig. 4b) and peach (Fig. 4c).

Control, severely and extremely Fe-deficient leaves had, respectively, J_{max} values of approximately 50, 26, and 18 (sugar beet), 245, 81, and 40 (pear) and 78, 30,

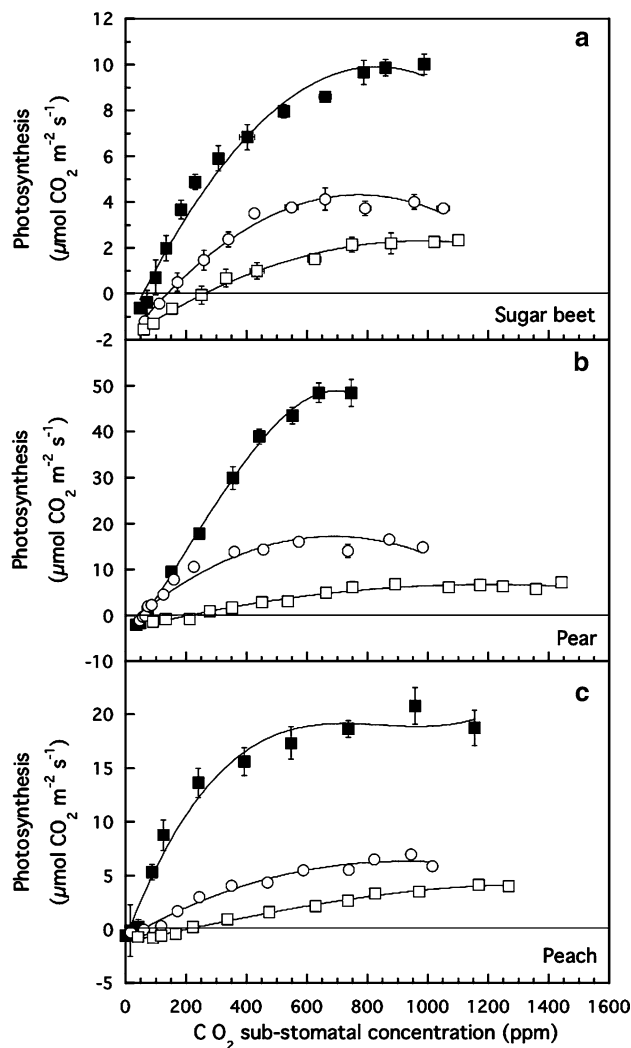


Fig. 4 Response of net photosynthetic rates to CO_2 sub-stomatal concentration (C_i) in control (solid squares), severely (open circles) and extremely (open squares) Fe-deficient sugar beet (**a**), pear (**b**) and peach (**c**) leaves. Data are mean \pm SE ($n = 3\text{--}25$)

Table 1 Calculations of *in vivo* maximum rates of electron transport driving regeneration of RuBP (J_{\max} ; $\mu\text{mol m}^{-2} \text{s}^{-1}$) and Rubisco carboxylation ($V_{\text{c,max}}$; $\mu\text{mol m}^{-2} \text{s}^{-1}$), apparent Rubisco carboxylation efficiency (ϵ ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1} \text{ ppm}^{-1}$), CO_2 compensation pressure (Γ ; ppm) and absorptance (α ; % of incident light) in hydroponically grown sugar beet and field-grown pear and peach leaves affected by Fe deficiency

	J_{\max}	$V_{\text{c,max}}$	ϵ	Γ	α
<i>Sugar beet</i>					
+Fe	49.80	21.70	0.032	69	80
Severe -Fe	25.84	11.88	0.012	136	50
Extreme -Fe	18.30	6.69	0.003	250	30
<i>Pear</i>					
+Fe	245.04	131.35	0.103	62	88
Severe -Fe	80.87	79.72	0.059	68	65
Extreme -Fe	39.67	17.22	0.011	210	55
<i>Peach</i>					
+Fe	78.40	56.18	0.064	35	75
Severe -Fe	29.70	14.57	0.016	60	60
Extreme -Fe	21.29	7.80	0.005	207	55

Values were calculated from Fig. 4 (α values were obtained from Morales et al. 1991 and Abadía et al. 1999)

and $21 \mu\text{mol m}^{-2} \text{s}^{-1}$ (peach) under their particular growing light conditions (Table 1). The $V_{\text{c,max}}$ values were approximately 22, 12, and 7 (sugar beet), 131, 80, and 17 (pear) and 56, 15, and $8 \mu\text{mol m}^{-2} \text{s}^{-1}$ (peach) in control, severely and extremely Fe-deficient leaves, respectively (Table 1). Also, the apparent Rubisco carboxylation efficiency (ϵ) was decreased by severe and extreme Fe deficiency, respectively, by approximately 63 and 91% (sugar beet), 43 and 89% (pear), and 75 and 92% (peach) (Table 1). All the initial slope values (ϵ) were obtained by linear regressions with R^2 values higher than 0.98.

The CO_2 sub-stomatal concentration where photosynthesis equals respiration (the CO_2 compensation pressure, Γ) was increased by Fe deficiency in the three species investigated. Γ values increased by approximately 2- and 3.6-fold in sugar beet, by 10% and 3.2-fold in pear and by 70% and 5.9-fold in peach in severely and extremely Fe-deficient leaves,

respectively, when compared to the controls (Table 1).

Effects of Fe deficiency on Chl fluorescence quenching parameters

In sugar beet, pear, and peach (Table 2), severe Fe deficiency (approximately 65–80% decrease in Chl when compared to the controls) did not cause any sustained decrease in PSII efficiency, as indicated by the relatively high dark-adapted F_V/F_M ratios found. In the case of extremely deficient leaves (approximately 82–92% decrease in Chl) there was a small decrease in the dark-adapted F_V/F_M ratio.

Severely and extremely Fe-deficient sugar beet, pear, and peach leaves had lower Φ_{PSII} (actual PSII efficiency at steady-state photosynthesis) values than those found in control leaves (Table 2). These decreases in Φ_{PSII} were caused by decreases in intrinsic

Table 2 Modulated chlorophyll fluorescence parameters of hydroponically grown sugar beet and field-grown pear and peach leaves affected by Fe deficiency

	F_V/F_M	Φ_{PSII}	$\Phi_{\text{exc.}}$	qP	NPQ
<i>Sugar beet</i>					
+Fe	0.78 ± 0.00	0.66 ± 0.01	0.74 ± 0.01	0.88 ± 0.01	0.18 ± 0.03
Severe -Fe	0.75 ± 0.00	0.34 ± 0.04	0.44 ± 0.03	0.77 ± 0.05	1.23 ± 0.20
Extreme -Fe	0.65 ± 0.03	0.17 ± 0.03	0.25 ± 0.04	0.75 ± 0.08	1.28 ± 0.20
<i>Pear</i>					
+Fe	0.76 ± 0.01	0.32 ± 0.02	0.47 ± 0.04	0.68 ± 0.06	1.35 ± 0.20
Severe -Fe	0.67 ± 0.02	0.18 ± 0.02	0.33 ± 0.02	0.56 ± 0.07	2.30 ± 0.07
Extreme -Fe	0.60 ± 0.04	0.09 ± 0.02	0.29 ± 0.05	0.33 ± 0.08	2.10 ± 0.30
<i>Peach</i>					
+Fe	0.76 ± 0.01	0.38 ± 0.02	0.46 ± 0.03	0.84 ± 0.07	1.32 ± 0.09
Severe -Fe	0.71 ± 0.01	0.16 ± 0.02	0.30 ± 0.03	0.57 ± 0.09	1.69 ± 0.05
Extreme -Fe	0.62 ± 0.01	0.10 ± 0.02	0.20 ± 0.03	0.55 ± 0.11	1.40 ± 0.08

+Fe, Severe -Fe and Extreme -Fe represent control, severely and extremely Fe-deficient leaves, respectively. Measurements are means \pm SE of between 6 and 8 replications

PSII efficiency ($\Phi_{exc.}$) and by decreases in the proportion of open, oxidized PSII reaction centers, estimated by qP (Table 2). NPQ increased in response to Fe deficiency in the three species investigated, excepting extreme Fe deficiency in peach (Table 2).

Effects of Fe deficiency on photosynthetic pigment composition

Changes induced by Fe deficiency in the photosynthetic pigment composition of sugar beet and field-grown pear leaves were similar to those reported in previous works (Morales et al. 1990, 1994). In the three species investigated, Fe deficiency decreased total Chl and carotenoids concentration, increased the Chl *a*/Chl *b* ratio, increased the molar ratio of V + A + Z pigments and lutein to Chl, and caused moderate (sugar beet and pear) or no changes (peach) in the neoxanthin to Chl molar ratio (Table 3).

Relationships between Rubisco carboxylation efficiency, light absorption, PSII photochemistry and pigment data assessed by principal component analysis

The two axes of the PCA explained approximately 85% (horizontal, axis I) and 12% (vertical, axis II) of the variance, respectively (Fig. 5). The axis I was negatively related to lutein and V + A + Z to Chl molar ratios, but positively related to the rest of variables. Control sugar beet leaves showed the highest scores along the axis I, whereas pear leaves affected by severe Fe deficiency showed the lowest scores along the axis I.

Data showed that Rubisco carboxylation efficiency (ϵ , estimated from the initial slope of the A/C_i curves; Table 1) was highly and positively correlated with α and significantly correlated with Φ_{PSII} (the correlation is positive when the angle between the arrows is narrow and negative when it is $> 90^\circ$) (Fig. 5). Both qP and $\Phi_{exc.}$, which together determine Φ_{PSII} (Genty et al. 1989), were linearly correlated with ϵ (Fig. 5).

The Fe deficiency-mediated increases in V + A + Z and lutein pigments to Chl ratio were associated to decreases in $\Phi_{exc.}$ in the three species investigated. This can be clearly seen in Fig. 5, where the angle between the arrows corresponding to pigment data and $\Phi_{exc.}$ is very large (close to 180°). Also, the angle between V + A + Z to Chl ratio and qP is nearly 180° (Fig. 5).

Discussion

This report provides evidence for the existence of high correlations between apparent Rubisco carboxylation efficiencies, leaf absorptances and PSII efficiencies, using Fe-deficient leaves of three different species growing in very different conditions (sugar beet grown in controlled growth chambers, and pear and peach grown in the field). Data presented here suggest that all these changes brought about by Fe deficiency are regulated and well coordinated, and that the three plant species tested are able to down-regulate Rubisco carboxylation to match the capacity of the rest of the photosynthetic machinery (photosynthetic pigments, photosynthetic electron transport carriers, etc.) to absorb light and use it photochemically. In line with these results, the NADPH/NADP⁺ ratio at steady-state photosynthesis only increased slightly (by 14%)

Table 3 Photosynthetic pigment composition of hydroponically grown sugar beet and field-grown pear and peach leaves affected by Fe deficiency

	Neo/Chl	Lut/Chl	VAZ/Chl	Σ Car	Chl <i>a</i> + <i>b</i>	Chl <i>a/b</i>
<i>Sugar beet</i>						
+Fe	28.4 ± 1.72	132.5 ± 3.4	53.2 ± 3.2	126.3 ± 4.5	388.5 ± 9.8	3.65 ± 0.1
Severe -Fe	28.6 ± 1.9	186.8 ± 0.9	89.8 ± 6.8	34.0 ± 2.0	79.0 ± 3.8	4.60 ± 0.27
Extreme -Fe	34.7 ± 3.3	242.2 ± 18.1	199.7 ± 15.5	19.2 ± 12.2	32.0 ± 3.2	5.72 ± 0.16
<i>Pear</i>						
+Fe	32.7 ± 1.1	137.8 ± 4.1	101.1 ± 8.5	95.2 ± 6.4	247.5 ± 14.5	3.50 ± 0.07
Severe -Fe	26.1 ± 1.3	184.7 ± 19.4	392.4 ± 26.9	60.1 ± 4.1	82.5 ± 5.8	5.70 ± 0.14
Extreme -Fe	42.8 ± 3.6	368.3 ± 24.6	580.0 ± 40.1	26.1 ± 1.8	23.7 ± 1.9	5.92 ± 0.3
<i>Peach</i>						
+Fe	39.7 ± 1.9	165.0 ± 3.4	169.5 ± 20.4	102.7 ± 9.3	199.4 ± 20.6	3.72 ± 0.18
Severe -Fe	39.1 ± 1.04	196.9 ± 1.2	307.0 ± 38.9	49.8 ± 2.9	70.3 ± 7.9	4.81 ± 0.29
Extreme -Fe	37.0 ± 3.1	231.5 ± 22.2	368.0 ± 31.2	28.6 ± 2.4	36.8 ± 2.4	4.92 ± 0.19

The total Chl and carotenoid values are given in $\mu\text{mol m}^{-2}$ and the neoxanthin, lutein and V + A + Z to Chl ratios in $\text{mmol pigment mol Chl}^{-1}$. +Fe, Severe -Fe and Extreme -Fe represent control, severely and extremely Fe-deficient leaves, respectively. Measurements are means ± SE of 6 replications

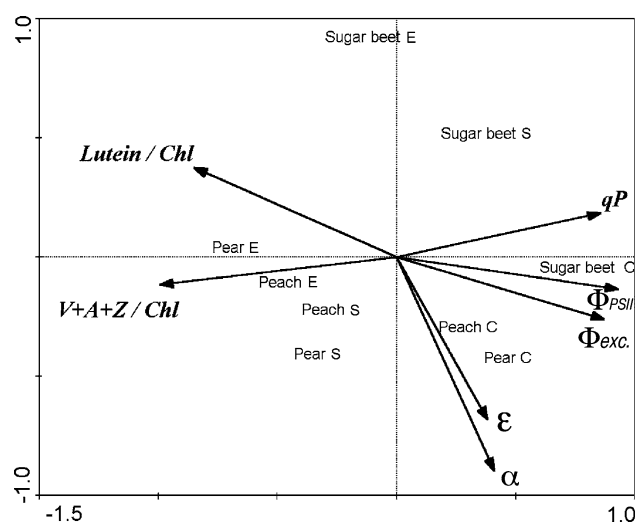


Fig. 5 Ordination diagram of the principal components analysis (PCA) based on the first two axes. Variables are indicated by arrows and italic letters. The sample scores correspond to the mean values for control (C), severely (S), and extremely (E) Fe-deficient leaves from hydroponically grown sugar beet, and field-grown pear and peach

in Fe-deficient leaves when compared to the controls (Belkhdja et al. 1998a), suggesting that there is not an excessive production of reducing power relative to its consumption in the reactions of the Calvin cycle. The question of how Fe deficiency down-regulates light absorption, photosystem II and Rubisco carboxylation efficiencies deserves further investigation. In that respect, it has been reported that Rubisco carboxylation efficiency is dependent on light (Vadell et al. 1993) and that the light-dependent Rubisco activation requires the presence of a Rubisco activase (Zhang et al. 2002). Our PCA data also point out to a close relationship between light absorbance and Rubisco carboxylation efficiency.

In a previous work, Winder and Nishio (1995) reported that Fe deficiency in sugar beet led to photosynthetic changes related to leaf Chl content. For instance, the amount of Rubisco protein was reduced by 60% in severely Fe-deficient leaves. This reduction was directly correlated to Chl content, as it was the rate of fully activated CO_2 fixation by Rubisco even in severely Fe-deficient leaves. The amount of both subunits of Rubisco (LSU and SSU) was linearly correlated to Chl content (Winder and Nishio 1995). Also, LSU and SSU mRNAs were linearly correlated to Chl content in Fe-deficient leaves (Winder and Nishio 1995). From these data, Winder and Nishio (1995) concluded that Fe deficiency in sugar beet down-regulated Rubisco gene expression.

Previous studies addressing the causes for a decreased photosynthetic rate in Fe-deficient leaves

obtained rather fragmentary information (Spiller and Terry 1980; Terry 1980, 1983; Davis et al. 1986; Miller et al. 1995; Pérez et al. 1995). Some of them were focused on the reduction in photosynthetic pigment concentrations that could limit photosynthetic rates by decreasing light absorption (Terry 1980; Morales et al. 1990, 1991; Masoni et al. 1996; Abadía et al. 1999). Also, it has been reported that the decrease in photosynthesis caused by Fe deficiency is not only associated with the decrease of photosynthetic units per unit leaf area (Spiller and Terry 1980) but also with a diminished efficiency at steady-state photosynthesis of the remaining PSII units (Morales et al. 1998a, 2000; this work). Thus, decreases in PSII efficiency should be considered as an important factor affecting photosynthetic rates under Fe deficiency.

Low photosynthetic rates could also be associated with an inefficient Rubisco activity, and the initial slope of net CO_2 assimilation rate versus CO_2 sub-stomatal partial pressure curve is a sensitive indicator of the photosynthetic capacity and is highly correlated to Rubisco activity (Evans and Seeman 1984). This is a good experimental approach to demonstrate *in situ*, in attached leaves, the extent of carboxylation efficiency modulation (Woodrow and Berry 1988). Our data from sugar beet, pear and peach indicate that Fe deficiency caused marked reductions in Rubisco carboxylation efficiency. Previous data showed that Rubisco activity was markedly reduced in Fe-deficient maize (Stocking 1975), and that Rubisco was 50 and 25% activated in control and severely Fe-deficient sugar beet leaves, respectively, at the same PPFD we used to grow and monitor photosynthesis (Taylor and Terry 1986). This is likely not caused by deactivation of Rubisco by low CO_2 concentrations, since Rubisco activation only falls markedly when C_i drops below 100 ppm (Badger 1985; Sage et al. 1990).

Our data show that Fe-deficient leaves were less water-efficient, and also that they have high C_i values. More water was transpired per unit C gained in response to Fe deficiency in sugar beet, peach, and pear, due to the fact that Fe deficiency causes larger decreases in photosynthetic rates than in transpiration rates, thus resulting in lower water use efficiency. On the other hand, C_i remained high or even increased in response to Fe deficiency, suggesting that photosynthesis of Fe-deficient leaves is limited by non-stomatal factors. If photosynthetic rates were decreased by a limited CO_2 access to the leaf, it would be possible to overcome it increasing the ambient CO_2 concentration (C_a). We therefore evaluated photosynthetic rates versus CO_2 sub-stomatal concentration (C_i) curves, and data found ruled out that a reduced stomatal

opening could be the main cause for the lower rates of photosynthesis under Fe deficiency, since increases in C_a (and therefore C_i) led to photosynthetic rates still low as compared to control values. Another way to distinguish between stomatal and non-stomatal limitations is to evaluate changes in photochemistry through the analysis of modulated Chl fluorescence quenching, because decreases in photosynthesis may have origin in an impaired PSII activity (monitored through changes in Chl fluorescence) with no changes in Rubisco CO_2 availability and/or Rubisco activity. These results pointed to a decreased Φ_{PSII} , due to both a decreased qP and $\Phi_{exc.}$, as one of the reasons for the low rates of photosynthesis in Fe-deficient leaves.

Our previous works with Fe-deficient plants have demonstrated a clear relationship between the down-regulation of intrinsic PSII efficiency and thermal energy dissipation by the xanthophylls cycle pigments (Abadía et al. 1999; Morales et al. 1998a, 2000) and/or lutein (Larbi et al. 2004). This has been reported for Fe-deficient and Fe-resupplied sugar beet grown in hydroponics (Morales et al. 1998a; Larbi et al. 2004) and Fe-deficient pear trees grown in the field (Abadía et al. 1999; Morales et al. 2000). In this work, strong negative correlations have been found between $V + A + Z$, lutein, and PSII-assessing parameters (Fig. 5). However, it should be taken into account that $V + A + Z$ and lutein are given on a Chl basis, and since the synthesis of Fe-containing cofactors of the electron transport chain and Chl biosynthesis are both directly impaired under Fe deficiency, it is likely that such negative relationships were partly due to the positive correlation between Chl concentration and PSII-assessing parameters. Even so, our previous works show the role of these xanthophylls in energy dissipation in Fe-deficient leaves, where strong linear correlations have been found between dissipation and the $A + Z/V + A + Z$ ratios (Morales et al. 1998a, 2000; Abadía et al. 1999; Larbi et al. 2004).

One possible role of xanthophylls under Fe deficiency may be as antioxidants against reactive oxygen species (ROS). At high PPF, the accumulation of excitation energy in the PSII antenna favors the production of triplet Chl that can interact with O_2 , generating reactive singlet oxygen (1O_2). Interestingly, the different ratios carotenoids/Chl increase markedly with Fe deficiency (Table 3), and these pigments can directly de-excite triplet Chl (Foyer et al. 1994). On the other hand, over-reduction of the photosynthetic electron carrier chain would also favor the direct reduction of O_2 by PSI, and the subsequent generation

of the ROS superoxide (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($\cdot OH$). Our PCA analysis showed an inverse relationship between qP (an estimation of the degree of oxidation/reduction of the PSII centers) and the $V + A + Z/Chl$ ratio (the angle between these variables is nearly 180° , Fig. 5). Possible photoprotective roles of zeaxanthin as an antioxidant (Havaux and Niyogi 1999) or as a ROS signaling modulating substance (Demmig-Adams and Adams 2002) have been reported. Thylakoids are very sensitive targets for photo-destruction by ROS, because of their unique lipid composition containing highly unsaturated (C18:3) fatty acids. Iron deficiency alters such lipid composition, decreasing the concentration of unsaturated (C18:3) and increasing those of saturated fatty acids (C18:0 and C16:0) (Abadía et al. 1988). This makes thylakoids from Fe-deficient plants less susceptible to be degraded by ROS. In fact, Iturbe-Ormaetxe et al. (1995) reported that oxidatively damaged lipids (and proteins) do not accumulate in Fe-deficient pea leaves. In severe Fe-deficient pea leaves, the concentration of catalytic Fe (leading to ROS) was virtually zero, and that of catalytic Cu did not change with Fe deficiency. Other antioxidant defenses (enzymes and small metabolites) seem to be well conserved in Fe-deficient plants (Iturbe-Ormaetxe et al. 1995). Furthermore, on a Chl basis, the concentration of these protecting enzymes increase largely in Fe-deficient sugar beet and pear leaves (Morales et al. 2006; Tobías and Abadía, unpublished data).

In a study with four tropical species, Thompson et al. (1992) suggested that plants are able to optimize the allocation of resources in order to preserve a balance between enzymatic (i.e., Rubisco) and light harvesting (i.e., Chl) capabilities across a wide range of light and nutrient regimes. In Fe-deficient leaves such a balance may form part of an adaptive mechanism to enable plants to survive periods of Fe stress at times when Fe supply from roots is not sufficient. On one hand, constant (or even higher) CO_2 sub-stomatal concentrations may aid to minimize photoinhibition during stomatal closure (Osmond and Björkman 1972; Osmond et al. 1980; Powles and Critchley 1980; Tenhunen et al. 1984). One possible cause is that respiration (and/or photorespiration) is less affected than photosynthesis by Fe deficiency (Morales et al. 1998a), which would contribute to maintain or even increase the sub-stomatal CO_2 concentrations. Alternatively, stomata might respond in order to maintain a constant ratio of leaf sub-stomatal CO_2 partial pressure to leaf surface partial pressure (Ball and Berry 1982). On the

other hand, down-regulation of intrinsic PSII efficiency ($\Phi_{exc.}$) under Fe deficiency seems to result from an enhanced, xanthophyll cycle- and/or lutein-related thermal energy dissipation (Morales et al. 1998a, 2000; Abadía et al. 1999; Larbi et al. 2004), which is considered to be an important protective mechanism of the photosynthetic apparatus from photodamage (Demmig-Adams 1990; Demmig-Adams and Adams 1992; Formaggio et al. 2001; Polivka et al. 2002). All these metabolic changes allow Fe-deficient leaves to survive in the field, exposed on clear days to PPFD as high as $2200 \mu\text{mol m}^{-2} \text{s}^{-1}$, for months without apparent major damage.

In summary, changes in light absorption, photochemistry and Rubisco carboxylation efficiency contributed to the observed low rates of photosynthesis in Fe deficiency-affected plants. This is a complex, regulated, and well-coordinated response of leaf metabolism to Fe deficiency.

Acknowledgments We thank Aurora Poc for her excellent technical assistance in growing the sugar beet plants, Dr. E. Gil-Pelegrín for use of equipment, Dr. J. Flexas for his advices in the analysis of the A/C_i response curves, and Dr. J.J. Camarero for his help with the PCA analysis. This work was supported by grants AGL 2003-01999 to A.A., AGL 2004-00194, and Isafruit from the Commission of European Communities to J.A. A.L. was recipient of a predoctoral fellowship from the Spanish Institute of International Cooperation (ICI-MAE).

References

- Abadía A, Ambard-Bretteville F, Remy R, Trémolières A (1988) Iron-deficiency in pea leaves: effect on lipid composition and synthesis. *Physiol Plant* 72:713–717
- Abadía J (1992) Leaf responses to Fe deficiency. A Review *J Plant Nutr* 15:1699–1713
- Abadía J, Abadía A (1993) Iron and plants pigments. In: Barton L, Hemming B (eds) *Iron chelation in plants and soil microorganisms*. Academic Press, San Diego, pp 327–344
- Abadía J, Morales F, Abadía A (1999) Photosystem II efficiency in low chlorophyll, iron-deficient leaves. *Plant Soil* 215: 183–192
- Andersen PC (1991) Leaf gas exchange of 11 species of fruit crops with reference to sun-tracking/non-sun-tracking responses. *Can J Plant Sci* 71:1183–1193
- Badger MR (1985) Photosynthetic oxygen exchange. *Annu Rev Plant Physiol* 36:27–53
- Ball JT, Berry JA (1982) The C_i/C_s ratio: a basis for predicting stomatal control of photosynthesis. *Carnegie Inst Washington Yearb* 81:88–92
- Belkhdja R, Morales F, Quílez R, López-Millán AF, Abadía A, Abadía J (1998a) Iron deficiency causes changes in chlorophyll fluorescence due to the reduction in the dark of the photosystem II acceptor side. *Photosynth Res* 25:173–185
- Belkhdja R, Morales F, Sanz M, Abadía A, Abadía J (1998b) Iron deficiency in peach trees: effects on leaf chlorophyll and nutrient concentrations in flowers and leaves. *Plant Soil* 203:257–268
- Chalmers DJ, Canterford RL, Jerie PH, Jones TR, Ugalde TD (1975) Photosynthesis in relation to growth and distribution of fruit in peach trees. *Aust J Plant Physiol* 2:635–645
- Crews CE, Williams SL, Vines HM (1975) Characteristics of photosynthesis in peach leaves. *Planta* 126:97–104
- Davis T, Jolley V, Walser R, Brown J, Blaylock A (1986) Net photosynthesis of Fe-efficient and Fe-inefficient soybean cultivars grown under varying iron levels. *J Plant Nutr* 9:671–681
- Demmig-Adams B (1990) Carotenoids and photoprotection: a role for the xanthophyll zeaxanthin. *Biochim Biophys Acta* 1020:1–24
- Demmig-Adams B, Adams III WW (1992) Photoprotection and other responses of plants to high light stress. *Annu Rev Plant Physiol Plant Mol Biol* 43:599–626
- Demmig-Adams B, Adams III WW (2002) Antioxidants in photosynthesis and human nutrition. *Science* 298:2149–2153
- Dubey RS (1997) Photosynthesis in plants under stressful conditions. In: Pessaraki M (ed) *Handbook of photosynthesis*. Marcel Dekker Inc, New York, pp 859–875
- Evans JR, Seeman JR (1984) Differences between wheat genotypes in specific activity of ribulose-1,5-bisphosphate carboxylase and the relationship to photosynthesis. *Plant Physiol* 74:759–765
- Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* 149:78–90
- Formaggio E, Cinque G, Bassi R (2001) Functional architecture of the major light-harvesting from higher plants. *J Mol Biol* 314:1157–1166
- Foyer CH, Lelandais M, Kunert KJ (1994) Photooxidative stress in plants. *Physiol Plant* 92:696–717
- Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim Biophys Acta* 990:87–92
- González-Vallejo EB, Morales F, Cistué L, Abadía A, Abadía J (2000) Iron deficiency decreases the Fe(III)-chelate reducing activity of leaf protoplasts. *Plant Physiol* 122:337–344
- Havaux M, Niyogi KK (1999) The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. *Proc Natl Acad Sci USA* 96:8762–8767
- Heras L (1960) Influence of light intensity on the redox potential in leaves in cases of iron-induced chlorosis. *Nature* 188: 335–336
- Hurley AK, Walser RH, Davis TD (1986a) Net photosynthesis and chlorophyll content in silver maple after trunk injection of ferrous sulfate. *J Plant Nutr* 9:683–693
- Hurley AK, Walser RH, Davis TD, Barney DL (1986b) Net photosynthesis, chlorophyll, and foliar iron in apple trees after injection with ferrous sulfate. *HortSci* 21:1029–1031
- Iturbe-Ormaetxe I, Morán JF, Arrese-Igor C, Gogorcena Y, Klucas RV, Becana M (1995) Activated oxygen and antioxidant defences in iron-deficient pea plants. *Plant Cell Environ* 18:421–429
- Larbi A (2003) *Clorosis férrica: Respuestas de las plantas y métodos de corrección*. PhD Thesis, University of Lleida, Spain
- Larbi A, Abadía A, Morales F, Abadía J (2004) Fe resupply to Fe-deficient sugar beet plants leads to rapid changes in the violaxanthin cycle and other photosynthetic characteristics without significant *de novo* chlorophyll synthesis. *Photosynth Res* 79:59–69
- Larbi A, Morales F, Abadía A, Abadía J (2003) Effects of branch solid Fe sulphate implants on xylem sap composition in field-grown peach and pear: changes in Fe, organic anions and pH. *J Plant Physiol* 160:1473–1481

- Larbi A, Morales F, López-Millán AF, Gogorcena Y, Abadía A, Moog PR, Abadía J (2001) Technical advance: reduction of Fe(III)-chelates by mesophyll leaf disks of sugar beet. Multi-component origin and effects of Fe deficiency. *Plant Cell Physiol* 42:94–105
- Le Roux X, Walcroft AS, Daudet FA, Sinoquet H, Chaves MM, Rodrigues A, Osorio L (2001) Photosynthetic light acclimation in peach leaves: importance of changes in mass:area ratio, nitrogen concentration, and leaf nitrogen partitioning. *Tree Physiol* 21:377–386
- Long SP, Bernacchi CJ (2003) Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *J Exp Bot* 54:2393–2401
- López-Millán AF, Morales F, Abadía A, Abadía J (2000) Effects of iron deficiency on the composition of the leaf apoplastic fluid and xylem sap in sugar beet. Implications for iron and carbon transport. *Plant Physiol* 124:873–884
- López-Millán AF, Morales F, Abadía A, Abadía J (2001a) Iron deficiency-associated changes in the composition of the leaf apoplastic fluid from field-grown pear (*Pyrus communis* L.) trees. *J Exp Bot* 52:1489–1498
- López-Millán AF, Morales F, Abadía A, Abadía J (2001b) Changes induced by Fe deficiency and Fe resupply in the organic acid metabolism of sugar beet (*Beta vulgaris*) leaves. *Physiol Plant* 112:31–38
- Masoni A, Ercoli L, Mariotti M (1996) Spectral properties of leaves deficient in iron, sulfur, magnesium and manganese. *Agron J* 88:937–943
- Miller GW, Jen Huang I, Welkie GW, Pushnik JC (1995) Function of iron in plants with special emphasis on chloroplasts and photosynthetic activity. In: Abadía J (ed) *Iron nutrition in soils and plants*. Kluwer Academic Publishers, Dordrecht, pp 19–28
- Morales F, Abadía A, Abadía J (1990) Characterization of the xanthophyll cycle and other photosynthetic pigment changes induced by iron deficiency in sugar beet (*Beta vulgaris* L.). *Plant Physiol* 94:607–613
- Morales F, Abadía A, Abadía J (1991) Chlorophyll fluorescence and photon yield of oxygen evolution in iron-deficient sugar beet (*Beta vulgaris* L.) leaves. *Plant Physiol* 97:886–893
- Morales F, Abadía A, Abadía J (1998a) Photosynthesis, quenching of chlorophyll fluorescence and thermal energy dissipation in iron-deficient sugar beet leaves. *Aust J Plant Physiol* 25:403–412
- Morales F, Abadía A, Abadía J (2006) Photoinhibition and photoprotection under nutrient deficiencies, drought and salinity. In: Demmig-Adams B, Adams III WW, Mattoo AK (eds) *Photoprotection, photoinhibition, gene regulation, and environment*. Springer, The Netherlands, pp 65–85
- Morales F, Abadía A, Belkhdja R, Abadía J (1994) Iron deficiency-induced changes in the photosynthetic pigment composition of field-grown pear (*Pyrus communis* L.) leaves. *Plant Cell Environ* 17:1153–1160
- Morales F, Belkhdja R, Abadía A, Abadía J (2000) Photosystem II efficiency and mechanisms of energy dissipation in iron-deficient, field-grown pear trees (*Pyrus communis* L.). *Photosynth Res* 63:9–21
- Morales F, Grasa R, Abadía A, Abadía J (1998b) Iron chlorosis paradox in fruit trees. *J Plant Nutr* 21:815–825
- Osmond CB, Björkman O (1972) Simultaneous measurements of oxygen effects on net photosynthesis and glycolate metabolism in C₃ and C₄ species of *Atriplex*. *Carnegie Inst Washington Yearb* 71:141–148
- Osmond CB, Björkman O, Anderson DJ (1980) Physiological processes in plant ecology. Toward a synthesis with *Atriplex*. (Ecological studies, vol. 36). Springer, Berlin Heidelberg New York
- Pérez C, Val J, Monge E (1995) Effects of iron deficiency on photosynthetic structures in peach (*Prunus persica* L. Batsch) leaves. In: Abadía J (ed) *Iron nutrition in soils and plants*. Kluwer Academic Publishers, Dordrecht, pp 183–189
- Pettigrew WT, Hesketh JD, Peters DB, Woolley JT (1990) A vapor pressure deficit effect on crop canopy photosynthesis. *Photosynth Res* 24:27–34
- Platt-Aloia KA, Thomson WW, Terry N (1983) Changes in plastid ultrastructure during iron nutrition-mediated chloroplast development. *Protoplasma* 114:85–92
- Polivka T, Zigmantas D, Sundström V, Formaggio E, Cinque G, Bassi R (2002) Carotenoid S1 state in a recombinant light-harvesting complex of photosystem II. *Biochemistry* 41: 439–450
- Powles SB, Critchley C (1980) Effect of light intensity during growth on photoinhibition of intact attached bean leaflets. *Plant Physiol* 65:1181–1187
- Römheld V (1997) The chlorosis paradox: Fe inactivation in leaves as a secondary event in Fe deficiency chlorosis. In: 9th International Symposium on Iron Nutrition and Interactions in Plants, Hohenheim, Stuttgart, Germany, p. 10 (Abstr.)
- Sage RF, Sharkey TD, Seemann JR (1990) Regulation of ribulose-1,5-bisphosphate carboxylase activity in response to light intensity and CO₂ in the C₃ annuals *Chenopodium album* L. and *Phaseolus vulgaris* L. *Plant Physiol* 94: 1735–1742
- Sanz M, Caverro J, Abadía J (1992) Iron chlorosis in the Ebro river basin, Spain. *J Plant Nutr* 15:1971–1981
- Spiller S, Terry N (1980) Limiting factors in photosynthesis. II. Iron stress diminishes photochemical capacity by reducing the number of photosynthetic units. *Plant Physiol* 65: 121–125
- Stocking CR (1975) Iron deficiency and the structure and physiology of maize chloroplasts. *Plant Physiol* 55:626–631
- Susín S, Abián J, Peleato ML, Sánchez-Baeza J, Abadía A, Gelpí E, Abadía J (1994) Flavin excretion from iron deficient sugar beet (*Beta vulgaris* L.). *Planta* 193:514–519
- Tagliavini M, Rombolà AD (2001) Iron deficiency and chlorosis in orchard and vineyard ecosystems. *Eur J Agron* 15:71–92
- Taylor SE, Terry N (1984) Limiting factors in photosynthesis. V. Photochemical energy supply colimits photosynthesis at low values of intracellular CO₂ concentration. *Plant Physiol* 75:82–86
- Taylor SE, Terry N (1986) Variation in photosynthetic electron transport capacity and its effect on the light modulation of ribulose bisphosphate carboxylase. *Photosynth Res* 8:249–256
- Taylor SE, Terry N, Huston RP (1982) Limiting factors in photosynthesis. III. Effects of iron nutrition on the activities of three regulatory enzymes of photosynthetic carbon metabolism. *Plant Physiol* 70:1541–1543
- Tenhunen JD, Lange OL, Braun M, Meyer A, Lösch R, Pereira JS (1980) Midday stomatal closure in *Arbutus unedo* leaves in a natural macchia and under simulated habitat conditions in an environmental chamber. *Oecologia* 47:365–367
- Tenhunen JD, Lange OL, Gebel J, Beyschlag W, Weber JA (1984) Changes in photosynthetic capacity, carboxylation efficiency, and CO₂ compensation point associated with stomatal closure and midday depression of net CO₂ exchange of leaves of *Quercus suber*. *Planta* 162:193–203
- ter Braak CJF (1994) Canonical community ordination. Part 1: Basic theory and linear methods. *Écoscience* 1:127–140
- ter Braak CJF, Šmilauer P (1998) *Canoco reference manual and user's guide to Canoco for Windows: Software for canonical*

- community ordination (version 4.5). Microcomputer Power, Ithaca
- Terry N (1980) Limiting factors in photosynthesis. I. Use of iron stress to control photochemical capacity in vivo. *Plant Physiol* 65:114–120
- Terry N (1983) Limiting factors in photosynthesis. I. Iron stress mediated changes in light harvesting and electron transport capacity and its effect on photosynthesis in vivo. *Plant Physiol* 71:855–869
- Terry N, Abadía J (1986) Function of iron in chloroplasts. *J Plant Nutr* 9:609–646
- Terry N, Low G (1982) Leaf chlorophyll content and its relation to the intercellular localization of iron. *J Plant Nutr* 5: 301–310
- Thomas D, Eamus D, Bell D (1999) Optimization theory of stomatal behaviour II. Stomatal responses of several tree species of north Australia to changes in light, soil and atmospheric water content and temperature. *J Exp Bot* 50:391–398
- Thompson WA, Huang LK, Kriedemann PE (1992) Photosynthetic response to light and nutrients in sun-tolerant and shade-tolerant rainforest trees. II. Leaf gas exchange and component processes of photosynthesis. *Aust J Plant Physiol* 19:19–42
- Vadell J, Sociás FX, Medrano H (1993) Light dependency of carboxylation efficiency and ribulose-1,5-bisphosphate carboxylase activation in *Trifolium subterraneum* L. leaves. *J Exp Bot* 44:1757–1762
- Winder T, Nishio J (1995) Early iron deficiency stress response in leaves of sugar beet. *Plant Physiol* 108:1487–1494
- Woodrow IE, Berry JA (1988) Enzymatic regulation of photosynthetic CO₂ fixation in C₃ plants. *Annu Rev Plant Physiol Plant Mol Biol* 39:533–594
- Zhang N, Kallis RP, Ewy RG, Portis AR (2002) Light modulation of Rubisco in *Arabidopsis* requires a capacity for redox regulation of the larger Rubisco activase isoform. *Proc Nat Acad Sci USA* 99:3330–3334