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Changes of donor and acceptor side in photosystem 2 complex induced by iron deficiency in attached soybean and maize leaves

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Abstract

Photosynthesis in iron-deficient soybean and maize leaves decreased drastically. The quantum yield of photosystem 2 (PS2) electron transport (Φ_{PS2}), the efficiency of excitation energy capture by open PS2 reaction centres (F_v'/F_m'), and photochemical quenching coefficient (q_P) under high irradiance were lowered significantly by iron deficiency, but non-photochemical quenching (NPQ) increased markedly. The analysis of the polyphasic rise of fluorescence transient showed that iron depletion induced a pronounced K step both in soybean and maize leaves. The maximal quantum yield of PS2 photochemistry (Φ_{po}) decreased only slightly, however, the efficiency with which a trapped exciton can move an electron into the electron transport chain further than Q_A (Ψ_0) and the quantum yield of electron transport beyond Q_A (Φ_{Eo}) in iron deficient leaves decreased more significantly compared with that in control. Thus not only the donor side but also the acceptor of PS2 was probably damaged in iron deficient soybean and maize leaves.

Additional key words: chlorophyll fluorescence kinetics; *Glycine max*; photosynthesis; *Zea mays.*

Introduction

Iron is an abundant component of the earth's crust, however, the deficiency of iron is one of the major abiotic problems limiting crop productivity in some area (Terry and Abadía 1986). Iron starvation leads in a series of processes to various morphological and physiological changes in plants due to the fundamental role of iron. Iron plays an important role in photosynthesis, and changes of photosynthetic apparatus induced by iron deficiency have been widely investigated. Iron deficient leaves have a reduced number of grana and stroma lamellae per chloroplast (Spiller and Terry 1980, Terry and Abadía 1986). This is accompanied by decreases in all membrane components, including electron carriers of the photosynthetic electron transport chain (Spiller and Terry 1980) and light-harvesting pigments (Terry and Abadía 1986, Morales *et al.* 1990, 1994). Also the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase shows a progressive decrease upon increase in iron deficiency (Bertamini *et al.* 2001).

Photosystem (PS) 1 and 2 complexes are both iron-containing proteins, the PS1 complex being particularly rich in iron due to its four Fe-S proteins. The content of iron in PS2 is lower than that in PS1. Sandmann and Malkin (1983) consider that PS1 in blue-green alga is mainly affected by iron depletion due to the decrease in the amount of Fe-S proteins A, B, and X. Iron in PS2 is important for water splitting (Hulsebosch *et al.* 1996), thus iron-deficiency may lead to decreased PS2 activity. Bertamini *et al.* (2001) proved that the significant decrease in photosynthetic electron transport is mainly due to the loss of PS2 activity in iron-deficient grapevine leaves, and the loss of PS2 activity is due to the loss of D1 protein and 33 kDa protein of the water-splitting complex. This opinion was concluded from *in vitro* experiments. In our study attached iron deficient soybean and maize leaves were used to explore how iron starvation affects PS2 by analysis of chlorophyll (Chl) *a* fluorescence transients and fluorescence quenching.

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Abbreviations: Chl – chlorophyll; P_N – net photosynthetic rate; RC – reaction centre.

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Materials and methods

Plants: The experiments were done in Tai-an City, Shandong Province, China, in July and August of 2001. Soybean (*Glycine max* cv. Ludou13) and maize (*Zea mays* cv. Luyu11) plants were grown in Hogland nutrient solutions with and without iron (as $FeSO_4$) in 1 000 cm^3 plastid bottles under natural irradiance. The nutrient solutions of control and iron-deficient treatments were replaced 3 times a week and adjusted to pH 5.0±0.5 with HCl. After 7 weeks serious symptoms of iron deficiency appeared. Then the youngest fully expanded leaves were used for measurements. Iron content of Hogland nutrient solutions with and without iron was measured by Atomic Absorption Spectroscopy (*WFD-Y*, Japan), and was 0.802 g m^{-3} in the control nutrient solution and undetected in iron-deficient nutrient solution, indicating iron content less than 0.010 g m^{-3} in iron-deficient nutrient solution.

$\boldsymbol{P_N}$ was measured at room temperature (25 °C) and 85 % relative humidity with a portable system (*CIRAS-1*, *PP Systems*, UK). P_N-PFD curves were measured in which photon flow density (PFD) was changed every 5 min in a sequence of 2 000, 1 600, 1 200, 800, 600, 400, 300, 200, 150, 100, and 0 μmol m^{-2} s^{-1}. PFD was controlled by the automatic control function of the *CIRAS-1* photosynthetic system.

Fluorescence quenching analyses under steady $\boldsymbol{P_N}$: *In vivo* Chl fluorescence was measured at room temperature (25 °C) using a Pulse-modulated Fluorimeter (*FMS2*, *Hansatech*, England) with attached soybean and maize leaves. The maximum PS2 quantum yield (F_v/F_m) was determined in dark-adapted (30 min) samples. After determining the initial Chl fluorescence yield (F_0) at low modulated measuring irradiance, a 0.7-s pulse of saturating "white light" (>3 000 μmol m^{-2} s^{-1}) was applied to obtain the maximum Chl fluorescence yield (F_m) and F_v/F_m (F_v, the variable Chl fluorescence yield, is defined as $F_m - F_0$). The leaf was then continuously irradiated with an actinic "white light" (1 000 μmol m^{-2} s^{-1}), provided by a *Fluorimeter* light source. When the steady state of fluorescence (F_s) was reached, a pulse of saturating "white light" was imposed to determine the maximum Chl fluorescence yield in the light-adapted state (F_m'). After the actinic radiation had been switched off, far-red radiation was applied for determination of the minimal level of fluorescence (F_0').

The actual PS2 efficiency (Φ_{PS2}) and efficiency of excitation captured by open PS2 centres (F_v'/F_m') were calculated as $(F_m' - F_s)/F_m'$ and $(F_m' - F_0')/F_m'$, respectively (Genty *et al.* 1989). Photochemical quenching coefficient (q_P) was calculated as $(F_m' - F_s)/(F_m' - F_0')$ (Genty *et al.* 1989). Non-photochemical quenching (NPQ) was calculated as $F_m/F_m' - 1$ (Bilger and Björkman 1990).

Polyphasic rise of fluorescence transients and the JIP test: Chl *a* fluorescence transients (OJIP) were measured at room temperature (about 25 °C) with a Plant Efficiency Analyzer (*PEA*, *Hansatech*, UK). All fluorescence transients were recorded during a 5-s pulse of red radiation [2 880 μmol(photon) m^{-2} s^{-1}] provided by an array of six light-emitting diodes. The fluorescence signals were recorded within a time scan from 10 μs to 1 s with a data acquisition rate of 10^5 readings per s for the first 2 ms, and 10^3 readings per s after 2 ms.

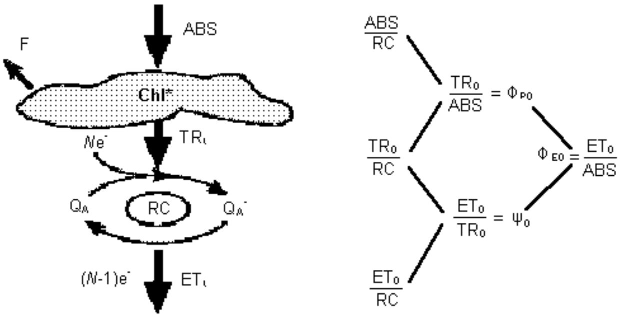

Fig. 1. Schematic energy flux model of PS2. ABS – photon absorption flux; ET_t, ET_0 – electron transport flux generated by reoxidation of Q_A^- to Q_A at times t and 0, respectively; ET_0/ABS – the quantum yield of electron transport beyond Q_A; ET_0/TR_0 – probability that a trapped exciton can move an electron into the electron transport further than Q_A^-; F – fluorescence emission; TR_t, TR_0 – energy flux trapped by PS2 reaction centres at time t and time 0; TR_0/ABS – maximum quantum efficiency to reduce Q_A to Q_A^-.

Based on the theory of energy fluxes in biomembranes of a photosynthetic apparatus and combined with data from measurements of the polyphasic rise of fluorescence transients, Strasser and Strasser (1995) developed the JIP test, from which formulae for calculation of the energy fluxes and flux ratios have been derived. According to the model of energy fluxes in this test (Fig. 1) photons absorbed by the antenna pigments are referred to as the absorption flux (ABS). Part of this excitation energy is dissipated as fluorescence, but most of it is transferred as the trapping flux (TR) to the reaction centres (RC). In the RCs, the exciton energy is converted to redox energy by reducing Q_A to Q_A^-, which is then reoxidised to Q_A, thus leading to an electron transport flux (ET) that maintains the metabolic reactions of the photosynthetic apparatus. Q_B-non-reducing, as well as the maximum pQ size were calculated as described by Cao and Govindjee (1990) and Strasser and Strasser (1995).

Each experiment was conducted at least three times independently.

Results

Changes of P_N in iron-deficient soybean and maize leaves: P_N in control leaves increased significantly by increasing PFD, however, in iron-deficient leaves it increased only little. Thus, the PFD-saturated P_N was drastically decreased by iron deficiency in soybean and maize leaves (Fig. 2). Also the saturating irradiance of iron-deficient leaves was markedly lowered.

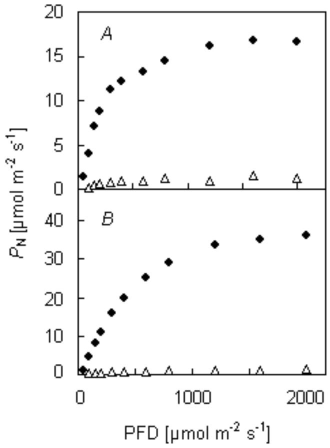

Fig. 2. Irradiance response curves of net photosynthetic rate (P_N) in control (●) and iron deficient soybean (*A*) and maize (*B*) leaves measured in air at room temperature (25 °C).

Changes of fluorescence transients: Polyphasic rise of fluorescence transient can give more information on PS2 photochemistry, such as the electron transport in both donor and acceptor sides of PS2 as well as efficiency of excitation utilisation per PS2 RC (Krüger *et al.* 1997, Srivastava *et al.* 1997). Fig. 3 shows the polyphasic rise of fluorescence transient in iron-sufficient and deficient leaves of soybean and maize plants. In iron deficient leaves, a more obvious characteristic of the polyphasic rise of fluorescence transient is the appearance of the K step. In addition, the maximal quantum yield of PS2 photochemistry (Φ_{po}), the efficiency with which a trapped exciton can move an electron into the electron transport chain further than Q_A^- (Ψ_0), and the quantum yield of electron transport beyond Q_A (Φ_{Eo}) decreased (Table 2). Obviously, iron deficiency had slight effect on Φ_{po} but induced a significant decrease in Ψ_0 and Φ_{Eo} both in soybean and maize leaves (Table 1). Meanwhile, PS2 reaction centres with Q_B-non-reducing in iron-deficient leaves increased distinctly compared with iron-sufficient leaves (Table 1).

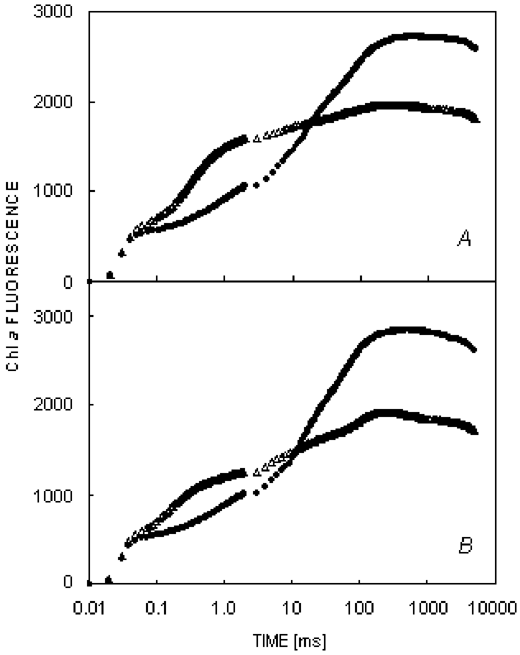

Fig. 3. The polyphasic chlorophyll fluorescence transients in iron sufficient (●) and deficient (Δ) soybean (*A*) and maize (*B*) leaves measured in air at room temperature (25 °C). Step K occurs at about 300 μs.

Changes of Chl fluorescence quenching under steady photosynthesis: In comparison with control leaves, iron-deficient leaves exhibited lower efficiency of excitation energy capture by open PS2 RCs (F_v'/F_m'), the quantum yield of PS2 electron transport (Φ_{PS2}), and in photochemical quenching coefficient (q_P), but higher values in non-photochemical quenching (NPQ) under the steady state of photosynthesis (Table 2). Hence iron starvation altered excitation energy distribution of photosynthetic apparatus owing to the role of iron in photosynthesis.

Discussion

Our results demonstrated that iron deficiency caused drastic lowering of photosynthesis both in soybean and maize leaves (Fig. 2). These values also strongly proved that drastic iron deficiency occurred in our plant materials.

In Fig. 3, a marked K step was observed in iron deficient soybean and maize plants. Phase K is caused by an inhibition of electron donor to the secondary electron donor of PS2, Y_Z, which is due to a damaged OEC. Phase K can, therefore, be used as a specific indicator of damage to the OEC (Guissé *et al.* 1995, Strasser and Strasser 1997). This result revealed that the donor side of PS2 might be damaged. Bertamini *et al.* (2001) found *in vitro* that iron deficiency induced changes on the donor side of PS2, and attributed this donor side damage to the diminished content of D1 and 33 kDa proteins of the water-splitting complex. Our experiments were a strong proof to this conclusion. But this might not be the only factor limiting the electron transport in PS2.

The slightly decreased Φ_{po} (F_v/F_m) caused by iron deficiency shows that the activity of PS2 RC in iron deficient leaves was damaged to certain extent, not very serious. However, the much more lowered Ψ_0 and Φ_{Eo} indicated that the activity of the electron transport beyond Q_A was inhibited in iron deficient soybean and maize leaves. Q_B-non-reducing is the state that lost the ability of electron transfer between Q_A^- and Q_B (Chylla and Whitmarsh 1989, Cao and Govindjee 1990). Obviously, it was the higher ratio of Q_B-non-reducing in iron deficient leaves that blocked the acceptor side of electron transport in PS2 complex.

Table 1. Changes of the experimental and phenomenological expressions of JIP-test and their values calculated from Fig. 3. Means of at least three measurements.

Parameter	Soybean		Maize	
	+Fe	–Fe	+Fe	–Fe
K	813±16 (100)	1092±12 (134)	964±13 (100)	1188±17 (123)
Φ_{po}	0.82±0.01 (100)	0.74± 0.02 (90)	0.83±0.01 (100)	0.72±0.01(87)
Ψ_o	0.71±0.01 (100)	0.25± 0.01 (35)	0.54±0.06 (100)	0.24±0.01 (44)
Φ_{Eo}	0.58±0.01 (100)	0.19±0.01 (33)	0.44± 0.04 (100)	0.18± 0.01 (41)
Non-Q_B	0.30±0.02 (100)	0.75±0.05 (250)	0.36± 0.04 (100)	0.76± 0.04 (210)

Table 2. Changes in the quantum yield of PS2, the efficiency of excitation energy capture by open PS2 reaction centres (F_v'/F_m'), photochemical quenching (q_P), and non-photochemical quenching (NPQ) in soybean and maize leaves grown under full and iron deficient Hogland nutrient solutions. Means of at least three measurements.

Plant	Treatment	F_v'/F_m'	Φ_{PS2}	q_P	NPQ
Soybean	+Fe	0.747±0.010 (100)	0.631±0.010 (100)	0.872±0.030 (100)	0.924±0.110 (100)
	–Fe	0.548±0.020 (73)	0.284±0.010 (45)	0.549±0.030 (63)	2.673±0.130 (289)
Maize	+Fe	0.716±0.010 (100)	0.619±0.010 (100)	0.863±0.010 (100)	0.694±0.150 (100)
	–Fe	0.531±0.010 (74)	0.288±0.010 (47)	0.524±0.010 (61)	2.462±0.110 (354)

q_P is a very important parameter under high irradiance, it reflects the balance between reduction of Q_A, which closes PS2 RCs, and oxidation of Q_A by the electron transport chain, which reopens the centres. This balance responds not only to excited energy capture, but also to factors influencing electron flow away from PS2 (Genty *et al.* 1989). Under high irradiance, the decreased q_P in iron deficient leaves implied that the oxidation of Q_A by further electron transport was suppressed. This result is consistent with that concluded from fluorescence transients. In addition, F_v'/F_m' in iron deficient leaves was also lowered significantly, therefore the Φ_{PS2} was decreased.

Higher values of NPQ indicated more excessive excitation energy was produced in iron deficient leaves than in iron sufficient leaves (Table 1). Low F_v'/F_m' in iron deficient leaves indicated that much more excessive excitation energy was dissipated in light-harvesting complexes (Table 2), which might correlate with the xanthophyll cycle (Morales *et al.* 1998, Jiang *et al.* 2001). The high NPQ and low Φ_{PS2} as well as q_P caused by iron deficiency under strong irradiance show that excitation energy distribution in iron deficient leaves was apparently altered.

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