BRIEF COMMUNICATION

Iron deficiency induced changes on electron transport rate in *Pisum sativum* chloroplasts

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Abstract

Iron deficiency induced decrease in the rate of whole electron transport chain in chloroplasts of pea (*Pisum sativum* L.). Such reduction was mainly due to the loss of photosystem (PS) 2 activity. The same result was obtained when the ratio of variable to maximum chlorophyll fluorescence (F_v/F_m) was evaluated. The loss in PS 2 activity was primarily due to a loss of 33, 23 and 17 kDa polypeptides. In contrast, iron deficiency induced the synthesis of 28 and 29 kDa polypeptides.

Additional key words: fluorescence, light harvesting chlorophyll-protein complexes, pea, photosystem.

Iron deficiency is a common abiotic stress for many photosynthetic organisms on earth (Terry and Abadia 1986, Straus 1994) from crops in arid and semi-arid regions (Mortvedt 1991) to phytoplankton in the sea (Behrenfeld *et al.* 1996). Iron deficiency markedly inhibited photosynthesis (Nedunchezhian *et al.* 1997, Morales *et al.* 2000). Plants grown under iron deficiency showed visible symptoms on their youngest leaves, which became yellow (chlorotic) due to a decrease in chlorophyll (Chl) content and had lower net photosynthetic rate (Terry 1980, Misra and Srivastava 1994, Abadia *et al.* 2000). Lack of iron reduced the formation of thylakoid membranes in chloroplasts. The number of granal and stromal lamellae per chloroplast was reduced, *e.g.*, in sugar beet (Spiller and Terry 1980, Platt-Aloia *et al.* 1983), maize (Stocking 1975), and barley (Pushnik and Miller 1982). This reduction was accompanied by a reduction in the number of thylakoids per granum (Spiller and Terry 1980), decrease in the photosynthetic electron transport rate (Alcaraz *et al.* 1985) and in the content of light-harvesting pigments (Terry 1980, Morales *et al.* 1990).

Spiller and Terry (1980) conclude that iron defciency in sugar beet leaves diminishes photochemical capacity reducing the number of photosystem (PS) 2 units per unit of leaf area, having no effect on the efficiency of photosynthetic energy conversion (Terry 1980). However, the efficiency of photosynthetic energy conversion in a response to iron deficiency decreased in other experiments with in sugar beet (Morales *et al.* 1990, 1998) and in cyanobacteria (Riethman and Sherman 1988). The explanation for these apparently conflicting results is that iron deficiency affects the efficiency of photosynthetic energy conversion only below a Chl threshold value (Morales *et al.* 1998, 2000). In the presented paper we have investigated the effect of iron deficiency induced changes on the photosynthetic activities and chloroplast proteins pattern in pea leaves.

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Abbreviations: BQ - p-benzoquinone; Chl - chlorophyll; cyt - cytochrome; DCPIP - 2,6-dicholrophenol indophenol; DPC - diphenyl carbazide; F_o - minimal fluorescence; F_m - maximum fluorescence; LHCP - light-harvesting chlorophyll protein; MV - methyl viologen; PPFD - photosynthetic photon flux density; PQ - plastoquinone; PS - photosystem; SDS-PAGE - sodium dodecylsulphate - polyacylamide gel electrophoresis.
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Pea (*Pisum sativum* L.) seeds were germinated and grown in vermiculite for two weeks. Seedlings were grown for 10 d in a 3/8 Hoagland nutrient solution with or without 22.4 mM Fe. Young, rapidly expanding leaves were harvested 10 d later. Plants were grown in a growth chamber under irradiance of 350 μmol(PAR) m^{-2} s^{-1}, 16-h photoperiod, temperature of 25 °C, and relative humidity of 80 %.

Chloroplasts were isolated from the 2nd and 3rd leaves from the top (chlorotic in the iron deficient plants) by grinding the leaves in a medium containing 330 mM sucrose, 10 mM $MgCl_2$, 5 mM NaCl and 20 mM Tris-HCl, pH 7.8. The slurry was filtered through eight-fold muslin and centrifuged at 7 000 *g* for 5 min. The pellet was washed once and resuspended in the same medium. Whole chain electron transport and partial reactions of photosynthetic electron transport mediated by PS 2 and PS 1 were estimated polarographically in isolated chloroplasts. PS 1 activity ($DCPIPH_2$ → MV) was measured as O_2 consumption whereas the whole chain (H_2O → MV) and PS 2 (H_2O → BQ) activities were measured as O_2 evolution (Nedunchezhian *et al.* 1997) at 27 °C using a Clark-type electrode (*Hansatech*, King's Lynn, UK) fitted with a circulating water jacket. Actinic light from a slide projector placed on the side of the electrode chamber was filtered through 9.5 cm of water. The irradiance was 1100 μmol(PAR) m^{-2} s^{-1} at the surface of water bath cell. The rate of DCPIP photoreduction was determined following the decrease in absorbance at 590 nm using a *Hitachi 557* (Tokyo, Japan) spectrophotometer (Nedunchezhian and Kulandaivelu 1991). As a exogenous electron donor for PS 2, 1 mM DPC was added.

Chl and protein contents were estimated according to Lichtenthaler (1987) and Lowry *et al.* (1951), respectively. SDS-PAGE was performed on gel system described by Laemmli (1970) using polyacrylamide gradient of 8 - 16 % gel. The gel bands were quantified by densitometry using *Bio-Image* apparatus (*Millipore*, Michigan, USA).

After 10 d of growth, leaves of control showed F_v/F_m ratio 0.830 will indicate high photochemical efficiency of PS 2. In contrast, iron deficient leaves showed the lower F_v/F_m ratio (Table 1). Similar results were also found in iron deficient pear and peach (Nedunchezhian *et al.* 1997, Morales *et al.* 2000). However, when photosynthetic electron transport was studied using isolated thylakoids from control and iron deficient leaves, photosynthetic electron transport from $DCPIPH_2$ to MV (PS1) was reduced by about 27 % in iron deficient leaves. The PS 2 mediated electron transport from H_2O to DCPIP and from H_2O to BQ was reduced by about 61 and 59 %, respectively (Table 2). A similar trend was also noticed for whole electron transport chain (H_2O → MV) activity (Table 2). PS 1 activity was less sensitive to iron deficiency than PS 2 activity as previously found Terry (1980), Sharma and Sanwal (1992), Foder *et al.* (1995), and Nedunchezhian *et al.* (1997).

Table 1. Changes in the parameters of chlorophyll fluorescence (F_0 - minimum fluorescence, F_v - variable fluorescence, and F_v/F_m - the ratio of variable to maximum fluorescence) in leaves from iron sufficient and iron deficient plants. F_0 was measured by switching on the modulated light 0.6 kHz; PPFD was less than 0.1 μmol m^{-2} s^{-1} at the leaf surface. F_m was measured at 20 kHz with a 1s pulse of 6000 μmol m^{-2} s^{-1} of white light.

	Fe sufficient	Fe deficient
F_0	2.00 ± 0.04	2.20 ± 0.05
F_v	10.00 ± 0.13	2.40 ± 0.06
F_v/F_m	0.83 ± 0.032	0.52 ± 0.005

Table 2. Changes in the whole electron transport chain, PS 1 and PS 2 activities in iron sufficient and iron deficient chloroplasts. Figures in parentheses are % of inhibition with reference to the iron sufficient plants. Mean ± SE, $n = 3$.

	Fe sufficient	Fe deficient
H_2O → MV [mmol(O_2) kg^{-1}(Chl) s^{-1}]	153.3 ± 8.1	54.4 ± 3.0 (65)
H_2O → BQ [mmol(O_2) kg^{-1}(Chl) s^{-1}]	174.3 ± 7.8	72.2 ± 6.2 (59)
H_2O → DCPIP [mmol(DCPIP) kg^{-1}(Chl) s^{-1}]	207.0 ± 8.2	81.1 ± 7.0 (61)
DCPIP → DPC [mmol(DCPIP) kg^{-1}(Chl) s^{-1}]	274.0 ± 7.3	191.9 ± 9.0 (30)
$DCPIPH_2$ → MV [mmol(O_2) kg^{-1}(Chl) s^{-1}]	316.0 ± 16.0	229.3 ± 9.9 (27)

DCPIP collects electron from PQ (Lien and Bannister 1971, Ouitrakul and Izawa 1973) but benzoquinone at the reducing side of PQ (Lien and Bannister 1971). As the PS 2 activity loss due to iron deficiency was similar in the systems H_2O → BQ and H_2O → DCPIP, the site of iron deficiency action must be prior to PQ in the electron transport. Diphenyl carbazide (DPC), as an artificial electron donor for PS 2 donates electrons close to the PS 2 reaction centre (Packham *et al.* 1982). Thus, the inhibition of PS 2 activity in iron deficient plants could be ascribed to an alteration of the water splitting complex, since the addition of DPC restored significantly its activity. Similar results was found by Nedunchezhian *et al.* (1997) in iron deficient peach leaves. According Sharma and Sanwal (1992) cyt *f* is the component that shows a maximum decrease at iron deficiency and thus the electron flow which through cyt *f* limits the rate of photosynthesis.

Supporting evidence for the damage of PS 2 activity was obtained from the chloroplasts polypeptide analysis:

a comparison of iron deficient chloroplasts with those of the control showed specific loss of 33, 26 - 24, 23, 17 and 10 kDa polypeptides (Table 3). The three extrinsic proteins of 33, 23 and 17 kDa associated with the lumenal surface of the thylakoid membranes are required for optimal functioning of the oxygen evolving machinery.

Table 3. Relative area [%] after integration of the densitometric scans of protein gels of pea chloroplasts isolated from iron sufficient and iron deficient plants. Each value is the mean of 3 measurements made in different gels; * - significant changes.

Protein [kDa]	Fe sufficient	Fe deficient
47	30.0 ± 0.9	30.4 ± 0.6
43	20.3 ± 0.4	20.2 ± 0.6
33	23.7 ± 1.4	10.6 ± 0.7*
29	14.0 ± 1.0	16.7 ± 1.5*
28	5.6 ± 0.5	8.6 ± 0.3*
26-24	89.0 ± 5.5	55.0 ± 4.5*
23	17.4 ± 0.8	10.4 ± 1.1
17	20.5 ± 1.5	14.2 ± 1.1*
10	30.0 ± 1.2	12.5 ± 0.6*

Loss of these polypeptides results in a simultaneous loss of oxygen evolution (Murata *et al.* 1984, Millner *et al.* 1987, Enami *et al.* 1994). Solubilization of the proteins is associated with partial or total inactivation of O_2 evolution. In particular removal of the 33 kDa protein from PS 2 membrane preparations by treatments with $CaCl_2$. NaCl treatment (Enami *et al.* 1994) results in strong inhibition of O_2 evolution and the loss is subsquently restored by reconstitution of the protein depleted membranes (Kuwabara *et al.* 1985). Our results indicate that the loss of 33, 23 and 17 kDa polypeptides could be the major reason for the loss of O_2 evolution induced in iron deficient plants. Also the 10 kDa polypeptide is a phosphoprotein located on the inner side of the thylakoid membrane with possible role in O_2 evolution (Ljungberg *et al.* 1984) and its loss (Table 3) may contribute to the loss O_2 evolution capacity in iron deficient plants. From the results we have confirmed that iron deficiency induced changes mainly located on the donor side of PS 2.

The reaction centre core 47 and 43 polypeptides were not affected by iron deficiency (Table 3). Similar results were found by Abadia *et al.* (1989) also in pea plants. A dramatic decrease of 26 - 24 kDa poly-peptides was noticed in iron deficient plants (Table 3). Iron deficiency also causes significant losses in LHCP complexes (Abadia *et al.* 1989, Foder *et al.* 1995, Nedunchezhain *et al.* 1997). In contrast, the relative contents of 29 and 28 kDa polypeptides were increased in iron deficient plants. These are Chl binding polypeptides that are intrinsic units of LHCP and probably protect the reaction centre from iron deficiency.

Our results show that chlorosis induced by iron deficiency is brought about by a complicated interaction of damage to and degradation of the photo-synthetic apparatus. Iron deficiency has induced a fast degradation of LHCP 2, which has become visible as yellowish pea leaves.

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