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Differential degradation of photosystem I subunits under iron deficiency in rice

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ABSTRACT

Rice (*Oryza sativa*) is one of the staple foods of the world. Iron (Fe) deficiency is a major abiotic stress factor that contributes world-wide to losses in crop yield and decline in nutritional quality. As cofactor for many enzymes and proteins, iron is an essential element. It plays a pivotal role in chlorophyll (Chl) biosynthesis, and iron deficiency may result in decreased Chl production and, thus, reduced photosynthetic capacity. Photosystem I (PSI) is a prime target of iron deficiency because of its high iron content (12 Fe per PS). To understand the protein level changes in the light-harvesting complex (LHC) of PSI (LHCI) under iron deficiency, rice seedlings were grown in Hoagland's nutrient medium with and without Fe. Chlorophyll content and photosynthetic efficiency decreased under iron deficiency. Protein gel blots probed with antibodies against the PSI core and Lhca 1–4 proteins revealed that the core subunits PsaA and PsaB remained stable under iron deficiency, whereas PsaC and PsaD decreased by about 50%, and PsaE was completely degraded. Among the LHCI subunits, Lhca1 and Lhca2 decreased by 40 and 50%, respectively, whereas Lhca3 and Lhca4 were completely degraded. We propose that the dissociation of LHCI subunits may be due to increased levels of reactive oxygen species, which is suggested by the increased activity of superoxide dismutase.

Introduction

Iron (Fe) is an essential element for almost all organisms. In humans, the occurrence of Fe-deficiency is estimated at about 30% of the world population (Lucca et al., 2002). As a transition metal, its ability to gain or lose an electron makes iron an important component of redox reactions taking place in proteins essential for photosynthesis, respiration and many other cellular functions, including DNA synthesis and hormone production. Although iron is abundant in soil, it is mainly present in the oxidized (Fe^{3+}) state, which is poorly soluble in neutral-to-alkaline soils and in water-logged conditions.

Fe-deficiency is a serious nutritional problem for virtually all forms of life. In higher plants, it causes a decrease in the abundance of photosynthetic proteins (Pushnik and Miller, 1982), reduction of electron transport chain components (Andaluz et al., 2006), decrease in photosystem (PS) I levels (Timperio et al., 2007), and decrease in the quantum yield of PSII (Samir, 2007; Msilini et al., 2011). Cyanobacteria respond to Fe deficiency *via* the degradation of light-harvesting phycobilisomes (Guikema and Sherman, 1984) and the expression of the "iron-stress-induced" operon *isiAB*. Fe deficiency results in decreased PSI antenna size in *Chlamydomonas reinhardtii* (Moseley et al., 2002). Roots of Fe-deficient plants show morphological and physiological changes. In dicots and non-grass monocots, Fe-deficiency is associated with inhibition of root elongation, increase in root tip diameter and abundant formation of root hairs (Schmidt et al., 2000). Young rice plants were shown to be highly susceptible to low Fe supply, being different from other cultivated grass species, such as oats, due to lower phytosiderophore production (Mori et al., 1991; Takahashi et al., 2001). Severe Fe deficiencies can lead to diminished productivity and even may lead to plant death, resulting in complete crop failure (Guerinot and Yi, 1994).

The photosynthetic machinery of higher plants contains two large membrane protein complexes, PSI and PSII, that harness incident light energy to initiate a series of electron transfer reactions across the thylakoid membrane in a coordinated manner. Each PSI core contains subunits designated PsaA to L or to N, respectively, in which PsaA and PsaB are highly conserved and forming the core of the PSI reaction centre. PsaD and PsaE are exposed to the stroma region of thylakoid membranes, where PsaD interacts strongly with Fd, and PsaE channels the electrons coming from PsaD to the rest

Abbreviations: Chl, chlorophyll; Cyt, cytochrome; HRP, horseradish peroxidase; LHC, light harvesting complex; PEA, plant efficiency analyzer; PI_{abs}, performance index on an absorption basis; PQ, plastoquinone; PS, photosystem; ROS, reactive oxygen species; SOD, superoxide dismutase.

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of the electron transport chain (Busch and Hippler, 2011; Yadavalli et al., 2011). Light harvesting complex (LHC) I subunits, consisting of Lhca1 to Lhca 4, form the light harvesting complex of PSI. Electron transfer within the PSI complex involves sequential reduction of Ao, A_1, and [4Fe–4S] iron–sulfur centers (F_X, F_A and F_B). Charge separation and stabilization through the secondary acceptor, A_1, and terminal iron-sulfur centers results in the reduction of ferredoxin, located at the stromal side of the membrane (Zaghdoudi et al., 2011).

PSI is a prime target for Fe deficiency because of its high Fe content (12 Fe per PSI). The ratio of PSI/PSII changes from 4:1 to 1:1 under Fe deficiency in cyanobacteria (Straus, 1994), and a diatom adapted to low ambient Fe has a constitutive PSII/PSI ratio of about 10:1 (Strzepek and Harrison, 2004). Further, Donnini et al. (2009) have studied the impact of iron deficiency on PSII in pear and quince and Msilini et al. (2011) in lettuce. However, there are no prior reports on complete characterization of PSI and LHCI subunits in higher plants under Fe deficiency.

In the present study we have chosen rice, which is often exposed to Fe deficiency because of its cultivation under waterlogged conditions, where Fe is not readily available for uptake by the plants. Here the rice seedlings were subjected to Fe-deficient conditions to explore the changes occurring in the PSI–LHCI complexes. The effect of Fe deficiency was studied by employing techniques such as chlorophyll fluorometry (Handy PEA), protein gel blot analysis and super oxide dismutase (SOD) activity assay.

Materials and methods

Growth of rice seedlings

Seeds of rice (*Oryza sativa* L.) cv. Raasi were obtained from the Directorate of Rice Research (DRR), Hyderabad, India. The seeds were sterilized in 10% H_2O_2 for 10 min and then in 70% ethanol for 5 min, followed by 5 thorough washings with sterile distilled water. The seeds were spread evenly in Petri dishes. Hoagland's Solution (Hoagland and Arnon, 1938) with 5 mg/L Fe (Fe-EDTA) (control) and without Fe (Fe-deficient) were separately prepared and kept at 4 °C. Equal volumes of the 2 solutions were poured onto the seeds, and the dishes covered. The seeds were kept in the dark until germination. Germinated seeds were placed in a growth chamber at 25 °C under a 16 h light and 8 h dark photoperiod with ~100 μmol of photons m^{-2} s^{-1} light supplied by fluorescent tubes. Media were regularly added to the seedlings, which were grown for a month under these conditions until analyzed.

Measurement of chlorophyll fluorescence with Handy PEA

Chlorophyll (Chl) fluorescence transients were measured with the Handy (Hansatech Instruments, UK) plant efficiency analyzer (PEA) at room temperature. The diodes provide red light with a peak wavelength of 650 nm and an intensity of 3000 μmol photons m^{-2} s^{-1}, which is readily absorbed by the chloroplasts. Second leaves of rice seedlings were dark-adapted for 10 min, after which fluorescence measurements were taken (Strasser et al., 1995). The fluorescence transient at 20 μs is designated as F_0, and the maximum fluorescence yield (F_m) occurs around 200 ms. The difference between F_0 and F_m is known as variable fluorescence (F_v).

Chlorophyll estimation

The Chl concentration of thylakoids was determined by adding 10 μL of thylakoid sample to 1 mL of 80% (v/v) acetone and absorption measured at 645 and 663 nm as described earlier (Arnon, 1949).

Protein extraction and SDS-PAGE

Protein was isolated from leaves of 30-d-old seedlings. About 200 mg leaves were homogenized in a pre-chilled mortar using 3 mL of sodium phosphate buffer (50 mM, pH 7.4) containing 1 mM EDTA, 1% polyvinylpyrrolidone and protease inhibitors (1 mM of amino caproic acid, benzamidine and phenyl methyl sulfonyl fluoride). This homogenate was centrifuged for 20 min at 18,000 × *g*. Protein was estimated by using the bicinchoninic acid method (Smith et al., 1985). Protein precipitation and solubilisation were carried out as described by Shine et al. (2011) with some modifications. Briefly, the soluble proteins were precipitated from 500 μL of buffered supernatant by adding equal volume of chilled 5% trichloroacetic acid and keeping at −20 °C for 2 h. The supernatant was discarded after centrifugation at 7800 × *g* for 10 min, and the pellet was dissolved by adding an equal volume of 2× sample buffer [62.5 mM Tris–HCl (pH 6.8), 10% glycerol, 2% sodium dodecyl sulfate (SDS), and 0.5% β-mercaptoethanol, containing traces of bromophenol blue], and heating for 5 min at 95 °C. Solubilized proteins (50 μg) were resolved on a 12% tricine-SDS-polyacrylamide gel.

Protein gel blotting

Protein gel blotting was carried out by electrophoretic transfer of proteins onto PVDF membranes. The membranes were probed with primary antibodies specific for PSI core subunits (PsaA, PsaB, PsaC, PsaD, and PsaE) and LHCI subunits (Lhca1, Lhca2, Lhca3, and Lhca4), which were purchased from Agrisera (Vännäs, Sweden). Secondary antibodies (Bio-Rad) conjugated with horseradish peroxidase (HRP) were diluted 1:3000 in TBST buffer. Chemiluminescence reagents specific for HRP were employed to develop the signal on the PVDF membrane. The images were recorded on a VersaDoc 5000 CCD camera (Bio-Rad).

Super oxide dismutase (SOD) activity

Fifty micrograms of leaf proteins were resuspended in 10 mM Tris–HCl (pH 7.5), 0.3 M sorbitol, 1.5 mM $CaCl_2$, and 10 mM $MgCl_2$. SOD activity was assayed according to Beauchamp and Fridovich (1971). SOD buffer and the protein sample were mixed in a cuvette and illuminated for 12 min using a comptalux bulb (Philips). Absorbance measured at 560 nm was used to calculate the SOD activity.

Results

Growth of seedlings under Fe-sufficient and Fe-deficient conditions

The seedlings under Fe-deficient conditions showed early symptoms of yellowing of the interveinal areas of the leaves, and the entire leaf turned yellow after one month. Stem and root lengths decreased under Fe deficiency by 20 and 50%, respectively, indicating that the roots were more sensitive than the stems. The decrease in root length also caused inefficient absorption of nutrients under Fe deficient conditions (Fig. 1A). Furthermore, Chl content decreased by 42%, compared to the control, in 30-d-old seedlings that were grown in the Fe deficient medium (Fig. 1B). Later, the primary photochemistry (light reactions) was also impaired by the decreased Chl content, as was the light harvesting capacity.