Characteristics of photosynthetic apparatus in Mn-starved maize leaves

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Abstract

The effects of Mn-deficiency on CO_2 assimilation and excitation energy distribution were studied using Mn-starved maize leaves. Mn-deficiency caused about 70 % loss in the photon-saturated net photosynthetic rate (P_N) compared to control leaves. The loss of P_N was associated with a strong decrease in the activity of oxygen evolution complex (OEC) and the linear electron transport driven by photosystem 2 (PS2) in Mn-deficienct leaves. The photochemical quenching of PS2 (q_P) and the maximum efficiency of PS2 photochemistry (F_v/F_m) decreased significantly in Mn-starved leaves under high irradiance, implicating that serious photoinhibition took place. However, the 'high-energy' fluorescence quenching (q_E) decreased, which was associated with xanthophyll cycle. The results showed that the pool of de-epoxidation components of the xanthophyll cycle was lowered markedly owing to Mn deficiency. Linear electron transport driven by PS2 de-creased significantly and was approximately 70 % lower in Mn-deficient leaves than that in control, indicating less trans-thylakoid pH gradient was built in Mn deficient leaves. We suggest that the decrease of non-radiative dissipation depending on xanthophyll cycle in Mn-starved leaves is a result of the deficiency of trans-thylakoid pH gradient.

Additional key words: chlorophyll fluorescence; net photosynthetic rate; photoinhibition; violaxanthin; violaxanthin de-epoxidase; xanthophyll cycle; *Zea*; zeaxanthin.

Introduction

Mn is important for photosynthesis (Fales and Ohki 1982, Ohki 1985, Jajoo *et al.* 2001). Four Mn ions are included in oxygen evolution complex (OEC). Through high redox potential between Mn^{2+} and Mn^{3+} (1.5 mV), water splitting occurs, then electrons are transferred to Q_A and Q_B (Barber and Andersson 1992). When Mn is depleted from OEC, the activity of OEC would decline, and electron flow from OEC to $P680^+$ would be blocked (Blubaugh and Cheniae 1990, Blubaugh *et al.* 1991), resulting in decrease of photosynthesis, so that more excess irradiance will be produced.

Thermal dissipation of excess absorbed photons measured as non-radiative quenching of chlorophyll (Chl) fluorescence (NPQ) is of paramount importance in the protection of the photosynthetic apparatus against the deleterious effect of excess irradiance. *In vivo*, at least two components of NPQ can be resolved by analysing dark relaxation kinetics (Krause and Weis 1991). The rapidly relaxing component is related to the state of energisation of the thylakoid membrane, and often is referred to as q_E (Briantais *et al.* 1979). The second, slowly reversible component of NPQ (often termed as q_I) is found after exposure to prolonged high irradiance or a combination of irradiance and another environment stress factor (Gilmore and Björkman 1995, Johns and Miehe 1996). q_E has been correlated with trans-thylakoid pH gradient (Briantais *et al.* 1980) and with the formation of the xanthophyll pigments zeaxanthin (Z) and antheraxanthin (A) (Demmig-Adams 1990, Choudhury *et al.* 1994, Gilmore 1997, Goss *et al.* 1998). Low lumen pH activates the violaxanthin de-epoxidase (VDE), which catalyses the conversion of violaxanthin (V) to A and then to Z by progressive de-epoxidation. The inhibition of VDE by dithiothreitol has usually been presented as demonstrating the participation of Z in NPQ formation (Yamamoto *et al.* 1972, Bilger *et al.* 1989). A linear relationship between Z formation and NPQ has frequently been observed in higher plants (Bilger and Björkman 1990, Demmig-Adams 1990, Gilmore and Yamamoto 1991). Two hypotheses try to explain the mechanism of the xanthophyll

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Abbreviations: A, antheraxanthin; Chl, chlorophyll; F_v/F_m, the maximum efficiency of PS2 photochemistry in the dark-adapted state; LHC2, light-harvesting complex of PS2; NPQ, non-photochemical quenching; OEC, oxygen evolving complex; PFD, photon flux density; P_N, net photosynthetic rate; q_E, the rapidly relaxing component of non-photochemical quenching; q_P, photochemical quenching; V, violaxanthin; VDE, violaxanthin de-epoxidase; Z, zeaxanthin; φ_{PS2}, the quantum yield of PS2 electron transport.

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cycle: (*a*) A and Z can directly quench the singlet-excited ^{1}Chl and the triplet-excited ^{3}Chl, while V would act as a light-harvesting pigment (Chow 1994, Frank *et al.* 1994). (*b*) A and Z do not directly quench ^{1}Chl and ^{3}Chl molecules but they facilitate the aggregation of LHC2 and, as a result, the quenching *via* Chl dimerisation will be enhanced (Horton *et al.* 1991, Horton 1996, Ruban *et al.* 1997).

In order to protect Mn-starved leaves against photodamage, excess irradiance trapped by the light-harvesting complexes of PS2 (LHC2) should be dissipated effectively. Serious Mn deficiency may block the linear electron transport driven by PS2 because of the loss of OEC activity (Blubaugh and Cheniae 1990, Blubaugh *et al.* 1991), thus trans-thylakoid pH gradient built up mainly by linear electron transport would be lost without Mn ion. Unfortunately, energy dissipation depending on xanthophyll cycle has a close relation with trans-thylakoid pH gradient. Therefore, can the xanthophyll cycle be stimulated when Mn-starved leaves are exposed to high irradiance? If not, why?

Materials and methods

Plants: Maize (*Zea mays* L.) plants (cv. Luyu11) were grown in Hogland nutrient solution with or without Mn (Mn was introduced into the medium as $MnCl_2 \times 4\ H_2O$) under natural irradiance. The concentration of Mn in control Hogland nutrient solution was about 390 g m^{-3}, and it was undetected in Mn-deficient nutrient solution by Atomic Absorption Spectroscopy (*WFD-Y*, Japan), which indicated that the Mn content was less than 10 g m^{-3}. The nutrient solution of control and Mn-deficient treatments were replaced 3 times a week and adjusted to pH 5.0±0.5 with HCl. The plants were grown for 7 weeks outdoors in summer until serious Mn-deficient symptom appeared. The youngest fully expanded leaves were used for all measurements.

Net photosynthetic rate (P_N): P_N-PFD curve was measured at room temperature (25 °C) with a portable photosynthetic system (*CIRAS-1*, *PP Systems*, UK). PFD was changed every 10 min in a sequence of 2 000, 1 600, 1 200, 800, 600, 400, 300, 200, 150, and 100 μmol m^{-2} s^{-1}. Irradiance was controlled by the automatic control function of the *CIRAS-1* photosynthetic system.

Chl fluorescence was measured at room temperature with a portable fluorometer (*FMS2*, *Hansatech*, UK). Initial fluorescence (F_0) was recorded with leaves adapted in the dark for 15 min. A single saturation pulse of radiation was applied to obtain the maximum fluorescence (F_m). Steady state fluorescence (F_s) was determined under actinic irradiation. Saturating pulse irradiation was applied to obtain F_m' following each actinic irradiation. The actinic radiation was removed and the minimal fluorescence of the light-adapted state (F_0') was determined by a 3-s far-red irradiation.

The maximum efficiency of PS2 photochemistry in dark-adapted state (F_v/F_m), quantum yield of PS2 electron transport (φ_{PS2}), photochemical quenching of PS2 reaction centres (q_P), and non-photochemical quenching (NPQ) were calculated using both light and dark fluorescence parameters according to Demmig-Adams and Adams (1996). NPQ was resolved into q_E and q_I components by extrapolation in semi-logarithmic plots of the maximum fluorescence yield *versus* time as described in Johnson *et al.* (1993).

Photosynthetic pigments were determined according to Thayer and Björkman (1990) with some modifications. After dark adaptation for 12 h, leaves were irradiated with 1 200 μmol(photon) m^{-2} s^{-1} for 0, 0.5, 1, 2, or 3 h, respectively, then were quickly frozen in liquid nitrogen and extracted with 100 % acetone. Pigment separation was performed in an HPLC system (*Waters*, USA) on a *Zorbax ODS-1* column protected by a guard column (*Lichrosorb RP18*). Concentrations of the pigments were estimated by using the conversion factors for peak area to nmol determined for this solvent mixture by Thayer and Björkman (1990).

Each experiment was performed at least three times independently.

Results

P_N: Mn-deficiency had a great influence on photosynthesis (Fig. 1). The PFD-saturated P_N values for control and Mn-starved maize leaves were 39 and 10 μmol m^{-2} s^{-1}, respectively, *i.e.* about 70 % reduction was induced by Mn-deficiency (Fig. 1).

The activity of PS2 and its quantum yield: F_v/F_m represents original activity of PS2 (Hulsebosch *et al.* 1996), because it reflects the maximum PS2 efficiency when the reaction centres are fully opened, *i.e.* when NPQ is absent. We found significant difference in F_v/F_m between control and Mn-starved leaves (Fig. 2*A*), 0.85±0.04 and 0.45±0.05, respectively. The almost 50 % decrease due to Mn-deficiency indicates that the activity of PS2 was significantly harmed. Because a more than 2-fold increase in F_0 resulted from Mn deficiency (120±35 and 310±30 in control and Mn starved leaves, respectively, Fig. 2*B*), we deduce that PS2 reaction centres were damaged owing to

Mn deficiency. The increase in F_0 indicates damage to PS2 reaction centres (Demmig *et al.* 1987). OEC is the primary site damaged by Mn deficiency (Blubaugh and Cheniae 1990, Blubaugh *et al.* 1991); this may further lead to loss of activity of PS2 reaction centres. Under a 3-h high irradiance (1 200 μmol m^{-2} s^{-1}), φ_{PS2} decreased significantly and was approximately 70 % lower in Mn-deficient leaves than that in control, indicating that linear electron transport driven by PS2 was harmed, too (Fig. 3).

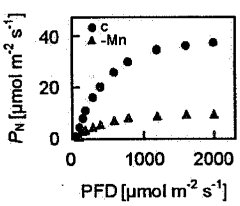

Fig. 1. Response of net photosynthetic rate (P_N) to irradiance in control (c) and Mn-starved maize (–Mn) leaves measured in air [340 μmol(CO_2) mol^{-1}] at room temperature (25 °C).

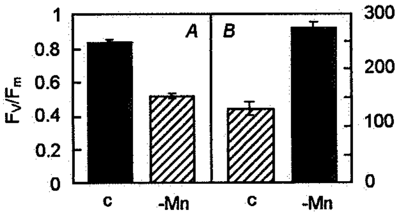

Fig. 2. Effect of Mn deficiency on F_v/F_m (*A*) and F_0 (*B*). Before measurement the leaves were dark-adapted for 24 h. c – control.

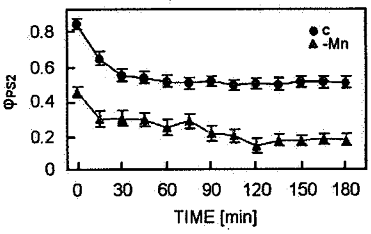

Fig. 3. Time course of quantum yield of PS2 electron transport (φ_{PS2}) in control (c) and Mn-starved (–Mn) maize leaves upon exposure to an irradiance of 1 200 μmol m^{-2} s^{-1} measured in air [340 μmol(CO_2) mol^{-1}] at room temperature (25 °C). Means ± SE of 3 replicates.

Photochemical quenching (q_P) and photoinhibition of PS2: After 2 h of high irradiance (1 200 μmol m^{-2} s^{-1}) treatment, q_P both in control and Mn-starved leaves were about 0.8 and 0.4, respectively (Fig. 4*A*). This indicated that the need of excitation energy for photochemical reaction was lowered by Mn-deficiency. When exposed to 1 200 μmol m^{-2} s^{-1}, F_v/F_m both in control and Mn-starved leaves decreased with prolonged time (Fig. 4*B*). However, the decrease was more significant in Mn-deficient leaves, and reached a value of about 0.2, which means a 65 % decrease after 3-h exposure to high PFD (Fig. 4*B*). In control leaves, however, the decrease was only about 20 % (Fig. 4*B*). Obviously, serious photoinhibition occurred in Mn-starved leaves.

Non-photochemical fluorescence quenching (NPQ): The dissipation of excess photons as heat was monitored by measuring NPQ, which can be resolved into q_E and q_I by extrapolation in semi-logarithmic plots of the maxi-

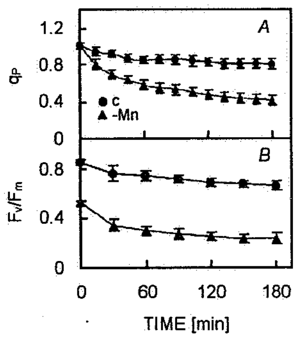

Fig. 4. Time course of changes in photochemical quenching, q_P (*A*) and photoinhibition, F_v/F_m (*B*) upon exposure to 1 200 μmol m^{-2} s^{-1} measured in air [340 μmol(CO_2) mol^{-1}] at room temperature (25 °C) in control (c) and Mn-starved (–Mn) maize leaves. Photoinhibition was determined as the change in photochemical efficiency of PS2 (F_v/F_m) measured after 15 min of dark adaptation. Means ± SE of 3 replicates.

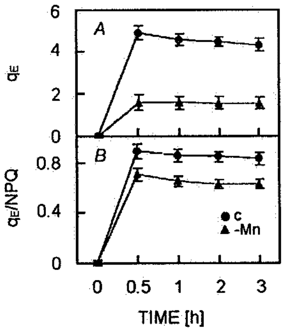

Fig. 5. Time course of the development of fast relaxing component, q_E of non-photochemical quenching, NPQ (absolute value in *A* and relative value in *B*) in control (c) and Mn-starved (–Mn) maize leaves upon exposure to 1 200 μmol m^{-2} s^{-1} measured in air [340 μmol(CO_2) mol^{-1}] at room temperature (25 °C). Means ± SE of 3 replicates.

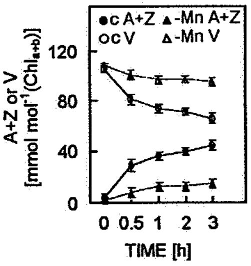

Fig. 6. Changes in antheraxanthin (A) + zeaxanthin (Z) and violaxanthin (V) contents [mmol mol^{-1}(Chl)] in control (c) and Mn-starved (–Mn) maize leaves upon exposure to 1 200 μmol m^{-2} s^{-1}. Means ± SE of 3 separate experiments.

mum fluorescence yield *versus* time (Johnson *et al.* 1993). To estimate the contribution of q_E to total NPQ, the dark relaxation kinetics of fluorescence were analysed both in control and Mn-starved leaves. Obviously, q_E was dramatically lowered at Mn-deficiency (Fig. 5*A*). Analysis of relative content of the component to total NPQ showed that q_E was 84 % in control leaves, but only 62 % in Mn-starved leaves (Fig. 5*B*). Thus Mn-deficiency caused a pronounced decrease in q_E.

Xanthophyll cycle pigments: The induction of q_E closely correlates with the formation of Z and A (Björkman 1987, Demmig-Adams 1990, Gilmore 1997, Goss *et al.* 1998). The pool of xanthophyll cycle pigments relative to Chl was hardly affected by Mn-deficiency (Fig. 6). The relative size of Z+A increased by up to about 43 mmol mol^{-1} within 3 h of high PFD exposure in control leaves; however, it was only 12 mmol mol^{-1} in Mn-starved leaves (Fig. 6). Significant difference in the relative size of Z+A between control and Mn-starved leaves revealed that the xanthophyll cycle was not fully triggered in Mn-deficienct leaves.

Discussion

In Mn-starved leaves, the photon-saturated P_N was dramatically lowered (Fig. 1), which indicated that the decrease in P_N was associated with a strong reduction in the activity of OEC of PS2 and in linear electron transport (Figs. 2 and 3). Obviously, it was Mn deficiency that lowered them (Blubaugh and Cheniae 1990, Blubaugh *et al.* 1991).

Mn deficiency resulted in lower q_P under high PFD (Fig. 4*A*) and thus we deduced that in Mn-starved leaves the photons were in excess. Thus, photoprotective mechanisms should be stimulated in order to protect Mn-starved leaves against photodamage. At excess irradiance, trans-thylakoid pH gradient plays a key role in the induction of energy dissipation, which is measured as q_E (Briantais *et al.* 1979, Briantais 1980, Terjung 1998). Linear electron transport driven by PS2 was depressed in Mn-starved leaves when exposed to 1 200 µmol m^{-2} s^{-1} PFD (Fig. 3), thus there would be less trans-thylakoid pH gradient built up, when the absolute or relative q_E were lowered (Fig. 5).

The q_E is correlated not only with trans-thylakoid pH gradient, but also with the formation of Z and A (Björkman 1987, Demmig-Adams 1990, Gilmore 1997, Goss *et al.* 1998). A positive relationship between Z formation and non-radiative dissipation has often been observed (Bilger and Björkman 1990, Demmig-Adams 1990, Gilmore and Yamamoto 1991). The pool of xanthophyll pigments relative to Chl in Mn-starved leaves was almost not changed, but the quantity of de-epoxidation components decreased because of Mn deficiency (Fig. 6). Thus, we consider that the lower content of A and Z may also be responsible for the lowered q_E in Mn-starved leaves. Violaxanthin de-epoxidase (VED), a key enzyme that catalyses V conversion into A and Z, is sensitive to trans-thylakoid pH gradient (Bratt *et al.* 1995, Rockholm and Yamamoto 1996). Therefore, we suggest that the deficiency in trans-thylakoid pH gradient was most probably responsible for the decrease of the xanthophyll cycle-dependent non-radiative dissipation in Mn-starved leaves.

Because the xanthophyll cycle was not fully stimulated in Mn-starved leaves, we presumed that large amount of excess photons was not successfully dissipated. Our experiments suggest that much more active oxygen was produced in Mn-starved leaves under high PFD, and PS2 reaction centres in Mn-starved leaves may be damaged by active oxygen, which resulted in serious photoinhibition (Fig. 4*B*).

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