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Full Length Article

Exogenous Malate Application Inhibits the Photochemical Activity of Photosystem II in Rice Leaves

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Abstract

Malate is a carrier that transfers CO_2 and NADPH from mesophyll cells (MC) to vascular bundle sheath cells (BSC) in C_4 plant photosynthesis. It likely plays a key role in regulating photosystem II (PSII) photochemical activity. We used the C_3 plant rice to study the effect of exogenous malate (200 μM) from roots on PSII activity. The malate treatment increased leaf malate content, NADPH/NADP ratio, and the initial fluorescence yield (F_0) and decreased photosynthetic oxygen evolution, the maximum fluorescence (F_m), the maximal efficiency of PS II photochemistry (F_v/F_m), and the number of active PSII reaction centers per excited cross section (RC/CS_m). Malate treatment also increased the initial fluorescence yield (F_0') and decreased the maximum fluorescence (F_m') and maximal efficiency of PS II photochemistry (F_v'/F_m') from light-adapted leaves. Based on these results, we concluded that higher concentrations of exogenous malate inhibit photosynthesis and PSII photochemical activity, perhaps in part because of the rise in NADPH/NADP ratio in chloroplasts in C_3 rice plants. © 2015 Friends Science Publishers

Keywords: Fluorescence parameter; C_3 plant; C_4 pathway; Photosynthesis; NADPH/NADP

Introduction

Plants that use C_4 carbon fixation possess elevated photosynthetic capacity, water-use efficiency, and nitrogen-use efficiency. Therefore, efforts are underway to incorporate these characteristics into C_3 plants. One approach is to transform the key genes for C_4 photosynthesis enzymes, such as PEP carboxylase (PEPC) (Hudspeth *et al*., 1992; Ku *et al*., 1999; Endo *et al*., 2008), NADP malic enzyme (NADP-ME) (Takeuchi *et al*., 2000; Tsuchida *et al*., 2001) and pyruvate orthophosphate dikinase (Fukayama *et al*., 2001; Wang *et al*., 2012) into C_3 plants. However, most such attempts have not achieved the expected goals (Matsuoka *et al*., 2001; Fukayama *et al*., 2006); some transgenic plants even exhibited lower photosynthetic rates (Tsuchida *et al*., 2001). Thus, the underlying mechanisms by which C_4 genes affect photosynthesis in C_3 plants must be investigated to further explore the possibility of modifying photosynthesis in C_3 plants.

C_3 and C_4 plants differ not only in their CO_2 fixation and reduction pathways, but also in leaf anatomical structure. In C_3 plants, CO_2 fixation and reduction via the Calvin cycle operates in the chloroplasts of mesophyll cells (MC), and the NADPH required for CO_2 reduction also comes from MC. In C_4 plants like maize, CO_2 is primarily fixed to form malate in MC and then transferred into bundle sheath cells (BSC) by the C_4 pathway to generate NADPH and release CO_2, which is reduced by Calvin cycle. Because BSC in maize lack PSII photochemical activity, the necessary NADPH for CO_2 reduction mainly comes from MC through malate transport (Taniguchi *et al*., 2004; Majeran *et al*., 2008). In the leaves of C_3 plants, CO_2 fixation and reduction are not compartmentalized as in C_4 plants, because BSC in C_3 plants do not photosynthesize. This difference in anatomy may be the main reason why some transgenic C_3 plants with C_4 key enzyme genes did not have enhanced photosynthesis. However, the detailed effects of C_4 gene expression on C_3 photosynthesis are not known.

Malate is the key intermediate product of the C_4 pathway; it not only carries CO_2 from MC to BSC, but also transfers NADPH generated in MC chloroplasts by the electron transport chain to BSC. Theoretically, an increase in malate will change the ratio of NADPH/NADP inside chloroplasts and affect the electron transport chain. In this experiment, we used C_3 rice plants to study the effect of exogenous malate on PSII photochemical activity.

Materials and Methods

Plant Materials, Growth Conditions and Treatments

The *Oryza sativa japonica* rice cultivar Kitaake was grown

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in sterilized quartz sands and watered with distilled water daily. When the second leaves were fully expanded, all seedlings were transplanted into a standard Espino nutrient solution (containing 10 mM KCl and 0.1 mM 2-(N-morpholino)-ethanesulfonic acid, pH 5.5). One day later, some seedlings were transferred into Espino solution with 200 μM malate. The nutrient solutions were replaced every day. All seedlings were grown in a growth chamber under 14 h photoperiod (500 μmol m^{-2} s^{-1} photon flux density (PFD)) and a day/night regime of 26/22°C. After 18 h of malate treatment, the middle sections of the second leaves were sampled for measurements. Design of the experiments was completely randomized with five replications.

Measurement of Photosynthetic Oxygen Evolution

The leaf samples were cut into 1 × 1 cm segments. The photosynthetic oxygen evolution rate was measured with a Clark-type oxygen electrode (Chlorolab 2, Hansatech, Norfolk, UK) after irradiation at 500 μmol m^{-2} s^{-1} PFD for 1 h at 25°C. The measurement was conducted in 0.4 mM $NaHCO_3$. The gross oxygen evolution rate is equal to the sum of net oxygen evolution and dark respiration.

Determination of Leaf Malate Content

To measure leaf malate content, 0.5 g fresh weight (FW) of leaf material was sampled, frozen immediately in liquid nitrogen, and stored at −80°C. Before malate analysis, extracts were prepared by grinding the frozen samples in 1.0 mL of 0.65 M HCl containing 0.1 mM EDTA, adding 2.5 mL distilled water, and then grinding the mixture again. The homogenate was heated at 80°C for 10 min with intermittent shaking, then centrifuged at 12 000 × *g* for 10 min. Supernatant (1.5 mL) was passed through a filter (0.45 μm) before HPLC analysis.

An Agilent 1100 HPLC system (Agilent, Santa Clara, CA, USA) equipped with a photodiode array detector (DAD) and a Spursil C18 column (5 μm, 4.6 mm × 250 mm, Dikma Technology, Lake Forest, CA, USA) was used. The mobile phase contained a 5% methanol and 95% phosphate buffer solution (containing 20 mM potassium dihydrogen phosphate adjusted to pH 2.5 with phosphoric acid). The flow rate was 0.6 mL min^{-1}, the detection wavelength was 215 nm, and the column temperature was 25°C.

Measurement of NADP and NADPH

The amounts of NADP and NADPH were determined by enzymatic cycling as described by Gerst *et al*. (1994). Leaf tissue (1 g FW) was ground in 6% $HClO_4$ in a liquid N_2-cooled mortar. The extracts were left on ice for 1 h and centrifuged at 10 000 × *g* for 10 min at 4°C. The supernatant was neutralized with 5 M K_2CO_3 and 0.25 M triethanolamine. The amount of NADP was determined in a reaction medium containing 33 mM Tris, 0.1 mM 2,6-dichlorophenolindophenol (DCPIP), 60 μM phenazine methosulfate, 2 mM EDTA, and 3.3 mM glucose-6-phosphate (G-6-P). The reaction was initiated by adding G-6-P dehydrogenase (2.5 units). The rate of DCPIP reduction was recorded by measuring the absorbance change at 625 nm using a Hitachi U-3010 spectrophotometer (Tokyo, Japan). The sum of NADP and NADPH was measured using the same procedure as for NADP except without $HClO_4$.

Chlorophyll Fluorescence in the Dark-adapted State

Intact leaves were subjected to dark for 15 min and then exposed to 3 000 μmol m^{-2} s^{-1} PFD generated by a Handy PEA portable fluorescence measurement system (Hansatech, UK) for 1 second (Strasser and Strasser, 1995). Using the JIP test (Strasser *et al*., 1995, 2004), we obtained the following parameters: (1) F_0, the initial fluorescence yield; (2) F_m, the maximum chlorophyll fluorescence; (3) F_v, the variable fluorescence; (4) F_v/F_m, the maximal efficiency of PS II photochemistry; (5) RC/CS_m, the number of active PSII reaction centers (RCs) per excited cross section (CS_m).

Chlorophyll Fluorescence in the Light-adapted State

Intact leaves were irradiated (500 μmol m^{-2} s^{-1} PFD) for 1 h and then excited by 2 200 μmol m^{-2} s^{-1} PFD for 1 s (Strasser *et al*., 2000). The initial fluorescence (F_0') and the maximum fluorescence (F_m') in the light-adapted state were obtained.

Data Analysis

Statistical analyses were performed using SPSS 11.5 (IBM, Chicago, IL, USA). Treatment means and variances were subjected to one-sample *t*-test analysis. These values and their significant differences are presented in the figures.

Results

Photosynthetic Oxygen Evolution

Oxygen evolution occurs in the O_2-evolving complex of PSII. As the primary electron donor, water is oxidized to release dioxygen and protons in the electron transport chain. The electron released from water is transported to NADP to produce NADPH. Thus, the rate of O_2 evolution can indicate the activity of photochemical reactions and electron transport in PSII. The O_2 evolution rate of malate-treated plants was 9.09% less than of the control (Fig. 1). These data suggested that malate inhibited the light reactions of photosynthesis.

Leaf Malate Content

The malate content in treated leaves was significantly higher by 17.29% compared with control plants (Fig. 2).

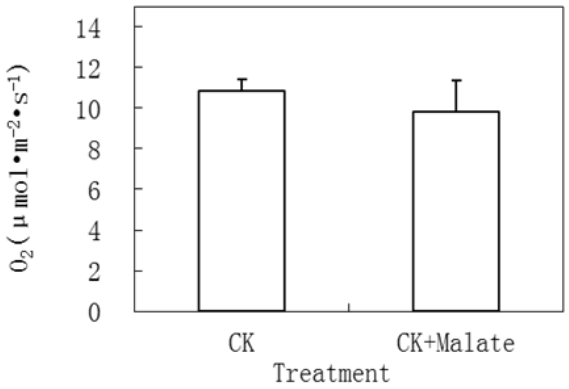

Fig. 1: Comparison of the O_2 evolution rates of photosystem II between malate-treated and control rice plants. The two treatments were not significantly different by the one-sample *t*-test. Values are means ± S.D. (n = 5)

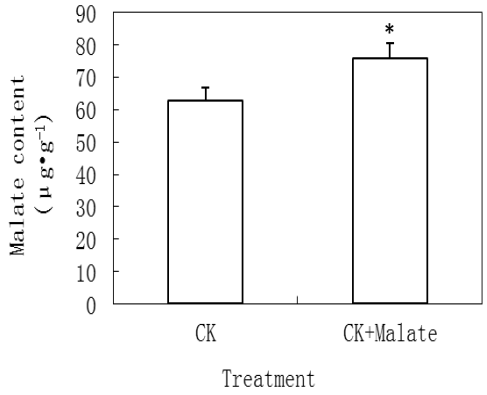

Fig. 2: Comparison of leaf malate contents between malate-treated and control rice plants. The asterisk (*) indicates a significant difference at $P < 0.05$ by the one-sample *t*-test. Values are means ± S.D. (n = 5)

This showed that malate in the nutrient solution could be absorbed by rice roots and transported to leaves. The rise of malate contents in treated leaves suggested that exogenous malate could affect leaf photosynthesis.

NADPH/NADP Ratio

In C_4 photosynthesis, malate is decarboxylated by NADP-ME to release CO_2 and produce NADPH in the BSC chloroplasts of the NADP-ME subtype of C_4 plants such as maize. Therefore, the malate content may affect the NADPH/NADP ratio in leaves. The NADPH/NADP ratio in malate-treated leaves was significantly higher by 84.62% compared with controls (Fig. 3). The results showed that exogenous malate enhanced malate catabolism and subsequently promoted the production of NADPH.

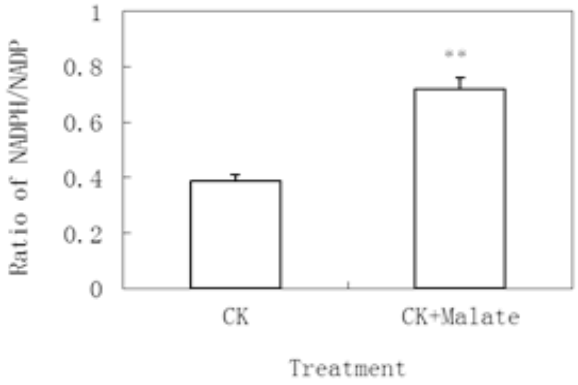

Fig. 3: Comparison of the leaf NADPH/NADP ratio between malate-treated and control rice plants. The double asterisks (**) indicate a significant difference at $P < 0.01$ by the one-sample *t*-test. Values are means ± S.D. (n = 5)

Chlorophyll Fluorescence Parameters

We observed significantly higher F_0 in dark-adapted leaves and F_0' in light-adapted leaves after malate treatment compared with control plants (Fig. 4A). F_0 is the amount of fluorescence released when all PSII RCs are completely open; it is affected by the RC/LHC II (light-harvesting complex II) ratio, the efficiency of excitation energy transfer from LHC II to PSII RC, and the activity of RC. F_0' is the F_0 of light-adapted leaves. The increases in F_0 and F_0' suggested that the exogenous malate treatment decreased the efficiency of energy transfer from harvesting pigments to PSII RC.

F_m is the amount of fluorescence released when all PSII RCs are entirely closed; it represents the maximal light absorption potential and is affected by the amount of harvesting pigments or the absorptivity of LHC II. F_m' is the F_m of light-adapted leaves. In this experiment, the data showed that the exogenous malate treatment decreased F_m and F_m' (Fig. 4B).

The ratio F_v/F_m shows the maximal efficiency of PSII photochemical reactions. F_v is the difference between F_m and F_0, which reflects the maximal potential of photochemical reactions. It is affected by the amount of RCs, photochemical reaction activity, and the efficiency of electron transfer. F_v'/F_m' is the F_v/F_m of light-adapted leaves. In this study, both F_v/F_m and F_v'/F_m' were reduced by malate treatment (Fig. 4C).

The ratio RC/CS_m is the amount of RCs per excited cross section (CS_m), which is the density of second plastoquinone acceptors (QA) reducing PSII RC per CS_m. RC/CS_m in malate-treated plants was less than in control plants, suggesting that exogenous malate could reduce the amount of PSII RC (Fig. 4D).

Discussion

Malate carries CO_2 from MC to BSC in the NADP-ME subtype of C_4 plants, which includes maize and sorghum.

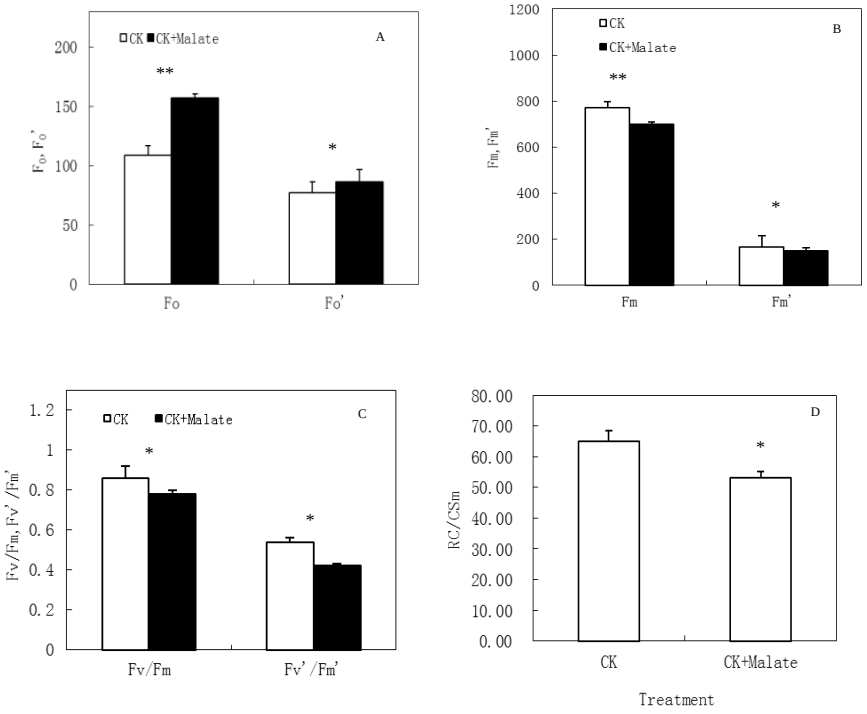

Fig. 4: Comparisons of basic fluorescence indices between malate-treated and control rice plants. (A) Initial fluorescence yield (F_0) and initial fluorescence in the light-adapted state (F_0'). (B) Maximum chlorophyll fluorescence (F_m) and maximum chlorophyll fluorescence in the light-adapted state (Fm′). (C) Maximum quantum yield of primary photochemistry (F_v/F_m) and maximum quantum yield of primary photochemistry in the light-adapted state (F_v'/F_m'). (D) Density of reaction centers per excited cross section (RC/CSm). Single (*) and double (**) asterisks indicate significant differences at $P < 0.05$ and $P < 0.01$ levels, respectively, by the one-sample *t*-test. Values are means ± S.D. (n = 5)

More malate synthesis enhances CO_2 reduction in BSC (Gietl, 1992). In contrast, in this study, exogenous malate treatment inhibited leaf photosynthetic activity (decreased O_2 evolution) in C_3 rice, although the treatment increased the leaf malate content. Hudspeth *et al.* (1992) transformed tobacco with maize C_4-PEPC cDNA and found that the PEPC activity and leaf malate content in transgenic plants were about twice as high as in wild-type plants. Moreover, some transgenic plants with a C_4 gene had lower photosynthetic rates, although the transgene was expressed (Tsuchida *et al*., 2001). These results showed that the accumulation of malate in MC of C_3 plants is not always beneficial to photosynthesis.

The decrease in photosynthesis in malate-treated rice plants was related to decreases in the efficiency of excitation energy transfer (F_0 rise; Fig. 4A), the maximal light absorption potential (F_m decline; Fig. 4B), the maximal efficiency of PSII photochemical reaction (F_v/F_m decline; Fig. 4C), and the amount of active PSII reaction centers per excited cross section (RC/CS_m decline; Fig. 4D). This effect of malate on PSII activity might be the consequence of the accumulation of malate and pyruvate produced by malate decarboxylation. It may also result from a change in the NADPH/NADP ratio, because a large increase in this ratio was observed in leaves of Mal-treated plants (Fig. 3). The overexpression of maize C_4 NADP-ME in rice was reported to severely compromise growth and cause photoinhibition and photodamage (Takeuchi *et al*., 2000; Tsuchida *et al*., 2001). Malate production appears to have different effects on photosynthesis in C_4 and C_3 plants.

In the NADP-ME subtype of C_4 plants, malate carries CO_2 from MC to BSC and transfers NADPH. NADPH is produced by the photosynthetic electron transport chain, which is inhibited when NADP is lacking inside chloroplasts. Hence, the NADPH/NADP ratio affects the electron transport chain (Takabayashi *et al*., 2005). The input of NADPH via malate decarboxylation in BSC does not inhibit photosynthesis, because it merely compensates for insufficient NADPH synthesis in BSC, which lack PSII activity (Kirchanscki, 1975). In C_3 plants, chloroplasts in MC have normal PSII activity, so the electron transport chain is inhibited by large amounts of malate entering the MC chloroplasts, leading to the generation of reactive oxygen species and photodamage to PSII complexes. This logic explains why the overexpression of NADP-ME and PEPC genes in some transgenic C_3 plants can decrease the photosynthesis rate. We also inferred that the deficiency of PSII activity in BSC of C_4 species may be related to the input of external malate during evolution.

Conclusion

In conclusion, higher concentrations of exogenous malate inhibited photosynthetic oxygen evolution and PSII photochemical activity in C_3 rice plants, which may have been partially caused by a rise in the NADPH/NADP ratio in chloroplasts.

Acknowledgements

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