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Different Effects of Malate on the Activities of Photosystem II in Detached Leaves of Maize and Tobacco

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Abstract

Malate is the first stable product after CO_2 is fixed in NADP-dependent malic enzyme (NADP-ME) type of C_4 plants, which transfers CO_2 and the reducing equivalent from mesophyll cell (MC) to vascular bundle sheath cell (BSC) chloroplasts and affects the redox state of BSC. The aim of this experiment is to investigate the effect of exogenous malate on the activity of photosystem II (PS II) in C_4 and C_3 plants. The leaf discs from the 5th fully expanded leaves of maize (NADP-ME type C_4 plants) and the 10th fully expanded leaves of tobacco (C_3 plants) were treated with malate of 50, 100 μM and the chlorophyll fluorescence parameters were measured. Malate treatments decreased the photochemical reaction efficiency (F_V/F_M) in maize leaves, as a result of rising in initial fluorescence (F_O) and decreasing in maximal fluorescence (F_M). The number of active PS II reaction center (RC) per excited cross section (RC/CS) declined in malate-treated maize, suggesting that malate inactivated PS II RC. Malate treatments also increased W_k, representing the severity of oxygen-evolving complex (OEC) damage, and decreased the rate of photosynthetic oxygen evolution. We conclude that exogenous malate regulates the activity and structure of PS II in C_4 plant maize. No significant changes in the activity of PS II were observed in malate-treated C_3 plant tobacco. It is suggested that the short term malate treatment will inhibit PS II of leaves which have C_4 anatomy and C_4 enzymes.

Keywords

Malate, Photosystem II, Chlorophyll Fluorescence, Maize, Tobacco

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1. Introduction

C_4 plants possess elevated photosynthetic ability, water use efficiency and nitrogen use efficiency compared with C_3 plants. They have better yield performance under high light intensity, high temperature and drought. Therefore, an effort is currently underway to incorporate these characteristics into C_3 plants by genetic engineering [1]-[7]. However, most such attempts have not achieved the expected goals [5] [8]. In typical C_4 plants, mesophyll cells (MC) and vascular bundle sheath cells (BSC) in the leaves are arranged to form the Kranz anatomy around vascular tissues. These two types of cells are different not only in structure but also in function. In MC chloroplasts, the activity of photosystem II (PS II) is normal, but the activity of ribulose bisphosphate carboxylase/oxygenase (Rubisco) is lower. On the other hand, in BSC chloroplasts, the activity of PS II is lower but the activity of Rubisco is higher [9]-[11]. For C_3 plants, leaves have not evolved the structure and function like C_4 plants. To this day, we know less about the regulation of the differentiation of chloroplast in structure and function in BSC and MC in C_4 plants. Several reports showed that the operation and efficiency of C_4 photosynthetic cycle were closely related to the stage of leaves. Maize is a C_4 plant of NADP-dependent malic enzyme (NADP-ME) type. Its leaves of from 1st to 3rd run the C_4 cycle with lower efficiency, and with lower activity of C_4 photosynthetic enzymes in MC and higher activity of PS II in BSC [12] [13]. The 4th leaf completes the differentiation of MC and BSC, with higher rate of C_4 photosynthesis. Tobacco is a C_3 plant which photosynthesizes in MC and not in BSC. Malate is the first stable product after CO_2 is fixed in maize, transferring CO_2 and the reducing equivalent from MC to BSC chloroplasts and affecting the redox state of BSC. The input of malate is paralleled with the changes in the structure and function of chloroplast in BSC. This suggests that the malate might contribute to the regulation in loss of grana and deficiency in PS II activity in BSC chloroplast of C_4 plants. The aim of this experiment is to investigate the effect of exogenous malate on the activity of PS II in C_4 plant maize and C_3 plant tobacco. The discs from 5th leaves of maize and 10th leaves of tobacco were treated with malate of 50 μM and 100 μM, and the chlorophyll fluorescence parameters were measured.

2. Materials and Methods

2.1. Plant Growth and Treatments

Maize hybrid Zhengdan 958 and tobacco K326 were grown in growth chamber, under a 14 h photoperiod (500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PFD) and a day/night regime of 26˚C/22˚C. The 5th fully expanded leaves of maize and the 10th fully expanded leaves of tobacco were used to sample. Six discs of 1cm diameter were taken from the middle section of leaves. The discs were infiltrated under vacuum for 60 min with malate and tartrate solution (0, 50, 100 μM), containing 10 mM KCl and 0.1 mM Mes/BTP, pH 5.5. Tartrate is a structural analog of malate.

2.2. Measurement of Chlorophyll Fluorescence

The discs were subjected to dark for 15 min and then exposed to 3000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PFD generated by Handy-PEA (Hansatech, UK) for 1 s [14]. According to the JIP-test [15] [16], the following parameters were obtained: 1) F_O: the initial fluorescence yield; 2) F_M: the maximum fluorescence; 3) F_V: the variable fluorescence; 4) F_V/F_M: maximal efficiency of PS II photochemistry; 5) RC/CS: the number of active PS II reaction center (RC) per excited cross section (CS); 6) ABS/RC: absorption flux per RC; 7) TR_O/RC: trapped energy flux per RC; 8) ET_O/RC: the electron transfer efficiency of active PS II reaction center; 9) W_K: the normalized relative variable fluorescence at the K step, $W_K = (F_K - F_O)/(F_J - F_O)$.

2.3. Measurement of Photosynthetic Oxygen Evolution

The discs were subjected to light of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 30 min at 25˚C before measurement. Photosynthetic oxygen evolution rate was measured with a Clark-type oxygen electrode (Chlorolab 2, Hansatech) at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 25˚C. The measurement was conducted in 0.4 mM $NaHCO_3$. Gross oxygen evolution is equal to the sum of net oxygen evolution and dark respiration.

3. Results

3.1. Changes in F_O, F_M, F_V and F_V/F_M in Maize and Tobacoo

F_O is the amount of fluorescence release when all PS II RCs are open, affected by ratio of active PS II reaction

center (RC)/light-harvesting complex II (LHC II) and the efficiency of excitation energy transfer to PS II RC. The efficiency of excitation energy flow to RC depends on the structure of LHCII and the connection between LHCII and RC. F_M is the amount of fluorescence release when all PS II RCs are closed, representing the maximal light absorption potential. This parameter is related to the total amount of light harvesting pigments. In malate treatment, tobacco has no significant difference in F_O and F_M, but maize has a significant increase in F_O and mild decline in F_M (**Table 1**). The decline in F_M can be explained by the decrease in light-harvesting pigment caused by malate treatment. The effect of malate on F_O is possibly caused by the change in the ratio RC/LHC II, the structure of LHC II and the connection between LHC II and RC. In fact, in this experiment, RC and F_M were reduced in malate solution (**Figure 1** and **Table 1**). Therefore, the increase in F_O in this study is mainly attributed to the change in structure of light-harvesting complex and the connection between LHC II and RC.

F_V is the difference between F_M and F_O, reflecting the photochemical reaction potential and affected by the amount of RC, the activity of photochemical reaction and the efficiency of electron transfer. In tobacco, F_V has no significant difference under 50 μM malate and mild decline under 100 μM malate. In maize, there was a significant decrease in F_V (**Table 1**). The decline of F_V is not only caused by the rise in F_O, but also by the drop in F_M.

F_V/F_M shows the maximal efficiency of PS II photochemistry. Under malate treatment, tobacco has a slight variety in F_V/F_M. But maize has a remarkable decrease under 100 μM malate (**Table 1**), which is mainly attributed to the reduction in F_V. The reduction in F_V/F_M mainly resulted from the increase in F_O. This means that the increase in Mal metabolism reduced PS II efficiency in maize leaves.

Comparing the same basic flourescence indices between tobacco and maize, besides F_V/F_M, the other indices of tobacco are all lower than maize. The high level of F_V/F_M in tobacco probably due to its low level of F_M.

Under tartrate treatment, these four basic flourescence indices didn't show significant difference in both tobacco and maize discs.

3.2. Changes in RC/CS and ET_O/CS in Maize and Tobacoo

RC/CS is the amount of QA-reducing PS II RC per excited cross section (CS), that is, the number of active PS II RC. In tobacco, RC/CS didn't show significant difference under malate treatment. Malate treated maize discs showed a remarkable decrease in RC/CS (**Figure 1(a)**), which suggested that exogenous malate has an effect of inactivating PS II RC. Because the activity of RC is affected by the structure of PS II complex, malate treatment must have changed the structure of PS II RC. Besides 100 μM malate treatment, RC/CS in tobacco were all lower than in maize.

ET_O/CS describes the electron transport flux of RC per excited cross section (CS), affected by RC/CS, the ability of RC to reduce Q_B and the efficiency of electron transfer chain. In this study, no remarkable difference were observed in tobacoo discs. Malate treatment significantly decreased ET_O/CS in maize discs (**Figure 1(b)**), which might be resulted from the decline in RC/CS (**Figure 1(a)**) and the Q_B-reducing ability of RC (**Figure 2(c)**). ET_O/CS in tobacco were all lower than in maize.

Under tartrate treatment, RC/CS and ET_O/CS didn't show significant difference in both tobacco and maize discs.

3.3. Changes in ABS/RC, TR_0/RC, ET_0/RC and W_K in Maize and Tobacoo

ABS/RC is the maximum light absorption per RC, reflecting the ratio of light harvesting pigment to RC. In tobacco, ABC/RC didn't show significant change under malate treatment (**Figure 2(a)**). In maize, the increase in ABS/RC in malate solution (**Figure 2(a)**) suggested that Mal treatment altered the ratio of ABS/RC. Because the F_M was decreased by Mal (**Table 1**), the rise in ABS/RC ratio must be attributed to the decline in RC amount. ABS/RC in tobacco were all higher than in maize.

TR_O/RC describes the trapped excitation energy per RC, representing the potential of excitation energy attained per RC. In tobacco, TR_O/RC didn't show significant change under malate treatment (**Figure 2(b)**). In maize, the increase in TR_O/RC in malate solution (**Figure 2(b)**) is attributed to the decline in RC amount. This means that, compared with the control, each RC can attain more supply of excitation energy in malate treatment leaves. TR_O/RC in tobacco was all higher than in maize.

ET_O/RC is the amount of excitation energy used per RC in photosynthetic electron transfer, affected by ability of RC to reduce Q_B and the efficiency of electron transfer chain. In this study, ET_O/RC describes the electron

Table 1. Changes in basic flourescence indices F_O, F_V, F_M and F_V/F_M in tobacco and maize discs treated with malate and tartrate (50, 100 μM).

Plant material	Flourescence indices	CK 0 μM	Malate		Tartrate	
			50 μM	100 μM	50 μM	100 μM
Tobacco	F_O	116.5 ± 5.61	119.5 ± 3.08	116.5 ± 3.83	117.3 ± 4.80	114.1 ± 4.41
	F_M	707.5 ± 30.09	719.2 ± 37.77	695.8 ± 28.15	696.8 ± 41.93	690.5 ± 49.75
	F_V	591.0 ± 26.33	599.7 ± 37.44	579.3 ± 25.48	579.5 ± 53.45	576.4 ± 38.74
	F_V/F_M	0.835 ± 0.006	0.833 ± 0.010	0.833 ± 0.005	0.832 ± 0.045	0.835 ± 0.023
Maize	F_O	246 ± 11.12	257 ± 28.55*	278 ± 10.52*	251 ± 14.58	250 ± 19.37
	F_M	1139 ± 77.60	1022 ± 76.30	950 ± 78.48	1108 ± 73.14	1167 ± 68.25
	F_V	893 ± 69.34	765 ± 58.71**	672 ± 60.68**	857 ± 64.97	917 ± 71.74
	F_V/F_M	0.784 ± 0.023	0.749 ± 0.223	0.706 ± 0.020**	0.774 ± 0.025	0.785 ± 0.028

Single asterisks (*) and double asterisks (**) indicate the significance of difference at $P < 0.05$ and $P < 0.01$ levels, respectively, by F test when compared with that in control plants. Values are means ± S.D. (n = 5).

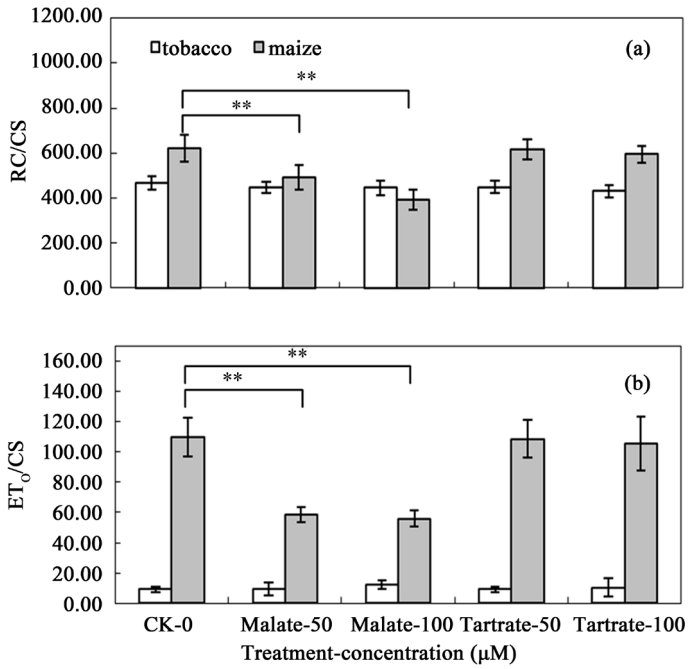

Figure 1. Changes in RC/CS and ET_o/CS in tobacco and maize discs treated with malate and tartrate (50, 100 μM). (a) RC/CS is the amount of QA-reducing PS II RC per excited cross section (CS); (b) ET_o/CS describes the electron transport flux of RC per excited cross section (CS). Single asterisks (*) and double asterisks (**) indicate the significance of difference at $P < 0.05$ and $P < 0.01$ levels, respectively, by F test when compared with that in control plants. Values are means ± S.D. (n = 5).

transport flux per RC. In tobacco, it didn't show significant change under malate treatment (**Figure 2(c)**). In maize, malate treatment significantly decreased ET_O/RC (**Figure 2(c)**), which showed that the Q_B-reducing ability of RC was inhibited and the flow of electron transfer was blocked. If chloroplasts in treatment leaves acquired CO_2 from malate as carbon source to operate Calvin Cycle, also accepted one molecule of NADPH. This means that Calvin Cycle only needs one NADP generated by linear electron transfer chain to reduce CO_2. Consequently, linear electron transfer was blocked. ET_O/RC in tobacco was all lower than in maize.

W_K is the variable fluorescence at K point of JIT curve, equal to the ratio of variable fluorescence F_V to the

amplitude F_J-F_O, representing the severity of oxygen-evolving complex (OEC) damage [17]. W_K didn't show significant change under malate treatment in tobacco but it increased in malate-treated maize leaf discs (**Figure 3**). The results showed that malate promoted the change in structure of OEC in maize leaf discs. This will reduce the activity of PS II, even inactivate the PS II. The significant rise in F_O can be partially explained by the increasing W_K. Besides 100 μM malate treatment, W_K in tobacco were all higher than in maize.

Under tartrate treatment, ABS/RC, TR_O/RC, ET_O/RC and W_K didn't show significant difference in both tobacco and maize discs.

3.4. Oxygen Evolution in Maize and Tobacoo

The O_2 evolution reaction is conducted in the O_2-evolving complex of PS II in chloroplasts. As the primary electron donor, water is oxidized and releases dioxygen and protons during linear photosynthetic electron transfer. The electron released from water is transported to NADP to produce NADPH. Thus, O_2 evolution can indicate the activity of PS II OEC and the electron transport activity of PS II. **Figure 4** shows the O_2 evolution of malate-treated discs, compared with the controls, the O_2 evolution didn't show remarkable change in malate-treated tobacco leaf discs. But it declined by 23.8% and 28.3% treated with 50 and 100 μM malate in maize leaf discs. These declines are consistent with the rise in W_K and decrease in RC/CS and ET_O/CS. O_2 evolution in tobacco was all lower than in maize.

Under tartrate treatment, O_2 evolution didn't show significant difference in both tobacco and maize discs.

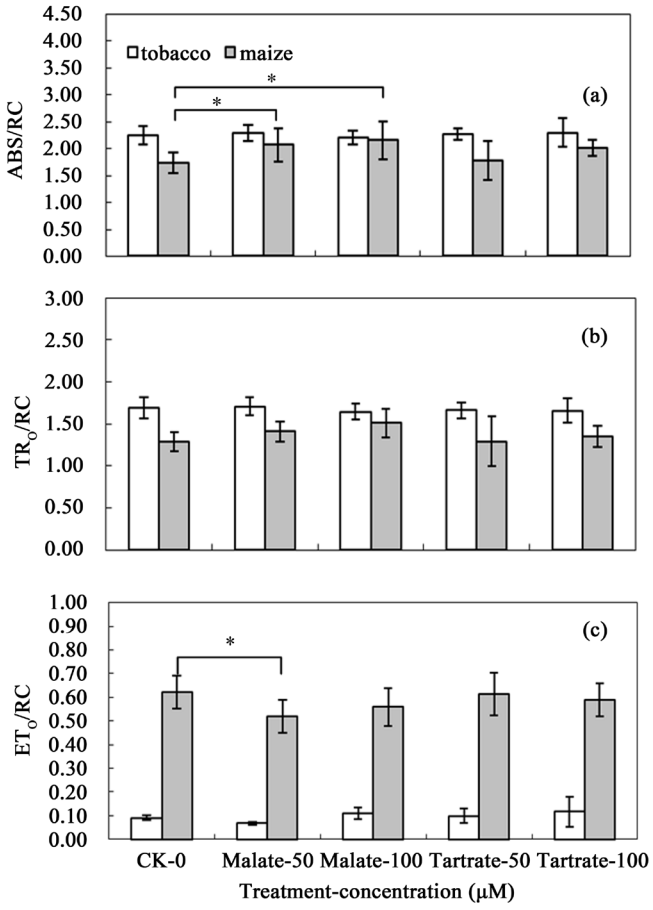

Figure 2. Changes in ABS/RC, TR_o/RC and ET_o/RC in tobacco and maize discs treated with malate and tartrate (50, 100 μM). (a) ABS/RC is the absorption flux per active PS II reaction center (RC); (b) TR_o/RC the trapped energy flux per RC; (c) ET_o/RC the electron transfer efficiency per RC. Single asterisks (*) and double asterisks (**) indicate the significance of difference at $P < 0.05$ and $P < 0.01$ levels, respectively, by F test when compared with that in control plants. Values are means ± S.D. (n = 5).

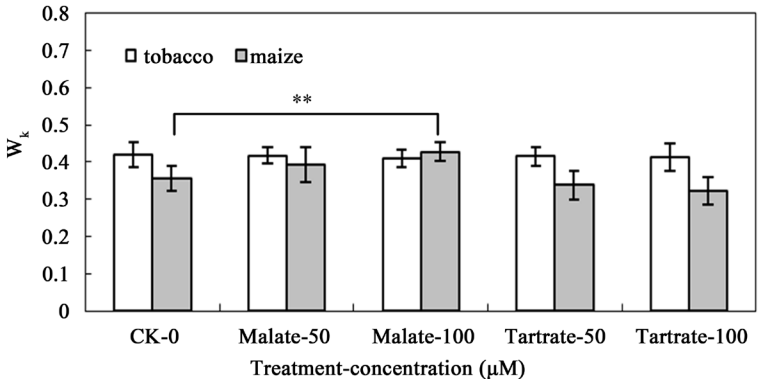

Figure 3. Changes in the normalized relative variable fluorescence at the K step (W_k) in tobacco and maize discs treated with malate and tartrate (50, 100 μM). Single asterisks (*) and double asterisks (**) indicate the significance of difference at $P < 0.05$ and $P < 0.01$ levels, respectively, by F test when compared with that in control plants. Values are means ± S.D. (n = 5).

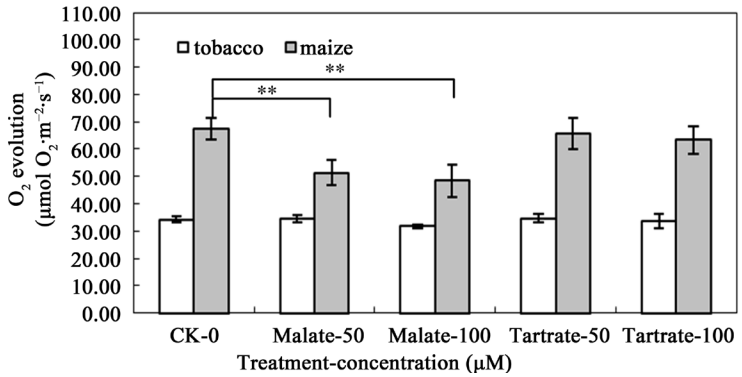

Figure 4. Changes in O_2 evolution of PS II in tobacco and maize discs treated with malate and tartrate (0, 50, 100 μM). Single asterisks (*) and double asterisks (**) indicate the significance of difference at $P < 0.05$ and $P < 0.01$ levels, respectively, by F test when compared with that in control plants. Values are means ± S.D. (n = 5).

4. Discussions

PS II supercomplex consists of reaction center, oxygen-evolving complex and light-harvesting complex. The activity of PS II depends on its structure, including the structure of each complex and the connection between complexes. The factors that disrupt the architecture of supercomplex will decrease the PS II activity. In this experiment, malate treatment inhibited the potential of trapping light energy (F_V) and reduction ability of reducing Q_B (ET_O/CS). Further analysis show that these changes are mainly resulted from the increase in F_O and inactivation of PS II RC. F_O represents the amount of useless energy absorbed by photosynthetic pigments. The increase in F_O means that the efficiency of excitation energy transfer to PS II RC decreases. In this study the rise in F_O is caused by the reduction in the ratio RC/LHC II (**Figure 1**) and the disrupt in the structure of OEC (W_K) (**Figure 3**). The inactivation of RC may be mainly attributed to the injury of OEC. Possibly the connection between LHCII and RC is affected by malate. However, until now, there is no any suitable parameter in fluorescence JIT cure to describe this change in connection between PS II complexes. In addition, the tartrate (a structural analog of malate) treatment was not affected PS II in both maize and tobacco. It confirmed that activities of PS II were affected directly by exogenous malate but not pH or other indirect factor.

NADP is the terminal acceptor of linear electron transfer. As a result, the efficiency of transfer is affected by NADP/NADPH. In malate treated leaves, a large amount of exogenous malate is imported into chloroplasts and decarboxylated by NADP-ME to produce CO_2, NADPH and pyruvate. This reaction will add NADPH into chloroplasts and decrease the NADP/NADPH ratio. The reduction of NADP molecules will result in the block of linear electron transfer. The pyruvate produced by malate decarboxylation may also play a role in change in the activity and structure of PS II in malate-treated leaves. We conclude that exogenous malate regulates the activity and structure of PS II in C_4 plant maize.

The operation of C_4 cycle in NADP-ME type C_4 plants depends on four key enzymes. For C_4 cycle, phosphoenolpyruvate carboxylase (PEPC), NADP-malate dehydrogenase (NADP-MDH), NADP-ME and pyruvate (PPDK), and regulated by the transporters of malate in cell plasma and chloroplast membrane. Malate treatments did not impose a significant effect on the activity and structure of PS II in tobacco detached leaves in this study. This may be attributed to the deficiency in NADP-ME and malate transporters with high capacity. Hudspeth *et al*. (1992) [1] reported that the transformation of C_4-PEPC into tobacco increased the content of malate, but not promoted the photosynthetic rate. The overexpression of maize C_4 NADP-ME in rice enhanced the decarboxylation of malate [3] [4]. We suppose that NADP-ME is a pivotal enzyme for the participation of malate in photosynthetic cycle in chloroplasts in tobacco. Because, in this experiment, leaf discs were treated with malate only for 60 min, we cannot deny that malate may impose effect on PS II activity and structure during a longer term treatment.

5. Conclusion

All in all, we conclude that exogenous malate regulates the activity and structure of PS II in C_4 plant maize. No significant changes in the activity of PS II were observed in malate-treated C_3 plant tobacco. It is suggested that the short term malate treatment will inhibit PS II of leaves which have C_4 anatomy and C_4 enzymes.

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