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BRIEF COMMUNICATION

Low concentration of bisulfite enhances photosynthesis in tea tree by promoting carboxylation efficiency in leaves

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Abstract

Tea tree (*Melaleuca alternifolia*) canopy was sprayed with low concentration of $NaHSO_3$ or mixture of $NaHSO_3$+ KH_2PO_4. The treatments significantly enhanced net photosynthetic rate (P_N), carboxylation efficiency (CE), and the maximum response of P_N to intercellular CO_2 concentration. The enhancement of P_N by foliar application of low concentrations of bisulfite was due to increasing CE relevant to ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase activity and regeneration rate of RuBP depending on ATP formation.

Additional key words: chlorophyll fluorescence; intercellular CO_2 concentration; *Melaleuca*; photorespiration; photosystem 2; regeneration of ribulose-1,5-bisphosphate; stomatal conductance.

Low concentration of bisulfite has often been used for enhancing photosynthesis to promote field crop yield (Wang *et al.* 2003, Chen *et al.* 2005, Guo *et al.* 2006). However, it is still unknown how it directly enhances carbon assimilation. Zelitch (1957) first reported that bisulfite could compose acetaldehyde acid to form α-hydroxysulfonates as inhibitors of the enzymatic oxidation of glycolate. Later on, researchers considered bisulfite as an inhibitor of photorespiration to enhance net photosynthetic rate, P_N (Zhou and Wang 2000, Chen *et al.* 2005), while the researches by Takemoto and Noble (1982) and Tan and Shen (1987) showed that bisulfite did not inhibit photorespiration. Although Wang and Shen (2002) stressed the increase relevant to enhancement of cyclic photophosphorylation, it is still a question whether or not bisulfite addition results in change in carboxylation efficiency (CE).

Tea is a traditional drink both in China and other countries. In spring the price of tea leaves depends on the date of picking. The earlier it is picked, the higher benefit the farmers get. Therefore, how to make tea bud in the spring sprout out earlier has become the key for increasing tea leaf yield. Chen *et al.* (2005) reported that low concentration of $NaHSO_3$ significantly enhanced bud growth and P_N of tea trees in the field, however, neither the photosystem 2 (PS2) activity nor the excitation energy distribution between photochemical and non-photochemical quenchings were changed.

To explore how bisulfite enhances photosynthesis directly, tea trees (*Melaleuca alternifolia* cv. Wuniuzao) grown outdoors in pots were moved to the sun-irradiated growth chamber at temperature of 20–25 ºC, relative humidity of 55–70 %, photosynthetic photon flux density (PPFD, 400–700 nm) of 800–1 000 μmol(photon) m^{-2} s^{-1} 1-d ago. The canopy was sprayed with distilled water (control, C), with 2 mM $NaHSO_3$, or with the mixture (2 mM $NaHSO_3$+7 mM KH_2PO_4). P_N and chlorophyll (Chl) fluorescence parameters, irradiance and CO_2 response curves, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) activity as well as photorespiration rate (R_P) were measured on the 4–5th top leaves on the next day after the treatment.

The steady gas exchange, Chl fluorescence parameters, the photosynthetic photon flux density (PPFD) and CO_2 concentration responses of P_N were determined with a portable photosynthesis system (*LiCor-6400*; *LiCor*, Lincoln, NE, USA) following the method of Huang *et al.* (2004) at PPFD of 1 000 μmol(photon) m^{-2} s^{-1}, leaf temperature of 25 ºC, and CO_2 concentration of 461±13 μmol mol^{-1} in the sample chamber.

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Table 1. Net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), maximal photochemical efficiency of photosystem 2, PS2 (F_v/F_m), excitation energy capture efficiency of PS2 (F_v'/F_m'), non-cyclic electron transport rate of PS2 (ETR), photorespiration rate (R_P), apparent quantum yield (AQY), carboxylation efficiency (CE), regeneration rate of ribulose-1,5-bisphosphate (J_{max}), and the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) activity of tea leaves affected by the $NaHSO_3$ and mixture treatments. Control was sprayed with distilled water. Values sharing different letters are statistically different at $p<0.05$.

Parameter	Control	$NaHSO_3$	Mixture
P_N [μmol(CO_2) m^{-2} s^{-1}]	3.89 [c]	6.80 [b]	8.64 [a]
g_s [μmol m^{-2} s^{-1}]	0.12 [b]	0.16 [b]	0.21[a]
C_i [μmol mol^{-1}]	389 [a]	401 [a]	409 [a]
F_v/F_m	0.76 [a]	0.76 [a]	0.78 [a]
F_v'/F_m'	0.53 [a]	0.40 [a]	0.47 [a]
ETR	42.16 [a]	39.51 [a]	45.25 [a]
R_P [μmol(CO_2) m^{-2} s^{-1}]	1.38 [a]	1.50 [a]	1.51 [a]
AQY	0.024 [a]	0.025 [a]	0.028 [a]
CE [mmol m^{-2} s^{-1}]	29.4 [b]	35.5 [a]	34.0 [ab]
J_{max} [μmol(CO_2) m^{-2} s^{-1}]	17.9 [b]	19.5 [a]	18.8 [a]
RuBPCO [μmol(CO_2) kg^{-1}(FM) s^{-1}]	115.7 [b]	146.5 [a]	97.7 [b]

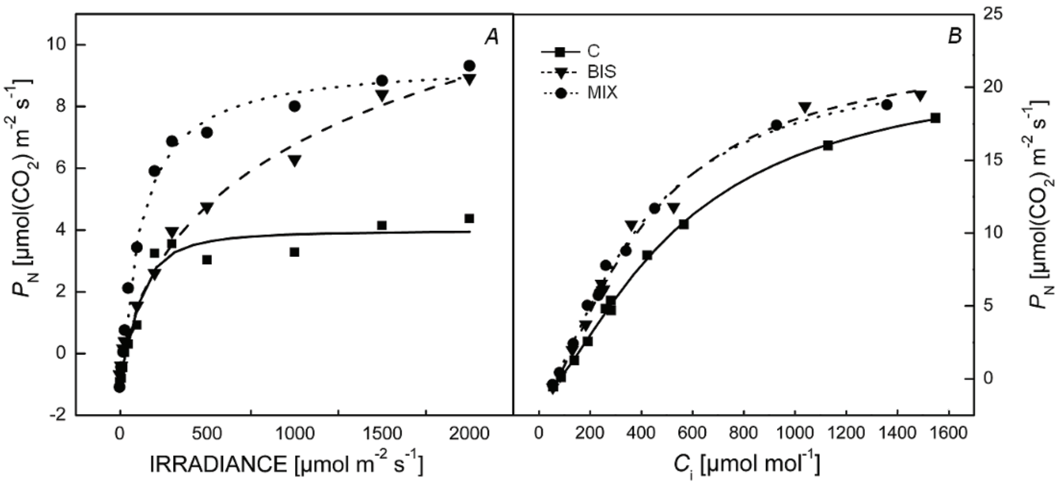

Fig. 1. Irradiance (*A*) and internal CO_2 concentration, C_i (*B*) responses of net photosynthetic rate (P_N) in tea leaves treated by $NaHSO_3$ (BIS) and mixture (MIX) of 2 mM $NaHSO_3$+7 mM KH_2PO_4; C – control.

The RuBPCO activity was determined by spectrophotometry (Jin *et al*. 2006). R_P was obtained as the photosynthetic difference between measurements at 21 and 2 % O_2.

All determinations were performed in at least three independent experiments. Statistical differences were analyzed by analysis of variance (ANOVA) using the *SPSS* package. Differences were considered significant at $p<0.05$ leve1.

$NaHSO_3$ treatment significantly promoted P_N of tea leaf in comparison with C treated with distilled water (Table 1). The mixture $NaHSO_3$+KH_2PO_4 further enhanced P_N. Stomatal conductance (g_s) was only enhanced by the mixture and not by $NaHSO_3$. Hence the increase in P_N by $NaHSO_3$ was not induced by increase in g_s. Chl fluorescence parameters, such as maximal photochemical efficiency of photosystem 2, PS2 (F_v/F_m), excitation energy capture efficiency of PS2 (F_v'/F_m'), and non-cyclic electron transport rate of PS2 (ETR) were not significantly influenced by either $NaHSO_3$ or the mixture (Table 1); hence $NaHSO_3$ did not stimulate PS2 activity in tea leaf. Under the steady condition, the gas exchange and Chl fluorescence characteristics caused by spraying low concentration of $NaHSO_3$ were similar to those found by other authors (Chen *et al.* 2005) for tea plants. Table 1 also shows that $NaHSO_3$ did not inhibit R_P. Wang (2000, 2003) found that P_N promotion by $NaHSO_3$ was in accordance with an increase in cyclic photophosphorylation and Chen (2007) reported $NaHSO_3$ made cyclic electron flow around PS1 rise, but these results did still not explain whether or nor it increases CE which is more direct to CO_2 assimilation.

Fig. 1 illustrates that all curves had a saturation shape at response to both irradiance and intercellular CO_2 concentration (C_i). Compared with C, $NaHSO_3$ increased significantly not only P_N under high irradiance, but also saturation irradiance (Fig. 1*A*). The mixture increased P_N more dramatically at middle irradiance, which might be partly caused by increasing g_s because K^+ plays very important role in stomata opening. Analysis of apparent quantum yield (AQY) under irradiance of <200 μmol(photon) m^{-2} s^{-1} showed no significant difference (Table 1). Thus low concentration of bisulfite could improve photon energy utilization only under high irradiance. The response curves of P_N to C_i illustrated that, at any C_i, P_N in the leaves sprayed by $NaHSO_3$ was higher than that in C. Farquhar and Sharkey (1982) pointed out that linear part of response curve of P_N to C_i, *i.e*. CE, is related to RuBPCO activity, and the maximum value of response curve of P_N to C_i, called regeneration rate of ribulose-1,5-bisphosphate (J_{max}), is related to ATP formation by photophosphorylation. Calculation showed that both CE and J_{max} of tea leaf were significantly enhanced by spraying $NaHSO_3$. Higher CE and J_{max} in the sprayed leaves indicated that low concentration of bisulfite can increase RuBPCO activity and ATP formation. Reports on enhancement of cyclic photophosphorylation by bisulfite in rice (Wang *et al.* 2000), wheat (Wang and Shen 2002), and algae (Wang *et al.* 2003) support our idea of ATP formation. In our experiment bisulfite enhanced P_N by increasing CE which reflected the RuBPCO activity. This result supports our conclusion that CE is due to increase in RuPCO activity by $NaHSO_3$ treatment (Table 1). Adding KH_2PO_4 to bisulfite can significantly enhance P_N at medium irradiance mainly because of higher g_s.

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