DOI: 10.1007/s11099-014-0044-7 PHOTOSYNTHETICA 52 (3): 475-478, 2014

BRIEF COMMUNICATION

Sodium bisulfite enhances photosynthesis in rice by inducing Rubisco activase gene expression

Y. CHEN[*], J.-H. JIN[*], Q.-S. JIANG[*], C.-L. YU[*], J. Chen[**], L.-G XU[*], and D.-A. JIANG[*,+]

State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou 310058, China[*]
Canal Street Agency, Yuhang District People's Government, Hangzhou 311102, China[**]

Abstract

To investigate how bisulfite promotes photosynthesis, a pot experiment was conducted with rice (*Oryza sativa* L.) plants to determine Rubisco activity and content, and Rubisco activase (RCA) gene expression after spraying $NaHSO_3$ on rice leaves. The $NaHSO_3$ treatment promoted significantly net photosynthetic rate (P_N), carboxylation efficiency, maximum carboxylation rate, ribulose-1,5-bisphosphate regeneration rate, initial Rubisco activity, and RCA protein and mRNA concentrations. Therefore, the $NaHSO_3$ enhancement of P_N could be directly attributed to induction of RCA gene expression both at the transcription and translation levels. Thus, the increased RCA regulated the initial Rubisco activity *in vivo*.

Additional key words: carboxylation efficiency; grain yield; maximum carboxylation rate; photorespiration; RuBP regeneration rate.

Photosynthesis is the basis of crop production. The efficiency of solar energy utilization by plants is usually < 2%, but it can reach 4.6% in C_3 and 6.0% in C_4 plants (Zhu *et al.* 2010). Therefore, there is a considerable effort to improve this efficiency to increase the productivity in the field.

Low concentrations of bisulfite has been used to enhance photosynthesis and field crop yield (Wang *et al*. 2003, Chen *et al*. 2005, Guo *et al*. 2006). However, it remains unknown how it enhances carbon assimilation (Yang *et al*. 2008). Zelitch (1957) first reported that bisulfite could induce glycoxylate to form α-hydroxysulfonate and inhibit the enzymatic oxidation of glycolate. Later, sodium bisulfite ($NaHSO_3$) was suggested to enhance P_N because it inhibited photorespiration (Zhou and Wang 2000, Chen *et al.* 2005). However, several reports showed that photorespiration was not inhibited by $NaHSO_3$ (Takemoto and Noble 1982, Tan and Shen 1987, Yang *et al.* 2008). Some studies have shown that $NaHSO_3$ enhanced P_N by increasing the rate of cyclic photophosphorylation (Wang *et al.* 2000a,b; Wang and Shen 2002, Wang *et al.* 2003, Chen *et al.* 2007). Yang *et al.* (2008) reported that improvement of P_N in tea plants was due to enhanced carboxylation efficiency (CE) and Rubisco activity. However, similarly as for carbon assimilation, it is not known how $NaHSO_3$ improves Rubisco activity.

Activation of Rubisco is regulated by Rubisco activase (RCA), one of the AAA^+ ATPase family, which may maintain a high Rubisco activation state. RCA facilitates carbamylation and the maintenance of Rubisco activity by removing inhibitors, such as tight-binding sugar phosphates, from Rubisco catalytic sites in an ATP-dependent manner (Spreitzer and Salvucci 2002, Potris 2003, Parry *et al.* 2008). In most species studied, RCA is a nuclear-encoded, chloroplast enzyme, usually present in two

Received 22 October 2013, *accepted* 24 January 2014.
[+]Corresponding author; tel: 0086 (0)571 88206461, fax: 0086 (0)571 88206461, e-mail: dajiang@zju.edu.cn
Abbreviations: AQY – apparent quantum yield; CE – carboxylation efficiency; ELISA – enzyme-linked immunosorbent assay; C_i – intercellular CO_2 concentration; g_s – stomatal conductance; J_{max} – RuBP regeneration rate; P_N – net photosynthetic rate; RCA – Rubisco activase; RCA_L – RCA large isoform; RCA_S – RCA small isoform; R_D – dark respiration rate; RLS – Rubisco large subunit; R_P – photorespiration rate; RSS – Rubisco small subunit; RuBP – ribulose-1,5-bisphosphate; V_{cmax} – maximum carboxylation rate.
Acknowledgements: This work was supported by National Science and Technology Support Plan (2012BAC09B01) and National Natural Science Foundation (30971703 and 31171462).

isoforms, the products of an alternate splicing event that generates two polypeptides: a large isoform of 45–48 kDa and the small isoform of 41–43 kDa (Zhang and Komatsu 2000, Portis 2002, Spreitzer and Salvucci 2002). One gene encodes two RCA polypeptides of 45 kDa (RCA_L) and 41 kDa (RCA_S) arising from a unique alternate splicing of pre-mRNA in rice plants (To *et al.* 1999). P_N in rice plants is most related to the initial Rubisco activity during leaf senescence (Jiang *et al.* 1995) and to regulation during diurnal changes of P_N (Jiang *et al.* 2001), indicating that *in vivo* initial Rubisco activity plays the most important role in determining P_N of the leaves.

Before the start of the experiment, rice (*Oryza sativa* L. cv. Zhenong 952) was grown to the heading stage in pots containing 10 kg of soil. The pots were kept outdoors and covered by nets in Zijingang Campus of Zhejiang University, where PPFD reached about 1,500 μmol (photon) m^{-2} s^{-1}, relative humidity (RH) about 50%, and temperature about 30ºC at noon on sunny days. To explore how $NaHSO_3$ enhances CE and Rubisco activity, the pots were divided into two groups. One, for the yield measurement, was kept outdoors after the $NaHSO_3$ treatment until harvest. Another was moved into a greenhouse under a PPFD of 1,300 μmol m^{-2} s^{-1} and RH of 60% controlled at day/night temperature of 30/22ºC. The canopy was either sprayed with distilled water (control), or with 2 mM $NaHSO_3$. P_N-light and P_N-CO_2 response curves, Rubico activity, and RCA content of the flag leaves were measured in the morning after the treatment. Apparent quantum yield (AQY), CE, maximum carboxylation rate (V_{cmax}), and regeneration rate of ribulose-1,5-bisphosphate (RuBP) (J_{max}) were calculated according to *Licor-6400* software package.

Steady gas exchange, PPFD and CO_2 concentration responses of P_N were determined with a portable photosynthesis system (*Licor-6400;Licor*, Lincoln, NE, USA) at PPFD of 2,000 μmol m^{-2} s^{-1}, leaf temperature of 30ºC, and CO_2 concentration of 400 μmol mol^{-1} in the sample chamber. The irradiance response of P_N was measured by *Licor-6400* with CO_2 concentration of 400 μmol mol^{-1} in the sample chamber. A serie of CO_2 reference concentrations (400, 300, 200, 100, 50, 0, 50, 100, 200, 300, 400, 600, 800, 1,000; 1,200; 1,500; and 2,000 μmol mol^{-1}) was used for a CO_2 response curve, and it was set up by the *Licor* CO_2 injection system (Huang *et al*. 2004, Yang *et al*. 2008). The initial and total Rubisco activity was measured spectrophotometrically (Wang *et al*. 2009). The photorespiration rate (R_P) was calculated on the basis of the photosynthetic difference between 21 and 2% O_2 conditions.

Both enzyme-linked immune sorbent assay (ELISA) and Western blot were employed to determine contents of Rubisco subunits and RCA isoforms (Wang *et al.* 2009). The accumulation of *RCA* mRNA was determined by both semiquantitative RT-PCR and real-time quantitative RT-PCR. Actin was used as an internal standard (F 5'-TCCATCTTGGCATCTCTCAG-3'; R 5'-GTACCCGCA TCAGGCATCTG-3'). The *RCA* gene-specific primers (F 5'-AGCTCGTCGTCCACATCTCCA-3'; R 5'-CTTGATG ATGTCTGCCGCCTC-3') were designed for a region that includes the shared region of *RCA* small isoform (RCA_S) and large isoform (RCA_L) mRNA. In real-time quantitative RT-PCR, *OsACTIN* (F 5'-CAACACCCCTGCTATGT ACG-3'; R 5'-CATCACCAGAGTCCAACACAA-3') and *OsEF-1a* (F 5'-CCGTGAGCACGCTCTTCTTG-3'; R 5'-GGGAATCTTGTCAGGGTTGTAG-3') were reference genes. The *RCA* primers (F 5'-CGTGACGGGCGTAT GGAGAAG-3'; R 5'-GCACGAAGAGCGCCGAAGAA ATC-3') and RCA_S specific primers (F 5'-TTCTGCGC CATCCAGCTGAA-3'; R 5'-CCTCCTCCTCCTATGCA GG-3') were designed for their real-time quantitative PCR.

All determinations were performed on at least three independent samples, and the statistical differences were expressed by analysis of variance (*ANOVA*) using *Origin* and *SPSS* statistical packages (Chicago, IL, USA). Differences were considered significant at $p<0.05$ level (Yang *et al*. 2008).

In comparison with control, the $NaHSO_3$ treatment significantly increased grain yields and promoted P_N, CE, V_{cmax}, J_{max}, and initial Rubisco activity of rice, but it did not influence significantly stomatal conductance (g_s), intercellular CO_2 concentration (C_i), respiration rate (R_D), R_P, AQY, and total Rubisco activity (Table 1). These results confirmed that the increase of P_N induced by $NaHSO_3$ was not due to an increase in g_s (Chen *et al.* 2005) and a decrease in R_P (Takemoto and Noble 1982, Tan and Shen 1987, Yang *et al.* 2008). Analysis of AQY under the irradiance of < 200 μmol(photon) m^{-2} s^{-1} showed no significant difference between controls and treated samples (Table 1), indicating that light-use efficiency was not enhanced by $NaHSO_3$ treatment under low light intensity.

The significant increase in J_{max} in the rice leaves after $NaHSO_3$ treatment showed the stimulation of RuBP regeneration rate, which depends on ATP formation rate during photophosphorylation. It seems probable that $NaHSO_3$ promotes cyclic electron flow (Chen *et al*. 2007) and photophosphorylation (Wang *et al.* 2000a,b; Wang and Shen 2002, Wang *et al.* 2003). The obvious increase in CE, calculated from P_N and C_i <400 μmol mol^{-1} (Table 1), suggested that $NaHSO_3$ treatment enhanced significantly Rubisco activity (Farquhar and Sharkey 1982, Yang *et al.* 2008).

To understand the reason for the significantly higher initial Rubisco activity in leaves of rice treated with $NaHSO_3$, the quantitative changes of two Rubisco subunits (Fig. 1*A*) and two RCA isoforms (Fig. 1*B*) were determined in the leaves sprayed with 2 mM $NaHSO_3$ by ELISA based on specific monoclonal antibodies to RLS, RSS, RCA_L, or both RCA isoforms according to the method of Wang *et al.* (2009). The ELISA showed that the content of the two Rubisco subunits was not influenced by $NaHSO_3$ treatment (Fig. 1*A*), while the content of the two RCA isoforms in leaves treated with $NaHSO_3$ significantly increased compared with that in the control leaves (Fig. 1*B*). Western blot assay further supported these results (Fig. 1*C*).

Table 1. The effect of $NaHSO_3$ on yield, photosynthetic and respiratory parameters, and Rubisco activity of rice leaves. Values with *different letters* are statistically different at $p<0.05$. P_N – net photosynthetic rate; g_s – stomatal conductance; C_i – intercellular CO_2 concentration; R_D – respiration rate; R_P – photorespiration rate; AQY – apparent quantum yield; CE – carboxylation efficiency; V_{cmax} – maximum carboxylation rate; J_{max} – RuBP regeneration rate.

Parameter	Control	$NaHSO_3$
Grain yield [kg pot^{-1}]	50.9 ± 4.5 [a]	61.5 ± 6.0[b]
P_N [μmol(CO_2) m^{-2} s^{-1}]	23.3 ± 0.3[a]	27.2 ± 0.1[b]
g_s [mmol(CO_2) m^{-2} s^{-1}]	0.69 ± 0.02[a]	0.71 ± 0.03[a]
C_i [μmol mol^{-1}]	388 ± 21[a]	397 ± 13[a]
R_D [μmol(CO_2) m^{-2} s^{-1}]	1.33 ± 0.02[a]	1.40 ± 0.03[a]
R_P [μmol(CO_2) m^{-2} s^{-1}]	11.16 ± 1.54 [a]	10.16 ± 1.73[a]
AQY [CO_2 photon^{-1}]	0.048 ± 0.020[a]	0.055 ± 0.010[a]
CE [mol m^{-2} s^{-1}]	0.12 ± 0.01[a]	0.17 ± 0.01[b]
V_{cmax} [μmol(CO_2) m^{-2} s^{-1}]	102.5 ± 0.2[a]	105.6 ± 0.2[b]
J_{max} [μmol m^{-2} s^{-1}]	83.0 ± 2.1[a]	102.6 ± 0.9[b]
Rubisco initial activity [μmol m^{-2} s^{-1}]	21.4 ± 0.2[a]	24.3 ± 0.3[b]
Rubisco total activity [μmol m^{-2} s^{-1}]	30.8 ± 0.1[a]	32.0 ± 0.2[a]

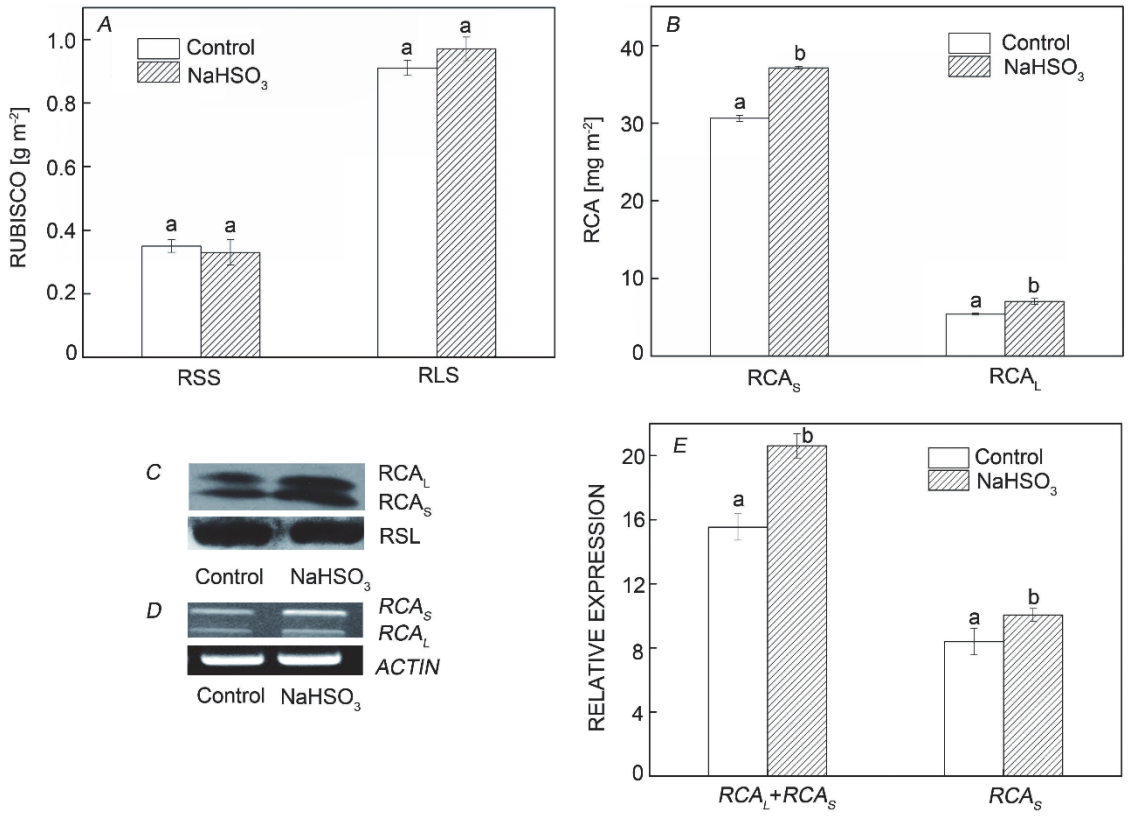

Fig. 1. Effect of $NaHSO_3$ on content of Rubisco and RCA, and accumulation of *RCA* mRNA. *A*: Rubisco content from ELISA. Error bars indicate standard deviations; $n = 3$. *B*: RCA content from ELISA. Extracted supernatant was diluted 60-fold for the RCA content assay using cover buffer. Error bars indicate standard deviations; $n = 3$. *C*: Content of two RCA isoforms (RCA_L and RCA_S) and Rubisco large subunit (RLS) from Western blot. Equal amounts of total protein extracted from flag leaves were separated by SDS-PAGE, and immune-detected by antibodies against Rubisco large subunit, RCA_L and RCA_S. *D*: RCA_S and RCA_L from semi-quantitative PCR. *ACTIN* was the internal control. *E*: Total *RCA* and RCA_S from real-time quantitative PCR. Expression levels were an average of three biological and three technical replicates of each sample, and *ACTIN* and *EF-1a* were internal controls. *Different letters* are statistically different at $p<0.05$.

The mRNA sequence of the two RCA polypeptides were 99% identical, the exception being an 82-bp addition near the 3'-end of the small isoform, which contained an early stop codon (To *et al.* 1999). Therefore, RCA_S had a large mRNA after transcription; and RCA_L had a small one. We designed two PCR primers, one for the total *RCA* content and another for RCA_S (Wang *et al.* 2010). Both RCA_S and RCA_L mRNA increased significantly after treatment with $NaHSO_3$ (Fig. 1*D,E*), suggesting that $NaHSO_3$ could regulate RCA protein at the transcriptional level.

Considering other reports and our results, we concluded that $NaHSO_3$ enhanced cyclic photophosphorylation and induced *RCA* gene expression both in transcription and translation, and then the increased RCA regulated *in vivo* initial Rubisco activity that enhanced directly P_N.

References

Chen, H.F., Hu, M.Q., Yang, Y. *et al.*: [Effect of foliage spraying by sodium bisulfite at low concentration on resistance to cold of tea trees.] – Acta Agr. Zhejiang **19**: 184-187, 2007. [In Chinese]

Chen, H.F., Hu, M.Q., Zhu, P.N. *et al.*: [Effects of spraying $NaHSO_3$ on bud growth and photosynthesis of tea trees.] – Acta Agr. Zhejiang **17**: 11-14, 2005. [In Chinese]

Farquhar, G.D., Sharkey, T.M.: Stomatal conductance and photosynthesis.– Annu. Rev. Plant Physiol. **33**: 317-345, 1982.

Guo, Y.P., Hu, M.J., Zhou, H.F. *et al.*: Low concentrations of $NaHSO_3$ increase photosynthesis, biomass, and attenuate photoinhibition in Satsuma mandarin (*Citrus unshiu* Marc.) plants. – Photosynthetica **44**: 333-337, 2006.

Huang, Z.A., Jiang, D.A., Yang, Y. *et al.*: Effects of nitrogen deficiency on gas exchange, chlorophyll fluorescence and antioxidant enzymes in leaves of rice plants. – Photosynthetica **42**: 357-364, 2004.

Jiang, D.A., Xu, Y.F.: [Internal dominant factors for declination of photosynthesis during rice leaf senescence.] – Acta Agric. Univ. Chekianensis **21**: 533-538,1995. [In Chinese]

Jiang, D. A., Lu, Q., Weng, X.Y. *et al.*: [Role of key enzymes for photosynthesis in the diurnal changes of photosynthetic rate in rice.] – Zuo Wu Xue Bao **27**: 301-307, 2001. [In Chinese]

Parry, M.A.J., Keys, A.J., Madgwick, P.J. *et al.*: Rubisco regulation: a role for inhibitors. – J. Exp. Bot. **59**: 1569-1580, 2008.

Portis, A.R., Salvucci M.E.: The discovery of Rubisco activase - yet another story of serendipity. – Photosynth. Res. **73**: 257-264, 2002.

Portis, A.R.: Rubisco activase - Rubisco's catalytic chaperone. – Photosynth. Res. **75**: 11-27, 2003.

Spreitzer, R.J., Salvucci, M.E.: Rubisco: Structure, regulatory interactions, and possibilities for a better enzyme. – Annu. Rev. Plant Biol. **53**: 449-475, 2002.

Takemoto, B.K., Noble, R.D.: The effects of short time SO_2 fumigation on photosynthesis and respiration in soybean Glycine max. – Environ. Pollut. A **28**: 67-74, 1982.

Tan, S., Shen, Y.G.: [The effects of sodium bisulfite on photosynthetic apparatus and its operation.] – Acta Phytophysiol. Sin. **13**: 42-50, 1987. [In Chinese]

To, K.Y., Suen, D.F., Grace-Chen, S.C.: Molecular characterization of ribulose-1,5-bisphosphate carboxylase/oxygenase in rice leaves. – Planta **209**: 66-76, 1999.

Wang, D., Li X.F., Zhou Z.J. *et al.*: Two Rubisco activase isoforms may play different roles in photosynthetic heat acclimation in the rice plant. – Physiol. Plantarum **139**: 55-67, 2010.

Wang, D., Lu, Q., Li, X.F. *et al.* : Relationship between Rubisco activase isoform levels and photosynthetic rate in different leaf positions of rice plant. – Photosynthetica **47**: 621-629, 2009.

Wang, H.W., Mi, H.L., Ye, J.Y. *et al.*: Low concentrations of $NaHSO_3$ increase cyclic photophosphorylation and photosynthesis in cyanobacterium *Synechocystis* PCC6803. – Photosynth. Res. **75**: 151-159, 2003.

Wang, H.W., Shen, Y.G.: How bisulfite enhances photosynthesis. – J. Plant Physiol. Mol. Biol. **28**: 247-252, 2002.

Wang, H.W., Wei J.M., Shen, Y.G.: Enhancement of wheat leaf photophosphorylation and photosynthesis by spraying low concentration of $NaHSO_3$. – Chinese Sci. Bull. **45**: 1308-1312, 2000a.

Wang, H.W., Wei, J.M., Shen, Y.G. *et al.*: Enhancement of photophosphorylation and photosynthesis in rice by low concentrations of $NaHSO_3$ under field conditions. – Acta Bot. Sin. **42**: 1295-1299, 2000b.

Yang, W.J., Chen H.F., Zhu, F.Y. *et al.*: Low concentration of bisulfite enhances photosynthesis in tea tree by promoting carboxylation efficiency in leaves. – Photosynthetica **46**: 615-617, 2008.

Zelitch, I.: α-hydroxysulfonates as inhibitors of the enzymatic oxidation of glycolic and lactic acids. – J. Biol. Chem. **224**: 251-260, 1957.

Zhang, Z.L., Komatsu, S.: Molecular cloning and characterization of cDNAs encoding two isoforms of ribulose-1,5-bisphosphate carboxylase/oxygenase activase in rice (*Oryza sativa* L.). – J. Biochem. **128**: 383-389, 2000.

Zhou, G.Y.; Wang, H.K.: [Study on effect of photorespiration inhibitor-sodium hydrogen sulfite on increase yield of corn.] – Soil. Fertil. Sci. China **6**: 35-38, 2000. [In Chinese]

Zhu, X.G., Long, S.P., Ort D.R.: Improving photosynthetic efficiency for greater yield. – Annu. Rev. Plant Biol. **61**: 235-261, 2010.