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BRIEF COMMUNICATION

Photosynthetic acclimation to CO_2 enrichment related to ribulose-1,5-bisphosphate carboxylation limitation in wheat

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Abstract

Net photosynthetic rate (P_N) measured at the same CO_2 concentration, the maximum *in vivo* carboxylation rate, and contents of ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (RuBPCO) and RuBPCO activase were significantly decreased, but the maximum *in vivo* electron transport rate and RuBP content had no significant change in CO_2-enriched [EC, about 200 µmol mol^{-1} above the ambient CO_2 concentration (AC)] wheat leaves compared with those in AC grown wheat leaves. Hence photosynthetic acclimation in wheat leaves to EC is largely due to RuBP carboxylation limitation.

Additional key words: maximum *in vivo* electron transport rate; net photosynthetic rate; ribulose-1,5-bisphosphate carboxylase/oxygenase activase; RuBP regeneration limitation; sucrose-phosphate synthase; *Triticum*.

After long-term exposure to elevated CO_2 concentration (EC) leaf net photosynthetic rate (P_N) is often significantly lower in the plants grown at EC than that in plants grown in ambient air (AC) when measured at a same CO_2 concentration. This phenomenon is so called photosynthetic acclimation to EC (Xu *et al.* 1994). Usually, this acclimation has been almost entirely attributed to the decrease in active ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) (Jacob *et al.* 1995, Rogers and Humphries 2000, Pérez *et al*. 2007). Nevertheless, Chen *et al.* (2005) reported that the photosynthetic acclimation in rice leaves is related to both RuBP carboxylation limitation and RuBP regeneration limitation. The similar result was also observed in Norway spruce (Urban *et al.* 2003). Furthermore, Zhang *et al.* (2008) has demonstrated that RuBP regeneration limitation is predominant within the two limitations. However, it is not known whether such mechanism of photosynthetic acclimation operating in rice is species-specific or universal. In order to answer the question the photosynthetic acclimation was investigated further in wheat.

The Chinese CO_2 enrichment facilities were located at Xiaoji village (119°42'0"E, 32°35'5"N), Yangzhou city in Jiangsu Province, East China. In the experimental field, there were six rings of 15 m diameter. Among them, three rings were sprayed with pure CO_2 as EC treatment, and the others were in the normal atmosphere as AC control. The distances between EC and AC rings were more than 90 m. Target CO_2 concentration in the centre of EC rings was about 200 µmol mol^{-1} above the AC. The EC treatment was made from emergence of wheat seedlings to harvesting. Seeds of wheat (*Triticum aestivum* L.cv. Ningmai 9) were sown in mid November. The cultivation of wheat was performed with typical agronomic management techniques for this region.

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Abbreviations: C_i – internal CO_2 concentration; Cyt – cytochrome; J_{max} – maximum *in vivo* electron transport rate; P_N − net photosynthetic rate; RuBP – ribulose-1,5-bisphosphate; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; SPS – sucrose-phosphate synthase; V_{cmax} – maximum *in vivo* carboxylation rate.

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Photosynthetic measurements with calculation of maximum *in vivo* carboxylation rate (V_{cmax}) and maximum *in vivo* electron transport rate (J_{max}), and determinations of RuBP and sucrose contents as well as sucrose-phosphate synthase (SPS) activity were made as described by Chen *et al.* (2005) and Zhang *et al.* (2008). RuBPCO and its activase contents were measured with a protein detector enzyme-linked immuno-sorbent assay (*ELISA*) kit, *ABTS* system (*KPL*, Gaithersburg, USA).

The effect of EC on photosynthesis was analyzed by the general liner model (*GLM*) procedure of the *SPSS 12* (*SPSS*, USA). Plot were arranged in a block design and data were analyzed for the effects of rings (blocks) and EC. This analysis showed no significant ring effect on gas exchange. Consequently, the factor of ring was dropped from the analyses of the biochemical parameters, and measurement data were then analyzed using a *t*-test for the EC effect.

When measured at their growth CO_2 concentration, leaf P_N of wheat plants grown in EC rings was significantly higher than that of those grown in AC rings at the jointing, heading, and filling stages (Fig. 1*A*). However, when measured at the same CO_2 concentration of 580 μmol mol^{-1}, P_N in EC leaves was significantly lower than that in AC leaves at the three development stages (Fig. 1*B*), indicating that acclimation of photosynthesis occurred under EC conditions. The P_N-internal CO_2 concentration (C_i) curve (Fig. 1*C*) showed that the EC leaves had a lower CO_2-saturated P_N and a smaller initial slope. V_{cmax} *in vivo* calculated from the data of P_N-C_i curve was significantly decreased, while J_{max} *in vivo* had no significant change in the EC leaves, compared with those in AC leaves (Fig. 1*D*). Moreover, the soluble protein, RuBPCO and its activase contents were also significantly decreased, implying that EC led to the decreased RuBPCO activity (Hrstka *et al.* 2005), but RuBP content did no significantly change in the EC leaves (Table 1). At the same time, sucrose content and SPS activity were also significantly increased in the EC leaves (Table 1).

Thus the photosynthetic acclimation to EC in wheat is largely due to RuBP carboxylation limitation without RuBP regeneration limitation. The following facts support this conclusion. First, in EC wheat leaves a significant decrease occurred in V_{cmax} but not in J_{max} (Fig. 1*D*). Second, the significant decreases in RuBPCO and its activase contents were observed in the EC wheat leaves (Table 1). Third, no significant decrease in RuBP content occurred in the EC leaves (Table 1), implying that there is no RuBP regeneration limitation to photosynthesis because the pool size of RuBP declines when photosynthesis is limited by RuBP regeneration (Cen and Sage 2005). Obviously, the mechanism of photosynthetic acclimation in wheat is different from that in rice (cf. Yong *et al*. 2007) where the photosynthetic acclimation is related to both RuBP carboxylation limitation and RuBP regeneration limitation and the latter is predominant one (Chen *et al.* 2005, Zhang *et al.* 2008). It appears that the mechanism for photosynthetic acclimation to EC treatment operating in rice is species-dependent but not universal.

The difference in the mechanism of photosynthetic acclimation between wheat and rice may be explained by the fact that wheat has a higher RuBP regeneration capacity than rice and the higher RuBP regeneration

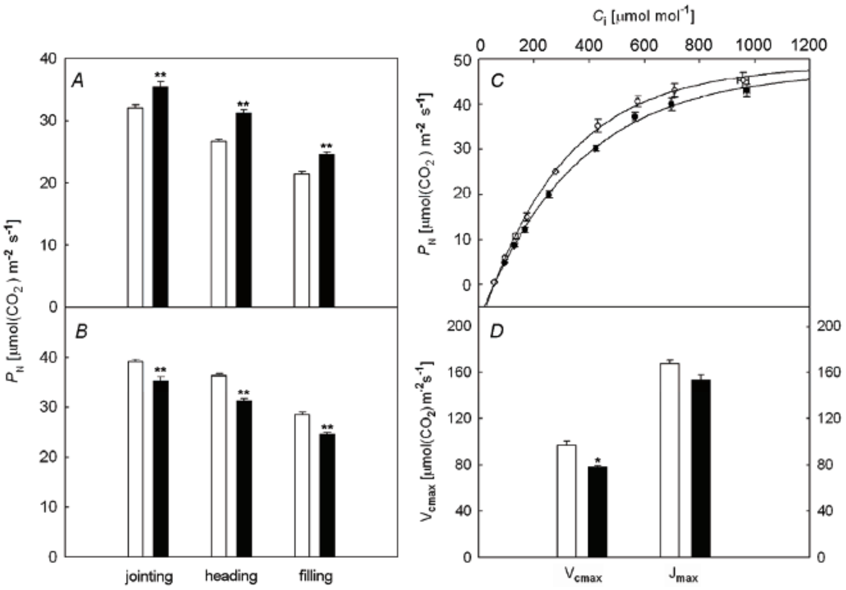

Fig. 1. The effect of elevated CO_2 concentration (EC) on photosynthesis in wheat leaves. (*A*): Net photosynthetic rate (P_N) was measured at wheat growth [CO_2] [380 and 580 μmol mol^{-1} for AC and EC leaves, respectively]. (*B*): P_N was measured at EC. (*C*): The curve of photon-saturated P_N to internal CO_2 concentration (C_i) in wheat leaves at heading stage. (*D*): V_{cmax} and J_{max} *in vivo* calculated from the P_N-C_i data. All P_Ns were measured at 1200 μmol photons m^{-2}s^{-1}. Means ± SE of 3 rings (ten and two leaves in each ring were measured for P_N and P_N-C_i curve, respectively). $^*p<0.05$, $^{**}p<0.01$. *Open circles and columns* represent the AC leaves.

Table 1. Effects of enriched CO_2 concentration (EC) on contents of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO), RuBPCO activase (RCA), RuBP, and sucrose (Saccharose), and SPS (sucrose-phosphate synthase) activity in wheat leaves at the H (heading) and F (filling) stage. Means of 6 leaves±SE. The amounts of RCA in EC leaves are expressed as the percentage of those of AC leaves at each growth stage. ${}^{*}p<0.05$, ${}^{**}p<0.01$. nd – not determined.

		RuBPCO [g m^{-2}]	RCA [%]	RuBP [μmol m^{-2}]	Saccharose [g m^{-2}]	SPS activity [mg m^{-2} s^{-1}]
H	AC	4.6±0.3	100±10	nd	0.35±0.04	0.28±0.06
	EC	3.8±0.2*	70±6**	nd	0.32±0.02	0.56±0.03*
F	AC	1.6±0.2	100±8	341±36	2.04±0.10	0.90±0.06
	EC	1.0±0.1**	76±5**	344±16	2.52±0.20*	1.31±0.08*

capacity is most closely related to a greater cytochrome (Cyt) *f* content (Sudo *et al.* 2003). Moreover, a significant decrease in the Cyt *f* content observed in CO_2-enriched rice leaves (Zhang *et al.* 2008) means that the EC rice leaves had a lower RuBP regeneration capacity. Although the Cyt *f* content in EC wheat leaves was not measured, the basically unchanged J_{max} (Fig. 1*D*) implied that there was no decrease in Cyt *f* content of EC wheat leaves as J_{max} was strongly correlated with Cyt *f* content (Onoda *et al.* 2005). Therefore, the higher and unchanged Cyt *f* content and the down-regulation of RuBPCO and its activase contents may prevent the occurrence of RuBP regeneration limitation in EC wheat leaves.

The down-regulation of RuBPCO and its activase contents in EC wheat leaves (Table 1) may be attributed to larger accumulation of soluble sugars. The fact that sucrose content in EC wheat leaves significantly increased (Table 1) supports the deduction. Also the hypothesis that high sugar content represses the expression of the RuBPCO small subunit gene involved in hexokinase acting as a flux sensor (Moore *et al.* 1999) has been extensively accepted (Long *et al.* 2004, Ainsworth and Rogers 2007).

In summary, the mechanism for photosynthetic acclimation to EC in wheat is different from that in rice. The photosynthetic acclimation in wheat is largely due to RuBP carboxylation limitation rather than RuBP regeneration limitation.

References

Ainsworth, E.A., Rogers, A.: The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. – Plant Cell Environ. 30: 258-270, 2007.

Cen, Y.-P., Sage, R.F.: The regulation of rubisco activity in response to variation in temperature and atmospheric CO_2 partial pressure in sweet potato. − Plant Physiol. 139: 979-990, 2005.

Chen, G.-Y., Yong, Z.-H., Liao, Y., Zhang, D.-Y., Chen, Y., Zhang, H.-B., Chen, J., Zhu, J.-G., Xu, D.-Q.: Photosynthetic acclimation in rice leaves to free-air CO_2 enrichment related to both ribulose-1,5-bisphosphate carboxylation limitation and ribulose-1,5-bisphosphate regeneration limitation. − Plant Cell Physiol. **46**: 1036-1045, 2005.

Hrstka, M., Urban, O., Marek, M.V.: Long-term effect of elevated CO_2 on spatial differentiation of ribulose-1,5-bisphosphate carboxylase/oxygenase activity in Norway spruce canopy. − Photosynthetica 43: 211-216, 2005.

Jacob, J., Greitner, C., Drake, B.G.: Acclimation of photosynthesis in relation to Rubisco and non-structural carbohydrate contents and in situ carboxylase activity in *Scirpus olneyi* grown at elevated CO_2 in the field. − Plant Cell Environ. 18: 875-884, 1995.

Long, S.P., Ainsworth, E.A., Rogers, A., Ort, D.R.: Rising atmospheric carbon dioxide: plant face the future. – Annu. Rev. Plant Biol. 55: 591-628, 2004.

Moore, B.D., Cheng, S.H., Sims, D., Seemann, J.R.: The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO_2. − Plant Cell Environ. 22: 567-582, 1999.

Onoda, Y., Hikosaka, K., Hirose T.: Seasonal change in the balance between capacities of RuBP carboxylation and RuBP regeneration affects CO_2 response of photosynthesis in *Polygonum cuspidatum*. − J. exp. Bot. 56: 755-763, 2005.

Pérez, P., Zita, G., Morcuende, R., Martínez-Carrasco, R.: Elevated CO_2 and temperature differentially affect photosynthesis and resource allocation in flag and penultimate leaves of wheat. – Photosynthetica 45: 9-17, 2007.

Rogers, A., Humphries, S.W.: A mechanistic evaluation of photosynthetic acclimation at elevated CO_2. – Global Change Biol. 6: 1005-1011, 2000.

Sudo, E., Makino, A., Mae, T.: Differences between rice and wheat in ribulose-1,5-bisphosphate regeneration capacity per unit of leaf-N content. − Plant Cell Environ. 26: 255-263, 2003.

Urban, O., Pokorný, R., Kalina, J., Marek, M.V.: Control mechanisms of photosynthetic capacity under elevated CO_2 concentration: evidence from three experiments with Norway spruce trees. − Photosynthetica **41**: 69-75, 2003.

Xu, D.-Q., Gifford, R.M., Chow, W.S.: Photosynthetic acclimation in pea and soybean to high atmospheric CO_2 partial pressure. − Plant Physiol. 106: 661-671, 1994.

Yong, Z.-H., Chen, G.-Y., Zhang, D.-Y., Chen, Y., Chen, J., Zhu, J.-G., Xu, D.-Q.: Is photosynthetic acclimation to free-air CO_2 enrichment (FACE) related to a strong competition for the assimilatory power between carbon assimilation and nitrogen assimilation in rice leaf? – Photosynthetica 45: 85-91, 2007.

Zhang, D.-Y., Chen, G.-Y., Gong, Z.-Y., Chen, J.,Yong, Z.-H., Zhu, J.-G., Xu, D.-Q.: Ribulose-1,5-bisphosphate regeneration limitation in rice leaf photosynthetic acclimation to elevated CO_2. − Plant Sci. 175: 348-355, 2008.