CO₂ Dependence of Gas Exchanges in Rice Leaves as Affected by Temperature, Light and Vapor Saturation Deficit

Katsu IMAI and Akio KANDA*

Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba 305, Japan

** Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan*

(Received June 19, 1995)

Rice plants were grown in a natural light phytotron. When plants had 12–13 leaves on the main stem, they were transferred into an artificially illuminated phytotron and acclimated a whole day to measurement conditions of net photosynthesis and transpiration. The gas exchange rates of attached, uppermost fully-expanded leaves were measured under combinations of ambient CO₂ concentration (5–500 $\mu\text{mol mol}^{-1}$), leaf temperature (20, 25, 35°C), light intensity (400, 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD) and vapor saturation deficit (1.1–1.5, 1.6–2.5 kPa). The maximum net photosynthetic rate with elevating CO₂ was obtained under conditions of high temperature, high light and low vapor saturation deficit. The water use efficiency of leaf expressed as the ratio of net photosynthetic rate to transpiration rate increased with elevating CO₂ concentration and the maximum values were obtained at 25°C under low vapor saturation deficit and at 20°C under high vapor saturation deficit, respectively, irrespective of light levels. The double-reciprocal plots of net photosynthetic rate and intercellular CO₂ concentration showed that the highest temperature (35°C) was effective to promote CO₂ fixation by mesophyll cells under high light conditions. Possible interactions among temperature, light and vapor saturation deficit on the gas exchanges of rice leaves under elevated CO₂ conditions were discussed.

INTRODUCTION

The elevated atmospheric CO₂ concentration (C_a) promotes both the growth and yield of rice plants (Baker *et al.*, 1990; Imai *et al.*, 1985) and, within limits, the promotion is larger at higher than lower temperature (Imai and Murata, 1979; Imai *et al.*, 1985). It is ascribed in part to the increased net photosynthetic rate of leaves. However, the combined effect of high C_a and high temperature on the water use efficiency of rice leaf is not clear (Imai and Okamoto-Sato, 1991) because high temperatures which increase evaporative demand tend to offset the effect of stomatal closure by high C_a (Baker and Allen, 1993). The other environmental factors such as light intensity and vapor saturation deficit (VSD) may, also, affect net photosynthetic rate at elevated C_a through the influences on stomatal and mesophyll conductances (Edwards and Walker, 1983; Löscher and Tenhunen, 1981; Pettigrew *et al.*, 1990; Yabuki, 1985).

Plants grown under elevated C_a often show decreased photosynthetic capacity in leaves with several possible causes (*e.g.*, accumulation of starch in chloroplasts, suppressed Rubisco activity, limitation of RuBP regeneration, *etc.*) and we call this phenomenon photosynthetic acclimation to elevated CO₂ (Imai, 1993). However, such acclimation hardly occurs for rice plants grown under moderately high C_a (500 $\mu\text{mol mol}^{-1}$) with sufficient nutrient and water supply (Imai and Okamoto-Sato, 1991). It is, therefore, worthwhile to estimate the interactions among important factors such as

C_a , temperature, light intensity and VSD by the instantaneous gas exchange measurements of rice leaves developed under sufficient nutrient and water supply to substitute the future conditions of elevated C_a ($<500 \mu\text{mol mol}^{-1}$ until 2030; Watson *et al.*, 1990) as a basis of dry matter production.

MATERIALS AND METHODS

An improved, photo-nonsensitive Thai rice (*Oryza sativa* L. cv. RD7) was used. Seedlings at the 4th leaf stage were planted singly on 1/5000 a Wagner pots with 2.5 kg soil and 5 g chemical compound fertilizer (N: P_2O_5 : K_2O =15: 20: 15, %), and were raised for 55–65 day in a natural light phytotron (25/20°C, day/night temperature, $65 \pm 7\%$ RH and $350 \pm 20 \mu\text{mol mol}^{-1} \text{CO}_2$). During the growth, plants were kept at paddy conditions (3-cm depth of water) and at day 50 they were supplemented with 5 g chemical compound fertilizer per pot.

The gas exchange rates of rice leaves developed in a natural light phytotron were measured in an artificially illuminated phytotron (Koito, KG-156-HL-D with $8 \times 400 \text{ W}$ metal halide, $16 \times 40 \text{ W}$ fluorescent and $3 \times 200 \text{ W}$ incandescent lamps), where plants were acclimated for a 12 h-day and a 12 h-night at 20, 25 or 35°C before measurements. The attached, fully-expanded uppermost leaf (12–13th from the bottom) of main stem was set in an acrylic leaf chamber ($18 \times 40 \times 8 \text{ cm}$) connected with a semi-closed gas exchange measurement system as described before (Kanda *et al.*, 1984).

At measurements, C_a ($5\text{--}500 \mu\text{mol mol}^{-1}$) was changed stepwise (high to low to high, with 11 steps for 30 min each) by adjusting the addition of CO_2 through a mass-flow controller into the stream of CO_2 -free air. Photosynthetic photon flux density (PPFD) at the leaf surface was kept at 400 or $800 \mu\text{mol m}^{-2} \text{s}^{-1}$. Leaf temperature (20, 25 or 35°C), vapor saturation deficit (VSD; 1.1–1.5 or 1.6–2.5 kPa) and wind velocity (1.7 m s^{-1}) inside the leaf chamber were controlled by the gas exchange measurement system adopted (Kanda *et al.*, 1984).

The rates of net photosynthesis and transpiration exhibited slight hystereses and tended to be higher when C_a was changed from the lower to higher levels than the reverse. The water use efficiency of leaf was expressed as the ratio of net photosynthetic rate to transpiration rate. Calculations of stomatal conductance and intercellular CO_2 concentration (C_i) were done after Gaastra (1959) and Wong *et al.* (1978). To estimate the activities of ribulose-1,5-bisphosphate (RuBP) carboxylase, kinetic constants (the Michaelis constant, K_m , for C_i and the maximum velocity, V_{max}) were obtained by making the Lineweaver-Burk double-reciprocal plots of net photosynthetic rates and $(C_i - \Gamma)$ levels. The interceptions of X and Y axes indicate $-1/(\Gamma + K_m)$ and $1/V_{\text{max}}$, respectively, where Γ is the CO_2 compensation concentration (Edwards and Walker, 1983).

The data are expressed as mean values of two sets of measurements.

RESULTS

Figure 1 shows the CO_2 dependence of net photosynthesis and transpiration measured at different leaf temperature (20, 25, 35°C), light intensity (400, $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD) and VSD (1.1–1.5, 1.6–2.5 kPa) levels.

In general, the effect of temperature on net photosynthetic rate was larger at higher than lower light intensity. At higher light, net photosynthetic rate above $200 \mu\text{mol mol}^{-1} C_a$ was the highest at 35°C, followed by 25 and 20°C. These tendencies

were similar when net photosynthetic rate-temperature relation was compared on C_i basis. The CO_2 compensation concentration elevated with temperature rise, suggesting more active photorespiration and dark respiration in such conditions. The response of transpiration to C_a was not so sensitive but at high VSD, the transpiration rate tended to be higher than at low VSD. The promotion of net photosynthetic rate by elevated C_a ($500/300 \mu\text{mol mol}^{-1}$) under high light and low VSD (HL-LD plot) was 44, 46 and 28% at 35, 25 and 20°C , respectively. On the other hand, it was 37, 34 and 30%, respectively, under low light and low VSD (LL-LD plot).

Figure 2 shows the relationship between stomatal conductance and net photosynthetic rate measured at $500 \mu\text{mol mol}^{-1} C_a$ and different light and temperature levels. Irrespective of different conditions measured, stomatal conductance had fairly a close

Fig. 1 Effects of CO_2 concentration, temperature, light intensity and vapor saturation deficit on gas exchange rates in rice leaves.

Open and closed symbols denote responses to ambient and intercellular CO_2 , respectively. $\circ$, $\bullet$, 20° ; $\triangle$, $\blacktriangle$, 25° ; and $\square$, $\blacksquare$, 35°C -leaf temperature. LL, HL: low (400) and high ($800 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD). LD, HD: low (1.1–1.5) and high (1.6–2.5) vapor saturation deficit (kPa).

Fig. 2 Relationship between stomatal conductance and net photosynthetic rate measured at $500 \mu\text{mol mol}^{-1}$ ambient CO_2 and different light, temperature and vapor saturation deficit levels.

Numerals in symbols indicate leaf temperatures ($^\circ\text{C}$). $\circ$, high light ($800 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD) and low vapor saturation deficit (1.1–1.5 kPa); $\triangle$, low light ($400 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD) and low vapor saturation deficit; $\square$, high light and high vapor saturation deficit (1.6–2.5 kPa); ∇ , low light and high vapor saturation deficit.

correlation with net photosynthetic rate ($r=0.641^*$). The low light intensity and the high VSD, generally, induced declines of both stomatal conductance and net photosynthetic rate under all temperatures.

Figure 3 shows the relationship between C_a and water use efficiency of leaves as affected by temperature, light and VSD.

In general, the water use efficiency increased with elevating C_a and light intensity and lowering VSD. Under low VSD, the water use efficiency above $300 \mu\text{mol mol}^{-1} C_a$ was the highest at the moderate temperature (25°C) irrespective of light levels. However under high VSD, this situation changed and the water use efficiency was the best at 20°C . The highest temperature (35°C) brought about the lowest water use efficiency because of a large increase in transpiration rate (Fig. 1). At 35 and 25°C , the high

Fig. 3 Effects of CO_2 concentration, temperature, light intensity and vapor saturation deficit on water use efficiency in rice leaves.

○, 20; △, 25; and □, 35°C -leaf temperature. LL, HL: low (400) and high (800) light ($\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD). LD, HD: low (1.1–1.5) and high (1.6–2.5) vapor saturation deficit (kPa).

Fig. 4 Double reciprocal plots of net photosynthetic rate and intercellular CO_2 concentration measured at different temperature and light levels.

C_i , Γ : intercellular and compensation concentration of CO_2 . 20, 25, 35: leaf temperature ($^\circ\text{C}$). LL, HL: low (400) and high (800) light ($\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD). 20·LL: $Y=1.063X+4.733$ ($R^2=0.980$); 25·LL: $Y=0.870X+3.448$ ($R^2=0.968$); 35·LL: $Y=0.994X+3.230$ ($R^2=0.749$); 20·HL: $Y=0.969X+3.426$ ($R^2=0.982$); 25·HL: $Y=0.755X+2.254$ ($R^2=0.984$); 35·HL: $Y=0.754X+1.660$ ($R^2=0.874$).

VSD induced lower water use efficiencies than those at the low VSD because of decreased net photosynthetic rate relative to increased transpiration rate. At 20°C, however, the effect of VSD was not clear (Fig. 3).

Figure 4 shows the double-reciprocal plots of net photosynthetic rates and $(C_i - \Gamma)$ values of leaves at different temperature and light levels. As there was no practical effect of VSD on C_i (data not shown), we incorporated data points at different VSDs into calculations of the regression lines.

Under low light intensity, the calculated K_m and V_{max} values at 20, 25 and 35°C were, respectively, 179, 202 and 250 $\mu\text{mol mol}^{-1}$ and 21.1, 29.0 and 31.0 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Under high light intensity, the K_m and V_{max} values at 20, 25 and 35°C were, respectively, 242, 284 and 397 $\mu\text{mol mol}^{-1}$ and 29.2, 44.3 and 60.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The K_m values increased with elevating temperature, especially at high light intensity, and coupled with the increases in V_{max} .

DISCUSSION

As shown in Fig. 1, it was obvious that the combination of high C_a (500 $\mu\text{mol mol}^{-1}$) and high temperature (35°C) brought about the highest net photosynthetic rate under conditions of high light (800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD) and low VSD (1.1–1.5 kPa) (HL-LD plot). The effect of VSD on net photosynthetic rate was apparent at 25 and 35°C. When compared with the high light and high VSD (1.6–2.5 kPa) (HL-HD plot), increased net photosynthetic rate in HL-LD plot was coupled with increased stomatal conductance to CO_2 transfer (0.334 *vs.* 0.248 $\text{mol m}^{-2} \text{s}^{-1}$ at 500 $\mu\text{mol mol}^{-1}$ C_a and 35°C, Fig. 2). However at 20°C, the effect of VSD on net photosynthetic rate was not large in our measurements probably because 1) sufficient water supply sustained the water potential of rice leaves at high level as shown in other species (Pettigrew *et al.*, 1990) and 2) rather small difference of VSD was adopted (0.5 kPa). On the other hand, the VSD during plant growth (3.0/0.8 *vs.* 0.5/0.5 kPa, day/night) did not affect net photosynthesis in cotton leaves probably due to increased leaf water potential (Wong, 1993). Therefore, some acclimation may occur if we conduct a long-term treatment.

The effect of temperature on water use efficiency of rice leaves in response to C_a was unclear in our previous experiment (Imai and Okamoto-Sato, 1991). Under low VSD, the water use efficiency was the highest at 25°C irrespective of light levels in the present experiment (Fig. 3). The promotion of transpiration by high temperature (35°C, Fig. 1) decreased the efficiency, although there was partial amelioration by low VSD. Under high VSD, the water use efficiency was the highest at 20°C due to relatively large decrease in stomatal conductance at both light levels (Figs. 1–3). In the present experiment, the differences between high and low VSDs were 0.5 kPa at 20°C and 1.0 kPa at 25 and 35°C. Therefore, the evaporative demand was smaller at 20°C than at 25 and 35°C when compared the effect of VSD. These above mentioned responses suggest the increased evaporative demand by high temperature and the stomatal closure by low temperature, which indicate the importance of stomatal adjustment in balancing carbon gain and water loss (Eams, 1991; Wong *et al.*, 1978).

It is known that rice plants often receive water stress during the daytime irrespective of the growth in paddy fields under submerged conditions (Ishihara *et al.*, 1971). This is often coupled with the enhancement of VSD especially in the afternoon (Pettigrew *et al.*, 1990). Therefore, an elevated C_a as high as 500 $\mu\text{mol mol}^{-1}$ would substantially ameliorate the water economy of paddy rice (Baker and Allen, 1993), at least on leaf area basis, because high C_a increases net photosynthesis while decreases

transpiration. On a plant or a community level, however, this amelioration may be partly offset due to increased leaf area by high C_a (Eams, 1991).

The double-reciprocal plots of net photosynthetic rate and $(C_i - I)$ level demonstrated that a high temperature (*e.g.*, 35°C) expanded the capacity of mesophyll cells to fix CO_2 (V_{max}) when coupled with high light levels (Fig. 4). If we adopted much higher light ($>800 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD), we might have clearer temperature effects because the net photosynthetic rate (Fig. 1) was far below the calculated V_{max} (29.2–60.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$, Fig. 4). The increase in K_m value (an inverse measure of the affinity of RuBP carboxylase for CO_2) with elevating temperature may be less important under conditions of elevated CO_2 because of sufficient substrate (CO_2) supply. These results suggest that the “greenhouse” conditions predicted for the future would reward to rice production under field conditions and, in fact, support our previous findings that under conditions of elevated C_a (700 $\mu\text{mol mol}^{-1}$) and natural light, rice growth and yield were greater at 33/26°C than at 28/21°C (Imai *et al.*, 1985). However, the temperature response curve of net photosynthesis tends to have narrower peak with elevating C_a (Hellmuth, 1971) and if the light factor is not sufficient, the preference of high temperature would be diminished (Figs. 1 and 2). Therefore, we need to identify the threshold temperature that becomes detrimental for rice ontogeny by considering both the cultivar characteristics and the other environmental factors, especially solar radiation.

This work was supported in part by a Grant-in-Aid for Scientific Research (No. 06454047) from the Ministry of Education, Science and Culture of Japan and was a part of the outcome of the Special Research Project on Global Environmental Change at the University of Tsukuba.

REFERENCES

- Baker, J. T., Allen, L. H., Jr., Boote, K. J. 1990. Growth and yield responses of rice to carbon dioxide concentration. *J. Agric. Sci.* **115**: 313–320.
- Baker, J. T., Allen, L. H., Jr. 1993. Effects of CO_2 and temperature on rice: A summary of five growing seasons. *J. Agric. Meteorol.* **48**: 575–582.
- Eams, D. 1991. The interaction of rising CO_2 and temperatures with water use efficiency. *Plant Cell Environ.* **14**: 843–852.
- Edwards, G., Walker, D. 1983. “ C_3 , C_4 : Mechanisms, and Cellular and Environmental Regulation, of Photosynthesis,” Blackwell Sci. Publ., Oxford. 542 pp.
- Gaastra, P. 1959. Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature, and stomatal diffusion resistance. *Meded. Landbouwhogeschool Wageningen* **59**: 1–68.
- Hellmuth, E. O. 1971. The effect of varying air- CO_2 level, leaf temperature, and illuminance on the CO_2 exchange of the dwarf pea, *Pisum sativum* L. var. Meteor. *Photosynthetica* **5**: 190–194.
- Imai, K. 1993. Global environmental change and field crops. *Environ. Control in Biol.* **31**: 129–137 (in Japanese).
- Imai, K., Murata, Y. 1979. Effect of carbon dioxide concentration on growth and dry matter production of crop plants. VII. Influence of light intensity and temperature on the effect of carbon dioxide-enrichment in some C_3 - and C_4 -species. *Jpn. J. Crop Sci.* **48**: 409–417.
- Imai, K., Coleman, D. F., Yanagisawa, T. 1985. Increase in atmospheric partial pressure of carbon dioxide and growth and yield of rice (*Oryza sativa* L.). *Jpn. J. Crop Sci.* **54**: 413–418.
- Imai, K., Okamoto-Sato, M. 1991. Effects of temperature on CO_2 dependence of gas exchanges in C_3 and C_4 crop plants. *Jpn. J. Crop Sci.* **60**: 139–145.
- Ishihara, K., Nishihara, T., Ogura, T. 1971. The relationship between environmental factors and behavior of stomata in the rice plant. II. On the diurnal movement of the stomata. *Proc.*

- Crop Sci. Soc. Jpn. **40**: 497–504 (in Japanese with English summary).
- Kanda, A., Imai, K., Moriya, T., Handa, S., Terajima, T. 1984. A gas exchange measurement system and its application to rice and cassava leaves. Jpn. J. Crop Sci. **53**: 472–478.
- Löscher, R., Tenhunen, J. D. 1981. Stomatal responses to humidity—phenomenon and mechanism. In “Stomatal Physiology” (ed. by Jarvis, P. G., Mansfield, T. A.), Cambridge Univ. Press, Cambridge. p 137–161.
- Pettigrew, W. T., Hesketh, J. D., Peters, D. B., Woolley, J. T. 1990. A vapor pressure deficit effect on crop canopy photosynthesis. Photosynth. Res. **24**: 27–34.
- Watson, R. T., Rodhe, R., Oeschger, H., Siegenthaler, U. 1990. Greenhouse gases and aerosols. In “Climate Change. The IPCC Scientific Assessment” (ed. by Houghton, J. T., Jenkins, B. A., Ephraums, J. J.), Cambridge Univ. Press, Cambridge. p 1–40.
- Wong, S. C. 1993. Interaction between elevated atmospheric concentration of CO₂ and humidity on plant growth: Comparison between cotton and radish. Vegetatio **104/105**: 211–221.
- Wong, S. C., Cowan, I., Farquhar, G. D. 1978. Leaf conductance in relation to assimilation in *Eucalyptus pauciflora* Sieb et Spreng. Plant Physiol. **62**: 670–674.
- Yabuki, K. 1985. “Dynamic Environment of Plants,” Asakura-shoten, Tokyo. 200 pp (in Japanese).

〈和文抄録〉

イネの葉におけるガス交換の CO₂ 濃度依存性と温度、光および飽差の影響

今井 勝・神田 昭夫*

筑波大学農林学系, * 東京大学農学部

自然光ファイトトロンを用いてイネをポット栽培し、主茎の葉数が12～13に達した時に人工光ファイトトロンに移し、1日間順化させた。その後、最上位完全展開葉につき、CO₂ 濃度 (5～500 μmol mol⁻¹)、葉温 (20, 25, 35°C)、光強度 (400, 800 μmol m⁻² s⁻¹ PPFD)、飽差 (1.1～1.5, 1.6～2.5 kPa) の組合せの下でガス交換速度を測定した。高 CO₂ 濃度に対する純光合成速度の最大値は高温、強光、低飽差 (高湿) 条件下で得られた。純光合成と蒸散の比から求めた葉の水利用効率は、CO₂ 濃度上昇に伴って増大し、最大値は光強度にかかわらず、低飽差下で 25°C、高飽差下で 20°C の場合に得られた。純光合成速度と細胞間隙 CO₂ 濃度の逆数プロットの解析から、本研究で用いた程度の高湿 (35°C) は強光下での葉肉細胞による CO₂ 固定を促進することが示された。得られた結果にもとづき、高 CO₂ 濃度下でのイネ葉のガス交換に対する温度、光および飽差の影響に関する論議を行った。