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BRIEF COMMUNICATION

Effect of elevated carbon dioxide concentration on the stomatal parameters of rice cultivars

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Abstract

The response of stomatal parameters of four rice cultivars to atmospheric elevated CO_2 concentration (EC) was studied using open top chambers. EC brought about reduction in stomatal conductance and increase in stomatal index, size of stomatal guard cells, stroma, and epidermal cells. Such acclimation helped the regulation of photosynthesis to EC. These changes in stomatal characters made rice cultivars adjustable to EC environment.

Additional key words: epidermal cell size and density; leaf area and dry mass; net photosynthetic rate; open top chamber; *Oryza sativa*; stomatal size and density; stomatal conductance; stroma.

An exponential rise in atmospheric CO_2 concentration has been observed since pre-industrial period and is expected to reach 600 μmol(CO_2) mol^{-1} by the middle of the 21st century (Keeling *et al.* 1995). Photosynthesis and plant water relations are significantly affected by the increasing atmospheric [CO_2] (Thomas and Harvey 1983). However, very little information is available concerning the effects of EC on stomata that are intimately associated with [CO_2] and water vapour exchange processes. EC significantly reduces stomatal conductance (g_s) in the leaves of higher plants including crops (Field *et al.* 1995, Uprety *et al.* 1995, Ulman *et al.* 2000, Kimball *et al.* 2002) affecting their water status, photosynthesis, and productivity (Pospíšilová and Čatský 1999). This stomatal response to EC requires understanding of the parameters that determine g_s. The experiment described here involves the measurement of stomatal characters in rice (*Oryza sativa* L.) cultivars to determine the effects of growth in EC.

Four rice cultivars, namely Pusa Basmati-1, P-677, P-834, and P-2503-6-693, were transplanted into open top chambers. N, P, and K fertilisers were applied as recommended, *i.e.* 9, 4, and 4 g m^{-2} (90, 40, and 40 kg ha^{-1}) in the form of urea, superphosphate, and potash, respectively, in two split doses. The open top chambers are 3 m in diameter, lined with transparent specialised polythene sheets, constructed and placed on the rice field with the base sealed on to a cement platform. The CO_2 supply to these chambers was regulated throughout the crop season from a manifold for five gas cylinders. Commercial CO_2 was pumped into the chambers along with the chamber air using blowers of 12″ diameter to ensure thorough mixing (Uprety 1998). The CO_2 concentration at the crop canopy level in EC chamber was maintained within 575-620 μmol mol^{-1} throughout the cropping season. The [CO_2] in the ambient CO_2 (AC) chamber ranged within 360-370 μmol mol^{-1} during the crop growing season. The photon flux density in the chamber was 95 % of that in the open field; however, thorough gentle washing of the polythene cover was frequently required to maintain its transparency by removing dust. The air temperature in the EC chamber was the same as in the AC chamber, however, both were 1 °C higher than in the surrounding field. Seedlings were flooded and thinned to 200 plants m^{-2} at the second leaf stage, 9 d after planting. Depth of floodwater was maintained at 5 cm above the soil surface. Relative humidity and temperature inside the chambers were recorded by thermohygrographs throughout the experimental period.

All the cultivars expressed maximum response to EC at flowering stage (Uprety *et al.* 2003). The stomatal measurements were recorded at this stage on uppermost fully expanded leaves. Net photosynthetic rate (P_N) and g_s were measured using a portable infrared gas analyser (*Li-6200*, *Licor*, Lincoln, NE, USA) inside the open top chambers. Leaf area was recorded using a *Licor 3000* leaf area meter. Stomatal density was measured by counting the number of stomata per unit of leaf area using a light microscope at 400× magnification. The stomatal index

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was calculated as number of stomata divided by number of stomata + number of epidermal cells (Woodward 1987). The number of stomata and its size in terms of guard cell length, width, and stroma (pore) length on the abaxial and adaxial surface of leaves were recorded using optical and scanning electron microscope as reported by Kubínová (1994). Four replicates were taken for each observation. Values were analysed statistically by analysis of variance (Snedecor and Cochran 1972).

There was a marked reduction in g_s in the rice cultivars grown in EC in comparison to those grown in AC. The reduction was maximal in Basmati-1 (61 %), followed by P-677 (58 %), P-2503-6-693 (56 %), and P-834 (55 %). The CO_2 enrichment brought about significant reduction in the stomatal density except in P-834 and adaxial surface of leaves of P-2503-6-693. In the abaxial surface of the leaf the reduction was maximal in P-677, followed by Basmati-1, and was minimal in P-2503-6-693. In the adaxial surface, the reduction was observed in Pusa Basmati-1 and P-677 only. It was minimal in Basmati-1 (5 %) compared to 11 % in P-677. The stomatal density was larger in adaxial surface than in the abaxial one. A decrease in stomatal density was found also by Tognetti *et al.* (2001).

The stomatal index was considerably increased in EC chamber. The increase was maximal in P-2503-6-693 in both the abaxial (23 %) and adaxial (33 %) surfaces. The enhancement in Basmati-1 was 18 % in abaxial surface and 20 % in adaxial surface, whereas the increase was less in P-677 (11 % in abaxial surface and 6 % in adaxial surface). In the case of P-834 the response was similar both at abaxial and adaxial surfaces (19 % increase) (Table 1).

The length and width of guard cells of stomata increased in EC and the increase was larger in width compared to length. The increase was larger in P-677 than in P-834 and P-2503-6-693. The stomatal pores were markedly longer in EC in Pusa Basmati-1, P-677 and P-2503-6-693 compared to P-834 (Table 1).

The epidermal cell density was considerably reduced

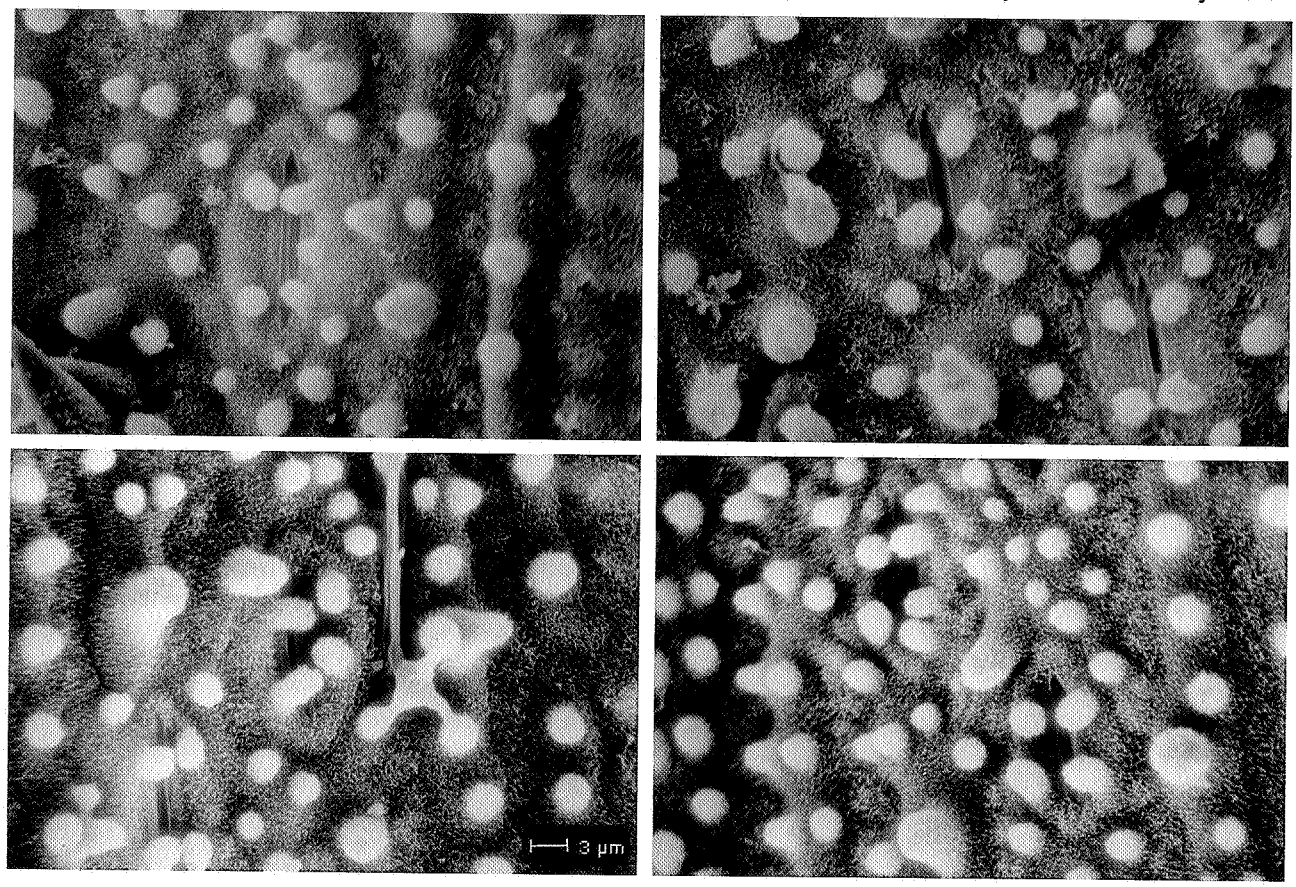

Fig. 1. Electron micrographs show the effect of elevated CO_2 concentration (*top*) on the size of stomata in abaxial (*left*) or adaxial (*right*) leaf surface in rice cultivar Basmati-1 in contrast to ambient CO_2 concentration (*bottom*).

in EC. The reduction was larger in Basmati-1 (35 % in abaxial and 28 % in adaxial surface), P-2503-6-693 (35 % in abaxial and 29 % in adaxial surface), and P-677 (30 % in abaxial and 19 % in adaxial surface) compared to P-834 (15 % in abaxial and 16 % in adaxial surface). The length and width of epidermal cells were markedly increased in EC in Pusa Basmati-1 and P-677 compared to the slight increase in P-834 and P-2503-6-693

(Table 1, Fig. 1).

Growth in EC brought about increase in leaf area which was significant in Pusa Basmati-1 (25 %) and the hybrid P-2503-6-693 (24 %), compared to P-677 (9 %) and P-834 (7 %). The dry mass of leaf per plant was significantly increased in all the four rice cultivars. The enhancement was greater in Basmati-1 (51 %) and hybrid P-2503-6-693 (27 %) than in P-677 (16 %) and P-834 (14 %). The leaf mass per unit area was significantly larger in EC in Basmati-1 compared to the other three cultivars. The specific leaf area, *i.e.* leaf area per unit dry mass, was reduced in EC and the reduction was 17 % in Basmati-1, whereas it was not significant in the other cultivars. P_N was significantly increased in all the cultivars. The increase ranged from 46 % (Basmati-1), 31 % in P-2503-6-693, and 27 % in P-834 to 25 % in P-677 (Table 1).

There have been relatively few studies on the long-

Table 1. Effect of elevated CO_2 concentration (EC) on the leaf, stomatal, and photosynthetic characteristics of four rice cultivars. g_s – stomatal conductance, P_N – net photosynthetic rate. n.s. = not significant.

		$[CO_2]$	Pusa Basmati-1	P-677	P-834	P-2503-6-693	C.D. at 5 % cultivation	treatment	interaction
g_s [mmol m^{-2} s^{-1}]		AC	0.187	0.208	0.170	0.187	0.013	0.013	n.s.
		EC	0.072	0.087	0.075	0.082			
Stomatal density [mm^{-2}]	Abaxial	AC	93.56	128.65	105.26	93.56	3.75	3.75	n.s.
		EC	81.87	105.26	119.88	87.74			
	Adaxial	AC	116.95	149.12	116.95	113.50	4.20	4.20	n.s.
		EC	111.11	132.42	131.57	149.12			
Stomatal index [%]	Abaxial	AC	28.6	31.9	23.9	24.4	1.20	1.20	n.s.
		EC	35.0	35.8	29.5	31.6			
	Adaxial	AC	29.8	29.8	28.1	26.6	1.60	1.60	n.s.
		EC	35.8	31.8	34.6	40.1			
Stomatal size (abaxial) [μm]	Length	AC	20.16	17.52	20.19	19.07	2.62	2.62	n.s.
		EC	21.07	24.00	22.58	19.29			
	Width	AC	10.38	8.87	9.18	8.39	n.s.	1.35	n.s.
		EC	10.95	11.69	11.12	11.50			
(adaxial)	Length	AC	19.80	17.29	18.11	18.14	1.25	1.25	n.s.
		EC	20.29	19.31	18.80	21.71			
	Width	AC	11.30	7. 04	7.51	9.61	1.10	1.10	n.s.
		EC	12.06	7.66	11.46	11.75			
Stroma [μm] (abaxial)	Length	AC	1.55	0.975	1.98	1.03	0.35	0.35	n.s.
		EC	2.09	2.240	2.18	1.66			
(adaxial)		AC	0.876	1.440	0.923	0.696	0.018	0.018	n.s.
		EC	1.860	1.720	0.976	1.660			
Epidermal cell density [mm^{-2}]	Abaxial	AC	233.91	214.85	336.25	290.63	7.25	7.25	9.61
		EC	152.04	192.98	286.54	190.05			
	Adaxial	AC	274.85	350.87	298.24	312.86	8.14	8.14	10.21
		EC	198.82	283.62	248.53	222.22			
Epidermal cell [μm]	Length	AC	82.3	81.30	80.00	102.00	4.85	4.85	n.s.
		EC	106.6	114.60	86.60	104.00			
	Width	AC	22.30	22.60	24.00	25.30	1.92	1.92	n.s.
		EC	26.30	27.30	24.00	29.30			
Leaf area [cm^2 $plant^{-1}$]		AC	781.43	708.53	897.10	88.80	52.06	52.06	73.65
		EC	980.49	775.25	960.20	104.70			
Leaf dry mass [g $plant^{-1}$]		AC	12.17	14.27	17.35	18.00	3.24	3.24	4.56
		EC	18.38	16.54	19.80	22.85			
Leaf area per unit mass [m^2 kg^{-1}]		AC	6.420	4.965	5.170	4.548	4.86	4.86	n.s.
		EC	5.334	4.687	4.849	4.440			
Leaf mass per unit area [g m^{-2}]		AC	15.5	20.1	19.3	22.0	2.1	2.1	n.s.
		EC	18.7	21.3	20.6	22.5			
P_N [μmol m^{-2} s^{-2}]		AC	18.07	13.55	15.26	21.75	4.54	4.54	6.42
		EC	26.45	16.92	19.40	28.50			

term consequences of EC on stomatal properties. The stomatal response to AC requires understanding of the parameters that determine g_s such as stomatal density, stomatal index, size of guard cells and stroma (pore), leaf area, *etc.* Basmati-1 and the hybrid P-2503-6-693 were more responsive to EC compared to P-677 and P-834 in some of the stomatal and leaf-area parameters. The stomata of lower epidermis were more sensitive to EC than those of the upper epidermis. This may result from different stomatal frequencies caused by their greater sensitivity to irradiance than on the upper surface (Turner 1974). The partial opening or closing of stomata results in the reduction of g_s to balance the resource use (Field *et al.* 1995) and to maintain the C_i:C_a ratio under EC (Wall *et al.* 2000). Such changes may occur in the cultivars of rice through the effects of stomatal size and number. These changes in the rice cultivars in the present study also compensate the increase in size of leaves due to EC. Adjustment in stomatal characters may provide a fine control on the rate of water loss and plant water use efficiency and also offer a mechanism for increasing survival and competition ability.

The earlier studies demonstrated that the variation in the CO_2 concentration alone may regulate stomatal density with a mean decrease of 67 % resulting from the 60 μmol mol^{-1} increase in [CO_2] after the pre-industrial period (Woodward 1987). The later changes to doubling [CO_2] could bring about 10-20 % decrease in stomatal density. The less reduction may probably be due to the adjustment and adaptation developed in the process of stomatal development in rising AC. The increase in leaf size generally affects the stomatal density by reducing the irradiance and spreading the stomata differentially. Stomatal density decrease in Basmati-1 and P-677 may be affected by the expansion of leaf area causing the stomata to be present less densely through changes in the size of epidermal cells. According to Tichá (1982) the effect on stomatal density was a consequence of increase in the size of epidermal cells due to greater turgor. Madsen (1975) also observed the reduction in stomatal density in tomato and related it to the [CO_2]-induced decline in leaf nitrogen status. In our study the length and width of epidermal cells was significantly increased by EC in Basmati-1 and P-677 although there was decrease in epidermal cell density indicating greater effect on cell expansion than on cell division. This increase in cell expansion has been attributed to the increased osmotic potential of leaf cells associated with their higher saccharide content which causes the cells to absorb more water and thus enlarge (Delucia *et al.* 1985). There was a consistent effect of EC on the stomatal density and stomatal index in the cultivars Basmati-1 and P-677 and partially in the hybrid rice and these appeared to adjust with EC through changes in stomatal number and size. However, the effect on stomatal density in P-834 was not in the same line. The size of epidermal cell in P-834 was also not significantly affected by EC. These changes in the stomatal parameters may help rice cultivars to regulate the gas exchange processes and adjust under rising atmospheric [CO_2].

References

Delucia, E.H., Sasek, T.W., Strain, B.R.: Photosynthetic inhibition after long term exposure to elevated levels of atmospheric carbon dioxide. – Photosynth. Res. **7**: 175-184, 1985.

Field, C.B., Jackson, R.B., Mooney, H.A.: Stomatal responses to increased CO_2: implications from the plant to global scale. – Plant Cell Environ. **18**: 1214-1225, 1995.

Keeling, C.D., Whorf, M., Wahlen, T.P., Van der Plicht, J.: Interannual extremes in the rate of rise of atmospheric carbon dioxide since 1980. – Nature **375**: 666-670, 1995.

Kimball, B.A., Kobayashi, K., Bindi, M.: Responses of agricultural crops to Free-Air CO_2 Enrichment. – Adv. Agron. **77**: 293-368, 2002.

Kubínová, L.: Recent stereological methods for measuring anatomical characteristics: estimation of the number and sizes of stomata and mesophyll cells. – J. exp. Bot. **45**: 119-127, 1994.

Madsen, E.: Effect of CO_2 enrichment on growth, development, fruit production and fruit quality of tomato – from a physiological view point. – In: Chouard, P., de Bilderling, N. (ed.): Phytotronics III. Pp. 318-330. Gauthier-Villars, Paris 1975.

Pospíšilová, J., Čatský, J.: Development of water stress under increased atmospheric CO_2 concentration. – Biol. Plant. **42**: 1-24, 1999.

Snedecor, G.W., Cochran, W.G.: Statistical Methods Applied to Experiments in Agriculture and Biology. – Iowa State Public., Ames 1972.

Thomas, J.F., Harvey, C.N.: Leaf anatomy of four species of gram under continuous CO_2 enrichment. – Bot. Gaz. **144**: 303-309, 1983.

Tichá, I.: Photosynthetic characteristics during ontogenesis of leaves. 7. Stomatal density and sizes. – Photosynthetica **16**: 375-471, 1982.

Tognetti, R., Sebastiani, L., Vitagliano, C., Raschi, A., Minnocci, A.: Responses of two olive tree (*Olea europaea* L.) cultivars to elevated CO_2 concentration in the field. – Photosynthetica **39**: 403-410, 2001.

Turner, N.C.: Stomatal response to light and water under field conditions. – Roy. Soc. New Zeal. Bull. **12**: 423-432, 1974.

Ulman, P., Čatský, J., Pospíšilová, J.: Photosynthetic traits in wheat grown under decreased and increased CO_2 concentration, and after transfer to natural CO_2 concentration. – Biol. Plant. **43**: 227-237, 2000.

Uprety, D.C.: Carbon dioxide enrichment technology: open top chambers, a new tool for global climate research. – J. sci. ind. Res. **57**: 266-270, 1998.

Uprety, D.C., Dwivedi, N., Jain, V., Mohan, R., Saxena, D.C., Jolly, M., Paswan, G.: Responses of rice cultivars to the elevated CO_2. – Biol. Plant. **46**: 35-39, 2003.

Uprety, D.C., Mishra, R.S., Abrol, Y.P.: Effect of elevated CO_2 on the photosynthesis, growth and water relation of *Brassica* species under moisture stress. – J. Agron. Crop Sci. **175**: 231-237, 1995.

Wall, G.W., Adam, N.R., Brooks, T.J., Kimball, B.A., Pinter, P.J., Jr., LaMorte, R.L., Adamsen, F.J., Hunsaker, D.J., Wechsung, G., Wechsung, F., Grossman-Clarke, S., Leavitt, S.W., Matthias, A.D., Webber, A.N.: Acclimation response of spring wheat in a free-air CO_2 enrichment (FACE) atmosphere with variable soil nitrogen regimes. 2. Net assimilation and stomatal conductance of leaves. – Photosynth. Res. **66**: 79-95, 2000.

Woodward, F.I.: Stomatal numbers are sensitive to increases in CO_2 from pre-industrial levels. – Nature **327**: 617-618, 1987.