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Responses of rice cultivars to the elevated CO_2

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Abstract

The effect of CO_2 concentration elevated to 575 - 620 μmol mol^{-1} on growth, tillering, grain yield, net photosynthetic rate, dark respiration rate, stomatal conductance, sugar content and protein profile of two rice (*Oryza sativa* L.) cultivars Pusa Basmati-1 and Pusa-677 at flowering stage was studied using open top chambers. The cultivar Pusa Basmati-1 responded more markedly for most of the growth and physiological parameters compared to Pusa-677. The increase in grain yield in Pusa Basmati-1 attributed largely to increased grain number. The increased net photosynthetic rate and greater accumulation of sugar contributed significantly to the accelerated development of leaves and tillers in both the cultivars. The reduction in the low molecular mass proteins including Rubisco and increase in high molecular mass photosystem 2 proteins was observed in both the cultivars. Additional sugars may possibly help in balancing the profile of photosynthetic proteins and sustain greater growth and productivity in rice cultivars.

Additional key words: net photosynthetic rate, open top chambers, *Oryza sativa*, protein, respiration rate, stomatal conductance, sugars.

Introduction

The exponential rise in the atmospheric concentration of CO_2 due to fossil fuel burning, industrialization and urbanization, and its expected doubling by the end of 21st century (Keeling *et al.* 1995) has generated considerable interest for the study of the response of crop plants to the elevated CO_2. Open top chamber and FACE technology are currently being used for these studies. Results from such studies have shown an increase in plant photosynthetic rate and crop yield in 430 plant species (Kimball 1983). Elevated CO_2 was highly significant in mitigating the adverse moisture stress effect on various processes in wheat and *Brassica* sp. (Gifford 1979, Uprety *et al.* 1995). Studies also demonstrated the transfer of CO_2 responsive characters from *Brassica campestris* to the hybrid *B. oxycamp* by interspecific hybridization (Uprety *et al.* 1998). However, few studies are available that address the effect of high CO_2 on the physiological and biochemical parameters of rice cultivars (Baker and Allen 1993, Uprety *et al.* 2000). Studies on the Indian rice cultivars demonstrated the significant increase in their growth and yield (Uprety *et al.* 2000) and the characterization of these responses in two rice cultivars has been attempted in the present study.

Materials and methods

Two Indian rice (*Oryza sativa* L.) cultivars Pusa Basmati-1 and Pusa-677 were transplanted in open top chambers. N, P and K fertilizers were applied as per recommended practice, *i.e.* 90, 40 and 40 kg ha^{-1}, in the form of urea, superphosphate and potash, respectively, in two split doses. These open top chambers were of 3 m diameter, lined with transparent specialized polythene sheets, constructed and placed on the rice field with sealed base on the cemented platform. The CO_2 gas was supplied to the chamber from 5 gas cylinders connected by a manifold through the pressure gauge line. Commercial CO_2 gas was pumped in the chambers along

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Abbreviations: CA - ambient CO_2 concentration; CE - elevated CO_2 concentration; g_s - stomatal conductance; P_N - net photosynthetic rate; PS - photosystem, R_D - dark respiration rate.
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with the ambient air using a blower to ensure thorough mixing (Uprety 1998). The air from the chamber at different positions of the crop canopy was measured by the infra red gas analyzer. CO_2 concentration at the crop canopy level was maintained between 575 - 620 μmol mol^{-1} throughout the cropping season. The irradiance in the chamber was 95 % of the open field, however, thorough gentle washing of the polythene cover was frequently required to maintain its transparency, by removing dust. The temperature of the elevated CO_2 chamber remained the same as in the ambient air chamber, however, 1 °C more than outside field. Seedlings were flooded and thinned to 200 plants m^{-2} at the second leaf stage, 9 d after planting. Flood water depth was maintained at 5 cm above the soil surface. Soil of the field was sandy loam. The relative humidity and the temperature inside the chamber were recorded by thermohygrograph throughout the experimental period.

Observations on growth, photosynthesis, respiration, yield and other physiological and biochemical parameters were recorded at vegetative, flowering and post-flowering stages. The response of rice cultivars was similar in these growth stages. The data of flowering stage are described in this paper to avoid repetition. This developmental stage was also chosen because the demand of photosynthate is likely to be high. Days to tillering, leaf initiation and flowering were recorded.

Net photosynthetic rate (P_N), and stomatal conductance (g_s) of the youngest fully developed top most leaf of main shoot was measured by photosynthetic system *Licor-6200* (*Lincoln*, USA), between 11:00 and 11:30. The dark respiration (RD) in terms of CO_2 efflux was measured as reported by Bunce (1990) using *Licor-6200*. Sugars and starch content were estimated on the fully expanded uppermost leaf of main shoot of rice plant. Sugars were extracted by the method of McCready *et al.* (1950). The reducing and total sugar were determined colorimetrically by arsenomolybdate method (Nelson 1944). Non reducing sugar was obtained by subtracting the amount of reducing sugar from that of total sugar. Starch content was determined by anthrone method (McCready *et al.* 1950).

Protein and protein profile study was done in fully expanded 3rd and 5th leaves of the main shoot at flowering stage. Leaf material (1 g) was frozen in liquid nitrogen and separately homogenized in extraction medium consisted of 50 mM Tris HCl (pH 8.8), 1 mM ethylene diamine tetra acetic acid (EDTA), 1 mM phenyl methyl sulfonyl floride (PMSF), 42.9 mM β-mercaptaethanol. Homogenized samples were centrifuged at 12 000 *g* for 10 min and supernatant was collected for the estimation of soluble protein by Bradford method (1976). Analysis of total proteins was done by using denaturing SDS-PAGE discontinous gel electrophoresis method (Laemmli 1970). 30 μg of protein samples were boiled in presence of sodium dodecyl sulphide (SDS) and 2-mercaptoethanol (to reduce disulfide bonds) before loading in the gel lane. The separating gel consisted of 12.5 % of acrylamide. Individual proteins were separated electrophoretically and stained with Coomassie brilliant blue R-250. The proteins were quantified by using two dimensional laser densitometer (*Pharmacia LKB Ultrascan XL*, Uppsala, Sweden).

Plants were sampled into leaf and stem and dried in an oven at 80 °C till constant mass. Plant height, tiller production and number of leaves were recorded. Leaf area was measured by leaf area meter (*Licor-3000*, Lincoln, USA). Seed yield, grain mass and spike number were recorded from harvested plants.

Observations were taken on per plant basis in triplicate. Statistical analysis of the data was done following the method of analysis of variance given by Snedecor and Cochran (1972).

Results

There was marked variation on the response of Basmati-1 and Pusa-677 to elevated CO_2 concentration (CE). The P_N was significantly increased under CE and the increase was greater in Pusa Basmati-1 (46 %) compared to Pusa-677 (25 %). The R_D was reduced and the reduction was 54 % in Pusa Basmati-1 and 31 % in Pusa-677. The g_s decreased in both the cultivars ranging 44 % in Pusa Basmati-1 and 34 % in Pusa-677 (Table 1).

Sugar composition of the rice leaves was significantly affected by CE. The increase in the amount was 26 and 22.7 % for total soluble sugars, 29 and 16 % for reducing sugars and 25 and 17 % for non-reducing sugars in Pusa Basmati-1 and Pusa-677, respectively. Starch content was increased 13 % in Pusa Basmati-1 and 18 % in P-677 (Table 1).

SDS PAGE study on protein profile showed 18 clearly marked protein bands in the gel stained with Coomasie brilliant blue R-250. Sharp decline in the quantity of less molecular mass proteins ranging from 29 to 43 kD under CE was observed in both the cultivars.

Pusa Basmati-1 had higher amount of Rubisco protein compared to Pusa-677. There was sharp decline in the concentration of Rubisco protein under CE (53 % in Pusa Basmati-1 and 69 % in Pusa-677 in the 3rd leaf) and 17 % in Pusa Basmati-1 and 27 % in Pusa-677 in the 5th leaf. The decline in the protein concentration was also observed in the 20 to 29 kDa molecular mass proteins under CE. CE brought about marked increase in the concentration of 68 to 97 kDa proteins in both cultivars (Fig. 1).

Table 1. Effect of elevated CO_2 concentration on net photosynthetic rate (P_N), dark respiration rate (R_D), stomatal conductance (g_s), and sugar and starch contents in rice cultivars.

Cultivar	CO_2	P_N [μmol m^{-2} s^{-1}]	R_D [μmol m^{-2} s^{-1}]	g_s [mmol m^{-2} s^{-1}]	Total sugars [mg g^{-1}(d.m.)]	Red. sugars [mg g^{-1}(d.m.)]	Non-red. sugars [mg g^{-1}(d.m.)]	Starch [mg g^{-1}(d.m.)]
Pusa	CA	18.07	7.46	0.187	56.65	15.68	40.97	26.67
Basmati-1	CE	26.45	3.39	0.105	76.90	22.07	54.84	34.87
Pusa-677	CA	13.55	3.17	0.132	48.87	12.02	40.18	22.19
	CE	16.92	2.17	0.087	63.27	14.39	48.85	28.05
$CD_{0.05}$	cultivars	4.54	1.51	0.013	2.76	1.39	2.15	1.82
	treatments	4.54	1.51	0.013	2.76	1.39	2.15	1.82
	interaction	6.42	n.s.	n.s.	3.54	n.s.	n.s.	2.70

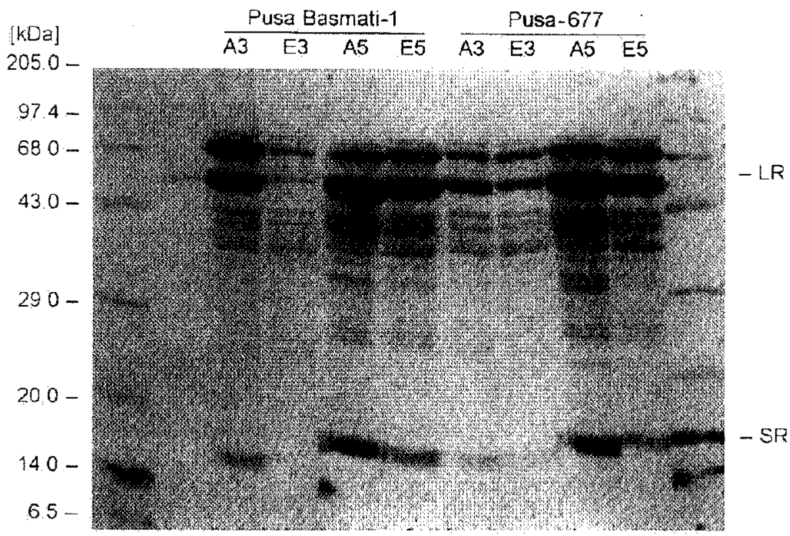

Fig. 1. Effect of elevated CO_2concentration on the protein profile in the leaves of rice cultivars (A3 - third leaf at ambient CO_2 concentration, A5 - fifth leaf at ambient CO_2 concentration, E3 - third leaf at elevated CO_2 concentration, E5 - fifth leaf at elevated CO_2 concentration, LR - larger sub-unit of Rubisco, SR - smaller sub-unit of Rubisco).

Table 2. Effect of elevated CO_2 concentration on the time of tillering, leaf initiation, flowering, and growth characteristics in rice cultivars.

Cultivar	CO_2	Tillering initiation [d]	Leaf initiation [d]	Flowering initiation [d]	Tiller number [plant^{-1}]	Plant height [cm]	Leaf number [plant^{-1}]	Leaf area [cm^2 plant^{-1}]
Pusa	CA	26.0	24.6	37.0	11.33	103.60	76.00	781.43
Basmati-1	CE	23.3	21.6	29.0	14.00	111.00	88.00	980.49
Pusa-677	CA	29.3	31.3	40.3	11.67	73.00	71.67	708.53
	CE	27.6	29.6	37.0	13.33	85.00	79.67	775.25
$CD_{0.05}$	cultivars	1.40	1.75	2.25	1.50	2.28	1.09	52.06
	treatments	1.40	1.75	2.25	1.50	2.28	1.09	52.06
	interaction	n.s.	n.s.	n.s.	2.13	3.32	n.s.	73.65

There was significant earliness in the appearance of tillers and leaves under CE (Table 2). The production of tillers increased 23.5 % in Pusa Basmati-1 and 14 % in Pusa-677. The dry mass of shoot and leaves increased 83.6 and 51 % in Pusa Basmati-1 and to 9 and 13 % in Pusa-677, respectively. The CE brought about significant increase in the production of leaves. Similarly the leaf area increased more in Pusa Basmati-1 (21 %) compared to Pusa-677 (9.4 %) (Table 2). CE brought about significant earliness in the anthesis, which was 8 d earlier in Pusa Basmati-1 compared to 3.3 d in P-677 (Table 2). The number of spike per plant increased in both the cultivars. The number of grains per spike increased more in Pusa-677 compared to Pusa Basmati-1. The grain yield per plant was significantly increased, as high as 40 % in Pusa Basmati-1 compared to 24 % increase in Pusa-677. There was no significant effect of CE on 1000 seed mass in Pusa-677, whereas, it increased to about 7.2 % in Pusa Basmati-1 (Table 3).

Table 3. Effect of elevated CO_2 concentration on the dry mass of shoots, leaves and yield components in rice cultivars.

Cultivar	CO_2	Leaf d.m. [g plant^{-1}]	Stem d.m. [g plant^{-1}]	Yield [g plant^{-1}]	Grain number [plant^{-1}]	Grain mass [g]	Spikelet number [plant^{-1}]
Pusa	CA	12.17	20.65	20.40	1850	25.16	11.97
Basmati-1	CE	18.38	29.66	28.70	2241	26.98	14.33
Pusa-677	CA	21.45	12.71	18.30	1085	25.25	11.67
	CE	23.39	14.32	22.74	1376	25.46	13.00
$CD_{0.05}$	cultivars	3.24	1.08	1.94	95.19	n.s.	2.24
	treatments	3.24	1.08	1.94	95.19	0.67	2.24
	interaction	4.56	1.53	2.75	n.s.	n.s.	n.s.

Discussion

Rice cultivars have been examined for their responses to the CE. It was apparent that at CE both Pusa Basmati-1 and Pusa 677 responded significantly for most of the growth and physiological parameters. The enhanced tillering, shoot growth and greater leaf area were the main cause of increase in dry mass and productivity of these cultivars. The rate of growth of leaves on the tillers and main shoot was faster at CE but the duration was shorter.

It was observed that in cv. Pusa Basmati-1 the increase in grain yield was attributed largely to increased grain number and marginally to grain mass, whereas, in cv. P-677 the increase was mostly due to greater number of grains per plant. The increased grain number was due to greater flux of sugars from the source leaves to the shoot apex which resulted into increased rate of sink development. Presumably there was sufficient flexibility to allow the rice plants to increase their sink capacity at CE during flowering stage. Similar flexibility and greater sink activity was observed in *Brassica oxycamp* hybrid in which CO_2 responsive characters were transferred from the *Brassica campestris* (Uprety *et al.* 1998). In the present study, rice cultivar Pusa Basmati-1 the CO_2 induced increase in P_N was proportionally more due to its greater sink capacity compared to P-677.

The electrophoresis study on protein profile demonstrated depression in the bands of low molecular mass proteins including larger and smaller subunits of Rubisco under CE and this reduction has not affected the CO_2 induced enhancement in the photosynthetic rate of rice cultivars. However, according to Bunce (1990) reduction in the dark respiration may be attributed to the depression in low molecular mass respiratory proteins as observed in the present investigation in rice. Stitt (1991) has suggested that decreased levels of low molecular mass proteins including Rubisco may have resulted from the accumulation of sugars. The increase in higher molecular mass proteins observed in the present study was found parallel to that of the increase in the net photosynthesis in rice cultivars at CE. Evans (1988) correlated the increase in higher molecular mass proteins to the increased light harvesting capacity and photosynthesis. Uprety *et al.* (1998) studied the effect of CE on the photosynthetic efficiency in terms of fluorescence parameters in *Brassica* species and demonstrated their greater light harvesting capacity contributed to the increase in P_N supporting the observations made on rice.

Thus the response of rice cultivars to CE was significant, leading to increase in their grain yield. Number of tillers and grains significantly contributed to this beneficial effect. Additional sugars helped in the production of more tillers and in balancing the protein profile to sustain greater growth and productivity in rice cultivars.

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