Photosynthetic acclimation to elevated CO_2 in wheat cultivars

Poonam SHARMA-NATU, F.A. KHAN and M.C. GHILDIYAL

Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi - 110012, India

Abstract

Wheat (*T. aestivum*) cvs. Kalyansona and Kundan grown under atmospheric (CA) and elevated CO_2 concentrations (650±50 $cm^3\ m^{-3}$ - CE) in open top chambers were examined for net photosynthetic rate (P_N), stomatal limitation (l_S) of P_N, ribulose-1,5-bisphosphate carboxylase (RuBPC) activity, and saccharide content of the leaves. The P_N values of both CA- and CE-grown plants compared at the same CO_2 concentration showed a down regulation under CE at the post-anthesis stage. The negative acclimation of P_N appeared to be due to both stomatal and mesophyll components, and the RuBPC activity got also adjusted. There was a decrease in activation state of RuBPC under CE. In connection with this, an increased accumulation of saccharides in wheat leaf under CE was observed. Kalyansona, owing to its larger sink potential in terms of the number of grains, showed a greater enhancement under CE in both post-ear emergence dry matter production and grain yield. Under CE, this cultivar also showed a lower down regulation of P_N than Kundan.

Additional key words: dry matter production; ear number; grain mass and yield; leaf area; ribulose-1,5-bisphosphate carboxylase; saccharides; starch; stomatal limitation.

Introduction

The rise of CO_2 concentration in the atmosphere has led to efforts to determine the impact of this change on crop species (Bowes 1993, Saralabai *et al.* 1997). However, a very few studies have dealt with the effect of CO_2 enrichment on plant species in a tropical environment (Ziska *et al.* 1991). Because in the present atmosphere CO_2 is a substrate which limits photosynthesis particularly in C_3 plants, an increase in P_N has been demonstrated in short-term studies (Kimball 1983). Photosynthetic response to CO_2 decreases under a long-term exposure, and plants acclimate to CE (Peet *et al.* 1986, Sage *et al.* 1989). The acclimation to CE usually results in a down regulation of CO_2 fixation, although long-term positive changes in carbon fixation rate have

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Fax: 91-011-5740722, 5751719, e-mail: Guest%bic-iari@dbt.ernet.in
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also been reported in some species (Campbell *et al.* 1990, Ziska *et al.* 1990). The information on photosynthetic acclimation within one species is, however, meagre.

One of the factors that may influence the photosynthetic acclimation to CE is the imbalance in supply and demand of the saccharides resulting in the end product inhibition (Neales and Incoll 1968, Herold 1980). The RuBPC activity may also be regulated accordingly (Sharma-Natu and Ghildiyal 1993, Anwaruzzaman *et al.* 1995). A high metabolic or storage sink capacity would, therefore, be required for sustained photosynthetic response to CO_2. Wheat cultivars show hysteresis in their response to CO_2 (Greiner de Mothes 1996, Greiner de Mothes *et al.* 1996). They also differ much in sink potentials owing to the number of grains. Furthermore, a negative correlation between the grain number and grain size has been frequently observed, and it is usually ascribed to a limitation in availability of assimilates per grain (Slafer *et al.* 1996, Sunita Kumari and Ghildiyal 1997). The present study, therefore, examines the photosynthetic acclimation to CE during grain development in two wheat cultivars with different grain numbers per ear, in an attempt to analyse the effect of sink demand on the photosynthetic acclimation to CE.

Materials and methods

Two wheat genotypes, *Triticum aestivum* cvs. Kalyansona and Kundan, were grown in the field inside open top chambers (200×160 cm). The construction of the open top chambers was based on the design of Leadley and Drake (1993). One chamber with an ambient CO_2 concentration served as a control, whereas CO_2 concentration in the other one was elevated to *ca.* 650±50 $cm^3\ m^{-3}$. Spacing of the plants was 20 cm between rows and 2.5 cm within a row. Fertilizers were applied to the soil at the rate of 12, 6, and 6 g m^{-2} of N, P, and K, respectively. N was supplied in two split doses, whereas P and K were given only at the time of sowing. The ear emergence and anthesis dates of the main shoot (MS) ear were recorded on tags placed on each plant. Dry matter components expressed per plant were determined at ear emergence and maturity stages. Three plants formed one replication. There were at least four replications for each observation at each sampling.

P_N of the flag leaf of MS of CA- and CE-grown plants was measured between 10.00 and 11.00 h, 10 d after anthesis at different CO_2 concentrations, using a *Li-Cor 6200* (*Li-Cor*, Lincoln, NE, USA) portable photosynthetic system, and P_N *versus* internal CO_2 concentration (C_i) curves were generated (McDermitt *et al.* 1989). The stomatal limitation (l_S) of P_N was calculated from this dependence using the formula described by Farquhar and Sharkey (1982). The incidental photosynthetic photon flux density during gas exchange measurement was saturating (>1200 μmol $m^{-2}\ s^{-1}$ upon the leaf surface). The comparable leaves were sampled for sugar and starch estimations at around 11.00 h, plunged into boiling 95 % ethanol for 2 min, and preserved. Reducing, non-reducing, total sugar, and starch contents were determined according to Ghildiyal and Sinha (1977). RuBPC activity of rapidly extracted enzyme (initial activity) and after activation (total activity) was determined in the flag leaf of main shoot at around 11.00 h.

RuBPC was rapidly extracted following the method of Servaites *et al.* (1984). The RuBPC activities were estimated by RuBP-dependent incorporation of $^{14}CO_2$ into an acid stable product. 'Initial' activities were measured at 25 °C by injecting 50 mm³ of 5 mM RuBP and 25 mm³ of soluble leaf extract into an assay mixture containing (final concentrations): 50 mM Tris-HCl (pH 8.0), 20 mM $MgCl_2$, 0.1 % (m/v) bovine serum albumin, 10 mM $NaH^{14}CO_3$ (74 kBq per assay) in a total volume of 0.5 cm³. The reaction was terminated after 60 s by adding 100 mm³ of 6 M acetic acid; the material was dried at 65 °C, and the acid stable ^{14}C was estimated by liquid scintillation counting. 'Total' activities were determined in a similar way except that 25 mm³ of the soluble leaf extract and 425 mm³ of the assay mixture were incubated together for 10 min at 25 °C before 50 mm³ of 5 mM RuBP were added. From the initial and total activities the % activation of the enzyme was calculated (Servaites *et al.* 1984).

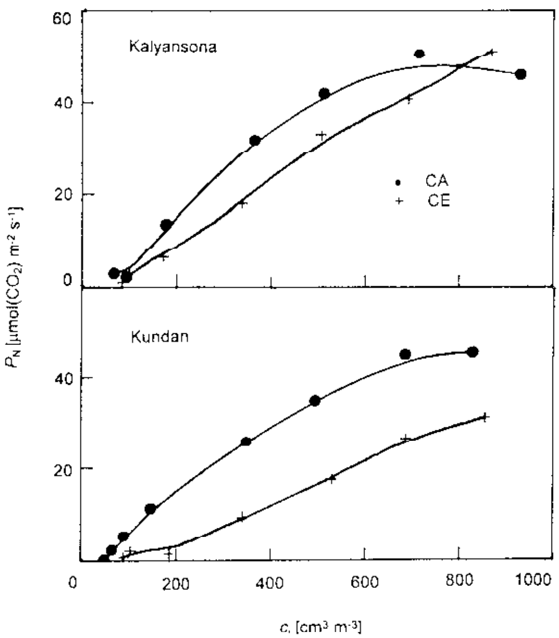

Fig. 1. The net photosynthetic rate (P_N) *versus* internal CO_2 concentration (C_i) of post-anthesis flag leaf of two wheat cultivars grown under control (CA) and elevated (CE) CO_2 concentrations.

Results

The P_N/C_i curves of CA- and CE-grown plants of Kalyansona and Kundan at the post-anthesis stage are shown in Fig. 1. The CE plants had a lower P_N value at any C_i except beyond 800 cm³(CO_2) m⁻³ in Kalyansona. This down regulation of P_N was, however, larger in Kundan than in Kalyansona. C_i's of 287 and 534 cm³ m⁻³ were, respectively, the mean C_i's of all plants measured at CA (350 cm³ m⁻³) and CE (650 cm³ m⁻³) (Table 1). The CE-grown plants at both low and high C_i showed a lower P_N than the CA plants. The decrease in P_N between CA and CE Kalyansona plants was

43 % when measured at a low C_i, and 19 % under a high C_i. In Kundan, the comparable decrease was much larger, 66 and 53 %. P_N measured at the respective CO_2 concentrations was, however, 43 % larger in CE-grown Kalyansona plants, whereas a 21 % decrease was observed in CE-grown Kundan (Table 1). The % limitation of P_N by stomatal resistance was higher in plants grown in CE at both measurement concentrations but the increase was larger when measured at CE. The cv. Kundan showed a greater increase than Kalyansona in this respect (Table 1).

Table 1. A comparison of properties of P_N/C_i curves of plants grown under control (CA) and elevated (CE) CO_2 concentration. P_N, net photosynthetic rate [μmol(CO_2) m^{-2} s^{-1}]; l_S, stomatal limitation [%]; C_i of 287 and 534 cm^3 m^{-3} are the mean C_i's of all plants measured at control and elevated CO_2 concentrations, respectively.

Cultivar	Measurements	CA	CE	CE/CA
Kalyansona	P_N at C_i 287 cm^3 m^{-3}	24.50	14.00	0.57
	P_N at C_i 534 cm^3 m^{-3}	43.00	35.00	0.81
	l_s at C_a 350 cm^3 m^{-3}	20.00	24.32	1.22
	l_s at C_a 650 cm^3 m^{-3}	7.29	10.25	1.41
Kundan	P_N at C_i 287 cm^3 m^{-3}	22.00	7.50	0.34
	P_N at C_i 534 cm^3 m^{-3}	37.50	17.50	0.47
	l_s at C_a 350 cm^3 m^{-3}	15.38	21.05	1.37
	l_s at C_a 650 cm^3 m^{-3}	11.76	27.08	2.30

Table 2. Initial and total RuBPC activity [μmol(CO_2) kg^{-1}(f.m.) s^{-1}] in the leaves of two wheat cultivars grown under control (CA) and elevated (CE) CO_2 concentrations.

Cultivar		Initial activity	Total activity	Activation [%]
Kalyansona	CA	171.50 ± 22.83	160.16 ± 6.00	107.08
	CE	90.00 ± 3.66	149.16 ± 14.83	60.33
	CE/CA	0.52	0.93	0.56
Kundan	CA	136.16 ± 18.16	153.33 ± 15.66	88.80
	CE	83.33 ± 3.66	137.66 ± 8.16	60.53
	CE/CA	0.61	0.90	0.68

The initial RuBPC activity was lower in the CE plants, while the total activity did not differ significantly between the treatments (Table 2). This confirmed a decrease in activation state of the enzyme under CE. Both Kalyansona and Kundan showed a more or less similar activation state under CE. A significant increase in the concentration of non-reducing sugars, total sugars, and starch in the flag leaf was observed under CE compared to CA plants of the cv. Kundan (Table 3). Kalyansona showed an increase of 119 % in non-reducing sugars, and 108 % in the total sugar content under CE, as compared to CA plants. Though the increase in leaf sugar concentration by CE was higher in Kalyansona than in Kundan, actual concentrations of sugars and starch in the leaves were higher in Kundan. The cv. Kalyansona showed a greater enhancement than Kundan in post-ear emergence dry matter production and grain yield under CE (Table 4). The increase in CE Kalyansona grain yield was due to a significant increase in ear number per plant and in seed size.

Table 3. Sugar and starch contents [g kg^{-1}(d.m.)] in the leaves of two wheat cultivars grown under control (CA) and elevated (CE) CO_2 concentrations.

Cultivar	Saccharides	CA	CE	CE/CA
Kalyansona	Reducing sugars	2.18 ± 1.21	3.77 ± 0.49	1.73
	Non-reducing sugars	7.24 ± 1.34	15.87 ± 1.74	2.19
	Total sugars	9.42 ± 1.14	19.64 ± 1.84	2.08
	Starch	144.88 ± 4.02	152.12 ± 4.61	1.05
Kundan	Reducing sugars	11.10 ± 0.55	11.80 ± 0.78	1.06
	Non-reducing sugars	24.07 ± 0.72	31.25 ± 2.26	1.30
	Total sugars	35.17 ± 1.28	43.05 ± 2.43	1.22
	Starch	193.61 ± 6.84	213.36 ± 6.64	1.10

Table 4. Dry matter production and grain yield components of two wheat cultivars grown under control (CA) and elevated (CE) CO_2 concentrations, measured at ear emergence (EE) or maturity (M).

	Cultivar	CA	CE	% increase
Leaf area, EE [cm^2 $plant^{-1}$]	Kalyansona	220.12 ± 12.88	258.28 ± 21.26	17.33
	Kundan	167.73 ± 3.09	221.64 ± 22.01	32.14
Dry matter, EE [g $plant^{-1}$]	Kalyansona	3.99 ± 0.15	4.51 ± 0.23	13.03
	Kundan	2.62 ± 0.24	3.72 ± 0.06	41.98
Dry matter, M [g $plant^{-1}$]	Kalyansona	11.86 ± 0.64	17.01 ± 1.44	43.42
	Kundan	8.74 ± 0.37	10.37 ± 0.25	18.65
Δdry matter (M - EE)	Kalyansona	7.87 ± 0.68	12.49 ± 1.18	58.70
	Kundan	6.12 ± 0.33	6.65 ± 0.19	8.66
Grain yield [g $plant^{-1}$]	Kalyansona	5.08 ± 0.36	7.46 ± 0.99	46.85
	Kundan	4.23 ± 0.44	4.63 ± 0.25	9.45
Ear number [per plant]	Kalyansona	3.00 ± 0.10	3.50 ± 0.29	16.66
	Kundan	3.50 ± 0.29	3.25 ± 0.25	-7.15
Grain number [per ear]	Kalyansona	55.65 ± 1.28	59.04 ± 1.93	6.09
	Kundan	27.34 ± 2.55	30.59 ± 2.64	11.89
1000 grain mass [g]	Kalyansona	30.44 ± 1.07	36.08 ± 0.53	18.53
	Kundan	44.18 ± 3.35	46.62 ± 3.50	5.52

Discussion

In the present study, the wheat cv. Kalyansona having a larger sink potential in terms of number of grains showed a greater enhancement in post-ear emergence dry matter production and grain yield when grown under CE (Table 4). This cultivar also exhibited a lesser down regulation of P_N under CE than the cv. Kundan (Fig. 1, Table 1).

The negative acclimation of P_N to CE appeared to be due to both stomatal and mesophyll components. There was an increase in l_s of P_N under CE in both the cultivars, with Kundan showing a greater increase than Kalyansona (Table 1). The comparison of P_N at the same C_i (when stomatal factor was eliminated) showed that P_N was still lower in CE plants, indicating photosynthetic acclimation or adjustment at the level of mesophyll component as well. Furthermore, the RuBPC activity was decreased due to a decrease in activation state of the enzyme under CE (Table 2). The lower activation state of the enzyme may decrease the *in vivo* performance of the enzyme at a sub-optimal CO_2 concentration under CA, but may not be so much detrimental under CE (Bowes 1991). This possibly could be one of the acclimation responses under CE to divert the energy towards the more limiting processes (Mott 1990, Bowes 1993). The decrease in activation state of RuBPC under CE has also been reported in other species (Sage *et al.* 1989, Rowland-Bamford *et al.* 1991).

Both cultivars showed a more or less similar activation state of RuBPC under CE, but this cannot explain the differential down regulation of photosynthesis. There was also a greater increase in % limitation of photosynthesis by stomatal resistance in CE plants of Kundan than Kalyansona.

Imbalance in supply and demand of the saccharides resulting in the end product inhibition may be one of the reasons of down regulation of P_N under CE (Arp 1991, Bowes 1993). The inhibition of P_N by saccharide accumulation could be effected *via* a decrease in activation state of RuBPC (Sharma-Natu and Ghildiyal 1993, Anwaruzzaman *et al.* 1995) or *via* regulation of the photosynthetic genes expression (Koch 1996). In the present study the saccharide content in the leaves was higher in CE compared to CA plants of both cultivars (Table 3). Yet the saccharides concentration was higher in Kundan than in Kalyansona which may possibly suggest a greater end product inhibition commensurate with greater down regulation of P_N in this cultivar. The study, therefore, showed that wheat cultivars with larger sink potentials would exhibit a relatively better photosynthetic acclimation and performance under CE.

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