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Photosynthetic response of wheat and sunflower cultivars to long-term exposure of elevated carbon dioxide concentration

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Abstract

Wheat (*Triticum aestivum* L. cv. HD 2329 and DL 1266-5) and sunflower (*Helianthus annuus* L. cv. MSFH 17 and MRSF 1754) plants were grown in field under atmospheric (360±10 $cm^3 m^{-3}$, AC) and elevated (650±50 $cm^3 m^{-3}$, EC) CO_2 concentrations in open top chambers for entire period of growth and development till maturity. Net photosynthetic rate (P_N) of wheat cvs. when compared at the same internal CO_2 concentration (C_i), by generating P_N/C_i curves, showed lower P_N in EC plants than in AC ones. EC-grown wheat cultivars also showed a lesser response to irradiance than AC plants. In sunflower cultivars, P_N/C_i curves and irradiance response curves were not significantly different in AC and EC plants. CO_2 and irradiance responses of photosynthesis, therefore, further revealed a down-regulation of P_N in wheat but not so in sunflower under long-term CO_2 enrichment. Wheat cvs. accumulated in leaves mostly sugars, whereas sunflower accumulated mainly starch. This further strengthened the view that accumulation of excess assimilates in the leaves under EC as starch is not inhibitory to P_N.

Additional key words: cultivar differences; feedback inhibition; *Helianthus*; photosynthetic acclimation; species differences; starch; sugars; *Triticum*.

Introduction

Atmospheric CO_2 concentration [CO_2] has risen from pre-industrial value of about 280 $cm^3 m^{-3}$ to present concentration of 372 $cm^3 m^{-3}$ and is expected to cross 700 $cm^3 m^{-3}$ by the end of this century (Prentice *et al.* 2001, Long *et al*. 2004). Attempts are, therefore, being made to analyse how crop plants are going to respond to such change in [CO_2] (Bowes 1993, Drake *et al.* 1997, Long *et al.* 2004). The information available on the effect of CO_2 enrichment on plant species in a tropical environment is, however, meager (Ziska *et al.* 1991, Fernández *et al*. 1999, Ghildiyal and Sharma-Natu 2000). Since CO_2 is a substrate limiting photosynthesis in C_3 plants in the present atmosphere, the impact of elevated [CO_2] (EC) would depend mainly on how photosynthesis acclimates or adjusts in long-term high CO_2 environment.

Photosynthetic response to EC decreases under long term exposure relative to short term exposure in many plant species (Cure and Acock 1986, Sage *et al.* 1989). This indicates that photosynthetic properties of leaves of EC grown plants differ from those of ambient [CO_2] (AC) grown plants. Such a change or adjustment in photosynthetic properties, consequently the photosynthetic efficiency of leaves due to long term exposure to EC is called photosynthetic acclimation. Acclimation has generally become synonymous with the word response, if long-term exposure to EC decreases P_N at a given [CO_2], it is called negative, if it stimulates the P_N at a given [CO_2], it is called positive acclimation. Photosynthetic acclimation is clearly revealed by comparing P_N of AC and EC plants at the same [CO_2] (Drake *et al.* 1997). Such a comparison of photosynthetic efficiency (P_N) of AC and EC plants at the same [CO_2] can be made more appropriately by generating P_N *versus* internal [CO_2] (C_i) curves (Sage 1994).

Acclimation to EC usually results in a down-regulation of CO_2 fixation, although long-term positive changes in carbon fixation rates have also been reported in some species (Sage 1994, Stitt 1996, Sharma-Natu *et al.* 1997, 2004). Plant species not showing down-regulation of P_N under EC may possibly utilize available high CO_2 resource more effectively. We have earlier observed different photosynthetic acclimation of EC in wheat and sunflower. The present study attempts to evaluate the differential photosynthetic acclimation of these species in terms of their irradiance and CO_2 responses.

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Materials and methods

Wheat (*Triticum aestivum* L.cv. HD 2329 and DL 1266-5) and sunflower (*Helianthus annuus* L. cv. MSFH 17 and MRSF 1754) were grown in field under atmospheric (360±10 $cm^3\ m^{-3}$, AC) and elevated (650±50 $cm^3\ m^{-3}$, EC) CO_2 concentrations inside open top chambers (OTCs) for entire period of growth and development till maturity. The construction of OTC's (300×200 cm) was based on the design of Leadley and Drake (1993). Standard cultural practices were followed (Singh 1983). Date of anthesis/flowering in the main shoot (MS) was recorded on the tags placed on each plant.

P_N of the flag leaf (uppermost fully expanded leaf) of main shoot (MS) of AC and EC plants was measured at different [CO_2] at anthesis stage using portable photosynthetic system (*CIRAS-2*, *PP Systems*, UK). *CIRAS-2* allows measurement of steady state photosynthesis rate at constant irradiance supplied by the LED light source at a given [CO_2] supplied by CO_2 cartridge. The sample leaf was enclosed in the assimilation chamber which received incident photons of constant saturating irradiance (>1 500 µmol $m^{-2}\ s^{-1}$ upon the leaf surface) from light source. The chamber CO_2 was programmed for different [CO_2]. The observations of P_N measured at different external [CO_2] (C_a) and computed values of C_i were recorded in AC and EC plants. The P_N *versus* C_i curves were then constructed. The chamber irradiances were programmed at constant [CO_2] of 360 or 650 $cm^3\ m^{-3}$. Irradiance response curves of both AC and EC plants were determined at the same [CO_2] of either 360 or 650 $cm^3\ m^{-3}$. There were three replications for each observation. Data were computed statistically by analysis of variance (ANOVA).

The comparable leaves were sampled for sugar and starch estimation, plunged into boiling 95 % ethanol for 2 min, and preserved. Contents of reducing, non-reducing, and total sugars and starch were determined as described by Ghildiyal and Sinha (1977). There were three replications for each determination.

Results

P_N *versus* C_i curves of AC and EC wheat cvs. HD 2329 (Fig. 1*A*) and DL 1266-5 (Fig. 1*B*) showed a down-regulation of P_N under EC except at around 100 $cm^3\ m^{-3}$ C_i. At any other C_i, P_N was significantly lower in EC plants than in AC-plants. Since a comparison of P_N of AC and EC plants at the same C_i eliminates stomatal component, this decrease in P_N under EC may be contributed by mesophyll limitation. P_N/C_i curves of sunflower genotypes MSFH 17 (Fig. 1*C*) and MRSF 1754 (Fig. 1*D*) showed no significant difference between AC and EC plants indicating no down-regulation of P_N in EC plants.

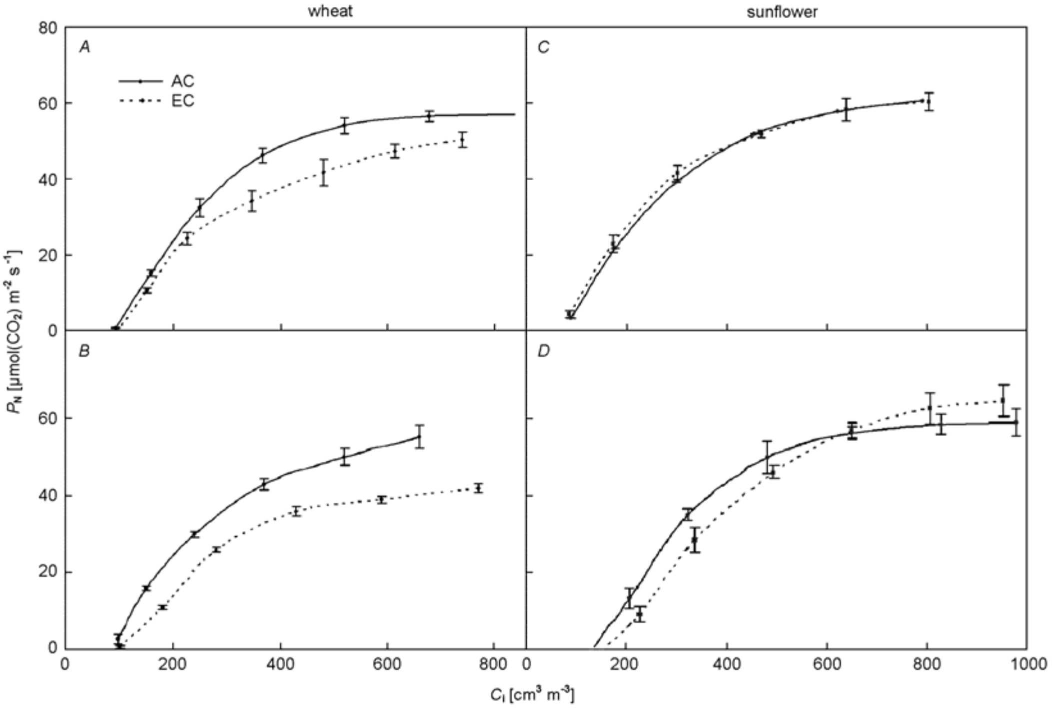

Fig. 1. Net photosynthetic rate (P_N) *versus* internal CO_2 concentration (C_i) in wheat genotypes HD 2329 (*A*) and DL 1266-5 (*B*) and sunflower genotypes MSFH 17 (*C*) and MRSF 1754 (*D*) grown in ambient (AC) and elevated (EC) CO_2 concentrations.

P_N *versus* irradiance curves showed that in wheat cv. HD 2329, P_N continued to respond to higher irradiances up to 1 500 µmol m^{-2} s^{-1} PAR when measurements of AC and EC grown plants were made at EC (Fig. 2*B*). In AC and EC plants measured at AC, P_N was also saturated at around 1 500 µmol m^{-2} s^{-1} PAR (Fig. 2*A*). In DL 1266-5, P_N of both AC and EC plants measured at EC continued to respond to higher irradiances even beyond 1 500 µmol m^{-2} s^{-1} PAR (Fig. 2*D*). In AC and EC plants measured at AC, P_N was saturated earlier (Fig. 2*C*). There were marked differences in irradiance response curves of AC and EC grown wheat cvs. when measured at AC or EC. AC-plants showed a greater response to increasing irradiance than EC-plants, even if measurements were taken at the same [CO$_2$], indicating a decrease in the photosynthetic capacity of EC plants.

In sunflower genotype MSFH 17, P_N of AC and EC plants when measured at EC continued to respond to higher irradiances (up to 1 500 µmol m^{-2} s^{-1} PAR) (Fig. 3*B*) compared to measurements at AC (Fig. 3*A*). P_N of AC and EC sunflower genotype MRSF 1754 also continued to respond to higher irradiance when measured at EC (Fig. 3*D*) compared to measurements at AC (Fig. 3*C*). Irradiance response curve of AC and EC grown sunflower genotypes when measured at the same [CO$_2$] did not differ significantly, showing no down regulation of P_N in EC sunflower plants.

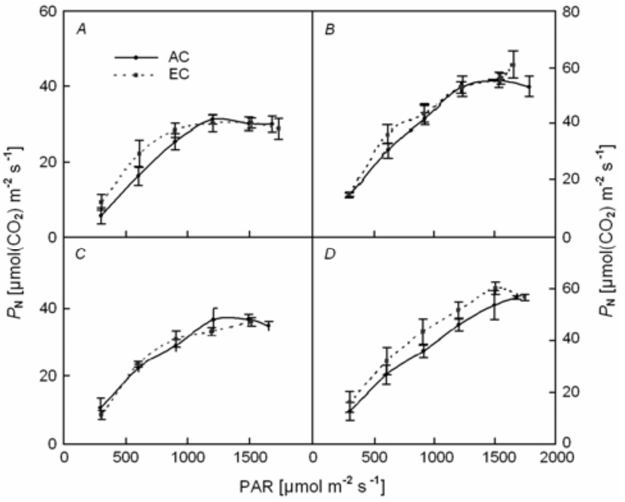

Fig. 3. The net photosynthetic rate (P_N) *versus* photosynthetically active radiation (PAR) in sunflower genotypes MSFH 17 (*A*, *B*) and MRSF 1754 (*C*, *D*) grown in ambient (AC) and elevated (EC) CO$_2$ concentrations and measured at AC (*A*,*C*) or EC (*B*,*D*).

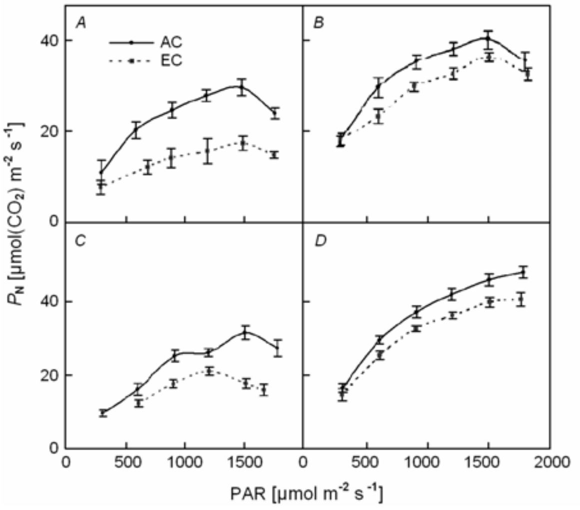

Fig. 2. The net photosynthetic rate (P_N) *versus* photosynthetically active radiation (PAR) in wheat genotypes HD 2329 (*A*, *B*) and DL 1266-5 (*C*, *D*) grown in ambient (AC) and elevated (EC) CO$_2$ concentrations and measured at AC (*A*,*C*) or EC (*B*,*D*).

The reducing, non-reducing, and total sugar contents in the leaves of EC plants were significantly higher than in AC plants in wheat cvs. (Table 1). There was no significant effect of EC on starch content in the leaves of HD 2329. However, in DL 1266-5, some increase in leaf starch content under EC compared to that of AC was observed. In sunflower cvs., leaf sugar content was low and did not increase, rather a decrease was observed under EC. Only in MRSF 1754, reducing sugar content in the leaves showed some increase under EC. This cv. also had relatively higher leaf sugar content than MSFH 17. Sunflower cvs. accumulated excess assimilates mainly as starch in the leaves. A significant increase in leaf starch content under EC compared to AC was observed in both cvs. of sunflower (Table 1).

Wheat cvs., therefore, showed a down-regulation of P_N in EC plants as revealed from P_N/C_i and irradiance response curves (Figs. 1 and 2) and also accumulated higher contents of sugars, particularly the non-reducing sugars in the leaves under EC. On the other hand, sunflower cvs. showed increased accumulation of starch in the leaves under EC and showed no down regulation of P_N under EC. Therefore, the accumulation of starch in the leaves was not associated with a decrease in P_N under EC.

Discussion

P_N of AC and EC grown wheat cvs., when compared at the same C_i, showed a lower P_N in EC-plants. Furthermore, EC plants of wheat cvs. showed also a lesser response to irradiance than AC-plants. On the other hand, in sunflower cvs. the P_N/C_i curves and irradiance response curves were not significantly different in AC and EC plants. This study, therefore, provided further evidence of a decrease in photosynthetic capacity of the leaves under long-term CO$_2$ enrichment in wheat but not so in sunflower.

A comparison of P_N of AC and EC plants at the same C_i eliminates stomatal component. Therefore, this decrease in P_N under EC in wheat may be due to mesophyll limitation. The initial slope of P_N/C_i curve indicates the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) limitation (Stitt 1991). In the present study, initial slope of P_N/C_i curve of wheat cvs. was lower in EC than AC plants, indicating RuBPCO limitation. These observations are in line with the reports that the most prominent change in leaf photosynthetic apparatus under EC is a decline in the amount of RuBPCO (Sage 1994, Drake *et al.* 1997, Ghildiyal and Sharma-Natu 2000, Long *et al.* 2004). Possibly due to this decrease in RuBPCO content, EC-wheat showed a lesser response to irradiance compared to AC-wheat. In sunflower, RuBPCO protein content remained unaffected in EC-plants relative to AC-plants (Ghildiyal *et al.* 2001). This possibly explains why sunflower showed no significant difference in AC and EC plants in P_N/C_i and irradiance response curves.

Table 1. Sugar and starch contents [g kg^{-1}(d.m.)] in the leaves of wheat and sunflower cultivars grown under ambient (AC) and elevated (EC) CO_2 concentrations. LSD at 5 % *p*; NS = not significant.

Plant	Cultivar	Saccharides	AC	EC	LSD
Wheat	HD 2329	Reducing sugars	1.11	6.83	1.79
		Non-reducing sugars	71.42	95.46	14.93
		Total sugars	72.53	102.29	13.55
		Starch	74.18	71.75	NS
	DL 1266-5	Reducing sugars	6.26	13.04	2.99
		Non-reducing sugars	66.91	86.58	12.46
		Total sugars	73.17	99.62	12.77
		Starch	77.11	87.51	6.20
Sunflower	MSFH 17	Reducing sugars	10.40	6.74	2.27
		Non-reducing sugars	10.02	6.44	3.50
		Total sugars	20.42	13.18	3.05
		Starch	103.32	165.08	22.81
	MRSF 1754	Reducing sugars	25.21	29.22	3.57
		Non-reducing sugars	19.10	14.37	4.59
		Total sugars	44.31	43.59	NS
		Starch	115.61	163.93	1.90

CO_2 enrichment causes imbalance in the supply and demand of saccharides resulting in their increased accumulation in leaves (Stitt 1991). Wheat accumulated in leaves mostly sugars, whereas sunflower accumulated mainly starch. Accumulation of sugars may inhibit P_N by decreasing of flux of P_i into the chloroplast (Sharkey 1990, Stitt 1996) and through repressing the expression of photosynthetic genes including those encoding small and large subunits of RuBPCO (Sheen 1994, Van Oosten and Besford 1996, Smeekens 2000, Rolland *et al.* 2002). The down regulation of P_N in EC grown wheat can therefore be explained on the basis of above mentioned effects of sugar accumulation in leaves.

An increased accumulation of starch in the leaves of EC-grown sunflower may not influence the above mentioned RuBPCO gene expression. Starch synthesis recycles P_i for photosynthesis and consequently sustains P_N unless starch granules start shading and disrupting the chloroplast. This may probably happen at extreme accumulation (Vu *et al.* 1989). A survey of literature showed that plant species showing little or no down regulation of P_N under EC such as soybean (Campbell *et al.* 1988, Vu *et al.* 1989, Xu *et al.* 1994), potato (Sage *et al.* 1989), and radish (Usuda and Shimogawara 1998), all happen to be leaf starch accumulators. However, this fact has not been emphasized adequately. Ludewig *et al.* (1998) using transgenic potato which is unable to accumulate transitory starch due to leaf mesophyll specific anti-sense expression of AGPase, also demonstrated that down-regulation of P_N is not caused by accumulation of starch. The study, therefore, strengthened the view that accumulation of excess assimilates in the leaves under EC as starch is not inhibitory to P_N. Since feedback inhibition of P_N in wheat has been observed also under AC (Azcón-Bieto 1983, Ghildiyal and Sirohi 1986, Sharma-Natu and Ghildiyal 1993, Evans and Wardlaw 1996), such a trait would, therefore, be useful in sustaining P_N not only under EC but also under AC. Such plants will be able to utilize the beneficial effects of high [CO_2] on P_N and consequently productivity more efficiently.

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