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# Photosynthetic response of wheat cultivar to long-term exposure to elevated temperature

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## Abstract

Wheat (*Triticum aestivum* L. cv. HD 2285) was grown in control (C) and heated (H) open top chambers (OTCs) for entire period of growth and development till maturity. The mean maximum temperature of the entire period was 3 ºC higher in H- compared to C-OTCs. Net photosynthetic rate ($P_N$) measured at different temperature (20−40 ºC) of C- and H-grown plants showed greater sensitivity to high temperature in H-plants. $P_N$ measured at respective growth temperature was lower in H- compared to C-plants. The $CO_2$ and irradiance response curves of photosynthesis also showed lesser response in H- compared to C-plants. The initial slope of $P_N$ *versus* internal $CO_2$ concentration ($P_N/C_i$) curve was lower in H- than C-plants indicating ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) limitation. In irradiance response curve, the plateau was lower in H- compared to C-plants which is interpreted as RuBPCO limitation. RuBPCO content in the leaves of C- and H-plants, however, was not significantly different. Ribulose-1,5-bisphosphate carboxylase (RuBPC) initial activity was lower in H-plants, whereas activity of fully activated enzyme was not affected, indicating a decrease in activation state of the enzyme. This was further substantiated by the observed decrease in RuBPCO activase activity in H- compared to C-plants. RuBPCO activase was thus sensitive even to moderate heat stress. The decrease in $P_N$ under moderate heat stress was mainly due to a decrease in activation state of RuBPCO catalysed by RuBPCO activase.

*Additional key words:* $CO_2$ and irradiance response curves; internal $CO_2$ concentration; ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) content and activation state; RuBPCO activase.

## Introduction

Inhibition of photosynthesis by heat stress is one of the important factors that adversely affect wheat productivity (Al-Khatib and Paulsen 1990, 1999). This has become all the more relevant in the light of rising $CO_2$ and other greenhouse gases in the atmosphere, the concentrations of which are expected to increase the global temperature (Bowes 1993, Ghildiyal and Sharma-Natu 2000, Ravi *et al.* 2001, Long *et al.* 2004). Under moderate heat stress, inhibition of photosynthesis is reversible, whereas under severe heat stress, damage to photosynthetic apparatus is permanent (Berry and Björkman 1980, Quinn and Williams 1985). Since moderate heat stress occurs more frequently and the effects are not permanent, attempts have been made to elucidate the component of photosynthesis, which is most sensitive to high temperature. Photosystem 2 (PS2) should be especially sensitive to heat stress (Havaux 1993, Enami *et al.* 1994, Havaux and Tardy 1996). However, it has now been shown that damage to PS2 only occurs at high temperature often above 45 ºC (Čajánek *et al.* 1998, Yamane *et al.* 1998, Sharkey 2005). The decrease in photosynthesis under moderate heat stress could, therefore, be through a decrease in $CO_2$ assimilation.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) is the key regulatory and rate limiting enzyme of $CO_2$ assimilation (Ogren 1984). Inhibition of $CO_2$ assimilation by high temperature is generally explained by greater rate of photorespiration at high temperature, which results from changes related with different solubility of $CO_2$ and $O_2$ and kinetic properties of RuBPCO (Ogren 1984, Long *et al.* 2004). However, the inhibition of $CO_2$ assimilation by high temperature has been observed under both photorespiratory and non-photorespiratory conditions (Kobza and Edwards 1987, Crafts-Brandner and Salvucci 2000, Pushpalatha *et al.* 2007), which indicated that reduced photosynthesis at elevated leaf temperature can only partially be explained by greater rate of photorespiration. The decrease in photosynthesis under moderate heat stress could, therefore, possibly be through a decrease in amount and activation state of

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RuBPCO. RuBPCO must be activated to function fully in $CO_2$ fixation. RuBPCO activase is the enzyme specifically involved in the activation of RuBPCO at physiological concentrations of $CO_2$ and $Mg^{2+}$. RuBPCO activase is, therefore, essential for activation of RuBPCO and consequently for efficient photosynthesis (Salvucci *et al.* 1985, Portis *et al.* 1986). Sharma-Natu and Ghildiyal (1993) observed that diurnal decline in $P_N$ in wheat was associated with diurnal decline in activation state of RuBPCO. A decrease in activation state of RuBPCO in plants grown in elevated $CO_2$ concentration [$CO_2$] was associated with down regulation of photosynthesis (Sharma-Natu *et al.* 1997, Ghildiyal *et al.* 2001). The present study attempts to elucidate the effect of moderate heat stress on wheat so as to elucidate the most sensitive component of photosynthetic carbon assimilation. Such information would provide the basis for improving sustenance of $P_N$ under high temperature conditions.

## Materials and methods

Wheat (*Triticum aestivum* L. cv. HD 2285) was grown in control (C) and heated (H) 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). In heated OTCs warm air was supplied by hot air blower, blown by an axial fan. The warm air entered the chamber through double walled plenum around the base perforated towards inside. To eliminate chamber environment effect, chambers in which only air is blown served as control. The maximum and minimum temperatures of C- and H-OTCs were recorded daily to assess the temperature difference. The H-OTCs maintained mean maximum temperature of around 3 ºC higher than C-OTCs. The mean maximum temperature of entire period from sowing to maturity in C- and H-OTCs was 38.3 and 41.2 ºC, respectively. Standard cultural practices were followed (Singh 1983). Date of anthesis in the main shoot (MS) was recorded on the tags placed on each plant.

Photosynthetic responses to temperature, $CO_2$, and irradiance were determined in the flag leaf of MS of C- and H-plants at anthesis stage using portable photosynthetic system (*CIRAS-2 PP Systems,* UK). *CIRAS-2* allows measurement of steady state photosynthesis rate at a given irradiance supplied by the LED light source at a given [$CO_2$] supplied by $CO_2$ cartridge and at a given temperature. The temperature control range is from 8 ºC below ambient up to 40 ºC. For temperature response curves, the sample leaf was enclosed in the assimilation chamber which received constant saturating irradiance (>1 500 μmol $m^{-2}$ $s^{-1}$ upon the leaf surface) from radiation source. The assimilation chamber temperature was programmed for different temperatures at constant [$CO_2$] of 360 $cm^3$ $m^{-3}$. For $CO_2$ response curves, the chamber $CO_2$ was programmed for different [$CO_2$] at constant temperature of 25 ºC. The observations of $P_N$ measured at different external $CO_2$ ($C_a$) and computed values of internal $CO_2$ concentration ($C_i$) were recorded in C- and H-plants. The $P_N$ *versus* $C_i$ curves were then constructed. For irradiance response curves, the chamber irradiance was programmed for different irradiances at constant $CO_2$ of 360 $cm^3$ $m^{-3}$ and temperature of 25 ºC.

Leaf samples for determination of RuBP carboxylase (RuBPC) activity and content were taken around 11:00 h and stored in liquid nitrogen. 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 ºC by injecting 50 $mm^3$ of 5 mM RuBP and 25 $mm^3$ 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, and 10 mM $NaH^{14}CO_3$ (74 kBq per assay) in a total volume of 0.5 $cm^3$. The reaction was terminated after 60 s by addition of 100 $mm^3$ of 6 M acetic acid; the material was dried at 65 ºC, and the acid-stable $^{14}C$ was estimated by liquid scintillation counting. 'Total' activities were determined in a similar way except that 25 $mm^3$ of the soluble leaf extract and 425 $mm^3$ of assay mixture were incubated together for 10 min at 25 ºC before 50 $mm^3$ of 5 mM RuBP were added. From the initial and total activities the % activation of the enzyme was calculated (Servaites *et al.* 1984). RuBPCO content was determined using SDS-PAGE (Laemmli 1970, Servaites *et al.* 1984). LSU and SSU bands of RuBPCO were quantified by using gel documentation program (*Alpha Imager*, *Alpha Innotech*). Soluble protein content was determined by the method of Bradford (1976).

RuBPCO activase activity was determined following Holbrook *et al.* (1991). Extraction buffer contained 50 mM *Hepes* KOH (pH 7.0), 5 mM $MgCl_2$, 1 mM EDTA, 1 mM ATP, 5 mM dithiothreitol (DTT), 50 mM 2-mercaptoethanol, 20 mM ascorbate, 2 % (m/v) polyvinylpolypyrrolidone, 10 % (v/v) glycerol, 1 mM PMSF, and 10 μM leupeptin. A coupled assay measuring the ATPase activity of activase was then performed at 25 ºC by adding the reaction mixture to a spectrophotometer cuvette containing a final volume of 1.0 $cm^3$ of solution comprising 100 mM Tricine (pH 8.0), 5 mM $MgCl_2$, 20 mM KCl, 0.2 mM NADH, 4.6 mM DTT, 1 mM ATP, 2 mM phospho*enol*pyruvate, 12 units of pyruvate kinase, and 12 units of lactic dehydrogenase. Oxidation of NADH was measured by the decrease in $A_{340}$.

There were three replications for each observation. Data were computed statistically by analysis of variance (ANOVA).

## Results

The temperature response curves of $P_N$ in C- and H-plants showed a decrease beyond 30 ºC. In C-plants, $P_N$ remained maximum between 20−30 ºC, whereas in H-plants optimum $P_N$ was observed only between 25−30 ºC. $P_N$ of C- and H-plants was more or less similar around optimum temperature. However, towards lower and higher temperatures, $P_N$ was lower in H-plants. H-plants, therefore, showed a greater sensitivity to temperature (Fig. 1*A*). The $CO_2$ and irradiance curves of photosynthesis showed lesser response in H- compared to C-plants. The initial slope of $P_N/C_i$ curve was lower in H- than in C-plants (Fig. 1*B*). In irradiance response curve, the plateau was lower in H- compared to C-plants (Fig. 1*C*).

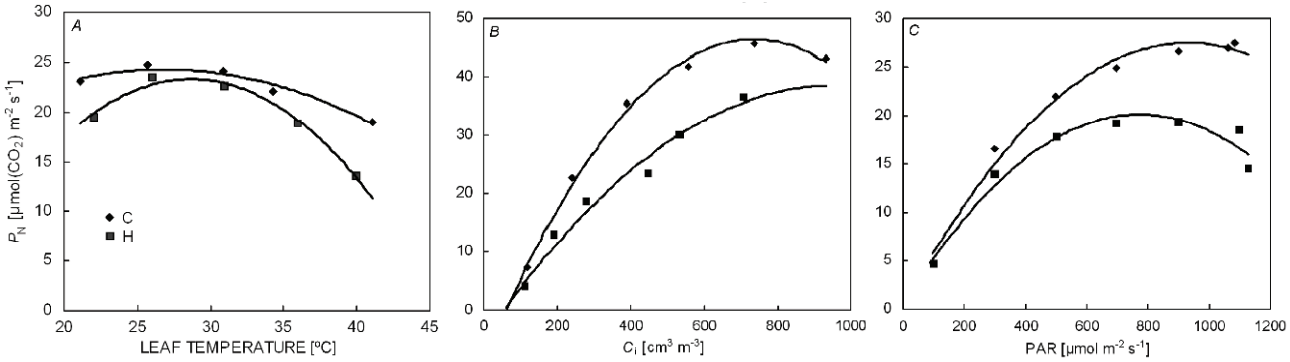


Fig. 1. (*A*) Net photosynthetic rate ($P_N$) *versus* leaf temperature; (*B*) $P_N$ *versus* internal $CO_2$ concentration ($C_i$), (*C*) $P_N$ *versus* photosynthetically active radiation (PAR) in the flag leaf of wheat cv. HD 2285 grown in control (C) and heated (H) open top chambers.

Table 1. Net photosynthetic rate [µmol($CO_2$) $m^{-2}$ $s^{-1}$], initial and total RuBPC activity [mmol($CO_2$) $kg^{-1}$(protein) $s^{-1}$], its activation state [%], and content [g $kg^{-1}$(f.m.)], and RuBPCO activase activity [mmol(ADP) $kg^{-1}$(protein) $s^{-1}$] in the flag leaf of wheat cultivars grown in control (C) and heated (H) open top chambers. NS = not significant.

| Measurements | | C | H | H/C |
|---|---|---|---|---|
| Net photosynthetic rate | | 24.50 | 17.00 | 0.69 |
| RuBPC | initial activity | 10.83 | 8.33 | 0.77 |
| | total activity | 15.17 | 14.33 | 0.94 NS |
| | activation state | 71.42 | 58.13 | 0.81 |
| | content | 7.85 | 7.54 | 0.96 NS |
| RuBPCO activase | activity | 5.67 | 5.00 | 0.88 |

The $P_N$ of C-plants measured at C and $P_N$ of H-plants at H showed significantly lower $P_N$ in H- compared to C-plants (Table 1). RuBPC initial activity was lower in H-plants, whereas activity of fully activated enzyme was not significantly affected. The activation state of RuBPC was lower in H-plants. This was further substantiated by the observed decrease in RuBPCO activase activity in H- compared to C-plants. RuBPC content in the leaves of C- and H-plants, however, was not significantly different (Table 1).

## Discussion

Optimum temperature of $P_N$ in C-grown wheat was 20−35 ºC and in H-grown plants it was between 25−30 ºC. Beyond 30 ºC $P_N$ decreased in both C- and H-plants, the decrease, however, was greater in H-plants. Since growth temperature of H-plants during the day at anthesis stage was greater than 30 ºC, a decrease in $P_N$ in H- compared to C-plants measured at respective growth temperatures was expected. Kobza and Edwards (1987) also reported optimum temperature of 20−30 ºC for $P_N$ in wheat. Temperature response curve of $P_N$ in oak leaves showed a decrease beyond 25 ºC (Haldimann and Feller 2004). The slope of temperature response curves differed in C- and H-plants. If the slope of curve remained the same in C- and H-plants, then the photosynthetic characteristics of leaves were not changed. In our study, slope of curves showed that $P_N$ of H-plants was more sensitive to temperature. This greater temperature sensitivity of $P_N$ of H-plants could be due to photosynthetic acclimation to elevated temperature. Lesser $CO_2$ and irradiance responses of $P_N$ in H- compared to C-plants provided further evidence of a decrease in photosynthetic capacity of the leaves in H-plants.

A comparison of $P_N$ of C- and H-plants at the same $C_i$ eliminates stomatal effects. Therefore, a lower $P_N$ in H-grown wheat reflects metabolic limitation. Other studies have also reported that inhibition of $P_N$ by heat stress does not arise because of stomatal limitation (Paulsen 1994, Jiao and Grodzinski 1996, Law and Crafts-Brandner 1999, Crafts-Brandner and Law 2000). In our study, the initial slope of $P_N/C_i$ curve was lower in H- than C-plants, thus indicating RuBPCO limitation (Woodrow and Berry 1988, Stitt 1991, Pandurangam *et al.* 2006). In irradiance response curves, the initial slope is interpreted as RuBP-regeneration limited (the low irradiance means the supply of ATP and NADPH is inadequate) and the plateau was interpreted as RuBPCO limitation (Stitt 1991, Pandurangam *et al.* 2006). The irradiance response curves in our study showed that the plateau was lower in H- compared to C-plants which is interpreted as RuBPCO limitation. The above observations are in line with the reports that $CO_2$ assimilation rather than electron transport is the primary functional limitation of $P_N$ at moderate heat stress normally encountered by plants (Haldimann and Feller 2005, Sharkey 2005).

In our study, H-plants showed no significant decrease in the amount of RuBPCO compared to C-plants indicating that RuBPCO limitation in H-plants is not due to amount of the enzyme. The activity of rapidly extracted RuBPC was determined without further activation (initial activity) and after activation with $CO_2$ and $Mg^{2+}$ (total activity). The initial activity thus determined represents the activity of enzyme *in vivo* (Portis 1992). RuBPC initial activity was lower in H-grown plants, whereas activity of fully activated enzyme was not affected, indicating a decrease in activation state of enzyme. This was further substantiated by the observed decrease in RuBPCO activase activity in H- compared to C-plants. Thus thermal denaturation of activase may play a role in the reduction of RuBPCO activation at high temperature. High temperature induces the formation of high-molecular-mass aggregates of activase (Rokka *et al.* 2001). The decrease in $P_N$ under elevated temperature thus appears to be due to a decrease in activation state of RuBPCO catalysed by RuBPCO activase. This is in agreement with results obtained for different species (Feller *et al.* 1998, Law and Crafts-Brandner 1999, Crafts-Brandner and Law 2000). Identification and incorporation of a thermostable RuBPCO activase would therefore be required to improve thermotolerance of photosynthesis.

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