# Diurnal changes in photosynthesis, sugars, and nitrogen of wheat and mungbean grown under elevated $CO_2$ concentration

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## Abstract

The diurnal changes in leaf net photosynthetic rate ($P_N$) and sugar and nitrogen contents in wheat [*Triticum aestivum* (L.) cv. HD 2285] and mungbean [*Vigna radiata* (L.) Wilczek cv. PS 16] were analysed under ambient, AC [350±25 $\mu$mol mol$^{-1}$] and elevated, EC [600±50 $\mu$mol mol$^{-1}$] $CO_2$ concentrations. In both mungbean and wheat $P_N$ of AC- and EC-grown plants compared at the same $CO_2$ concentration showed that $P_N$ was higher under EC. However, increased $P_N$ in EC-plants declined in the afternoon and approached $P_N$ of AC-plants. Depression in $P_N$, however, was less in mungbean compared with the large depression in wheat. Greater down regulation of $P_N$ in wheat was associated with the accumulation of large amount of sugars and low nitrogen content in wheat leaves. Mungbean leaves accumulated mostly starch under EC and the difference in N content in AC- and EC-plants was relatively less than in wheat.

*Additional key words*: net photosynthetic rate; non-protein nitrogen; non-structural saccharides; protein nitrogen.

## Introduction

Leaf level studies have shown that photosynthetic responses to $CO_2$ partial pressure vary greatly with crops, growth environments, and plant developmental stages (Delucia *et al.* 1985, Gunderson and Wullschleger 1994, Sage 1994, Griffin and Luo 1999). This large variation poses a challenge for plant scientists wishing to extrapolate from leaf level studies to predict the stimulation in global terrestrial carbon influx resulting from rising atmospheric $CO_2$ partial pressure. The photosynthetic responses of plants depend upon duration of exposure to high $CO_2$ concentration (EC). Enhanced photosynthesis may not remain high for longer duration under long-term exposure to EC and may decrease (Sharma-Natu *et al.* 1997). Short-term (minutes to hours) increase in ambient $CO_2$ concentration (AC) from 350 to 700 $\mu$mol mol$^{-1}$ typically increases photosynthesis by 30-70 % due to photosynthetic sensitivity (Sharma and Sengupta 1990, Bowes 1991, Stitt 1991, Sage 1994, Luo and Mooney 1996). Nevertheless, many species will not maintain this

stimulation of photosynthesis when grown in EC for weeks or longer as a result of photosynthetic acclimation (Sage *et al.* 1989, Druţă 2001). For example, the 78 % average initial stimulation of photosynthesis in mungbean reported by Sharma and Sengupta (1990) decreased to an average of 29 % after long-term exposure to EC.

The suppression of photosynthesis by $CO_2$ enrichment is also associated with a decrease in total leaf nitrogen content (Conroy and Hocking 1993, Delgado *et al.* 1994, Roumet *et al.* 1996, Gielen *et al.* 2000). Since photosynthesis is determined by the absolute N content and N partitioning in a leaf, change in $P_N$ by $CO_2$ enrichment must be evaluated in relation to reduced leaf N content. The present study examines change in photosynthetic pattern due to EC in leguminous (mungbean) and non-leguminous (wheat) crops in order to analyse interactive impact of change in N and non-structural saccharides on photosynthesis.

## Materials and methods

Mungbean [*Vigna radiata* (L.) Wilczek cv. PS16] and wheat [*Triticum aestivum* (L.) cv. HD 2285] were grown

in pots at Plant Physiology Division, Indian Agricultural Research Institute, New Delhi (India) from 10 April to 18

June 1999 and from 12 November 1999 to 25 March 2000. A total of 16 pots were used in each chamber for the treatment. In the experiment, a modified open top chamber (OTC), as described by Rogers *et al.* (1983), was developed to study crop responses to EC. The height and diameter of the open top chamber was 1.8 and 1.6 m, respectively. One set of plants was grown under AC ($350\pm25$ $\mu$mol mol$^{-1}$) and other group of plants was grown under EC ($600\pm50$ $\mu$mol mol$^{-1}$) in a naturally lit OTC. These chambers were lined with transparent polyvinyl chloride (PVC) film (120 $\mu$m thickness) which allowed 85 % solar irradiation. Pure $CO_2$ gas (99.7 % v/v $CO_2$ and less than 10 $\mu$mol mol$^{-1}$ CO) was purchased from m/s *Gas Associates*, New Delhi. The flow of pure gas was controlled through a regulator and a flow meter. The concentration of $CO_2$ in the chamber was monitored by an infrared gas analyser (IRGA) (*LiCOR 6200*, Lincoln, USA). $P_N$ was measured at hourly intervals from 08:00 to 17:00 in the fully expanded top-most leaf using IRGA. Observations on the $P_N$, and saccharide and nitrogen contents in wheat leaves were taken at 40 and 85 d after emergence of the plants. In mungbean, observations were taken at 20 and 40 d after germination.

Leaflet blades of the same physiological age for analysing non-structural saccharides and N were sampled during forenoon (10:00) and afternoon (15:00). Reducing sugar content was estimated by the arsenomolybdate method of Nelson (1944) using improved copper reagent of Somogyi (1952). Absorbance was measured at 630 nm in *Spectronic-20* (*Baush and Lamb*, Rochester, USA). Content of non-reducing sugars was calculated by subtracting the reducing sugar content from the total sugar content. Dried samples of the residue leaf after extraction for reducing sugars were used for starch content (Pucher *et al.* 1948).

Non-protein and protein nitrogen portions in the form of reduced N in the trichloroacetic acid (TCA) preserved leaf material were determined by using *N-Kjeltech Auto 1030* analyser, following the procedure detailed in *Tecator* manual, 1987 (*Tecator Company*, Hoganas, Sweden).

Leaf samples were collected randomly between plants in polyethylene bags. 0.5 g of leaf from each sample was preserved in 10 cm$^3$ of 10 % TCA. Homogenised leaves were centrifuged twice at 20 124×$g$ for 10 min. Supernatant (non-protein nitrogen) and residues (protein nitrogen) were subjected to digestion and distillation.

Statistical analysis of the data was done by analysis of variance (ANOVA) given by Panse and Sukhatme (1967). The critical difference (CD) values were calculated at 5 % probability level.

## Results

A significant increase in $P_N$ in mungbean was observed under EC. Major difference in $P_N$ had occurred at vegetative stage in the first half of the day, where average $P_N$ was 7.35 and 19.86 $\mu$mol($CO_2$) m$^{-2}$ s$^{-1}$ for AC- and EC-grown plants, respectively (Fig. 1*A*). The superiority in $P_N$ of the EC-plants was sustained over AC-plants throughout the day. However, under both $CO_2$ concentrations, mungbean showed a decline in $P_N$ towards the afternoon. In contrast to vegetative stage, increase in $P_N$ at flowering stage was observed in the forenoon only and no significant difference in $P_N$ was seen in the afternoon due to EC.

Similar to mungbean, significant increase in $P_N$ observed in EC-grown wheat plants was superior throughout the day and in the early part of the day (08:00 to 12:00), $P_N$ of the EC-grown plants was fairly high. However, $P_N$ declined in the later part of the day and we observed less difference in $P_N$ of AC- and EC-grown plants. In wheat, $P_N$ at flowering stage was greater under EC throughout the day (Fig. 1*B*).
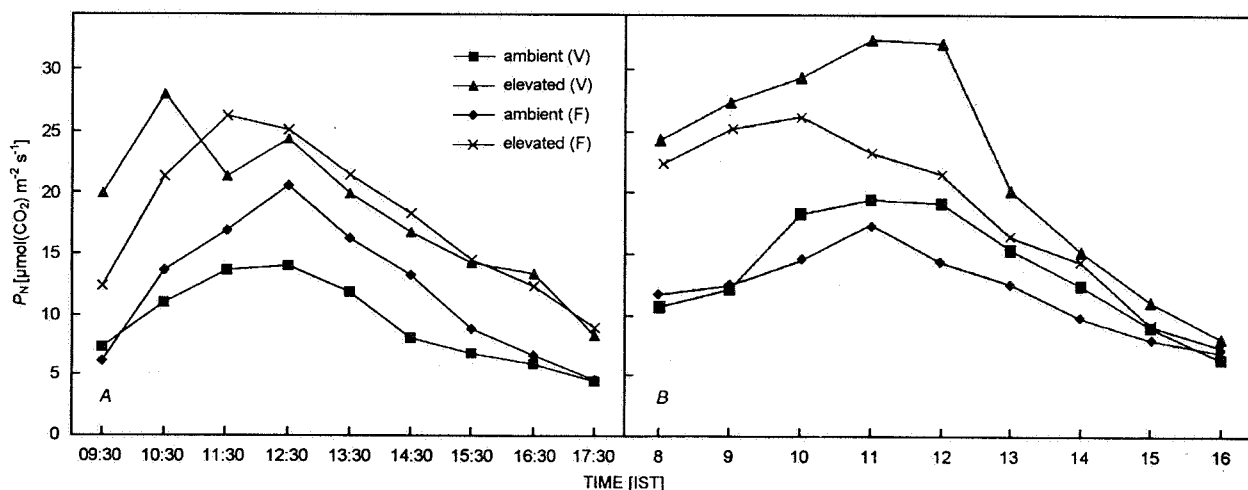


Fig. 1. Diurnal changes in net photosynthetic rate, $P_N$ of mungbean (*A*) and wheat (*B*) exposed to long term elevated $CO_2$ conditions. V – vegetative stage, F – flowering.

No significant difference in contents of reducing and non-reducing sugars was observed in mungbean leaves when they were grown under EC. However, significant increase in starch content was found at vegetative and flowering stage throughout the day. We recorded opposite results for wheat under EC. Sugar contents (reducing and non-reducing) of wheat leaves were significantly higher in the afternoon at vegetative and flowering stages but no significant difference in the starch content was observed under EC (Table 1).

We observed low contents of non-protein and protein nitrogen at vegetative and flowering stages in mungbean and wheat leaves when they were grown under EC. Protein N content in mungbean and wheat leaves harvested at vegetative stage (forenoon) was significantly lower in EC than in AC. Interestingly, in wheat, we recorded less protein N throughout the day at all growth stages when plants were grown under EC. How-ever, in mungbean no significant difference in protein N content was observed except in the early part of the day at vegetative stage (Table 1).

Table 1. Contents of non-structural saccharides [g $kg^{-1}$(d.m.)] and nitrogen [g $kg^{-1}$(d.m.)] in the leaves of wheat and mungbean at vegetative and flowering stages under elevated $CO_2$ condition. The contents of sugars and nitrogen were measured at 10:00 and 15:00 IST. RS = reducing sugars; NRS= non-reducing sugars; TS = total sugars; PN = protein nitrogen; NPN = non-protein nitrogen. Means ± SD of five replications. *$p<0.05$, ns = non-significant.

| Crop stage | Session | Parameter | Wheat Ambient | Elevated | Mungbean Ambient | Elevated |
|---|---|---|---|---|---|---|
| Vegetative | Forenoon | RS | 9.56±1.00 | 12.20±0.11* | 0.90±0.18 | 0.99±0.02$^{ns}$ |
| | | NRS | 32.70±1.21 | 32.97±0.92ns | 1.48±0.22 | 1.96±0.07$^{ns}$ |
| | | TS | 43.26±0.10 | 46.20±7.93$^{ns}$ | 2.38±0.04 | 2.95±0.09$^{ns}$ |
| | | Starch | 44.52±0.10 | 54.40±0.04$^{ns}$ | 182.00±9.8 | 295.00±11.79* |
| | | PN | 30.60±2.03 | 24.60±2.16* | 18.36±0.48 | 12.30±0.37* |
| | | NPN | 22.90±0.98 | 18.90±0.73* | 13.39±0.91 | 11.18±0.46$^{ns}$ |
| | Afternoon | RS | 21.35±0.99 | 36.12±0.52* | 1.79±0.62 | 2.74±0.83$^{ns}$ |
| | | NRS | 56.11±0.08 | 77.11±1.67* | 1.70±0.26 | 2.40±0.57$^{ns}$ |
| | | TS | 81.47±1.11 | 113.21±1.38* | 3.49±0.86 | 5.15±0.66$^{ns}$ |
| | | Starch | 82.58±7.30 | 118.40±8.19$^{ns}$ | 306.00±13.05 | 490.00±9.30* |
| | | PN | 28.03±1.89 | 22.29±1.71* | 13.85±0.45 | 11.89±0.29$^{ns}$ |
| | | NPN | 17.35±1.22 | 17.09±1.23$^{ns}$ | 12.39±0.31 | 10.38±0.41$^{ns}$ |
| Flowering | Forenoon | RS | 8.00±0.02 | 10.72±0.66$^{ns}$ | 2.14±0.22 | 2.85±0.06$^{ns}$ |
| | | NRS | 41.65±1.50 | 42.10±3.20$^{ns}$ | 3.09±0.69 | 4.80±0.17$^{ns}$ |
| | | TS | 49.55±4.48 | 54.82±2.51$^{ns}$ | 5.23±0.79 | 7.65±0.28$^{ns}$ |
| | | Starch | 66.12±2.09 | 70.03±1.40$^{ns}$ | 279.00±9.94 | 450.00±12.80* |
| | | PN | 25.20±1.11 | 17.72±1.65* | 11.03±0.56 | 10.69±0.35$^{ns}$ |
| | | NPN | 17.30±0.80 | 14.78±1.07$^{ns}$ | 14.43±0.62 | 10.97±0.41* |
| | Afternoon | RS | 21.84±1.54 | 33.15±2.37* | 4.83±0.33 | 5.66±0.38$^{ns}$ |
| | | NRS | 62.79±1.01 | 79.22±1.22* | 5.05±0.19 | 5.29±0.29$^{ns}$ |
| | | TS | 83.24±4.74 | 111.77±2.88* | 9.88±0.45 | 10.95±0.67$^{ns}$ |
| | | Starch | 78.83±0.55 | 103.18±1.71* | 486.00±13.12 | 666.00±10.37* |
| | | PN | 22.33±1.39 | 15.25±0.73* | 10.09±0.22 | 9.89±0.17$^{ns}$ |
| | | NPN | 14.41±0.72 | 13.12±0.99$^{ns}$ | 12.37±0.82 | 9.87±0.35* |

## Discussion

$CO_2$ enrichment of wheat plants enhanced $P_N$ and the enhancement was observed throughout the day but the trend of photosynthetic enhancement under EC differed entirely with the diurnal changes in $P_N$. Maximum diurnal increase in $P_N$ was observed when the enhancement in $P_N$ due to EC was lowest (Fig. 1B). Highest enhancement (146 %) of $P_N$ due to EC was observed between 08:00 and 09:00 in the morning. As the $P_N$ increased, EC-mediated enhancement in $P_N$ decreased and reached its minimum around 11:00-12:00. Thus we suggest that the EC-mediated enhancement in $P_N$ is larger when $P_N$ is low. This was even true during afternoon hours when $P_N$ de-clined to its minimum. However, the EC-mediated enhancement of $P_N$ was highest during morning hours (08:00-09:00) as compared to the late afternoon (14:00-16:00). Such variations in $P_N$ enhancement and diurnal change may be due to the export of saccharides from the leaves which declined in the afternoon (Azcón-Bieto 1983) or increased content of saccharides later in the day (Rufty and Huber 1983). Increased content of sugars in wheat leaves may inhibit $P_N$ by tying up $P_i$ resulting in $P_i$ limitation of RuBP regeneration capacity (Harley et al. 1992, Socias et al. 1993). Inhibition of $P_N$ by leaf sugar accumulation could also be through a decrease in the state

of activation of RuBP carboxylase (Sharma-Natu and Ghildiyal 1993, Urban and Marek 1999).

In mungbean, pattern of diurnal changes in $P_N$ and enhancement in $P_N$ due to EC was similar to that in wheat in the forenoon, but in the afternoon a *ca.* 100 % increase in $P_N$ was found under EC. These findings suggest that change in pattern of diurnal $P_N$ and $P_N$ enhancement due to EC was probably mediated by saccharide-regulated suppression of gene expression. According to this hypothesis, intracellular sugar-sensing mechanism responds to excess saccharides by decreasing transcripts for photosynthetically associated genes such as the small subunit of RuBPCO (Jang and Sheen 1997). In the first half of the day at vegetative stage, we recorded significant difference in protein N content under EC but as the day progressed, protein N and non-protein N contents relatively increased. The reason for less difference in protein nitrogen concentration under AC and EC could be

increase in nitrogenase activity due to high $CO_2$ concentrations (Finn and Brun 1982, Srivastava *et al.* 2001) which apparently increase N content and allocate more N to RuBPCO protein and total Chl for maintaining constant enhancement in $P_N$ throughout the growth period. However, the reason for significantly low protein N in the early part of the day at vegetative stage could be low nitrogenase activity in the morning hours (Rainbird *et al.* 1983). The lesser sensitivity of $P_N$ to leaf starch accumulation could possibly be another reason for less down regulation of $P_N$ in mungbean, similar to earlier reports on soybean (Porter and Grodzinski 1984, Vu *et al.* 1989).

In nutshell, pattern of $P_N$ enhancement due to EC and diurnal changes in $P_N$ differed in non-leguminous (wheat) and leguminous (mungbean) crop due to difference on their N (non-protein and protein) contents and accumulation of non-structural saccharides.

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