The effect of elevated CO_2 concentration on leaf chlorophyll and nitrogen contents in rice during post-flowering phases

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Abstract

The effect of elevated CO_2 concentration (CE) on leaf chlorophyll (Chl) and nitrogen (N) contents and photosynthetic rate (P_N) was evaluated during the post-flowering stages of rice grown at CE (570 ± 50 µmol mol^{-1}) in open top chamber (OTC), at ambient CO_2 concentration (~ 365 µmol mol^{-1}) in OTC and at open field. Thirty-five day old seedlings were transplanted in OTCs or in field and allowed to grow till maturity. Chl and N contents were highest at the time of flowering and thereafter it started to decline. The rate of decline in Chl and N contents was faster in plants grown under CE mostly in later part of growth. Irrespective of treatment difference, flag leaf contained the highest amount of Chl and N than penultimate and third leaf. The higher P_N was observed in leaves under CE than in the leaves in other two growing conditions. Considering growth stage, P_N was the highest at flowering which reduced at the later part of growth due to degradation of Chl and N content of the leaf. Under CE it was 40.02 µmol m^{-2} s^{-1} at flowering and it reduced to only 14.77 µmol m^{-2} s^{-1} at maturity stage. The beneficial effect of CE in increasing leaf P_N may be maintained by applying extra dose of nitrogen at the later stages of plant growth.

Additional key words: open top chamber, photosynthesis, *Oryza sativa* L.

Introduction

Atmospheric concentration of CO_2 is steadily increasing and expected to double sometimes in the middle of present century (Watson *et al*. 1990). The impact of the changes in atmospheric CO_2 concentration and other greenhouse gases, and consequential rise in temperature - known as global change, is generally predicted to be disastrous. But CO_2 being the substrate of photosynthesis, the increase in CO_2 concentration increases the net photosynthetic rate (P_N) and crop productivity (Kimball 1983, Cure and Acock 1986, Ziska *et al*. 1997). The effect of rising atmospheric CO_2 concentration on photosynthesis and productivity is reported to be more pronounced in C_3 plants (Jones *et al*. 1984, Atkinson 1996). Baker *et al*. (1990) and Ziska and Teramura (1992) reported significantly positive response of rice plants to elevated CO_2 concentration (CE) in respect to photosynthesis, growth and grain yield of rice.

Grain yield of rice depends almost entirely on the current photosynthesis. Several authors (*e.g*., King *et al*. 1967, Patil *et al*. 1976, Evans 1972) showed that in the cereals, most of the assimilates are translocated to the developing grains from the flag leaf during the grain-filling periods. Current P_N of the penultimate leaf and the third leaf from the top may also contribute substantially to developing grains. P_N is regulated by Chl and N contents of the leaf (Cock and Evans 1983, Makino *et al*. 1988, Greenwood *et al*. 1991). Sinclair and Horie (1989) provide a comprehensive account of the relationships among leaf N, P_N and radiation use efficiency. Manalo *et al*. (1994) demonstrated that CE enhances crop maturity and the onset of senescence. However, information on the effect of CE on the Chl and N contents

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Abbreviations: CE - elevated CO_2 concentration; Chl - chlorophyll; DAF - days after flowering; OTC - open top chambers; P_N - net photosynthetic rate.
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of the flag, penultimate and third leaf of rice during post-flowering phases has not been reported. This paper evaluates the effect of CE on the changes in leaf Chl and N concentrations in relation to P_N during the post-flowering phases in rice plants.

Materials and methods

An experiment was conducted at the Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur (Bangladesh) during the wet season of 2000. 35-d-old seedlings of rice (*Oryza sativa* L. cv. BRRIdhan39) were transplanted in OTC placed on a previously prepared lowland. Details of the construction and operation of OTC have been provided elsewhere (Uprety 1998). In one chamber CO_2 concentration was raised to 570 ± 50 µmol mol^{-1} while in another chamber ambient CO_2 concentration, *i.e.*, ~360 µmol mol^{-1} was maintained. Single seedling per hill was used. Seedlings were also transplanted in a plot of similar dimension in the open field close to the OTC's as control treatment. The diameter of the chamber was 3 m and 280 hills of rice were accommodated maintaining a planting configuration of 25 × 10 cm. A fertilizer dose of 90 kg N, 20 kg P, 60 kg K, 20 kg S and 3.5 kg Zn was used. Entire amount of all the fertilizers excepting N was applied prior to transplanting while N was applied in three split doses at 4, 21, and 52 d after transplanting. Adequate measures were taken to keep the insect pest infestation and weed growth to a minimum.

Leaf Chl and N concentrations were determined non-destructively using *SPAD* meter (*Minolta Co*., Japan). *SPAD* values were transformed into Chl and N contents based on standard curves determined previously analyzing a large number of leaf samples representing wide ranges of Chl and N contents. Leaf samples were analyzed for Chl content following Yoshida *et al*. (1976) and N contents using Kjeldahl procedure. *SPAD* values were taken on flag leaf, penultimate leaf and the third leaf during the post-flowering period until maturity. P_N of flag leaves were determined on four occasions during post-flowering stages with a portable photosynthesis device (*LiCor 6200*, Lincoln, NE, USA). Measurements were taken on clear sunny days between 10:00 and 13:00. Replicated data were analyzed statistically and mean values were used with ± standard errors.

Results and discussion

Elevated CO_2 concentration increased leaf Chl slightly as found by De Costa *et al*. (2003) in rice. Chl content in the flag leaf was generally higher than in the penultimate and third leaf irrespective of environmental conditions. Chl content remained nearly unchanged for about 12 d following anthesis and thereafter it started declining (Fig. 1). There were gradual changes in Chl although third leaf had a great deal of fluctuations in Chl contents. From 13 d after flowering (DAF) and onwards, Chl content in all three leaves declined sharply. At maturity, the flag leaf and penultimate leaves showed a reduction of Chl to 90 % and 62 %, respectively. Overall, the decline in leaf Chl since the beginning of senescence was highest in the CE treatment suggesting that CE enhanced senescence. In absence of environmental stress, rice grain growth is supported by the current P_N when leaves are the major source and the spikelets are the major sink. In the present study, most grain growth occurred during the three weeks following anthesis (Kato 1993) while the leaf Chl content was also relatively high. It is possible that on completion of grain growth, the leaf Chl declined sharply in the absence of active sink. Such a feed-back mechanism has also been reported by Makino *et al*. (1988). Fluctuations in Chl contents of the third leaf indicate that chlorophyll degradation due to sink demand might have followed a quick recovery perhaps through translocation of metabolites from the subtending leaves.

Nitrogen contents of the leaves and the effect of CE thereon followed the trend similar to that observed in Chl contents. Leaf N content was highest at flowering and decreased gradually with the increase of leaf age (Fig. 1). Dey and Chandra (1994) also observed the highest leaf N content at flowering stage in several rice cultivars. Plants grown at CE had a bit higher leaf N concentration in beginning (flowering stage) compared with other two treatments; but the difference narrowed down over time. Irrespective of treatment difference, however, the flag leaf displayed the highest leaf N concentration during the active spikelet growth period (1 - 12 DAF). The leaf N concentration in the penultimate leaf closely followed the flag leaf. But from 13 DAF the trend changed and leaf N in flag leaf declined sharply giving the penultimate leaf to take the lead. The third leaf showed the lowest N concentration almost throughout the grain growth period, but in the late maturity phase it had maintained identical or more N than those of the uppermost leaves. This was mainly because of the greater rate of N reduction in the upper leaves of the plants grown in OTC with ambient CO_2 and field. Leaf N concentrations are generally lowered due to CE (Conroy 1992, Fangmeier *et al*. 1999) and our results are in disagreement with the earlier findings. The discrepancy might be due to the timing of recording of the leaf N concentrations, for the concentration changes not only over time but also depending on growth stage. The differential response of leaf N, particularly the fluctuation of N concentration in the third leaf, in OTC with ambient CO_2 concentration and field grown plants might be due to chamber effect (Uprety 1998).

P_N of flag leaf was measured on four occasions at flowering and post-flowering stages. Plants grown at CE exhibited significantly higher P_N compared with other two treatments throughout the study period (Table 1). Higher P_N of crops grown under CE has also been reported (Uprety and Mahalaxmi 2000, Madan Pal *et al.* 2003/4, Pal *et al.* 2005). Plants grown in OTC with ambient CO_2 concentration or in the field had consistently lower P_N. Irrespective of treatment differences,

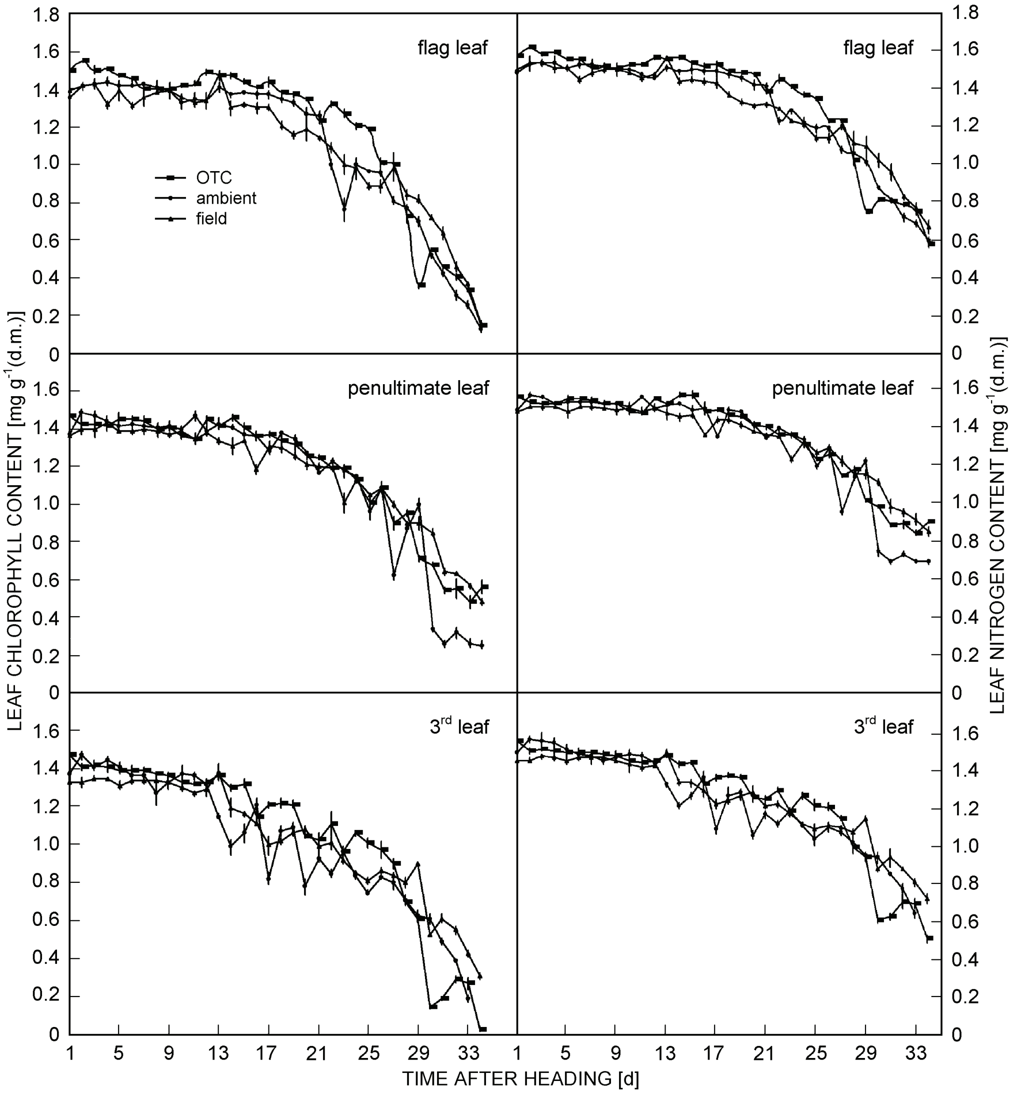

Fig. 1. Changes in leaf chlorophyll and nitrogen contents during post-heading period of rice. *Bars* indicate SE of the mean (n = 6), and are shown when greater than the symbol size.

Table 1. Influence of CE on the P_N in flag leaf during post flowering period of rice. Means ± SE, n = 9.

Treatments	Leaf P_N [μmol m^{-2} s^{-1}]			
	flowering	early maturity	mid maturity	maturity
OTC elevated CO_2 concentration	40.02 ± 0.62	29.89 ± 0.32	18.44 ± 0.43	14.77 ± 0.58
OTC ambient CO_2 concentration	25.58 ± 0.53	19.08 ± 0.72	11.93 ± 0.39	10.45 ± 0.32
Field (control)	25.30 ± 0.52	19.97 ± 0.61	14.33 ± 0.51	9.09 ± 0.47

P_N was the highest at flowering, which decreased progressively over time. The leaf P_N of the plants grown at CE was 40.02 μmol m^{-2} s^{-1} at flowering stage and was reduced to 14.77 μmol m^{-2} s^{-1} at maturity stage. The corresponding P_N of field grown plants were 25.30 μmol m^{-2} s^{-1} and 9.09 μmol m^{-2} s^{-1}, respectively. When Fig. 1 and Table 1 are compared in conjunction, the changes in leaf P_N can be explained from the progressive changes in leaf Chl and N contents. The decrease in P_N at the later phase of rice growth may be associated with the loss of integrity of leaf chloroplast as the leaf age advanced (Thornton and Wample 1980). However, the extent of reduction in P_N was more than the drop in leaf Chl or N contents over time. Takano and Tsunoda (1971), for the time, explained the non-linear relationship between N content and leaf P_N. Peng *et al.* (1995) also showed strong relationship between leaf P_N and N content of field grown rice. Rapid degradation of chlorophyll and leaf N under elevated CO_2 might be attributed to the sharp drop in leaf P_N that resulted in narrowing the difference between the treatments. The plausible reasons for the sharp decline in P_N might be that on completion of grain growth the sink demand for photosynthesis was also minimal that might have eventually terminated the growth hastening the leaf senescence.

The results revealed that the Chl and N contents and P_N rates in rice leaves started declining soon after flowering. The extent and rate of drop was slow at the beginning but sharp on 12 DAF and afterwards in both CE and ambient CO_2 concentration. Chl degradation and reduction in leaf N content 13 DAF was faster at CE eventually resulting to drastic reduction in leaf P_N. Studies conducted elsewhere showed that after full leaf expansion, the Chl content of wheat leaves increased (Demothes and Knoppik 1994), remained unaffected (Nie *et al*. 1995, Manderschied and Weigel 1997) or decreased (Tuba *et al*. 1994) by CE. Nie *et al*. (1995) and Ommen *et al*. (1999) observed that the decline in Chl concentration during senescence was accelerated due to CO_2 enrichment. Our results indicate that the senescence of rice leaves was slightly enhanced by CE. Sharp decline in the leaf Chl and N concentrations might have been associated with the rapid senescence. Nitrogen demand of the spikelet causes faster leaf senescence under CE. The redistribution of nitrogen from Chl binding proteins is considered to be the main cause of Chl degradation (Matile *et al*. 1996). Our results suggest that the beneficial effect of CE in increasing leaf P_N of rice may be maintained applying nitrogen at the later stages of crop growth.

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