PHOTOSYNTHETICA 48 (4): 530-536, 2010

Effect of panicle removal on photosynthetic acclimation under elevated CO_2 in rice

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Abstract

To examine the role of sink size on photosynthetic acclimation under elevated atmospheric CO_2 concentrations ($[CO_2]$), we tested the effects of panicle-removal (PR) treatment on photosynthesis in rice (*Oryza sativa* L.). Rice was grown at two $[CO_2]$ levels (ambient and ambient + 200 μmol mol^{-1}) throughout the growing season, and at full-heading stage, at half the plants, a sink-limitation treatment was imposed by the removal of the panicles. The PR treatment alleviated the reduction of green leaf area, the contents of chlorophyll (Chl) and Rubisco after the full-heading stage, suggesting delay of senescence. Nonetheless, elevated $[CO_2]$ decreased photosynthesis (measured at current $[CO_2]$) of plants exposed to the PR treatment. No significant $[CO_2]$ × PR interaction on photosynthesis was observed. The decrease of photosynthesis by elevated $[CO_2]$ of plants was associated with decreased leaf Rubisco content and N content. Leaf glucose content was increased by the PR treatment and also by elevated $[CO_2]$. In conclusion, a sink-limitation in rice improved N status in the leaves, but this did not prevent the photosynthetic down-regulation under elevated $[CO_2]$.

Additional key words: acclimation; allocation; elevated CO_2; photosynthesis; rice; senescence.

Introduction

Elevated atmospheric CO_2 concentrations ($[CO_2]$) can stimulate photosynthesis of C_3 plants in a short term, but after prolonged exposure to elevated $[CO_2]$, plants could acclimate. Acclimation to elevated $[CO_2]$ has been reported for many C_3 plant species, including rice (Nakano *et al*. 1997, Makino *et al*. 2000, Seneweera *et al*. 2002, Shimono and Bunce 2009, Shimono *et al*. 2009), wheat (Farage *et al*. 1998, Wall *et al*. 2000), soybean (Xu *et al*. 1994, Sawada *et al*. 2001, Ainsworth *et al*. 2004), barley (Fangmeier *et al*. 2000), cotton (Delucia *et al*. 1985) and other plant species (Moore *et al*. 1998).

It is generally accepted that the balance between sink and source strengths plays a pivotal role in regulating photosynthetic down-regulation under elevated $[CO_2]$. At elevated $[CO_2]$, carbohydrate can accumulate in leaves due to the higher source strength, leading to a feedback-based inhibition of photosynthesis (Moore *et al*. 1999). Considering a sink-source balance, plants with a relatively limited sink strength can therefore suffer from more severe photosynthetic down-regulation than those with stronger sinks (Arp 1991, Ainsworth *et al*. 2004). However, N levels in leaves can also affect the magnitude of photosynthetic down-regulation under elevated $[CO_2]$ (Makino *et al*. 1997, Farage *et al*. 1998, Fangmeier *et al*. 2000, Seneweera *et al*. 2002). With increasing sink strength as growth progresses, the N demand by the large

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Abbreviations: C_i – intercellular CO_2 concentration; Chl – chlorophyll; $[CO_2]$ – atmospheric CO_2 concentration; DAT – days after transplanting; DM – dry mass; FM – fresh mass; g_s – stomatal conductance; LA – leaf area; PPFD – photosynthetic photon flux density; PR – panicle removal; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; VPD – vapor pressure deficit.
Acknowledgements: We thank H. Nakamura, T. Saitoh, Y. Ookawara, M. Abe, and Y. Sato of the National Agricultural Research Center for Tohoku region for their technical assistance, and K. Tamura, K. Kudo, Y. Ohtsubo, and S. Kikuchi of the Center's Field Management Divisions for setting up the growth chambers. We also thank Dr. K. Ohashi (University of Tokyo) and Dr. J. Sakurai (National Agricultural Research Center for Tohoku region) for their advice on our Rubisco measurements. This study was supported by the Japan Society for the Promotion of Science. We would like to acknowledge to anonymous reviewers for invaluable comments and suggestions.

reproductive organs such as panicles and pods increases N translocation out of leaves and can therefore lead to photosynthetic down-regulation under elevated [CO_2] as a result of N limitations (Farage *et al*. 1998, Sims *et al*. 1998).

In terms of N dynamics, a plant with limited sink strength might alleviate the photosynthetic down-regulation that occurs under elevated [CO_2] by maintaining a high leaf N content by preventing N translocation from the leaves and maintaining a high level of photosynthetic activity (Makino *et al*. 1997, Farage *et al*. 1998, Fangmeier *et al*. 2000, Seneweera *et al*. 2002). It is therefore difficult to predict the effect of sink size on the magnitude of photosynthetic down-regulation that occurs under elevated [CO_2].

To determine the physiological effects of sink size on photosynthesis, artificial sink-removal treatments have proven to be useful. A number of studies under ambient [CO_2] have examined the effects of the sink-removal treatment on the photosynthesis of wheat (Guitman *et al*. 1991, Koide and Ishihara 1992, Wang *et al*. 1998), rice (Nakano *et al*. 1995, Shinano *et al*. 2006), soybean (Crafts-Brandner *et al*. 1984, 1991, Sawada *et al*. 1990), bean (Nakano *et al*. 2000), and peanut (Nautiyal *et al*. 1999), but these authors have reported contradictory, species-dependent responses. The treatment can decrease photosynthesis of legumes by increasing leaf carbohydrate concentrations (Crafts-Brandner *et al*. 1984, 1991, Sawada *et al*. 1990, Nautiyal *et al*. 1999, Nakano *et al*. 2000), but for cereals, can increase photosynthesis by increasing leaf N contents (Guitman *et al*. 1991, Koide and Ishihara 1992, Nakano *et al*. 1995, Wang *et al*. 1998, Shinano *et al*. 2006). This difference presumably relates to the different capacities of each species to develop alternative sinks for carbohydrates (Nakano *et al*. 2000). Cereals, but not legumes, can store carbohydrates in the leaf sheath and culm to prevent excessive accumulation of carbohydrates in their leaves.

Only few studies examined the effects of sink removal on photosynthetic down-regulation under elevated [CO_2]. For example, under elevated [CO_2], Xu *et al*. (1994) found that pod removal increased the down-regulation of photosynthesis in soybean. Jifon and Wolfe (2002) also reported that the sink limitation induced by heat stress in beans led to photosynthetic down-regulation under elevated [CO_2]. However, to our knowledge, no study has examined the effects of the sink removal on photosynthetic down-regulation in cereals under elevated [CO_2]. The present paper describes the first study to test the effects of the sink-removal treatment on photosynthetic down-regulation in rice under long-term-elevated [CO_2].

Materials and methods

On May 21th, 2005, seedlings of the rice cultivar 'Kirara 397', a local japonica and early mature cultivar from northern Japan, were transplanted in four sunlit growth chambers (6 m × 4.5 m × 2 m in height) at the National Agricultural Research Center for Tohoku region, Morioka (39°74'N, 141°13'E) under two [CO_2] levels. The cultivar 'Kirara 397' has higher N concentration per dry mass than late mature cultivars throughout the season (Shimono *et al*. 2009). There were two replicates for [CO_2]; two chambers were maintained at ambient [CO_2] and the other two were maintained at ambient [CO_2] plus 200 μmol mol^{-1} during the day. Outdoor air was continuously introduced into the chambers by ventilation fans. Pure gaseous CO_2 was added to the incoming air in the chambers with elevated [CO_2]. Seasonal daytime [CO_2] in the elevated [CO_2] chambers was 577 ± 10 μmol mol^{-1} (average ± SD), versus 378 ± 10 μmol mol^{-1} in the ambient [CO_2] chambers. Seasonal air temperature in the chambers was 22.9°C, and was on average 1.7°C higher than the outside air temperature. Seasonal mean solar radiation was 15.9 MJ m^{-2} d^{-1} outside the chambers. The light transmittance of the chambers was estimated at around 72% based on measurements of a similar type of chamber (Okada *et al*. 1995). Plants were grown in 9-L pots containing a paddy soil (typic gray lowland soil, Eutric Fluvisols) with one plant per pot grown under submerged conditions (to a depth less than 5 cm) throughout the growing season. Fertilizer was applied as the basic fertilizer (N, P_2O_5 equivalent, K_2O equivalent = 0.6, 0.9, 0.9 g per pot), and at the full-heading stage (60 d after transplanting, DAT), ammonium sulfate was top-dressed at a rate of 0.3 g of N per pot to minimize the effects of a potential N shortage during the grain-filling stage since plant N status is a critical factor for photosynthetic down-regulation under elevated [CO_2]. Note that without N fertilization, a final whole-rice-plant N content at harvesting was equivalent to 0.17 to 0.19 g per pot (data not shown) which came from soil *per se* additionally. We used 26 plants per [CO_2] treatment divided equally between the two growth chambers in each [CO_2] (including four plants that underwent panicle removal). Heading date was 53 DAT under ambient [CO_2] and 51 DAT under elevated [CO_2]. At the full-heading stage (61 DAT), four plants per [CO_2] treatment (two plants per growth chamber) had all of their panicles removed. The oven-dried mass of the trimmed panicles averaged 8 g per plant in the ambient [CO_2] treatment, *vs*. 14 g at elevated [CO_2]. To minimize the effects of environmental difference in the chambers, plants were rotated twice a week.

Plant dry mass (DM) (including roots) and leaf area (LA) of three plants (control) or one plant (PR treatment) per chamber was measured at the full heading (60 DAT) and maturity (100 DAT). After grinding the samples, the N concentrations of each organ were determined by Kjeldahl analysis. Leaf photosynthetic rate (P_N), stomatal

conductance (g_s), and intercellular CO_2 concentration (C_i) were measured at ambient levels of external [CO_2] of 350 μmol mol^{-1}, photosynthetic photon flux density (PPFD) at 2,000 μmol m^{-2} s^{-1}, vapor pressure deficit (VPD) at the leaf surface at 1.3–2.2 kPa, and leaf temperature at 25°C at mid-grain filling stage (76 to 78 DAT) using a portable photosynthesis system (*LI-6400, LI-COR, Lincoln*, NE, USA). Measurements were conducted in the flag leaf of two plants per chamber. After measuring photosynthesis, we measured leaf greenness using a SPAD meter (*SPAD-502, Minolta*, Tokyo, Japan), and the leaves were each cut into three segments and LA estimated (from leaf length and width). One leaf segment was used for the measurement of fresh mass (FM), and oven-dried mass (80°C for 72 h), and leaf N content per unit LA was measured by C/N analyzer. The other segments were stored at –80°C and used to measure Chl-, Rubisco- and carbohydrate contents. Chl and Rubisco were extracted by grinding in a chilled mortar with an extraction buffer [20 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA-Na_2, 1% polyvinyl-pyrolidone, 20% (v/v) glycerol, 1 mM dithiothreitol and 1 mM phenylmethylsulfonyl fluoride] and leaves stored. To measure the Chl content, 0.2 mL of the extract was added to 80% acetone and the absorbance of the supernatant was measured at 646.6 and 663.6 nm after centrifugation (10,000 × *g* for 5 min). The Chl content was estimated from the following equation (Porra *et al*. 1989):

$$\text{Chl}\ [\mu\text{M}] = 19.54\ A_{646.6} + 8.29\ A_{663.6} \qquad (1)$$

To measure the Rubisco content, 0.5 mL of the extract was transferred into a 1.5-mL test tube containing a sample buffer composed of 50 mM Tris-HCl, 2% SDS, 5% 2-mercaptoethanol, 0.014% bromphenol blue, and 40 mM phenylmethylsulfonyl fluoride. Samples were then boiled for 2 min. After centrifugation (10,000 × *g* for 10 min), the supernatant was used for SDS-PAGE with bovine albumin as the standard. After staining gels with Coomassie Brilliant Blue *R-250* (*Fluka AG*, Buchs, Switzerland), gel photographs were analyzed to determine their protein contents using the *Image J* software (http://rsbweb.nih.gov/ij/). To measure carbohydrate content, we ground another two leaf segments per growth chamber (one segment per leaf of plant) in a chilled mortar and pestle containing 80% ethanol. The supernatant (after centrifugation at 12,000 × *g* for 5 min) was used for HPLC analysis (*8020 system, Tosoh Corporation*, Tokyo, Japan) to determine the amounts of glucose, fructose and sucrose using the standards of these sugars. The precipitate was used to determine the starch content using the *F-kit* (*Roche*) following the manufacturer's instructions from the standard regression line.

Statistical analysis: To test significant differences for the [CO_2] and PR treatments, we used two-way analysis of variance using data from two replicates for [CO_2] and PR treatment. Analysis was conducted for mean values averaging for two to four plants at each chamber. Statistical analyses were performed with the *SPSS* statistical software (*SPSS Inc*., Chicago, IL, USA).

Results

The PR treatment tended to decrease total dry mass by 6–9% at both [CO_2] at maturity (Table 1), but the magnitude of the reduction was much smaller than the proportion of panicle mass which accounted for 43–46% of total DM in the control. As alternative sinks, the PR treatment increased stem mass (leaf sheath and culm) by 80% (P<0.001), and also green leaf by 36–37% (P<0.05) and root dry mass by 11–34% (P<0.05). Elevated [CO_2] tended to increase total DM by 7% (control) and 3% (PR). Green LA of whole plant, an indicator of plant senescence, largely decreased after the full heading under ambient [CO_2], but the magnitude of the reduction was decreased by the PR treatment. Elevated [CO_2] significantly decreased green LA of plants at harvest for either control or PR plants (P < 0.01) without interaction with the PR treatment by 28% (control) and 20% (PR). Whole-plant N allocation to leaves was increased by the PR treatment (P<0.001), and decreased by the elevated [CO_2] of 33% (control) and 27% (PR) (P<0.01) without interaction.

Leaf photosynthesis under ambient [CO_2] tended to be higher for the PR treatment than for the control by 11% (Table 2). The photosynthesis was decreased by elevated [CO_2] by 23% (control) or 37% (PR) (P<0.05) without interaction with the PR treatment. Although g_s was decreased by elevated [CO_2] (P<0.1) without interaction with the PR treatment, C_i concentration was not consistently affected by both [CO_2] and PR treatment.

Rubisco content, tending to be higher under the PR treatment, was significantly reduced by elevated [CO_2] (P<0.05) without interaction with the PR treatment (Table 2). Similar trend was observed for Chl content and SPAD readings.

Glucose, fructose and sucrose contents were significantly increased by the PR treatment (Table 3). Elevated [CO_2] significantly increased especially glucose content (P<0.05). Starch content tended to increase by the PR treatment and [CO_2].

Table 1. Dry mass (DM) of total (including roots), panicle, green leaf, stem (leaf sheath and culm) and root, and whole-plant N allocation to leaves of rice cultivar 'Kirara397' affected by $[CO_2]$ and panicle-removal (PR) treatment. *** $P<0.001$, ** $P<0.01$, + $P<0.1$, ns – not significant. Mean ± standard error ($n = 2$). Ratio E/A indicates the relative values at elevated $[CO_2]$ to those at ambient $[CO_2]$. Whole-plant N allocation to leaves = N content in leaves/N content of whole-plant (including roots). FH – full-heading stage; LA – leaf area.

$[CO_2]$	PR	Total DM [g plant^{-1}]		Panicle DM [g plant^{-1}]		Green leaf DM [g plant^{-1}]		Stem DM [g plant^{-1}]		Root DM [g plant^{-1}]		Green LA [cm^2 plant^{-1}]		Whole-plant N allocation to leaves	
		FH	Maturity	FH	Maturity	FH	Maturity	FH	Maturity	FH	Maturity	FH	Maturity	FH	Maturity
Ambient	Control	62 ± 2	114 ± 4	9 ± 0.2	48 ± 0.2	9.7 ± 0.0	8.6 ± 0.1	34 ± 0.6	46 ± 3.0	6.1 ± 0.4	6.4 ± 0.0	2377 ± 96	1775 ± 44	0.35 ± 0.01	0.12 ± 0.00
	PR	–	107 ± 4	–	–	–	11.8 ± 0.4	–	83 ± 3.5	–	7.1 ± 0.2	–	2348 ± 138	–	0.25 ± 0.00
Elevated	Control	68 ± 1	122 ± 11	11 ± 0.8	55 ± 4.6	9.3 ± 0.1	6.6 ± 0.2	38 ± 0.7	48 ± 5.1	6.0 ± 0.0	6.5 ± 0.6	2133 ± 17	1274 ± 69	0.30 ± 0.00	0.08 ± 0.01
	PR	–	110 ± 1	–	–	–	9.1 ± 0.7	–	86 ± 0.6	–	8.7 ± 0.5	–	1883 ± 114	–	0.18 ± 0.01
Ratio E/A	Control	1.09	1.07	1.30	1.15	0.95	0.76	1.10	1.04	0.98	1.01	0.90	0.72	0.88	0.67
	PR	–	1.03	–	–	–	0.77	–	1.04	–	1.23	–	0.80	–	0.73
$[CO_2]$		ns	ns	+	ns	+	ns	+	ns	ns	ns	ns	**	+	**
PR		–	ns	–	–	–	*	–	***	–	*	–	**	–	***
$[CO_2]$ × PR		–	ns	–	–	–	ns	–	ns	–	ns	–	ns	–	ns

Table 2. Leaf photosynthesis (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), Rubisco, chlorophyll (Chl) contents and SPAD reading of rice plants of cultivar 'Kirara 397' grown under two $[CO_2]$ levels and in the panicle-removal (PR) treatment. * $P<0.05$, ns – not significant. Mean ± standard error ($n = 2$). Ratio E/A indicates the relative values at elevated $[CO_2]$ to those at ambient $[CO_2]$.

$[CO_2]$	Treatment	P_N [μmol m^{-2} s^{-1}]	g_s [mol m^{-2} s^{-1}]	C_i [μmol mol^{-1}]	Rubisco [g m^{-2}]	Chl [mmol m^{-2}]	SPAD reading
Ambient	Control	15.4 ± 2.4	0.33 ± 0.10	228 ± 43	1.09 ± 0.25	0.31 ± 0.05	43.6 ± 2.5
	PR	17.1 ± 1.7	0.49 ± 0.04	264 ± 11	2.27 ± 0.16	0.35 ± 0.03	47.8 ± 2.0
Elevated	Control	11.9 ± 0.0	0.29 ± 0.04	256 ± 8	0.77 ± 0.09	0.27 ± 0.03	39.9 ± 0.2
	PR	10.9 ± 1.3	0.20 ± 0.02	238 ± 3	1.49 ± 0.08	0.32 ± 0.03	45.1 ± 2.2
Ratio E/A	Control	0.77	0.87	1.12	0.71	0.86	0.92
	PR	0.63	0.41	0.90	0.66	0.91	0.94
$[CO_2]$		*	+	ns	*	ns	ns
PR		ns	ns	ns	ns	ns	ns
$[CO_2]$ × PR		ns	ns	ns	ns	ns	ns

Discussion

The experiment demonstrated that long-term-elevated [CO_2] induced photosynthetic down-regulation of plants without significant [CO_2] × PR interaction despite the PR treatment delayed the leaf senescence and maintained higher leaf N status (Table 2). The present result used for rice was different from that used for legumes; sink-removal treatment in legumes has been reported to induce a greater photosynthetic down-regulation under elevated [CO_2] (Xu *et al*. 1994, Jifon and Wolfe 2002).

It is well accepted that while low N supply below the optimal level can accelerate the photosynthetic down-regulation under elevated [CO_2] (Farage *et al*. 1998, Sims *et al*. 1998), high N input above the optimum mostly does not alleviate the photosynthetic down-regulation in rice (Seneweera *et al*. 2002, Shimono and Bunce 2009, Shimono *et al*. 2009). Considering the present results that PR treatment increased leaf N content but caused photosynthetic acclimation, increased N supply to leaves *per se* would not be a major regulating factor for photosynthetic down-regulation under elevated [CO_2].

Table 3. Leaf carbohydrate contents of rice plants of cultivar 'Kirara 397' grown under two [CO_2] levels and in the panicle-removal (PR) treatment. ** P<0.01, * P<0.05, + P<0.1, ns – not significant. Mean ± standard error (n = 2). Ratio E/A indicates the relative values at elevated [CO_2] to those at ambient [CO_2].

[CO_2]	Treatment	Glucose [g m^{-2}]	Fructose [g m^{-2}]	Sucrose [g m^{-2}]	Starch [g m^{-2}]
Ambient	Control	0.001 ± 0.001	0.013 ± 0.008	4.61 ± 1.13	0.61 ± 0.36
	PR	0.039 ± 0.008	0.259 ± 0.128	4.16 ± 0.13	0.76 ± 0.07
Elevated	Control	0.024 ± 0.000	0.173 ± 0.077	7.63 ± 0.66	0.86 ± 0.14
	PR	0.100 ± 0.020	0.318 ± 0.065	3.46 ± 0.31	0.99 ± 0.08
Ratio E/A	Control	18.62	12.83	1.65	1.41
	PR	2.56	1.23	0.83	1.30
[CO_2]		*	ns	ns	ns
PR		**	+	*	ns
[CO_2] × PR		ns	ns	+	ns

On the other hand, whole-plant N allocation to leaves was significantly decreased by elevated [CO_2] without interaction with PR treatment (Table 1), in agreement with Makino *et al*. (1997). Changes of whole-plant N allocation to leaves even under PR treatment would be a key factor for photosynthetic down-regulation-elevated [CO_2]. Currently, our understanding of whole-plant N allocation during the senescence and the associated photosynthetic response under elevated [CO_2] is limited.

A possible signal is reported that hexose (glucose plus fructose) accumulation in leaves under elevated [CO_2] can trigger a signal for a plant to reduce its Rubisco content (Dai *et al*. 1999, Moore *et al*. 1999, Pourtau *et al*. 2006). In the present study, elevated [CO_2] increased glucose content (Table 3). There are many studies reporting close correlation between photosynthetic acclimation to elevated [CO_2] and increase of sugar content in leaves (reviewed in Moore *et al*. 1999). The increase of glucose content in the present study might partially be a key factor for photosynthetic down-regulation by elevated [CO_2]. However, it should be noted that the PR treatment under ambient [CO_2] increased glucose as well (Table 3) but this increase did not decrease whole-plant N allocation to leaves (Table 1) and also photosynthesis (Table 2). Miller *et al*. (1997) reported that elevated [CO_2] fastened individual leaf developmental stages as a cause of photosynthetic down-regulation by elevated [CO_2]. Although we did not measure the developmental stage of a single leaf, but the heading date was apparently enhanced by elevated [CO_2] by two days for all cultivars. Fasten life cycle of individual leaf by elevated [CO_2] might be another factor for regulating photosynthetic down-regulation.

Starch accumulation under elevated [CO_2] and PR treatment is the major factor that hinders CO_2 diffusion and decreases photosynthesis (Delucia *et al*. 1985, Nakano *et al*. 2000). In the present study, the starch content tended to be increased by elevated [CO_2], from 0.6 to 0.9 g m^{-2} in the control plants and from 0.8 to 1.0 g m^{-2} in the plants with their panicles removal (Table 3), but the magnitude was much smaller than observed in soybean (Xu *et al*. 1994) and bean (Jifon and Wolfe 2002). This small increase of starch in rice might be attributed to the fact that rice as well as other cereals (Guitman *et al*. 1991, Koide and Ishihara, 1992, Nakano *et al*. 1995, Wang *et al*. 1998, Shinano *et al*. 2006), but not legumes, had a large capacity for accumulating carbohydrates in stem as an alternative sink (Table 1). Additionally, the observed range of starch increase in the present study was much smaller than the threshold carbohydrate content for decreasing photosynthesis in rice of 6.0 g m^{-2} (Weng and Chen 1991). Physical resistance to CO_2 diffusion to chloroplasts through starch accumulation would not appear to be responsible for the

photosynthetic differences.

In conclusion, the present study revealed that the PR treatment under ambient [CO_2] increased N and Rubisco contents in leaves in rice. However, elevated [CO_2] induced photosynthetic down-regulation even under the PR treatment by changing N allocation within the plant although further physiological and molecular studies will be necessary to identify the causal factors responsible for the interactions between PR and elevated [CO_2] and their effects on photosynthetic acclimation.

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