Effect of CO₂ Enrichment on the Translocation and Partitioning of Carbon at the Early Grain-filling Stage in Rice (*Oryza sativa* L.)

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**Abstract**: Rice plants (*Oryza sativa* L.) were grown under normal (350 µL L⁻¹ CO₂) and CO₂-enriched (660 µL L⁻¹ CO₂) conditions, and ¹³CO₂ was supplied to the rice plants after heading to examine the translocation and partitioning of photosynthate at the early grain-filling stage. At 2 days after supplying ¹³CO₂, no difference in the ¹³C content of the whole plant was observed between the plants grown under normal and CO₂-enriched conditions, but translocation of ¹³C from the leaf blade to other plant organs seemed to be accelerated by CO₂ enrichment. Up to 9 days after supplying, ¹³CO₂ fixed into sucrose was mainly used to synthesize starch in the stem rather than translocated to the ear in plants grown under normal conditions. In contrast, the supplied ¹³C was rapidly translocated to the ear, and ¹³C stored as starch in the stem was also translocated to the ear in plants grown under CO₂-enriched conditions. Therefore, we concluded that CO₂ enrichment accelerated the translocation of carbohydrates to the ear.

**Keywords**: ¹³C, CO₂ enrichment, Grain-filling, Partitioning, Rice, Sink, Translocation.

The global atmospheric CO₂ concentration, presently averaging 360 µL L⁻¹, are increasing and are predicted to double by the end of this century. Though CO₂ enrichment can stimulate photosynthesis, crop production is affected not only by photosynthesis but also by translocation or partitioning of photosynthate in the plant. It is important to know how elevated CO₂ concentrations influence photosynthetic carbon fixation, the translocation and partitioning of photosynthates, and the grain-filling process in rice (*Oryza sativa* L.).

The photosynthetic responses of higher plants to CO₂ enrichment, including acclimation, have mainly been studied during vegetative growth (Webber et al., 1994; Koch, 1996; Wolfe et al., 1998), and CO₂ enrichment has been reported to increase the total concentration of non-structural carbohydrates in leaf blades, leaf sheaths, and culms (Rowland-Bamford et al., 1996). The enhancement of growth and yield by CO₂ enrichment has been reported for many plant species, including rice (Baker et al., 1990a; Baker and Allen, 1993; Rowland-Bamford et al., 1996). However, the effect of CO₂ enrichment on the translocation of photosynthates has only been examined during short-term CO₂ enrichment (Grodzinski et al., 1998).

Grain filling can be limited by the photosynthetic activity of the plant (the source), and the uptake capacity of the grain or the ear (the sink), or a combination of the two. Both the photosynthetic products transferred directly from the leaf blades and the repartitioned from vegetative tissues contribute to grain filling in rice (Ho, 1988). In many experiments designed to analyze these processes, the photosynthetic rate, photosynthate translocation, or spikelet number was studied (Iwasaki et al., 1992; Wada et al., 1993; Conocono et al., 1998). These experiments, however, were conducted under unnatural conditions (e.g., removal of panicles, spikelets or leaves), and the results might have been affected by the resulting physiological changes in the plant. Translocation has been evaluated based on the dry weight or carbohydrate content but this approach may not show real translocation rates, because newly produced or used carbohydrates in each organ were not taken into consideration (Gebbing et al., 1998). For such investigations, ¹³C is useful because it is a stable carbon isotope and safe to use. For this reason, we used labeling with ¹³C for our analysis of the partitioning of carbon in the plants. Although it is not possible to study the flow of newly fixed photosynthate in plants based solely on the measurement of dry...
weight or carbohydrate content in each organ, labeling with \(^{13}\)C provides a powerful tool for monitoring the partitioning of newly fixed carbon. Furthermore, it may reveal the translocation of fixed carbohydrates from the culm and leaf sheath to the ears.

The carbohydrates redistributed from vegetative tissues contribute to grain filling in rice. The maximum starch content was attained in the leaf sheaths at 4 days after heading, and thereafter, starch in the stem was digested into sucrose and translocated to the ears (Hirose et al., 1999). The translocation of fixed carbohydrates from the culms and leaf sheaths to the ears may be examined by the \(^{13}\)C content of the stems and the ears in the form of starch at several days after flowering and at the active grain-filling stage. Therefore, we examined the translocation and partitioning of \(^{13}\)C at the early grain-filling stage.

The objective of this study was to directly determine the effect of \(\text{CO}_2\) enrichment on the translocation and partitioning of carbohydrates in rice plants at the early grain-filling stage by examining the uptake, use, and translocation of \(^{13}\)C.

**Materials and Methods**

1. **Cultivation of rice plants**

   Rice (Oryza sativa L. cv Nipponbare) was grown in 1998 at the National Institute of Agro-Environmental Sciences in Tsukuba, Japan. Rice seeds were sown in plug pots at 25 °C, a relative humidity of 80 %, and a \(\text{CO}_2\) concentration of 350 or 660 µL L\(^{-1}\) on 20 April. One month later, the seedlings were transplanted into paddy boxes (150 × 150 × 30 cm high), with three seedlings per hill at a plant density of 20 × 20 cm in 16-m\(^2\) computer-controlled environmental chambers. Each chamber contained two paddy boxes and was exposed to natural light, with air conditioning to maintain ambient air temperatures, a relative humidity of 80 %, and \(\text{CO}_2\) concentrations of 350 and 400 µL L\(^{-1}\) (day and night, respectively) for the normal \(\text{CO}_2\) conditions, and 660 and 700 µL L\(^{-1}\) for the \(\text{CO}_2\)-enriched conditions (Sakai et al., 2001). The plants were supplied with fertilizer solution (5 g N m\(^{-2}\), 15 g P\(_2\)O\(_5\) m\(^{-2}\), 15 g K\(_2\)O m\(^{-2}\)) just before transplanting, and an additional 3 g N m\(^{-2}\) was supplied 56 days after transplanting (DAT). Heading occurred on 23 August (100 DAT) and 22 August (99 DAT) under normal and \(\text{CO}_2\)-enriched conditions, respectively.

2. **Measurements of air temperature and PAR**

   Air temperature was measured with a platinum resistance thermometer that was shielded, aspirated and placed outside the chambers, and PAR was measured with an infrared compact sensor (IKS-25, Koito, Tokyo, Japan). Air temperature and PAR were monitored every 10 s, and 5 min means were recorded.

3. **Overall \(\text{CO}_2\)-exchange rate**

   Plant \(\text{CO}_2\)-uptake in the day time \((C_{\text{uptake}})\) and release at night \((C_{\text{release}})\) were estimated from the \(\text{CO}_2\) injection rate required to maintain a constant \(\text{CO}_2\) concentration in the chamber, according to the method of Sakai et al. (2001); this method takes account of the leakage rate of the chamber and the \(\text{CO}_2\) flux out of the paddy water and the soil.

4. **Determination of sucrose and starch contents**

   The contents of soluble sugars and of starch were determined enzymatically according to the method reported by Nakamura and Yuki (1992).

5. **Supply of \(^{13}\)C and sampling**

   \(^{13}\)CO\(_2\) was supplied to six plants grown under normal conditions and six plants grown under enriched \(\text{CO}_2\) conditions on 24 August (101 DAT). Each plant was covered with a transparent bag made of 0.10 mm-thick polyvinylchloride film that neither transmitted nor absorbed air or \(\text{CO}_2\). Plants took up \(^{13}\)CO\(_2\) gas liberated from 100 mg of Ba\(^{13}\)CO\(_3\) powder mixed with 7.3 M H\(_3\)PO\(_4\) inside the bag. To allow plants to entirely absorb the liberated \(^{13}\)CO\(_2\), we sealed the bag with water and exposed the plants to \(^{13}\)CO\(_2\) at about 1400 µmol m\(^{-2}\) s\(^{-1}\) PAR for 90 min. Three of the six plants supplied with \(^{13}\)CO\(_2\) under each \(\text{CO}_2\) condition were harvested 2 days (26 August) and 9 days (2 September) after supplying the \(^{13}\)CO\(_2\). The plants were then divided into culms, leaf sheaths, leaf blades, and ears. Each plant part was immediately stored at −80 °C, then freeze-dried.

6. **Extraction of structural and non-structural carbohydrates**

   The dried plant materials were weighed, then ground to a fine powder. Samples (approximately 500 mg) were incubated in 80 % ethanol at 80 °C for 1 h, then centrifuged at 3000 g for 10 min, after which the ethanol-soluble fraction was decanted. The ethanol-soluble fraction was dried using a centrifugal dryer in vacuum, then further fractionated using a mixture of 2 mL of distilled water and 2 mL of chloroform, and the aqueous phase was passed through a cation-exchange resin (Dowex-50, Dow Chemical, USA) to remove amino acids. The efflux was collected, and used as the soluble sugar fraction. Distilled water was added to the ethanol-insoluble fraction which was dried using a centrifugal dryer in vacuum, and the suspension was boiled for 4 h. Thereafter, 20 units of amylase-glucosidase in 0.5 mL of 100 mM acetate buffer (pH 4.6) was added to the suspension, which was then incubated for 2 h at 55 °C to digest starch into glucose. After centrifugation at 3000 g for 10 min, the water-soluble fraction was collected, then passed through Dowex-50 and a nitrocellulose filter (Advantec, Tokyo, Japan) to remove protein. The filtrate was collected...
and used as the insoluble sugar fraction (i.e., starch). Both the soluble sugar and the sugar from starch were dried in vacuum using a centrifugal dryer. Thereafter, the water-insoluble fraction (structural components) was washed twice with distilled water at 40 ºC and dried in an oven at 80 ºC.

7. Measurement of $^{13}$C content

The total carbon and $^{13}$C contents were determined using an elemental analyzer (NC2500, Thermoquest, San Jose, CA, USA) and a mass spectrometer (Delta Plus System, Thermoquest, San Jose, CA, USA). Each fraction was dried completely, as described above, and the $^{13}$C content was determined. The $^{13}$C content was calculated in each organ, according to equation 1:

$\frac{(^{13}\text{C content})}{(\text{total carbon atom content})} \times (^{13}\text{C atom excess }%) \times 13 \quad (1)$

where $^{13}$C atom excess % is the difference in the $^{13}$C /($^{12}$C+$^{13}$C) ratio between the plants supplied with $^{13}$CO$_2$ and those supplied with ordinary $^{12}$CO$_2$. The increase in $^{13}$C content from 26 August to 2 September was calculated by subtracting the $^{13}$C content on 26 August from that on 2 September.

Results

Figure 1 shows the change in ear weight after heading. During the early grain-filling stage (2 September), the ear weight under CO$_2$-enriched conditions increased more rapidly than under normal CO$_2$ conditions, and the ear weight at maturity (13 October) under CO$_2$-enriched conditions (31.2 ± 0.9 g hill$^{-1}$) was significantly ($P<0.05$, Student’s t-test) heavier than under normal conditions (25.6 ± 0.9 g hill$^{-1}$). The sink strength, which is potentially responsible for this difference can be divided into two components: sink size and sink activity (including the activities of enzymes). Table 1 shows the influence of CO$_2$ enrichment on spikelet number, which is an indication of sink size. The spikelet number per ear or per hill was not significantly higher under the CO$_2$-enriched conditions (Table 1).

Though the difference in ear weight was not seen on 26 August, the influence of CO$_2$ enrichment on ear
weight was marked on 2 and 12 September (Fig. 1). To examine the translocation of fixed carbohydrate from the stems to the ears, we measured the carbon budget (carbohydrates) during the early grain-filling stage for a week (from 26 August to 2 September). Figure 2 shows the changes in air temperature and incident PAR from 24 August to 2 September. It was sunny and PAR was above 20 mol m\(^{-2}\) day\(^{-1}\) from 24 to 26 August. Thereafter (from 28 August to 2 September), it was cloudy or rainy, hence PAR and air temperature were lower than those during the first 3 days. \(C_{\text{uptake}}\) was high under both normal and CO\(_2\)-enriched conditions during the first 3 days (24, 25 and 26 August), when PAR was above 20 mol m\(^{-2}\) day\(^{-1}\) (Fig. 3). However, when PAR was below 13 mol m\(^{-2}\) day\(^{-1}\) (from 28 August to 2 September), \(C_{\text{uptake}}\) was low under both normal and CO\(_2\)-enriched conditions; there was no significant

![Fig. 3. Change in CO\(_2\) uptake and release from 24 August to 2 September. CO\(_2\) uptake in the day time (\(C_{\text{uptake}}\)) and CO\(_2\) release at night (\(C_{\text{release}}\)) represent the amounts of absorbed and released CO\(_2\), respectively. The plants grown under normal and CO\(_2\)-enriched conditions are shown by the broken and solid lines, respectively. Bars indicate the standard errors of three replications. Differences between the means under the two CO\(_2\) conditions were analyzed using Student's t-test (*, \(P<0.05\)).](image)

![Fig. 4. Increase in total and structural weights of each plant organ from 26 August to 2 September, and increases in starch and sucrose contents over this period. W, L, S, and E indicate the whole plant, leaf blades, stems (including leaf sheaths and culms), and ears, respectively. Closed and open columns indicate CO\(_2\)-enhanced and normal conditions, respectively, plus the standard errors for three replications. The increase in total and structural weights was calculated by subtracting the weight on 26 August from that on 2 September. Differences between the means were analyzed using Student's t-test (**, \(P<0.01\); *, \(P<0.05\)).](image)
difference in \( C_{\text{uptake}} \) between the two conditions from 24 August to September 2. \( C_{\text{release}} \) was lower than \( C_{\text{uptake}} \). Although the daily CO\(_2\) budget was significantly higher under the CO\(_2\)-enriched conditions during the first 2 days, the total CO\(_2\) budget from 26 August to 2 September was not significantly different (4.3±0.2 and 4.4±0.2 g CO\(_2\) hill\(^{-1}\) under normal and CO\(_2\)-enriched conditions, respectively).

The dry weight of the ears (total weight in Fig. 4) increased from 26 August to 2 September by 2.4 and 5.1 g hill\(^{-1}\) under normal and CO\(_2\)-enriched conditions, respectively, and the difference was significant (Fig. 4). The dry weights of the other plant organs did not increase significantly during this period. The starch content in the ears under CO\(_2\)-enriched conditions increased significantly (to 1.45 g hill\(^{-1}\)), and this increase was approximately three times the increase observed under normal conditions (0.45 g hill\(^{-1}\)).

Rice plants were supplied with \(^{13}\)CO\(_2\) to examine the translocation and partitioning of photosynthate in the plants. We preliminarily examined the absorption of \(^{13}\)CO\(_2\) liberated from 100 mg of Ba\(^{13}\)CO\(_3\) powder into the rice plant (cv Fujihikari) at the heading stage under 900 µmol m\(^{-2}\) s\(^{-1}\) PFD of artificial lights, which was lower than PFD at the 24 August in 1998 (Fig. 5). Though 95 % of supplied \(^{13}\)C was absorbed into a plant at 60 min after the start of supplying \(^{13}\)CO\(_2\), all \(^{13}\)C was absorbed at 90 min. To absorb \(^{13}\)CO\(_2\) entirely, we exposed the plants to \(^{13}\)CO\(_2\) for 90 min in this experiment. At 2 days after supplying \(^{13}\)CO\(_2\), no difference was observed in the \(^{13}\)C content of the

Table 2. Partitioning of \(^{13}\)C supplied to plants on 24 August for the structural components of each organ and to sucrose and starch on 26 August.

| CO\(_2\) condition | Organ      | Total | Component          |
|-------------------|------------|-------|--------------------|
|                   |            | %     | Structural | % | Sucrose | % | Starch | % |
| Normal            | Leaf blade | 9.0 ± 1.1 * | 3.8 ± 0.4 * | 1.2 ± 0.1 ns | 0.1 ± 0.1 ns |
|                   | Stem       | 31.5 ± 1.6 ns | 14.4 ± 0.7 ns | 4.2 ± 0.5 ns | 3.7 ± 0.5 ns |
|                   | Ear        | 18.8 ± 1.2 ns | 14.1 ± 0.4 ns | 0.5 ± 0.1 ns | 0.8 ± 0.5 ns |
|                   | Whole plant| 59.3 ± 2.2 ns | 30.9 ± 0.4 ns | 5.9 ± 0.3 ns | 4.6 ± 1.0 ns |
| Enriched          | Leaf blade | 6.2 ± 0.4 | 2.4 ± 0.2 | 1.0 ± 0.1 | 0.1 ± 0.0 |
|                   | Stem       | 29.6 ± 4.4 | 13.5 ± 1.7 | 5.2 ± 0.6 | 4.7 ± 0.9 |
|                   | Ear        | 19.3 ± 1.2 | 11.9 ± 1.4 | 0.5 ± 0.2 | 0.7 ± 0.6 |
|                   | Whole plant| 55.1 ± 3.7 | 27.9 ± 1.7 | 6.7 ± 0.4 | 5.5 ± 0.4 |

The amount of \(^{13}\)C supplied to the plants was set to 100 %. The stem component included leaf sheaths and culm. Values represent the mean ± standard error for three replications. Differences between the means under the two CO\(_2\) conditions were analyzed using Student’s t-test (*, \( P < 0.05 \); ns, non-significant).
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Whole plant under normal (59.3 %) and CO$_2$-enriched conditions (55.1 %), but the $^{13}$C content of leaf blades under CO$_2$-enriched conditions (6.2 %) was lower than under normal conditions (9.0 %) (Table 2). In the whole plant, the $^{13}$C content of sucrose fraction at 2 days after supplying $^{13}$CO$_2$ was about 6 % under both conditions. Thereafter, the $^{13}$C labeling in sucrose of the whole plant decreased under both conditions, accompanied by an increase in $^{13}$C labeling of starch during the period we examined (from 26 August to 2 September) (Fig. 6). This indicates that sucrose was converted starch during this period for the plant as a whole. In stems, $^{13}$C in starch increased significantly during this period under normal conditions, but decreased or did not change under CO$_2$-enriched conditions. In the ears, the $^{13}$C content of starch under CO$_2$-enriched condition increased by 5.3 % during this period, but only by 0.8 % under normal conditions.

**Discussion**

In most investigations concerned with the contribution of stored and newly produced photosynthate to grain-filling, researchers have analyzed the long-term reserves and the growth of grains and other plant parts. However, estimates of translocation based solely on changes in dry-matter weight or carbohydrate content may not represent actual translocation, because newly produced or used carbohydrates in each organ are not taken into consideration (Gebbing et al., 1998). In the present study, we analyzed the growth of each plant organ together with the partitioning of photosynthate labeled with $^{13}$C to let us estimate the contribution of the reserves in each organ to grain-filling. Under normal conditions, the starch content of the stem did not change for 1 week after heading; thus, it seems that carbohydrates stored in the stem were not decomposed and translocated to the grains (Fig. 4). However, based on the results of the $^{13}$C analysis, it appears that a large amount of the soluble sugars in the stem was used to synthesize starch and was stored in the stem during this period (Fig. 6). These findings indicate that carbohydrates stored in the stem are translocated to the grains, and newly fixed carbon is stored in the stem.

In the present paper, the roles of the roots and of dead leaves were not considered. In another study conducted in a paddy field (Kim et al., 2001), the amount of $^{13}$C translocated to the roots was less than 1 % of the $^{13}$C supplied at the time of heading (data not shown). We also measured the amount of fallen leaves every 30 days, but the data cannot be presented here, because the difference between plants grown under normal and CO$_2$-enriched conditions was so small and because the amount of leaves fallen during the 9-day study period was negligible in our analysis of $^{13}$C translocation. Though about 40% of the $^{13}$C supplied on 24 August was released from the whole plant before 26 August, the $^{13}$C content of the whole plant did not decrease from 26 August to 2 September (Table 2, Fig.6). Since about 50 % of the $^{13}$C supplied at 3 days
after heading day was used for respiration within 2 days and thereafter, little $^{13}$C in the whole plant was released (Hara et al. 1999), we thought that about 40 % of the $^{13}$C supplied on 24 August was used for respiration within 2 days and little $^{13}$C was used for respiration after 26 August.

Heading day under CO$_2$-enriched condition was earlier than that under normal CO$_2$ condition, and it might affect the translocation and partitioning of carbohydrate. However, since the difference in heading day was only one day and the differences were not observed in partitioning of $^{13}$C to starch fractions in the stems and ears between under normal and CO$_2$-enriched conditions (Table 2), the influence of heading day was thought to be little at 2 days after supplying CO$_2$. The translocation and partitioning of carbohydrate at 9 days after supplying CO$_2$, active grain filling stage, might be affected by the difference in heading day. However, since the difference in heading days was only one day and the plants under both conditions were grown under the same light and temperature conditions, the influence of heading day was also thought to be little at even 9 days after supplying CO$_2$.

Our finding that ear weight was increased by CO$_2$ enrichment agrees with previous reports (Kimball, 1983, 1986; Kim et al., 2001). Although increased photosynthesis has been reported under long-term CO$_2$ enrichment (Baker et al., 1990b; Monje and Bugbee, 1998; Sakai et al., 2001), the influence of CO$_2$ enrichment on carbohydrate translocation has been reported by only a few researchers (e.g., Grüters, 1999). At 2 days after supplying CO$_2$, the $^{13}$C content of leaf blades under CO$_2$-enriched conditions was lower than under normal conditions (Table 2); thus the carbon fixed in leaf blades under CO$_2$-enriched conditions was translocated to other organs more rapidly than under normal conditions. This indicated that CO$_2$ enrichment accelerated the translocation of carbohydrates from leaf blades. CO$_2$ enrichment has been reported to increase the activity of leaf sucrose-phosphate synthase (SPS, EC2.4.1.14), which would promote the translocation of photosynthates in rice plants (Hussain et al., 1999; Seneweera et al., 1995, Ono et al. 2003). These findings suggest that translocation might be increased because CO$_2$ enrichment promotes the activities of enzymes such as SPS that are necessary for photosynthetic translocation.

Although effects of CO$_2$ enrichment on the final grain yield have been reported (Kimball, 1983, 1986; Kim et al., 2001), its influence on grain-filling has not been investigated in detail. The balance between source and sink was thought to determine carbohydrate storage in the grains during the grain-filling stage (Ho, 1988). However, our study revealed little difference between the total CO$_2$ budget (source activity) and the spikelet number per plant or per ear (sink size) between plants grown under normal and CO$_2$-enriched conditions (Fig. 3, Table 1). We hypothesize that CO$_2$ enrichment promotes the efficiency of translocation of carbon. Newly fixed carbon was translocated more rapidly from leaf blades under CO$_2$-enriched conditions, but the $^{13}$C content in the form of starch in the ears at 2 days after supplying CO$_2$ under a CO$_2$-enriched condition was not greater than under normal conditions (Table 2). We thought that sink activity was too low to transport a large amount of carbon fixed in the leaf blades to the ear, and excess carbon was stored as starch in the stem or as sucrose in a whole plant. At 9 days after supplying CO$_2$, the $^{13}$C content of sucrose in the whole plant decreased under both CO$_2$ conditions, but the $^{13}$C content of starch in both the stem and the ear increased under normal conditions (Fig. 6). In contrast, the $^{13}$C content of starch in the ears increased more under CO$_2$-enriched conditions than under normal conditions, but the $^{13}$C content of starch decreased in the stem under CO$_2$ enrichment. The sink activity in the plant under normal CO$_2$ conditions was thought to be lower than that under CO$_2$-enriched conditions and excess carbon under normal CO$_2$ conditions was stored in the stem. Considering that $^{13}$C content of starch decrease in the stem under CO$_2$-enriched conditions, CO$_2$ enrichment is considered to promote the translocation of carbohydrate from the culms and leaf sheaths accompanied by an increase in sink activity. We suggest that CO$_2$ enrichment promotes sink activity and metabolic activities related to the translocation of carbon to other plant organs. We also suggest that the heavier ear weight at maturity under CO$_2$-enriched condition results from the increased translocation of carbon to the ear during grain-filling period.

The effect of CO$_2$ enrichment on enzymes related to carbohydrate metabolism has been studied in the leaf blade but not in the stem. In a previous study, we found that the levels of mRNA for cytosolic fructose 1,6-bisphosphatase (EC3.1.13.11) and for SPS were increased in the leaf blade by CO$_2$ enrichment (Aoki et al., 2003). We also found that CO$_2$ enrichment accelerated the translocation of carbohydrates from the leaf blade. Although we also found that CO$_2$ enrichment accelerated the translocation of carbohydrate from the stem to the ears, whether it increased enzymatic activity in the stem has not been investigated in sufficient depth. It is nonetheless possible that increased activities of enzymes related to debranching and degradation of starch and to translocation will be observed under CO$_2$ enrichment conditions.

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References

Aoki, N., Sasaki, H., Ono, K., Seneweera, S., Sakai, H., Kobayashi, K. and Ishimaru, K. 2003. Effects of elevated CO₂ concentration on rice before and after heading: changes in carbohydrate contents and transcript levels of enzymes involved in carbon fixation and sucrose synthesis in flag-leaf blades. Plant Prod. Sci. 6 : 52-58.

Baker, J.T., Allen, L.H. Jr. and Boote, K.J. 1990a. Growth and yield responses of rice to subambient, ambient and superambient carbon dioxide concentration. J. Agric. Sci. (Camb.) 115 : 313-320.

Baker, J.T., Allen, L.H. Jr., Boote, K.J., Jones, P. and Jones, J.W. 1990b. Rice photosynthesis and evapotranspiration in subambient, ambient, and superambient carbon dioxide concentration. Agron. J. 82 : 834-840.

Baker, J.T. and Allen, L.H. Jr. 1993. Contrasting crop species responses to CO₂ and temperature: rice, soybean and citrus. Vegetatio 104/105 : 299-320.

Conocono, E.A., Egdane, J.A. and Setter, T.L. 1998. Estimation of canopy photosynthesis in rice by means of daily increases in leaf carbohydrate concentrations. Crop Sci. 38 : 987-995.

Gebbing, T., Schnyder, H. and Kühbauch, W. 1998. Carbon mobilization in shoot parts and roots of wheat during grain filling: assessment by ¹³C/¹²C steady-state labeling, growth analysis and balance sheets of reserves. Plant Cell Environ. 21 : 301-313.

Grodzinski, B., Jiao, J. and Leonardos, E.D. 1998. Estimating photosynthesis and concurrent export rates in C₃ and C₄ species at ambient and elevated CO₂. Plant Physiol. 117 : 207-215.

Grüters, U. 1999. On the role of wheat stem reserves when source-sink balance is disturbed by elevated CO₂. J. Appl. Bot. 73 : 55-62.

Hara, T., Sasaki, H., Yamagishi, T. and Ishii, T. 1999. The fate of assimilated ¹³C in rice. 1. Seasonal changes in the distribution of assimilated ¹³C and its subsequent redistribution. Jpn. J. Crop Sci. 68 (extra 2) : 120-121.

Hirose, T., Endler, A. and Ohsugi, R. 1999. Gene expression of enzymes for starch and sucrose metabolism and transport in leaf sheaths of rice (Oryza sativa L.) during the heading period in relation to the sink to source transition. Plant Prod. Sci. 2 : 178-183.

Ho, L.C. 1988. Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. Ann. Rev. Plant Physiol. Plant Mol. Biol. 39 : 355-378.

Hussain, M.W., Allen, L.H. Jr. and Bowes, G. 1999. Up-regulation of sucrose phosphate synthase in rice grown under elevated CO₂ and temperature. Photosynth. Res. 60 : 199-208.

Iwaska, Y., Maé, T., Makino, A., Ohira, K. and Ojima, K. 1992. Nitrogen accumulation in the inferior spikelet of rice ear during ripening. Soil Sci. Plant Nutr. 38 : 517-525.

Kim, H.Y., Lieffering, M., Miura, S., Kobayashi, K. and Okada, M. 2001. Growth and nitrogen uptake of CO₂-enriched rice under field conditions. New Phytol. 150 : 223-229.

Kimball, B.A. 1983. Carbon dioxide and agricultural yield: an assemblage and analysis of 430 prior observations. Agron. J. 75 : 779-788.

Kimball, B.A. 1986. Influence of elevated CO₂ on crop yield. In H.Z. Enoch and B.A. Kimball eds., Carbon Dioxide Enrichment of Greenhouse Crops, vol. 2 : Physiology, Yield, and Economics. CRC Press, Boca Raton, FL. 105-115.

Koch, K.E. 1996. Carbohydrate-modulated gene expression in plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. 47 : 509-540.

Monje, O. and Bugbee, B. 1998. Adaptation to high CO₂ concentration in an optimal environment: radiation capture, canopy quantum yield and carbon use efficiency. Plant Cell Environ. 21 : 315-324.

Nakamura, Y. and Yuki, K. 1992. Changes in enzyme activities associated with carbohydrate metabolism during the development of rice endosperm. Plant Sci. 82 : 15-20.

Ono, K., Sasaki, H., Harai, T., Kobayashi, K. and Ishimaru, K. 2003. Changes in photosynthetic activity and export of carbon by overexpressing a maize sucrose-phosphate synthase gene under elevated CO₂ in transgenic rice. Plant Prod. Sci. 6 : 281-286.

Rowland-Bamford, A.J., Baker, J.T., Allen, L.H. Jr. and Bowes, G. 1996. Interactions of CO₂ enrichment and temperature on carbohydrate accumulation and partitioning in rice. Environ. Exp. Bot. 36 : 111-124.

Sakai, H., Yagi, K., Kobayashi, K. and Kawashima, S. 2001. Rice carbon balance under elevated CO₂. New Phytol. 150 : 241-249.

Seneweera, S.P., Basra, A.S., Barlow, E.W. and Conron, J.P. 1995. Diurnal regulation of leaf blade elongation in rice by CO₂: is it related to sucrose-phosphate synthase activity? Plant Physiol. 108 : 1471-1477.

Wada, Y., Miura, K. and Watanabe, K. 1993. Effects of source-to-sink ratio on carbohydrate production and senescence of rice flag leaves during the ripening period. Jpn. J. Crop Sci. 62 : 547-553.

Webber, A.N., Nie, G.Y. and Long, S.P. 1994. Acclimation of photosynthetic proteins to rising atmospheric CO₂. Photosynth. Res. 39 : 413-425.

Wolfe, D.W., Gifford, R.M., Hilbert, D. and Luo, Y. 1998. Integration of photosynthetic acclimation to CO₂ at the whole-plant level. Global Change Biol. 4 : 879-893.

* In Japanese.