Elevated CO2 Could Induce Heat Stress in Two Japanese Cultivars of Rice (Oryza Sativa L.) via Reduction in Transpiration Under High Air Temperature Conditions

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Abstract

Two Japanese cultivars of rice (Oryza sativa L.), Hinohikari and Nikomaru, were planted using potting soil on June 13, 2018, and were exposed to elevated CO₂ from June 26 to October 9 using open-top chambers. The study was conducted in Nagasaki, in the Kyushu region of Japan, where the air temperature is relatively high. There were two treatments: ambient CO₂ treatment with approximately 400 µmol mol⁻¹ (ppm) CO₂, and elevated CO₂ treatment with approximately 550 ppm CO₂. The elevated CO₂ treatment significantly increased the net photosynthetic rate and whole-plant dry mass of the two rice cultivars. However, this treatment did not produce significant effects on grain yield and adversely affected grain appearance quality of both cultivars. Among the yield components, spikelet fertility was significantly reduced by exposure to elevated CO₂. These adverse effects were typical manifestations of heat stress in rice. Even under ambient CO₂ treatment, there was relatively low spikelet fertility and grain appearance quality, because air temperature during the cultivation period was higher than the standard climatological normal of air temperature. Furthermore, under elevated CO₂ treatment, significant reductions in transpiration rate of flag leaves were observed during the flowering period. This may cause an increase in temperature of the canopy, including the panicle, and induce heat stress. These results suggest that elevated CO₂ could induce heat stress in rice via reduction in transpiration under high air temperature conditions.

1. Introduction

Atmospheric CO₂ concentration has increased because of industrial activities, such as fossil fuel combustion and cement production (Myhre et al. 2013). The increase in atmospheric CO₂ concentration is beneficial for plant growth and crop production because photosynthesis is a CO₂-dependent process (Körner 2003). According to a meta-analysis of the results of free-air CO₂ enrichment (FACE) experiments, the elevation of atmospheric CO₂ concentration from ambient levels in the 1990s to the 2000s, to 550–600 µmol mol⁻¹ (ppm), increased net photosynthetic rate and dry matter production of C₃ plants by approximately 34% and 20%, respectively (Long et al. 2004). Ainsworth (2008) reported that, based on the results of a meta-analysis, elevated CO₂ (627 ppm) increased the yield of rice (Oryza sativa L.) by 23%, varying with the magnitude of increase and the fumigation technique. Elevated CO₂ induces stomatal closure in addition to increasing net photosynthesis, thus increasing the leaf-level water use efficiency, which results in the improvement of the water status of C₃ plants (Ainsworth and Long 2005). As the induction of stomatal closure reduces transpiration from the leaves, elevated CO₂ increases the canopy temperature (Long et al. 2006; Bernacchi et al. 2007; Kimball 2016). For example, Yoshimoto et al. (2005) observed an elevated CO₂-induced reduction in stomatal conductance in the leaves of rice in their FACE experiment in China, resulting in increased leaf and panicle temperatures. These results suggest that elevated CO₂ could affect the growth and physiological functions of plants by increasing the plant temperature.

Rice is an important agricultural crop in Japan. Rice is sensitive to heat stress and shows heat stress-induced damage, such as reductions in grain quality and spikelet fertility (e.g., Jagadish et al. 2015; Morita et al. 2016). Several studies have shown that elevated CO₂ exacerbates the heat stress in rice. Kim et al. (1996) and Matsui et al. (1997) reported that the heat-induced reduction in spikelet fertility of rice was intensified by elevated CO₂ in the Japanese (japonica) rice cultivar ‘Akihikari,’ cultivated in a greenhouse-type chamber in Japan, and indica rice cultivar ‘IR72,’ cultivated in an open-top chamber (OTC) in the Philippines, respectively. Based on the results of the 5-year FACE experiment, Kobayasi et al. (2019) observed a negative relationship between air temperature on flowering day and spikelet fertility of the Japanese rice cultivar ‘Koshihikari’ under elevated CO₂ conditions, but was not observed under ambient CO₂ conditions. These results suggest that elevated CO₂ can reduce spikelet fertility under high-temperature conditions.

In western Japan, especially in the Kyushu region, the quality of rice grains began to decline in the 1980s (Okada et al. 2009; Ishigooka et al. 2011). It has been suggested that the temperature increase caused a trend of declining grain quality in this region, while the cumulative solar radiation during the grain filling period affected the reduction in grain quality (Okada et al. 2009; Uno et al. 2012). These results suggest that the temperature in this region has reached the point of inducing heat stress in rice. It is projected that atmospheric CO₂ concentration will continue to increase in the future (Myhre et al. 2013). Because of this continued increase in CO₂ levels in the atmosphere, the canopy temperature, including the panicle temperature, will increase via elevated CO₂-induced transpiration reduction. In the Kyushu region, therefore, elevated CO₂ could induce additional heat stress outcomes, such as reduced grain quality and diminished spikelet fertility of rice.

In warm temperate areas of Japan, including the Kyushu region, the primary rice cultivar is ‘Hinohikari.’ Since Hinohikari rice is relatively sensitive to heat stress, the heat-tolerant cultivar ‘Nikomaru’ has been introduced in this region (Tanaka et al. 2009; Tanamachi et al. 2016). Using these two cultivars with varying heat tolerances, we investigated the effects of elevated CO₂ on rice cultivated in OTCs. The study was conducted in Nagasaki in the Kyushu region of Japan, where heat stress on rice has become increasingly evident. Under these conditions, we hypothesized that exposure to elevated CO₂ could induce heat stress in rice via elevated CO₂-induced reduction in transpiration.
2. Materials And Methods

2.1 Plant material

Two Japanese cultivars of japonica rice, ‘Hinohikari’ and ‘Nikomaru,’ were used. Hinohikari rice is a common cultivar in warm temperate zones such as the Kyushu region in Japan, and Nikomaru rice is also cultivated in warm temperate areas and is a recently developed cultivar with higher heat tolerance. The seedlings were planted on June 13, 2018, in 1/5000 a Wagner's pots (ø159 mm × 300 mm in height, approximately six L) filled with a flooded soil mixture of Andisol and Akadama soils (1:1) at three hills per pot and two seedlings per hill. Before planting, 1.013 g of N-P-K fertilizer (N-P-K = 15:15:15) (i.e., 76 kg N ha⁻¹) and silica fertilizer (5.0 g) were applied to the pots. The seedlings were grown in six OTCs (60 cm in width, 120 cm in height, and 82.5 cm in length) located at Nagasaki University (Nagasaki, Japan) from June 26 to October 9. Inside each OTC, ambient air was introduced using a fan (MRS18V2-B, ORIENTAL MOTOR Co., Ltd., Japan) and was blown in an upward direction from the bottom of the chamber. For each cultivar, three pots were assigned to each chamber, and a total of six pots were placed on the floor of each chamber. The N-P-K fertilizer (1.013 g) was also applied on July 17 and August 25. Irrigation was conducted to keep the soil flooded during the cultivation period, except during drainage at the end of July. The air temperature (T_air) and relative air humidity (RH) both inside and outside of each chamber were continuously measured using a TR-72-wf Thermo Recorder (T&D Corporation, Nagano, Japan). The sensor of the recorder was set at a height of 115 cm from the bottom of each OTC, which corresponded to the canopy height after rice heading. Each sensor was installed inside a ventilated two-layer radiation shield consisting of a fan (MU92SS-11, ORIENTAL MOTOR Co., Ltd., Japan) and two polyvinyl chloride pipes with different diameters, the outer pipe being covered with an aluminum foil.

2.2 CO₂ treatment

The rice plants were exposed to ambient or elevated CO₂ concentrations in the OTCs from June 26 to October 9. Ambient air was introduced into three of the six OTCs assigned to the ambient CO₂ treatment. In addition to ambient air, CO₂ gas was introduced into the other three OTCs, assigned to the elevated CO₂ treatment. To introduce CO₂ gas, a polyethylene tube connected to a CO₂ cylinder was inserted into the chamber near the outlet of the fan located at the lower part of the chamber. The target CO₂ concentration in the elevated CO₂ treatment was 550 ppm during the day, from before sunrise to after sunset. The introduction of CO₂ gas was controlled manually by a valve with a flow meter, and the flow was stopped at night. The CO₂ concentration inside the OTCs was monitored using a CO₂ gas analyzer (LI-820, Li-Cor Inc., USA) and was continuously calibrated with standard CO₂ gases (601 ppm and 374 ppm). To measure the CO₂ concentration inside the chamber, the air inside each chamber at a height of 110 cm above the bottom was sampled sequentially using an electric valve system for a period of 5 min and introduced into the CO₂ gas analyzer. The seasonal mean CO₂ concentrations in ambient CO₂ and elevated CO₂ treatments during the day were 409.4 ± 0.6 ppm and 546.9 ± 3.1 ppm (mean of three chamber replications ± standard deviation), respectively. Although we did not measure the distribution of CO₂ concentration inside the chamber throughout the experimental period, the range of the horizontal distribution at a height of 80 cm inside the chamber was approximately 95%–105% of the average. In each treatment, the pots were rotated within and among the chambers at 10–14-day intervals to minimize variation in chamber effects among the chambers.

2.3 Measurement of the leaf gas exchange rates

During the flowering period from August 22 to 27, 2018, the light-saturated net photosynthetic rate (A), stomatal conductance (g_s), and transpiration rate (E) of the flag leaves were measured using an infrared gas analyzer system (LI-6400, Li-Cor Inc., USA). For each cultivar, three or four plants from each OTC were randomly selected for measurements. While the measurements were taken, air temperature, relative air humidity, and the photosynthetic photon flux density in the leaf chamber were maintained at 30 °C, 65%, and 1500 µmol m⁻² s⁻¹, respectively. For the measurements of A, g_s, and E, the atmospheric CO₂ concentration in the leaf chamber was 400 ppm for the ambient CO₂ treatment and 550 ppm for the elevated CO₂ treatment.

2.4 Measurements of the growth, yield, yield components, and grain appearance quality

To determine the heading date, we counted the stem and panicle numbers per plant and calculated the heading rate every day from August 21 to September 4. The heading date was defined as the day on which the mean heading rate reached 50% for each treatment. To measure the dry mass (DM) of plant organs, yield, and yield components, all rice plants of both Hinohikari and Nikomaru cultivars were harvested on October 7 and 9, 2018, respectively. The harvested plants were divided into panicles, leaf blades, stems (including leaf sheaths), and root parts. The separated plant organs, except for the panicle, were dried in an oven at 80 °C for 5 days and then weighed. The panicles were counted to obtain the panicle number per plant and then air-dried in the field for 5 days. Whole-plant DM was calculated as the sum of the DM of all plant organs. Grains were separated from dried panicles and counted to obtain the grain number per panicle. The grains were manually categorized into two groups, filled grains and unfilled grains, and counted. Filled grains were defined as fertile grains, including
ripened and partially filled grains, and unfilled grains were defined as unfertilized grains. To evaluate spikelet fertility, the percentage of filled grains was calculated from the total grain number and the filled grain number for each plant. Filled grains were unhusked and weighed to obtain the yield per plant, and then the 1000-grain mass was calculated with the filled grain number per plant. The harvest index (HI) was the ratio of grain mass (yield) per plant to shoot (panicle, leaf blade, and stem) DM. Grain appearance quality was determined using a rice grain image analyzer (ES-1000, Shizuoka Seiki Co., Ltd., Japan), which classifies grains into perfect, immature, damaged, abortive, and colored grains (Sawada et al., 2016). Grain appearance quality was expressed as the percentage of the number of each quality class to the total grain number.

2.5 Statistical analysis

The mean of each parameter for each OTC was used for the statistical analyses (n = 3). Two-way analysis of variance (ANOVA) was used to test the effects of elevated CO₂ treatment and cultivar. When there was a significant interaction between CO₂ and the cultivar, Tukey’s HSD test was performed to identify significant differences among the four values. The HI, spikelet fertility, and grain quality were analyzed after logit transformation. All statistical analyses were performed using IBM SPSS Advanced Statistics 22.

3. Results

Table 1 shows the $T_{\text{air}}$ and RH inside and outside the OTCs during the experimental period. Inside the OTCs, the mean $T_{\text{air}}$ during the experimental period was 28.3 °C, and 0.9 °C higher than that outside the OTCs, which resulted in a lower RH inside the OTCs than outside the OTCs. The 30-year average (1981−2010) (i.e., standard climatological normal) of mean $T_{\text{air}}$ from July to September in Nagasaki, Japan is 26.5 °C. The mean $T_{\text{air}}$ outside and inside the OTCs from July to September 2018 were 28.0 °C and 28.9 °C, respectively. These results indicate that $T_{\text{air}}$ in the year of this study was higher than the standard climatological normal.

The effects of elevated CO₂ on the DMs of plant organs and whole plants, yield per plant, and HI of the two rice cultivars are shown in Table 2. Exposure to elevated CO₂ significantly increased the DMs of stem and whole plant, but significantly reduced HI. There was no significant effect of elevated CO₂ on the DMs of the panicle, leaf blade, root, and yield per plant, although the yield tended to be reduced by elevated CO₂ ($p = 0.065$). The DMs of the stem and whole plant of Nikomaru were significantly higher than those of Hinohikari, but the DM of the roots in Nikomaru was significantly lower than that in Hinohikari. No significant interactions were detected between CO₂ and the cultivar for DMs of plant organs, whole plant, yield, and HI. However, the elevated CO₂-induced increase in whole-plant DM tended to be higher for Nikomaru ($p = 0.057$).

The effects of elevated CO₂ on yield components are shown in Fig. 1. Exposure to elevated CO₂ significantly reduced spikelet fertility but did not significantly affect panicle number per plant, grain number per panicle, and 1000-grain mass. The grain number per panicle of Nikomaru was significantly higher than that of Hinohikari. There were no significant interactions between CO₂ and the cultivars for any yield component.

Table 3 shows heading date and mean $T_{\text{air}}$ during flowering period for one week around the heading date. In the ambient CO₂ and elevated CO₂ treatments, the heading dates of Hinohikari were August 22 and 21, respectively, and that of Nikomaru were August 26 and 25, respectively. During flowering period, the difference in the $T_{\text{air}}$ between the CO₂ treatments was within the range of variation among chamber replications.

The effects of elevated CO₂ on $A$, $g_s$, and $E$ in the flag leaves of the two rice cultivars are shown in Fig. 2. Exposure to elevated CO₂ significantly increased $A$ and significantly reduced $g_s$ and $E$. There were no significant cultivar differences and significant interactions between elevated CO₂ and cultivars for $A$, $g_s$, and $E$.

Table 4 shows the effect of elevated CO₂ on the grain appearance quality of the two rice cultivars. Exposure to elevated CO₂ significantly reduced the percentage of perfect grains and significantly increased the percentage of immature grains. For Nikomaru, the percentage of perfect grains was significantly higher, and the percentages of immature and damaged grains were significantly lower than those for Hinohikari. There was a significant interaction between elevated CO₂ and cultivars for the percentage of colored grains, but there was no significant effect of elevated CO₂ on the percentage in either cultivar.

4. Discussion

Consistent with previous studies (e.g., Ainsworth 2008), elevated CO₂ significantly increased whole-plant DM in both rice cultivars in this study (Table 2). This increase in whole-plant DM could be caused by an increase in $A$ (Fig. 2). Regardless of the beneficial effects of elevated
**CO}_2 on rice, exposure to elevated CO}_2 did not significantly increase yield, and thus there was a significant reduction in HI (Table 2). Among the yield components, spikelet fertility was significantly reduced by exposure to elevated CO}_2 in both cultivars (Fig. 1), which could result in inconsistent results between whole-plant DM and yield responses to elevated CO}_2 (Table 2). In addition to the reduction in spikelet fertility, we also found another adverse outcome of elevated CO}_2 in rice: the significant deterioration of grain appearance quality in both cultivars (Table 4). Lower spikelet fertility and grain quality of rice are typical manifestations of heat stress in rice plants (Jagadish et al. 2015; Morita et al. 2016). Jagadish et al. (2007) reported that spikelet fertility decreases with an increase in the panicle temperature during the flowering stage. It is possible that elevated CO}_2-induced acceleration of growth rate, such as early heading, makes the difference in temperature regime during the flowering stage between ambient and elevated CO}_2 treatments. In the present study, however, no differences were detected in the air temperature regime between the treatments during the flowering stage (Table 3), although the elevated CO}_2 hastened heading date in both cultivars. In contrast, according to a meta-analysis by Kimball (2016), elevated CO}_2 caused an increase in canopy temperature of approximately 0.7 °C because of a decrease in evapotranspiration by approximately 10% when averaged across several crops, including rice. In this study, elevated CO}_2 significantly reduced the E of the flag leaf by approximately 19% on average across the cultivars (Fig. 2). These results suggest that the temperature of the canopy, including the panicle, might increase because of the elevated CO}_2-induced reduction in transpiration, which induces heat stress in rice, such as reductions in spikelet fertility and grain appearance quality.

Hasegawa et al. (2016) analyzed the results of their FACE experiments over 11 years. They indicated that elevated CO}_2 reduced spikelet fertility with increasing air temperature within the range of high air temperatures. This result suggests that elevated CO}_2 could reduce spikelet fertility under high-temperature conditions, although conditions other than CO}_2 concentration differed among the years in their analysis. In 2018, the year of this study, the growth conditions included relatively high temperatures, sufficient to induce heat stress on rice, because the mean T\textsubscript{air} was higher than the standard climatological normal (Table 1), and the spikelet fertility and grain appearance quality were relatively low even in the ambient CO}_2 treatment (Fig. 1 and Table 4). Because the growth conditions other than CO}_2 concentration were almost the same between the two treatments, the results obtained in the present study support the hypothesis that elevated CO}_2 could reduce spikelet fertility under high temperature conditions, sufficient to induce heat stress. Using the FACE system, several researchers have reported the adverse effects of elevated CO}_2 on rice, such as deterioration of grain quality (Yang et al. 2007; Usui et al. 2014, 2016; Jing et al. 2016) and reduction of spikelet fertility in warm years (Cai et al. 2016). It is projected that the atmospheric CO}_2 concentration will increase (Myhre et al. 2013), and consequently, air temperature could rise to the level at which the effect of heat stress on rice could become more evident (e.g., Okada et al. 2011; Jagadish et al. 2015; Morita et al. 2016). Therefore, in the future, the effects of elevated CO}_2 on rice might become harmful rather than beneficial; thus, more significant heat stress might be observed in paddy fields.

According to a meta-analysis by Ainsworth (2008), the response of above-ground biomass and net photosynthetic rate of rice to elevated CO}_2 was approximately 39% by +304 ppm and 23% by +258 ppm, respectively. The typical beneficial effect of elevated CO}_2 on the growth and net photosynthesis of rice in this study was significant. However, the impact was considerably low: 8% and 12% by +136 ppm CO}_2 for above-ground DM (sum of the panicle, leaf blade, and stem DMs) and A\textsubscript{r} respectively, on average across the cultivars grown in the OTCs (Table 2 and Fig. 2). Long et al. (2004) and Ainsworth (2008) reported inconsistent results that OTCs could exaggerate the effects of elevated CO}_2. However, a lower growth and yield response to elevated CO}_2 has been reported under low N fertilizer treatments (Ainsworth 2008; Kimball 2016). In the present study, the fertilizer was applied following the local practical procedure based on the soil surface area; however, nutrients might be insufficient because of the limited soil volume in the pot. These results suggest that the existing growth conditions for rice could be relatively low N conditions. Kimball (2016) reported that under N-limited conditions, the elevated CO}_2-induced reduction in evapotranspiration was greater than that under N-sufficient conditions, resulting in a higher increase in the canopy temperatures of wheat. The magnitude of reduction in E in the present study was approximately 19% by +136 ppm CO}_2, which was greater than the ~ 7% reduction in evapotranspiration of rice by +190 ppm CO}_2 (Kimball 2016). These results suggest that because of the relatively low N condition in this study, the considerable reduction in spikelet fertility by elevated CO}_2 might be due to a greater increase in canopy temperature caused by a greater reduction in transpiration. Further research is needed to elucidate whether N fertilization can mitigate the adverse effects of elevated CO}_2 on spikelet fertility. It could be beneficial for future rice breeding practices to consider countermeasures to heat stress on rice under the expected growth conditions of elevated CO}_2 and air temperature.

Even under ambient CO}_2 treatment, the percentage of perfect grains for the Hinohikari cultivar was very low because of the high air temperature conditions. The percentage of perfect grains for the Nikomaru cultivar was significantly higher in both treatments (Table 4), which could be caused by the heat-tolerant trait of this cultivar (Tanaka et al. 2009; Tanamachi et al. 2016). Therefore, introducing a heat-tolerant cultivar, such as Nikomaru rice, could be an effective countermeasure to the possible adverse effects of elevated CO}_2 on grain quality, although the degree of elevated CO}_2-induced reduction in grain appearance quality did not significantly differ between the cultivars (Table 4).
There were no significant cultivar differences and significant interactions between cultivars and elevated CO$_2$ for spikelet fertility (Fig. 1).

Matsui et al. (2001) reported that Hinohikari grain fertility was susceptible to heat stress during the flowering period. Maruyama et al. (2013) reported that heat tolerance from the viewpoint of spikelet fertility did not differ between Hinohikari and Nikomaru. These results suggest that Nikomaru rice is a heat-tolerant cultivar from the perspective of grain quality, but not from that of spikelet fertility. Several researchers have reported cultivar differences in heat tolerance from the perspective of spikelet fertility (Matsui et al. 2001; Maruyama et al. 2013). To avoid the possible adverse effects of elevated CO$_2$ on rice production in the future, elucidation of heat tolerance and development of a heat-tolerant cultivar from the perspectives of both grain quality and spikelet fertility would be necessary.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions**

MY and YK contributed to the study conception and design. SK and DK performed cultivation, measurement of growth and gas exchange rates, and data curation. TN measured atmospheric CO$_2$ concentration inside the chamber. TY conducted measurement of grain appearance quality. MY analyzed the data and was a major contributor in writing the manuscript. YK was major contributor in the revision of first draft of the manuscript. All authors read and approved the final manuscript.

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### Tables

#### Table 1
Air temperature and relative air humidity inside and outside the open-top chambers from June 26 to October 9, 2018.

|              | Air temperature (°C) | Relative air humidity (%) |       |       |
|--------------|----------------------|---------------------------|-------|-------|
|              | Daily mean           | Daily max.\(^a\)          | Daily min.\(^b\) | Daily mean | Daily max.\(^a\) | Daily min.\(^b\) |
| Inside       | 28.3 (0.1)           | 33.5 (0.2)                | 24.5 (0.1)      | 69.4 (0.4) | 87.3 (0.4)     | 49.3 (0.5)     |
| Outside      | 27.4                 | 31.0                      | 24.4          | 72.4       | 88.3           | 55.7           |

Each value for inside the chambers is the mean of six chambers, and its standard deviation is shown in parentheses. \(^a\) Mean of daily 1-h maximum value. \(^b\) Mean of daily 1-h minimum value.

#### Table 2
Effects of elevated CO\(_2\) on the dry masses of plant organs and whole plant, yield per plant and harvest index (HI) of two Japanese cultivars of rice in October 2018.

| Cultivar   | CO\(_2\)   | Dry mass (g) | Yield\(^a\) | HI\(^b\) |
|------------|------------|--------------|-------------|--------|
|            | treatment | Panicle      | Leaf blade  | Stem   | Root   | Whole plant | (g)      | (g g\(^{-1}\)) |
| Hinohikari | Ambient   | 5.9 (0.9)    | 4.7 (0.8)   | 13.4 (0.8) | 6.0 (0.5) | 30.0 (0.3)  | 3.5 (1.7) | 0.148 (0.075) |
|            | Elevated  | 5.1 (0.4)    | 4.3 (0.0)   | 15.8 (0.7) | 5.0 (0.4) | 30.3 (0.8)  | 2.0 (0.6) | 0.082 (0.023) |
| Nikomaru   | Ambient   | 5.7 (1.0)    | 4.8 (0.3)   | 15.3 (0.2) | 4.8 (0.6) | 30.5 (1.7)  | 3.0 (1.4) | 0.115 (0.051) |
|            | Elevated  | 5.1 (0.2)    | 5.0 (0.2)   | 18.5 (0.8) | 4.9 (0.2) | 33.5 (0.9)  | 1.7 (0.2) | 0.057 (0.008) |

ANOVA CO\(_2\) n.s. n.s. *** n.s. * n.s. (\(p = 0.065\)) *

Cv. n.s. n.s. *** * * n.s. n.s.

Cv. x CO\(_2\) n.s. n.s. n.s. n.s. n.s. (\(p = 0.057\)) n.s. n.s.

Each value is the mean of three chambers, and its standard deviation is shown in parentheses. \(^a\) Filled grain mass per plant. \(^b\) Ratio of filled grain mass per plant to shoot (panicle, leaf blade and stem) dry mass. Two-way analysis of variance (ANOVA): *\(p<0.05\), ***\(p<0.001\), n.s. = not significant.
Table 3
Heading date and air temperature inside the open-top chambers during flowering period in each gas treatment.

| Cultivar | CO₂ treatment | Heading date<sup>a</sup> | Daily mean | Daily max.<sup>b</sup> | Daily min.<sup>c</sup> |
|----------|---------------|--------------------------|------------|------------------------|------------------------|
| Hinohikari | Ambient       | Aug. 22                  | 30.5       | (0.1) 36.2 (0.6)       | 26.6 (0.1)             |
|           | Elevated      | Aug. 21                  | 30.3       | (0.1) 36.0 (0.2)       | 26.5 (0.1)             |
| Nikomaru  | Ambient       | Aug. 26                  | 30.4       | (0.3) 36.0 (1.2)       | 26.9 (0.2)             |
|           | Elevated      | Aug. 25                  | 30.5       | (0.3) 35.9 (0.6)       | 27.4 (0.2)             |

Each value is the mean of two (400 ppm CO₂) or three (550 ppm CO₂) chambers, and its standard deviation is shown in parentheses. Flowering period: one week around the heading date. <sup>a</sup> Day on which mean heading rate reached 50%. <sup>b</sup> Mean of daily 1-h maximum value. <sup>c</sup> Mean of daily 1-h minimum value.

Table 4
Effects of elevated CO₂ on the percentage of grain appearance qualities of two Japanese cultivars of rice in October 2018.

| Cultivar | CO₂ treatment | Perfect (%) | Immature (%) | Damaged (%) | Abortive (%) | Colored (%) |
|----------|---------------|-------------|--------------|-------------|--------------|-------------|
| Hinohikari | Ambient       | 34.1 (11.2) | 40.8 (2.1)   | 23.1 (8.7)  | 0.2 (0.3)    | 1.8 (0.8)   | a           |
|           | Elevated      | 18.1 (4.2)  | 58.6 (8.9)   | 22.0 (13.8) | 0.2 (0.1)    | 1.2 (0.7)   | a           |
| Nikomaru  | Ambient       | 78.4 (5.2)  | 17.8 (6.4)   | 3.3 (1.7)   | 0.3 (0.3)    | 0.3 (0.3)   | b           |
|           | Elevated      | 70.5 (0.9)  | 24.1 (1.8)   | 3.9 (1.4)   | 0.5 (0.5)    | 1.0 (0.8)   | ab          |

ANOVA

| CO₂ | Perfect (%) | Immature (%) | Damaged (%) | Abortive (%) | Colored (%) |
|-----|-------------|--------------|-------------|--------------|-------------|
| CO₂ | *           | **           | n.s.        | n.s.         | n.s.        |
| Cv. | ***         | ***          | **          | n.s.         | *           |
| Cv. x CO₂ | n.s. | n.s. | n.s. | n.s. | * |

Each value is the mean of three chambers, and its standard deviation is shown in parentheses. Two-way analysis of variance (ANOVA): *p<0.05, **p<0.01, ***p<0.001, n.s. = not significant. Values with different letters are significantly different at p<0.05 (Tukey’s HSD test).

Figures
Figure 1

Effects of elevated CO2 on the panicle number per plant, grain number per panicle, 1000-grain mass, and spikelet fertility of two Japanese cultivars of rice. Hino: Hinohikari; Niko: Nikomaru. Two-way analysis of variance (ANOVA): *p<0.05, n.s. = not significant. Each value represents the mean of three chamber replicates, and error bars represent the standard deviations.

Figure 2

Effects of elevated CO2 on the net photosynthetic rate (A), stomatal conductance (gs), and transpiration rate (E) in the flag leaf of two Japanese cultivars of rice in late August 2018. Hino: Hinohikari; Niko: Nikomaru. Two-way analysis of variance (ANOVA): *p<0.05, n.s. = not significant. Each value represents the mean of three chamber replicates, and error bars represent the standard deviations.