

Elevated CO₂ could reduce spikelet fertility and grain appearance quality of rice (*Oryza sativa*) grown under high-temperature conditions

Masahiro Yamaguchi (✉ masah-ya@nagasaki-u.ac.jp)

Nagasaki University: Nagasaki Daigaku <https://orcid.org/0000-0003-0682-9832>

Shoma Kamiya

Nagasaki University: Nagasaki Daigaku

Dai Kokubun

Nagasaki University: Nagasaki Daigaku

Tomoki Nakayama

Nagasaki University: Nagasaki Daigaku

Tetsushi Yonekura

Center for Environmental Science in Saitama

Yoshihisa Kohno

Central Research Institute of Electric Power Industry: Denryoku Chuo Kenkyujo

Research Article

Keywords: Elevated CO₂, Heat stress, Japanese rice, Open-top chamber, Spikelet fertility, Stomatal conductance, Transpiration rate

Posted Date: March 8th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-576481/v2>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published at Asian Journal of Atmospheric Environment on August 31st, 2022. See the published version at <https://doi.org/10.5572/ajae.2022.044>.

Abstract

- It is projected that atmospheric CO₂ concentrations will increase because of industrial activities. Elevated CO₂ reduces transpiration from leaves via stomatal closure, which increases plant temperature; therefore, higher levels of CO₂ could adversely affect crop production via increasing plant temperature, particularly for plants that are susceptible to heat stress, such as rice (*Oryza sativa*).
- Two Japanese rice cultivars with different heat-tolerance, Hinohikari and Nikomaru, were grown in pots inside open-top chambers and exposed to ambient CO₂ (400 μmol mol⁻¹) or elevated CO₂ (550 μmol mol⁻¹) from the beginning of the tillering stage to maturity. The study was conducted in Nagasaki, in the Kyushu region of Japan, where heat stress on rice has become increasingly evident.
- During the flowering period, the air temperature was sufficiently high to induce heat stress effects in rice, such as low spikelet fertility, even under ambient CO₂ conditions for both cultivars. Although elevated CO₂ significantly improved the net photosynthesis and whole-plant growth of the cultivars, there were no significant effects on grain yield, which in turn reduced harvest index. In both cultivars, adverse effects on other processes occurred with elevated CO₂, such as reductions in spikelet fertility and grain appearance quality, which are typical manifestations of heat stress in rice. The elevated CO₂ treatment showed significant reductions in stomatal conductance and transpiration rate of flag leaves, which could increase plant temperature.
- Under high-temperature condition, therefore, elevated CO₂ could induce manifestations of heat stress in Japanese rice via reduction in transpiration due to stomatal closure.

Introduction

Atmospheric carbon dioxide (CO₂) concentration has increased because of industrial activities, such as fossil fuel combustion and cement production (Myhre *et al.* 2013). Generally, an increase in atmospheric CO₂ concentration is beneficial for plant growth and crop production because photosynthesis is a CO₂-dependent process (Körner 2003) and is enhanced by elevated CO₂ levels. According to a meta-analysis of the results of free-air CO₂ enrichment (FACE) experiments, the elevating the atmospheric CO₂ concentration from the ambient levels in the 1990s and 2000s to 550–600 μmol mol⁻¹ (ppm), increased the 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%, with some variation depending on the magnitude of increase and the fumigation technique. In addition to increasing net photosynthesis, elevated CO₂ induces stomatal closure (Ainsworth & Long 2005), which reduces transpiration from the leaves, causing the canopy temperature to rise (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 during a FACE experiment in China, resulting in increased leaf and panicle temperatures. These results suggest that by increasing the plant temperature, elevated CO₂ could affect the growth and physiological functions of plants, with possible adverse effects under sufficiently high-temperature conditions that can induce heat stress.

Rice is sensitive to heat stress, which can cause damage, such as reductions in spikelet fertility and grain quality (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. Therefore, it is possible that elevated CO₂ induces manifestations of heat stress, such as a reduction in spikelet fertility, by increasing the plant temperature. Several studies have shown that elevated CO₂ exacerbates 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 chamber in Japan, and the indica rice cultivar “IR72,” cultivated in an open-top chamber (OTC) in the Philippines. Based on the results of a 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, which was not observed under ambient CO₂ conditions. These results suggest that elevated CO₂ can reduce spikelet fertility under high-temperature conditions.

In the present study, we conducted an experimental study on the effects of elevated CO₂ on rice using OTCs located in Nagasaki in the Kyushu region, which is a warm temperate area of Japan. In this 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 quality (Okada *et al.* 2009; Uno *et al.* 2012). These results suggest that the temperature in this region reached the level for heat stress induction in rice. Therefore, in the present study, we hypothesized that exposure to elevated CO₂ in Nagasaki, where heat stress on rice has become increasingly evident, could reduce spikelet fertility and grain appearance quality via elevated CO₂-induced transpiration reduction.

Materials And Methods

Plant material

The primary rice cultivar in the Kyushu region, where the present study was conducted, is “Hinohikari.” Since Hinohikari rice is relatively sensitive to heat stress, the heat-tolerant cultivar “Nikomaru” has been introduced to Nagasaki in this region (Tanaka *et al.* 2009; Tanamachi *et al.* 2016). The present study used both cultivars. 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 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) (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; 32.79 N, 129.87 E), from June 26 to October 9. Inside each OTC, ambient air was introduced using a fan (MRS18V2-B, ORIENTAL MOTOR Co., Ltd., Japan) and blown in an upward direction from the bottom of the chamber. For each chamber, three pots (i.e., nine hills) for each cultivar, for a total of six pots, were assigned and placed on the floor of the chamber. The N-P-K fertilizer (1.013 g) was again 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 outside and inside 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 approximate canopy height after flag leaf emergence in mid-

August. Each sensor was installed inside a ventilated two-layer radiation shield consisting of a fan (MU925S-11, ORIENTAL MOTOR Co., Ltd., Japan) and two polyvinyl chloride pipes with different diameters; the outer pipe was covered with aluminum foil.

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 the 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, which were assigned to the elevated CO₂ treatment. For each treatment, three-chamber replications were performed. To introduce CO₂ gas, a polyethylene tube connected to a CO₂ cylinder was inserted into the chamber near the outlet of the fan 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 chambers, the air at 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 the ambient and elevated CO₂ treatments during the day were 409.4 ± 0.6 ppm and 546.9 ± 3.1 ppm (mean of three chamber replications \pm 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.

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 hills from each OTC were randomly selected for measurements. When the measurements were taken, the air temperature, relative air humidity, and the photosynthetic photon flux density in the leaf chamber were maintained at 30 °C, 65%, and $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$, 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.

Temperatures of flag leaf and panicle

At the end of the flowering period on August 24 and 28 for Hinohikari and Nikomaru, respectively, the temperatures of the flag leaf and panicle were measured at 10:00 and 13:00 using a thermography camera (FLIR

i5, FLIR Systems Japan, Japan). Both measurement days were sunny. For each cultivar, three hills from each OTC, and one flag leaf and panicle from each hill were randomly selected for measurements.

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, as well as the yield, and yield components, all rice plants of both Hinohikari and Nikomaru cultivars were harvested from each hill 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 weighed. The panicles were counted to obtain the panicle number per hill and air-dried in the field for 5 days. Whole-plant DM per hill was calculated as the sum of the DM of all plant organs. Spikelets were separated from dried panicles and counted to obtain the spikelet number per panicle. The spikelets were categorized by hand inspection into two groups, sterile and fertile, and counted. Fertile spikelets consists of filled and partially filled spikelets. To evaluate spikelet fertility, the percentage of fertile spikelets was calculated from the total and fertile spikelet numbers for each plant. Fertile spikelets were weighed to obtain the yield per hill, and the 1000-grain mass was calculated using the fertile spikelet number per hill. Since there were few partially filled spikelets, we defined the mass of fertile spikelets per hill as yield per hill, although partially filled spikelets were included. The harvest index (HI) is the ratio of yield per hill to shoot (panicle, leaf blade, and stem) DM. After husking fertile spikelets, 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 (Sawada *et al.* 2016). Grain appearance quality was expressed as the percentage of the number of each quality class to the total grain number.

Statistical analysis

The means of each parameter for the OTCs was used for the statistical analyses ($n = 3$ for each treatment). A two-way analysis of variance (ANOVA) was used to test the effects of the 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. For leaf and panicle temperatures, a two-way ANOVA was used to test the effect of elevated CO₂ treatment and time of day for each cultivar. In this analysis, we did not test the effect of cultivar, because the measurement date was different between the cultivars. All statistical analyses were performed using IBM SPSS Advanced Statistics 22.

Results

Air temperature during cultivation

Inside the OTCs, the mean T_{air} during the experimental period was 28.3 °C, which was 0.9 °C higher than that outside the OTCs, resulting in a lower RH than that of outside air (Table 1). The 30-year average (1981–2010) (standard climatological normal) of mean T_{air} from July to September in Nagasaki, Japan was 26.5 °C. The mean T_{air} outside and inside the OTCs from July to September 2018 were 28.0 °C and 28.9 °C, respectively (data not shown). These results indicate that T_{air} in the year of this study was higher than the standard climatological normal.

To identify the flowering period during which spikelet fertility is affected by air temperature (Maruyama *et al.* 2013), we determined the heading date. In the ambient CO₂ treatment, heading dates were August 22 and 26 for Hinohikari and Nikomaru, respectively (Table 2). Increasing the CO₂ level accelerated heading by one day for both cultivars. During the flowering period for one week around the heading date, the difference in the T_{air} between the CO₂ treatments was within the range of variation among chamber replications. In contrast, Maruyama *et al.* (2013) reported that the daily T_{air} maximums during the flowering period when spikelet fertility was reduced to 75% were 35.9 °C and 34.3 °C for Hinohikari and Nikomaru, respectively. In the present study, the air temperature during the flowering period was higher than the values for both cultivars (Table 2). These results suggest that the air temperature during flowering was sufficiently high to induce heat stress effects in rice, such as low spikelet fertility.

Growth, yield and grain quality

The DM of stems and whole-plant was increased by exposure to elevated CO₂ for both cultivars, and they were significantly higher in Nikomaru than in Hinohikari (Table 3). Across the cultivars, the enhancement of whole-plant growth by elevated CO₂ averaged 5.4%. There were no significant effects of elevated CO₂ and cultivar and their significant interaction for the DM of panicles and leaf blades and yield per hill. The root DM of Hinohikari was significantly higher than that of Nikomaru without significant effects of elevated CO₂ or significant interaction of CO₂ and cultivar. A significant reduction in the HI was observed in the elevated CO₂ treatment without significant difference between the cultivars or significant interaction of CO₂ and cultivar. Across the cultivars, an average 47.1% reduction in HI was observed in the elevated CO₂ treatment compared with that in the ambient CO₂ treatment.

Among the yield components, spikelet fertility was significantly reduced by exposure to elevated CO₂ in both cultivars, with no significant difference between the cultivars or significant interaction of CO₂ and cultivar (Fig. 1). No significant effects of elevated CO₂ and cultivar and their significant interaction were observed for panicle number per hill and 1000-grain mass. Although a higher grain number per panicle was observed in Nikomaru than in Hinohikari, there was no significant effect of elevated CO₂ or interaction of CO₂ and cultivar.

As shown in Table 4, grain appearance quality was significantly deteriorated by the exposure to elevated CO₂ in both cultivars, showing significant reductions in the percentage of perfect grains and significant increases in immature grains without significant interaction between CO₂ and cultivar. The percentage of perfect grains across the cultivars was reduced from an average of 56.2% in the ambient CO₂ treatment to 44.3% in the elevated CO₂ treatment. Although the effects of elevated CO₂ on the percentage of perfect and immature grains were not

significantly different between the cultivars, a significantly higher percentage of perfect grains and lower percentage of immature grains were observed in Nikomaru than in Hinohikari. On average, across the treatments, the percentage of perfect grains was 26.1% in Hinohikari and 74.5% in Nikomaru. The percentage of damaged grains in Nikomaru was significantly lower than that in Hinohikari without a significant effect of elevated CO₂ or interaction of CO₂ and cultivar. There were no significant effects of elevated CO₂ and cultivar and their interaction on the percentage of abortive grains. A significant interaction between elevated CO₂ and cultivars was observed in the percentage of colored grains, but there was no significant effect of elevated CO₂ on the percentage in either cultivar.

Leaf gas exchange rates and temperatures of leaf and panicle

Exposure to elevated CO₂ significantly increased the A and reduced g_s and E in late August during the flowering period (Fig. 2). There were no significant cultivar differences or interactions between elevated CO₂ and cultivars for A , g_s , and E . An average 12.2% increase in A , 26.4% reduction in g_s , and 18.7% reduction in E were observed in the elevated CO₂ treatment across the cultivars.

We measured the temperatures of the flag leaf and panicle at the end of the flowering period on August 24 and 28 for Hinohikari and Nikomaru, respectively (Table 5). Hinohikari revealed no significant effects of elevated CO₂ and time of day or their interaction on the temperatures of the flag leaf and panicle. The average panicle temperature over the treatments and times for Hinohikari was 37.1 °C. For Nikomaru, significant differences in the temperatures of flag leaves and panicles between times of day were observed without a significant effect of elevated CO₂ or interaction between CO₂ and time. On average across the treatments, panicle temperatures of Nikomaru at 10:00 and 13:00 were 36.1 °C and 39.9 °C, respectively. Maruyama *et al.* (2013) reported that panicle temperature during the flowering period at which spikelet fertility was reduced to 75% were 34.9 °C and 33.2 °C for Hinohikari and Nikomaru, respectively. In the present study, panicle temperatures were considerably higher than the values for both cultivars in both treatments (Table 5). These results suggest that the growth conditions during the flowering period included sufficiently high temperatures to induce heat stress symptoms in rice, such as low spikelet fertility, resulting in low grain yield even in the ambient CO₂ treatment.

Discussion

Consistent with previous studies (Ainsworth 2008), elevated CO₂ significantly increased whole-plant DM in both rice cultivars in this study (Table 3). This could be caused by an increase in A (Fig. 2). However, regardless of the beneficial effects of elevated CO₂, exposure to elevated CO₂ did not significantly increase the yield, and thus, there was a significant reduction in HI (Table 3). Among the yield components, spikelet fertility was significantly reduced by exposure to elevated CO₂ for both cultivars (Fig. 1), which could result in inconsistent results between the effects on whole-plant DM and yield (Table 3). Hasegawa *et al.* (2016) suggested that increased CO₂ could reduce spikelet fertility under high-temperature conditions. Those authors analyzed the results of their FACE experiments over 11 years with different climatic conditions and indicated that the percent change in spikelet fertility due to elevated CO₂ for each year decreased with increasing air temperature within a range of high air temperatures. In 2018, the year of the present study, the growth conditions included high temperatures that were

sufficient to induce heat stress on rice, because the mean T_{air} throughout the exposure period and maximum T_{air} and panicle temperature during the flowering period were high (Tables 1, 2, and 5), and the spikelet fertility and grain appearance quality were relatively low even in the ambient CO_2 treatment (Fig. 1 and Table 4). The growth conditions other than CO_2 concentration were the same between the two treatments; therefore, the results support the hypothesis that elevated CO_2 can reduce spikelet fertility under high temperature conditions.

Another adverse outcome observed from increased CO_2 levels was the significant deterioration of rice grain appearance quality for both cultivars (Table 4), which is also manifestation of heat stress in rice (Jagadish *et al.* 2015; Morita *et al.* 2016). Using the FACE system, several researchers have also 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). Therefore, elevated CO_2 could induce or exacerbate the manifestations of heat stress in rice under high-temperature conditions. It is projected that atmospheric CO_2 concentrations will increase (Myhre *et al.* 2013), and consequently, air temperatures may rise to a level at which the effect of heat stress on rice could become more prevalent (Jagadish *et al.* 2015; Morita *et al.* 2016; Okada *et al.* 2011). Therefore, the effects of elevated CO_2 on rice in the future could be harmful as opposed to beneficial, and substantial manifestations of heat stress could be observed in paddy fields. The present study was a case study conducted in a specific year with two genotypes and provided limited data for testing the hypothesis that exposure to elevated CO_2 could induce manifestations of heat stress in rice. Although the sample size and replications were sufficient for experimental study in one particular year, further studies with other cultivars in different year are required.

Spikelet fertility is sensitive to air temperature during the flowering period, and high temperatures reduce this fertility (Maruyama *et al.* 2013). In the present study, the air temperature during this period was sufficiently high to induce low spikelet fertility (Table 2). Therefore, it is possible that elevated CO_2 -induced acceleration of heading leads to a higher temperature regime during the flowering stage in the elevated CO_2 treatment as compared with the ambient CO_2 treatment. In the present study, however, no differences were detected in the air temperature regime between the treatments during the flowering stage (Table 2). Jagadish *et al.* (2007) reported that spikelet fertility decreases with an increase in the panicle temperature during the flowering stage. According to a meta-analysis by Kimball (2016), elevated CO_2 caused an increase in canopy temperature of approximately 0.7°C because of an approximate 10% decrease in evapotranspiration when averaged across several crops, including rice. In this study, elevated CO_2 significantly reduced the average E of the flag leaf by approximately 19% across the cultivars (Fig. 2). Although no significant increase was detected in temperatures of the flag leaf and panicle in the elevated CO_2 treatment (Table 5), the temperature of the canopy, including the panicle, might increase because of the elevated CO_2 -induced reduction in transpiration (Fig. 2). Since the temperatures of the air and panicle during the flowering period were high enough to reduce spikelet fertility, even in the ambient CO_2 treatment (Tables 2 and 5), a small increase in the panicle temperature in the elevated CO_2 treatment (Table 5) could cause a significant reduction in spikelet fertility. We measured the panicle temperature on one day at the end of the flowering period and did not detect a significant increase in temperature due to exposure to elevated CO_2 , but further research is required to clarify whether elevated CO_2 increases panicle temperature via reduction in transpiration, and the mechanism involved. To verify the hypothesis, furthermore, it is necessary to clarify the effects of elevated CO_2 on the determinants of spikelet fertility, such as pollen viability and germination, and those of grain appearance quality, such as translocation of photosynthate and synthesis of starch in the grain. It

is also possible that elevated CO₂-induced changes in transpiration and plant growth (e.g., leaf area) could affect the humidity inside the chamber, light capture of individual leaves and panicles, and optimum nutrient amounts for plant growth. To ensure a more confident conclusion, the adverse effects of elevated CO₂ should be observed with relation to these traits.

According to a meta-analysis by Ainsworth (2008), the response of above-ground biomass and net photosynthetic rate of rice to elevated CO₂ was approximately 39% with +304 ppm and 23% with +258 ppm, respectively. In the present study, the typical beneficial effect of elevated CO₂ on the growth and net photosynthesis of rice was significant. However, the impact was considerably low: 8% and 12% with +136 ppm CO₂ for above-ground DM (sum of DM of the panicle, leaf blade, and stem) and *A*, respectively, on average (Table 3 and Fig. 2). Long *et al.* (2004) and Ainsworth (2008) reported inconsistent results in terms of OTCs exaggerating the effects of elevated CO₂. However, lower growth and yield responses to elevated CO₂ have 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 have relatively low N levels. Kimball (2016) reported that under N-limited conditions, the elevated CO₂-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% with +136 ppm CO₂, which was greater than the ~ 7% reduction in evapotranspiration of rice with +190 ppm CO₂ (Kimball 2016). These results suggest that because of the relatively low N condition in this study, a greater reduction in transpiration by elevated CO₂ could cause a greater increase in canopy temperature, which might result in a considerable reduction in spikelet fertility. Further research is needed to elucidate whether N fertilization can mitigate the adverse effects of elevated CO₂ on spikelet fertility. Future rice breeding practices should consider heat stress countermeasures for rice under the expected future growth conditions of elevated CO₂.

The extent of the effects of elevated CO₂ on all parameters investigated in the present study was not significantly different between Hinohikari and Nikomaru, although we observed significant cultivar differences in the grain appearance quality. Even under ambient CO₂ treatment, the percentage of perfect grains for the Hinohikari cultivar was low because of the high temperature conditions in the present study. The percentage of perfect grains for the Nikomaru cultivar was significantly higher in both treatments (Table 4), which could be caused by the heat tolerance 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 CO₂ on grain quality, although the degree of elevated CO₂-induced reduction in grain appearance quality did not significantly differ between the cultivars (Table 4). In contrast, there were no significant cultivar differences and significant interactions between cultivars and elevated CO₂ for spikelet fertility (Fig. 1). Maruyama *et al.* (2013) reported that heat tolerance with regard to spikelet fertility did not differ between Hinohikari and Nikomaru. These results suggest that Nikomaru rice is a heat-tolerant cultivar in terms of the effects on grain quality, but not in relation to spikelet fertility. Several studies have reported cultivar differences in heat tolerance from the perspective of spikelet fertility (Maruyama *et al.* 2013; Matsui *et al.* 2001). To avoid the possible adverse effects of elevated CO₂ on future rice production, elucidation of heat tolerance and development of a heat-tolerant cultivar for the preservation of both grain quality and spikelet fertility would be necessary.

Conclusion

In the present study, we observed adverse effects of elevated CO₂, including reductions in spikelet fertility and grain appearance quality, which are major manifestations of heat stress, in two Japanese rice cultivars. The growth conditions included sufficiently high temperatures to induce heat stress, especially during the flowering period, and the elevated CO₂-induced reduction in transpiration might have increased the plant temperature. These results suggest that, under high-temperature conditions, elevated CO₂ could induce or exacerbate the manifestations of heat stress in rice via a reduction in transpiration. Although we did not observe significant cultivar differences in the effects of elevated CO₂, elucidation of heat tolerance and development of a heat-tolerant cultivar from the perspectives of both grain quality and spikelet fertility would be necessary to avoid the possible adverse effects of elevated CO₂ on rice production in the future.

Declarations

Data Availability

The data reported in the present study are available from the corresponding author upon reasonable request.

Acknowledgements

The authors are indebted to Professor Takeshi Izuta of Tokyo University of Agriculture and Technology for his invaluable advice on using thermography camera. The authors are grateful to Mr. Kentaro Nakashima, Ms. Akane Tsujimatsu, Mr. Taketomo Matsushita, and Ms. Mai Sakai (Faculty of Environmental Science, Nagasaki University) for their technical support in the cultivation and treatment of rice. We thank Margaret J. Sporck-Koehler, Ph.D., from Edanz Group (www.edanzediting.com/ac) for editing the draft of this manuscript. We would like to thank Editage (www.editage.com) for two times of English language editing.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing Interests

The authors declare that they have no competing interests.

References

Ainsworth E.A., Long S.P. (2005) What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytologist*, **165**, 351–371.

- Ainsworth E.A. (2008) Rice production in a changing climate: a meta-analysis of responses to elevated carbon dioxide and elevated ozone concentration. *Global Change Biology*, **14**, 1642–1650.
- Bernacchi C.J., Kimball B.A., Quarles D.R., Long S.P., Ort D.R. (2007) Decreases in stomatal conductance of soybean under open-air elevation of [CO₂] are closely coupled with decreases in ecosystem evapotranspiration. *Plant Physiology*, **143**, 134–144.
- Cai C., Yin X., He S., Jiang W., Si C., Struik P.C., Luo W., Li G., Xie Y., Xiong Y., Pan G. (2016) Responses of wheat and rice to factorial combinations of ambient and elevated CO₂ and temperature in FACE experiments. *Global Change Biology*, **22**, 856–874.
- Hasegawa T, Sakai H, Tokida T, Usui Y, Yoshimoto M, Fukuoka M, Nakamura H, Shimono H, Okada M (2016) Rice free-air carbon dioxide enrichment studies to improve assessment of climate change effects on rice agriculture. In: Hatfield J.L., Fleisher D. (Eds.), *Improving Modeling Tools to Assess Climate Change Effects on Crop Response*. American Society of Agronomy, Crop Science Society of America & Soil Science Society of America, USA: 45–68.
- Ishigooka Y., Kuwagata T., Nishimori M., Hasegawa T., Ohno H. (2011) Spatial characterization of recent hot summers in Japan with agro-climatic indices related to rice production. *Journal of Agricultural Meteorology*, **67**, 209–224.
- Jagadish S.V.K., Craufurd P.Q., Wheeler T.R. (2007) High temperature stress and spikelet fertility in rice (*Oryza sativa* L.). *Journal of Experimental Botany*, **58**, 1627–1635.
- Jagadish S.V.K., Murty M.V.R., Quick W.P. (2015) Rice responses to rising temperatures - challenges, perspectives and future directions. *Plant, Cell and Environment*, **38**, 1686–1698.
- Jing L., Wang J., Shen S., Wang Y., Zhu J., Wang Y., Yang L. (2016) The impact of elevated CO₂ and temperature on grain quality of rice grown under open-air field conditions. *Journal of the Science of Food and Agriculture*, **96**, 3658–3667.
- Kim H.Y., Horie T., Nakagawa H., Wada K. (1996) Effects of elevated CO₂ concentration and high temperature on growth and yield of rice. II. The effect on yield and its components of Akihikari rice (in Japanese with English summary). *Japanese Journal of Crop Science*, **65**, 644–651.
- Kimball B.A. (2016) Crop responses to elevated CO₂ and interactions with H₂O, N, and temperature. *Current Opinion in Plant Biology*, **31**, 36–43.
- Kobayasi K., Eydi M.J., Sakai H., Tokida T., Nakamura H., Usui Y., Yoshimoto M., Hasegawa T. (2019) Effects of free-air CO₂ enrichment on heat-induced sterility and pollination in rice. *Plant Production Science*, **22**, 374–381.
- Körner C. (2003) Atmospheric CO₂ enrichment - an ecological perspective. In Larcher W. (Ed.), *Physiological Plant Ecology*, 4th ed. Springer-Verlag Berlin Heidelberg New York: 177–184.
- Long S.P., Ainsworth E.A., Rogers A., Ort D.R. (2004) Rising atmospheric carbon dioxide: plants FACE the future. *Annual Review of Plant Biology*, **55**, 591–628.

- Long S.P., Ainsworth E.A., Leakey A.D.B., Nösberger J., Ort D.R. (2006) Food for thought: lower-than-expected crop yield stimulation with rising CO₂ concentrations. *Science*, **312**, 1918–1921.
- Maruyama A., Weerakoon W.M.W., Wakiyama Y., Ohba K. (2013) Effects of increasing temperatures on spikelet fertility in different rice cultivars based on temperature gradient chamber experiments. *Journal of Agronomy and Crop Science*, **199**, 416–423.
- Matsui T., Namuco O.S., Ziska L.H., Horie T. (1997) Effects of high temperature and CO₂ concentration on spikelet sterility in *indica* rice. *Field Crops Research*, **51**, 213–219.
- Matsui T., Omasa K., Horie T. (2001) The difference in sterility due to high temperatures during the flowering period among japonica-rice varieties. *Plant Production Science*, **4**, 90–93.
- Morita S., Wada H., Matsue Y. (2016) Countermeasures for heat damage in rice grain quality under climate change. *Plant Production Science*, **19**, 1–11.
- Myhre G., Shindell D., Bréon F.-M., Collin W., Fuglestedt J., Huang J., Koch D., Lamarque J.-F., Lee D., Mendoza B., Nakajima T., Robock A., Stephens G., Takemura T., Zhang H. (2013) Anthropogenic and natural radiative forcing. In: Stocker T.F., Qin D., Plattner G.-K., Tignor M., Allen S.K., Boschung J., Nauels A., Xia Y., Bex V., Midgley P.M. (Eds.), *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, USA: 659–740.
- Okada M., Hayashi Y., Iizumi T., Yokozawa M. (2009) A climatological analysis on the recent declining trend of rice quality in Japan. *Journal of Agricultural Meteorology*, **65**, 327–337.
- Okada M., Iizumi T., Hayashi Y., Yokozawa M. (2011) Projecting climate change impacts both on rice quality and yield in Japan. *Journal of Agricultural Meteorology*, **67**, 285–295.
- Sawada H., Tsukahara K., Kohno Y., Suzuki K., Nagasawa N., Tamaoki M. (2016) Elevated ozone deteriorates grain quality of *japonica* rice cv. Koshihikari, even if it does not cause yield reduction. *Rice*, **9**, 7.
- Tanaka K, Onishi R, Miyazaki M, Ishibashi Y, Yuasa T, Iwaya-Inoue M (2009). Changes in NMR relaxation of rice grains, kernel quality and physicochemical properties in response to a high temperature after flowering in heat-tolerant and heat-sensitive rice cultivars. *Plant Production Science*, **12**, 185–192.
- Tanamachi K., Miyazaki M., Matsuo K., Suriyasak C., Tamada A., Matsuyama K., Iwaya-Inoue M., Ishibashi Y. (2016) Differential responses to high temperature during maturation in heat-stress-tolerant cultivars of *Japonica* rice. *Plant Production Science*, **19**, 300–308.
- Uno F., Iizumi T., Nishimori M., Hayashi Y. (2012) Time trends and variations in mean and accumulated solar radiation for the ripening period of paddy rice in Kyushu for 1979–2007. *Journal of Agricultural Meteorology*, **68**, 69–76.
- Usui Y., Sakai H., Tokida T., Nakamura H., Nakagawa H., Hasegawa T. (2014) Heat-tolerant rice cultivars retain grain appearance quality under free-air CO₂ enrichment. *Rice*, **7**, 6.

Usui Y., Sakai H., Tokida T., Nakamura H., Nakagawa H., Hasegawa T. (2016) Rice grain yield and quality responses to free-air CO₂ enrichment combined with soil and water warming. *Global Change Biology*, **22**, 1256–1270.

Yang L., Wang Y., Dong G., Gu H., Huang J., Zhu J., Yang H., Liu G., Han Y. (2007) The impact of free-air CO₂ enrichment (FACE) and nitrogen supply on grain quality of rice. *Field Crops Research*, **102**, 128–140.

Yoshimoto M., Oue H., Takahashi N., Kobayashi K. (2005) The effects of FACE (free-air CO₂ enrichment) on temperatures and transpiration of rice panicles at flowering stage. *Journal of Agricultural Meteorology*, **60**, 597–600.

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		Daily		Daily		Daily		Daily		Daily	
	mean	max. ^a	max. ^a	min. ^b	min. ^b	mean	max. ^a	max. ^a	min. ^b	min. ^b	mean	max. ^a
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 Heading date and air temperature inside the open-top chambers during flowering period in each gas treatment.

Cultivar	CO ₂ treatment	Heading date ^a	Daily		Daily		Daily	
			mean	max. ^b	max. ^b	min. ^c	min. ^c	
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. ^a Day on which mean heading rate reached 50%. ^b Mean of daily 1-h maximum value. ^c Mean of daily 1-h minimum value.

Table 3 Effects of elevated CO₂ on the dry masses of plant organs and whole plant, yield per hill and harvest index (HI) of two Japanese cultivars of rice in October 2018.

Cultivar	CO ₂ treatment	Dry mass (g per hill)										Yield ^a		HI ^b	
		Panicle		Leaf blade		Stem		Root		Whole plant		(g per hill)	(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. (<i>p</i> = 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.	n.s. (<i>p</i> = 0.057)		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 4 Effects of elevated CO₂ on the percentage of grain appearance qualities of two Japanese cultivars of rice in October 2018.

Cultivar	CO ₂ 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	CO ₂	*	**	n.s.	n.s.	n.s.
	Cv.	***	***	**	n.s.	*
	Cv. x CO ₂	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).

Table 5 Effects of elevated CO₂ on temperatures of flag leaf and panicle of two Japanese cultivars of rice at the end of flowering period.

Time of day	CO ₂ treatment	Hinohikari (August 24)		Nikomaru (August 28)	
		Leaf	Panicle	Leaf	Panicle
10:00	Ambient	34.6 (0.5)	36.2 (1.2)	32.9 (0.9)	35.4 (1.5)
	Elevated	34.9 (1.9)	36.6 (2.3)	34.5 (1.8)	36.8 (2.0)
13:00	Ambient	34.8 (0.6)	37.8 (1.3)	37.2 (1.5)	39.6 (1.1)
	Elevated	35.4 (1.5)	37.7 (1.0)	38.2 (1.5)	40.2 (0.7)
ANOVA	CO ₂	n.s.	n.s.	n.s.	n.s.
	Time	n.s.	n.s.	**	**
	CO ₂ x Time	n.s.	n.s.	n.s.	n.s.

Each value is the mean of three chambers for each treatment, and its standard deviation is shown in parentheses. Two-way analysis of variance (ANOVA): ** $p < 0.01$, n.s. = not significant.

Figures

Fig. 1

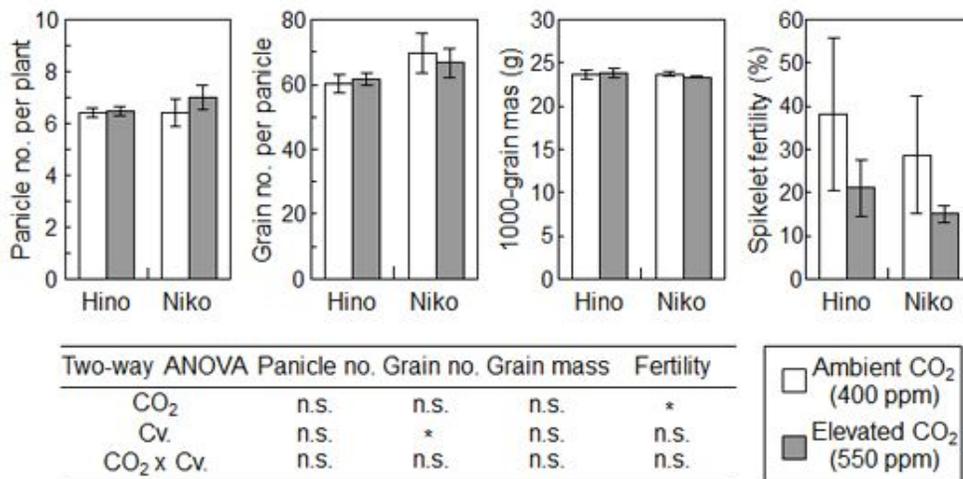


Figure 1

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

Fig. 2

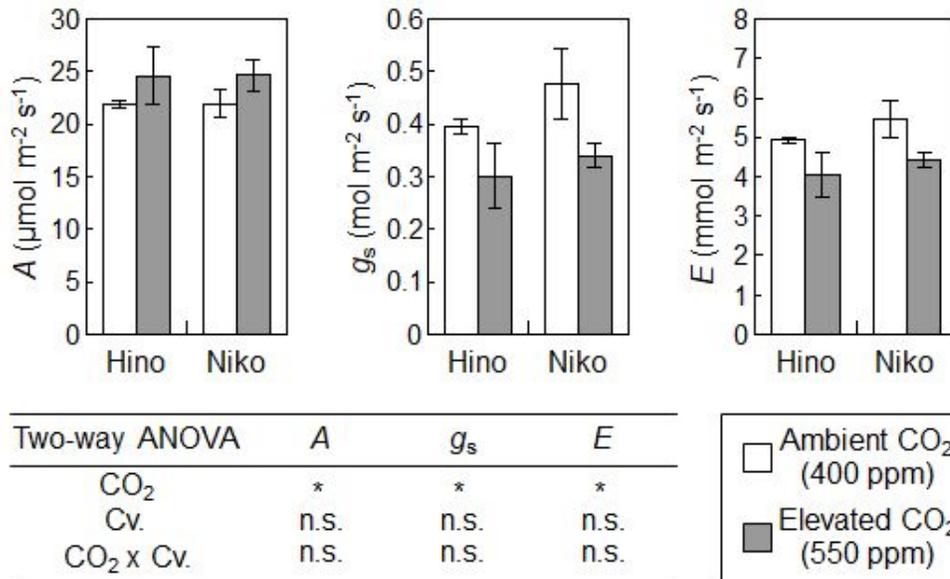


Figure 2

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