

Research Article

Elevated CO₂ Could Reduce Spikelet Fertility and Grain Appearance Quality of Rice (*Oryza sativa* L.) Grown under High-temperature Conditions

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ABSTRACT Two Japanese rice cultivars with different heat-tolerance, Hinohikari (sensitive) and Nikomaru (tolerant), 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. 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 occurred with elevated CO₂, such as reductions in spikelet fertility and grain appearance quality, which are typical manifestations of heat stress in rice. During the flowering period, the air temperature was high that spikelet fertility was reduced even under ambient CO₂ conditions for both cultivars. These results suggest that, under high-temperature conditions, elevated CO₂ could induce or exacerbate the manifestations of heat stress in rice. Because transpiration rate in the flag leaf was significantly reduced by the exposure to elevated CO₂, it is possible that elevated CO₂ increased plant temperature via a reduction in transpiration during flowering period, although we did not detect significance of the increase in leaf and panicle temperature. To ensure a more confident conclusion, further studies focusing on the effects of elevated CO₂ on the determinants of spikelet fertility and grain appearance quality with other cultivars in different year are required.

KEY WORDS Heat stress, Japonica rice, Open-top chamber, Stomatal conductance, Transpiration rate

1. INTRODUCTION

Atmospheric carbon dioxide (CO₂) concentration has increased mainly due to the fossil fuel combustion and land use change (Hartmann *et al.*, 2013). Although an increase in atmospheric CO₂ concentration is generally beneficial for plant growth, the effects of elevated CO₂ differed among plant species, cultivars and growth conditions (Hasegawa *et al.*, 2013; Ainsworth, 2008; Ainsworth and Long, 2005). 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 $\mu\text{mol mol}^{-1}$ (ppm), increased the net photosynthetic rate and dry matter production of C_3 plants by approximately 34% and 20%, respectively (Long *et al.*, 2004). For rice (*Oryza sativa* L.), which is the primarily consumed as a major food staple in Asia (OECD/FAO, 2022), elevated CO_2 (627 ppm) increased the yield by 23%, with some variation depending on the magnitude of the elevation and the fumigation technique (Ainsworth, 2008). From the results of FACE experiments on rice conducted in Japan, yield enhancement due to elevated CO_2 by approximately 200 ppm differed among the cultivars and varied from year to year, ranging from 0 to 36% (Hasegawa *et al.*, 2016, 2013). In addition to increasing net photosynthesis, elevated CO_2 induces stomatal closure (Hasegawa *et al.*, 2016; Ainsworth and Long, 2005), which reduces transpiration from the leaves, causing the canopy temperature to rise (Kimball, 2016; Bernacchi *et al.*, 2007; Long *et al.*, 2006). For example, Yoshimoto *et al.* (2005) observed an elevated CO_2 -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 CO_2 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 (Morita *et al.*, 2016; Jagadish *et al.*, 2015). 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 CO_2 induces manifestations of heat stress by increasing the plant temperature. It has been reported that the heat-induced reduction in spikelet fertility of rice was intensified by elevated CO_2 in the Japanese (japonica) rice cultivar “Akihikari” cultivated in a greenhouse chamber in Kyoto, Japan (Kim *et al.*, 1996), and the indica rice cultivar “IR72” cultivated in an open-top chamber (OTC) in Los Baños, Philippines (Matsui *et al.*, 1997). Based on the results of a multi-year FACE experiment conducted in Shizukuishi and Tsukuba, Japan, Hasegawa *et al.* (2016) and Kobayasi *et al.* (2019) reported the exposure to elevated CO_2 reduces spikelet fertility and exacerbated the reduction in japonica rice cultivar “Akitakomachi” and “Koshihikari”, respectively, under high-temperature conditions. Using the FACE system in Jiangsu, China and Tsukuba, Japan, several research-

ers have also reported that the exposure to elevated CO_2 induced deterioration of grain quality of japonica rice cultivars (Jing *et al.*, 2016; Usui *et al.*, 2016, 2014; Yang *et al.*, 2007). Therefore, it is expected that the detrimental effects of elevated CO_2 could become prominent in the warmer region.

In the present study, we conducted an experimental study on the effects of elevated CO_2 on rice using OTCs located in Nagasaki in the Kyushu region, which is a warm temperate area and warmer than Kyoto, Shizukuishi and Tsukuba where the studies reporting the detrimental effects on japonica rice were conducted in Japan. In this region, the quality of rice grains began to decline in the 1980s (Ishigooka *et al.*, 2011; Okada *et al.*, 2009). 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 (Uno *et al.*, 2012; Okada *et al.*, 2009). These results suggest that the temperature in this region reached the level for heat stress induction in rice. Although it is unclear whether the estimated impact of the elevated CO_2 on rice quality and yield were greater than such effects, adverse effects of elevated CO_2 could occur in this region. In the present study, therefore, we hypothesized that exposure to elevated CO_2 in this region could reduce spikelet fertility and grain appearance quality of japonica rice via elevated CO_2 -induced transpiration reduction.

2. MATERIALS AND METHODS

2.1 Plant Material

In Nagasaki, the primary rice cultivar is “Hinohikari.” Since Hinohikari rice is relatively sensitive to heat stress, the heat-tolerant cultivar “Nikomaru” has been introduced (Tanamachi *et al.*, 2016; Tanaka *et al.*, 2009). The present study used both cultivars. The seedlings were planted on June 13, 2018, in 1/5000 a Wagner’s pots ($\phi 159 \text{ mm} \times 300 \text{ mm}$ 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 to the chamber. The planting density were 150 and 50 hills m⁻² against soil surface area and chamber floor area, respectively. 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. Vapor pressure deficit (VPD, kPa) was calculated from the T_{air} and RH. Hourly mean global solar radiation measured at the Nagasaki Local Meteorological Observatory, which is about 5.8 km south of the experimental site, was obtained from website of the Japan Meteorological Agency (<https://www.data.jma.go.jp/gmd/risk/obsdl/index.php>).

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 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. 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. The CO₂ concentration inside the OTCs was monitored using a CO₂ gas analyzer (LI-820, Li-Cor Inc., USA) which 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. Observed diurnal variations in ambient CO₂ concentrations from July 1 to September 30 in 2018 typically show their maxima between 5:00 and 7:00 and their minima between 14:00 and 17:00 with the average difference of 28.6 ppm. The range of the horizontal distribution of CO₂ concentration inside the chamber 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.

2.3 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 $1,500 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. For the measurements of A , g_s , and E , the atmospheric CO₂ concentration inside the leaf chamber was 400 ppm for the ambient CO₂ treatment and 550 ppm for the elevated CO₂ treatment.

2.4 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. To identify the flag leaf and panicle in the infrared images, black acrylic board warmer than plant tissue was put behind a target leaf or panicle immediately before taking the images. The minimum temperature within the area of leaf or panicle in the images was analyzed and assumed to be the temperatures of leaf and panicle. For each cultivar, three hills from each OTC, and

one flag leaf and panicle from each hill were randomly selected for measurements.

2.5 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 Hino-hikari 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 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 hill. 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. 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.

2.6 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 and time of day for each cultivar. In this analysis, we did not test the effect of cultivar, because the measurement date differed between the cultivars. All statistical analyses were performed using IBM SPSS Advanced Statistics 22.

3. RESULTS

3.1 Air Temperature during Cultivation

Fig. 1 shows time course of temperature outside and inside the OTCs during the experimental period. Number of hours exceeded the 35°C outside and inside the OTCs during the period were 39 and 240, respectively. 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 (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. Because 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), 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

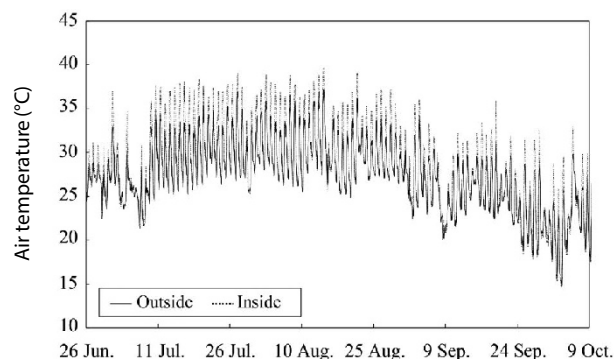


Fig. 1. Time course of temperature outside and inside open-top chambers (OTCs) during the experimental period from 26 June to 9 October 2018. Number of hours exceeded the 35°C outside and inside the OTCs during the period were 39 and 240, respectively.

Table 1. Air temperature (T_{air}), relative air humidity (RH) and vapor pressure deficit (VPD) inside and outside the open-top chambers from June 26 to October 9, 2018.

	T_{air} (°C)			RH (%)			VPD (kPa)		
	Daily mean	Daily max. ^a	Daily min. ^b	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)	1.34 (0.02)	2.77 (0.05)	0.42 (0.01)
Outside	27.4	31.0	24.4	72.4	88.3	55.7	1.11	2.06	0.39

Each value for inside the chambers is the mean of six chambers, and its standard deviation is shown in parentheses. ^aMean of daily 1-h maximum value. ^bMean of daily 1-h minimum value.

Table 2. Heading date and air temperature (T_{air}), vapor pressure deficit (VPD) and global solar radiation during flowering period in each gas treatment.

Cultivar	CO ₂ treatment	Heading date ^a	T_{air} (°C)		VPD (kPa)		Global solar radiation (W m ⁻²)
			Daily mean	Daily max. ^b	Daily mean	Daily max. ^b	
Hinohikari (heat-sensitive)	Ambient	Aug. 22	30.5 (0.1)	36.2 (0.6)	1.73 (0.08)	3.44 (0.31)	221
	Elevated	Aug. 21	30.3 (0.1)	36.0 (0.2)	1.78 (0.06)	3.47 (0.07)	222
Nikomaru (heat-tolerant)	Ambient	Aug. 26	30.4 (0.3)	36.0 (1.2)	1.49 (0.16)	3.11 (0.50)	238
	Elevated	Aug. 25	30.5 (0.3)	35.9 (0.6)	1.58 (0.07)	3.22 (0.20)	224

Each value of T_{air} and VPD is the mean of two (Ambient CO₂ treatment) or three (Elevated CO₂ treatment) chambers, and its standard deviation is shown in parentheses. Flowering period: one week around the heading date. ^aDay on which mean heading rate reached 50%. ^bMean of daily 1-h maximum value.

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 differences in the T_{air} and VPD 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. Because the air temperature in the present study was higher than the values for both cultivars (Table 2), the air temperature during flowering could be sufficiently high that spikelet fertility was reduced. Mean global solar radiation during the flowering period in the ambient CO₂ treatment was similar and less in the elevated CO₂ treatment for Hinohikari and Nikomaru, respectively.

3.2 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. 2). 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. The percentage of perfect grains across

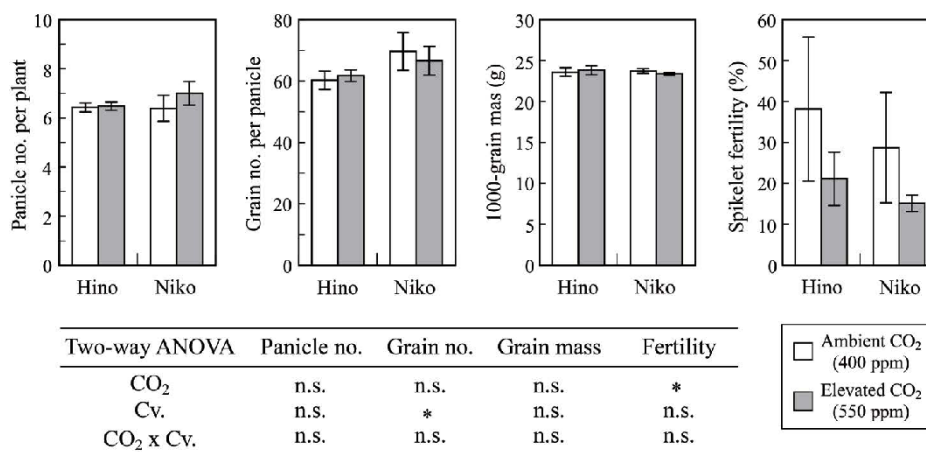


Fig. 2. Effects of elevated CO₂ on the panicle number per hill, grain number per panicle, 1000-grain mass, and spikelet fertility of two Japanese cultivars of rice. Hino: Hinohikari (heat-sensitive); Niko: Nikomaru (heat-tolerant). Each value represents the mean of three chamber replicates, and error bars represent the standard deviations. Two-way analysis of variance (ANOVA): **p* < 0.05, n.s. = not significant.

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 (g per hill)	HI ^b (g g ⁻¹)
		Panicle	Leaf blade	Stem	Root	Whole plant		
Hinohikari (heat-sensitive)	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 (heat-tolerant)	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. (<i>p</i> = 0.057)	n.s.	n.s.

Each value is the mean of three chambers, and its standard deviation is shown in parentheses. ^aFilled grain mass per plant. ^bRatio 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 (heat-sensitive)	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 (heat-tolerant)	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).

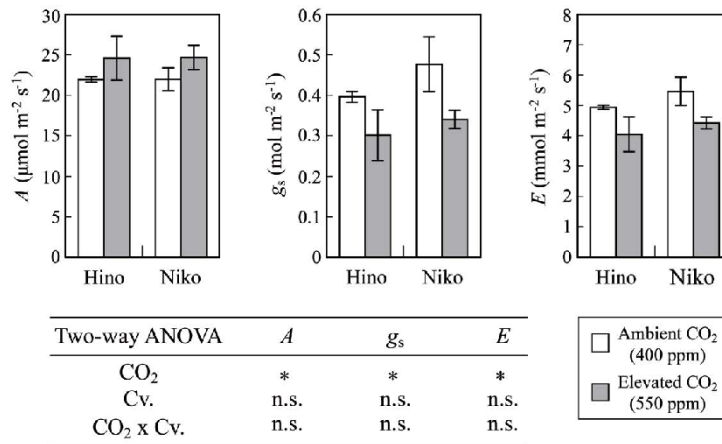


Fig. 3. Effects of elevated CO₂ on the net photosynthetic rate (A), stomatal conductance (g_s), and transpiration rate (E) in the flag leaf of two Japanese cultivars of rice in late August 2018. Hino: Hinohikari (heat-sensitive); Niko: Nikomaru (heat-tolerant). Each value represents the mean of three chamber replicates, and error bars represent the standard deviations. Two-way analysis of variance (ANOVA): * $p < 0.05$, n.s. = not significant.

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 (heat-sensitive) (August 24)		Nikomaru (heat-tolerant) (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 × 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.

the cultivars was reduced from an average of 56.2% in the ambient CO₂ treatment to 44.3% in the elevated CO₂ treatment, which was mainly caused by significant increase in immature grains. 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.

3.3 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. 3). 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 CO_2 treatment across the cultivars.

As shown in Table 5, Hinohikari revealed no significant effects of elevated CO_2 and time of day or their interaction on the temperatures of the flag leaf and panicle at the end of the flowering period. 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 CO_2 or interaction between CO_2 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 high temperatures that were able to induce heat stress symptoms in rice, such as low spikelet fertility, resulting in low grain yield even in the ambient CO_2 treatment.

4. DISCUSSION

Consistent with previous studies (Hasegawa *et al.*, 2016), elevated CO_2 significantly increased dry mass growth in both rice cultivars in this study (Table 3). This could be caused by an increase in A (Fig. 3). However, regardless of the beneficial effects of elevated CO_2 , exposure to elevated CO_2 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 CO_2 for both cultivars (Fig. 2), which could result in inconsistent results between the effects on whole-plant DM and yield (Table 3). 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 (Morita *et al.*, 2016; Jagadish *et al.*, 2015). These results support the hypothesis that exposure to elevated CO_2 in warm region including high temperatures, which were able to induce heat stress on rice, could reduce spikelet fertility and grain appearance quality of japonica rice. However, the present study was a case study conducted in a specific year with

two genotypes and provided limited data for testing the hypothesis. 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.

The air temperature during the flowering period was high that spikelet fertility was reduced (Table 2). Although 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, no differences were detected in the regime between the treatments (Table 2). Jagadish *et al.* (2007) reported that spikelet fertility decreases with an increase in the panicle temperature during the flowering stage. It has been suggested that VPD, solar radiation and wind speed, as well as air temperature, affect the panicle temperature (Yoshimoto *et al.*, 2022; Tian *et al.*, 2010; Matsui *et al.*, 2007). However, there were no differences in the VPD and solar radiation (Table 2), and wind speed inside the chamber was same among the chambers. According to a meta-analysis by Kimball (2016), on the other hand, 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. 3). Although no significant increase was detected in temperatures of the flag leaf and panicle in the elevated CO_2 treatment (Table 5), a small increase in the panicle temperature in the elevated CO_2 treatment could cause a significant reduction in spikelet fertility, since the panicle temperature during the flowering period were high enough to reduce spikelet fertility (Table 5). The panicle temperatures were much higher than those calculated using the integrated micrometeorology model for panicle and canopy temperature (Meteo-Crop DB, <https://meteocrop.dc.affrc.go.jp/real/quickmodel.php>) (Yoshimoto *et al.*, 2011, 2005a, b). Difference in temperature deviation of panicle from air between measured and modeled values was negatively correlated with difference in the wind velocity between inside the OTCs (approximately 0.5 m s⁻¹) and in the model (up to 5 m s⁻¹), suggesting lower wind velocity caused higher temperature deviation of panicle from air. Therefore, low wind velocity in the OTCs might explain why the elevated CO_2 -induced increase in panicle was not detected whereas the reduction in the transpiration rate was observed.

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 CO₂, but further research is required to clarify whether elevated CO₂ 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 CO₂ 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.

Based on the results of FACE experiments in Japan, Hasegawa *et al.* (2016) reported that, the response of above-ground biomass and net photosynthetic rate of rice to elevated CO₂ by approximately 200 ppm were around 12 to 13% and 18%, respectively. In the present study, we observed comparable beneficial effects of elevated CO₂ on the growth and net photosynthesis: 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. 3). However, Ainsworth (2008) reported that yield and photosynthetic responses of rice to elevated CO₂ were greater in the OTC experiments than those in the FACE experiments, suggesting the lower growth and photosynthetic responses in our present study as compared with other studies using OTC. Lower growth and yield responses to elevated CO₂ have been reported under low N fertilizer treatments (Kimball, 2016; Ainsworth, 2008; Kim *et al.*, 2003). 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). 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. Because of this, a greater reduction in transpiration by elevated CO₂ might cause a greater increase in canopy temperature and a considerable reduction in spikelet fertility. Otherwise, elevated CO₂-induced growth stimulation could exacerbate N deficient in the plant tissue, and consequently might cause poor grain filling, although we didn't measure N concentration in plant tissue in the present study. Further research is needed to elucidate whether N fertilization can mitigate the adverse effects of elevated CO₂ on spikelet fertility.

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 (Tanamachi *et al.*, 2016; Tanaka *et al.*, 2009). 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. In contrast, there were no significant cultivar differences and significant interactions between cultivars and elevated CO₂ for spikelet fertility (Fig. 2). 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.

5. CONCLUSION

In the present study, we observed adverse effects of elevated CO₂, including reductions in spikelet fertility and grain appearance quality, which are typical manifestations of heat stress, in two Japanese rice cultivars. The growth

conditions included high temperatures that able to induce heat stress, especially during the flowering period. These results suggest that, under high-temperature conditions, elevated CO₂ could induce or exacerbate the manifestations of heat stress in japonica rice. Because transpiration rate in the flag leaf was significantly reduced by the exposure to elevated CO₂, it is possible that elevated CO₂ increased plant temperature via a reduction in transpiration during flowering period, although we did not detect significance of the increase in leaf and panicle temperature. To ensure a more confident conclusion, further studies focusing on the effects of elevated CO₂ on the determinants of spikelet fertility and grain appearance quality with other cultivars in different year are required.

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