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Effects of free-air CO₂ enrichment on heat-induced sterility and pollination in rice

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ABSTRACT

Global climate changes may cause heat-induced sterility in rice, threatening the global production of this important crop. Although little is currently known about the combined effects of the concentration of atmospheric CO₂ ([CO₂]) and temperature on heat-induced sterility, elevated [CO₂] (E-[CO₂]) will likely increase the panicle temperature and thereby exacerbate heat-induced sterility, but this was not tested in open fields. Therefore, we investigated the effect of E-[CO₂] on heat-induced sterility and sterility-related traits in rice by increasing E-[CO₂] by approximately 200 μmol mol⁻¹ above ambient levels using a free-air CO₂ enrichment (FACE) facility for six growing seasons with variable growing season temperatures. The percentage fertility was not significantly correlated with the air temperature (T_a) between 09:00 and 12:00 on each flowering day, but it did significantly vary among the years, with 2011 experiencing cool temperatures resulting in chilling-induced mild sterility. When data from 2011 were removed, there was a significant negative correlation between fertility and T_a between 09:00 and 12:00 on each flowering day under E-[CO₂], whereas no such effect was seen under ambient [CO₂]. E-[CO₂] also significantly reduced the number of pollen grains deposited on the stigma by 10%, but it slightly increased the anther length by 1.3%, indicating that it had both negative and positive effects on heat-induced sterility. These findings suggest that E-[CO₂] affects many traits related to heat-induced sterility and may sometimes exacerbate sterility by reducing pollen grain deposition.

Abbreviations: A-[CO₂]: ambient CO₂; [CO₂]: the concentration of carbon dioxide; E-[CO₂]: elevated [CO₂]; FACE: free-air CO₂ enrichment; T_a : air temperature

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1. Introduction

According to the 5th Assessment Report of the Intergovernmental Panel on Climate Change (IPCC, 2013), the atmospheric concentrations of greenhouse gases have increased since 1750 as a result of human activities, with the concentration of carbon dioxide ([CO₂]) in 2011 (391 μmol mol⁻¹) exceeding preindustrial levels by approximately 40%. This has led to a combined global average increase in land and ocean surface temperatures of 0.85°C (IPCC, 2013), which threatens the production of many crops, including rice, an important staple food in Asia and Africa.

Heat-induced sterility occurs when the air temperature (T_a) at anthesis is above 35°C in growth chambers (Jagadish et al., 2007; Satake & Yoshida, 1978; Weerakoon et al., 2008), temperature gradient chambers (Kim et al., 1996; Maruyama et al., 2013), open-top chambers (Chaturvedi et al., 2017), and the field (Ishimaru et al., 2016; Matsui et al., 2014; Tian et al., 2010). This is mainly caused by low pollination due to anther indehiscence (Kobayasi et al., 2011; Satake & Yoshida, 1978). Not only anther indehiscence but also

the length of anther dehiscence at the basal part of the theca plays a critical role in stable pollination and fertilization under high temperature conditions (Matsui et al., 2005; Shah et al., 2011). For example, in a growth chamber experiment, Kobayasi et al. (2011) demonstrated that the relative contribution of anther indehiscence and the basal dehiscence length to pollination (percentage of sufficiently pollinated florets) changed according to T_a , with the percentage of dehisced anthers becoming more important with increasing T_a .

However, the combined effects of [CO₂] and heat stress on sterility, pollination, and anther dehiscence under open-air field conditions remain unclear. Although some studies have shown that heat-induced sterility is exacerbated by elevated [CO₂] (E-[CO₂]) (Chaturvedi et al., 2017; Kim et al., 1996; Matsui et al., 1997), no such effect has been observed by others (Madan et al., 2012), and all of these experiments were conducted under enclosed conditions. E-[CO₂] increases the panicle temperature by causing the leaf stomata to close and by reducing leaf stomatal conductance (Oue et al., 2005; Shimono et al., 2010). A model simulation showed that the panicle temperature increased by 0.5–1.0°

C on a flowering day as a result of E-[CO₂] alone, which is sufficient to exacerbate heat-induced sterility (Yoshimoto et al., 2005). Therefore, an assessment of the effects of E-[CO₂] on sterility, pollination, and anther dehiscence is important for accurately predicting the effect of heat stress on rice production and for evaluating the performance of heat-tolerant cultivars under heat stress conditions.

The objective of this study was to reveal the effects of E-[CO₂] on floret sterility and sterility-related traits, such as pollination and anther dehiscence, in rice. To do this, we used the Tsukuba free-air CO₂ enrichment (FACE) facility (Hasegawa et al., 2013) because it represents the most reliable and feasible experimental platform for evaluating the effect of [CO₂] on the physiological and ecological traits of crops under open-air field conditions (Hasegawa et al., 2016; Kobayashi, 2001). We conducted the FACE experiments for six growing seasons with different temperatures, some of which exceeded the critical temperature for spikelet sterility.

2. Materials and methods

2.1. Experimental site, plant materials, and culture methods

This study was conducted at the Tsukuba FACE facility in Ibaraki Prefecture, Japan (35°58'N, 139°60'E; 10 m above sea level) from 2011 to 2016 using the standard japonica rice cultivar 'Koshihikari'. This area has a humid subtropical climate, with an average annual temperature of 13.8°C and annual precipitation of 1280 mm. Details of weather conditions and soil properties at the site are described by Hasegawa et al. (2013).

The crop cultivation practices that were used at Tsukuba FACE during the 2011–2016 growing seasons were essentially identical to those reported by Hasegawa et al. (2013). Briefly, all plots received equal amounts of P and K in early April, before ploughing, when PK compound fertilizer was applied at a rate of 4.36 g m⁻² of P and 8.30 g m⁻² of K. The fields were kept submerged after late April. Three kinds of N fertilizer were applied just prior to puddling: 2 g m⁻² as urea, 4 g m⁻² as one type of controlled-release fertilizer-coated urea (type LP100, JCAM Agri. Co. Ltd, Tokyo, Japan), and 2 g m⁻² as another type of controlled-release fertilizer (type LP140, JCAM Agri. Co. Ltd) in 2011, 2012, and 2013. In 2014, 2015, and 2016, controlled-release fertilizer (type LP140) was changed to controlled-release fertilizer (type LP40).

Rice seeds were germinated in water and sown into seedling trays, each with 448 circular cells (16 mm in diameter and 25 mm in depth, Minoru Pot 448, Minoru Industrial Co. Ltd, Okayama, Japan). Each cell was filled with sterilized soil amended with fertilizer at the rate of 0.4 g N,

0.35 g P, and 0.5 g K per 1 kg of soil. Three seeds were sown in each cell. After seedling emergence, the seedling trays were transferred to the puddled open field and protected with a tunnel cloche or floating mulch for the first two weeks. The seedlings were then manually transplanted into hills (30 × 15 cm) on May 25, May 23, May 22, May 22, May 26 and May 25 in 2011, 2012, 2013, 2014, 2015 and 2016, respectively. Planting density was 22.2 hills m⁻² (three seedlings per hill). After the transplanting, the fields were kept flooded until August 20, about 3 weeks after heading, when the ponding water was drained for harvesting. We applied flush irrigations on several occasions to keep the soil moist.

2.2 CO₂ treatment

The method for controlling [CO₂] in an open field is described by Nakamura et al. (2012). Briefly, four blocks were established in paddy fields, with each block consisting of two octagonal plots (204 m², 17 m across): an ambient CO₂ (A-[CO₂]) plot and an elevated CO₂ (E-[CO₂]) treatment plot. The E-[CO₂] plots were equipped with emission tubes on the perimeter, where CO₂ was released from the windward side to keep the [CO₂] measured at the central point at about 200 μmol mol⁻¹ above the ambient control level.

Over the entire growing season (June–September), the mean A-[CO₂] at the site was 379, 383, 384, 386, 383, and 391 μmol mol⁻¹ in 2011, 2012, 2013, 2014, 2015 and 2016, respectively. The actual season-long mean [CO₂] in the FACE plots was 560, 578, 576, 580, 579, and 586 μmol mol⁻¹ in those years, respectively.

2.3 Measurements of fertility, pollination, and anther dehiscence

Once plants reached the heading stage, we selected five panicles in each plot and marked the lemmas of all opened florets with an acrylic gouache paint 3–4 h after flower opening. We changed the paint color each day to distinguish the day of flowering and marked the florets on more than three flowering days per year, excluding rainy days. We then collected the painted panicles at maturity and examined their fertility by manually inspecting each floret for ovary development.

To examine pollination, we sampled more than five florets from the primary rachis-branches of different panicles in each plot at around 14:00 on more than three flowering days per year. The stigmata were extracted from the florets and stained with 0.1% cotton blue solution (0.1 g of cotton blue, 75 mL of 2% acetic acid, and 25 mL of glycerol), and the number of pollen grains deposited on

the stigma was counted under an optical microscope (OPTIPHOTO; Nikon, Tokyo, Japan).

To examine the percentage of dehiscent anthers and the length and width of the anther dehiscence at the basal part of the theca, we sampled more than five florets (different florets sampled for measuring pollination as mentioned before) from the primary rachis-branches of different panicles in each plot at around 14:00 on more than three flowering days per year (Matsui et al., 2005). The anthers were air-dried, and we counted the number of indehiscent anthers and measured anther length (both dehiscent and indehiscent anthers) under a digital USB microscope at 100 × magnification (Anyty 2.4; 3R System C., Fukuoka, Japan). The length and width of the basal anther dehiscence were measured under the same digital USB microscope (Matsui et al., 2005).

2.4 Measurement of weather conditions

We measured T_a , relative humidity, wind speed, and solar radiation in the center at the Tsukuba FACE field. A temperature and humidity sensor (HMP45D; Vaisala Inc., Helsinki, Finland) was installed 2 m above the soil surface with a forced-ventilated radiation shield, and an anemometer and a wind vane (Model 03001-5 Wind Sentry; R. M. Young Co., Traverse City, MI, USA) were placed at a height of 2.5 m. Solar radiation was measured with a pyranometer (LI-200SA; LI-COR Inc., Lincoln, NE, USA). Each variable was measured every 10 s, and 10 min averages were recorded in a data logger (CR1000; Campbell Scientific Inc., Logan, UT, USA). T_a , relative humidity, wind speed, and solar radiation are common in four replicates.

2.5 Statistical analysis

We conducted an analysis of variance by applying a randomized block design, in which year, $[CO_2]$ treatment, and block are the factors, and year × $[CO_2]$ is the interaction factor with four replications, using R (version 3.2.5, R Development Core Team, 2016). We analyzed our data by means of correlation procedures using R. In all tests, statistical significance is indicated for $p < 0.001$, $p < 0.01$, or $p < 0.05$, and the actual p -values are presented where $0.05 < p < 0.1$.

3. Results

3.1 Weather conditions during the flowering periods

The daily mean T_a during the flowering periods of 2011–2016 ranged between 24.8 and 29.2°C (Table 1),

being the highest in 2015. The daily maximum T_a sometimes reached 35°C in 2014, 2015 and 2016, which is the temperature at which heat-induced floret sterility occurs. By contrast, in 2011, the average daily mean T_a was <25°C and the daily minimum T_a did not exceed 25°C during the flowering period.

The daily mean wind speed was low during all six flowering periods, remaining below 3 m s⁻¹ (Table 2). The average daily mean relative humidity varied from 73.2% in 2014 to 85.8% in 2013, whereas the daily mean solar radiation ranged from 10.0 MJ m⁻² in 2011 to 23.6 MJ m⁻² in 2014.

3.2 Fertility and pollination

The percentage fertility significantly differed among the years ($p < 0.001$), but it was not significantly affected by $[CO_2]$ or the interaction between the treatment and the year (Table 3). Fertility was below 90% in 2011 and higher than 90% in all other years in the control and treatment plots.

The number of pollen grains deposited on the stigma also significantly differed among the years ($p < 0.001$) and was significantly affected by $[CO_2]$ ($p < 0.05$), with no significant interaction between the year and $[CO_2]$. The number of deposited pollen grains was the highest in 2015 and the lowest in 2011, when T_a was the lowest, and decreased by approximately 10% under E- $[CO_2]$.

3.3 Anther dehiscence and length

Anther indehiscence was rarely observed in this experiment, so data on the percentage anther dehiscence are

Table 1. Daily mean, maximum and minimum air temperatures (T_a , °C) during the flowering periods from 2011 to 2016.

Year	Daily mean T_a	Daily maximum T_a	Daily minimum T_a
2011	22.3–27.0 (24.8)	24.0–31.6 (28.1)	20.6–24.4 (22.3)
2012	25.7–28.8 (27.2)	28.7–32.7 (31.3)	23.0–25.4 (23.6)
2013	22.9–27.7 (25.6)	26.7–32.2 (29.9)	20.4–24.2 (22.5)
2014	26.3–30.1 (28.4)	31.4–35.0 (33.4)	20.8–26.8 (23.9)
2015	28.6–29.6 (29.2)	33.9–35.1 (34.4)	23.5–24.9 (24.4)
2016	27.2–29.5 (28.1)	31.0–35.1 (32.9)	23.3–24.0 (23.7)

Values are ranges, with means in parentheses.

Table 2. Daily mean wind speed, relative humidity, and solar radiation during the flowering periods from 2011 to 2016.

Year	Daily mean wind speed (m s ⁻¹)	Daily mean relative humidity (%)	Daily solar radiation (MJ m ⁻²)
2011	0.2–1.3 (0.7)	83.2–88.3 (85.0)	4.0–13.9 (10.0)
2012	0.4–2.1 (1.1)	73.9–92.6 (82.0)	8.5–26.1 (21.1)
2013	0.7–1.9 (1.0)	77.1–92.9 (85.8)	12.2–25.6 (17.7)
2014	0.7–2.4 (1.4)	66.7–78.3 (73.2)	20.6–25.9 (23.6)
2015	0.7–1.7 (1.0)	75.1–79.7 (76.5)	21.4–26.4 (22.9)
2016	0.8–1.7 (1.1)	62.4–79.4 (73.9)	17.1–26.8 (23.3)

Values are ranges, with means in parentheses.

Table 3. Fertility and pollination (number of pollen grains deposited on the stigma) under enhanced and ambient concentrations of CO₂ (E-[CO₂] and A-[CO₂], respectively).

Year	Treatment	Fertility (%)	Pollination
2011	E-[CO ₂]	86.1	49.2
	A-[CO ₂]	88.5	48.2
2012	E-[CO ₂]	94.4	34.8
	A-[CO ₂]	94.3	36.7
2013	E-[CO ₂]	94.4	47.1
	A-[CO ₂]	94.3	51.3
2014	E-[CO ₂]	96.9	76.6
	A-[CO ₂]	97.0	85.8
2015	E-[CO ₂]	95.4	102.1
	A-[CO ₂]	96.1	110.3
2016	E-[CO ₂]	91.1	86.0
	A-[CO ₂]	90.8	108.1
Mean	E-[CO ₂]	93.1	66.0
	A-[CO ₂]	93.5	73.4
ANOVA	Year	***	***
	Treatment	ns	*
	Block	ns	ns
	Year × treatment	ns	ns

Statistically significant effects are indicated: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$. The value indicates the probability between 0.05 and 0.1; ns, not significant (ANOVA).

not shown. Both the length and the width of the anther dehiscence at the basal part of the theca significantly differed among the years ($p < 0.001$) but were not significantly affected by [CO₂] or the interaction term (Table 4). The basal dehiscence length was the greatest in 2012 and was also high in 2011, when T_a was the lowest, and was smaller in 2014, 2015, and 2016, when the daily mean T_a was relatively high. The width of the anther dehiscence zone showed similar trends to the length.

The anther length significantly differed among the years ($p < 0.001$) and with [CO₂] ($p < 0.01$), and the interaction

Table 4. Lengths and widths of the anther dehiscence at the basal part of the theca and anther lengths under enhanced and ambient concentrations of CO₂ (E-[CO₂] and A-[CO₂], respectively).

Year	Treatment	Basal dehiscence length (mm)	Basal dehiscence width (mm)	Anther length (mm)
2011	E-[CO ₂]	0.511	0.109	2.014
	A-[CO ₂]	0.590	0.120	1.988
2012	E-[CO ₂]	0.571	0.099	1.916
	A-[CO ₂]	0.562	0.101	1.933
2013	E-[CO ₂]	0.542	0.099	1.926
	A-[CO ₂]	0.561	0.104	1.961
2014	E-[CO ₂]	0.491	0.067	1.906
	A-[CO ₂]	0.493	0.068	1.875
2015	E-[CO ₂]	0.391	0.094	1.925
	A-[CO ₂]	0.394	0.093	1.905
2016	E-[CO ₂]	0.333	0.070	1.901
	A-[CO ₂]	0.298	0.061	1.781
Mean	E-[CO ₂]	0.473	0.090	1.931
	A-[CO ₂]	0.483	0.091	1.907
ANOVA	Year	***	***	***
	Treatment	ns	ns	**
	Block	ns	ns	ns
	Year × treatment	ns	ns	***

Statistically significant effects are indicated: ***, $p < 0.001$; **, $p < 0.01$. The value indicates the probability between 0.05 and 0.1; ns, not significant (ANOVA).

between the year and [CO₂] was also significant ($p < 0.001$). Anthers were the longest in 2011, when T_a was the lowest, and were particularly short in 2016. E-[CO₂] averaged across the six years slightly increased the anther length by approximately 1.3% although E-[CO₂] slightly decreased the anther length in 2011 and 2012.

3.4 Relationship between weather factors and traits related to fertility and pollination

There was no significant correlation between the percentage fertility and T_a between 09:00 and 12:00 on each flowering day. However, there was a significant difference in fertility among the years, with relatively high sterility occurring in 2011 despite the moderate T_a during the flowering period. This may have been due to the extraordinarily low minimum T_a that was experienced approximately 10 days before heading ($<17.5^\circ\text{C}$), at which stage cool temperatures may cause mild sterility (Nishiyama, 1995). When the 2011 data were removed, there was a significant negative correlation between fertility and T_a between 09:00 and 12:00 under E-[CO₂] but not under A-[CO₂] (Figure 1).

No significant correlation was observed between percentage fertility and relative humidity between 9:00 and 12:00 on each flowering day (data not shown). Solar radiation between 9:00 and 12:00 was not also significantly correlated with the percentage fertility (data not shown).

The number of pollen grains deposited on the stigma was not correlated with T_a between 09:00 and 12:00 on each flowering day under E-[CO₂] or A-[CO₂] (Figure 2). However, the number of deposited pollen grains was significantly positively correlated with wind speed between 09:00 and 12:00 in both the treatment and the control plots (Figure 3), with a wind speed of $>1.5 \text{ m s}^{-1}$ resulting in more than 100 pollen grains being deposited.

The basal dehiscence length was not significantly correlated with T_a between 09:00 and 12:00 on each flowering day (Figure 4), but it did significantly vary among years.

4. Discussion

We found that an increased T_a between 09:00 and 12:00 on each flowering day slightly decreased the percentage fertility of rice plants under E-[CO₂] but had no effect under A-[CO₂] (Figure 1). Previous experiments conducted under enclosed conditions have similarly shown that heat-induced sterility is often exacerbated by E-[CO₂] (Chaturvedi et al., 2017; Kim et al., 1996; Matsui et al., 1997), which increases the panicle temperature by inducing leaf stomatal closure and reducing leaf stomatal

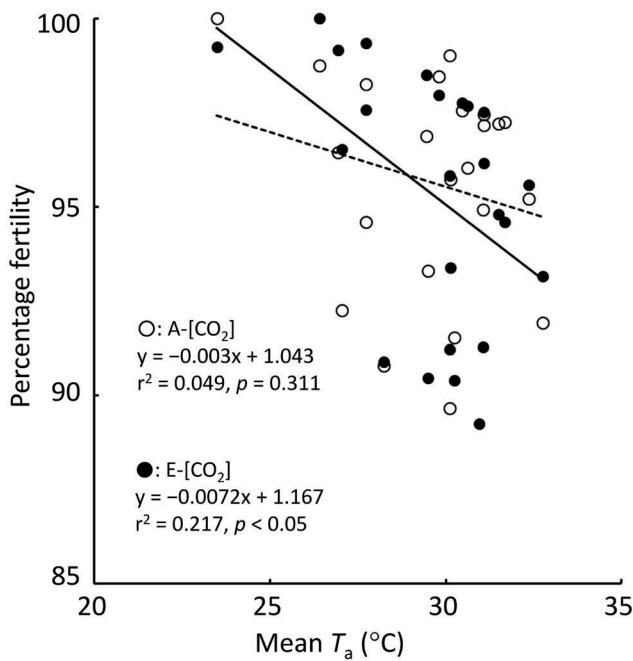


Figure 1. Relationship between the percentage fertility and the mean air temperature (T_a) between 09:00 and 12:00 on each flowering day from 2012 to 2016. Open circles and dotted line: ambient concentrations of CO_2 (A-[CO_2]); closed circles and solid line: elevated concentrations of CO_2 (E-[CO_2]).

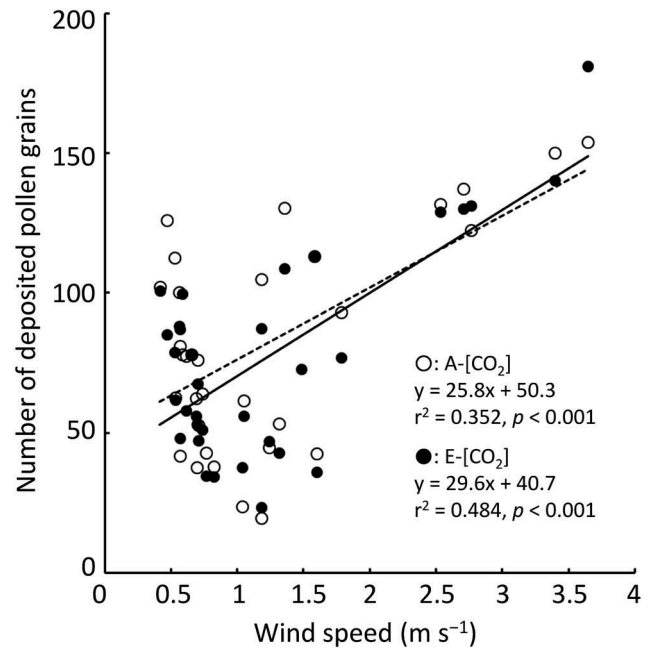


Figure 3. Relationship between the number of pollen grains deposited on the stigma and the mean wind speed between 09:00 and 12:00 on each flowering day from 2011 to 2016. Open circles and dotted line: ambient concentrations of CO_2 (A-[CO_2]); closed circle and solid line: elevated concentrations of CO_2 (E-[CO_2]).

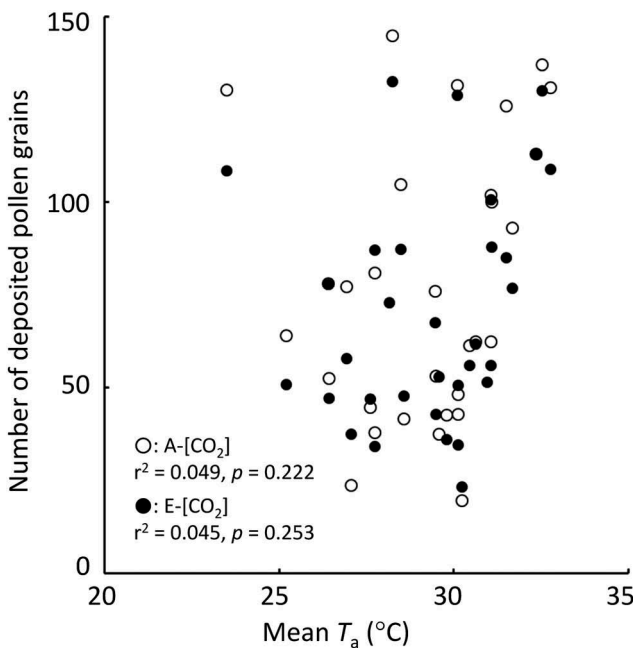


Figure 2. Relationship between the number of pollen grains deposited on the stigma and the mean air temperature (T_a) between 09:00 and 12:00 on each flowering day from 2011 to 2016. Open circles: ambient concentrations of CO_2 (A-[CO_2]); closed circles: elevated concentrations of CO_2 (E-[CO_2]).

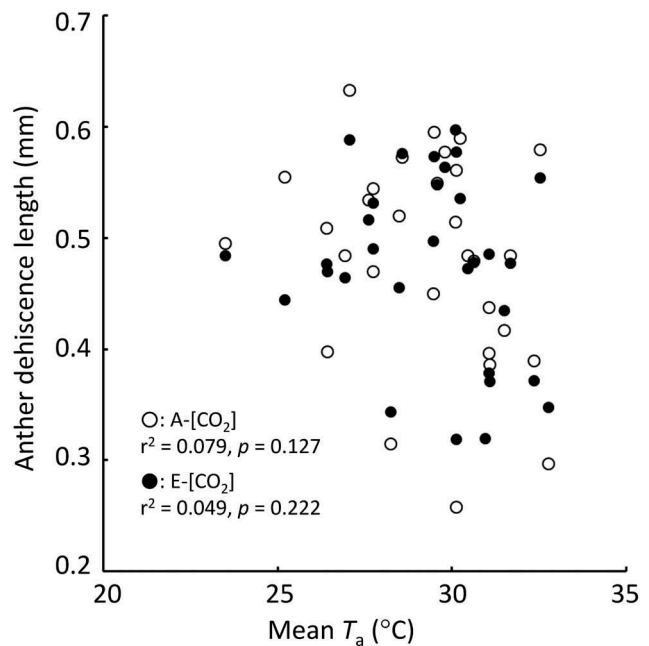


Figure 4. Relationship between the length of the anther dehiscence zone in the basal part of the theca and the mean air temperature (T_a) between 09:00 and 12:00 on each flowering day from 2011 to 2016. Open circles: ambient concentrations of CO_2 (A-[CO_2]); closed circles: elevated concentrations of CO_2 (E-[CO_2]).

conductance. Although we did not measure the panicle temperature here, if the 0.5–1.0°C increase estimated by Yoshimoto et al. (2005) under E-[CO₂] is valid, the panicle temperature will sometimes exceed 35°C, where heat-induced sterility occurs.

E-[CO₂] had varied effects on traits related to heat-induced sterility and pollination, decreasing the number of pollen grains deposited on the stigma (Table 3) but increasing the anther length (Table 4) and having no effect on anther dehiscence in the basal part of the theca. Matsui et al. (1997) similarly observed a reduction in pollen grain deposition under E-[CO₂] conditions in an open-top chamber experiment. In terms of the other environmental conditions measured, the number of deposited pollen grains was not significantly affected by T_a between 09:00 and 12:00 on each flowering day under E-[CO₂] or A-[CO₂] (Figure 2), but it was significantly positively correlated with wind speed (Figure 3), with a wind speed of >1.5 m s⁻¹ resulting in more than 100 pollen grains being deposited. Similarly, Tian et al. (2010) previously observed significant losses in seed set due to sterility under a high relative humidity and a low wind speed of <1 m s⁻¹, although the maximum T_a was approximately 35°C. In our experiment, the correlation coefficient between daily mean T_a and wind speed during the flowering periods was 0.372 ($p < 0.001$), indicating that high temperatures usually accompany higher wind speeds. Therefore, it is likely that only mild sterility was observed at E-[CO₂] in our experiment because the negative effect of increased panicle temperature was offset by the stronger wind under increased T_a , highlighting the importance of considering wind speed when evaluating heat-induced sterility.

The effects of E-[CO₂] on anther dehiscence and length were not clear in our study. Although E-[CO₂] significantly increased the anther length (Table 4), it did not improve pollination (Table 3). Cool-weather-tolerant rice varieties have longer and wider anthers (Tanno et al., 1999), and Matsui and Omasa (2002) argued that longer anthers can hold more pollen grains, making it an effective trait for tolerating heat-induced sterility. Zhao et al. (2010) also reported that the percentage of sufficiently pollinated florets was correlated with the basal dehiscence length and the percentage seed set. However, we only used one cultivar ('Koshihikari') in our study, so variations in the anther length and dehiscence in response to changes in the environment may have been small. Since the anther length and dehiscence are highly heritable traits (Tanno et al., 1999; Zhao et al., 2016), genetic improvement would improve the tolerance of this cultivar to heat-induced sterility.

Fertility was less than 90% in 2011, likely due to the minimum T_a being below 17.5°C approximately 10 days before heading, when cool temperatures may cause slight sterility (Nishiyama, 1995), and E-[CO₂] further increased sterility in this year (Table 3). Okada et al., (2005) similarly reported that E-[CO₂] increased sterility under low temperatures indirectly by increasing the spikelet number. However, we did not observe any difference in the number of spikelets per panicle between A-[CO₂] and E-[CO₂] in 2011, and we found a little difference in the number of deposited pollen grains (Table 3). Therefore, a possible explanation for the mild sterility that was observed in this year is that the cool temperature affected pollen maturation during the booting stage, resulting in pollen sterility, unstable pollen germination and pollen tube elongation after pollination.

In our study, E-[CO₂] had only a small effect on sterility. In addition to the effect of wind speed mentioned above, this was likely due to only a slight increase in panicle temperature occurring because of decreased transpiration during the flower opening periods, when the sensitivity to heat-induced sterility is highest (Satake & Yoshida, 1978). During the vegetative stage, leaf stomatal conductance under E-[CO₂] in Shizukuishi FACE was low at 09:00 but became higher around noon (Shimono et al., 2010). The flower opening time in our study was around 10:00–11:00, so there may have been a little difference in T_a between E-[CO₂] and A-[CO₂] just before this time.

Although we only detected small changes in sterility and traits related to heat-induced sterility and pollination under E-[CO₂], this treatment had both negative and positive effects on heat-induced sterility, suggesting that the evaluation and prediction of E-[CO₂] effects require data obtained from open-field experiments as well as under controlled conditions. However, now that Tsukuba FACE project has finished, it will be difficult to conduct additional experiments under E-[CO₂] in the open field. Therefore, the development of a model to estimate heat-induced sterility under E-[CO₂] using various datasets, including the data obtained here for six growing seasons with variable growing season temperatures, and application of the micrometeorological model that has been developed for simulating panicle temperature (Yoshimoto et al., 2011) will be useful for predicting the effects of E-[CO₂] on the sterility and pollination of rice.

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References

- Chaturvedi, A. K., Bahuguna, R. N., Shah, D., Pal, M., & Jagadish, S. V. K. (2017). High temperature stress during flowering and grain filling offsets beneficial impact of elevated CO₂ on assimilate partitioning and sink-strength in rice. *Scientific Reports*, 7, 8227.
- Hasegawa, T., Sakai, H., Tokida, T., Usui, Y., Yoshimoto, M., Fukuoka, M., ... Okada, M. (2016). Rice free-air carbon dioxide enrichment studies to improve assessment of climate change effects on rice agriculture. In J. L. Hatfield & D. Fleisher (Eds.), *Improving modeling tools to assess climate change effects on crop response: Vol. 7. Advances in agricultural system model*: (pp. 45–68). Madison, WI: American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America.
- Hasegawa, T., Sakai, H., Tokida, T., Nakamura, H., Zhu, C., Usui, Y., & Makino, A. (2013). Rice cultivar responses to elevated CO₂ at two free-air CO₂ enrichment (FACE) sites in Japan. *Functional Plant Biology*, 40, 148–159.
- IPCC. (2013). 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, UK: Cambridge University Press.
- Ishimaru, T., Xaiyalath, S., Nallathambi, J., Sathishraj, R., Yoshimoto, M., Phoudalay, L., ... Jagadish, S. V. K. (2016). Quantifying rice spikelet sterility in potential heat-vulnerable regions: Field surveys in Laos and southern India. *Field Crops Research*, 190, 3–9.
- 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.
- Kim, H. Y., Horie, T., Nakagawa, H., & Wada, K. (1996). Effects of elevated CO₂ concentration and high temperature on growth and yield of rice. The effect on yield and its components of Ahihikari rice. *Japanese Journal of Crop Science*, 65, 644–651. In Japanese with English abstract.
- Kobayashi, K. (2001). FACE (free-air CO₂ enrichment) experiment. *Japanese Journal of Crop Science*, 70, 1–16. In Japanese.
- Kobayashi, K., Matsui, T., Murata, Y., & Yamamoto, M. (2011). Percentage of dehisced thecae and length of dehiscence control pollination stability of rice cultivars at high temperatures. *Plant Production Science*, 14, 89–95.
- Madan, P., Jagadish, S. V. K., Craufurd, P. Q., Fitzgerald, M., Lafarge, T., & Wheeler, T. R. (2012). Effect of elevated CO₂ and high temperature on seed-set and grain quality of rice. *Journal of Experimental Botany*, 63, 3843–3852.
- 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., Kobayashi, K., Kagata, H., & Horie, T. (2005). Correlation between viability of pollination and length of basal dehiscence of the theca in rice under a hot-and-humid condition. *Plant Production Science*, 8, 109–114.
- Matsui, T., Kobayashi, K., Nakagawa, H., Yoshimoto, M., Hasegawa, T., Reinke, R., & Angus, J. (2014). Lower-than-expected floret sterility of rice under extremely hot conditions in a flood-irrigated field in New South Wales, Australia. *Plant Production Science*, 17, 245–252.
- 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. (2002). Rice (*Oryza sativa* L.) cultivars tolerant to high temperature at flowering: Anther characteristics. *Annals of Botany*, 89, 683–687.
- Nakamura, H., Tokida, T., Yoshimoto, M., Sakai, H., Fukuoka, M., & Hasegawa, T. (2012). Performance of the enlarged Rice-FACE system using pure CO₂ installed in Tsukuba, Japan. *Journal of Agricultural Meteorology*, 68, 15–23.
- Nishiyama, I. (1995). Factors and mechanisms causing cool weather damage. In T. Matsuo, K. Kumazawa, R. Ishii, K. Ishihara, & H. Hirata (Eds.), *Science of the rice plant* (Vol. 2 Physiology, pp. 776–793). Tokyo: Food and Agriculture Policy Research Center.
- Okada, M., Shimono, H., Yamakawa, Y., Inoue, M., & Kobayashi, K. (2005). Effects of elevated CO₂ on floral sterility of rice plants caused by low temperature. *Journal of Agricultural Meteorology*, 60, 589–592.
- Oue, H., Yoshimoto, M., & Kobayashi, K. (2005). Effects of free-air CO₂ enrichment on leaf and panicle temperatures of rice at heading and flowering stage. *Phyton*, 45, 117–124.

- Satake, T., & Yoshida, S. (1978). High temperature-induced sterility in indica rices at flowering. *Japanese Journal of Crop Science*, 47, 6–17.
- Shah, F., Huang, J., Cui, K., Nie, L., Shah, T., Chen, C., & Wang, K. (2011). Impact of high-temperature stress on rice plant and its traits related to tolerance. *Journal of Agricultural Science*, 149, 545–556.
- Shimono, H., Inoue, M., Nakamura, H., Kobayashi, K., & Hasegawa, T. (2010). Diurnal and seasonal variations in stomatal conductance of rice at elevated atmospheric CO₂ under fully open-air conditions. *Plant Cell and Environment*, 33, 322–331.
- Tanno, H., Xiong, J. H., Dai, L. Y., & Ye, C. R. (1999). Some characteristics of cool weather-tolerant rice varieties in Yunnan Province, China. *Japanese Journal of Crop Science*, 68, 508–514.
- Tian, X. H., Matsui, T., Li, S. H., Yoshimoto, M., Kobayasi, K., & Hasegawa, T. (2010). Heat-induced floret sterility of hybrid rice (*Oryza sativa* L.) cultivars under humid and low wind conditions in the field of Jiangnan Basin, China. *Plant Production Science*, 13, 243–251.
- Weerakoon, W. M. W., Maruyama, A., & Ohba, K. (2008). Impact of humidity on temperature-induced grain sterility in rice (*Oryza sativa* L.). *Journal of Agronomy and Crop Science*, 194, 134–140.
- Yoshimoto, M., Fukuoka, M., Hasegawa, T., Utsumi, M., Ishigooka, Y., & Kuwagata, T. (2011). Integrated micrometeorology model for panicle and canopy temperature (IM²PACT) for rice heat stress studies under climate change. *Journal of Agricultural Meteorology*, 67, 233–247.
- 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.
- Zhao, L., Kobayasi, K., Hasegawa, T., Wang, C. L., Yoshimoto, M., Wan, J. M., & Matsui, T. (2010). Traits responsible for variation in pollination and seed set among six rice cultivars grown in a miniature paddy field with free air at a hot, humid spot in China. *Agriculture Ecosystems & Environment*, 139, 110–115.
- Zhao, L., Zhu, Z., Tian, X. H., Kobayasi, K., Hasegawa, T., Zhang, Y. D., ... Matsui, T. (2016). Inheritance analysis of anther dehiscence as a trait for the heat tolerance at flowering in japonica hybrid rice (*Oryza sativa* L.). *Euphytica*, 211, 311–320.