Effects of Elevated CO₂ on Floral Sterility of Rice Plants Caused by Low Temperature

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Abstract

To evaluate the effects of elevated CO₂ concentration on low-temperature induced sterility, we conducted chamber and FACE (Free-Air CO₂ Enrichment) experiments under two levels of CO₂ concentration; ambient and elevated (ambient + 200ppmV). In the chamber experiment for three rice seasons from 2001 through 2003, rice (*Oryza sativa* L., cv. Sasanishiki) plants were submerged up to 25 cm above the soil surface in a water pool. The young panicles near the soil surface were thus subjected to a critical temperature of 19.5 °C throughout the susceptible period from panicle initiation to anthesis. The effects of elevated CO₂ on floral sterility were inconsistent among the three years, but the relationship between the floral sterility and the total spikelet number was consistent. As the spikelet number increased, the sterility increased, whether the change was caused by CO₂ or nitrogen. Similar results were obtained in the FACE experiment in 2003, when summer temperatures were extremely low. The earliest cultivar Kirara397 increased in spikelet number under elevated CO₂ but decreased in yield due to floral sterility. These results suggest that elevated CO₂ concentration may increase floral sterility under low temperature indirectly via the increase in spikelet number.

Key words: Cold summer damage, Elevated CO₂, Floral sterility, Low temperature, Rice

1. Introduction

Elevated atmospheric CO₂ concentration, in general, increases biomass accumulation and its resultant yield of crops with enhanced photosynthesis as reviewed by Kimball *et al.* (2002). There are, however, significant interactions between CO₂ and other environmental factors such as temperature, soil moisture and fertility, and hence the CO₂ effects vary largely. Kim *et al.* (1996) reported no effect of CO₂ enrichment on rice yield under high temperatures, where the yield loss due to higher floral sterility cancelled out the increase in biomass production under elevated CO₂. Floral sterility of rice plants is also caused by low temperature (*e.g.* below 20 °C) during the booting stage, especially at the time of microspore formation with the pollen development being hindered (Satake and Hayase, 1970). This form of sterility is of the greatest concern in rice production in cool-temperate regions worldwide.

Floral sterility due to low-temperature is altered by the nutrient conditions, especially nitrogen, of the rice plant. High nitrogen supply increases sterility, (Otani *et al.*, 1948) with a very high positive correlation between the sterility and the nitrogen concentration in leaf blades (Satake *et al.*, 1987). Under elevated CO₂, increased carbohydrate accumulation dilutes the nitrogen concentration of plants (see Kimball *et al.*, 2002) leading to lower nitrogen concentration of the rice leaves (Kim *et al.*,

2003). With the lower nitrogen concentration in the leaves, the elevated CO₂ can mitigate cold summer damage due to floral sterility. The purpose of the present study is to test if the floral sterility due to low temperature is ameliorated by CO₂ enrichment in the field.

2. Materials and Methods

2.1 Cold water treatment in the chamber experiment

We used temperature-gradient chambers for the CO₂ enrichment. One chamber had an ambient CO₂ concentration, and the other was controlled at 200 ppmV above the ambient level (Okada *et al.*, 2000).

Cold water treatment during a rice-booting stage has been widely used to select rice varieties for tolerance to low-temperature induced floral sterility or to evaluate the effectiveness of agronomical countermeasures. In this study, we submerged the young panicles at the susceptible stage in deep flooded water, the temperature of which was controlled at a certain critical level by supplying cold underground water.

The water pool designed for the treatment was 1 m x 1 m and 60 cm deep. The bottom and side panels were insulated by 10-cm thick foam polystyrene (Fig. 1). When the water temperature exceeded the target, cold water at a temperature of approximately 16 °C was introduced to the pool through an

electromagnetic valve. A carefully calibrated Pt100 thermometer was used to measure the water temperature. A datalogger was employed for measuring the thermometer signals and operating the valve at an interval of two seconds and for recording the average of water temperature every 30-min. Water was circulated by a pump to minimize the spatial distribution of water temperature. The system was able to control the temperature at the target level: 19.5 °C, with an accuracy of ± 0.1 °C both temporarily and spatially

Fig. 1. Experimental layout of cold water treatment.

A rice (*Oryza sativa* L.) cultivar, Sasanishiki, was used in the experiments. In the preliminary experiment in 2001, soil was packed into the pool up to 40 cm above the bottom, leaving 20 cm to submerge plants in cold water. Sixteen seedlings were transplanted in each pool and grown at a shallow water level before cold water treatment. Two pools were used for repetition. In 2002 and 2003 two plants were transplanted in a 15-litre pot and grown outside the pool before cold water treatment. Four pools were prepared for repetition. Nutrients were supplied as listed in Table 1.

Table 1. Levels of nutrient supply.

(g plant ⁻¹)				
year	N treatment	N	P ₂ O ₅	K ₂ O
2001	low N	0.63	1.56	0.83
2002	low N	0.6	1.2	0.8
	middle N	0.9		
	high N	1.2		
2003	low N	0.6	1.2	0.8

At the time of panicle initiation, the water level was raised to 20 cm in 2001 and 25 cm in 2002 and 2003. The water temperature control started at the same time. The supply of cold water to regulate the water temperature was terminated at anthesis. To

avoid floral sterility caused by unexpected low air temperature, the water pools were placed at the warmer end of two temperature gradient chambers (Okada et al., 2000).

In 2003, twelve pots were prepared for each CO₂ level and divided into three groups; each group consisting of four pots in correspondence to the 4-pool replicates. Two of the three groups were moved from the ambient (A) CO₂ chamber to the elevated (E) chamber and vice versa at the beginning and the middle of the cold water treatment (see Table 2). Thus, each of the three groups was exposed to different CO₂ concentrations during the three periods. This test was designed to separate the CO₂ effects in the growth period prior to the cold water treatment (pre-treatment effect) and those during the treatment.

Table 2. CO₂ exposure calendar of the groups in the experiment in 2003 where the location of the pots was changed between the elevated and the ambient chambers.

group	prior to panic initiation (by Jun. 25)	before mid-booting (Jul. 25 to Aug. 8)	after mid-booting (from Aug. 8)
AAA	ambient		
AAE	ambient		elevated
AEE	ambient	elevated	
EEE	elevated		
EEA	elevated		ambient
EAA	elevated	ambient	

2.2 FACE experiment in 2003

Four rice cultivars (Kirara397, Kakehashi, Akitakomachi and Hitomebore), each having different growth periods, were grown in the FACE (Free-Air CO₂ Enrichment) field at Shizukuishi, Iwate, Japan to compare the CO₂ effects on growth responses. Details of the FACE facility are described by Okada et al. (2001). Briefly, it consists of four FACE rings and four companion ambient rings; each having a nominal usable area of ca 80 m². The CO₂ concentration at the center of the FACE ring is controlled at a level of 200ppmV above the ambient.

Rice seedlings were transplanted with a density of 19 hill m⁻² in the experimental field on May 21, 2003. In total, 8 g m⁻² of nitrogen was supplied as a basal dressing with 6 g m⁻² as 70-day type release controlled urea and 2 g m⁻² as ammonium sulfate. Application rates of phosphorous and potassium were 30 g P₂O₅ m⁻² and 15 g K₂O m⁻².

We had an extremely cool summer in northern Japan in 2003. According to the AMEDAS automated meteorological observation near the FACE site, the monthly mean temperature was 18.7 °C in July and 21.3 °C in August in 2003, while that for a normal year is 21.0 °C in July and 22.4 °C in August.

Fig. 2. Effect of CO₂ enrichment on the sterility percentage.

The treatments; AAA and EEE in Table 2 are shown for 2003. Labels on the horizontal axis denote the N supply level as defined in Table 1. Asterisks denote statistically significant difference between the CO₂ levels;

* $p < 0.05$, ** $p < 0.1$.

Fig. 3. Effect of CO₂ enrichment on the number of spikelets per plant.

The treatments; AAA and EEE in Table 2 are shown for 2003. Labels on the horizontal axis denote the N supply level as defined in Table 1. Asterisks denote statistically significant difference between the CO₂ levels;

* $p < 0.05$.

3. Results and Discussion

3.1. Chamber experiments

Figure 2 compares the sterility percentage observed in the experiments over three years. The elevated CO₂ increased the spikelet sterility significantly in 2001, and that in the low N plot in 2002. However, no difference was observed in the sterility between the CO₂ treatments in the middle and high N plots in 2002. In 2003, when the weather was unusually cool in summer, the ambient CO₂ increased the spikelet sterility. The effects of elevated CO₂ on spikelet sterility were thus inconsistent among the three years.

Figure 3 compares the number of spikelets per plant. The spikelet number increased as the nitrogen supply increased in 2002. Comparing the three years

for similar nitrogen levels, the spikelet number was much larger in 2001 than the other two years. This may be attributed to a larger soil mass in 2001 than that in the pot experiments in the other two years. The elevated CO₂ increased the spikelet number significantly in the low N plot in 2001 and 2002, but decreased in 2003. Similarly to spikelet sterility, the effects of elevated CO₂ on the spikelet number were not consistent over the three years. The spikelet number was increased to a large extent by cold water treatment when compared to that of the untreated plants placed outside the water. This may be because the low temperature decelerated the reduction of spikelets during the young panicle development stage. Although the mechanism causing this increase is not clearly known, it affected presumably those inconsistent results.

Figures 2 and 3 show correspondence between the spikelet sterility and the spikelet number in response to CO₂, water temperature, and N. When every pair of treatments is compared, the sterility increased only when the total spikelet number significantly increased. Namely, as the total spikelet number increased, the sterility increased whether the change was caused by CO₂, nitrogen or cold water treatment. This suggests that spikelet number is one of the determinants of the low-temperature induced floral sterility of rice, though the year-to-year fluctuations, or the fluctuations among the treatments, are not entirely explained by the spikelet number alone.

Fig. 4. Comparison of the pre-treatment effects of CO₂ and those during the cold water treatment on the sterility percentage.

Labels on the horizontal axis are as defined in Table 2.

The experiment in 2003 was designed to separate the pre-treatment effects of CO₂ from those during the cold water treatment on floral sterility. No significant difference was observed between the CO₂ levels in the pot exchange treatments during the cold water treatment both on the spikelet sterility (Fig. 4) and the spikelet number (data not shown), but the differences were significant between the CO₂ concentrations prior to the cold water treatment. Hence, the influence of the CO₂ enrichment on floral

sterility is exerted via its effect on plant growth prior to the low-temperature damage rather than the effect on plant sensitivity to low-temperature during the susceptible stage from panicle initiation to anthesis.

3.2. FACE experiment

The cold spell in the summer of 2003 increased the spikelet sterility more in the earlier varieties than the later ones (Fig. 5).

The earliest variety Kirara 397 was exposed to a low temperature below the critical level for the longest time during the booting stage and thus the sterility was the largest. Increases in sterility through CO₂ enrichment were observed in all cultivars except the latest cultivar, Hitomebore, whose sterility was close to the level usually observed in warmer years. The increase of the sterility under the elevated CO₂ was associated with the increase of the spikelet number (Figs. 5 and 6). This association was most obvious in Kirara 397.

Fig. 5. Comparison of the sterility percentage of the four cultivars in the FACE experiment.

The labels denote the cultivars, KR; Kirara 397, KK; Kakehashi, AK; Akitakomachi and HB; Hitomebore. The dates of heading for the ambient plot were, KR; Jul. 27, KK; Aug. 2, AK; Aug. 12 and HB; Aug. 20.

Fig. 6. Comparison of the spikelet number of the four cultivars in the FACE experiment.

The leaf N concentrations at heading were consistently lower under the elevated than the ambient CO₂, both in the chamber and in the FACE experiments. Except for the results of the chamber experiment in 2003, where the CO₂ enrichment decreased the sterility, the lower N concentration of

the CO₂ enriched plants did not mitigate the spikelet damage due to low temperature. Comparing each pair of treatment, the changes of the sterility were always associated with those of the spikelet number.

The results obtained in this study suggest that elevated CO₂ concentration may increase floral sterility under low temperature indirectly via the increase in spikelet number. Since the increases of the sterility and the spikelet number are comparable, no yield increase is expected when the sterility is high, even though dry matter production is enhanced under the elevated CO₂.

References

- Kim, H.Y., Horie, T., Nakagawa, H., and 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. *Jpn. J. Crop Sci.*, **65**, 644-651.
- Kim, H.Y., Liefferring, M., Kobayashi, K., Okada, M., and Miura, S., 2003: Seasonal changes in the effects of elevated CO₂ on rice at three levels of nitrogen supply: a free air CO₂ enrichment (FACE) experiment. *Global Change Biology*, **9**, 826-837.
- Kimball, B.A., Kobayashi, K., and Bindi, M., 2002: Responses of agricultural crops to free-air CO₂ enrichment. *Advances in Agronomy*, **77**, 293-368.
- Okada, M., Hamasaki, T., and Sameshima, R., 2000: Pre-air-conditioned temperature gradient chambers for research on temperature stress in plants. *Biotronics*, **29**, 43-55.
- Okada, M., Liefferring M., Nakamura, H., Yoshimoto, M., Kim, H.Y., and Kobayashi, K., 2001: Free air CO₂ enrichment (FACE) using pure CO₂ injection: system description. *New Phytologist*, **150**, 251-260.
- Otani, Y., Doi, Y., and Izumi, S., 1948: Physiological studies on the injuries of cool weather in rice plant (preliminary report). XI. Relation between ammonium sulphate, and heading and injuries of low temperature at the different stages after transplanting. *Proc. Crop Sci. Soc. Japan*, **16**, 3-5.
- Satake, T., and Hayase, H., 1970: Male sterility caused by cooling treatment at the young microspore stage in rice plants. V. Estimation of pollen developmental stage and the most sensitive stage to coolness. *Proc. Crop Sci. Soc. Japan*, **39**, 468-473.
- Satake, T., Lee, S.Y., Koike, S., and Kariya, K., 1987: Male sterility caused by cooling treatment at the young microspore stage in rice plants. XXVII. Effect of water temperature and nitrogen application before the critical stage on the sterility induced by cooling at the critical stage. *Jpn. J. Crop Sci.*, **56**, 404-410.