

Plant Architecture and Flag Leaf Morphology of Rice Crops Exposed to Experimental Warming with Elevated CO₂

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ABSTRACT Projected increases in atmospheric CO₂ concentration ([CO₂]) and temperature (T_a) have the potential to alter in rice growth and yield. However, little is known about whether T_a warming with elevated [CO₂] modify plant architecture. To better understand the vertical profiles of leaf area index (LAI) and the flag leaf morphology of rice grown under elevated T_a and [CO₂], we conducted a temperature gradient field chamber (TGC) experiment at Gwangju, Korea. Rice (*Oryza sativa* L. cv. Dongjin1ho) was grown at two [CO₂] [386 (ambient) vs 592 ppmV (elevated)] and three T_a regimes [26.8 (≈ ambient), 28.1 and 29.8°C] in six independent field TGCs. While elevated T_a did not alter total LAI, elevated [CO₂] tended to reduce (c. 6.6%) the LAI. At a given canopy layer, the LAI was affected neither by elevated [CO₂] nor by elevated T_a, allocating the largest LAI in the middle part of the canopy. However, the fraction of LAI distributed in a higher and in a lower layer was strongly affected by elevated T_a; on average, the LAI distributed in the 75-90 cm (and 45-60 cm) layer of total LAI was 9.4% (and 35.0%), 18.8% (25.9%) and 18.6% (29.2%) in ambient T_a, 1.3°C and 3.0°C above ambient T_a, respectively. Most of the parameters related to flag leaf morphology was negated with elevated [CO₂]; there were about 12%, 5%, 7.5%, 15% and 21% decreases in length (L), width (W), L:W ratio, area and mass of the flag leaf, respectively, at elevated [CO₂]. However, the negative effect of elevated [CO₂] was offset to some extent by T_a warming. All modifications observed were directly or indirectly associated with either stimulated leaf expansion or crop phenology under T_a warming with elevated [CO₂]. We conclude that plant architecture and flag leaf morphology of rice can be modified both by T_a warming and elevated [CO₂] via altering crop phenology and the extent of leaf expansion.

Keywords : plant architecture, leaf morphology, elevated CO₂, global warming, rice

There are obvious concerns about the impact of a projected increase in atmospheric CO₂ concentration ([CO₂]) and temperature (T_a) on agricultural field crop species. Many studies highlighted that elevated [CO₂] (i.e. 200-300 ppmV above current ambient [CO₂]) increases yield of major C₃ cereal crops such as rice, wheat and so on (see reviews by Ainsworth and Long, 2005; Ziska and Bunce, 2007). However, far less attention has been paid to whether a projected increase in [CO₂] and T_a will modify plant architecture and morphology, which play critical role in plant photosynthesis and hence in crop yield. In a recent review, it is becoming more evident that an ideal plant type of rice is necessary to break current stagnation of the yield (Peng *et al.*, 2008). This concept will also be just the case for the future high [CO₂] world to take full advantage of the high [CO₂] into the yield increase, and to minimize a disadvantage of T_a warming. In this context, understanding of the plant architecture and morphology of rice crops grown under elevated [CO₂] and T_a is the first step to develop an effective plant type of rice which is highly adaptable to projected climate change.

Plant architecture of rice is defined as three-dimensional distribution of plant component parts, such as plant height, tiller and leaf area. Since these all traits determine the potential yield of rice, plant architecture has been recognized as a major agronomic importance (Khush, 2001; Peng *et al.*, 2008; Ariyaratna *et al.*, 2011). The architecture of rice

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plants can be affected by several environmental factors (e.g. T_a , $[CO_2]$), as well as by genetic backgrounds. For example, elevated $[CO_2]$ had little affect plant height of inbred rice (cv. Akihikari) (Kim *et al.*, 1996), but strong positive affect that of hybrid rice (cv. Liangyoupeijiu) (Yang *et al.*, 2009). However, T_a warming universally appears to have a stimulatory effect on plant height of rice plants (Oh-e *et al.*, 2007). Early studies reported that although elevated $[CO_2]$ increases tiller number of rice crops, this stimulatory effect is only significant up to heading but a relatively minor afterward (Kim *et al.*, 2003). A similar response fashion to elevated $[CO_2]$ was also reported for leaf area of rice plants (Kim *et al.*, 2003; Sakai *et al.*, 2006). Such changes in plant height, tiller and leaf area under elevated $[CO_2]$ and/or T_a will have the potential to modify in plant architecture at whole-plant or canopy level. However, there are no reports addressing the vertical profiles of leaf area of rice crops exposed to elevated $[CO_2]$ and T_a . In addition to plant height and tiller, the vertical profiles of leaf area are key factors for integrating and understanding the plant architecture at whole-plant level. Hence, it remains to be determined whether a projected warming with elevated $[CO_2]$ alters vertical profiles of leaf area of rice crops in order to better understand plant architecture at whole-plant level.

Unlike wheat and barley, which have a greater capability of ear photosynthesis (Evan and Rawson, 1969; Zhu *et al.*, 2008), in rice the flag leaves play a crucial role in plant photosynthesis during grain filling period, thus determining grain yield. For example, rice flag leaves at heading stage contribute about 19% of total leaf area (Yoshida *et al.*, 1972), and contribute about 45% of total grain yield (Abou-khalifa *et al.*, 2008). To date, however, it has rarely been reported for the morphology of the flag leaf in rice crops grown under expected warming with elevated $[CO_2]$. To better understand the changes that occur in plant architecture under changes in $[CO_2]$ and climate, it is needed to consider not only the vertical profiles of leaf area, but also the flag leaf morphology.

In the present study, we examined whether elevated T_a in combination with elevated $[CO_2]$ would affect plant architecture of rice crops by employing temperature gradient field chambers (TGCs). The objectives of this study were;

(i) to determine the vertical profiles of leaf area of rice crops under elevated T_a and $[CO_2]$; (ii) to identify the changes in the effect of elevated $[CO_2]$ and T_a on flag leaf morphology of rice crops.

MATERIALS AND METHODS

Experimental site and system descriptions

The experimental site was located in the experimental paddy fields of Chonnam National University, Gwangju, Korea (126°53' E, 35°10'N, alt. 33 m). The area is typical of the agro-climate that grows a large proportion of Korean rice crops, occupying > 20% of total area harvested (approximately one million ha). This site was subject to temperate monsoon climate with an annual mean temperature of 13.7°C over the past 30 years. Annual mean precipitation was about 1520 mm, with > 60% rainfall occurring from June to August (rainy season). During the rice growing season (May to September), the mean T_a was 22.7°C, with a monthly mean minimum and maximum T_a of 12.8°C (May) and 30.5°C (August), respectively.

The experiment was performed using TGCs established in paddy fields, covered by a highly transparent film, with or without CO_2 fumigation. TGCs have been described in detail by Kim *et al.* 2004 (also see Horie *et al.*, 1995; Okada *et al.*, 2000). Briefly, the TGCs consisted of six independent field chambers that had a tunnel shape of 24.0 m in length, 2.4 m in width and 2.0 m in height. Of these, three TGCs were allocated for ambient $[CO_2]$ and the other three were allocated for elevated $[CO_2]$. The target $[CO_2]$ in the TGCs assigned to the elevated $[CO_2]$ was 600 ppmV and controlled by fumigating CO_2 mixed with air in a mixing cylinder. One side of the TGC in the longitudinal direction was fully open (air inlet); while the other side (air outlet) was closed, where three exhaust fans [two small (17.7 m³ min⁻¹) and one large (125.0 m³ min⁻¹)] were mounted. Air from the inlet traveled towards the outlet by the exhaust fans and was gradually warmed by either incident solar radiation (clear day) or warm air provided by an electronic heating blower at nighttime (including cloudy and rainy day), creating a linear T_a gradient along the longitudinal axis of the TGCs. The T_a difference between the air inlet (~ambient) and outlet of the TGC was set at

3°C with natural diurnal fluctuations. To minimize the enclosure effects expected, we used highly transparent films (F-clean[®], Asahi Glass, Japan) with a transmittance > 90% in a wide range of radiation from short- to long-wave. In addition, an oscillating fan was placed on one side wall inside the TGCs with 4.0 m distance intervals in order to disperse CO₂ quickly throughout the TGCs and to imitate wind conditions similar to an open field.

Environmental variables and data collection

To maintain a target [CO₂] of 600 ppmV for elevated [CO₂], CO₂ was mixed with air in a mixing cylinder equipped at the air inlet of the TGCs fumigated into the TGCs for 24 h. The fumigating rate of CO₂ was computer-controlled with the solenoid valve. The [CO₂] was monitored (GMT 222, Vaisala, Switzerland) every 5 s at 1.0 m, 11.0 m and 22.0 m distances from the air inlet of the TGCs, and stored in the data logger (CR100, Campbell Scientific, USA) as an average value of 5 min. For ambient [CO₂], the [CO₂] was also monitored as was the case for the elevated [CO₂], though neither was fumigated nor controlled. On average (\pm s.d.) over the season, the [CO₂] in elevated and ambient [CO₂] resulted in 592 \pm 24 ppmV and 386 \pm 15 ppmV, respectively.

For T_a control and monitoring, dry-bulb thermo couples were housed in a ventilated radiation shield placed at 20 cm above plant canopy at distances of 0.6 m, 8.0 m, 15.5 m and 23.0 m from the air inlet of the TGCs. As with [CO₂], T_a was continuously measured every 5 s and stored in the data logger as an average value of 5 min. Based on these data, the T_a at arbitrary distances from the air inlet of the TGCs was calculated by linear interpolation. In the current study, experimental plots were designed at 0.6-1.8 m (1.2 m in the plot center) from the air inlet of the TGCs, 11.4-12.6 m (12.0 m) and at 21.0-22.2 m (21.6 m). The mean T_a from transplanting (4 June) to the full heading stage (18 August) resulted in 26.8°C (~ambient T_a) at 1.2 m, 28.1°C (1.3°C above ambient) at 12.0 m and 29.8°C (3°C above ambient) at 21.6 m. Over the season, PAR both inside and outside the TGC was also measured by a quantum sensor (LI-COR, LI-190, Lincoln, NB, USA) every 5 s and stored in the data logger as an average value of

5 min. The PAR was transmitted through TGC by 91%, and the daily PAR inside the TGC over the season.

Crop culture and measurements

On 4 June 2005, 3 seedlings per hill of 30-day-old rice (*Oryza sativa* L., cv. Dongjin1ho) were hand-transplanted in rice soil under either ambient [CO₂]-TGCs or elevated [CO₂]-TGCs. Row and hill space were 30 cm and 15 cm, respectively (22.2 hills m⁻²). In paddy surroundings outside of the TGCs, the seedlings were also transplanted with the same planting density to create canopy conditions. Application rates of nitrogen (N, as Urea)-P₂O₅ (as fused phosphate)-K₂O (as KCl) fertilizers were 11.0-4.5-5.7 g m⁻². N was applied as basal dressing 2 days prior to transplanting (60% of the total), at mid-tillering (20%), and at panicle initiation (20%). Paddies were flooded through most of the growing season, but treated to mid-drainage for 7 days before panicle initiation in mid-July. All other agronomic practices including weeding and pest management were similar to those adopted by local farmers.

At full heading stage (18 August) when plant height fully developed, 5 hills were destructively sampled from each combination treatment plot of [CO₂] and T_a (2 [CO₂] × 3 T_a factorial plots). At plant sampling, a block of soil 30 cm wide, 15 cm long and 15 cm deep around each individual hill for 2 hills was extracted, whereas only above ground parts were sampled for the rest of the hills. For the former 2 hills, to determine vertical profiles of leaf area, all leaves were cut at intervals of 15 cm along the stem from ground level to the top, and the leaf area of each layer (i.e. with a 15 cm layer) was measured separately using leaf area meter (AMM-9, Hayashi Denko Co., Ltd. Japan). The remaining above ground parts of the plant were also cut by the same way, and the dry mas of each layer was determined separately after drying at 80°C in a forced air oven for > 72 hrs. Finally, soil and other extraneous matter were carefully cleansed from the root system under running water and root biomass was determined. For the later 3 hills, all flag leaves were collected, and the individual length, width, area and mass were determined. For all hills (i.e. 5 hills) the plant height and tiller number were also measured.

Experimental design and statistical analysis

In the basic design, which was a split-plot with the whole plot arranged in randomized complete blocks, two levels of [CO₂] (ambient or elevated) were the whole plot treatment and three levels of T_a (~ambient T_a, 1.3°C and 3.0°C above ambient) were the split-plot treatment. There were three blocks, each consisting of an elevated [CO₂]- and an ambient [CO₂]-TGC located in experimental paddy fields. To test a significant difference among treatments, analysis of variance (ANOVA) in a split-plot design was performed with mean values from each treatment with triplications. Statistical analyses were conducted with a general linear model procedure using a SPSS software package (version 19.0, SPSS Inc., Chicago, IL).

RESULTS

General features

Elevated [CO₂] and T_a, each alone, and their interactive effects were not significant for plant height at full heading stage when it developed fully (Table 1). Elevated [CO₂] had a minor and insignificant effect on tiller number (P=0.156), whereas elevated T_a had a significant positive effect (P=0.009). Interactive effect between [CO₂] and T_a on tiller number was a marginally significant (P=0.103). As a result, the stimulatory effect of elevated T_a was greater in elevated [CO₂] than in ambient [CO₂]; under 3°C

warming tiller number increased by 23% in elevated [CO₂] and by only 9% in ambient [CO₂] (Table 1). Elevated [CO₂] did not alter root to shoot ratio of rice crops at full heading stage, while elevated T_a reduced it (p=0.076); this negative effect of T_a was amplified with increasing T_a from ambient T_a to 3.0°C above ambient T_a in both [CO₂] treatments. However, no [CO₂] × T_a interaction was observed for the root to shoot ratio.

Vertical profiles of leaf area index (LAI)

Overall, while elevated T_a did not alter total LAI at full heading stage, elevated [CO₂] tended to reduce the LAI (P=0.091). On average, total LAI was reduced by 6.6% in elevated [CO₂] (Fig. 1a). At a given canopy layer (i.e. every 15 cm layer from ground level to the top), the LAI was affected neither by elevated [CO₂] (P=0.211-0.645) nor by elevated T_a (P=0.116-0.681), allocating the largest LAI in the middle part (45-75 cm) of the canopy (Fig 1a; Table 2). However, the fraction of LAI distributed in a higher (75-90 cm) and in a lower layer (45-60 cm) was strongly affected by elevated T_a, but not by elevated [CO₂] (Fig. 1a; Table 2). On average, the LAI distributed in the 75-90 cm (and 45-60 cm) layer of total LAI was 9.4% (and 35.0%), 18.8% (25.9%) and 18.6% (29.2%) in ambient T_a, 1.3°C and 3.0°C above ambient T_a, respectively. Cumulative LAI to a given canopy layer from the top was also unaffected both by elevated [CO₂] and T_a (Fig 1b;

Table 1. Plant height, tiller number and root to shoot ratio (mean ± standard error of three replicates) at full heading stages of rice crops exposed to either ambient or elevated CO₂ with three regimes of air temperature (T_a). Analysis of variance (ANOVA) results (P-value only) are shown.

CO ₂	T _a	Plant height (cm)	Tillers (no. m ⁻²)	Root/shoot ratio
Ambient	Ambient*	88.4 ± 2.0	266.6 ± 8.5	0.097 ± 0.016
	+1.3°C	90.3 ± 8.5	253.7 ± 15.2	0.103 ± 0.010
	+3.0°C	96.4 ± 3.2	290.7 ± 17.7	0.075 ± 0.001
Elevated	Ambient	94.2 ± 2.3	246.3 ± 13.0	0.123 ± 0.026
	+1.3°C	100.1 ± 0.4	287.0 ± 15.2	0.098 ± 0.010
	+3.0°C	98.3 ± 2.2	327.7 ± 9.6	0.081 ± 0.002
ANOVA results				
CO ₂		0.366	0.156	0.586
T _a		0.223	0.009	0.076
CO ₂ × T _a		0.505	0.103	0.467

* indicates mean ambient T_a and was a 26.8°C.

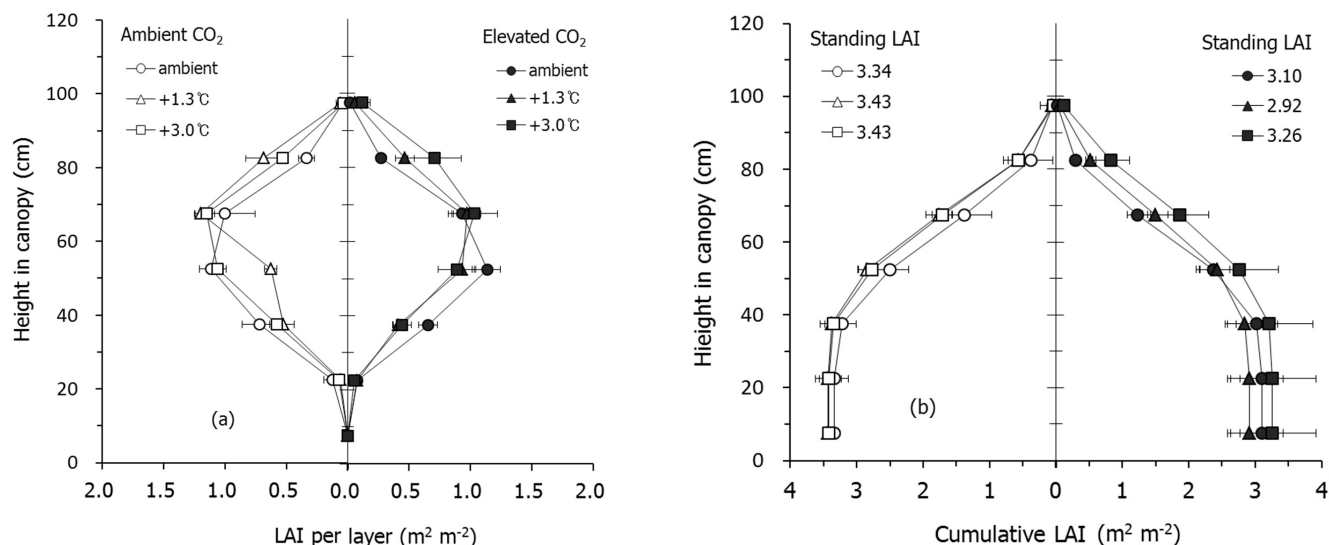


Fig. 1. (a) Vertical profiles of leaf area (expressed by leaf area index, LAI) and (b) cumulative LAI at a given canopy layer within canopy at full heading stage of rice (cv. Dongjin1ho) grown at either elevated or ambient [CO₂] with three regimes of air temperature [ambient T_a (26.8°C), 1.3°C and 3.0°C above ambient]. Canopy height was from the ground level. Data are represented as means (\pm standard error bars) of three replicates. See table 2 for the statistical significance.

Table 2. The results (P-values) of analysis of variance in split-plot design on leaf area (expressed by leaf area index, LAI) in terms of vertical arrangement (LAI), distribution (%) and cumulative LAI at a given canopy layer at full heading stage of rice grown under either elevated or ambient [CO₂] (main-plot) with three regimes of air temperature (T_a, sub-plot).

Effects	parameters	Height in canopy (cm)						
		> 90	75-90 (> 75) ^a	60-75 (> 60)	45-60 (> 45)	30-45 (> 30)	15-30 (> 15)	0-15 (> 0)
LAI								
CO ₂		0.464	0.645	0.386	0.390	0.210	0.544	-
T _a		0.448	0.122	0.681	0.361	0.116	0.460	-
CO ₂ ×T _a		0.262	0.625	0.881	0.593	0.937	0.639	-
LAI distribution (%)								
CO ₂		0.474	0.443	0.839	0.914	0.559	0.640	-
T _a		0.324	0.015	0.289	0.001	0.122	0.436	-
CO ₂ ×T _a		0.213	0.389	0.887	0.026	0.939	0.628	-
Cumulative LAI								
CO ₂		0.520	0.580	0.386	0.334	0.146	0.088	-
T _a		0.473	0.155	0.333	0.717	0.899	0.919	-
CO ₂ ×T _a		0.288	0.545	0.788	0.877	0.882	0.909	-

^a is only applicable for the cumulative LAI.

Table 2).

Flag leaf morphology

The length of flag leaf (LFL) decreased by 12.1% (P=0.037) in elevated [CO₂] and significantly increased by

21.0% (p=0.026) in elevated T_a (+3.0°C) (Fig 2a). The negative effect of elevated [CO₂] on LFL was reduced with increasing T_a, though no [CO₂] × T_a interaction was found. Under elevated [CO₂], the width of flag leaf (WFL) also decreased by 5.0%, whereas it slightly but significantly

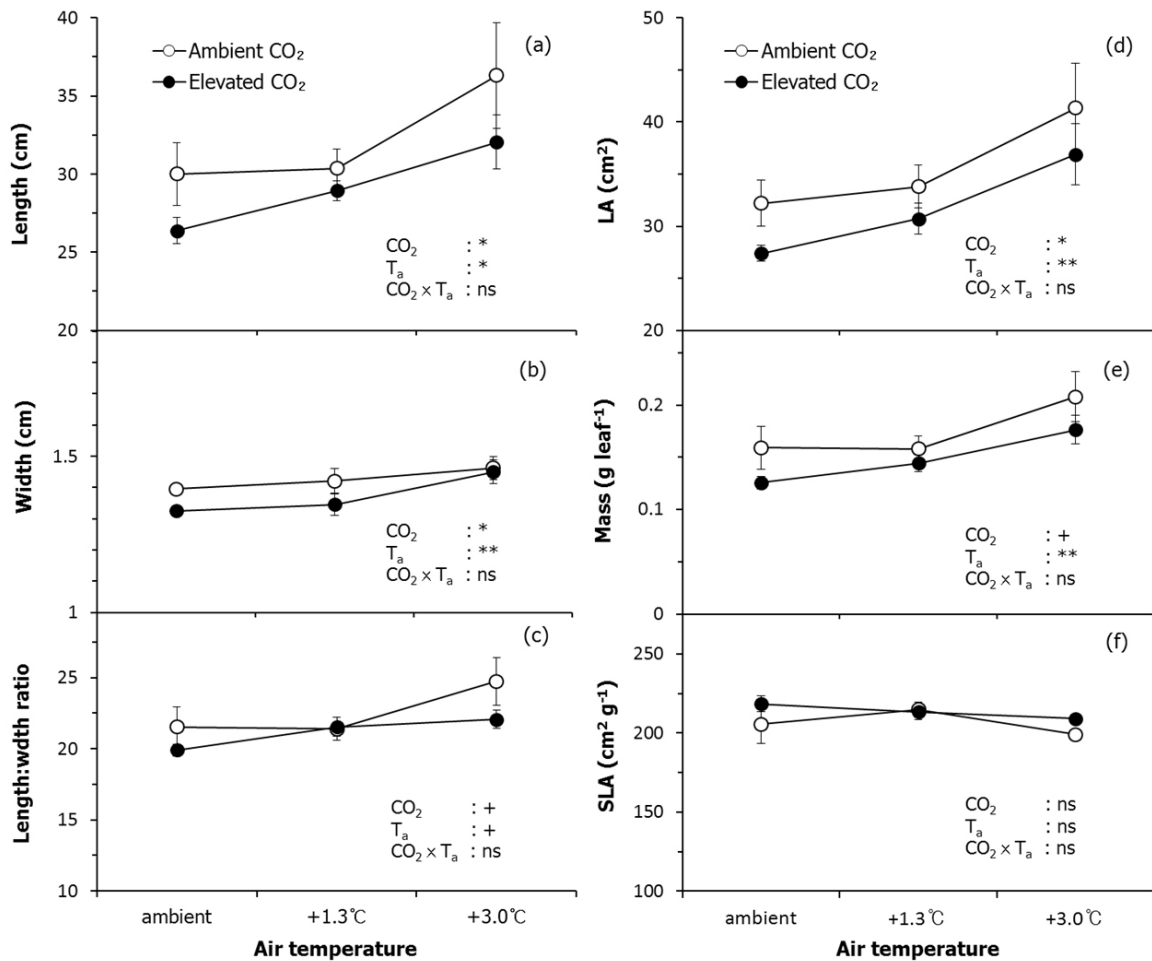


Fig. 2. Morphological traits [(a) length, (b) width, (c) length : width ratio, (d) leaf area, (e) leaf mass and (f) specific leaf area, SLA] of flag leaf of the rice plants grown at either elevated or ambient [CO₂] with three regimes of air temperature (T_a) [ambient T_a (26.8°C), 1.3°C and 3.0°C above ambient]. Data are represented as means (± standard error bars) of three replicates. **, *, + and ns indicate P<0.01, P<0.05, P<0.1 and no significance, respectively.

increased (1.9-4.8%) under elevated T_a (Fig 2b). Overall, the effect of elevated [CO₂] and T_a on WFL was smaller than that on LFL. As a result, the response of length to width (L : W) ratio in flag leaf to elevated [CO₂] and T_a was also smaller compared to that of LFL, and only marginally significant (P=0.083-0.101) (Fig. 2c); the L : W ratio resulted in no change (in elevated [CO₂] and combined with 1.3 -3.0°C warming), decrease 7.5% (in elevated [CO₂] alone) and increase 15.0% (in 3.0°C warming). The response of leaf area (FLA) of single flag leaf to elevated [CO₂] and T_a showed a similar fashion to that of LFL (Fig. 2d), exhibiting a greatest increase by 28.4% in 3.0°C warming (P=0.006) and decrease by 14.9% in elevated [CO₂] alone (P=0.017). Reflecting this, elevated [CO₂] resulted in a

significant decrease in flag leaf mass (FLM) by 20.9% (P=0.098), whereas elevated T_a (3.0°C warming) led to a significant increase by 30.7% (P=0.011) (Fig. 2e). However, specific leaf area (SLA; leaf area per unit leaf dry mass) was not altered both at elevated [CO₂] and T_a (Fig 2f). Specific leaf mass (SLM; leaf dry mass per unit leaf area, and a reciprocal of the SLA) was also the case that unchanged with elevated [CO₂] and T_a (data not shown).

DISCUSSION

This study was simply designed to evaluate the consequences of global warming with elevated [CO₂] on key plant morphological parameters of rice crops. Overall, plant

height, tiller number and root to shoot ratio tended to be less affected by elevating $[\text{CO}_2]$ rather than by elevating T_a (Table 1). Indeed, earlier studies also showed that elevated $[\text{CO}_2]$ had little affect plant height of inbred japonica rice species (Kim *et al.*, 1996). Recently, however, Yang *et al.* (2009) reported that elevated $[\text{CO}_2]$ could increase plant height of hybrid rice in China. These divergent results may suggest that plant height of rice crops in response to elevated $[\text{CO}_2]$ could vary depending on their genotypes. On the other hand, higher T_a can stimulate cell division and expansion in plant organs (Hamamoto *et al.*, 2006), thereby increasing plant height (Oh-e *et al.*, 2007). We also observed a slight increase (but not significant) in plant height by rising T_a ; the magnitudes of the increase were by 2.1% (1.3°C above ambient) and 9.0% (3.0°C above ambient) under ambient $[\text{CO}_2]$ and by 13.2% and 11.2% under elevated $[\text{CO}_2]$ (Table 1). Taken together, it is therefore possible that ongoing rising T_a could have the potential to increase in plant height of rice crops. Considering the plant lodging, this would be of undesirable plant architectures under expected warming in future climate. Alternatively, this can be advantageous for CO_2 diffusion inside rice canopy, thereby increasing photosynthesis (Kuroda *et al.*, 1989), unless there is concern about the plant lodging.

In the present study, there was no increase in the tiller number of rice crops at elevated $[\text{CO}_2]$. However, it is well documented that elevated $[\text{CO}_2]$ had a significant positive effect on tiller number of rice (Kim *et al.*, 1996; Jitla *et al.*, 1997; Yang *et al.*, 2009). In particular, the stimulatory effect of elevated $[\text{CO}_2]$ on tiller number was pronounced in vegetative growth stage, while the effect became significantly small in reproductive stage due to the fact that a large portion of tiller produced was expired during reproductive stage (Kim *et al.*, 2003). The latter likely supported our result that at full heading stage tiller number showed the lack of response to elevated $[\text{CO}_2]$. This was probably attributed to the strong carbon (C) and nitrogen (N) sink of the vigorous reproductive tiller, which may have depressed the supply of C and N to the smaller tillers (Daepf *et al.*, 2001), eventually leading to the annihilation of the small tillers. Unlike elevated $[\text{CO}_2]$, elevated T_a had a still significant positive effect on tiller number even at full heading stage ($P=0.009$; Table 1); the stimulatory

effect of T_a was more pronounced in 3.0°C than in 1.3°C above ambient, and at elevated $[\text{CO}_2]$ than at ambient $[\text{CO}_2]$, resulting in a marginally significant positive $[\text{CO}_2] \times T_a$ interaction ($P=0.103$). Higher T_a was expected to produce more tiller than ambient T_a during the vegetative growth (Oh-e *et al.*, 2007; Kim *et al.*, 2011). However, the higher- T_a -induced increase in tiller was not always linked to the increase in final tiller number at harvest (Oh-e *et al.*, 2007). Even so, the increased tillers with rising T_a , irrespective of whether or not they would be productive, may have the potential to change canopy architecture in terms of horizontal-spatial distribution of tiller densities.

Under elevated $[\text{CO}_2]$, despite a significant increase in root biomass (data not shown), as has open been reported ((Kim *et al.*, 2003; Ziska and Bunce, 2007; Yang *et al.*, 2007), the root : shoot ratio was not altered in this study. This suggested that elevated $[\text{CO}_2]$ is unlikely to change the balance between above- and below-ground developments of rice cultivar used (i.e. Dongjin1ho) in this study. However, some other reported that elevated $[\text{CO}_2]$ increases root : shoot ratio in rice cultivar IR72 (Baysa *et al.*, 2003) and Akitakomachi (Kim *et al.*, 2003), suggesting that there may be a genotypic variation in root : shoot ratio in response to elevated $[\text{CO}_2]$.

A notable feature of the results was that, despite the reduction in total LAI under elevated $[\text{CO}_2]$ ($P=0.091$), the vertical profiles of LAI remained unchanged under elevated $[\text{CO}_2]$ and T_a treatments (Fig. 1a). Early studies have addressed the fact that leaf area or LAI of rice crops grown at elevated $[\text{CO}_2]$ increased during the vegetative stage, but decreased during the grain filling (Kim *et al.*, 2003; Sakai *et al.*, 2006). Our result that total LAI observed at full heading stage reduced with elevated $[\text{CO}_2]$ was in line with previous findings. Taken together, our results suggested that, although elevated $[\text{CO}_2]$ could affect total LAI, it could not lead to a significant alteration of rice canopy structure in terms of at least vertical profiles of the LAI. However, this does not mean that leaf angle would also be unchanged. Assuming that leaf angle is in inverse relation to leaf nitrogen (N) content (Chen *et al.*, 2003), rice crops grown in elevated $[\text{CO}_2]$ will be expected to have more erect leaves than those in ambient $[\text{CO}_2]$ because rice plants showed a significant reduction in leaf

N content at elevated $[\text{CO}_2]$ (Kim *et al.*, 2003, 2011; Seneweera *et al.*, 2011; Yang *et al.*, 2007). On the other hand, elevated T_a significantly increased the fraction of LAI distributed into the upper layer (75-90 cm), but decreased that distributed into the lower layer (45-60 cm) both under ambient and elevated $[\text{CO}_2]$ (Fig. 1a; Table 2). This is most likely associated with the effect of elevated T_a both on leaf expansion and crop phenology, i.e. (i) an expansion of the leaf area can be greater in elevated T_a than in ambient T_a (Hamamoto *et al.*, 2006), resulting in more LAI in the upper layer that has a relatively new leaves, (ii) by contrast, the senescence of the leaves in the lower layer can be commenced earlier in elevated T_a than in ambient T_a due to the hastened crop phenology (Craufurd and Wheeler, 2009), resulting in a less LAI in the lower canopy layer at warmer T_a .

The most striking result was that most of the parameters related to flag leaf morphology is negated with elevated $[\text{CO}_2]$ (Fig. 2). There were about 12%, 5%, 7.5%, 15% and 21% decreases in LFL, WFL, L:W ratio, FLA and FLM, respectively, in elevated $[\text{CO}_2]$ in comparison with ambient $[\text{CO}_2]$. This result is likely to partly reflect advanced crop phenology under elevated $[\text{CO}_2]$. In the present study, under elevated $[\text{CO}_2]$, the timing of the flowering was earlier 2 days at current ambient T_a (14 August in ambient $[\text{CO}_2]$ vs 12 August in elevated $[\text{CO}_2]$), and 3 days at two levels of warmer T_a (11 August vs 8 August). Such a phenology shift was also the case at warmer T_a under current ambient $[\text{CO}_2]$, and has been reported for many crops species grown under elevated $[\text{CO}_2]$ and/or T_a (Kim *et al.*, 1996; Craufurd and Wheeler, 2009). Under elevated $[\text{CO}_2]$, earlier flowering indicates a shortening the duration of crop growth from transplanting to the flowering. This could have the potential to reduce in the period of development of the flag leaves, leading to shorter and narrower flag leaves, and eventually to less FLA. However, our result showed that T_a warming could offset to some extent of the negative effects of elevated $[\text{CO}_2]$ on flag leaves. As discussed earlier, this was most likely attributed to the enhanced leaf expansion with elevated T_a (Hamamoto *et al.*, 2006).

The flag leaves of rice plant play a critical role in plant or canopy photosynthesis during the ripening phase, occupying

of about 19% of total leaf area at heading (Yoshida *et al.*, 1972), and contributing about 45% of grain yield (Abou-khalifa *et al.*, 2008). Despite such an importance of the flag leaf in rice yield processes (Peng *et al.*, 2008), there is no report addressing the flag leaf morphology of rice plants under changes in $[\text{CO}_2]$ and climate. This is the first study, of which we are aware, that has investigated flag leaf morphology of field-grown rice under elevated $[\text{CO}_2]$ and T_a , though we did not determine grain yield. It appears, however, that it is difficult to assess how the modified flag leaf morphology affects grain yield. This is because the yield processes will likely to be further complicated by the fact that elevated $[\text{CO}_2]$ and T_a also can modify the rates of net photosynthesis (Ainsworth and Long, 2005) and the life span of leaves (Seneweera *et al.*, 2011), as well as the flag leaf morphology. Therefore, it is needed to further study in integration of whole-plant and flag leaf morphology responses and models, in order to better understand of rice yield processes under changes in $[\text{CO}_2]$ and climate.

In conclusion, the data presented here suggests that plant height of rice (cv, Dongjin1ho) among the components of plant architecture is not affected by elevating $[\text{CO}_2]$, but will be more or less increased by temperature warming (1.3-3.0°C above ambient). In addition, the temperature warming could have the potential to alter in canopy structure via modifying both the horizontal-spatial distribution of rice tillers and the vertical profiles of LAI. More importantly, the results of this study clearly demonstrate that most of the parameters with respect to flag leaf morphology is negated with elevated $[\text{CO}_2]$, and that the negative effect of elevated $[\text{CO}_2]$ can be offset to some extent by temperature warming.

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