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RESEARCH PAPER

Effect of elevated CO₂ and high temperature on seed-set and grain quality of rice

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Abstract

Hybrid vigour may help overcome the negative effects of climate change in rice. A popular rice hybrid (IR75217H), a heat-tolerant check (N22), and a mega-variety (IR64) were tested for tolerance of seed-set and grain quality to high-temperature stress at anthesis at ambient and elevated [CO₂]. Under an ambient air temperature of 29 °C (tissue temperature 28.3 °C), elevated [CO₂] increased vegetative and reproductive growth, including seed yield in all three genotypes. Seed-set was reduced by high temperature in all three genotypes, with the hybrid and IR64 equally affected and twice as sensitive as the tolerant cultivar N22. No interaction occurred between temperature and [CO₂] for seed-set. The hybrid had significantly more anthesed spikelets at all temperatures than IR64 and at 29 °C this resulted in a large yield advantage. At 35 °C (tissue temperature 32.9 °C) the hybrid had a higher seed yield than IR64 due to the higher spikelet number, but at 38 °C (tissue temperature 34–35 °C) there was no yield advantage. Grain gel consistency in the hybrid and IR64 was reduced by high temperatures only at elevated [CO₂], while the percentage of broken grains increased from 10% at 29 °C to 35% at 38 °C in the hybrid. It is concluded that seed-set of hybrids is susceptible to short episodes of high temperature during anthesis, but that at intermediate tissue temperatures of 32.9 °C higher spikelet number (yield potential) of the hybrid can compensate to some extent. If the heat tolerance from N22 or other tolerant donors could be transferred into hybrids, yield could be maintained under the higher temperatures predicted with climate change.

Key words: Elevated CO₂, flowering, grain quality, high temperature, rice, spikelet fertility.

Introduction

Atmospheric CO₂ concentration [CO₂] could increase to almost 700 ppm by the end of the century (IPCC, 2007). At the plant level, a higher [CO₂] increases photosynthesis, growth, development, and yield of a wide range of cultivated crops, including rice (Long *et al.*, 2004, 2006; Ainsworth and Long, 2005; Ainsworth *et al.*, 2008). To date, the majority of experiments with elevated [CO₂] and rice have tested conventionally bred *japonica* (Kim *et al.*,

2001, 2003a, b; Sasaki *et al.*, 2005, 2007; Shimono *et al.*, 2007; Yang *et al.*, 2007) and *indica* cultivars (Weerakoon *et al.*, 2005; De Costa *et al.*, 2007). However, modern hybrid rice cultivars exhibit higher seedling vigour, rate of tillering, relatively higher growth rate, and higher yield potential than conventional inbred rice cultivars (Ling *et al.*, 1994; Xie *et al.*, 1996). Yield enhancement under elevated [CO₂] may, therefore, be greater for hybrids than for conventional

rice cultivars. Recently, the response of two hybrid rice cultivars to CO₂ enrichment was tested under *in situ* Free Air CO₂ Enrichment (FACE) systems (Liu *et al.*, 2008; Yang *et al.*, 2009). A three-line *indica* hybrid, Shanyou 63, grown at 570 ppm CO₂ increased yield by 34% (Liu *et al.*, 2008). Similarly, an inter-specific rice hybrid, Liangyoupei-jiu, grown at approximately 580 ppm CO₂ had 24% and 20% higher grain yield and biomass, respectively, than plants grown at ambient [CO₂] (Yang *et al.*, 2009). However, the response of hybrids to a combination of elevated [CO₂] and high temperature has not been studied.

Studies on both high day temperatures (Jagadish *et al.*, 2010a, b) and high night temperatures (Peng *et al.*, 2004; Nagarajan *et al.*, 2010; Welch *et al.*, 2010) have demonstrated negative effects on rice spikelet fertility and yields. High day temperatures beyond the critical threshold during sensitive developmental stages like gametogenesis and flowering leads to low seed-set (Prasad *et al.*, 2006; Jagadish *et al.*, 2007, 2008, 2010a, b, 2011). The impact of high night temperatures leading to either increased respiration or other mechanisms which lower rice yields has not been studied in detail. Moreover, [CO₂] is relatively high during the night, so a further increase could lead to smaller beneficial effects and a fall in the domain of diminishing returns on plant processes (Pinter *et al.*, 2000). Recently, it was shown that the major part of the night had 500–700ppm of CO₂ even under ambient level in a soybean FACE system (Bunce, 2011). Further, most FACE sites including the DUKE forest face (<http://face.env.duke.edu/description.cfm>), aspen FACE (<http://aspenface.mtu.edu/>), soy FACE (<http://soyface.illinois.edu/technology.htm>) and others don't fumigate CO₂ at night. Hence the ideal and most practical combination of high day temperature and elevated CO₂ conditions at sensitive flowering stage was tested in this study.

Although elevated [CO₂] *per se* should increase productivity and yield in rice, the increasing frequency and intensity of short-duration high temperature events (>33 °C) pose a serious threat to agricultural production, especially in cereals such as wheat (Modarresi *et al.*, 2010) and rice (Wassmann *et al.*, 2009). The threat is highest when high temperatures coincide with flowering (Yoshida *et al.*, 1981; Matsui *et al.*, 1997; Prasad *et al.*, 2006; Jagadish *et al.*, 2007, 2008, 2010a, b; Rang *et al.*, 2011) and grain-filling (Fitzgerald and Resurreccion, 2009). These effects may be exacerbated by higher tissue/canopy temperature associated with stomatal response to elevated [CO₂] (Vara Prasad *et al.*, 2006; Long and Ort, 2010). Although the effects of [CO₂] and high temperature over the entire life-cycle have been studied in rice (Baker *et al.*, 1992; Ziska *et al.*, 1996; Matsui *et al.*, 1997; Baker, 2004; De Costa *et al.*, 2006; Cheng *et al.*, 2009), the effect of more realistic impacts of short episodes of high temperature at flowering at different [CO₂] has not been studied.

Furthermore, studies related to [CO₂] and temperature (FACE or controlled environments) have been restricted to phenological and yield parameters; their effects on spikelet fertility and grain quality are not known. Acceptable grain quality is essential for any new cultivar to be accepted by

consumers and thereby adopted by farmers. The main traits that define market price are the proportion of chalk and broken grains in the sample (Cooper *et al.*, 2008). Chalkiness causes grains to break, and this increases with higher temperature during grain-filling (Lisle *et al.*, 2000; Fitzgerald and Resurreccion, 2009). Amylose concentration and gelatinization temperature contribute to texture and cooking time; with high temperature, amylose concentration declines in many rice varieties (Chen *et al.*, 2008) and gelatinization temperature increases (Cuevas *et al.*, 2010).

The objective of this paper was to determine the responses of a rice hybrid, a standard *indica* and a heat-tolerant *aus* cultivar to a short episode of elevated temperature (5 d) under ambient and elevated [CO₂] at flowering on (i) seed-set and seed number and (ii) rice grain characteristics and quality. The hypotheses to be tested were that (i) seed-set is reduced by high tissue temperature with no interaction between tissue temperature and [CO₂]; (ii) high [CO₂] increases spikelet and seed number at all temperatures; (iii) there is no difference in the response of seed-set, seed yield or grain characteristics and quality traits of the hybrid and *indica* cultivar to high temperature and [CO₂]; and (iv) short episodes of high temperature at anthesis at ambient and high [CO₂] have no effect on grain characteristics and quality traits.

Materials and methods

The experiment was carried out between April and September 2007 using controlled environment facilities at the Plant Environment Laboratory, Department of Agriculture, University of Reading, UK (51°27' N, 00°56' W). Plants were raised inside growth cabinets under optimum temperature and photoperiod conditions, and transferred to adjacent growth cabinets to impose high-temperature treatments at flowering.

Plant growth and maintenance

Plants were grown in a medium without soil exactly as previously described (Jagadish *et al.*, 2007; 2008). Two rice varieties (*Oryza sativa* L.), the *aus* type N22 and the *indica* cv. IR64 and one hybrid, IR75217H (IRRI138-IR68897A×IR60819-34-2 R), were tested. For each, five seeds were sown in each pot at a depth of 2–2.5 cm and thinned to one plant per pot at the three-leaf stage. Plants were maintained under fully watered conditions with a complete nutrient solution containing 100 mg l⁻¹ inorganic nitrogen throughout the crop growth cycle. The nutrient solution was acidified to pH 5 to avoid Fe deficiency (Yoshida *et al.*, 1976). Plants were sprayed twice with foliar feed (Miracle-Gro, The Scotts Company, UK Ltd.) at 3.75 g l⁻¹ at 7 d intervals until panicle emergence. There were no major pest or disease problems.

Growth chambers

The three varieties were grown in controlled environment chambers (internal size 1.4×1.4×1.5 m). Aspirated temperature and relative humidity (RH) were measured every 10 s using copper-constantan thermocouples and a data logger (Delta T Devices, Burwell, Cambridge, UK) and averaged over 10 min for the entire crop growth period. An optimum day/night temperature (29±0.57 °C/21±0.34 °C) and RH (60±1.45%/80±1.21%) with a short inductive photoperiod of 11 h (Summerfield *et al.*, 1992) from 08.00 h to 19.00 h with a thermo period of 13 h (07.00 h to 20.00 h) was imposed. A photosynthetic photon flux density of

650 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was maintained at the floor of the chamber using a combination of cool white fluorescent tubes and incandescent lamps. Lamps were balanced to ensure uniform flux densities throughout the cabinet. A centrally placed fan circulated air uniformly throughout the chamber.

CO₂ and temperature treatments

The [CO₂] in the chambers was controlled by a 12-channel measurement and control system using ADC WA 526 IRGA (Infra-Red Gas Analyser) manufactured by ADC, Unit 35, Hoddesdon Industrial Centre, Pindar Road, Hoddesdon, Herts., UK. The system sampled each chamber in turns and delivered (pure) CO₂ to each chamber via 12 solenoid valves, according to a calculation based on the difference between the reading and the set point. The sampling 'dwell' on each chamber was 40 s, so it sampled each chamber once every 5.3 min (8 chambers \times 40 s) and CO₂ was delivered after recalculating the valve opening based on the reading and the set point. Two independent sets of plants were maintained at ambient (380 ppm) or elevated (760 ppm) [CO₂] for the entire growth period except during anthesis, when one of the two sets was exposed to either 35 °C or 38 °C for 5 d. The transition time from day-time maximum to night-time minimum was 5 h. A square wave heat treatment was applied to overcome the potentially confounding effects of gradually increasing temperature (Jagadish *et al.*, 2007, 2008, 2010a, b). There were therefore six combinations of temperature and [CO₂]: (i) ambient temperature and CO₂ (29 °C and 380 ppm CO₂), (ii) ambient temperature and elevated CO₂ (29 °C and 760 ppm CO₂), (iii) 35 °C at anthesis and 380 ppm CO₂, (iv) 35 °C at anthesis and 760 ppm CO₂, (v) 38 °C at anthesis and 380 ppm CO₂, and (vi) 38 °C at anthesis and 760 ppm CO₂. Apart from the change in conditions during the treatment period, all other aspects relating to the environmental conditions in the growth chamber were identical to those described earlier. For the high-temperature treatments, at the initiation of anthesis (identified by the protruding anthers), four replicate plants from both ambient and elevated [CO₂] were transferred into growth chambers maintained at either 35 °C or 38 °C between 09.00 h and 15.00 h to impose treatments (iii) to (vi), while another set of four plants was left undisturbed in the chambers to impose treatments (i) and (ii). The experimental design for the analysis of temperature and [CO₂] treatments was therefore six treatments in two replicated blocks (growth chambers) each with four replicate plants (pots) per growth cabinet, i.e. $n=8$ observations per treatment.

Main tiller panicles were tagged and seed-set was measured on these panicles. Any spikelets that underwent anthesis (i.e. with a visible anther) outside the 5 d temperature treatment period were marked with acrylic paint and were not included in further analyses (Jagadish *et al.*, 2008). The majority of the unmarked spikelets on the main tiller exposed to the stress treatments were located toward the top of the panicle and had finished flowering within the experimental treatment period of 5 d (Table 1). In all three genotypes, across each treatment more than 100 spikelets were used for determining the seed-set, except in IR64 under 29 °C ($n=92$) and 35 °C ($n=89$) under ambient [CO₂]. Around 100 spikelets are considered as a threshold level for estimating seed-set under high temperature stress in rice.

Spikelet tissue temperature for IR64 was measured by placing copper–constantan micro-thermocouples inside the spikelets of three independent plants in all treatments. The data on spikelet tissue temperatures were recorded by a data logger (Delta T Devices, Burwell, Cambridge, UK) every 10 s and averaged over 10 min for the entire period (Table 2).

Analyses

Yield parameters and phenology: Spikelets on the main tiller panicles were separated into unmarked (anthesed during temperature treatments) and marked (anthesed under control conditions) and seed-set was calculated from the number of unmarked filled

Table 1. Average number of spikelets on the main tiller exposed to stress treatments (unmarked) at flowering and the remaining number of spikelets flowering under ambient conditions (marked)

CO ₂ (ppm)	Temperature (°C)	Genotype/hybrid	No. of unmarked spikelets	No. of marked spikelets ^a
380	29	IR64	94.0 \pm 5.28	*
		IR75217H	169.3 \pm 9.79	*
		N22	116.4 \pm 4.18	*
	35	IR64	78.6 \pm 2.65	34.0 \pm 3.0
		IR75217H	172.0 \pm 5.67	53.2 \pm 2.94
		N22	93.7 \pm 3.25	23.4 \pm 2.31
	38	IR64	93.7 \pm 4.81	25.9 \pm 2.47
		IR75217H	156.6 \pm 4.27	71.1 \pm 3.17
		N22	98.5 \pm 2.89	26.4 \pm 1.94
760	29	IR64	129.9 \pm 5.71	*
		IR75217H	209.6 \pm 12.2	*
		N22	118.7 \pm 4.53	*
	35	IR64	100.3 \pm 5.20	31.3 \pm 2.43
		IR75217H	168.1 \pm 5.50	68.5 \pm 2.90
		N22	104.9 \pm 2.94	28.1 \pm 2.00
	38	IR64	111.9 \pm 2.53	28.1 \pm 1.70
		IR75217H	170.1 \pm 5.66	77.3 \pm 2.71
		N22	104.4 \pm 2.74	30.6 \pm 1.77

^a Indicates that spikelets that flowered on the main panicle under ambient conditions were not marked.

Table 2. Spikelet tissue temperature measured using micro-thermocouples from replicated growth chambers in IR64 under three different air temperatures and two CO₂ concentrations; numbers are \pm SD

CO ₂ (ppm)	Spikelet tissue temperature (°C)		
	29	35	38
380	28.4 \pm 0.42	32.6 \pm 0.45	34.0 \pm 0.69
760	28.2 \pm 0.44	32.9 \pm 1.11	35.0 \pm 0.67
Δ	-0.18	0.28	0.97

spikelets as a proportion of the total number of unmarked spikelets. Plants from all six treatments were harvested as replicate samples and separated into root, stems, and leaves. Plant parts were dried separately at 60 °C until a constant weight was obtained. The root–shoot ratio and total plant biomass were calculated from the components. The total number of tillers per plant was recorded while the tillers with a productive panicle (i.e. bearing filled grains) were considered for the determination of panicle number.

Grain quality: Filled grains from the main-tiller spikelets used to calculate seed-set were dried at 38 °C in a forced-air oven for 8–10 d until they attained a constant dry weight. Individual replicate samples for each genotype/treatment were bulked to give sufficient material for the analysis of quality traits. From the bulked sample, two replicate sub-samples of seeds from each treatment and entry were separated and analysed for amylose content, gel consistency, chalkiness (%), and length, width, and percentage of whole grain at the Grain Quality and Nutrition Center, IIRRI, Philippines.

Physical characteristics of the grain were measured using a 1625 Grain Inspector (Foss, Denmark). In order to measure amylose

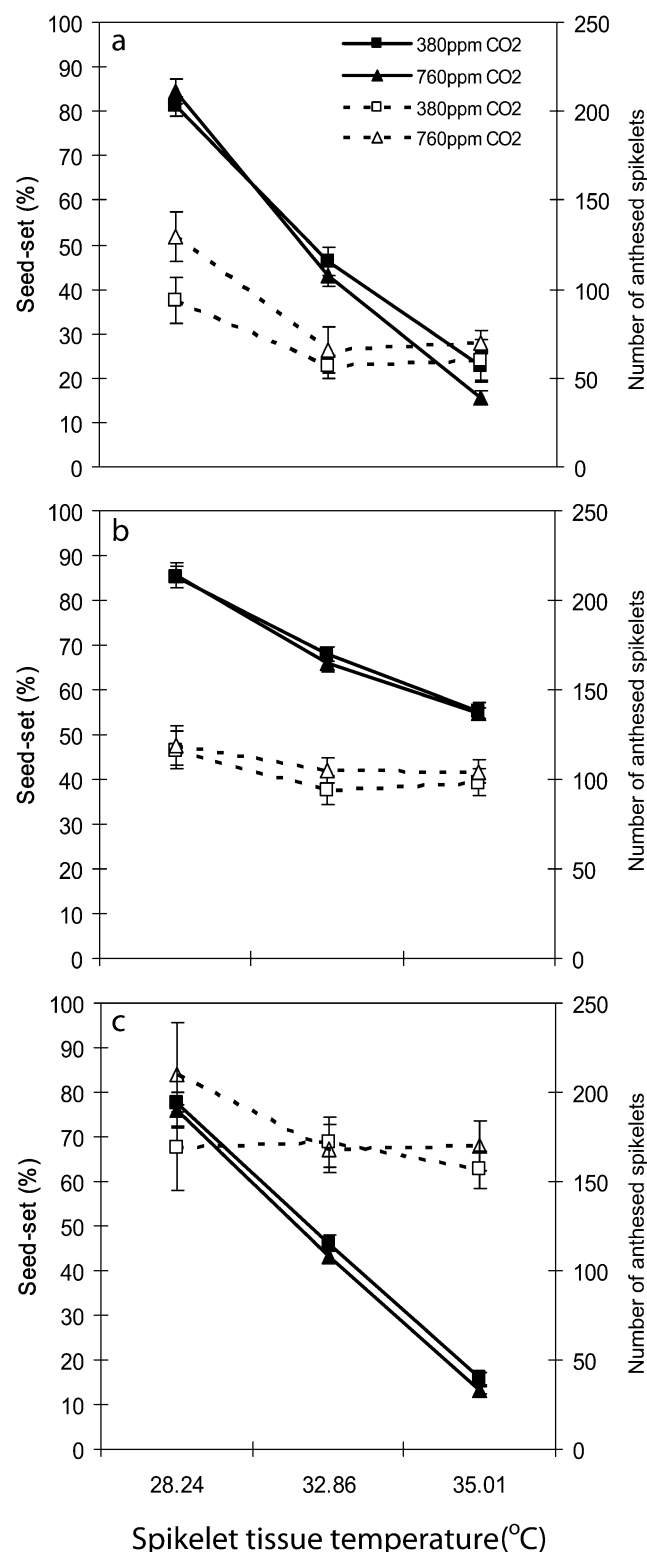


Fig. 1. Seed-set and number of spikelets on the main tiller in IR64 (a), N22 (b), and a hybrid, IR75217H (c), exposed to high-temperature stress for five consecutive flowering days in combination with either ambient or elevated CO₂ concentration. Solid symbols represent seed-set and open symbols the number of spikelets. Bars indicate ± SE.

and gel consistency, polished grains were ground to pass through a 0.5 mm sieve in a cyclone mill (Udy Cyclone Sample Mill 3010-030, Fort Collins, CO). Amylose concentration and gel consistency were measured as previously described (Juliano, 1971; Tran *et al.*, 2011).

Statistical analysis

All the yield parameters, phenological traits, and grain quality components were analysed as a completely randomized design with growth chambers as blocks and four pots as replicates using Genstat Version 11 (Rothamsted Experimental Station, UK). Comparison of regressions was also carried out with the same software.

Results

Spikelet tissue temperature was lower than the air temperature at 29, 35, and 38 °C by 0.67, 2.28, and 3.48 °C, on average, respectively. These effects were similar to those observed in the same growth chambers for rice cultivar Azucena (Jagadish *et al.*, 2007). On average, higher [CO₂] reduced spikelet tissue temperature by 0.18 °C at 29 °C, but increased spikelet temperature by 0.28 °C and 0.97 °C at 35 °C and 38 °C, respectively (Table 2).

Growth and development

The overall effects of [CO₂] on the growth and development of the three genotypes were as expected based on other [CO₂] studies and so are only summarized here. The biomass of IR64 increased from 34.5 g plant⁻¹ at 380 ppm to 51.4 g plant⁻¹ at 760 ppm CO₂ at 29 °C, an increase of 49%. Comparable figures for the hybrid were 57.9 and 82.2 g plant⁻¹ (an increase of 42%). The short 5 d episodes of high temperature did not affect overall growth or development (see Supplementary Table S1 at JXB online). Elevated [CO₂] influenced the phenology and days to flowering and harvest were delayed in all three entries at high [CO₂]. The biggest delay in days to flowering was recorded with the hybrid (+7 d) and the least with N22 (+3 d). However, the influence of elevated CO₂ on the grain-filling phase was smaller, with both IR64 and the hybrid having an increase by a day while it was 3 d with N22, compared with ambient [CO₂].

Anthesis

In all three genotypes, irrespective of the temperature regimes or CO₂ levels, the majority of the spikelets on the main tiller panicle completed flowering in 5 d (Table 1). The total number of spikelets on the main tiller panicle differed significantly with variety, temperature, and [CO₂] ($P < 0.001$) with a significant interaction between [CO₂] and variety ($P < 0.05$) and temperature and variety ($P < 0.001$). In both IR64 and N22 the number of spikelets on the main tiller panicle were not significantly affected by increasing temperatures ($P > 0.05$). However, the hybrid recorded a significant increase in the spikelet numbers with higher temperatures compared with the control under both ambient and elevated [CO₂] ($P < 0.001$). The hybrid had more

Table 3. Regression parameters for seed-set and seed-yield using tissue temperatures in two rice genotypes and a hybrid; values are \pm SE

CO ₂	N22 Intercept	Slope	IR64 Intercept	Slope	Hybrid Intercept	Slope
Seed-set						
380 ppm	–	–	–	–	–	–
Single line	209.56 \pm 6.80	–4.352 \pm 0.211	362.40 \pm 10.7	–9.776 \pm 0.333	326.64 \pm 9.60	–8.798 \pm 0.299
780 ppm	–	–	–	–	–	–
Temperature (T)	$P < 0.001$		$P < 0.001$		$P < 0.001$	
CO ₂ (parallel lines)	$P > 0.244$		$P > 0.450$		$P > 0.270$	
T \times CO ₂ (separate lines)						
Seed-yield						
380 ppm	–	–	6.091 \pm 0.534	–0.162 \pm 0.017	10.782 \pm 0.987	–0.290 \pm 0.030
Single line	4.618 \pm 0.333	–0.105 \pm 0.01	–	–	–	–
780 ppm	–	–	11.196 \pm 1.289	–0.311 \pm 0.041	15.872 \pm 2.387	–0.440 \pm 0.073
Temperature (T)	$P < 0.001$		$P < 0.001$		$P < 0.001$	
CO ₂ (parallel lines)	$P > 0.970$		$P < 0.001$		$P < 0.05$	
T \times CO ₂ (separate lines)			$P < 0.001$		$P < 0.01$	

($P < 0.001$) spikelets opening on the main tiller than N22 or IR64 at all temperatures and [CO₂]: 176 to 236 compared with 110 to 142 in IR64 at 380 ppm and 760 ppm, respectively (Fig. 1). Higher temperature reduced the number of anthesing spikelets ($P < 0.05$) in all three genotypes, with the effect being more prominent under elevated [CO₂], especially in IR64 ($P < 0.001$) and the hybrid ($P < 0.05$). Spikelet numbers were generally greater with higher [CO₂], notably at 29 °C in IR64 and the hybrid. By contrast, the number of spikelets anthesing in N22 was not influenced by higher [CO₂].

Seed-set

Seed-set in the three genotypes was reduced by temperature ($P < 0.001$) and [CO₂] ($P < 0.05$), with a significant interaction only between the temperature and genotype ($P < 0.001$) (Fig. 1). There was no effect of [CO₂] \times temperature interaction, nor did the growth cabinets have any significant effect on percentage seed-set ($P > 0.55$). Temperature had a large effect on seed-set in all genotypes, with seed-set declining from between 75% and 80% at 29 °C to $< 20\%$ in the hybrid at 38 °C. N22 was the most tolerant genotype, achieving 57% seed-set at 38 °C. The interaction between genotype and temperature mainly reflected the response of IR64 when seed-set was higher at 29 °C and lower at 38 °C. A comparison of regressions for seed-set against tissue temperature and [CO₂] by genotype showed effects of temperature ($P < 0.001$) alone and in interaction with genotype, but no effect of [CO₂] ($P > 0.05$). Hence, across [CO₂] treatments, responses to temperature and [CO₂] were best described by three separate lines with different intercepts and slopes (Table 3). N22 was the most tolerant of temperature, with a slope of -4.35 ± 0.21 (SE) while IR64 (slope -9.78 ± 0.33) and the hybrid (slope -8.80 ± 0.30), were approximately twice as sensitive to temperature as N22 (Table 3).

Seed yield and seed number

Seed yield per main tiller was highly correlated with seed number ($r = 0.98$) and with seed-set ($r = 0.71$) (Fig. 2). Seed yield and seed number were significantly affected by temperature ($P < 0.001$), [CO₂] and their interaction ($P < 0.01$) in both IR64 and the hybrid. In N22, both seed yield and seed number were affected by temperature ($P < 0.001$) but not [CO₂] or their interaction ($P > 0.40$). At 29 °C and 380 ppm CO₂, the hybrid gave a yield advantage of about 1 g per panicle (*c.* 65%) over N22 and IR64. However, at the highest temperature (38 °C), this yield advantage was lost and N22 yielded more than IR64 and the hybrid. High [CO₂] increased seed yield at 29 °C, especially in IR64 and the hybrid, but this advantage was also completely lost at 35 °C and 38 °C. Individual kernal weight increased with increasing temperature ($P < 0.01$) and with elevated [CO₂] ($P < 0.001$) with no significant interaction ($P > 0.70$). The ANOVA from a comparison of regressions for seed yield against temperature, [CO₂] and genotype revealed that the seed yield of N22 at different tissue temperatures and [CO₂] could be described by a single line (slope -0.105). By contrast, in IR64 and the hybrid, the response to temperature varied with [CO₂] and individual regressions were better (Table 3). Thus, the sensitivity of seed yield to temperature at ambient [CO₂] increased from -0.105 ± 0.01 g °C⁻¹ in N22 to -0.162 ± 0.02 in IR64 and -0.29 ± 0.03 in the hybrid. At higher [CO₂], the values were -0.311 ± 0.04 g °C⁻¹ and -0.44 ± 0.07 in IR64 and the hybrid, respectively (Table 3).

Grain quality parameters

Amylose concentration was affected by temperature and [CO₂] and their interaction ($P < 0.05$), but the differences were only within 0.5–1%. At 380 ppm [CO₂], amylose concentration decreased very slightly with increasing temperature, particularly in IR64 (Fig. 3). For the other two

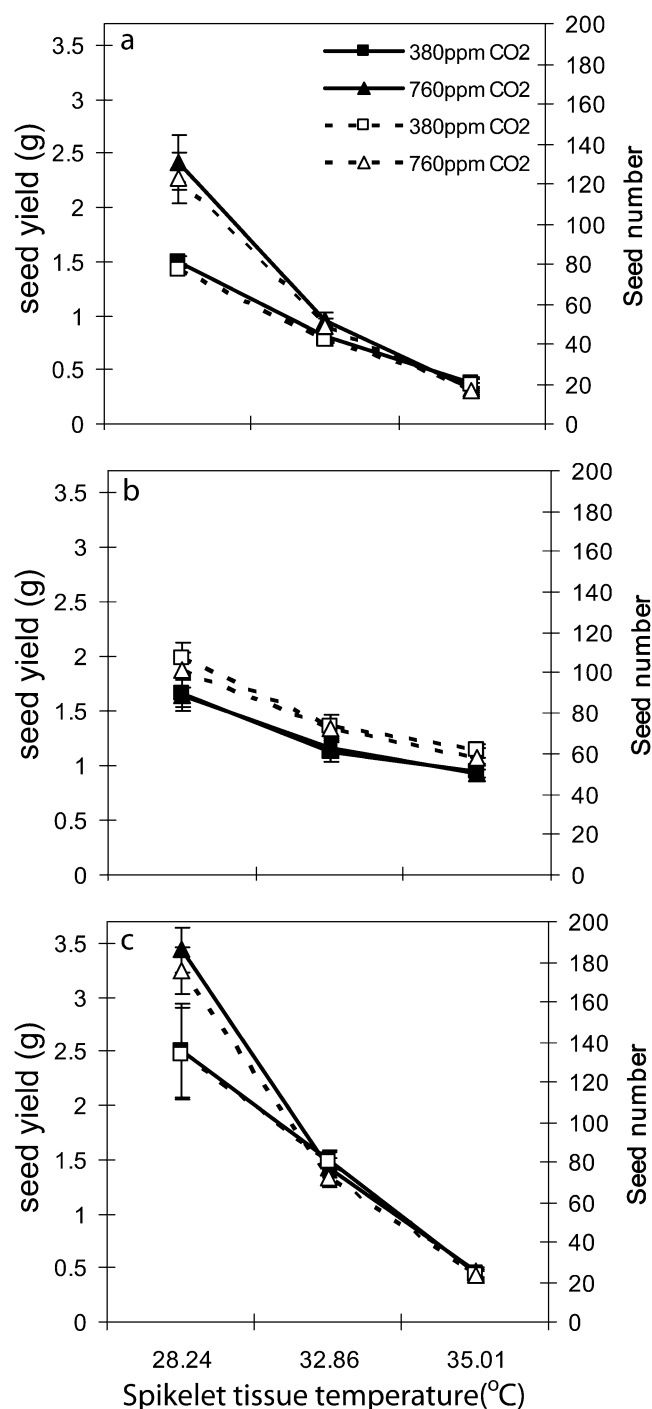


Fig. 2. Seed yield and seed number on the main tiller in IR64 (a), N22 (b), and a hybrid, IR75217H (c), exposed to high-temperature stress for five consecutive flowering days in combination with either ambient or elevated CO_2 concentration. Solid symbols represent seed weight and open symbols the seed number. A single line was the best fit for seed yield response in N22 ($y=4.62+(-0.105x; r^2=68.5)$), unlike IR64 [at 380 ppm CO_2 ($y=6.091+(-0.162x)$; at 760 ppm ($y=\{6.091+(5.105)\}+(-0.162)+(-0.149)x$)] with $r^2=90.9$ and the hybrid [at 380 ppm CO_2 ($y=10.78+(-0.290x)$; at 760 ppm ($y=\{10.78+(5.09)\}+(-0.290)+(-0.150)x$)] with $r^2=86.4$, wherein two separate lines having different slopes explained the variation better. Bars indicate $\pm \text{SE}$.

genotypes, there was no effect of $[\text{CO}_2]$ or temperature on amylose content.

There were also differences between genotypes for gel consistency ($P < 0.001$) but no effect of temperature or interactions with temperature at ambient $[\text{CO}_2]$. The hybrid had slightly harder gel consistency values at ambient $[\text{CO}_2]$ than the other two genotypes. High $[\text{CO}_2]$ increased ($P < 0.05$) gel consistency in IR64 and in the hybrid. High temperature reduced gel consistency at high $[\text{CO}_2]$ but not at ambient $[\text{CO}_2]$.

The proportion of broken grains (milling quality) was reduced by $[\text{CO}_2]$ ($P < 0.001$) in the two long-grain genotypes, IR64 and the hybrid. At ambient $[\text{CO}_2]$, higher temperature increased the proportion of broken grains in IR64, and especially in the hybrid, in which the proportion went from 10% at 29 °C to nearly 35% at 38 °C, but the effect was not statistically significant. The broken grain % for N22 could not be estimated due to the technical difficulties with very short grains.

Higher $[\text{CO}_2]$ increased the grain width of all three genotypes ($P < 0.01$) while temperature and its interaction with $[\text{CO}_2]$ had no effect (Fig. 3). Chalk concentration differed among the genotypes ($P < 0.01$) but temperature and $[\text{CO}_2]$ had no effect. Temperature had no effect on the grain physical parameters, i.e. length and width (see Supplementary Table S2 at *JXB* online).

Discussion

The effects of $[\text{CO}_2]$ on biomass (increased by 50%) and seed yield (increased by 24% to 30%) observed in this study are similar to those of other studies in controlled environments (Baker, 2004; Ainsworth, 2008). Recent FACE (Free Air CO_2 Enrichment) studies under near-field conditions have, however, demonstrated the yield increase in response to elevated CO_2 to be less than that obtained under enclosure studies (Long *et al.*, 2006; Ainsworth, 2008; Long and Ort, 2010).

Our first hypothesis was that no interaction would be found between tissue temperature and $[\text{CO}_2]$ on seed-set, and the results support this. There appears to be a very small effect of $[\text{CO}_2]$ on seed-set in IR64, but the interaction between temperature and $[\text{CO}_2]$ was not significant. Thus, a small increase in tissue temperature can lead to a large decline in seed-set and yield. Tissue temperature increases due to a decrease in transpiration cooling at higher $[\text{CO}_2]$ and genetic variation for this trait exists (Weerakoon *et al.*, 2008) and is influenced by vapour pressure deficit (Gholipour *et al.*, 2010). It has also been hypothesized that $[\text{CO}_2]$ would increase spikelet numbers at all temperatures, which would result in greater seed numbers. Although $[\text{CO}_2]$ increased yield potential (number of spikelets) at 29 °C, this response was not observed at 35 °C or 38 °C. Moreover, an increase in the total number of spikelets on the main tiller panicle of the hybrid was observed which could indicate a possible developmental mechanism in response to increasing temperature. In any case, greater

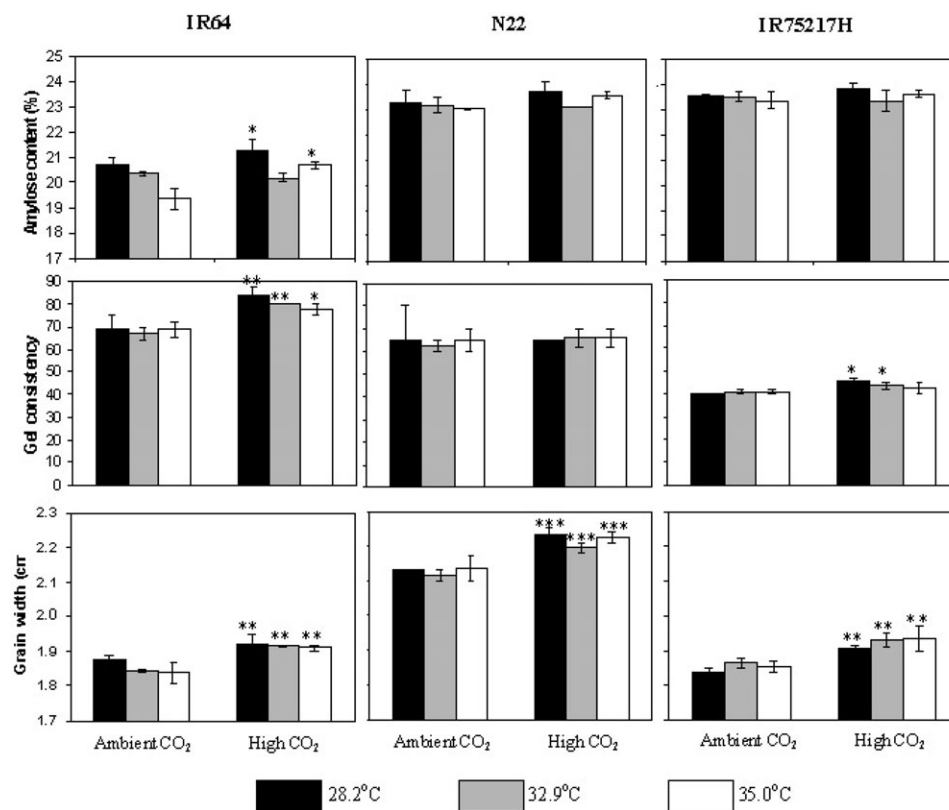


Fig. 3. Amylose content, gel consistency, and grain width for IR64, N22, and IR75217H after exposure to high temperature for five consecutive days starting on the first day of anthesis in combination with ambient and elevated [CO₂]. Bars indicate \pm SE. ***, **, and * indicate significance at 0.1%, 1%, and 5%, respectively.

growth rates or yield potentials at higher [CO₂] and also high temperatures, particularly in the case of the hybrid, cannot compensate for the direct effects of high temperature at anthesis on spikelet fertility and these episodes will remain a major constraint in the future as well as under current climates (Wheeler *et al.*, 1996; Wassmann *et al.*, 2009). The observed increase in individual kernel weight with increasing temperature is associated with greater spikelet sterility and hence compensation for a smaller sink size.

Another hypothesis concerned the effect of these treatments on quality traits, including broken grains, amylose, gel consistency, and chalkiness. Different concentrations of [CO₂] have not been shown previously to have an effect on quality, but temperature has (Fitzgerald and Resurreccion, 2009; Chen *et al.*, 2008). Grain development undergoes two main stages: cell enlargement and granule initiation, and starch and protein accumulation. Two alleles of the gene responsible for amylose content, are sensitive to high temperature (Chen *et al.*, 2008). IR64 carries one of these sensitive alleles (M Fitzgerald, unpublished data), and the amylose concentration of the other two indicates that they probably carry the temperature-tolerant allele. The enzyme responsible for amylose synthesis, Granule Bound Starch Synthase (GBSS) I, expresses strongly at 5 d after flowering (Hirose and Terao, 2004), and the short bursts of high temperature obviously affected that expression at ambient and high [CO₂]. Such responses to short bursts of heat

could explain the degree of season-to-season variability in amylose concentration within the low and intermediate classifications of amylose, which are both under the control of the temperature-sensitive alleles (Larkin and Park, 1999; Chen *et al.*, 2008). However, the negative effect on chalkiness at high temperature was not captured in this experiment since peak starch granule formation generally occurs >5 d after anthesis and hence after the high temperature episode (Fitzgerald and Resurreccion, 2009). The small differences in quality are likely to lead to small differences in texture of the cooked rice.

The increase in grain width seen in the high [CO₂] treatments suggests that substrate supply to the panicle was higher at elevated [CO₂] or that the spikelets were larger, allowing wider grains to develop. This increase in width is likely to explain the decrease in broken grains at high [CO₂], since wider grains are more capable of withstanding the frictional forces of milling (Jindal and Seibenmorgen, 1994). High temperature overrode the beneficial [CO₂] effect on broken grains, which suggests that temperature had an effect on the internal structure of the grain. High temperature at the early stages of filling can lead to a decrease in the number of granules initiated (Fitzgerald and Resurreccion, 2009), which could lead to insufficient capacity to synthesize densely packed grains, leading to a more fragile grain that breaks more easily. Taken together, these data indicate that [CO₂] has small positive effects on some quality traits,

but all advantage is lost with high-temperature stress, even with only short bursts during the early stage of grain development.

Our final hypothesis tested whether the hybrid responded quantitatively the same as the conventional genotype IR64. N22, as expected, was clearly more tolerant of high temperature and seed-set declined less with high temperature (-4.4% , $^{\circ}\text{C}^{-1}$) in this genotype compared with the hybrid (-8.8% , $^{\circ}\text{C}^{-1}$) and IR64 (-9.8% , $^{\circ}\text{C}^{-1}$). N22 has been shown to be highly tolerant of high temperatures in many studies (Yoshida *et al.*, 1981; Prasad *et al.*, 2006; Jagadish *et al.*, 2008, 2010a). With regard to the hypothesis on the response of the hybrid compared with IR64, there was no evidence that the response of seed-set in the hybrid to temperature at ambient or high $[\text{CO}_2]$ was any different from that of the other genotypes. However, it is highly possible that the same diversity seen with the two inbreds is also present in hybrids. Higher spikelet numbers at 29 $^{\circ}\text{C}$ and 35 $^{\circ}\text{C}$ in the hybrid did translate into higher seed numbers and hence higher main-tiller seed yields. This might suggest that, at moderately warm temperatures, just above the typical critical temperature for seed-set of about 33–35 $^{\circ}\text{C}$ (Yoshida *et al.*, 1981; Nakagawa *et al.*, 2002; Jagadish *et al.*, 2010a), the greater yield potential of the hybrid (evidenced by greater spikelet number) is still advantageous. At 38 $^{\circ}\text{C}$ any advantages of hybrid vigour were lost. Given the much greater spikelet production of the hybrid at 35 $^{\circ}\text{C}$ and 38 $^{\circ}\text{C}$ (>150 spikelets compared with <100 spikelets per main tiller), introgressing the heat-tolerance genes of N22 (Jagadish *et al.*, 2010a) or another heat-tolerant donor should provide a substantial yield benefit under warming environments.

In conclusion, the tested hybrid was highly susceptible to high temperatures coinciding with anthesis and neither elevated $[\text{CO}_2]$ nor the generally accepted hybrid vigour alleviated the sensitivity, resulting in low seed-set and poor quality grain. N22, the heat-tolerant *aus* check variety, by contrast, maintained its tolerance of high temperatures even under elevated $[\text{CO}_2]$. Elevated $[\text{CO}_2]$ did not compensate for the negative effects of high temperature on yield. With poor grain quality already being a major bottleneck for the wider adoption of hybrids, further deterioration of quality under predicted future climates could result in slowing the adoption rate of hybrids, which has serious repercussions for sustaining rice yields. Hence, steps to address reduced seed-set and grain quality among inbreds, and more so for hybrids under high temperature and elevated $[\text{CO}_2]$ conditions, are needed to sustain rice yields under future climates.

Supplementary data

Supplementary data can be found at *JXB* online.

Supplementary Table S1. Effect of $[\text{CO}_2]$ and temperature on growth and yield parameters of two rice genotypes and a hybrid.

Supplementary Table S2. Effect of ambient and elevated CO_2 with high temperatures coinciding with anthesis for five consecutive days on grain width and length, chalkiness and broken grain (excluding N22).

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