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RESEARCH PAPER

Effect of carbohydrates and night temperature on night respiration in rice

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Abstract

Global warming causes night temperature (NT) to increase faster than day temperature in the tropics. According to crop growth models, respiration incurs a loss of 40–60% of photosynthate. The thermal sensitivity of night respiration (R_n) will thus reduce biomass. Instantaneous and acclimated effects of NT on R_n of leaves and seedlings of two rice cultivars having a variable level of carbohydrates, induced by exposure to different light intensity on the previous day, were investigated. Experiments were conducted in a greenhouse and growth chambers, with R_n measured on the youngest fully expanded leaves or whole seedlings. Dry weight-based R_n was 2.6-fold greater for seedlings than for leaves. Leaf R_n was linearly related to starch (positive intercept) and soluble sugar concentration (zero intercept). Increased NT caused higher R_n at a given carbohydrate concentration. The change of R_n at NT increasing from 21 °C to 31 °C was 2.4-fold for the instantaneous response but 1.2- to 1.7-fold after acclimation. The maintenance component of R_n (R_m'), estimated by assimilate starvation, averaged 28% in seedlings and 34% in leaves, with no significant thermal effect on this ratio. The acclimated effect of increased NT on R_m' across experiments was 1.5-fold for a 10 °C increase in NT. No cultivar differences were observed in R_n or R_m' responses. The results suggest that the commonly used Q10=2 rule overestimates thermal response of respiration, and R_n largely depends on assimilate resources.

Key words: Acclimation, *Oryza sativa* L., maintenance and growth respiration, Q10, shading, soluble sugars, starch.

Introduction

Mitochondrial respiration is an important component of plant and crop carbon balance and productivity, fuelling growth and maintenance processes (Penning de Vries, 1972; McCree, 1982; for a review, see Amthor, 2000). It is also a limiting factor for biomass production because it consumes a large fraction of available assimilates. While growth respiration (R_g) is mainly determined by the quantity and chemical composition of the new biomass generated (McCree, 1970; Penning de Vries *et al.*, 1989), maintenance respiration (R_m) covers the costs of keeping existing tissues functional (McCree, 1970; Thornley, 1970; Penning de Vries, 1975; Penning de Vries *et al.*, 1989). As such, R_m increases with the weight of the organism and its metabolic activity, and is described as increasing exponentially with temperature (McCree, 1970). This has two important consequences: (i) non-woody plants can only attain a finite weight at which photosynthesis and the R_m burden break even; and (ii) at higher temperatures, such as those caused by global warming, the attainable biomass of crops becomes smaller.

Many studies confirmed that respiration rates (R) increase under elevated night temperature (NT) in diverse species including *Heteromeles arbutifolia* and *Lepechinia fragans* (Villar *et al.*, 1995), soybean (Bunce, 2005; Frantz *et al.*, 2004), lettuce and tomato (Frantz *et al.*, 2004), and rice (Cheng

et al., 2009; Kanno et al., 2009; Kanno and Makino, 2010; Mohammed and Tarpley, 2010). Whether or not the yield decline reported by Peng et al. (2004), Nagarajan et al. (2010), and Welch et al. (2010) can be attributed to NT effects on R is unclear because many environmental variables vary along with NT and temperature effects on R do not always affect crop biomass (Peraudeau et al., 2015). Crop biomass and yield may be unaffected by increased NT because it probably also affects other processes such as the photosynthetic rate which increased as reported for *Populus deltoides* (Turnbull et al., 2002) and rice (Kanno et al., 2009), or not, as reported for lettuce, tomato, and soybean (Frantz et al., 2004), and rice (Mohammed and Tarpley, 2010); and plant phenology which is under thermal control and also affects productivity (Yin et al., 1996).

Conditions that increase carbon assimilation and growth increase R. Indeed, R was reported to be positively correlated with soluble sugar (SS) and starch concentration in leaves of *Quercus rubra* (Whitehead et al., 2004) and wheat (Azcon-Bieto and Osmond, 1983). Dark or night R are thus, at least in part, driven by assimilate availability, but it is unclear to what extent this reflects the energy demand for stimulating growth (R_g) when resources abound, or wasteful respiration directly caused by substrate abundance (Thornley,1971). Night respiration (R_n) of crop canopies was reported to be proportional to canopy nitrogen content, the linear correlation being the same for several developmental stages and agronomic practices (Ingram et al., 1991). Nitrogen is also the driver of photosynthetic potential (Archontoulis et al., 2011). No such generic correlation was found between R_n and plant dry matter, indicating that R_n is a function of metabolic activity rather than of biomass *per se*.

Physiological plant models (e.g. ORYZA 2000; Bouman et al., 2001) divide respiration into R_g and R_m, where R_g is not directly driven by temperature because it depends on growth and, ultimately, resource acquisition. According to van Iersel and Seymour (2000), 1.39 g of glucose is needed to produce 1 g of non-lignified plant dry weight, involving 28% loss of the assimilate invested through R_g, regardless of temperature. In contrast, R_m, which is required to maintain ionic gradients, turnover of membrane components, and molecular housekeeping within cells (Penning de Vries, 1975), increases exponentially with temperature according to Q10=2, resulting in doubling in R_m for each 10 °C increase in temperature (Atkin and Tjoelker, 2003). The instantaneous (without acclimation to temperature) exponential response of R to temperature was reported to be valid for large temperature ranges, namely from 5 °C to ~50 °C in *Eucalyptus pauciflora* (O'Sullivan et al., 2013) or 10 °C to 35 °C in semi-arid temperate steppe (Chi et al., 2013). However, in the context of global warming increasing NT faster than day temperature (IPCC, 2013), changes in NT predicted by climate models for South East Asia (Chotamonsak et al., 2011) are much smaller than these temperature ranges, and NT evolution involves acclimation because it is long term. Such effects on R are not well known. For rapid temperature changes, Q10 equals ~2 within the range of the species' thermal adaptation (Yoshida, 1981; Penning de Vries et al., 1989; Bouman et al., 2001), but is smaller under hot conditions (Yoshida, 1981; Atkin and Tjoelker, 2003). Other studies reported that after acclimation, Q10 for R dropped to between 1.35 and 1.55 in *Tagetes patula* (van Iersel, 2006) and to 1.77 for semi-arid temperate steppe (Chi et al., 2013), compared with values >2 without acclimation. Atkin and Tjoelker (2003) proposed two acclimation types for R to temperature. In Type I, R is constant at low temperature ranges regardless of growth temperature and responds to higher temperatures with a lower Q10 if plants are acclimated to them. In Type II, R is lower for warm-acclimated plants regardless of the temperature studied and Q10 does not necessarily change with growth temperature. The temperature dependency of R might thus be subjected to a moderating acclimation effect, but information is too limited to generalize. For lack of better evidence, many crop models use the Q10=2 paradigm for acclimated R_m, although it may give inaccurate predictions of the impact of global warming.

The objectives of this study were to characterize (i) instantaneous and acclimated effects of NT on R_n of rice leaves and whole seedlings under non-limiting water and nitrogen supply; and (ii) interactions of R_n with assimilate resources resulting from differential illumination on the day preceding measurement of R_n. Furthermore, an attempt was made to (iii) estimate the R_m component of R_n, considered to be equal to R when photosynthesis of the previous day approaches zero. The work was conducted in thermally controlled environments. The purpose was to provide information that may help in improving rice crop models that simulate R but face uncertainty on thermal response.

Materials and methods

The two cultivars (N22 and an indica rice hybrid) studied are different in terms of architecture (N22 is taller), growth duration (longer for the hybrid), and yield potential (greater for the hybrid) (Peraudeau et al., 2015). Furthermore, N22 is heat tolerant (Prasad et al. 2006; Jagadish et al., 2008). Cultivars were grown in three experiments. A greenhouse experiment (GH) was conducted in 2013 at CNRS, Montpellier (43°38'N, 3°51'E), France. Two artificially lit controlled-environment chamber experiments were carried out, one in 2013 (GC1) at the International Rice Research Institute (IRRI), Los Baños, Philippines and the second in 2014 (GC2) at the Agricultural Research Center for International Development (CIRAD), Montpellier, France.

Plant management

In GH and in GC2, seed dormancy was broken by exposure to 29 °C for 4 d. Pre-germinated seed was sown at two seedlings per pot and thinned to one plant after 1 week. Pots were arranged in flat basins with 5 cm water maintained throughout the experiment, plants being irrigated by capillarity. In GH, four tables were used per compartment, each containing 84 pots (3 litres). The tables were moved and re-oriented twice a week to control heterogeneity effects. In GC2, one table containing 150 pots (1 litre) was used. Pots were randomly rearranged three times a week to control chamber heterogeneity. In both GH and GC2, the commercial soil EGO 140 (17N-10P-14K, pH 5, Jiffy International AS, Norway) was used. In GH, Basacot 6 M+ (2g l^{-1}, 11N-9P-19K+2Mg, Compo, Germany) was added and incorporated before planting. Foliar manganese (2%) was applied whenever deficiency appeared.

In GC1, seed dormancy was broken by exposure to 50 °C for 3 d, followed by pre-germination and sowing in seedling trays. Fourteen-day-old seedlings were transplanted at one seedling per pot (7 litres) filled with clay loam soil and maintained in a greenhouse. Basal fertilizer was applied at 2-1-1 g of N-P-K per pot. Topdressing was done at 2.5 g pot^{-1} ammonium sulphate. Pots were flooded with 2 cm of water throughout the experiment. On 1 April 2013, pots were moved to controlled environments. Pots were frequently rearranged to control heterogeneity effects.

In the three experiments, nitrogen was not limiting, as confirmed by a chlorophyll meter (SPAD-502, Konica Minolta, Japan) (GH and GC1) or Kjeldahl analysis of leaf nitrogen content (GC2). The SPAD (Soil Plant Analysis Development) value in GH was 33 ± 2 (mean ±SD) for the hybrid and N22, and in GC1 it was 41 ± 2 for the hybrid and 38 ± 2 for N22. In GC2, seedling nitrogen content was 4.9 ± 0.4% (g g^{-1}) for the hybrid and 4.6 ± 0.3% (g g^{-1}) for N22.

Temperature treatments and relative humidity

In GH, two naturally lit independent compartments were used (6.2 m×6.2 m). Temperature was controlled continuously at 29 °C during the day, and 21 °C (control, T_C) or 26 °C (increased NT, T_I) at night.

In GC2, the same artificially lit compartment was used three times consecutively, namely from 25 March 2014 to 11 April 2014 for T_C, from 14 April 2014 to 2 May 2014 for T_I, and from 5 May 2014 to 22 May 2014 for high NT (T_H). Temperature treatments were 29 °C during the day, and 21, 25, and 29 °C at night for T_C, T_I and T_H, respectively.

In both GH and GC2, actual air temperature was measured with a PT1000 probe (Campbell Scientific, Logan, UT, USA) combined with a fan-cooled shield. Relative humidity was measured with an MP100 probe (Rotronic, Switzerland). Both probes were connected to a CR1000 data logger (Campbell Scientific).

In GC1, two artificially lit controlled-environment compartments were used simultaneously (3 m×3 m). Air temperature was controlled at 29 °C during the day, and 21 °C (T_C) and 26 °C (T_I) at night. Plants were transferred from a greenhouse to these environments at 32 d (hybrid) or 38 d (N22)s after sowing and then grew there continuously. Actual air temperature and relative humidity were measured with a MINCER (Micrometeorological Instrument for Near Canopy Environment of Rice, developed by the National Institute of Agrobiological Sciences, Japan; Yoshimoto *et al.*, 2012).

For the three experiments, the actual air temperature, relative humidity, and vapour pressure deficit (VPD) are presented in Table 1. The VPD was calculated based on the equation published by the Food and Agriculture Organization of the United Nations (Allen *et al.*, 1998).

Light treatments

In GH, photosynthetically active radiation (PAR) measured at canopy level with PAR sensors (SKP215, Campbell Scientific) was 26.0 mol m^{-2} d^{-1} (Table 1). During each day preceding the nights when R_n was measured, three light treatments were implemented, one control (PAR_C for control, 100%) and two using mosquito nets reducing incident PAR to 30% (PAR_M, medium shading) and 12% (PAR_S, severe shading) (Table 2).

In GC1, average PAR measured at canopy level with PAR sensors (3415FXSE Spectrum® Technologies, Inc.) was 17.3 mol m^{-2} d^{-1} (Table 1). During each day before the nights when R_n was measured, two light treatments were implemented, control (PAR_C, 100%) and mosquito nets reducing incident PAR to 4% (PAR_S) (Table 2).

In GC2, average PAR measured at canopy level with PAR sensors (SKP215, Campbell Scientific) was 22.2 mol m^{-2} d^{-1} (Table 1). During each day before the nights when R_n was measured, four light treatments were implemented, one control (PAR_C, 100%) and three using mosquito nets reducing incident PAR to 51% (PAR_L, low shading), 12% (PAR_S), and 0% (PAR_N, no light) (Table 2).

Gas exchange measurements

Leaf R_n measurements were performed during the night-time, at least 1 h after sunset or light extinction, with a gas analyser GFS-3000 (model 3000-C or 3100-C, Walz, Germany) combined with a gas exchange chamber 3010-GWK1 (Walz, Germany). The last fully expanded leaf was chosen to measure R_n, except in GC2 where the whole seedling was inserted in the chamber, designed with a large area for leaf measurement (14 cm×10 cm) to minimize signal noise by reducing air leakage and exposing a large leaf area. The temperature in the chamber was controlled at 21, 25, 26, or 29 °C according to the temperature treatment. Humidity was set to obtain a VPD of 1.2 kPa. Air flow and fan speed were optimized to avoid condensation and minimize noise (air flow ~1100 µmol s^{-1}). The time until stability of readings was reached was 6–15 min.

Respiration measurements were performed at different plant ages. Two sets of leaf respiration measurements in GH and GC1, and one set in GC2, were conducted for each temperature treatment. The period of R_n measurements is presented in Table 3.

Immediate temperature responses of R_n (temperature response curve) were performed: (i) in GH, 61–64 days after sowing (DAS) for the hybrid [2–5 d before panicle initiation (PI)] and 53–56 DAS for N22 (10–13 d after PI); and (ii) in GC1, 82 and 83 DAS for the hybrid (7 d and 8 d after PI) and 74 and 75 DAS for N22 (1 d and 2 d before flowering).

Ascending steps by 3 °C were used between 19 °C and 31 °C. Before and after this series of measurements, the leaf was exposed to 22 °C until steady state was observed, and R_n measured. This

Table 1. *Climatic data of experiments in the greenhouse (GH) in France, growth chambers (GC1) in the Philippines, and growth chambers in France (GC2)*

Experiment	Year	Temperature treatment	PAR (mol m^{-2} d^{-1})	Average temperature			Relative humidity	VPD (kPa)
				Day (°C)	Night (°C)	Δ*T*°C	Day (%)	
GH	2013	T_C	26.0 ± 9.1	29.1 ± 0.3	21.4 ± 1.4		63 ± 11	1.1 ± 0.4
		T_I		29.3 ± 0.7	26.5 ± 0.5	+5.1	56 ± 9	1.6 ± 0.3
GC1	2013	T_C	17.3 ± 0.0	27.0 ± 0.2	21.1 ± 0.1		78 ± 1	0.8 ± 0.1
		T_I		26.3 ± 0.2	24.7 ± 0.0	+3.6	79 ± 1	0.7 ± 0.1
GC2	2014	T_C	22.2 ± 3.7	28.9 ± 0.2	21.4 ± 0.1		73 ± 3	0.7 ± 0.1
		T_I		28.8 ± 0.5	24.9 ± 0.3	+3.5	77 ± 2	0.6 ± 0.1
		T_H		28.5 ± 0.3	29.0 ± 0.7	+7.6	72 ± 2	0.7 ± 0.1

Treatments: T_C, control temperature; T_I, increased night temperature; T_H, high night temperature; Δ*T*°C, average difference between night temperature in T_I or T_H and T_C. PAR, photosynthetic active radiation; VPD, vapour pressure deficit.
Mean values ±SD.

Table 2. *Average PAR at plant tops during the day before night respiration measurements in the greenhouse in France (GH), growth chambers at IRRI in the Philippines (GC1), and growth chambers in France (GC2)*

Experiment	Cultivar	Time to measurement	Temperature treatment	Light treatment (mol m^{-2} d^{-1})				
				PAR_C	PAR_L	PAR_M	PAR_S	PAR_N
GH	Hybrid	55 DAS	T_C	33.8±0.5	–	10.3±0.2	4.4±0.1	–
			T_I	33.8±0.5	–	9.7±0.2	3.6±0.1	–
		83 DAS	T_C	25.4±4.9	–	7.7±1.5	3.3±0.6	–
			T_I	25.4±4.9	–	7.3±1.4	2.7±0.5	–
	N22	47 DAS	T_C	29.6±2.6	–	9.0±0.8	3.8±0.3	–
			T_I	29.6±2.6	–	8.5±0.7	3.1±0.3	–
		75 DAS	T_C	22.7±10.4	–	6.9±3.2	6.5±3.0	–
			T_I	22.7±10.4	–	3.0±1.4	2.4±1.1	–
GC1	Both cultivars	Both set of measurements	T_C	25.7±0.0	–	–	1.0±0.0	–
			T_I	27.2±0.0	–	–	1.0±0.0	–
GC2	Both cultivars	Seedling	Three treatments	29.2±0.0	14.9±0.0	–	3.6±0.0	0.0±0.0

T_C, control temperature; T_I, increased night temperature; T_H, high night temperature; PAR_C (100%); PAR_L (51%); PAR_M (30%); PAR_S (12% in GH and GC2; 4% in GC1); PAR_N (0%).
Mean values ±SD.

Table 3. *Identification of series of measurements, days after sowing for measurements (DAS), and physiological stage when respiration measurements were performed*

Experiment	Cultivar	Identification	DAS	Physiological stage
GH	Hybrid	55 DAS	54–57	9–12 d before PI
		83 DAS	82–85	13–16 d before flowering
	N22	47 DAS	46–49	3–6 d before PI
		75 DAS	74–77	1–4 d after flowering
GC1	Hybrid	49 DAS	47–52	23–28 d before PI
		75 DAS	70–79	5 d before to 4 d after PI
	N22	49DAS	47–52	4 d before to 1 d after PI
		66 DAS	61–71	5–15 d before flowering
GC2	Both cultivars	22 DAS	18–22	Seedling stage

PI, panicle initiation.

standard procedure was applied to all plants regardless of NT treatment. Respiration rates observed at 22 °C before and after the temperature response measurements were not significantly different.

For GH and GC1, leaf area-based R_n was converted into leaf dry matter-based R_n using measurements of specific leaf area (SLA) of the same portion of the leaf.

In GC2, R_n of whole seedlings was measured on a dry matter basis only, as the leaf area had not been measured.

Non-structural carbohydrate analysis

Two different techniques were used to analyse the non-structural carbohydrate (NSC) content, according to the facilities available for GC1 at IRRI (Philippines) and for GH at CIRAD (France).

In GC1, leaf samples were treated with a heat burst in a microwave for 1 min (Pelletier *et al.*, 2010) to stop enzymatic reactions and then dried at 70 °C for 48 h. In GH, leaf samples were submersed in liquid nitrogen, conserved at –30 °C, and then freeze-dried. In both experiments, leaf samples were ground finely before NSC extraction.

In GC1, 10 mg of dry powder was extracted three times with 0.7 ml of ethanol (80% v/v) for 10 min at 80–85 °C. The supernatant after each extraction was recovered by centrifugation. After the last extraction, the pellet was washed with 0.5 ml of 80% ethanol. All supernatants were transferred to a test tube and the combined volume was adjusted to 2.5 ml with 80% ethanol. SS content was determined by a colorimetric method with anthrone reagent (Yoshida *et al.*, 1976). The remaining ethanol-insoluble residue was dried at 70 °C for 24 h. Then, 0.2 ml of water was added and the tube was placed in a boiling water bath for 15 min. After cooling, 0.2 ml of 9.2 N $HClO_4$ was added. The solution was stirred occasionally for 15 min, and the suspension adjusted to a volume of 0.6 ml with water, and centrifuged for 10 min at 3000 rpm. The supernatant was transferred to a test tube and 0.2 ml of 4.6 N $HClO_4$ was added. These steps were repeated twice, the residue washed with 0.5 ml of water, the supernatants combined, and the volume adjusted to 5 ml with water. The glucose content, equivalent to the starch content before hydrolysis, was measured by a colorimetric method with anthrone reagent (Yoshida *et al.*, 1976).

In GH, 20 mg of dry powder was extracted three times with 1 ml of ethanol for 30 min at 75 °C. After each extraction the supernatant was recovered by centrifugation (10 min, 10 000 rpm) and transferred to a test tube. Then, 0.5 ml of ethanol (80% v/v) was added to the remaining ethanol-insoluble residue and stored at –30 °C. Combined supernatants were filtered through a column composed of 50 mg of polyvinyl polypyrrolidone and 50 mg of activated charcoal prepared in 80% ethanol. After filtration, the column was washed with 1 ml of ethanol (80%). Ethanol of the filtrate was evaporated and 1 ml of distilled water added to dissolve the dry residue. Quantification of SSs was done by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC). Tubes containing the ethanol-insoluble residue were thawed and centrifuged (5 min, 10 000 rpm). Supernatants were discarded and the pellets solubilized using 1 ml of 0.02 N NaOH solution at 90 °C for 90 min and stirred every 30 min. Starch was hydrolysed to glucose by addition of 100 µl of amyloglucosidase (5 mg ml^{-1}) solution and incubated at 50 °C for 90 min. Glucose was quantified by enzymatic assay with hexokinase/glucose-6-phosphate dehydrogenase (Bergmeyer *et al.*, 1974).

Statistical analysis

The significance of the effect of cultivar (C), temperature (T), and light (L) treatments on leaf respiration, leaf starch content, and leaf SS content was analysed using a two- or three-way analysis of variance (ANOVA) with R (version 2.15.2, R Foundation for Statistical

Computing). Where requirements for ANOVA were not satisfied, especially for residual normality, the non-parametric test of Kruskal and Wallis was performed using the same software.

Results

Relationship between leaf respiration rate and leaf starch or soluble sugar content

Leaf night respiration rate was significantly, positively, linearly correlated with leaf starch and SS content in GH at 55 DAS for the hybrid and 47 DAS for N22 (Fig. 1). Correlations were significant at $P<0.001$ (Pearson's test), except for N22 for T_C with $P<0.01$ (starch) and $P<0.05$ (SS) (Table 4). Similar results were observed in GH at 75 DAS for N22 (the hybrid was non-significant at 83 DAS) and in GC1 for both cultivars (data not shown). Thus, there was a consistent pattern of R_n being proportional to leaf starch and SS concentration across developmental stages, experiments, and treatments, although slopes of the correlation varied (Table 4).

In all cases, decreased light intensity during the day before R_n measurement caused a decrease in leaf starch and SS content that was about proportional to the decrease in R_n (Fig. 1A–D). The correlations observed between R_n and starch or SS were greater for the hybrid ($r^2=0.75$ for R_n versus starch and 0.62 for R_n versus SS) than for N22 ($r^2=0.51$ for R_n versus starch and 0.40 for R_n versus SS). The correlations observed between R_n and starch was greater than those between R_n and SS for both genotypes (Fig. 1; details of statistics in Table 4).

The slope of the correlations between R_n and starch or SS across the different levels of the previous day's light was greater under T_I than under T_C (Fig. 1E–H; Table 4). In all cases, slopes and determination coefficients were greater under T_I than under T_C for both cultivars, in both GH and GC1 experiments. Consequently, higher temperatures increased the leaf respiration rate per unit of leaf starch or SS concentration.

Effect of previous day's radiation level and night temperature on leaf respiration rate

Leaf (GH and GC1) and whole seedling (GC2) R_n were significantly lower when plants were exposed to low light during the day before R_n measurement, for both cultivars ($P<0.001$; Figs 2, 3; Table 5).

GH experiment, hybrid

At 55 DAS, R_n of the hybrid was significantly lower for PAR_M and PAR_S than for PAR_C ($P<0.05$; Fig. 2; Table 5). A significant interaction between light and temperature on R_n was found ($P<0.01$). Thus, R_n for PAR_C under T_I was significantly greater than under T_C ($P<0.05$), whereas for PAR_M and PAR_S no significant differences were observed among temperature treatments. At 83 DAS, R_n was significantly lower for PAR_S than for PAR_C, but no significant difference was observed under T_I between PAR_C and PAR_M (Table 5). The R_n was

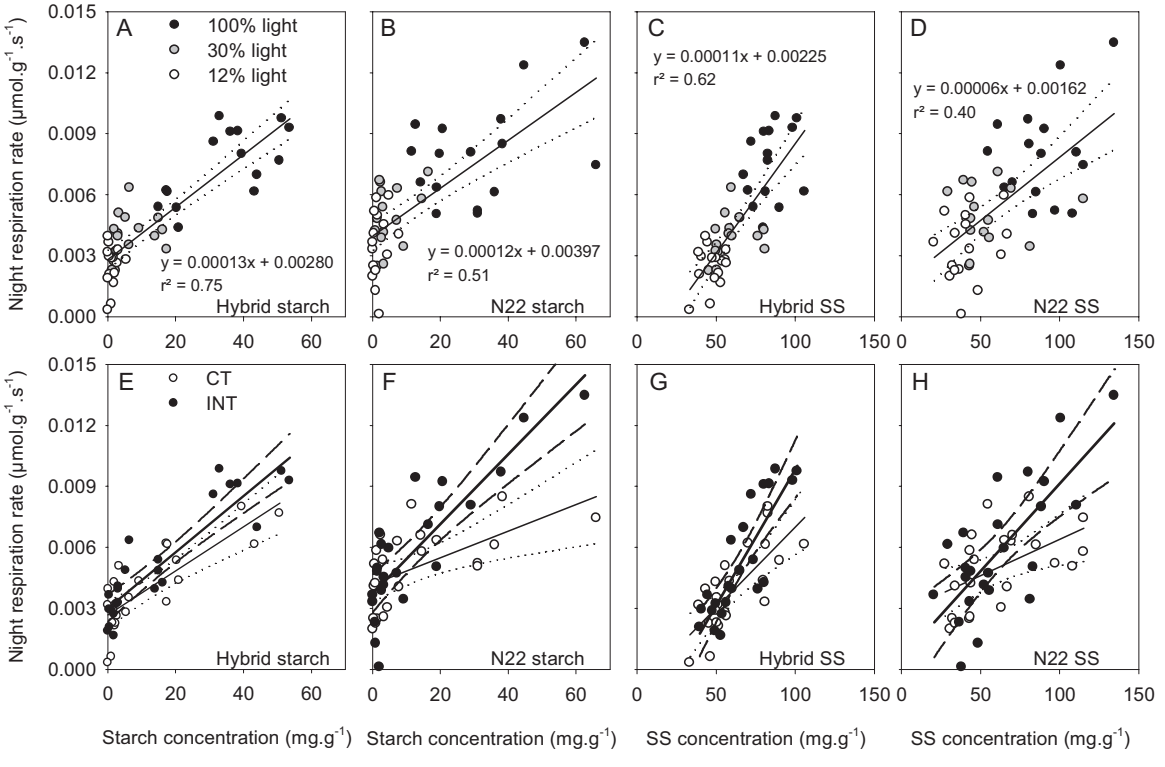

Fig. 1. Leaf night respiration rate in relation to starch (A–F) and SS (soluble sugar; C–H) concentrations for the hybrid (A, C, E, G) and N22 (B, D, F, H) rice cultivars at 55 DAS and 47 DAS, respectively, in the GH experiment. Upper four graphs: observations grouped by light treatments with 100%, full light (black symbols); 30%, intermediate shading (grey); and 12%, severe shading (white). Dotted lines represent the confidence interval of linear regression (95%). Lower four graphs: observations grouped by temperature treatment with T_C, control at 21 °C (white symbols); and T_I, increased night temperature at 26 °C (black). Linear regression lines are shown for T_C and T_I treatments.

Table 4. *Coefficients of linear regressions* (y=ax+b) *between leaf respiration rate and starch or soluble sugar content*

Cultivar	Code	Starch or soluble sugar	Temperature	No. of measurements	Linear regression *y=ax+b*			
					a	*b*	r^2	Significance
GH								
Hybrid	55 DAS	Starch	T_C	23	1.1E-04	2.7E-03	0.65	***
			T_I	24	1.4E-04	3.0E-03	0.82	***
		Soluble sugar	T_C	23	0.8E-04	–1.0E-03	0.56	***
			T_I	24	1.3E-04	–3.5E-03	0.72	***
N22	47 DAS	Starch	T_C	23	0.7E-04	4.2E-03	0.34	**
			T_I	24	1.7E-04	3.7E-03	0.73	***
		Soluble sugar	T_C	23	0.4E-04	2.8E-03	0.26	*
			T_I	24	0.9E-04	0.6E-03	0.54	***
GC1								
Hybrid	75 DAS	Starch	T_C	24	3.9E-04	0.7E-03	0.45	***
			T_I	24	4.4E-04	1.4E-03	0.48	***
		Soluble sugar	T_C	24	1.7E-04	0.2E-03	0.44	***
			T_I	24	3.6E-04	–1.4E-03	0.63	***
N22	66 DAS	Starch	T_C	24	3.5E-04	1.7E-03	0.46	***
			T_I	24	4.4E-04	2.3E-03	0.47	***
		Soluble sugar	T_C	24	2.1E-04	1.3E-03	0.53	***
			T_I	24	4.0E-04	–0.3E-03	0.60	***

T_C, control temperature; T_I, increased night temperature; T_H, high night temperature.
Level of significance is expressed as *, **, ***, for $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

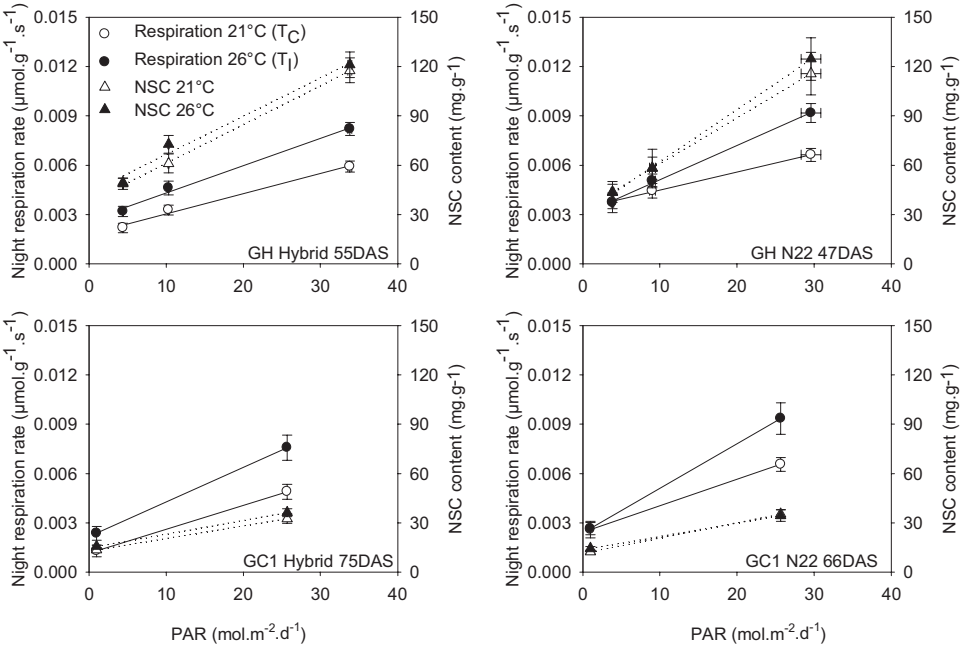

Fig. 2. Relationship between leaf night respiration rate (circles) and non-structural carbohydrates (NSC, triangles) versus PAR (photosynthetically active radiation) for the hybrid and N22 rice cultivars grown under 21 °C (open symbols) or 26 °C (filled symbols) night temperature. Upper graphs: the GH experiment at 55 DAS for the hybrid and 47 DAS for N22. Bottom: the GC1 experiment at 75 DAS for the hybrid and 66 DAS for N22. Error bars=SE of 16 measurements for the hybrid and 13 for N22 in GH; and 12 measurements in GC1. Error bars of NSC=SE of 8 measurements (GH) and 12 measurements (GC1).

significantly greater under T_I than under T_C for PAR_C and PAR_M light treatments ($P<0.05$).

GH experiment, cv. N22

Similar to the hybrid, at 47 DAS, N22 showed a significantly lower R_n for PAR_M and PAR_S than for PAR_C ($P<0.05$), and a higher R_n under T_I than under T_C for PAR_C only (interaction light×temperature) (Fig. 2; Table 5). At 75 DAS, R_n of N22 was significantly lower for PAR_S than for PAR_C, and no significant difference was observed under T_C between PAR_C and PAR_M (Table 5). However, unlike for the hybrid, no significant difference of R_n was observed between the NT treatments for

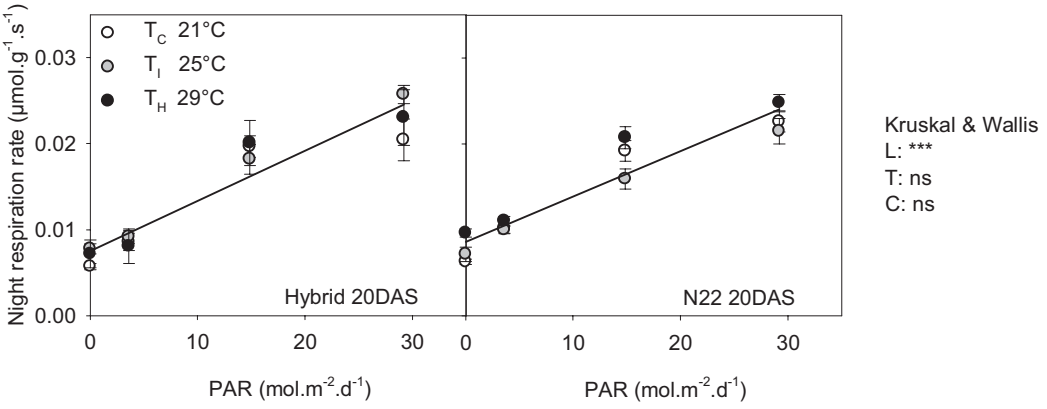

Fig. 3. Relationship of night respiration rate on whole seedlings versus PAR (photosynthetically active radiation) for the hybrid and N22 rice cultivars grown under 21 °C (white symbols), 25 °C (grey), or 29 °C (black) night temperature. T_C, control temperature; T_I, increased night temperature; T_H, high night temperature. Different light treatments during the previous day: PAR_C (100%); PAR_L (51%); PAR_M (30%); PAR_S (12%); PAR_N (0%). Level of significance is expressed as *, **, ***, and ns for $P<0.05$, $P<0.01$, $P<0.001$, and $P>0.05$, respectively, using Kruskal and Wallis non-parametric test. Error bars=SE of 6 measurements.

Table 5. *Results of three-way ANOVA for the factors light treatment (L), night temperature treatment (T), and cultivar (C) on night respiration rate (R_n) and leaf non-structural carbohydrate (NSC) content*

Experiment/series of measurement										Hybrid		N22	
GH		**L**	**T**	**C**	**L×T**	**C×T**	**L×C**	**L×C×T**		T_C	T_I	T_C	T_I
55 DAS hybrid	R_n	***	***	***	**	NS	NS	NS	PAR_C	cd	ab	bc	a
47 DAS N22									PAR_M	ef	de	de	cde
									PAR_S	f	ef	ef	ef
GH	NSC	***	NS	*	NS	NS	NS	NS	PAR_C	a	a	ab	a
83 DAS Hybrid									PAR_M	cd	bc	cd	cd
75 DAS N22									PAR_S	cd	cd	d	d
	R_n	***	*	NS	NS	***	NS	NS	PAR_C	bcd	a	ab	ab
									PAR_M	e	abc	bcde	de
									PAR_S	e	bcde	cde	e
	NSC	***	NS	***	NS	NS	NS	NS	PAR_C	a	a	bcde	abcd
									PAR_M	ab	a	def	cdef
									PAR_S	ab	abc	ef	f
										Hybrid		**N22**	
GC1		**L**	**T**	**C**	**L×T**	**C×T**	**L×C**	**L×C×T**		T_C	T_I	T_C	T_I
49 DAS hybrid	R_n	***	NS	NS	/	/	/	/	PAR_C	/	/	/	/
49 DAS N22									PAR_S	/	/	/	/
GH	NSC	***	NS	NS	NS	NS	NS	NS	PAR_C	a	a	a	a
75 DAS Hybrid									PAR_S	b	b	b	b
66 DAS N22	R_n	***	***	**	**	NS	NS	NS	PAR_C	cd	ab	bc	a
									PAR_S	e	e	de	de
	NSC	***	NS	NS	NS	NS	NS	NS	PAR_C	a	a	a	a
									PAR_S	b	b	b	b

T_C, control temperature; T_I, increased night temperature; T_H, high night temperature.
Plants were grown under three intensities of light during the day preceding R_n: PAR_C (100%); PAR_M (30%); PAR_S (12% in GH and 4% in GC1).
Annotations *, **, ***, and NS stand for $P<0.05$, $P<0.01$, $P<0.001$, and $P>0.05$, respectively.
Different lower case letters show significant difference ($P<0.05$) according to post-hoc testing of Tukey-HSD.
'/' stands for 'unknown' in cases where normality of residue of ANOVA was not observed.

N22 ($P>0.05$), resulting in a significant interaction between temperature and cultivar ($P<0.001$; Table 5).

GC1 experiment, hybrid

Results at 75 DAS were similar to those of the GH experiment, but at 49 DAS no significant temperature effect on R_n was observed. At both temperatures, significantly lower R_n was observed for PAR_S than for PAR_C (at 75 DAS, Tukey HSD post-hoc test, $P<0.05$; Table 5). At 75 DAS, R_n was significantly higher under T_I than under T_C ($P<0.05$) for PAR_C only, resulting in an interaction light×temperature.

GC1 experiment, cv. N22

Compared with the hybrid, N22 showed similar results with a significant decrease in R_n for PAR_S (at 49 and 66 DAS) and a significant increase in R_n under T_I for PAR_C only at 66 DAS (Fig. 2; Table 5).

GC2 experiment: whole-plant light response of R_n

Four light level treatments, including zero light on the day preceding R_n measurement, were applied to whole seedlings acclimated to three NTs (Fig. 3). The response was linear and near-identical between cultivars and also near-identical among temperature levels. A common intercept on the *y*-axis for the R_n versus PAR correlations was observed, describing R_n in the absence of light, and photosynthesis, on the previous day.

Synopsis of GH, GC1, and GC2 experiments

Overall, in both GH and GC1 experiments, reduced light level on the previous day strongly reduced R_n, regardless of NT. Increased NT increased leaf-level R_n in GH and GC1, resulting in a mostly additive effect of light and temperature on R_n, although factor interactions were also present in some data sets. The two genotypes behaved similarly in terms of absolute R_n and its responses. For whole-seedling R_n, light effects were dominant and linear, with no effects of temperature and cultivar observed. An increase in R_n by a factor of 1.14–1.67 under PAR_C (1.17–1.58 all light treatment combined) was calculated between 21 °C and 31 °C for the three experiments combined when applying the assumption of linear response of R_n to NT. R_n increased by a factor of 1.14–1.82 under PAR_C (1.18–1.75 all light treatments combined) when applying the assumption of an exponential response of R_n to NT.

Effect of treatments and cultivar on leaf non-structural carbohydrate content

Leaf NSC (sum of starch and SS) concentration decreased similarly after reduced light treatment in GH and GC1 experiments ($P<0.001$; Fig. 2; Table 5), but no such measurements were made on the whole seedlings in GC2. No interaction between NT, light treatment, and cultivar on leaf NSC content was observed, indicating additive factor effects.

GH experiment

Leaf NSC content for PAR_C light treatment was significantly greater than for PAR_M and PAR_S ($P<0.05$; Table 5) at 55 DAS for the hybrid and 47 DAS for N22, but no significant difference was observed between PAR_M and PAR_S. However, for the later sampling dates (83 DAS for the hybrid and 75 DAS for N22), no significant difference in leaf NSC content was observed between the three light treatments, except for N22 under T_I where leaf NSC content was larger for PAR_C than for PAR_S ($P<0.05$; Table 5).

In contrast to the light factor, NT did not affect leaf NSC regardless of cultivar ($P>0.05$; Table 5).

There were significant cultivar differences in NSC content. For the later sampling, the hybrid had significantly greater NSC content than N22 ($P<0.001$). At the earlier sampling (55 DAS for the hybrid and 47 DAS for N22), cultivars also differed significantly in leaf NSC, with hybrid leaves containing higher concentrations than N22 leaves ($P<0.05$).

GC1 experiment

In all cases in GC1, leaf NSC content for PAR_C was significantly greater than for PAR_S ($P<0.05$; Table 5). Similar to the GH experiment, NT did not affect leaf NSC in GC1. Concentrations were generally lower than in the GH experiment, possibly related to different growth conditions.

Instantaneous leaf respiration response to temperature

In both GH and GC1, instantaneous temperature response curves of leaf R_n were measured on plants acclimated to the different NT treatments and pre-treated with different light levels on the preceding day (Fig. 4; Table 6). Temperature responses (between 19 °C and 31 °C at steps of 3 °C) were strictly linear and no hysteresis was observed, as indicated by unchanged R_n observed at 22 °C before and after the response cycle (not presented). In all cases, coefficients of determination (r^2) were always ≥0.98 (Table 6).

In both GH and GC1, the slopes of the linear R_n versus temperature correlations were highly significantly different among light levels ($P<0.001$) in both experiments, whereas effects of NT treatments and cultivars were only observed in the GH experiment (Table 6). An increase in R_n by an average factor of 2.4 under PAR_C (same factor for all light treatments combined) was calculated between 21 °C and 31 °C (cultivars, experiments, and temperature conditions confounded).

The differences in R_n versus T slopes, regardless of the treatment factor responsible for them, did not change the intercept on the *x*-axis where the curves converged. This intercept can be interpreted as a non-acclimated base temperature (T_b). The thus estimated T_b for R_n (Table 6) averaged at 12.9 °C for GH and 14.3 °C for GC1. It was on average 13.9 °C for the hybrid and 13.3 °C for N22.

Estimating maintenance respiration

Maintenance respiration (R_m) was estimated as the intercept on *y* of the correlation of R_n versus PAR applied on the previous day (GH and GC1), or as the value of R_n measured when PAR on the previous day was zero (GC2). Figures 2 and 3 show examples of such correlations. Because of the uncertainty surrounding R_m, this variable was called R_m'.

Observed ranges of R_m' were between 0.0017 and 0.0031 μmol g^{-1} s^{-1} in GH, 0.0011 and 0.0044 μmol g^{-1} s^{-1} in GC1, and 0.0057 and 0.0096 μmol g^{-1} s^{-1} in GC2 (Fig. 5). On average, R_m' was about twice higher in GC2 than in the two other experiments. Plants in GC2 were much younger (seedling stage) and measurements were conducted on whole plants, as opposed to sections of fully grown leaves in GH and GC1.

Error terms were large for R_m' because of error accumulation from the many measurements constituting the R_m' estimate (Fig. 5). NT treatments had no significant impact on R_m' observed in GH and GC1 ($P>0.05$). In GC2, a significant

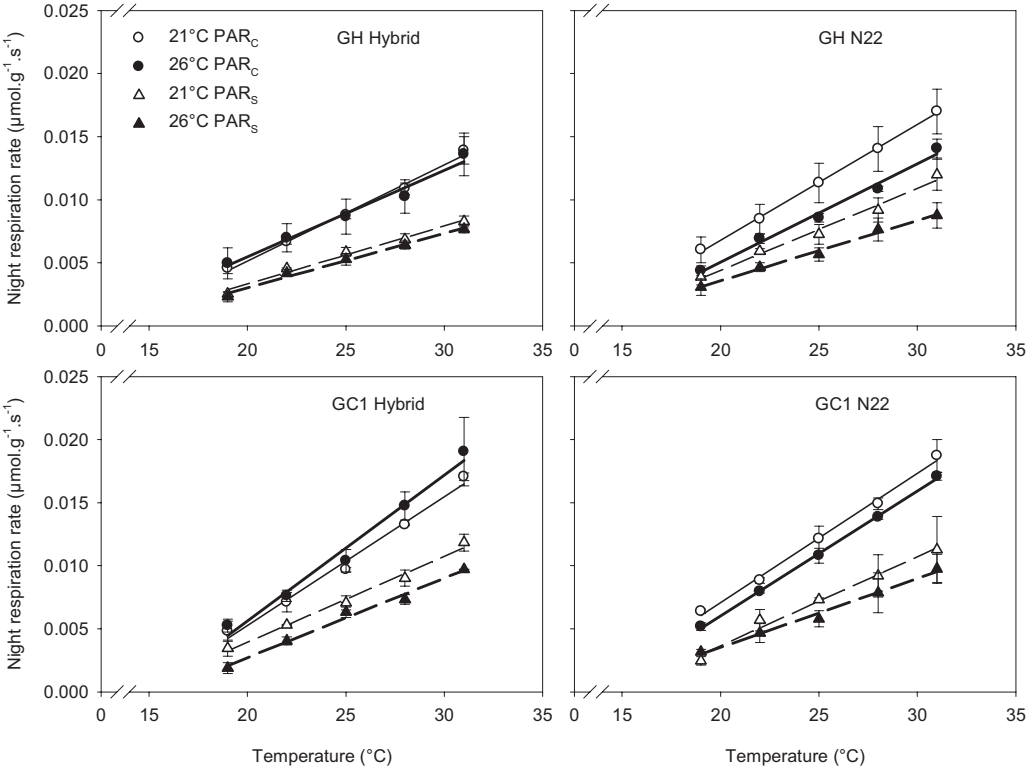

Fig. 4. Immediate night leaf respiration response curves to temperature for the hybrid and N22 rice cultivars exposed to T_C (21 °C, open symbols) or T_I (26°C, filled symbols) under PAR_C (circles, 100%) or PAR_S (triangles, 12% in GH and 4% in GC1) in GH and GC1 experiments. Linear regressions are represented by a fine solid line (21 °C PAR_C); thick solid line (26 °C PAR_C); fine dashed line (21 °C PAR_S); and thick dashed line (26 °C PAR_S). Linear regression ($y=ax+b$) details are show in Table 6. Error bars of respiration response curves to temperature=SE of 2 and 4 replications for GC1 and GH, respectively.

Table 6. *Characteristics of linear regression of instantaneous night respiration rate (µmol g^{-1} s^{-1}) responses to temperature between 19 °C and 31 °C*

Site	Cultivar	Temperature	Light	Linear regression: $y=ax+b$			Apparent T_{base}	Slope (a) analysis ANOVA
				a	*b*	r^2		
GH	Hybrid	T_C	PAR_C	7.6E-04	–0.010	0.99	13.3	L: ***
			PAR_S	4.6E-04	–0.006	0.99	12.0	T: *
		T_I	PAR_C	6.9E-04	–0.008	0.98	12.8	C: **
			PAR_S	4.4E-04	–0.006	0.99	13.0	L×T: NS
	N22	T_C	PAR_C	9.2E-04	–0.012	1.00	12.6	T×C: NS
			PAR_S	6.5E-04	–0.009	0.99	13.2	L×C: NS
		T_I	PAR_C	7.8E-04	–0.011	0.99	13.5	L×T×C: NS
			PAR_S	4.8E-04	–0.006	0.99	12.5	
GC1	Hybrid	T_C	PAR_C	10.2E-04	–0.015	0.99	14.8	L: ***
			PAR_S	6.8E-04	–0.010	0.99	14.2	T: NS
		T_I	PAR_C	11.6E-04	–0.018	0.98	15.1	C: NS
			PAR_S	6.3E-04	–0.010	0.99	15.7	L×T:NS
	N22	T_C	PAR_C	10.3E-04	–0.013	0.99	13.1	T×C: NS
			PAR_S	7.1E-04	–0.010	0.99	13.9	L×C: NS
		T_I	PAR_C	9.9E-04	–0.014	1.00	13.9	L×T×C: NS
			PAR_S	5.5E-04	–0.007	0.99	13.5	

Plants were acclimatized to 21 °C (T_C) or 26 °C (T_I) at night.
Light intensities during the previous day were PAR_C (100%) and PAR_S (12% GH and 4% GC1).
Three-way ANOVA was performed for slope coefficients. Level of significance is expressed as *, **, ***, and NS for $P<0.05$, $P<0.01$, $P<0.001$, and $P>0.05$, respectively.

effect of NT was observed by ANOVA (P<0.05), but the post-hoc test of Tukey-HSD did not pick up a significant effect (Fig. 5).

The R_m' was not significantly different between the hybrid and N22 across all experiments for any given temperature treatment (P>0.05). The measured and instantaneous R_m' was converted to weight of CH_2O consumed per weight of plant dry matter during 1 d, while assuming that R_m' continued during the day and night (summary in Table 7). Neither daytime temperature effects on R_m' (which might increase it) nor photosynthesis effects (which might decrease it in leaves) were considered. Thus estimated values varied among experiments, growth stage, and treatments between 2.9 mg g^{-1} d^{-1} and 25 mg g^{-1} d^{-1}, or 0.29% and 2.50% weight loss per day (0.43–0.80% in GH, 0.29–1.15% in GC1, and 1.48–2.50% in GC2). Thus, R_m' represented 0.23–0.50 of R_n with an average of 0.33 for all experiments combined (Table 7).

In order to obtain a measure of temperature dependency of R_m' across all data sets, the relative R_m' was calculated as a fraction of values obtained at 21 °C (Fig. 6), which was the control treatment common to all experiments. This resulted in a four-point (including control), R_m' (rel.) versus NT relationship with NT values of 21, 25, 26, and 29 °C. Despite the large error, an approximately linear, positive relationship was found having a slope of 0.05 $°C^{-1}$. This slope would translate into a 1.49-fold (or +49%) increase of R_m' for an increase of temperature from 21 °C (control) to 31 °C (+10 °C).

Discussion

Major findings

Mitochondrial respiration, the main source of energy fuelling growth and maintenance processes in plants, can also be seen as a metabolic cost that limits carbon availability for crop productivity. This study investigated rice night respiration (R_n) of plants acclimated to different NTs. Because much of R_n is related to growth (Penning de Vries *et al.*, 1989; Amthor, 2000), and thus depends on assimilate availability, variation of radiation, on the preceding day, was included as a second

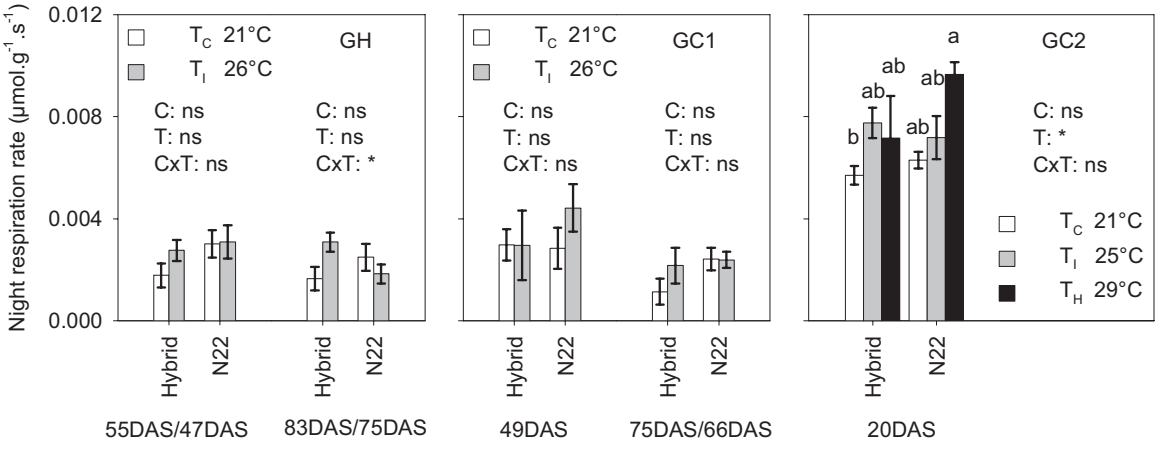

Fig. 5. Estimated maintenance respiration (R_m'); that is, night respiration rate under conditions of zero incident light on previous day, of leaves acclimated to different night temperatures (T) (T_C, control temperature, 21 °C; T_I, increased night temperature, 26 °C GH and GC1; 25 °C GC2; T_H, high night temperature, 29 °C); for the hybrid and N22 rice cultivars (C) in GH, GC1, and GC2 experiments. In GH and GC1, R_m' values were extrapolated from light response by linear regressions as in Fig. 2 for measurements on leaf segments, whereas in GC2, R_m' values were measured directly on whole seedlings having received no light on the previous day as in Fig. 3. Error bars=SE of three, four, and six replications for GC1, GH, and GC2, respectively.

Table 7. *Estimated maintenance respiration rates (R_m') expressed as CH_2O lost per unit dry matter per day and R_m'/R_n ratio for the hybrid and N22 rice cultivars exposed to different night temperatures (T_C, 21 °C; T_I, 26 °C GH and GC1; 25 °C GC2; T_H, 29 °C) in GH, GC1, and GC2 experiments*

Site	Cultivar	Code	R_m' (mg CH_2O g^{-1} d^{-1})			R_m'/R_n		
			T_C	T_I	T_H	T_C	T_I	T_H
GH	Hybrid	55 DAS	4.6±1.2	7.1±1.1	–	0.30	0.33	
		83 DAS	4.3±1.2	8.0±1.0	–	0.39	0.50	
	N22	47 DAS	7.8±1.4	8.0±1.7	–	0.45	0.34	
		75 DAS	6.4±1.4	4.8±1.0	–	0.50	0.38	
GC1	Hybrid	49 DAS	7.7±1.6	7.7±3.5	–	0.24	0.23	
		75 DAS	2.9±1.3	5.6±1.8	–	0.23	0.29	
	N22	49 DAS	7.4±2.1	11.5±2.4	–	0.26	0.28	
		66 DAS	6.3±1.1	6.2±0.8	–	0.37	0.26	
GC2	Hybrid	20 DAS	14.8±0.9	20.1±1.5	18.6±4.3	0.28	0.30	0.31
	N22	20 DAS	16.3±0.8	18.6±2.2	25.0±1.3	0.28	0.33	0.39

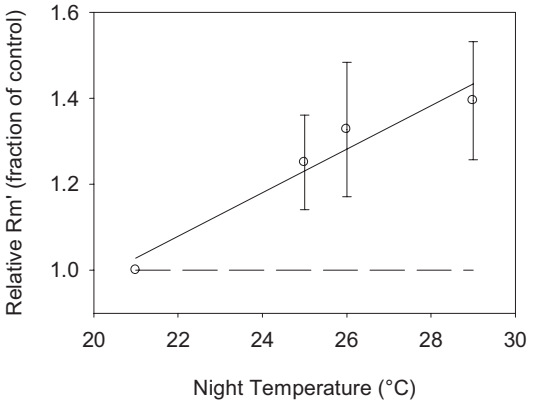

Fig. 6. Relationship between relative (fraction of control, 21 °C) estimated maintenance respiration rate (R_m'), and night temperature to which the plants were fully acclimated. Symbols represent means for experiments and cultivars combined. Error bars=SE.

environmental factor, resulting in a reduction of the production of assimilate available as respiratory substrate.

Five major findings are reported. First, R_n both of expanded leaves and of whole seedlings, was positively and linearly correlated with the previous day's incident light resources. Secondly, SS and starch concentration in the leaf during R_n measurement were linearly correlated with the previous day's light dose and the resulting R_n, indicating that R_n is at least partly resource driven. However, increased NT increased the slope of R_n versus starch and SS concentrations. Thirdly, instantaneous response of R_n to temperature change was strictly linear, with a converging base temperature of ~13–14 °C regardless of long-term temperature treatment and light history. This instantaneous temperature response of R_n was very strong, with R_n increasing by a factor of 2.4 between 21 °C and 31 °C. Fourthly, acclimation to different NTs causes much smaller differences in R_n: in whole young plants, the effect of a 10 °C increase was 1.20, whereas in fully grown leaves whose R_n probably had a higher proportion of R_m, it averaged at 1.67. Lastly, R_m' based on full acclimation constituted a daily loss of biomass between 0.3% in fully grown leaves and 2.5% in whole seedlings, and increased by a factor of only 1.49 as temperature increased by 10 °C. No consistent cultivar effects on R_n were observed.

Respiration rates were much higher in whole seedlings than in mature leaves of developed plants, as suggested by Villar *et al.* (1995). Similarly, it was reported that respiration rates for young leaves are higher than for mature leaves, an effect that is not related to photosynthetic rate (Osaki *et al.* 2001). At the whole-plant level, higher R_n was observed for young than for older lettuce plants, which was explained by the growth rate (van Iersel, 2003). The higher R_n observed here in rice seedlings can thus be explained by the presence of growth processes in meristems, tiller buds, and expanding organs (Osaki *et al.*, 2001; van Iersel, 2003), whereas growth respiration in mature leaves probably only served assimilate export activity but not local growth.

The observed increase in the linear slope of R_n versus starch and SS concentrations under increased NT in acclimated plants indicates that higher temperatures increase respiratory assimilate demand at a given level of supply.

Methodological and conceptual uncertainties

Time of night versus R_n measurement

In this study, time of night of measurement, conducted at least 1 h after sunset, did not affect R_n (Supplementary Fig. S1 available at *JXB* online). In soybean leaves, depletion of SS content occurs within the first hour of the night, followed by a steady state (Mullen and Koller, 1988). Errors in the present study from the assumption of constant R_n during the night, if any, are small because (i) no temporal trends were observed in the experiments and (ii) sequential R_n measurements during the night were grouped by replication and not treatment or cultivar, avoiding temporal bias.

Estimation of R_m from R_n response to previous day's PAR

The assumption here was that growth is absent if no fresh assimilates are available. This is true in terms of biomass growth because assimilate gain is only possible in the presence of light. However, even if plant biomass growth is zero, stored assimilates (Lafarge *et al.*, 2010) or degradation products from senescing tissue can fuel some structural growth, then involving R_g (Gifford, 1995; van Iersel, 2003). This is conceptually a grey area: the distinction of R_g and R_m is impossible where 'reshuffling' of internal resources occurs. Severe assimilate starvation would also incur new physiological costs for cellular crisis management which would not be part of normal maintenance (Amthor, 2000). Consequently, R_m and R_g can not be fully distinguished experimentally because suppressing one would affect the other.

The main arguments for R_m' being similar to actual functional R_m are that (i) the response of R_n to light resources was linear, thus giving credence to the intercept on y (when PAR=0) as being a baseline respiration rate; and (ii) the mobilizable reserves (starch) in the leaf had disappeared under those conditions. Any longer exposure to assimilate starvation would probably trigger compensatory structural growth such as etiolation that fundamentally changes the system's behaviour.

Extrapolation of R_n losses from night to day and leaf to plant

Respiration was measured only at night, although it is a continuous process. The estimation of the carbon cost of R_m under different temperatures, expressed as a fraction of dry matter loss per day, may be inaccurate when used as generic coefficients in plant models. Indeed, leaf respiration continues during daytime at a lower rate than at night (Brooks and Farquhar, 1985; Villar *et al.*, 1994; Pärnik and Keerberg, 1995; Villar *et al.*, 1995). At 800 µmol photon m^{-2} s^{-1} and 25 °C, the ratio of daytime respiration rate over R_n was 0.4 in snow gum (Atkin *et al.*, 2000) and 0.72 in the field for sunflower, kenaf, and *Cynara* on average (Archontoulis *et al.*, 2011). In snow gum, the leaf daytime respiration is inhibited by light between 0 and 200 µmol photon m^{-2} s^{-1}, higher light intensities having no additional effect (Atkin *et al.*, 2000). Increased temperature also caused an increase in leaf daytime respiration. These reports indicate that daytime respiration behaves differently from R_n due to interactions with photosynthesis occurring in the same organ.

For the extrapolation of R_n to 24 h, a distinction must thus be made between the leaf and the photosynthetically inactive tissues. Much greater R_n was observed in whole seedlings than in fully expanded (thus, not growing) leaves, although they had similar R_n response to the previous day's PAR. Because whole-plant R_n is proportional to plant nitrogen content (Ingram *et al.*, 1991; Hirai *et al.*, 1997), Penning de Vries *et al.* (1989) suggested to predict organ respiration rates based on nitrogen content. This assertion probably needs to be qualified, given the low R_n rates observed in young but fully expanded leaves, which have a high nitrogen content (Dingkuhn *et al*., 1992a, b). The respiratory activity of the plant is therefore probably concentrated in parts of the plant that are both high in nitrogen content and are growing.

Extrapolation of observed R_m' (night-time) to the whole day for whole seedlings, while ignoring daytime interactions with photosynthesis (which would reduce it) and variable temperature (which would increase it), gave values of fraction of dry matter loss per day of 1.5–2.5%. This is similar to estimations by Penning de Vries (1972) and Penning de Vries *et al.* (1989) and slightly below values reported by McCree (1982).

Temperature, the Q10 rule, and acclimation

Short-term temperature effects on leaf R_n were linear between 19 °C and 31 °C. Rates increased 2.4-fold from 21 °C to 31 °C but the Q10 exponential rule did not apply because of the observed linearity. Other authors reported exponential temperature responses for both daytime respiration and R_n (e.g. Chi *et al.*, 2013; O'Sullivan *et al.*, 2013). Short-term temperature change, in contrast to long-term change, is reported to have a greater effect on respiration. In wheat, respiration measured at 30 °C was lower for plants acclimated to 30 °C than to 25 °C (Gifford, 1995). The acclimation effect was strong in our study. Acclimated temperature effects for a 10 °C increase were between 1.14 and 1.67 (linear fitting) or between 1.14 and 1.82 (exponential). The instantaneous temperature response of R_n was thus about twice as strong as the acclimated response. Plants grown under low temperature are more sensitive to a short-term increase in temperature than plants grown under higher temperature (Atkin and Tjoelker, 2003; Silim *et al.*, 2010). The widely used but poorly tested rule of Q10=2 (doubling of rate as T increases by 10 °C) may thus have limited validity in the field (Amthor, 2000; Atkin and Tjoelker, 2003).

In rice crop models (Bouman *et al.*, 2001; Heinemann, 2008), Q10=2 is frequently used to calculated R_m. Our results indicate Q10=1.49 for R_m' but with a large error caused by error propagation and inclusion of several experiments. Similarly, van Iersel (2006) reported acclimated Q10 to be between 1.35 and 1.55 for *Tagetes*.

The surprisingly uniform intercept on the x-axis for the R_n versus temperature relationship is similar to the base temperature (T_b) reported for development processes in rice (Dingkuhn and Miezan, 1995). Since the apparent T_b for R_n was similar for plants acclimated to different temperatures, it is proposed that, at least in rice, temperature acclimation acts more on the slope of the temperature response than its intercept.

Is dark respiration substrate driven?

Respiration might be affected by three parameters. One, the respiratory capacity, which can increase with temperature as suggested in this study. Two, product demand, which was highlighted through the correlation between R_n and plant nitrogen content (Ingram *et al.*, 1991). However, because much nitrogen is in enzymes, this relationship may also be an indicator of the respiratory capacity. Three, the substrate availability, as reported in this study with the dependence of R_n on the previous day's incident light resources.

A linear relationship was reported here between R_n and the previous day's light resources via the tissue's sugar and starch concentration. Similar results were reported for *Quercus rubra* (Whitehead *et al.*, 2004) and wheat (Azcon-Bieto and Osmond, 1983). In the assimilate starvation experiments conducted here, this relationship had a large positive intercept for starch (R_n continues when starch is used up), but zero intercept for SS (R_n stops when SSs are missing). At least under severe starvation, R_m becomes substrate dependent and R_g is probably nil.

Crop models generally assume that R_m constitutes a fixed physiological demand at a given temperature and chemical tissue composition (e.g. Bouman *et al.*, 2001). This assumption is inaccurate because physiological stresses, for example, increase the physiological costs of maintenance. For example, Asch *et al.* (2000) observed that rice produced far less biomass per unit of photosynthesis under salinity stress. Conversely, it cannot be excluded that R_m (or the fraction of R_n that is not used for growth) might depend on assimilate supply when it is high, and may to some extent be wasteful.

As pointed out by Gifford (2003), the R_g/R_m paradigm is a notional functional construct. For a modeller, the issue is to predict the organism's carbon balance accurately, and from a crop breeder's perspective it would be to minimize respiratory carbon losses, if genetic diversity for it exists. The present study found no genotypic differences, and it demonstrated a near proportionality between R_n and assimilate resources. The strong parallel decline in carbohydrates and respiration rates with decreased irradiance lends support for the hypothesis that carbohydrate supply controls respiration rates in rice. However, the possibility cannot be excluded that in parallel with reduced substrate production, shade changed processes such as demand for respiratory product. At a given carbohydrate level, respiration in warmed plants was higher, suggesting a role in demand for ATP or other respiratory products in controlling respiration rates.

Conclusion

This study showed that R_n in rice is driven by tissue carbohydrate status and is much more dependent, in these environmental conditions, on short-term light history than temperature if plants are acclimated. The estimated maintenance component R_m' was ~33% of R_n. Both R_n and its component R_m', when

acclimated, were less sensitive to temperature than predicted with the Q10=2 rule, although the instantaneous thermal response of R_n was nearly twice as steep. The Q10=2 paradigm thus overestimates temperature effects on respiration in acclimated plants. Predictions of climate change impacts on vegetation biomass and crop production need more accurate models of respiration. This study provides some evidence but is insufficient to propose better models. Field-based studies are necessary to confirm these results.

Supplementary data

Supplementary data are available at *JXB* online.

Figure S1. Leaf night respiration rate for the hybrid (circles) and N22 (triangles).

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