

Sufficient leaf transpiration and nonstructural carbohydrates are beneficial for high-temperature tolerance in three rice (*Oryza sativa*) cultivars and two nitrogen treatments

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Abstract. To determine whether variations in high-temperature (HT) tolerance in three rice (*Oryza sativa* L.) cultivars and two N treatments are related to leaf transpiration rate (E), and whether the involvement of nonstructural carbohydrates (NSC) in HT tolerance is related to E , a pot experiment supplied with two N levels (low N, 0.077 g urea kg⁻¹ soil; sufficient N, 0.538 g urea kg⁻¹ soil) was conducted under ambient temperature (AT) and HT with three cultivars, N22, Zhenshan 97B and Koshihikari. HT significantly decreased grain yield and seed setting percentage in Koshihikari and ZS97, which could be partly offset by a sufficient N supply. The most HT-tolerant cultivar, N22, had the highest E and stem NSC concentrations under both N treatments, whereas the most sensitive cultivar, Koshihikari, had the lowest E and stem NSC concentrations. A sufficient N supply significantly increased E in the three cultivars under the HT treatment. Grain yield and seed-setting percentage were positively related to E and plant NSC concentration under HT, and E was positively related to NSC concentration under both AT and HT. Therefore, variations in HT tolerance among rice cultivars and nitrogen treatments were related to E , and possibly to NSC concentration.

Additional keywords: heat stress, nitrogen levels, seed setting percentage, yield.

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Introduction

The world's population is expected to increase to 9 billion by 2050 (Godfray *et al.* 2010), with the most significant increases taking place in Africa and Asia (Lutz *et al.* 1997). To feed this rapid growing population, the world will need to produce 70–100% more food than at present (Godfray *et al.* 2010; Tomlinson 2013). Rice (*Oryza sativa* L.) is the most important crop in Asia and accounts for 35–60% of the calories consumed by 3 billion Asians (Khush 1997). It will be necessary to increase rice production at a rate of 1% annually to achieve sustainable food security (Ray *et al.* 2013). However, achieving this improvement will be a great challenge in a changing climate and without an increase in available arable land.

Global warming is one of the most important factors in climate change. The global average temperature has risen by 0.13°C per decade since 1950 and an even more rapid pace of 0.2°C per decade is expected in the next two or three decades (Lobell *et al.* 2011). This ongoing global warming is threatening rice production. It has been reported that each 1°C increase in the growing-season minimum temperature will lead to a 10% decrease in rice yield (Peng *et al.* 2004). In addition to long-term global warming scenarios, extreme high temperature (HT) events

can also threaten rice production, especially in the reproductive stage of rice.

Yield decline under extreme HT is mainly caused by a relatively low seed setting percentage as a result of poor pollen germination and spikelet fertility (Matsui and Omasa 2002; Jagadish *et al.* 2007; Rang *et al.* 2011). It has been reported that spikelet fertility significantly decreases when the canopy temperature exceeds a threshold of ~35°C at the heading and flowering stages (Jagadish *et al.* 2007). HT at the flowering stage inhibits the swelling of pollen grains, and results in anther indehiscence and defective pollen release (Matsui *et al.* 2000). The resulting poor pollination is the major cause of spikelet sterility observed at HT.

Different rice cultivars show great varietal differences in HT tolerance (Matsui and Omasa 2002; Jagadish *et al.* 2010). Matsui *et al.* (2001) reported that variation in sterility-inducing HT among nine japonica cultivars can reach 3°C. Differences in morphological, structural and molecular traits are related to varietal differences in HT tolerance. Yan *et al.* (2010) showed that cultivars with erect panicles and with panicles above the flag leaves were more tolerant than those with droopy panicles and with panicles below the flag leaves, respectively. Matsui

and Omasa (2002) demonstrated that cultivars with more cell layers, which separate anther locule from lacunae formed between the septum and stomium, were more HT-sensitive. Jagadish *et al.* (2010) proposed that the greater HT tolerance of N22 could be due to its accumulation of stress-responsive cold shock proteins and heat shock proteins in anthers.

Transpiration can substantially cool the canopy, and is significantly and positively related to the canopy–air temperature difference (Yan *et al.* 2012; Suralta *et al.* 2013). Therefore, variations in leaf transpiration rate per unit leaf area (E) could be related to HT tolerance. However, there are limited data available on this subject. Nitrogen is the most important fertilizer used in rice production. Yan *et al.* (2010) showed that N application can improve HT tolerance in rice plants, because canopy temperature under sufficient N supply was lower than that under deficient N supply (Ferguson *et al.* 1973; Seligman *et al.* 1983; Yan *et al.* 2010). However, whether the enhanced HT tolerance under sufficient N supply is related to high E is poorly understood.

Recently, the stem nonstructural carbohydrate (NSC) concentration has been demonstrated to be capable of improving the HT tolerance of rice plants at the full heading stage (Samonte *et al.* 2001; Kobata and Uemuki 2004; Morita and Nakano 2011). NSC, particularly sugar, has proven to be capable of maintaining plant hydraulic conductance under drought stress (Sala *et al.* 2012). Whether the involvement of NSC under HT tolerance in rice plants is related to E is unknown.

In the present study, a pot experiment with HT-tolerant and -sensitive cultivars, which were selected according to the results of a preliminary screening experiment (data not shown), was conducted under two N levels. The objectives of the study were (1) to clarify whether varietal difference in HT tolerance are related to E ; (2) to study whether N supply can regulate rice E and whether this is related to enhanced HT tolerance under sufficient N supply; and (3) to study whether the involvement of NSC in HT tolerance is related to E .

Materials and methods

Plant materials and temperature treatments

Three rice (*Oryza sativa* L.) cultivars, N22 (HT-tolerant), Zhenshan 97B (ZS97, moderately HT-tolerant), and Koshihikari (HT-sensitive), were grown in a pot experiment at Huazhong Agricultural University (114.37°E, 30.48°N). After germination on moist filter on 15 April 2013, the seeds were transferred to nursery plates. When the seedlings had developed an average of 2.5 leaves, they were transplanted into 15.0-L pots (diameter, 31 cm; height, 23 cm) with a density of three hills per pot and one seedling per hill. Each pot was filled with 13.0 kg soil; P and K were applied as basal fertilisers at a rate of 1.95 g per pot. Under the low N treatment, 0.5 g urea per pot was applied as a basal fertiliser and another 0.5 g urea per pot was applied at the panicle initiation stage (~55 days after germination) as a top-dressing fertiliser; under sufficient N treatment, the basal and top-dressing fertilisers were 3.0 and 4.0 g urea per pot, respectively. Basal fertilisers were applied by incorporating them into the soil, whereas top-dressed fertiliser was dissolved in water and irrigated to pots. After transplanting, rice plants

under the HT treatment were placed in a naturally irradiated greenhouse until harvest; under the AT treatment, they were placed outdoors. HT in daytime was achieved through the effects of the greenhouse and night temperature fell to the ambient temperature (AT) because of the lack of radiation. The mean day–night temperatures of the AT and HT treatments were 29.1–25.4°C and 35.2–26.1°C (Fig. 1); the humidity was 78.8–86.1% and 79.2–85.9%, respectively. The maximum PFDs under AT and HT at noon were $1621 \pm 24 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and $1557 \pm 42 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively. During the entire growth season, rice plants were well watered and a minimum 2-cm water layer was maintained to avoid drought stress. There were, therefore, four treatments: AT-low N, AT-sufficient N, HT-low N and HT-sufficient N, with three pots per treatment.

Measurement of leaf photosynthesis and leaf transpiration

About 74 days after germination (at the heading stage), one flag leaf per pot was chosen to measure leaf photosynthesis (A) and leaf transpiration using a Li-Cor 6400XT portable infrared gas

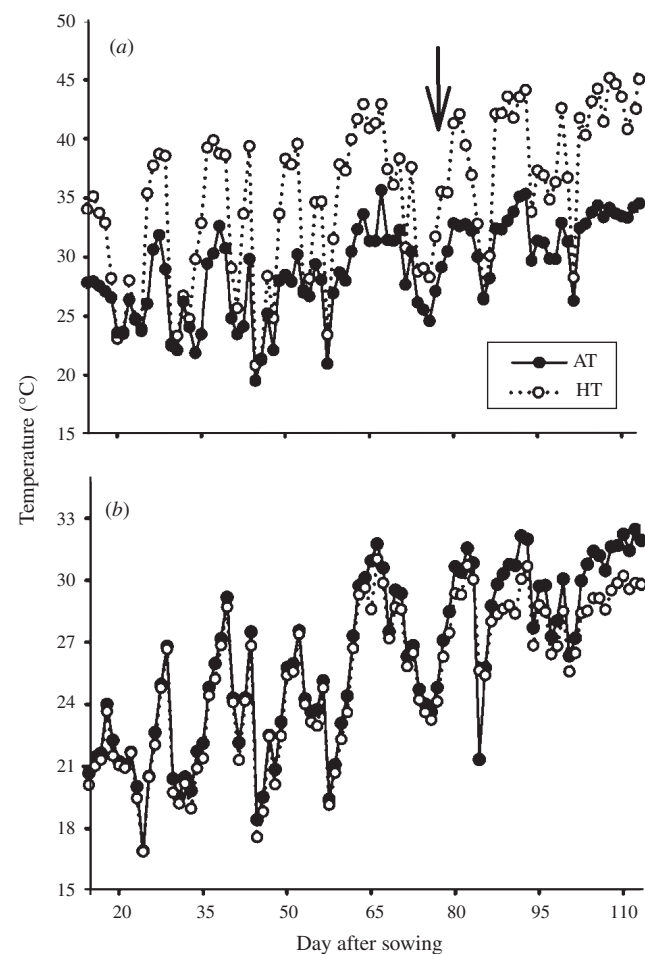


Fig. 1. Daily variations in (a) day (0700–1900 hours) and (b) night (1900–0700 hours) temperatures. Seeds were germinated on 15 April 2013 and were harvested on 13 August. Arrows indicate the heading date. AT, ambient temperature; HT high temperature.

Table 1. Effects of growth temperature and N levels on grain yield and yield components in three different rice cultivars

Data were presented as means \pm s.d. with three replications. The mean day–night temperatures of the ambient temperature (AT) and high temperature (HT) treatments were 29.1–25.4°C and 35.2–26.1°C. N application rates were 0.077 g urea kg⁻¹ soil and 0.538 g urea kg⁻¹ soil under the LN and SN treatment, respectively. LN, low N supply; SN, sufficient N supply; T, temperature; V, variety; ns, non-significant at $P < 0.05$; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$

Cultivars	Treatments	Biomass (g per hill)	Panicle no. per hill ⁻¹	Spikelet no. per hill ⁻¹	Seed setting (%)	Grain yield (g per hill)
Koshihikari	AT-LN	48.3 \pm 2.7	7.0 \pm 1.0	626 \pm 93	73.1 \pm 3.6	9.7 \pm 2.3
	AT-SN	87.1 \pm 1.2	13.3 \pm 1.5	1465 \pm 90	72.5 \pm 2.7	22.0 \pm 1.6
	HT-LN	43.4 \pm 2.4	6.7 \pm 0.6	587 \pm 53	6.8 \pm 3.0	0.8 \pm 0.2
	HT-SN	78.0 \pm 4.2	13.3 \pm 1.2	1465 \pm 113	20.3 \pm 3.1	6.4 \pm 1.3
ZS97	AT-LN	47.7 \pm 3.8	6.7 \pm 1.5	790 \pm 65	89.3 \pm 2.8	14.5 \pm 3.7
	AT-SN	89.3 \pm 4.6	13.3 \pm 1.5	1495 \pm 79	86.0 \pm 4.8	31.3 \pm 4.3
	HT-LN	39.5 \pm 3.8	6.0 \pm 0.0	768 \pm 69	12.7 \pm 3.3	1.5 \pm 0.5
	HT-SN	80.9 \pm 5.6	13.7 \pm 0.6	1467 \pm 184	41.8 \pm 4.4	17.4 \pm 1.9
N22	AT-LN	44.5 \pm 4.5	7.7 \pm 0.6	705 \pm 159	91.9 \pm 3.3	11.6 \pm 0.9
	AT-SN	83.5 \pm 9.0	13.3 \pm 1.5	1814 \pm 56	92.5 \pm 2.6	23.7 \pm 2.8
	HT-LN	41.1 \pm 2.0	7.3 \pm 0.6	598 \pm 21	82.2 \pm 2.3	10.3 \pm 1.2
	HT-SN	78.5 \pm 4.9	13.3 \pm 0.6	1607 \pm 165	91.8 \pm 2.4	22.8 \pm 0.8
ANOVA	T	**	ns	ns	***	***
	V	*	ns	*	***	***
	N	***	***	***	***	***
	T \times N	ns	ns	ns	***	***
	T \times V	**	ns	ns	***	***
	V \times N	*	ns	ns	**	**

analyser (LI-COR, Lincoln, NE, USA) from 0900 hours to 1500 hours. PPFD during measurements was set to 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ using a red–blue light-emitting diode artificial light source, leaf–air vapour pressure deficit was controlled at $1.5 \pm 0.3 \text{ kPa}$ and CO_2 concentration in leaf chamber was $\sim 390 \pm 10 \mu\text{mol mol}^{-1}$. Leaf temperatures under AT and HT were set to 28°C and 35°C, respectively, which were near their daytime mean growth temperatures. Data were recorded after equilibration to a steady state, ~ 20 min after attaching the leaves to the leaf chamber.

Measurements of N and NSC concentration

One day later, three hills per treatment were harvested from three different pots and separated into leaves (all leaves included), stems and panicles. They were then oven-dried at 80°C to achieve a constant weight and were ground with a mixer mill homogeniser (MM400, Retsch, Haan, Germany). Thereafter, ~ 50 mg was used to measure N concentration using an NC analyser (IsoPrime100 IRMS, Isoprime, Cheadle, UK).

NSC concentration was determined according to the method described by Yoshida *et al.* (1976). Briefly, NSC was extracted from 0.25 g oven-dried plant tissue in 10 mL distilled H₂O in a boiling water bath for 2 h. Next, 2 mL of the extraction was reacted with 2 mL of a phosphate buffer for 24 h at 38°C. The composition of the phosphate buffer was as follows: 0.1 M KH₂PO₄, 0.2 M Na₂HPO₄·12H₂O, 1.0 mM NaNO₃, 0.5 mg mL⁻¹ α -amylase and 0.25 mg mL⁻¹ amyloglucosidase. Afterwards, 1.5 mL of this reaction solution was transferred to a cuvette, followed by the addition of 1.5 mL distilled water, 0.5 mL anthrone reagent (1 g anthrone was dissolved in 50 mL ethyl acetate) and 5 mL H₂SO₄. The

Table 2. Effects of growth temperature and N levels on gas exchange parameters and leaf water potential in three different rice cultivars

Data were presented as means \pm s.d. with three replications. The mean day–night temperatures of ambient temperature (AT) and high temperature (HT) were 29.1–25.4 °C and 35.2–26.1 °C. N application rates were 0.077 and 0.538 g urea kg⁻¹ soil in the low N (LN) and sufficient N (SN) treatment, respectively; *E*, leaf transpiration rate; *A*, leaf photosynthesis; *g_s*, stomatal conductance; T, temperature; V, variety; ns, nonsignificant at $P < 0.05$; *, significant at $P < 0.01$; **, significant at $P < 0.05$; ***, significant at $P < 0.001$ levels

Cultivars	Treatments	<i>A</i> ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	<i>g_s</i> ($\text{mol m}^{-2} \text{s}^{-1}$)	<i>E</i> ($\text{mmol m}^{-2} \text{s}^{-1}$)
Koshihikari	AT-LN	19.7 \pm 0.7	0.25 \pm 0.01	8.6 \pm 0.3
	AT-SN	25.7 \pm 0.9	0.32 \pm 0.01	10.1 \pm 0.4
	HT-LN	17.1 \pm 2.2	0.21 \pm 0.08	6.0 \pm 1.0
	HT-SN	22.0 \pm 0.9	0.29 \pm 0.08	7.5 \pm 0.9
ZS97	AT-LN	21.9 \pm 0.5	0.43 \pm 0.01	8.9 \pm 0.1
	AT-SN	26.5 \pm 1.0	0.65 \pm 0.02	9.7 \pm 0.3
	HT-LN	16.9 \pm 0.8	0.44 \pm 0.02	8.0 \pm 0.6
	HT-SN	24.3 \pm 0.5	0.76 \pm 0.01	10.3 \pm 0.8
N22	AT-LN	23.4 \pm 0.9	0.32 \pm 0.00	10.0 \pm 0.3
	AT-SN	25.1 \pm 0.5	0.36 \pm 0.01	11.4 \pm 0.4
	HT-LN	21.2 \pm 0.7	0.24 \pm 0.03	13.1 \pm 0.5
	HT-SN	24.0 \pm 0.7	0.57 \pm 0.11	14.4 \pm 0.1
ANOVA	T	*	ns	*
	V	ns	**	***
	N	***	***	***
	T \times N	*	**	*
	T \times V	ns	***	***
	V \times N	ns	ns	***

cuvettes were then heated in a boiling water bath for 2 min. Finally, the absorbance of each solution at 630 nm was measured (Infinite M200, Tecan, Morrisville, NC, USA).

Measurements of shoot biomass, grain yield and yield components

At physiological maturity (~120 days after germination), three hills per treatment were sampled from three different pots to measure shoot biomass, grain yield and yield components. All panicles were hand-threshed, and filled grains were separated from unfilled grains by submersion in tap water. The dry weights of leaves, stems and panicles were determined after oven drying

at 80°C to achieve a constant weight. The number of spikelets per panicle was counted, and seed setting percentage was calculated as the ratio of filled grains to total grains.

Statistical analysis

One- and two-way ANOVAs were applied to assess the differences between treatments using SAS ver. 9.2 (SAS Institute, Cary, NC, USA). The statistical model used included sources of variation due to temperature, N and variety treatment and the interaction of temperature × variety, temperature × N and variety × N. Data from each sampling date were analysed separately. Linear regression analysis was performed to test

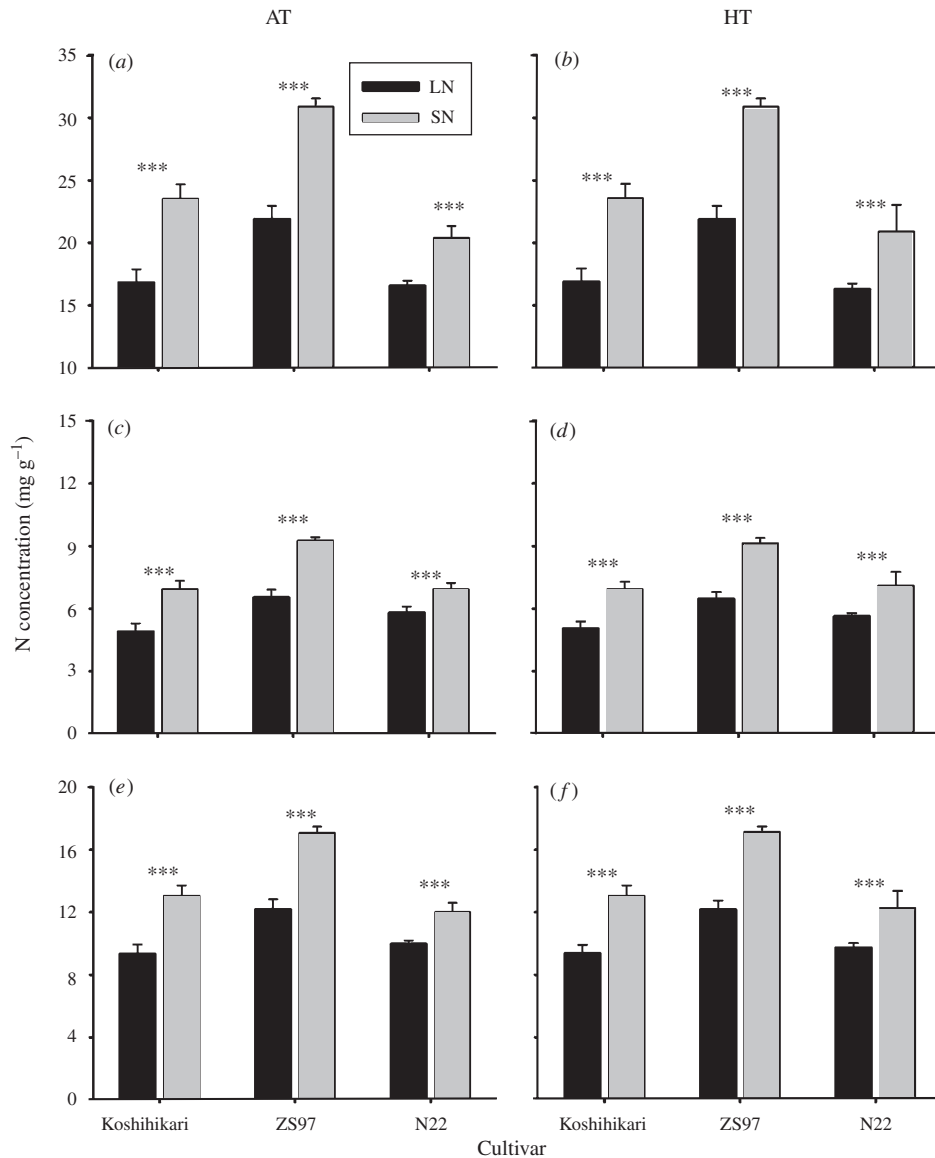


Fig. 2. Effects of growth temperature and nitrogen levels on nitrogen concentration of (a, b) leaf, (c, d) stem and (e, f) panicle in three rice cultivars. The mean day–night temperatures of (a, c, e) ambient temperature (AT) and (b, d, f) high temperature (HT) were 29.1–25.4°C and 35.2–26.1°C, respectively. N application rates were 0.077 and 0.538 g urea kg⁻¹ soil under low N (LN) and sufficient N (SN) treatments, respectively. ***, significant at $P < 0.001$.

the correlations between parameters using SigmaPlot ver. 12 (SPSS Inc., Chicago, IL, USA).

Results

Effects of growth temperature and N levels on rice grain yield and yield formation

To determine whether variations in HT tolerance among rice cultivars and N treatments are related to *E* and NSC, three rice cultivars (N22, Koshihikari and ZS97) were selected according to their HT tolerance observed in a preliminary screening experiment. HT significantly decreased grain yield and seed

setting percentage in Koshihikari and ZS97, although it had no detrimental effect on N22, except for its seed setting percentage under low N supply (Table 1). Under low N supply, the grain yield of Koshihikari and ZS97 under HT decreased by 91.8% and 89.7%, respectively; under sufficient N supply, significantly less severe decreases of 70.9% and 44.4% were obtained. Under low N supply, the seed setting percentages of Koshihikari and ZS97 in HT decreased by 90.7% and 85.8%, respectively; similarly, relatively less severe decreases of 72.0% and 51.4% were obtained under sufficient N supply. These data indicate that sufficient N supply can, to some extent, offset the detrimental effects of

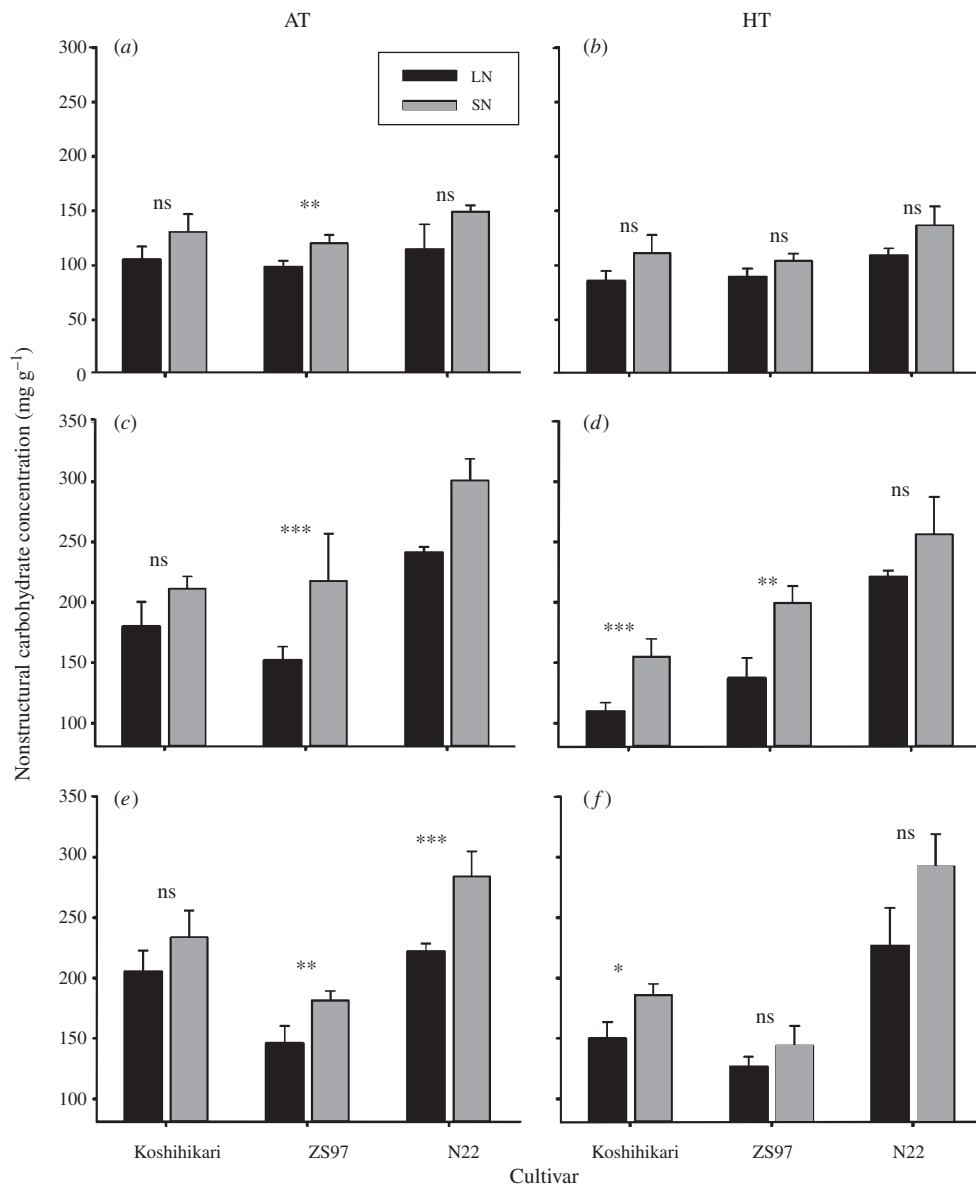


Fig. 3. Effects of growth temperature and nitrogen levels on the nonstructural carbohydrate (NSC) concentration of (a, b) leaf, (c, d) stem and (e, f) panicle in three rice cultivars. The mean day–night temperatures of (a, c, e) ambient temperature (AT) and (b, d, f) high temperature (HT) were 29.1–25.4°C and 35.2–26.1°C. N application rates were 0.077 and 0.538 g urea kg⁻¹ soil under the low N (LN) and sufficient N (SN) treatments, respectively. ns, not significant at $P < 0.05$; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$.

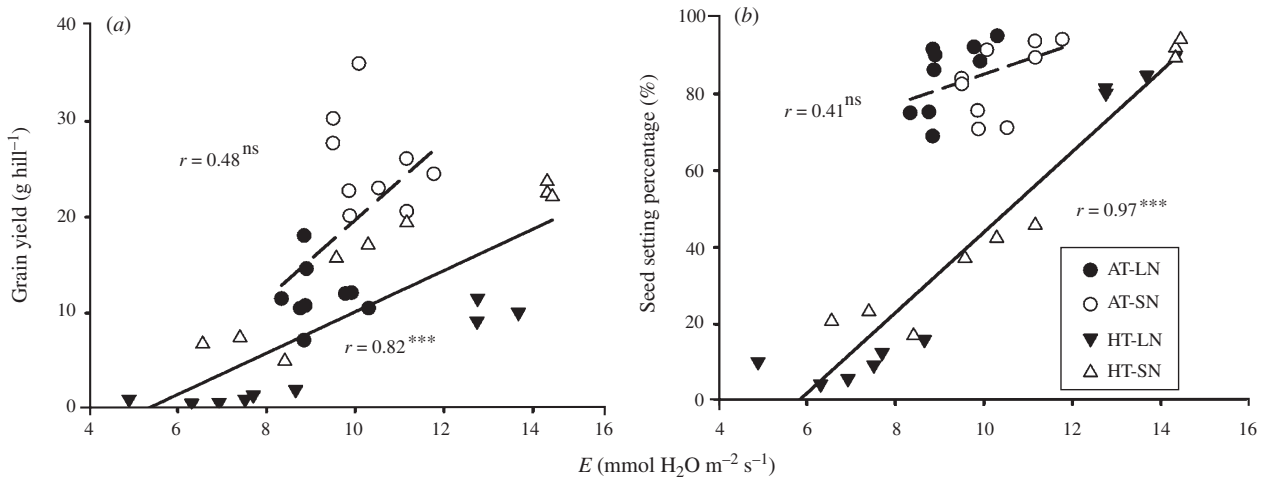


Fig. 4. Relationships of (a) yield and (b) seed setting percentage with leaf transpiration rate (E) under ambient temperature (AT, filled circles) and high temperature (HT, open circles) treatments in three rice cultivars. The mean day–night temperatures of AT and HT were 29.1–25.4°C and 35.2–26.1°C. N application rates were 0.077 and 0.538 g urea kg⁻¹ soil under the low N (LN) and sufficient N (SN) treatments, respectively. ns, not significant at $P < 0.05$; ***, significant at $P < 0.001$.

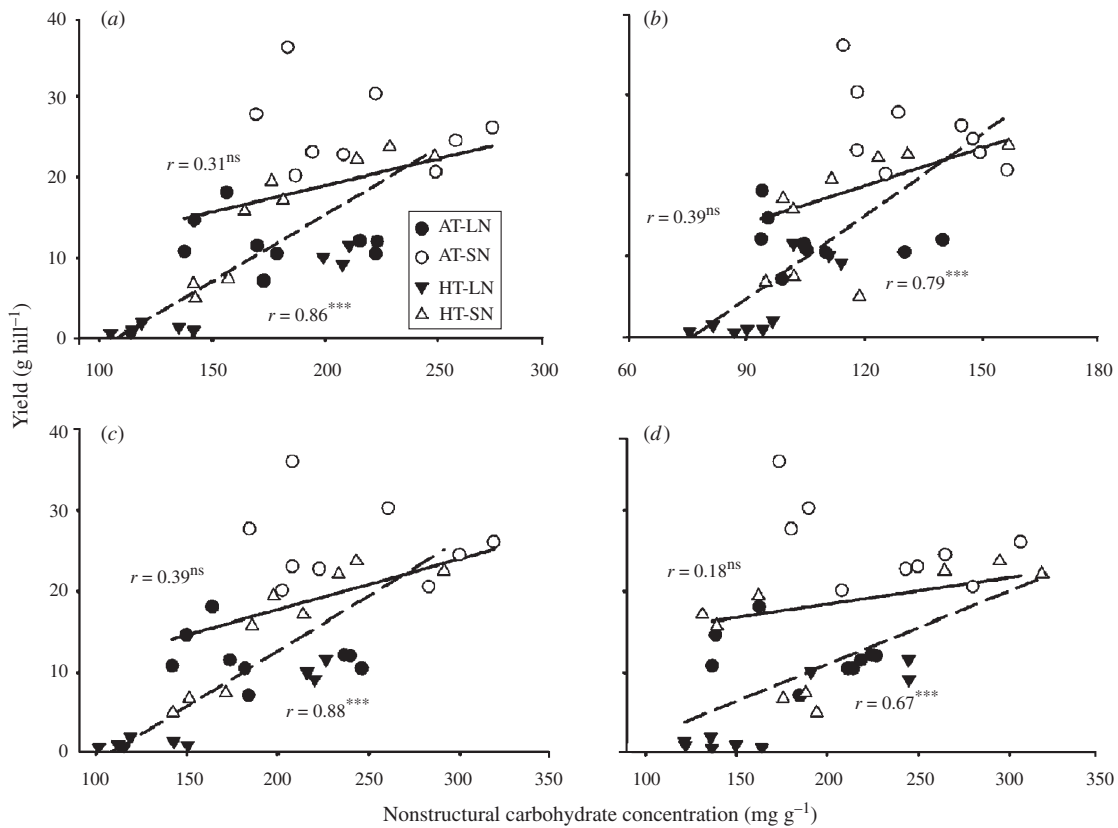


Fig. 5. Relationships between yield and (a) shoot, (b) leaf, (c) stem and (d) panicle nonstructural carbohydrate concentration under ambient temperature (AT, circles) and high temperature (HT, triangles) treatments in three rice cultivars. The mean day–night temperatures of AT and HT were 29.1–25.4°C and 35.2–26.1°C. N application rates were 0.077 and 0.538 g urea kg⁻¹ soil under the low N (LN) and sufficient N (SN) treatments, respectively. ns, not significant at $P < 0.05$; ***, significant at $P < 0.001$.

HT on grain yield and seed setting percentage. Shoot biomass was significantly decreased under HT but panicle number and spikelet number per hill were similar under both the AT and HT treatments.

Effects of growth temperature and N levels on gas exchange parameters

HT significantly decreased A but there was no significant varietal difference among the three cultivars (Table 2). Under low N supply, the A of Koshihikari, ZS97 and N22 decreased by 13.20%, 22.83% and 9.40%, respectively; under sufficient N supply, less severe decreases of 14.4%, 8.30% and 4.38% were obtained. Growth temperature had no significant effect on stomatal conductance in the three cultivars.

E was highest in N22 under the AT and HT treatments (Table 2). The responses of E to HT also varied with cultivar and N level. HT significantly decreased E in Koshihikari under both N supply levels. In contrast, HT significantly increased E in ZS97 and N22 under both N supply levels. Sufficient N supply significantly improved A , stomatal conductance and E in the three cultivars.

Effects of growth temperature and N levels on nitrogen and NSC concentrations

The HT treatment had no significant effect on leaf, stem, or panicle N concentration in the three cultivars (Fig. 2). Regardless of N and temperature treatments, N concentration was higher in ZS97 than in the other two cultivars.

There was no significant effect of the HT treatment on leaf NSC concentration in any of the three cultivars (Fig. 3). The HT treatment significantly decreased stem NSC concentration in Koshihikari and N22, but not in ZS97, whereas panicle NSC concentration decreased under the HT treatment in Koshihikari and ZS97, but not in N22.

Sufficient N supply significantly increased leaf, stem and panicle NSC concentrations in the three cultivars. Regardless of the N supply, leaf, stem, and panicle NSC concentrations were higher in N22 than in the other two cultivars under both the AT and HT treatments.

Correlations among grain yield, seed setting percentage, leaf transpiration rate and NSC concentrations

When pooled together, the data from the two N supplies and three cultivars showed that grain yield and seed setting percentage were positively correlated with E and shoot NSC

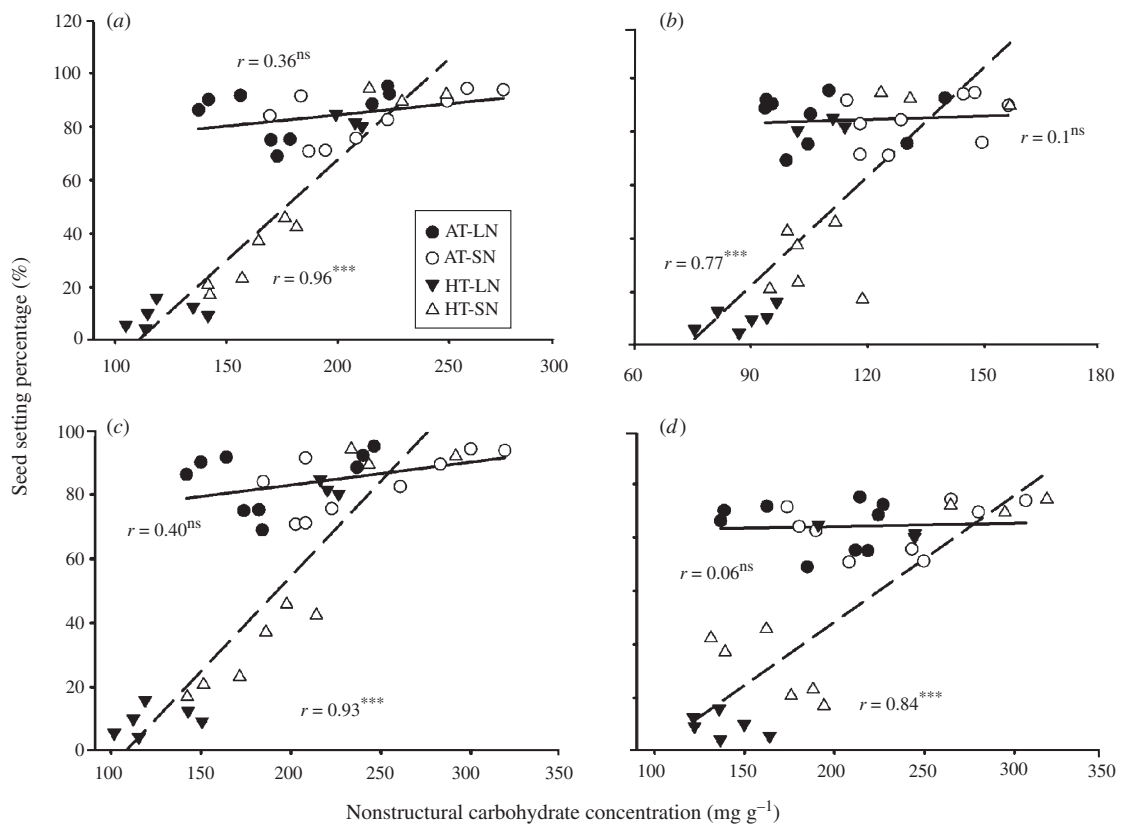


Fig. 6. Relationships between seed setting percentage and (a) shoot, (b) leaf, (c) stem and (d) panicle nonstructural carbohydrate concentration under ambient temperature (AT, circles) and high temperature (HT, triangles) treatments in three rice cultivars. The mean day–night temperatures of AT and HT were 29.1–25.4°C and 35.2–26.1°C. N application rates were 0.077 and 0.538 g urea kg⁻¹ soil under the low N (LN) and sufficient N (SN) treatments, respectively. ns, not significant at $P < 0.05$; ***, significant at $P < 0.001$.

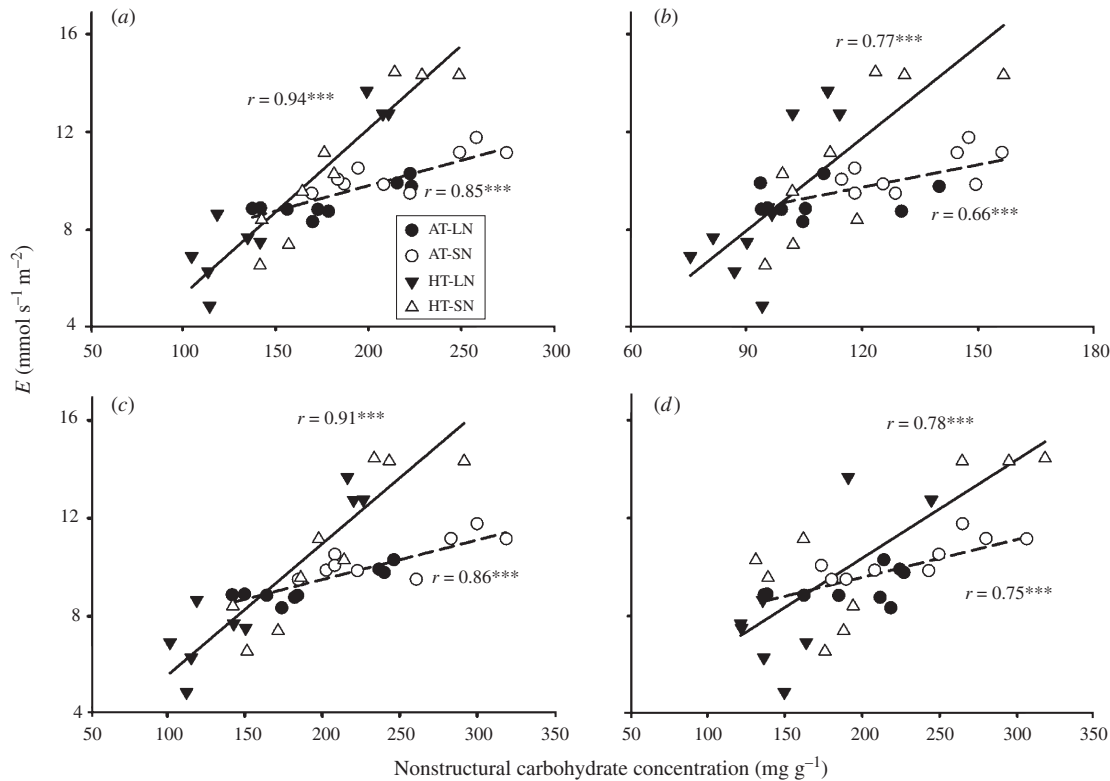


Fig. 7. Relationships between leaf transpiration rate (E) and (a) shoot, (b) leaf, (c) stem and (d) panicle nonstructural carbohydrate concentration under ambient temperature (AT, circles) and high temperature (HT, triangles) treatments in three rice cultivars. The mean day–night temperatures of AT and HT were 29.1–25.4°C and 35.2–26.1°C. N application rates were 0.077 and 0.538 g urea kg⁻¹ soil under the low N (LN) and sufficient N (SN) treatments, respectively. ***, significant at $P < 0.001$.

concentration under the HT treatment but not under the AT treatment (Figs 4–6). Under both the AT and HT treatments, E was positively correlated with shoot NSC concentration and the correlation was closer under the HT treatment than under the AT treatment (Fig. 7).

Discussion

Both long-term global warming and extreme HT stress can suppress rice yield under cultivation, but the underlying mechanisms are different. Yield decline under long-term global warming is mainly caused by decreased biomass and spikelet formation (Peng *et al.* 2004). In contrast, poor seed setting percentage is the most significant cause of yield decline under extreme HT stress (Matsui *et al.* 2000). In the present study, although HT significantly suppressed shoot biomass by inhibition of photosynthesis, it had no negative effect on panicle number and spikelet number per hill (Tables 1 and 2). Therefore, poor seed setting percentage is also the primary reason for yield decline under the HT treatment in the present study. When we compare responses of grain yield and seed setting percentage to the HT treatment in the three cultivars, it is clear N22 is the most HT-tolerant cultivar and Koshihikari is the most HT-sensitive cultivar. Furthermore, sufficient N supply can improve seed setting percentage and grain yield under the HT treatment, which suggests that sufficient N supply can improve HT tolerance in rice plants.

Under the HT treatment, grain yield and seed setting percentage were usually negatively related to canopy or organ (e.g. panicles and leaves) temperatures in field or pot experiments (Amani *et al.* 1996; Ayeneh *et al.* 2002; Yan *et al.* 2010). Because leaf transpiration is the determinant for canopy or organ temperature, a high leaf transpiration rate will be beneficial for yield formation. Therefore, positive correlations between grain yield, seed setting percentage and E were observed under HT stress (Fig. 4). Additionally, the most HT-tolerant cultivar, N22, showed the highest E , whereas the most HT-sensitive cultivar, Koshihikari, had the lowest E under the HT treatment (Table 2). These results suggest that varietal differences in HT tolerance are related to E .

Water absorbed by the root contributes to the overall water balance of the shoot. Because of the existence of apoplastic transport barriers, the hydraulic conductivity of rice root is much lower and less adaptable to a changing environment than that of other herbaceous species (Miyamoto *et al.* 2001). This could explain why rice plants usually suffer from water deficiency, even when growing in paddy fields. Water channels (aquaporins) in the plasma membrane of root cells play an important role in the symplastic pathways. Their density and activity are reported to be dependent on the growing environment (Martre *et al.* 2002; Luu and Maurel 2005; Ahamed *et al.* 2012).

The phenomenon of N application improving root hydraulic conductivity and E has been frequently observed in previous

studies (Radin and Matthews 1989; Clarkson *et al.* 2000). In the present study, enhanced HT tolerance under sufficient N supply in the three rice cultivars was accompanied by increased *E* (Tables 1 and 2), which suggests that sufficient N supply can increase HT tolerance, at least partially, through increasing leaf *E*. The underlying mechanism of why sufficient N supply can increase *E* is mostly attributed to the enhanced root or leaf hydraulic conductivity through increasing aquaporin expression or activity, or changing xylem vascular development (Clarkson *et al.* 2000; Ligaba *et al.* 2011; Ishikawa-Sakurai *et al.* 2014). Stomata make a large contribution to plant hydraulic conductivity, so varietal differences in stomatal density and aperture would possibly be one of the reasons for different *E* among rice cultivars.

Stem NSC is of vital importance in grain filling and nearly 25% of carbohydrates supplied to rice kernels is from the reserve stem NSC before heading (Cock and Yoshida 1972; Pan *et al.* 2011). A large stem NSC at full heading is capable of maintaining a highly ripened grain, especially at high temperatures (Morita and Nakano 2011). In the present study, the positive relationships among NSC, grain yield and seed setting percentage were observed under HT rather than AT (Figs 5 and 6), which suggests that variations in HT tolerance among rice cultivars and N treatments are also related to plant NSC concentration.

However, the underlying mechanism that enables NSC to improve the HT tolerance of rice plants has not yet been fully exploited. Miyazaki *et al.* (2013) suggested that higher expression of the sucrose transporter gene in rice is beneficial for its HT tolerance. In wood species, it has been suggested that NSC helps to maintain hydraulic conductivity under drought stress (Sala *et al.* 2012). In the present study, positive relationships between NSC and *E* were observed (Fig. 7). However, further investigation into the interaction of NSC and *E* in rice plants is needed. McDowell (2011) suggested that carbon starvation under drought was the determinant for hydraulic failure. Whether this interpretation is applicable to HT stress is unknown.

Leaf photosynthesis is very sensitive to growth temperature and is depressed under conditions that are lower or higher than the optimal growth temperature. In the present study, a decrease in *A* was also observed. Nitrogen can alleviate this depression (Table 2). Depression in *A* may be the cause of inhibited shoot biomass formation.

In conclusion, *E* played an important role in improving the HT tolerance of rice plants. The most HT-tolerant cultivar, N22, had a higher *E* than the other two cultivars, and the improved *E* under sufficient N supply at least partially accounted for the enhanced HT tolerance. Although NSC was positively related to *E* and grain yield, the underlying mechanism is still not fully understood.

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