

Differential response of two important Southern US rice (*Oryza sativa* L.) cultivars to high night temperature

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Abstract

The impact of climatic change on crop production is a major global concern. The climatic factor, night temperature, increasing as a result of global warming, can alter rice productivity and quality. As the initial step in development of high night temperature (HNT; 30 °C)-tolerant rice cultivars for the Southern United States (US) and similar climates, in this study we screened two popular Southern US rice cultivars 'Cocodrie' (inbred pure line) and 'XL723' (hybrid) for variation in tolerance to HNT. Plants were exposed to ambient night temperature (ANT; 25 ± 0.5 °C) and HNT (30 ± 0.5 °C) from 30 days after emergence (DAE) until harvest in the greenhouse. Growth, morphological and physiological traits of Cocodrie and XL723 were examined. The HNT did not affect plant morphology, leaf photosynthetic rate or leaf pigments for rice cultivars 'Cocodrie' and 'XL723'. For 'Cocodrie', HNT increased leaf internal CO₂ concentration (ICO₂; 3%), respiration rate (R_s; 21%) and relative injury to the leaf membrane (RI; 35%) and decreased pollen germination (49%), spikelet fertility (40%), and yield (10%). For 'XL723', HNT increased ICO₂ (3%), R_s (36%) and RI (28%) and decreased pollen germination (36%), spikelet fertility (13%), and yield (6%). In this study, 'XL723' yielded (4%) more than 'Cocodrie' under HNT as a result of lower RI (7%) and higher pollen germination (13%) compared to 'Cocodrie'. The RI and pollen germination, individually or in combination, can be used as screening tools for HNT tolerance in future breeding efforts to enhance HNT tolerance in Southern US rice cultivars.

Key words: Cocodrie; High night temperature; Relative injury to the membrane; Respiration rate; Rice; Pollen germination; XL723.

Abbreviation: ANT_Ambient night temperature, DAE_Days after emergence, HNT_High night temperature, ICO₂_Leaf internal CO₂ concentration, P_N_ Leaf photosynthetic rate, RI_Relative injury to the leaf membrane, R_s_Respiration rate, WUE_Water use efficiency.

Introduction

Rice (*Oryza sativa* L.) is a primary source of food for more than half of the world's population and provides food security for many countries (Krishnan et al., 2011). High night temperature (HNT) is one of the major environmental stresses limiting rice productivity. In the past century, the daily minimum (night) temperature has increased faster than the daily maximum (day) temperature (Easterling et al., 1997). The faster increase in night temperature (NT) compared to day temperature is due to less radiant heat loss as a result of increased cloudiness (Alward et al., 1999). High temperatures are a major constraint to rice productivity, especially when temperature extremes coincide with critical stages of plant development (Kobata and Uemuki, 2004). High temperatures have been implicated to cause reductions in rice yield in many rice-growing areas (Mohammed and Tarpley, 2009a; Welch et al., 2010). High night temperature significantly reduced yields in many crop species (Mohammed and Tarpley, 2009b; Morita et al., 2005) by increasing respiration (Frantz et al., 2004; Mohammed and Tarpley, 2009a) and relative injury to the membrane (Halford, 2009; Mohammed and Tarpley, 2009a), decreasing sugar and starch content (Turnbull et al., 2002), suppressing floral bud development (Ahmed and Hall, 1993), and causing male sterility, low pollen viability and low pollen germination (Endo et al., 2009; Mohammed and Tarpley, 2009b). In addition, high temperatures at flowering or during grain-filling phase can cause spikelet sterility and shortening of the grain-filling duration, thus decreasing yield

(Mohammed and Tarpley, 2009b; Prasad et al., 2006). A previous study has reported that each 1 °C increase in day maximum/night minimum in temperature from the optimum temperature (28/21 °C) can decrease rice yields by 7-8% (Baker et al., 1992). Management practices for heat stress includes early sowing of rice cultivars and/or selection of early maturing cultivars to avoid heat stress during grain-filling and/or chemical applications at sensitive stages of rice plant to prevent heat damage. The application of chemicals (1-methylcyclopropane, glycine betaine, salicylic acid) can partially alleviate negative effects of HNT (Mohammed and Tarpley, 2009a, b). However, management practices may not be adequate as heat stress events are becoming more frequent and severe. The progress in developing crop cultivars for tolerance to abiotic stresses has been slow because of lack of knowledge of mechanisms of tolerance, poor understanding of inheritance of resistance or tolerance, low heritability, and lack of efficient techniques for screening the cultivars and germplasm (Khush, 2005). Developing stress tolerant cultivars requires a thorough understanding of genetics, biochemical, and physiological processes for identification, selecting traits and enhancing tolerance mechanisms in cultivars. The screening of rice cultivars for high night temperature tolerance in the Southern US and similar subtropical humid regions would be accelerated if the physiological traits related to HNT stress tolerance could be identified for southern US rice cultivars. With this background, we hypothesize that the magnitude of cultivar

Table 1. Responses of southern US rice cultivars to night temperatures with respect to morphology and dry weights

Parameters	Cocodrie		% difference	XL723		% difference
	ANT	HNT		ANT	HNT	
Plant height, cm	78.0 ± 3.0 a ¹	74.5 ± 0.5 a	NS	83.0 ± 1.0 x ¹	85.0 ± 4.0 x	NS
Number of tillers per plant	11.5 ± 2.5 a	9.5 ± 0.5 a	NS	12.0 ± 3.0 x	15.5 ± 0.5 x	NS
Number of panicles per plant	6.5 ± 0.5 a	7.5 ± 0.5 a	NS	7.0 ± 1.0 x	7.5 ± 0.5 x	NS
Leaf dry weight per plant, g	10.3 ± 0.2 a	10.0 ± 0.1 a	NS	10.6 ± 0.4 x	11.0 ± 0.2 x	NS
Stem dry weight per plant, g	19.7 ± 0.6 a	22.0 ± 2.7 a	NS	21.3 ± 1.1 x	21.7 ± 0.9 x	NS
Panicle dry weight per plant, g	16.7 ± 0.2 a	15.0 ± 0.2 b	-10	16.2 ± 0.2 x	15.2 ± 0.2 y	-6

Each value is the mean with standard error (\pm S.E.) (n = 36). ¹Means within a row for a cultivar followed by a different letter differed significantly ($P < 0.05$). NS = not significant. Analysis for differences between the night temperatures was performed separately for each cultivar.

tolerance to enhanced HNT will differ between the inbred pure line and hybrid with respect to morphological, phenological and physiological traits. Therefore, the objectives of the present study were (1) to examine the effects of high night temperature on growth, morphological, and physiological traits of an inbred pure line and a hybrid; and (2) to determine which screening trait might be a useful tool in future breeding efforts to enhance HNT tolerance.

Results

Leaf photosynthetic parameters and leaf biochemistry

In the present study, there were no significant differences among the experiments with respect to measured parameters. There was no difference between the night temperatures with respect to P_N for both the cultivars (Fig. 1a). However, ‘Cocodrie’ plants grown under HNT showed increased stomatal conductance (47%), internal CO_2 concentration (ICO_2 ; 3%) and transpiration (17%) and decreased water use efficiency (WUE; 11%), compared to plants grown under ANT (Fig. 1b, c, d, e). The ‘XL723’ plants grown under HNT showed no difference between NT with respect to stomatal conductance, transpiration and WUE (Fig. 1b, d, e). However, ‘XL723’ plants grown under HNT showed increased ICO_2 (3%) compared to ‘XL723’ plants grown under ANT (Fig. 1c). For ‘Cocodrie’ plants, there was no difference between the NT with respect to leaf biochemistry, except for F_o/F_m (Fig. 2a, b, c, d, e). The ‘Cocodrie’ plants grown under HNT showed 23% increase in F_o/F_m , compared to ‘Cocodrie’ plants grown under ANT (Fig. 2a). The ‘XL723’ plants grown under HNT showed 45% decrease in ETR and 10% decrease in quantum yield, compared to ‘XL723’ plants grown under ANT (Fig. 2c, d).

Leaf respiration rate and relative injury to the membrane

The ‘Cocodrie’ plants grown under HNT showed increased R_S (21%) and relative injury to the leaf membrane (RI; 36%) compared to ‘Cocodrie’ plants grown under ANT (Fig. 3a, b), whereas ‘XL723’ plants showed 34% and 28% increase in R_S and RI, compared to ‘XL723’ plants grown under ANT (Fig. 3a, b).

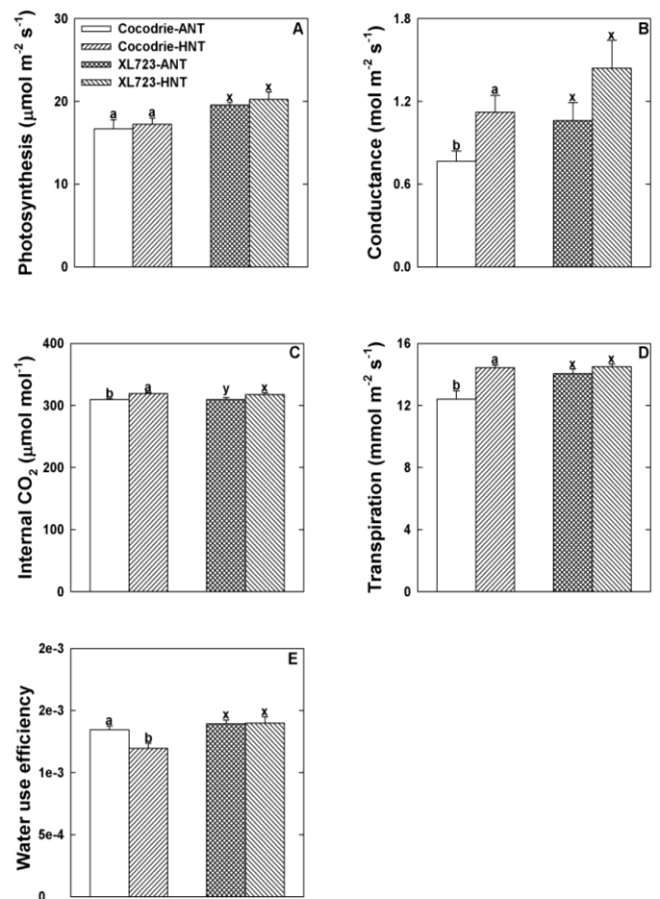


Fig 1. Responses of rice cultivars to night temperatures with respect to photosynthetic parameters. Analysis for differences between the night temperatures was performed separately for each cultivar. The values represent means with standard error bars, n = 36. Bars for a cultivar with different letters are significantly different at $P \leq 0.05$.

Table 2. Responses of southern US rice cultivars to night temperatures with respect to leaf characteristics and pigments.

Parameters	Cocodrie		% difference	XL723		% difference
	ANT	HNT		ANT	HNT	
Number of leaves	27.5 ± 2.5 a ¹	14.0 ± 0.1 b	-49	22.5 ± 6.5 x ¹	27.5 ± 2.5 x	NS
Leaf area, cm ² plant ⁻¹	181.3 ± 14.1 a	138.5 ± 26.8 b	-24	237.6 ± 74.8 x	234.1 ± 27.8 x	NS
Leaf length, cm	35.5 ± 1.1 a	31.5 ± 6.5 a	NS	36.5 ± 3.5 x	30.5 ± 2.5 x	NS
Leaf width, cm	1.3 ± 0.1 a	1.4 ± 0.1 a	NS	1.4 ± 0.2 x	1.1 ± 0.1 x	NS
Leaf nitrogen content, %	1.6 ± 0.1 a	1.5 ± 0.1 a	NS	1.2 ± 0.1 y	1.6 ± 0.1 x	37
Chlorophyll a, µg cm ⁻²	6.5 ± 0.5 a	6.5 ± 0.3 a	NS	8.9 ± 0.4 x	9.4 ± 1.0 x	NS
Chlorophyll b, µg cm ⁻²	1.9 ± 0.2 a	1.8 ± 0.1 a	NS	2.8 ± 0.2 x	3.2 ± 0.6 x	NS
Total chlorophyll, µg cm ⁻²	8.4 ± 0.7 a	8.3 ± 0.3 a	NS	11.6 ± 0.6 x	12.6 ± 1.6 x	NS
Chlorophyll a/b	3.4 ± 0.7 a	3.6 ± 0.4 a	NS	3.2 ± 0.4 x	2.9 ± 0.5 x	NS
Carotenoids, µg cm ⁻²	1.6 ± 0.1 a	1.6 ± 0.1 a	NS	1.2 ± 0.1 x	1.1 ± 0.2 x	NS
Phenolics, µg cm ⁻²	3.4 ± 0.3 a	3.3 ± 0.2 a	NS	4.8 ± 0.6 x	4.7 ± 0.7 x	NS

Each value is the mean with standard error (±S.E.) (n = 36). ¹Means within a row for a cultivar followed by a different letter differed significantly (P < 0.05). NS = not significant. Analysis for differences between the night temperatures was performed separately for each cultivar.

Morphology and dry weights

There was no difference between the night temperatures with respect to plant height, number of tillers per plant, number of panicles per plant, leaf dry weight and stem dry weight, for both the cultivars (Table 1). However, 'Cocodrie' and 'XL723' plants grown under HNT showed 10% and 6% decrease in panicle dry weight, respectively, compared to plants grown under ANT (Table 1).

Leaf and panicle characteristics

For both the cultivars, there was no difference between night temperatures for leaf length, leaf width, chlorophyll a, chlorophyll b, total chlorophyll concentration, chlorophyll a/b ratio, and carotenoid and phenolic concentrations (Table 2). However, 'Cocodrie' plants grown under HNT showed 49% and 24% decrease in number of leaves per plant and leaf area per plant, respectively, compared to 'Cocodrie' plants grown under ANT (Table 2). The 'XL723' plants grown under HNT showed 37% increase in leaf nitrogen content, compared to 'XL723' plants grown under ANT (Table 2). For both the cultivars, there were no differences between night temperatures for panicle length and primary branches per panicle (Table 3). However, 'Cocodrie' plants grown under HNT showed 21%, 25% and 29% decrease in total grains per panicle, filled grains per panicle and grain weight per panicle, respectively, compared to 'Cocodrie' plants grown under ANT (Table 3). For cultivar 'XL723', plants grown under HNT showed 29% and 9% decrease in unfilled grains per panicle and grain weight per panicle, respectively, compared to plants grown under ANT (Table 3).

Pollen germination, spikelet fertility and grain characteristics

The 'Cocodrie' and 'XL723' plants grown under HNT showed 49% and 36% decrease in pollen germination and 39% and 13% decrease in spikelet fertility, compared to

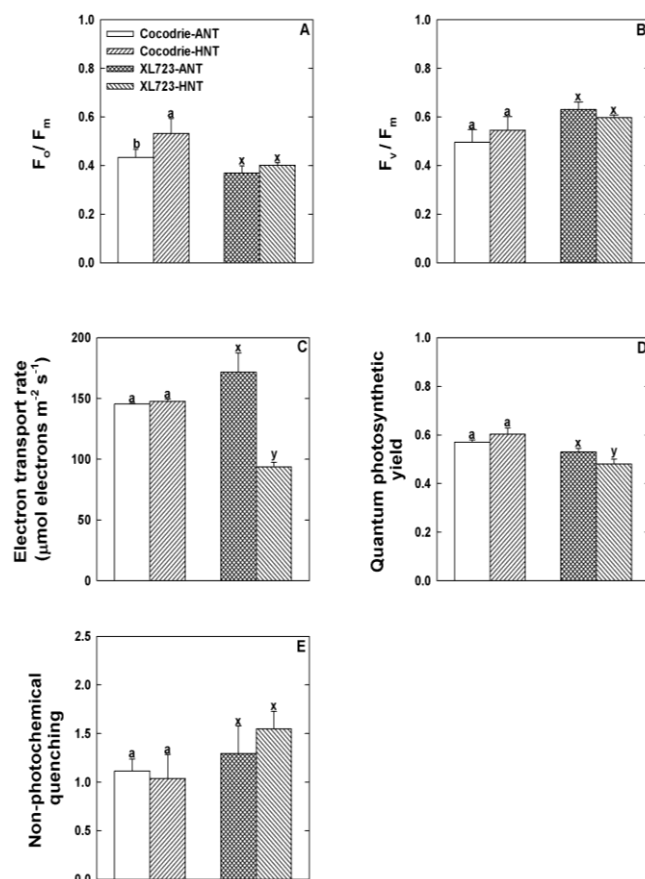


Fig 2. Responses of rice cultivars to night temperatures with respect to leaf chlorophyll fluorescence. Analysis for differences between the night temperatures was performed separately for each cultivar. The values represent means with standard error bars, n = 36. Bars for a cultivar with different letters are significantly different at $P \leq 0.05$.

Table 3. Responses of southern US rice cultivars to night temperatures with respect to panicle characteristics.

Parameters	Cocodrie		% difference	XL723		% difference
	ANT	HNT		ANT	HNT	
Panicle length, cm	17.1 ± 0.4 a ¹	16.8 ± 1.0 a	NS	18.9 ± 0.8 x ¹	17.6 ± 0.6 x	NS
Number of 1 ^o branches per panicle	12.0 ± 0.9 a	10.5 ± 0.6 a	NS	10.6 ± 0.4 x	9.8 ± 1.0 x	NS
Total grains per panicle	101.3 ± 3.5 a	80.0 ± 9.1 b	-21	87.4 ± 7.9 x	74.3 ± 7.4 x	NS
Filled grains per panicle	62.9 ± 4.4 a	47.4 ± 7.5 b	-25	36.6 ± 5.5 x	38.0 ± 9.1 x	NS
Unfilled grains per panicle	37.6 ± 5.4 a	32.6 ± 8.7 a	NS	50.8 ± 5.2 x	36.3 ± 3.2 y	-29
Grain weight per panicle, g	1.3 ± 0.1 a	0.9 ± 0.1 b	-29	0.74 ± 0.01 x	0.67 ± 0.01 y	-9

Each value is the mean with standard error (\pm S.E.) (n = 36). ¹Means within a row for a cultivar followed by a different letter differed significantly (P < 0.05). NS = not significant. Analysis for differences between the night temperatures was performed separately for each cultivar.

plants grown under ANT (Fig. 4a, b). In addition, plants grown under HNT showed decreased grain width and grain surface area. The ‘Cocodrie’ and ‘XL723’ plants grown under HNT showed 2% and 2.5%, and 2.4% and 3.8% decreases in grain width and grain surface area, respectively, compared to plants grown under ANT (Table 4). Strong association occurred between leaf respiration, membrane injury, pollen germination, spikelet fertility and yield (Fig. 5).

Discussion

Figuring out crop plant responses to various stresses and their underlying physiological mechanisms can provide insights into how plants may be modified to become more stress tolerant (Mohammed and Tarpley, 2011; Ni et al., 2009; Wang et al., 2010). Several studies have screened rice cultivars for genetic variation in response to increased day temperature (Baker, 2004; Matsui et al., 2001), carbon dioxide (Decosta et al., 2006), drought (Venuprasad et al., 2007) and UV-B radiation (Mohammed and Tarpley, 2011). To our knowledge, this is one of the first studies reporting genetic variation in rice cultivars in response to high night temperature. In the present study, cultivar ‘Cocodrie’ and cultivar ‘XL723’ showed differential responses to HNT, with respect to several morphological and physiological parameters. Both the cultivars showed increased ICO_2 , R_S and RI and decreased pollen germination, spikelet fertility, grain width, grain surface area and plant grain yield, when exposed to HNT. However, the degree of increase or decrease for a parameter varied between the cultivars. In the present study, HNT had no effect on P_N for both the cultivars. Similar results were seen in a previous study where HNT had no effect on P_N (Mohammed and Tarpley, 2009b). Under HNT, both the cultivars showed increase in internal CO_2 concentration, which indicates the inability of the leaf to fix the available carbon due to heat stress. As in our previous study, the increase in internal CO_2 concentration did not affect P_N (Mohammed and Tarpley, 2009b), indicating that the activity of Rubisco enzyme and the photosystems (Pastenes and Horton, 1996) might play an important role in governing P_N under HNT. Water use efficiency (WUE), described as the ratio of P_N and transpiration, decreased in cultivar ‘Cocodrie’ due to the increased transpiration. Ben-

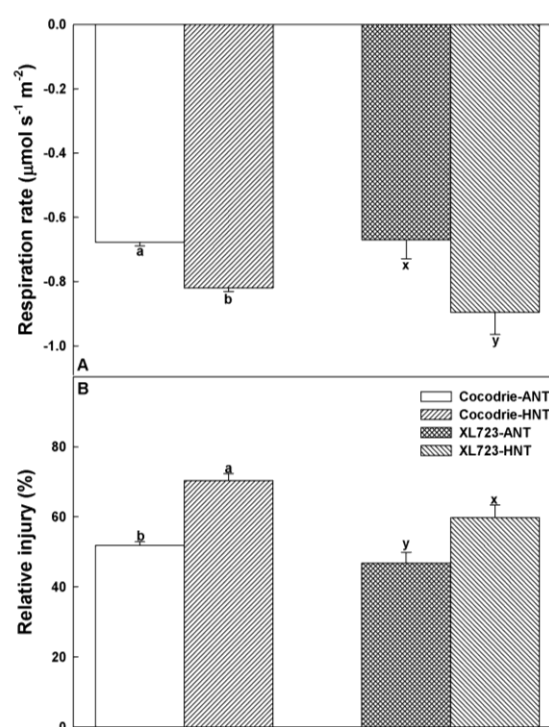


Fig 3. Responses of rice cultivars to night temperatures with respect to respiration rate and relative injury to the membrane. Analysis for differences between the night temperatures was performed separately for each cultivar. The values represent means with standard error bars, n = 36. Bars for a cultivar with different letters are significantly different at $P \leq 0.05$.

Asher et al., (2008) reported decreased WUE in sweet corn (*Zea mays* L. var. rugosa) under heat stress. There were cultivar differences with respect to leaf photochemistry. The HNT increased F_o/F_m in cultivar ‘Cocodrie’, which indicates the damage to the thylakoid membranes (Ristic et al., 2007), and decreased electron transport rate and quantum yield in cultivars ‘XL723’. In the present study, HNT increased

Table 4. Responses of southern US rice cultivars to night temperatures with respect to grain characteristics.

Parameters	Cocodrie		% difference	XL723		% difference
	ANT	HNT		ANT	HNT	
Grain length, mm	7.01 ± 0.04 a ¹	6.99 ± 0.04 a	NS	6.79 ± 0.08 x ¹	6.89 ± 0.03 x	NS
Grain width, mm	2.29 ± 0.01 a	2.25 ± 0.01 b	-2	2.22 ± 0.01 x	2.17 ± 0.01 y	-2.5
Grain surface area, mm ²	15.55 ± 0.15 a	15.18 ± 0.17 b	-2.4	14.58 ± 0.23 x	14.03 ± 0.21 y	-3.8
Grain volume, mm ³	36.11 ± 0.24 a	35.72 ± 0.27 a	NS	34.47 ± 0.36 x	34.12 ± 0.32 x	NS
Grain nitrogen content, %	1.46 ± 0.05 a	1.41 ± 0.10 a	NS	1.46 ± 0.07 x	1.56 ± 0.08 x	NS
Grain chalkiness, %	4.92 ± 0.56 a	5.94 ± 0.56 a	NS	8.15 ± 0.70 x	9.09 ± 0.84 x	NS

Each value is the mean with standard error (±S.E.) (n = 36). ¹Means within a row for a cultivar followed by a different letter differed significantly (P < 0.05). NS = not significant. Analysis for differences between the night temperatures was performed separately for each cultivar.

respiration rate and relative injury to the membrane for both the cultivars. However, cultivar ‘Cocodrie’ had higher membrane injury compared to cultivar ‘XL723’, under HNT. Previous studies reported increased respiration rate and relative injury to the membrane as a result of HNT (Cheng et al., 2009; Loka and Oosterhuis, 2010; Mohammed and Tarpley, 2009a). Higher respiration increases the proportion of assimilates respired, thereby affecting plant carbon status (Turnbull et al., 2002), whereas a leaky membrane as a result of injury to the membrane can impair the properties of the photosynthetic system, including key enzymes and thylakoid membrane activities (Björkman et al., 1980). In the present study, increase in night temperature from 25 °C to 30 °C had no effect on plant morphology and pigment concentration. However, increase in night temperature from 25 °C to 30 °C decreased pollen germination, spikelet fertility, grain width, grain surface area and yield. Previous studies reported decreased pollen germination, spikelet fertility, grain dimensions and yield as a result of heat stress (Cheng et al., 2009; Mohammed and Tarpley, 2010; Prasad et al., 2006). The decrease in pollen germination under heat stress is associated with poor anther dehiscence and pollen reception (Prasad et al., 2006) and reduced pollen swelling and decreased anther pore size (Endo et al., 2009), whereas decreased spikelet fertility is associated with impaired hormonal balance in the floret (Micheal and Beringer, 1980) and/or decreased availability of photosynthates to the kernel (Afuakwa et al., 1984) and/or inability of floral buds to mobilize carbohydrates under heat stress (Dinar and Rudich, 1985). The decreases in grain dimensions observed under HNT are associated with a reduction in average endosperm cell area (Morita et al., 2005), endosperm cell number (Commuri and Jones, 1999) and duration of dry matter accumulation (Zakaria et al., 2002). In the present study, rice yield showed a negative association with leaf respiration rate and relative injury and a positive association with pollen germination and spikelet fertility. Lambers (1985) reported a negative association between yield and respiration rates under heat-stressed conditions. In addition, several studies have also reported a negative association between yield and leaf membrane injury and a positive association between yield and pollen germination under heat-stress conditions (Al-Khatib and Paulsen, 1990; Reynolds et al., 1994; Ismail and Hall, 1999). In this study, relative injury to the leaf membrane along with pollen germination stood out as

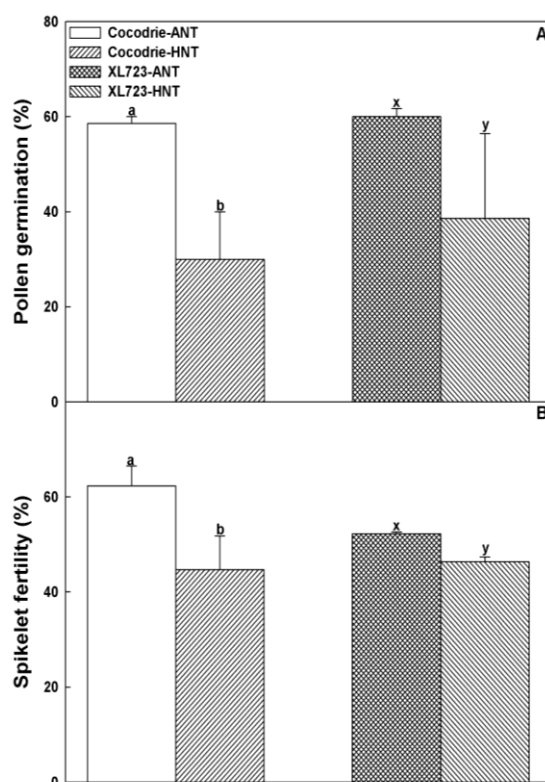


Fig 4. Responses of rice cultivars to night temperatures with respect to pollen germination and spikelet fertility. Analysis for differences between the night temperatures was performed separately for each cultivar. The values represent means with standard error bars, n = 36. Bars for a cultivar with different letters are significantly different at $P \leq 0.05$.

potential screening tools among various parameters for screening of HNT tolerance in Southern US rice cultivars.

Material and Methods

Plant materials

‘Cocodrie’ is a semidwarf long-grain variety developed from

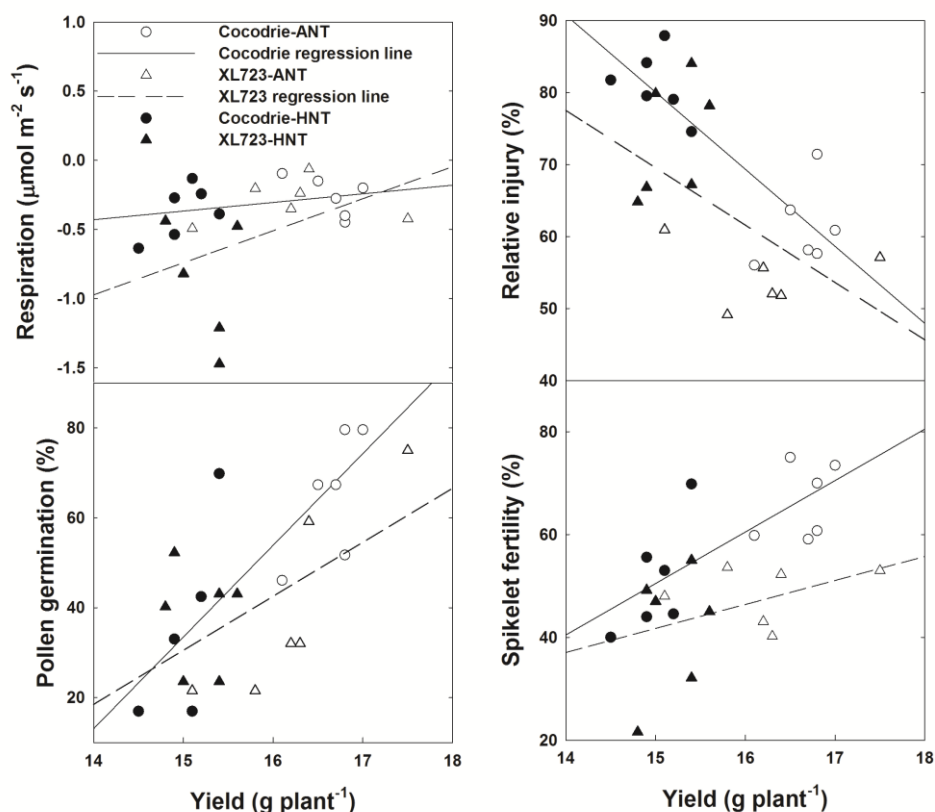


Fig 5. Scatter plots between yield and yield-determining parameters at different night temperatures. Regression line for each cultivar is also shown.

a cross of Cypress//L202/Tebonnet (Linscombe et al., 2000), which was used here to represent inbred pure lines due to its high popularity in the Southern US for many years after its release. ‘XL723’, is a product of RiceTec Co. (Alvin, Texas, USA) is a conventional (no herbicide tolerance) semidwarf long-grain hybrid of proprietary genetics, which, like Cocodrie, was used here due to its high popularity in the Southern US for many years.

Plant culture

Three experiments (repeats) were conducted in the greenhouse at the Texas A&M AgriLife Research Center at Beaumont, Texas, USA. Rice inbred pure line ‘Cocodrie’ and hybrid ‘XL723’ were used in all experiments. Plants were grown in 4.65 L pots filled with a clay-rich soil that is common to rice farms in the area which were then placed in a box. Five seeds per pot were sown at a 2.5-cm depth. After emergence, plants were thinned to one plant per pot, which were maintained until maturity. At 20 days after emergence (DAE), the boxes were filled with water to approximately 3 cm above the top of the soil in each pot. All the plants were maintained at ambient conditions until 30 DAE. At 30 DAE, randomly selected plants (half of the plants) were moved under the heat lamps (HNT). A reflective insulation with foil cover (ASTRO-E, Heartland Insulation Supply, Wichita, KS, USA) was placed over the water surface to prevent direct infrared heating of the water. A three-way split application of nitrogen was done as described by Mohammed and Tarpley (2011) in both the experiments. Nitrogen was applied in the form of urea and ammonium sulfate, and phosphorus in the form of P₂O₅. Urea-N was applied at the rate of 50 kg ha⁻¹ along with 34 kg ha⁻¹ of phosphorus (P₂O₅) at planting. The

remaining nitrogen fertilizations (101 and 90 kg ha⁻¹ of nitrogen in the form of ammonium sulfate) were applied 20 DAE and at the panicle-differentiation stage.

Night temperature treatments

There are three experiments presented in the study. The assignment of heat treatment to greenhouse location was random within each experiment. The average night temperatures at Beaumont, Texas, USA (Longitude: 94° 16' 59" W; Latitude: 30° 4' 0" N) during the reproductive growth of the rice plants often ranged between 25–27 °C (Yang et al., 2010). Hence, the ANT was set at 25 °C and HNT as 30 °C (ambient + 5 °C). The greenhouse was maintained at 25 °C night temperature and, within this, plants of the HNT treatment were subjected to a high night temperature (30 °C) through the use of nearly continuously controlled (sub-second response) infrared heaters (1100 W, Chromalox, Ogden, UT, USA), as described by Mohammed and Tarpley (2009c), starting 30 DAE. The infrared heaters were positioned 1.0 m above the topmost part of the plants and provided infrared radiation enrichment. In ANT treatments, dummy heaters were provided to account for shading. The night temperatures were imposed from 2000 h until 0600 h, starting from 30 DAE and maintained until harvest. The set-up and control of the infrared heating system used in this study (Mohammed and Tarpley, 2009c) minimizes direct heating of the plant tissue, including the use of a fast-response air-temperature thermocouple as input as opposed to tissue or tissue-surface temperature thermocouples, and an extremely rapid feedback/control system (i.e. 9 ms time base) with a proportional (as opposed to on-off) control of the heating element. In our previous study, the difference

between the leaf temperature of the plants growing under HNT and ANT was far less than the air temperature difference between the HNT and ANT, which is qualitatively similar to that found in most natural systems. This indicates that infrared heating of the water in foliage is not driving the heating of air in the system. A pair of infrared heaters provided heating to the box (0.84 m²).

Greenhouse conditions

Temperatures treatments in the greenhouse were monitored independently of the temperature-control system through the use of standalone sensor/loggers (HOBO, H08-003-02, Onset Computer Corporation, Bourne, MA, USA) in both portions of the study in each experiment. The HOBOs were placed in the canopy (few centimeters into the canopy), and the temperatures were logged every 15 min. The ANT and HNT were maintained at the set temperature plus or minus 0.5 °C. The mean day/night temperatures were 33.61/25.33 and 33.89/30.43 °C in ANT and HNT treatments and day/night humidity was 15.4/15.6 and 15.3/15.4 g m⁻³ in ANT and HNT treatments, respectively. The light intensity in the greenhouse varied between 600 to 1200 μmol m⁻² s⁻¹ on a clear sunny day.

Photosynthetic parameters

The P_N, stomatal conductance, internal CO₂ concentration and transpiration were measured on the penultimate leaves using a LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) between 1000 h and 1200 h, 40 DAE (10 days after heat treatment). When measuring P_N, the photosynthetic photon flux density (PPFD) was set to 1500 μmol m⁻² s⁻¹. The temperature, CO₂ concentration and flow rate in the leaf cuvette were set to 25°C, 390 μmol mol⁻¹ and 500 μmol s⁻¹, respectively. Water use efficiency (WUE) was also determined. In this study, WUE is a ratio between P_N and transpiration.

Chlorophyll fluorescence

Chlorophyll fluorescence is often used to evaluate the functionality of the photosynthetic system in chloroplast membranes under various stresses (Chen et al., 2010). Chlorophyll a fluorescence parameters, maximum quantum efficiency of photosystem-II (F_v/F_m), thylakoid membrane stability (F_o/F_m), quantum yield of PSII (Y), electron transport rate (ETR) and non-photochemical quenching (NPQ) were assessed by measuring fluorescence with a pulse-modulated fluorometer (OS5p, Opti-Sciences, Hudson, NH, USA). The minimal fluorescence (F_o), maximum fluorescence (F_m) and F_v/F_m were measured in 30 min dark-adapted leaves. For Y and ETR, plants were under a steady state of photosynthesis (plants were exposed to ambient sunlight for more than 5 hours), a prerequisite for measuring Y and ETR. A photosynthetically active radiation (PAR) clip (OS5p PAR Clip, Opti-Sciences, Hudson, NH, USA) provides the PAR measurements while measuring Y and ETR. While measuring the Y and ETR the range of PAR was 600-700 μmol m⁻² s⁻¹. The coefficient of non-photochemical quenching of excitation energy (NPQ) was calculated using Klughammer and Schreiber's equations, where NPQ is [(F_m-F_{ms})/F_{ms}] (Klughammer and Schreiber, 2008). The leaf chlorophyll fluorescence was measured 40 DAE.

Leaf respiration rates

Leaf-level respiration rates were measured using a LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE). Respiration rates were measured on the penultimate leaves, 40 DAE. While measuring respiration rates, the PPFD, provided by a 6400-02 LED light source, was set at 0 μmol m⁻² s⁻¹ (dark environment). The temperature and CO₂ concentration in the leaf cuvette were set at 25°C and 390 μmol mol⁻¹ (ambient CO₂ concentration in the greenhouse), respectively.

Relative injury to the leaf membrane (RI) and leaf pigments

The relative injury to the leaf membrane was measured using the procedure described by Mohammed and Tarpley (2009a), 40 DAE. The leaf chlorophyll and carotenoid contents were determined on the penultimate leaves 40 DAE using the procedures from (Chappelle et al., 1992). The pigment concentrations were expressed on a leaf area basis, μg cm⁻². Total chlorophyll was calculated by summing up the values of chlorophyll a and chlorophyll b. The leaf phenolic analysis was done using the procedures from Mohammed and Tarpley (2011). The values were expressed as *p*-coumaric acid units, which were expressed on a leaf area basis (μg cm⁻²).

Morphology and dry weights

At harvest, leaf length (from the point of contact of the leaf blade and the stem to the tip of the leaf) and width (widest part of the leaf) of the penultimate leaf was measured and viable leaf area per plant was measured using a LI-3100C area meter (LI-COR Inc., Lincoln, NE, USA). The leaf nitrogen content (LNC) was measured using a FP-528 Nitrogen/Protein analyzer (LECO Corporation, St. Joseph, MI, USA) at harvest and expressed as percentage (%; w/w).

Destructive sampling procedures were adopted at harvest: plants were dissected; plant height was measured, numbers of tillers, leaves and panicles were counted and dry weights were determined.

Pollen germination

The pollen germination (PG) was determined through *in vitro* germination on a culture medium prepared in accordance with the media of Song et al. (2001), with minor modifications. The components of the culture medium in the present study are 15 g sucrose, 0.3 g calcium nitrate, and 0.1 g boric acid dissolved in 100-mL deionised water. Agar, 6 g, was added to this solution and heated on a hot plate to dissolve the agar. When the agar was dissolved in the solution, 10-mL medium was poured per Petri dish (diameter 8.5 cm) and allowed to cool for 15 minutes. The pollen grains from the different cultivar X NT treatment combinations were dusted on the culture medium as soon as the floret opened in the morning. The Petri dishes were incubated at 25 °C in the dark for 48 h under controlled temperature and humidity (Convion CMP3244, Controlled Environments Ltd., Winnipeg, Manitoba, Canada). Pollen was considered as germinated when the tube length was at least equal to or greater than the grain diameter as determined using a microscope. The total number of pollen grains and germinated and non-germinated pollen in each Petri dish from five fields were counted, and the percentage of germinated pollen grains was recorded.

Panicle and grain characteristics

Main-stem panicles were harvested separately from the rest of the panicles and panicle length and number of primary branches on the main-stem panicle were determined. Total number of grains, and filled and unfilled grains per panicle were determined. Spikelet fertility was estimated using the procedures of Mohammed and Tarpley (2011). Spikelet fertility in this study was defined as the ratio of the number of filled grains to total number of reproductive sites (florets) at ANT treatment. This method was adopted because of floret abortion at HNT treatments. Each floret was pressed between the thumb and forefinger to determine if the grain was filled or not. The number of filled grains included both completely and partially filled grains. Spikelet fertility was expressed as percentage.

The paddy rice was dehulled manually and the grain length, width, surface area and volume of the brown (dehulled) rice were determined using a Winseedle (Regent Instruments, Inc. Quebec, Canada), which uses image analysis of scanned color images of the grain to calculate these parameters. The chalkiness in the brown grain was also determined using the Winseedle. The grain length and width are expressed in millimeters (mm), surface area in mm², volume in mm³ and grain chalkiness as percentage (%) of total grain area as observed in the Winseedle image. The GNC was measured using a FP-528 Nitrogen/Protein analyzer (LECO Corporation, St. Joseph, Michigan, USA). For GNC analysis, brown rice grains were packed in a capsule (QUIK CAPS, LECO Corporation, St. Joseph, Michigan, USA) for nitrogen analysis. To determine the weight of grain per capsule, each capsule was weighed with and without the grains. The GNC was expressed as percentage (%; w/w).

Experimental design and data analysis

The study was set up as a randomized block design (experiments as blocks). In each experiment, 12 randomly selected plants were exposed to ANT and the other 12 plants were exposed to HNT. In total, there were 36 plants per cultivar X night temperature treatment. To test the significance of experiments, night temperatures, and their interactive effects on morphology, leaf photosynthetic parameters, chlorophyll fluorescence, leaf pigments, respiration, pollen germination, spikelet fertility, yield and grain characteristics, data was statistically analyzed using analysis of variance (ANOVA; SAS statistical analysis package version 9.2, SAS Institute, Cary, North Carolina, USA). The means were separated using *Tukey's* Honest Significant Difference (HSD) at an alpha level of 0.05. Differences among the experiments, night temperature treatments, and their interactive effects were also tested. If there was no significant difference among the experiments for a parameter, then the values from all the experiments for that parameter were used to obtain the mean and error. The standard errors of the mean were also calculated and presented in the graphs as error bars. Analysis for differences between the night temperatures was performed separately for each cultivar.

Conclusion

This study indicated differential sensitivity of the cultivars to HNT for a number of parameters, thus implying that genetic diversity might exist in Southern US modern rice germplasm with regard to HNT tolerance. High night temperature decreased yield in 'Cocodrie' and 'XL723', which was

associated with increased respiration rate and relative injury to the leaf membrane and decreased pollen germination and spikelet fertility. In this study, 'XL723' yielded better than 'Cocodrie' under HNT as a result of less injury to the leaf membrane and greater pollen germination and spikelet fertility compared to 'Cocodrie'. The RI or pollen germination, individually or in combination, can be used as screening tools for HNT tolerance in future breeding efforts to enhance HNT tolerance in Southern US rice cultivars.

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