High night temperature induces contrasting responses for spikelet fertility, spikelet tissue temperature, flowering characteristics and grain quality in rice

Abridged title – Night temperature affects flowering, yield & quality in rice

Title

High night temperature induces contrasting responses for spikelet fertility, spikelet tissue temperature, flowering characteristics and grain quality in rice

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List of abbreviations

DAA, days after anthesis; DBA, days before anthesis; DT, day temperature; HDT, high day temperature; HNT, high night temperature; IRRI, International Rice Research Institute; NT, night temperature; NTc, critical night temperature; UoR, University of Reading.

ABSTRACT

Climate change is increasing night temperature (NT) more than day temperature (DT) in rice-growing areas. Effects of combinations of NT (24 - 35 °C) from microsporogenesis to anthesis at one or more DT (30 or 35 °C) at anthesis on rice spikelet fertility, temperature within spikelets, flowering pattern, grain weight per panicle , amylose content, and gel consistency were investigated in contrasting rice cultivars under controlled environments. Cultivars differed in spikelet fertility response to high NT, with higher fertility associated with cooler spikelets ($P<0.01$). Flowering dynamics were altered by high NT and a novel high temperature tolerance complementary mechanism, shorter flower open duration in cv N22, was identified. High NT reduced spikelet fertility, grain weight per panicle, amylose content and gel consistency, whereas high DT reduced only gel consistency. Night temperature >27 °C was estimated to reduce grain weight. Generally, high NT was more damaging to grain weight and selected grain quality traits than high DT, with little or no interaction between them. The critical tolerance and escape traits identified i.e., spikelet cooling, relatively high spikelet fertility, earlier start and peak time of anthesis and shorter spikelet anthesis duration can aid plant breeding programs targeting resilience in warmer climates.

Key words – Flowering dynamics, grain quality, high night temperature, rice, spikelet fertility

Summary text for the table of contents (80 words max)

High night temperature (HNT) can significantly reduce rice yield and quality. Flowering dynamics, spikelet tissue temperature determines seed-set among diverse rice genotypes exposed to early and/or late night temperature stress. Documented for the first time is a critical night temperature threshold of 27 ^{o}C beyond which reduction in spikelet fertility is induced. The different sensitivities to HNT of cultivars for spikelet fertility and quality will complement plant breeding efforts targeted towards enhancing resilience of rice cultivars to warmer climates.

Introduction

A greater increase in night-time minimum than daytime maximum temperature appears to be a common global phenomenon (Vose *et al.* 2005) and there are predictions that this trend will continue throughout the 21st century (IPCC 2013). Greater increase in mean night temperature (NT) together with hotter maximum DT (IPCC 2013) are detrimental to rice production and are important factors to be considered in crop improvement. High day temperature (HDT) and high night temperature (HNT) affect rice (Peng *et al.* 2004; Nagarajan *et al.* 2010; Shi *et al.* 2013; and other crops (Lobell and Field 2007). The response of rice to HDT during flowering, the most-sensitive reproductive stage, has been the subject of many studies (Satake and Yoshida 1978; Matsui *et al.* 2001; Jagadish *et al.* 2007; 2008; 2010*a*,*b*; Rang *et al.* 2011;). Knowledge of the effect of HNT at flowering is more limited, and the few reported studies aimed at critical reproductive stages (Cheng *et al.* 2009; Mohammed and Tarpley 2009*a*,*b*) are limited to the performance of solitary cultivars. Hence, an understanding of the diversity in HNT tolerance or sensitivity amongst contrasting genotypes is required in order to indicate the likelihood of differences in mechanisms amongst genotypes.

Rice flowering traits, within a day, are affected by that day and the previous night's temperatures. The previous night's temperature is negatively associated with time of peak anthesis (Julia and Dingkuhn 2012). Time of anthesis is strongly is strongly associated with temperatures between 0600 and 0900 than that from midnight till dawn (Kobayasi *et al.* 2010). High day temperature from late in the morning to noon, coinciding with peak anthesis in many cultivars (Sheehy *et al.* 2005; Prasad *et al.* 2006; Jagadish *et al.* 2008), reduces spikelet fertility (Jagadish *et al.* 2007; 2008; 2010*a*). High temperature earlier in the morning advances start of anthesis and flower opening by 1 to 2 h in some cultivars (Kobayasi *et al.* 2010). Heat escape by changes in flowering pattern has received much attention, particularly early morning flowering (Sheehy *et al.* 2005; Ishimaru *et al.* 2010), but a more holistic approach, including, for example individual spikelet anthesis duration is necessary to understand HNT effects at anthesis on rice.

Two important grain quality traits (amylose content and gel consistency) are affected by high temperature stress (Fitzgerald and Resurreccion 2009; Madan *et al.* 2012). But these studies like others, have either considered HNT effects over the entire crop life cycle (Nagarajan *et al.* 2010), during the reproductive and grain filling stages (Lanning *et al.* 2011),

or only from grain filling onwards (Cooper *et al.* 2008; Li *et al.* 2011). Also, investigations of grain quality at HDT have similarly mostly been during grain filling (Resurreccion *et al.* 1977; Li *et al.* 2011). The gel consistency of cultivars exposed to HDT, HNT or both, is variable with cultivars responding uniquely (Resurreccion *et al.* 1977; Li *et al.* 2011). Hence, the possible susceptibility of grain quality to high temperature stress, whether day or night, just before grain filling in contrasting cultivars requires systematic investigation.

These and other studies related to response of spikelet fertility, grain yield and grain quality to HDT during reproductive development and grain filling (Prasad *et al.* 2006; Jagadish *et al.* 2010*a*) or HNT (Mohammed and Tarpley 2009*a,b*, Nagarajan *et al.* 2010) have not considered the effect of HNT from microsporogenesis to anthesis combined with HDT at anthesis, or any possible interaction. Results from separate studies of HDT or HNT suggest similar effect (>70% reduction) on spikelet fertility and grain yield: for example, compare HDT effects in Jagadish *et al*. (2008; 2010*a,b*) with those of HNT in Mohammed and Tarpley (2009*a,b*). Ziska and Manalo (1996) hypothesized that cumulative exposure to HNT could affect rice yield as much as short-term HDT. This remains untested until now.

One way to compare HDT and HNT would be to determine their effects just above critical temperatures, but whilst DT beyond 33 °C can be said to be critical for spikelet fertility and ultimately grain yield (Satake and Yoshida 1978; Jagadish *et al.* 2008) reports of critical NT (NTc) vary from 23 °C (Nagarajan *et al.* 2010) to 25 - 33 °C (Ziska and Manalo 1996) and could conceivably be DT dependent (Lyman *et al.* 2013). Moreover, Peng *et al*. (2004) and Sheehy *et al*. (2006), using the same dataset, provided contrasting estimates of the effects of HNT and HDT on grain yield. The determination of NTc across a range of genetically-diverse rice cultivars is thus necessary.

Hence, to quantify the effect of HNT on rice spikelet fertility and to identify traits influencing HNT tolerance and HDT escape, the objectives were to:

1. Quantify differences in spikelet fertility across tested cultivars under HNT
2. Ascertain the association between spikelet fertility under HNT and night-time spikelet temperature
3. Explore the impact of HNT affects on subsequent daytime flowering traits
4. Investigate if early (1830 to 0000) and late (0000 to 0530) HNT affect subsequent daytime flowering traits differently.
5. Document the cumulative effect of HDT and HNT on amylose content and gel consistency

Materials and methods

Crop husbandry

Four experiments were carried out in the early wet season of 2011 at the International Rice Research Institute (IRRI), Philippines, one of which (Experiment 4) was also carried out in 2010 at the University of Reading (UoR), United Kingdom. A total of ten photoperiod insensitive cultivars (Table 1) identified for contrasting tolerance of HDT at flowering were selected, with seeds from the IRRI genebank.

At IRRI, seeds were sown into trays filled with clay loam soil. Fourteen-day-old-seedlings were transplanted into 10-L plastic pots filled with 7.5 kg of this soil, previously soaked for 40 h before transplanting. Basal fertilizer of 2 g urea [$CO(NH_2)_2$], 1 g phosphorus pentoxide (P_2O_5), and 1 g muriate of potash (KCl) per pot was applied at transplanting. Seedlings were thinned from four to two (Experiment 1) or one (Experiments 2-3) per pot 9 days later. Eight (Experiment 1) or four (Experiments 2 and 3) replicate pots were maintained per treatment combination. Each pot was top dressed with 1 g urea at mid-tillering (21 days after transplanting), watered daily, and maintained flooded from transplanting till maturity. Weeds were removed manually and plants sprayed 21 days after transplanting with Cypermethrin (Cymbush) and every other week with Cartap to control white flies (*Bemisia* spp.) and brown-back rice plant hoppers (*Nilaparvata lugens* Stål.). Transplanted materials were kept in a greenhouse at optimal conditions until transfer to growth chambers for the various treatments described below. Greenhouse temperature (30/26 °C day/night) was close to the control treatment (30/24 °C day/night) in the growth chambers. Actual greenhouse temperature and relative humidity conditions are given in subsection *Environments.*

At UoR, 12.5 cm diameter pots were filled with a soilless medium of steam-sterilized sand and acid-washed gravel mixed with peat compost and vermiculite in the proportions of 2:4:1:4. A controlled-release fertilizer, (Osmocote Pro 3-4 month feed, The Scotts Company, UK) was added to the soilless medium. Pots were soaked overnight and then four or five seeds of each cultivar sown. Seedlings were thinned to one per pot at the three-leaf stage. Pots were placed on automated trolleys in a glasshouse, as described in Jagadish *et al*. (2007), maintained at 30/24 °C day/night, with a 12 h thermo- and photoperiod. Automatic irrigation drips supplied approximately 65ml of acidified water (pH 4.5 to 5.0; acidified by addition of HCl) at 20 °C six times a day to each pot throughout the investigation.

Environments

Night temperatures of 24, 30, or 35 °C were selected, based on studies of earlier investigations (Ziska and Manalo 1996; Peng et al. 2004; Cheng et al. 2009; Mohammed and Tarpley, 2009a,b).

At IRRI, walk-in growth chambers [3.3 x 3.2 x 2.7 m (10.6 m^2)] set to common day and different NT of 30/24, 30/30, and 30/35 °C (11 h/11 h day/night) from 0630 to 1730 and 1830 to 0530, respectively, were used. A linear change in temperature from day to night and *vice versa* was programmed between 1730 and 1830 and 0530 and 0630. A constant day and night RH of 70-75% was maintained throughout the treatment period with atmospheric CO_2 concentration of about 380 µmol CO_2 mol^{-1}. Chamber CO_2 was not controlled or monitored. Aspirated air temperature and RH in the chambers were measured and recorded every 10 minutes using MINCER (Fukuoka *et al.* 2012). Light, supplied by six 1 kw high-intensity discharge lamps, was provided for 11 h d^{-1} at a photosynthetic photon flux density of 650 µmol m^{-2} s^{-1} at plant height and about 215 µmol m^{-2} s^{-1} during the temperature change periods, providing a photoperiod of 13 h d^{-1}. Photosynthetically active radiation measured 1.2 m above the base of the chamber/cabinet at the start of the experiments was 700 (UoR) and 650 µmol m^{-2} s^{-1} (IRRI). Greenhouse temperature and relative humidity during the day (0630 – 1730) were 29.8 °C (±2.66) and 80.9% (±8.69) respectively. Early night (1830 – 0000) temperature (26.7 °C [±1.15]) and relative humidity (87.7% [±4.44]) were similar to those of late night (0000 – 0530) at 25.5 °C (±0.67) and 92.0% (±2.36) respectively. Temperature and relative humidity error terms given alongside in parenthesis are standard deviations unless otherwise stated.

At UoR, very similar experimental environments were provided by growth cabinets, described in Jagadish *et al*. (2008). Glasshouse temperature was maintained close to the target 30/24 °C day/night at 28.7 °C (±1.99)/24.1 °C (±1.18), by a combination of heating and venting (day) and by air-conditioning units (night). Relative humidity was not controlled and so varied with day/night values of 46.2% (±12.87)/53.3% (±8.11).

Temperature treatments for the four experiments were given at growth stages corresponding to between R0 and R8 in the rice staging nomenclature proposed by Counce et al., 2000. The exact stage(s) for each experiment are given along with the titles of the experiment below.

Experiment 1: Spikelet fertility and spikelet tissue temperature (Stages R4 – R8)

Plants of the nine cultivars selected (Table 1) were moved into the chambers when 50% of panicles had at least one spikelet at anthesis. They were exposed to experimental

regimes for 24 days. Panicles with spikelets that had anthesed well before transfer were tagged and excluded from the investigation. Thereafter, the most developmentally-advanced panicle from each of the eight replicate pots that began and completed anthesis within the chambers was harvested at the end of the treatment period to record spikelet fertility. To estimate fertility, spikelets were pressed between thumbs to determine whether they were filled (fertile) or unfilled (sterile) (Jagadish *et al.* 2007).

Temperature inside the spikelet (hereafter spikelet temperature) was recorded for 48 h, commencing 2 days after the plants were moved into chambers using a 0.2-mm copper-constantan thermocouple (T PTFE twin twist fine thermocouple, RS Components Ltd., Northamptonshire, UK). The insulated thermocouple wire was clipped to the base of the panicle with tiny insulated paper clips and exposed thermocouple ends inserted between clasped lemma and palea of spikelet. Thermocouple wires were matched to within 0.1 °C in preliminary trials. Temperatures were monitored every 5 seconds and means logged every 5 minutes (CR1000 data logger, Campbell Scientific, Inc., USA).

Experiment 2: Daily flowering and spikelet anthesis duration (Stage R4)

Three cultivars (Table 1) were investigated at three NT (24, 30 and 35 °C) with a common day of 30 °C provided for four consecutive nights in a 3 x 3 factorial experiment. Of the three cultivars chosen, two showed contrasting responses to HNT in Experiment 1 (IR64, HNT susceptible; and N22, HNT tolerant) and the third was selected for its early morning flowering trait (CG14). Plants on the first day of anthesis were transferred from the greenhouse to the chambers for the inclusive period 1830 to 0530 each night and then returned to the greenhouse. Spikelet anthesis duration was defined as the period from the start of the separation to the re-joining of the lemma and palea. Daily flowering duration per panicle (number of hours within a day during which some spikelets on a panicle flower) was taken as the difference in time between the first spikelet undergoing lemma-palea movement and the last spikelet lemma and palea re-joining within the target panicle in a day. Anthesis duration of two spikelets from the mid-section of four main panicles were recorded using digital stopwatches (SNS Professional, JS-307; Guangdong, China) from 0600 to 0800 (CG14), 1000 to 1200 (IR64) and 0830 to 1210 (N22) on the first, second and fourth day, after imposing HNT treatments. These observation times differed due to the inherent differences in flowering times of the cultivars. Pots were moved carefully out of the chambers on push carts to minimize shaking and neither the spikelet nor panicles were touched to prevent external stimuli inducing anthesis (Kobayasi *et al.* 2010).

Experiment 3: Times of start of and peak anthesis (Stage R4)

Cultivars CG14, N22 and WAB56-104 were exposed to three NT (24, 30 and 35 °C) and two night period treatments (early, 1830 to 0000; or late, 0000 to 0530) in a 3 x 3 x 2 factorial design with ambient temperature in the greenhouse during the day and the half of the night when plants were not in the chambers.

Treatments began the night following the day the first spikelet on the main panicle anthesed. Plants were transferred in and out of the chambers to provide a 5.5 h treatment (as above) on four consecutive nights. On each subsequent day (i.e. after each night of treatment) the flowering pattern (timing) was recorded. The number of spikelets that completed anthesis was recorded at 30 min intervals from 0500 until 1400 each day from four replicate main panicles. Start and peak anthesis time were defined as when the tip of the lemma began to move away from the palea of the first anthesing spikelet and when most spikelets were open, respectively. Similar to Experiment 2 care was taken to avoid inducing external stimuli.

Experiment 4: Spikelet fertility, grain weight per panicle and grain quality (Stage R0 - R4)

Each investigation, i.e. at UoR or IRRI, was a 2 x 3 x 10 factorial, with two DT (30 °C throughout, or 30 °C with 35 °C provided only briefly at anthesis, see below), three NT (24, 30 or 35 °C each provided from microsporogenesis to 3 days after anthesis (DAA) of the main panicle (i.e. first panicle to emerge), and ten cultivars differing in HDT tolerance (Table 1). The microsporogenesis stage was determined based on two criteria (i) estimated 12 days before the date of anthesis or (ii) when the distance between the collar of the fully-opened leaf and the collar of the flag leaf (yet to emerge) was 8 to 9cm (Jagadish *et al.* 2013). On the first day of anthesis (or the following day if anthesis started after treatment would have begun), half the plants remained at 30 °C DT and half were moved to 35 °C DT from 0900 to 1400 (UoR) or 0800 to 1400 (IRRI) and then returned to the original regime. Day/night thermo- and photoperiod regimes of 0700 to 2000 /2000 to 0700 at UoR and 0530 to 1830 /1830 to 0530 at IRRI were used for the 30/24, 30/30 and 30/35 °C day/night regimes for this period. Gradual changes in temperature and lighting to simulate natural conditions were programmed from 0700 to 0800 and 1900 to 2000 (UoR) or 0530 to 0630 and 1730 to 1830 (IRRI).

The NT and DT treatments were provided for 15 nights and 5-6 h, respectively, based on previous studies. As little as 1 h of high day temperature at anthesis results in spikelet sterility (Jagadish *et al.* 2007). We extended the period to 5 or 6 h to encompass the entire

flowering period in a day and to ensure that all spikelets opening received at least 1 hour of stress coinciding with anthesis, considered critical, as recommended by Jagadish *et al*. (2007). Longer (>4 – 12 h) nights at relatively higher temperatures over a greater period are required to achieve spikelet sterility levels similar to that from HDT (Satake and Yoshida, 1978; Yoshida *et al.* 1981). The factorial design provided one NT-DT treatment combination with a NT 5 °C warmer than the DT. This would be an unusual combination for a field environment, but such experimental designs help to elucidate the separate effects of HNT and HDT and enabled us to test the proposal from Ziska and Manalo (1996) that cumulative exposure to HNT would affect rice yield as much as short-term high DT.

Data were collected for the main panicle only. Spikelet fertility, grain weight per panicle and grain quality (amylose content and gel consistency) were determined after harvest at maturity. Polished grains for amylose content and gel consistency determination were ground to pass through a 0.5 mm sieve in a cyclone mill (Udy Cyclone Sample Mill 3010-030, Fort Collins, CO). Grain quality traits for both cultivars and both years were determined at the Grain Quality and Nutrition Center, IRRI, as described by Juliano (1971) and Tran et al. (2011), gel consistency being estimated indirectly by the alkali-spreading method (Little *et al.* 1958).

Data analyses

All statistical analyses were carried out with GenStat (GenStat® 11th Edition, VSN International Ltd., UK) including the Shapiro-Wilk test for normality and Bartlett's test for homogeneity of variances.

Experiment 1: ANOVA was used to investigate the effects of the treatments on spikelet fertility and spikelet temperature. Vapour pressure deficit was included as a covariate for the fertility analysis. Spearman's rank correlation coefficient was used to test spikelet fertility response across temperatures.

Experiment 2: Spikelet anthesis duration was analyzed by the Generalized Linear Model procedure with cultivar and temperature as variables in the model. Thereafter, individual cultivars were subjected to one-way ANOVA with temperature as a factor; means were separated by LSD ($P = 0.05$). Means of two spikelets each, from four plants on the first, second and fourth day following treatment application were used as replicates. Comparative regressions were carried out on spikelet anthesis duration with 24 °C as reference.

Experiment 3: Start of anthesis and peak anthesis times, cumulative percentage of anthesed spikelets at the time of peak anthesis and duration of flowering in the main panicle were analyzed by Generalized Linear Model. Cultivar, time of treatment (early or late night), and temperature were included in the model. Means of four plants each for the first, second and third day of flowering were used as replicates.

Experiment 4: Spikelet fertility and grain weight per panicle were analyzed by residual maximum likelihood with cultivar, NT and DT as fixed models, pot number the random model and residual terms calculated for each year. Amylose contents were not distributed normally (Shapiro Wilk's W=0.974, P=0.002) even after transformation, but the variances were homogenous (Bartlett's $\chi 2$= 15.8, P=0.071) and so data were analyzed by Generalized Linear Model with a square root link function. Gel consistency was analysed by the generalized linear mixed models as a normal distribution with an identity link function. The fixed models were the same as for spikelet fertility and grain weight per panicle but the random model included pot number and year.

Results

Spikelet fertility and spikelet tissue temperature responses to high night temperatures (Stages R4 – R8; Experiment 1)

Spikelet fertility was negatively correlated with spikelet temperature (r=-0.51; P<0.01; Fig. 1) and night temperature (r=-0.52; P<0.001). In all nine cultivars, the difference between within spikelet and chamber temperature reduced with increase in NT from 24 to 35 °C. The strength of correlation between spikelet fertility and spikelet temperature for individual genotypes were WAB56-104 (-0.827) > Lemont (-0.786) > IR64 (-0.785) > Bala (-0.777) > Azucena (-0.690) > Moroberekan (-0.689) > N22 (-0.512) > IR36 (-0.509) > Co39 (-0.429), with an average correlation (r=-0.51) across all genotypes (Fig. 1). Although chamber vapour pressure deficit during the night varied between 0.708 – 1.713 (kPa) across cultivars, it did not explain genotypic difference (P>0.05) in spikelet fertility under varying temperature regimes.

Significant differences in spikelet fertility were detected amongst cultivars (P<0.001) and NT (P<0.001), with considerable interaction between them (P<0.001): spikelet fertility varied between extremes of 92% (N22, coolest NT) and only 6% (Lemont, warmest NT) (Fig. 2A). Cultivars were classified into four groups based on spikelet fertility in the warmest regime (35 °C): highly tolerant (spikelet fertility >80%), tolerant (>50 to ≤80%), susceptible (>20 to ≤50%), and highly susceptible (≤20%) (Fig. 2A).

Cultivar N22 had the highest spikelet fertility at all temperatures and even that at 35 °C was also greater (P<0.05) than all other cultivars at all temperatures (24 to 35 °C). Cultivars Azucena, WAB56-104, Moroberekan, IR64 and Lemont had very low spikelet fertility (≤20%) at 35 °C. An increase in NT from 24 to 30 °C did not reduce spikelet fertility, except in WAB56-104 (declined from 64 to 45%). The apparent increases in spikelet fertility at 30 °C compared with 24 °C for the highly tolerant N22, tolerant IR36, and susceptible Co39 were not significant (P>0.05).

Cultivar (P<0.001), NT (P<0.001), and their interaction (P<0.001) resulted in contrasting outcomes for spikelet temperature relative to air temperature in chambers (warming or cooling depending upon treatment combination, Fig. 2B). Spikelets of the tolerant cultivars IR36 and Bala and also the highly susceptible WAB56-104 had temperatures significantly warmer than those of the air in chambers at both 24 and 30 °C. At

35 °C, all cultivars had spikelets cooler than the chamber temperature except Bala (Fig. 2B; 1.1 °C warmer).

Night temperature effects on spikelet anthesis duration (Stage R4; Experiment 2)

High NT (as main effect) increased the following day's spikelet anthesis duration on the main panicle in IR64 ($P<0.001$; Fig. 3) and CG14 ($P<0.05$) but not N22 (P>0.05), although this main effect was comparatively slight. Regression analysis was applied to identify the effect of HNT. Comparison of regressions within each cultivar identified variation in temperature responses for the different days of observation ($P<0.05$) in CG14 ($F=7.03_{[4,30]}$) and N22 ($F=14.20_{[4,30]}$), whereby responses for observations in days 1 and 4 in CG14 and day 2 in N22 were not significant (Suppl. Table 1). Spikelet anthesis duration of IR64 showed an inverted "V" pattern with NT. It was longest at 30 °C, the greatest value recorded in this experiment (86 ± 4.5 min). The HNT-tolerant N22 had the shortest mean spikelet anthesis duration. Its spikelet anthesis duration on Days 1 and 4 decreased and increased respectively, the greater the NT.

Night temperature induced changes in daily flowering duration, time of start and peak anthesis (Stage R4; Experiment 2 and 3)

Increase in early NT from 24 to 30 or to 35 °C advanced start of anthesis by 2 or 2.5 h respectively in WAB56-104, but no effect was detected in the other two cultivars (Suppl. Table 2). Late HNT advanced the start of anthesis in N22 by 30 min at both 30 and 35 °C, while a delay and advance in flowering by 30 min at 30 and 35 °C, respectively, was recorded in WAB56-104, with no change in CG14. CG14 opened its first flower before 0530 h consistently (Suppl. Table 2) and because observations started at 0500 h and every 30 min thereafter no HNT effect could be detected.

The interaction of NT and the time of exposure to NT on peak anthesis time was most marked in WAB56-104 ($P<0.001$), with the biggest shift observed in plants exposed to a late NT of 35 °C, when peak anthesis time was advanced by 5.5 h (Fig. 4A,B). Peak times of anthesis for CG14 and N22 treated to early NT were 0800 and 1000, respectively, with advances of about 1 to 2 h in timing with late-NT-treated plants.

The cumulative percentage of anthesed spikelets at time of peak anthesis (Suppl. Table 2) was not affected by NT ($P=0.13$) or time of exposure to NT ($P=0.59$), but cultivars differed ($P<0.05$). Although WAB56-104 appeared to be the most sensitive to HNT and also to late night exposure to HNT, the values of cumulative anthesed spikelets at the peak did not

differ greatly across all treatment combinations. Cultivars differed in daily anthesis duration on the main panicle ($P<0.001$) and in response to temperature at the different periods of the night ($P<0.05$). Across all temperatures, N22 had the briefest duration of flowering in the main panicle (c. 4 h) differing ($P<0.05$) from both CG14 (c. 6.5 h) and WAB56-104 (c. 7 h) (Fig. 4C). In WAB56-104, early HNT increased ($P<0.05$) the following day's flowering duration from 5 h (at 24 °C) to 8 h (at 35 °C) and late HNT also increased it from 6 to 8 h (Fig. 4C, D). In contrast, estimates for N22 and CG14 were not affected ($P>0.05$).

Day and night temperature effects on spikelet fertility, grain weight per panicle and grain quality (Stage R0 - R4; Experiment 4)

Although the recorded environments at UoR and IRRI were not identical, e.g. greenhouse RH was higher at IRRI (day 70.9% ±7.4; night 89.6% ±4.7) than UoR (day 47.5 % ±12.6; night 53.3% ±9.9), the two runs of the experiment provided similar results and were combined. For example, in the analysis of variance for spikelet fertility the estimate of the residual term for 2010 (380.8±48.5; UoR) was only slightly larger than for 2011 (301.5±29.2; IRRI).

Mean spikelet fertility at 30 and 35 °C DT were almost identical at 62% (Table 2). Although the main effect of DT imposed for 5 h (UoR) or 6 h (IRRI) on spikelet fertility was not significant, those of cultivar and NT from microsporogenesis to 3 DAA were, while the first order interaction of NT and DT and the second order interaction of NT, cultivar and DT approached significance ($0.10 > P > 0.05$). Increase in NT from 24 to 30 °C at either 30 or 35 °C DT had comparatively minor effect on spikelet fertility overall (Table 2) or within individual cultivars (Fig. 5A). The reduction in spikelet fertility with further increase to 35 °C NT was however substantial both on average (more than halved, Table 2) and in most individual cultivars (Fig. 5B). At 35 °C NT, the effect of brief increase from 30 to 35 °C DT was nil or negligible in most cultivars, but reduced spikelet fertility further in cvs Moroberekan, IR64 and Co39 (Fig. 5B). Cultivars CG14 and N22 were the most tolerant to increase in NT from 24 to 35 °C for spikelet fertility with Azucena, Co39, Lemont, IR64 and WAB56-104 being the most sensitive (Fig. 5B). No association was detected between cultivars' spikelet fertility responses to high DT (35/24 °C) with combined high DT and NT (35/30 and 35/35 °C), but high NT responses were associated with those to combined high DT and NT (Suppl. Table 3).

When averaged across cultivars, the effects of DT and NT on grain weight per panicle (Table 2) showed a similar pattern of differences to those for spikelet fertility (Table 2)

whereby the relative reduction in grain weight per panicle was of the same order as for spikelet fertility. Both DTs provided 2.5 g dry matter per panicle, but 35 °C NT provided only a third of the yield of 24 or 30 °C NT (Table 2; Fig 5 C, D). As with spikelet fertility, analysis of variance showed no effect of DT alone or in interaction with NT on grain weight per panicle (Table 2). NT, cultivar, and the first order interactions of cultivar with each of NT and DT did affect grain weight per panicle, however.

Comparison of regressions was employed to compare cultivar responses of grain weight per panicle to NT. These curvilinear relations were best described with linear and quadratic terms (Suppl. Table 4) for each cultivar separately ($P<0.001$). Estimates of NTc, i.e. the value where grain weight per panicle was calculated from the fitted relations to decline with further increase in NT, varied from 26.1 to 28.5 °C amongst cultivars, albeit not significantly (Suppl. Table 4). Most cultivars showed no difference in this curvilinear response between 30 and 35 °C DT but differences within this group of cultivars in the magnitude of grain weight per panicle decline between 30 and 35 °C NT were apparent: for example, grain weight per panicle was reduced far more in WAB56-104 than in CG14 (Suppl. Fig. 1). The responses for cvs IR64, Moroberekan, and Co39 differed with DT (Suppl. Table 4) with grain weight per panicle in cv. IR64 being particularly more sensitive to high NT at 35 compared with 30 °C DT (Suppl. Fig. 2).

With regard to grain quality, the ten cultivars differed ($P<0.001$) in amylose content and its response to NT (Table 3). In Azucena, Bala, IR36, IR64 and WAB56-104, amylose content declined ($P<0.05$) with increase in NT but not in CG14, N22 and Co39 ($P>0.05$). There was an interaction of cultivar and NT for amylose content ($P<0.001$) but no other interactions were detected. CG14 and N22 had amylose content ≥23% across all NT whereas Azucena, IR64 and WAB56-104 showed <20%. The decline in amylose content with increase in NT from 24 to 35 °C was greatest in Azucena, IR36 and WAB56-104 (0.4% per °C). Generally, amylose content decreased with increase in NT and also with increase in DT although these differences were small in the context of those amongst cultivars (Table 3).

Gel consistency varied significantly across cultivars, was reduced with increase in DT, and was reduced considerably at 35 °C NT compared with cooler NT (Table 4). Cultivars Co39 and CG14 had the greatest gel consistency, with Bala by far the lowest. No first or second order interactions amongst the effects of cultivar, NT and DT on gel consistency were detected ($P>0.05$).

Discussion

In rice-growing regions, days at 33 °C and above are considered critical (Prasad *et al.* 2006; Jagadish *et al.* 2007; 2008). HDT at anthesis reduces anther dehiscence, anther pore size, pollen viability and arrests fertilization, which all lead to reduced spikelet fertility (Matsui *et al.* 2001; Prasad *et al.* 2006; Jagadish *et al.* 2010 *a,b*). HNT also results in poor spikelet fertility and grain weight per plant by reducing pollen germination (Mohammed and Tarpley 2009 *a,b*; 2010), grain endosperm cell size and grain growth rate (Morita *et al.* 2005), reducing grain size and weight (Cooper *et al.* 2008; Mohammed and Tarpley 2010).

In this study, substantial differences in the sensitivity of spikelet fertility to HNT were identified within the rice germplasm investigated. The sensitivity of spikelet fertility to HNT varied considerably from the highly-tolerant N22 to the highly-susceptible Lemont. The latter's high susceptibility is similar to the report of approximately 90% reduction in spikelet fertility at 32 ^{o}C for "Cocodrie" – a cultivar from USA (Mohammed and Tarpley 2009*a*). Studies which have used single cultivars like "Cocodrie" and reported very large reductions in spikelet fertility (~90%) could lead to overestimation of the impact of HNT when generalised. Unless, by coincidence a tolerant cultivar is selected, single cultivar studies will fail to identify tolerant cultivars like N22, whose trait through breeding options can be included in background populations of present popular cultivars (e.g. IR64). Using different entries as done in study puts the impact of HNT on rice in perspective and identifies cultivars that could aid breeding programmes.

The temperature recorded within the spikelet was negatively associated with spikelet fertility, indicating that cooling spikelets was an effective mechanism to cope with warmer nights. During the day, spikelet-ambient temperature differences of 1.8 °C (Jagadish *et al.* 2007), 2 °C (Nishiyama 1981), and 3.5 °C (Madan *et al.* 2012) above 30 °C have shown that rice spikelets employ cooling mechanisms in response to HDT. Similar cooling here at night may have helped to reduce HNT stress among eight of the nine cultivars investigated. The exception was Bala (spikelet temperature 1.1 °C warmer than the 35 °C regime) despite being relatively tolerant of HNT. Hence, cooling is probably not the only mechanism to maintain fertility under HNT stress. Better pollen viability, pollen germination, and fertilization have been invoked to explain HDT tolerance (Jagadish *et al.* 2010*a*) and may be applicable to HNT tolerance also.

Significant variation in flowering characteristics among the cultivars was observed in the day after HNT, with a greater effect of late than early HNT. Differences in spikelet anthesis characteristics of the cultivars, sometimes considerable, were noted for duration and

for the pattern across the days of observation. Spikelet anthesis duration of N22 reduced with increase in NT on the first day of anthesis, when most spikelets anthesed. Moreover, this duration showed the least variation across all observations in N22 and was also shorter than other cultivars. In addition to the comparatively early start to daily flowering (Yoshida *et al.* 1981), we identified a novel complementary mechanism of shorter flower open duration in N22 which could reduce the risk of longer duration of exposure to hotter ambient temperature, further enhancing fertility aided by its superior high temperature tolerance at anthesis (Jagadish *et al.* 2010*b*).

Times of start and peak anthesis of WAB56-104 were advanced by 3 to 5 h at HNT. Start of anthesis of CG14, which flowers 1.5 to 2.5 h earlier than *O. sativa* cultivars (Yoshida *et al.* 1981; Sheehy *et al.* 2005; Prasad *et al.* 2006), was unaffected by HNT. The cessation of HNT stress, especially late NT, might be due to attainment of the required thermal time to flowering or due to a range of mechanistic aspects such as induced conversion of starch to sugars, which could possibly be driven by increased pollen respiratory demand in response to stress (Parish *et al.* 2012) or increasing water absorption in pollen (Matsui *et al.* 2000). Some of the above reasons could possibly influence advancement of times of start and peak anthesis in the subsequent days. Advancing times of start of anthesis and peak anthesis could minimize yield losses by increasing spikelet fertility under HDT (Ishimaru *et al.* 2010; Kobayasi *et al.* 2010). On the other hand, the advantage from the early start and peak anthesis times could be lost due to changes in other flowering characters from HNT detected here. These include increased spikelet anthesis duration, increased daily flowering duration per panicle and reduced cumulative percentage of anthesed spikelets at time of peak anthesis. Such changes in flowering characteristics might partly explain the susceptibility of WAB56-104 to HDT (Jagadish *et al.* 2008): more spikelets would be exposed to longer periods of HDT, reducing spikelet fertility and yield.

In the HNT-tolerant N22, all flowering components acted harmoniously to facilitate escape from HDT after exposure to HNT. An earlier start and peak time of anthesis accompanied by a shorter spikelet anthesis duration and high cumulative percentage of anthesed spikelets at the time of peak anthesis would ensure a high percentage of spikelets escaped HT the following day (Sheehy *et al.* 2005; 2006; Prasad *et al.* 2006; Ishimaru *et al.* 2010). In previous research, HNT-induced flowering changes have been linked with poor anther dehiscence (Kobayasi *et al.* 2010) as have HDT-induced changes (Matsui *et al.* 2000; Matsui and Omasa 2002).

The particular combination of long exposure to HNT combined, or not, with brief exposure to HDT at anthesis was designed to mimic two different aspects of climate change scenarios whilst providing new insight into HNT effects which cannot be provided from field investigations (e.g., HNT > HDT are not common in the field). High NT from microsporogenesis to three days after anthesis was far more damaging than HDT imposed for only 5-6 h at anthesis, the magnitude of the effect varying amongst cultivars. High NT (35 °C) resulted in spikelet fertility and grain weight per panicle reductions of up to 80 and 95%, respectively, similar to the effect of extreme high DT (Yoshida *et al.* 1981; Prasad *et al.* 2006; Jagadish *et al.* 2008). This further confirms the threat future climates with warmer NT pose to rice production (Peng *et al.* 2004).

The response of grain weight per panicle to interactions between NT and DT varied with genotype. It was negative in cv. IR64, additive in cvs Co39 and Moroberekan, and had no DT effect in the remaining seven cultivars (Suppl. Table 4). No NT by DT interactions was detected for either estimate of grain quality. Exposure to a longer high NT from microsporogenesis onwards could have resulted in acclimatization or acquired thermotolerance (Sung *et al.* 2003) to a short high DT exposure provided only at anthesis. Despite high NT ceasing soon after anthesis, high NT affected amylose content and gel consistency. This indicates a lack of plasticity within the panicle for recovery from stresses experienced just before grain filling and is compatible with reports for chilling stress on maize (*Zea mays* L.) tassels (Tranel *et al.* 2009) and high DT stress on rice (Madan *et al.* 2012).

The effects of high NT from microsporogenesis to anthesis and of high DT at anthesis on amylose content and gel consistency were similar to those reported for high temperature stress either during grain filling or throughout the rice crop life cycle (Cooper *et al.* 2008; Fitzgerald and Resurreccion 2009). Although the initial amylose content of cultivars varied, high NT increased variation further (Table 3). This reduction in amylose content by high NT means stickier less-desirable grains. The vulnerability of amylose content to high NT cannot be said to be sub-species specific as the three most–sensitive (Azucena, WAB56-104 and IR36) and the four insensitive cultivars (CG14, N22, Co39 and Moroberekan) each include indicas and japonicas. Unlike other traits examined, gel consistency remained within the preferred range (60 to 100 mm gel length), except in cv. Bala which was consistently poor (Table 4).

Estimates of critical DT for spikelet fertility and grain yield have been reported (Satake and Yoshida 1978; Jagadish *et al.* 2008). In this study we have estimated NT*c* for rice

grain weight per panicle using diverse cultivars: irrespective of DT or cultivar, increase in NT above about 27 °C was calculated to reduce grain weight per panicle. Cultivars did vary substantially however in the magnitude of the decline in grain weight per panicle above NT*c* (e.g. Suppl. Fig. 1). Tashiro and Wardlaw (1989) working with rice cv. Calrose reported a 4.4% decline in grain weight per 1 °C rise in temperature beyond NT of 26.7 °C: here the equivalent gradients varied amongst cultivars from as little as a fifth to almost three times the above estimate.

We have shown substantial genotypic differences in the sensitivity of spikelet fertility, grain weight per panicle and quality to HNT. Moreover, heat-tolerant cultivars generally employed spikelet cooling under HNT whilst HNT also altered subsequent patterns of flowering in the day. Cultivars in this study were common to the study by Jagadish *et al.* (2008) of spikelet fertility in response to HDT. Cultivar ranking for tolerance of HDT and HNT across the two studies showed many similarities, with genotypes more tolerant of HNT also being more tolerant to HDT, with N22 the most tolerant to both HDT and HNT.

The three most resilient cultivars (best retention of spikelet fertility and least grain weight per panicle reduction at 35 °C NT), namely CG14 (an early-morning flowering cultivar), N22 (a high-day temperature tolerant cultivar), and Bala (with N22 in its pedigree), are all upland adapted ecotypes. The good performance of N22 under HNT is confirmed by a recent field study (Shi *et al.* 2013). The tolerance of CG14 to high NT contrasts with reports of its susceptibility to high DT (Prasad *et al.* 2006; Jagadish *et al.* 2008). The former could result from traits not previously examined such as high NT tolerant pollen, high pollen production compensating for reduction in viable pollen count, and normal pollen tube growth through the ovule under HNT, all of which are associated with good fertility in drought tolerant cv. Moroberekan (Liu and Bennett 2011) and HDT tolerant cv. N22 (Rang *et al.* 2011) which warrants further investigation.

The critical traits determining the cultivars tolerance were spikelet cooling and relatively high spikelet fertility under stress. In addition, flowering traits such as earlier start and peak time of anthesis, shorter spikelet anthesis duration and reduced daily flowering duration per panicle could help escape subsequent HDT. The considerable differences in sensitivity to HNT identified amongst cultivars provides a route forward to mitigating negative climate change impacts on food security in the major rice-producing regions through plant breeding utilising HNT-tolerant cultivars (such as N22 and possibly CG14) which, crucially, show good resilience in terms of both grain yield and grain quality.

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FIGURE LEGENDS

Fig. 1. Mean spikelet fertility by spikelet temperature relationship of nine rice cultivars treated to night temperatures between 24 and 35 ºC for 24 consecutive nights from flowering. y = -3.0304x + 144.59; *r*=-0.51 (Experiment 1).

Fig. 2. Variation in spikelet fertility (A) and difference between spikelet and chamber temperature (B) for nine rice cultivars at night temperatures of 24, 30, or 35 °C. In (A) cultivars are classified for HNT tolerance on the basis of spikelet fertility at 35 °C: HT = highly tolerant (spikelet fertility>80%); T = tolerant (spikelet fertility>50 to ≤80%); S = susceptible (spikelet fertility >20 to ≤ 50%); HS = highly susceptible (spikelet fertility ≤20%). Bars represent ±standard error of means (Experiment 1).

Fig. 3. The effect of night temperature on spikelet anthesis duration (main panicle) of rice cultivar IR64. Responses for cvs CG14 was comparatively slight and N22 not significant, however linear regression models are provided where significant (Suppl. Table 1). Symbols represent observations on different days after night temperature treatment: day 1 (solid lines, circles); day 2 (broken lines, squares); day 4 (dotted lines, triangles). Vertical bars represent ±s.e. about the mean (*n*=8) where larger than symbols (Experiment 2).

Fig. 4. Times of peak anthesis (A, B) and daily flowering duration per panicle (C,D) of three rice cultivars (CG14 = broken lines, diamonds; N22 = solid lines, squares; WAB56-104 = dotted lines, circles) following different night temperatures provided from 1830 to 0000 or 0000 to 0530 for four consecutive nights at anthesis. Vertical bars represent ±s.e. about the mean (*n*=8) where larger than symbols (C and D) (Experiment 3).

Fig. 5. Spikelet fertility (A, B) and grain weight per panicle (C, D) of ten rice cultivars treated to combinations of day/night temperatures. Temperatures during day were 30 ºC (control temperature) or 35 ºC (high day temperature) at anthesis and nights of 24 (black), 30 ºC (light grey) or 35 ºC (dark grey) from microsporogenesis to anthesis. Bars are standard error of means (Experiment 4).

Table 1. Cultivars selected for study

Cultivar[A]	Genotype	Experiment	HDT tolerance[B]	Accession number[C]	Adaptation	Origin	Days to anthesis[D]
N22[E]	*O. sativa* (L.) *aus*	1, 2, 3, 4	Highly tolerant	IRTP-03911	Upland	India	50
Bala	*O. s.* (L.) indica	1, 4	Tolerant	IRGC-50927	Upland	India	55
Co39	*O. s.* (L.) indica	1, 4	Tolerant	IRGC-51231	Lowland	India	42
IR36	*O. s.* (L.) indica	1, 4	Tolerant	IRGC-30416	Lowland	Philippines	59
Lemont	*O. s.* (L.) indica	1, 4	Tolerant	IRGC-66756	Lowland	U.S.A.	63
WAB56-104	*O. s.* (L.) japonica	1, 3, 4	Susceptible	IRTP-19463	Upland	Côte d'Ivoire	45
CG14	*O.* glaberrima (Steud.)	2, 3, 4	Susceptible	IRGC-96717	Upland	Senegal	57
IR64	*O. s.* (L.) indica	1, 2, 4	Susceptible	IRTP-12158	Lowland	Philippines	61
Moroberekan	*O. s.* (L.) japonica	1, 4	Susceptible	IRGC-12048	Upland	Guinea	65
Azucena	*O. s.* (L.) japonica	1, 4	Susceptible	IRGC-0328	Upland	Philippines	66

[A]Germplasm sourced from the International Rice Research Institute (IRRI), Philippines

[B]Selected by spikelet fertility under high day temperature (HDT) stress following Jagadish *et al*. (2008)

[C]IRTP (International Rice Testing Program, now International Network for Genetic Enhancement of Rice); IRGC (International Rice Germplasm Center)

[D]From transplanting to first anthesis, Experiment 1; personal observation for CG14

[E]Accession IRTP-03911 sown at IRRI in 2011 and IRTP-19379 sown at UoR in 2010, are both equally tolerant to high DT at anthesis (Rang *et al.* 2011).

Table 2. Mean spikelet fertility (%) and grain weight per panicle (g) across all cultivars at different night and day temperatures across both years (Experiment 4).

Day temperature (°C)	Night temperature (°C)[A]			
	24	30	35	Mean±s.e.m.
Spikelet fertility				
30	76.8±1.9	72.0±2.2	32.7±3.0	60.7±1.9
35	81.3±1.5	76.6±2.4	30.4±2.7	62.7±2.0
Mean±s.e.m.	79.0±1.2	74.3±1.6	31.6±2.0	
Grain weight panicle^{-1}				
30	3.32±0.15	3.08±0.16	1.14±0.15	2.51±0.11
35	3.29±0.15	3.25±0.15	0.97±0.10	2.49±0.10
Mean±s.e.m.	3.30±0.11	3.17±0.11	1.05±0.09	

	Effects of treatments						
Factor (*P*. value)	NT	cv	DT	NT x cv	NT x DT	cv x DT	NT x cv x DT
Spikelet fertility	<0.001	<0.001	0.109	0.016	0.076	0.840	0.051
Grain weight panicle^{-1}	<0.001	<0.001	0.722	0.001	0.566	0.024	0.641

[A]= mean values are ± s.e.m.

Table 3. Quality classification and ranking of ten rice cultivars for amylose content (AC) (Experiment 4).

Cultivar	Amylose content[A]	Relative loss of AC (%) compared to 24 °C NT[B]		Rank[C]	Quality at 35 °C NT[D]
	24 °C NT[E]	30 °C NT	35 °C NT		
CG14	25.2 (±0.6)	2.4	0.8	1^{NS}	Intermediate
N22	24.0 (±0.3)	2.9	3.8	2^{NS}	Intermediate
Co39	22.8 (±0.8)	1.8	7.0	3^{NS}	Intermediate
Moroberekan	20.2 (±0.6)	0.0	9.9	$4^{P=0.07}$	Low
Lemont	20.2 (±0.8)	2.0	11.9	$5^{P=0.06}$	Low
IR64	19.2 (±1.1)	1.0	14.1	6*	Low
Bala	22.1 (±1.0)	1.4	14.5	7*	Low
IR36	24.7 (±0.4)	4.0	17.8	8**	Intermediate
WAB56-104	18.8 (±0.5)	10.6	22.3	9**	Low
Azucena	19.1 (±0.6)	5.8	24.1	10**	Low
LSD(*P*=0.05)	1.3				
Night temperature (°C)					
24	21.6 (±0.3)				
30	20.9 (±0.4)				
35	19.9 (±0.4)				
LSD (*P*=0.05)	0.3				
Day temperature (°C)					
30	21.3 (±0.3)				
35	20.6 (±0.4)				
LSD(*P*=0.05)	0.4				

[A]=Percentage by weight

[B]=night temperature

[C]=Ranks are based on slopes of linear regression of amylose content at NT between 24 and 35 °C with 1 the least and 10 the most steep. Superscripts indicate significance of slopes; *=P≤0.05; **=P≤0.01; NS=not significant at *P*≤0.05

[D]=Quality is based on amylose content classification = waxy (1 to 2%), non-waxy (>2%), very low (2 to 9%), low (10 to 20%), intermediate (20 to 25%), and high (25 to 30%). Generally, the intermediate classification is preferred (WARDA/FAO/SAA, 2008)

[E]=Values in parenthesis are s.e.m.

Table 4. Gel consistency of grains of ten rice cultivars exposed to different night and day temperatures at and around anthesis (Experiment 4).

Factors			Gel consistency (mm)
Night temperature (NT)	24 °C		84.4±1.7
	30 °C		82.3±1.8
	35 °C		72.6±2.7
LSD=3.4; *P*<0.001			
Cultivar (C)	Co39		90.1±1.7
	CG14		89.0±2.7
	Moroberekan		87.5±2.7
	IR64		86.3±2.0
	Azucena		85.6±2.3
	N22		79.7±2.5
	IR36		79.1±3.2
	Lemont		84.7±3.2
	WAB56-104		75.2±2.7
	Bala		48.9±2.3
LSD=6.1; *P*<0.001			
Day temperature (DT)	30 °C		82.5±1.6
	35 °C		79.0±1.7
LSD=2.7; *P*=0.030			
Interactions	LSD	*P*. value	
NT x C	10.6	0.102	
NT x DT	4.7	0.548	
C x DT	8.6	0.087	
NT x C x DT	15.0	0.217	

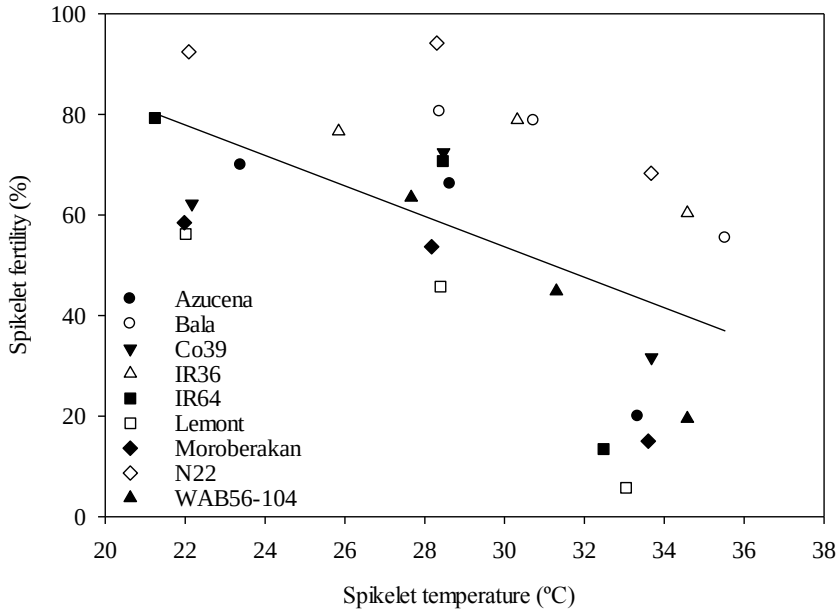


Fig. 1. Mean spikelet fertility by spikelet temperature relationship of nine rice cultivars exposed to night temperatures between 24 and 35 ºC for 24 consecutive nights from flowering. y = -3.0304x + 144.59; *r*=-0.51 (Experiment 1).

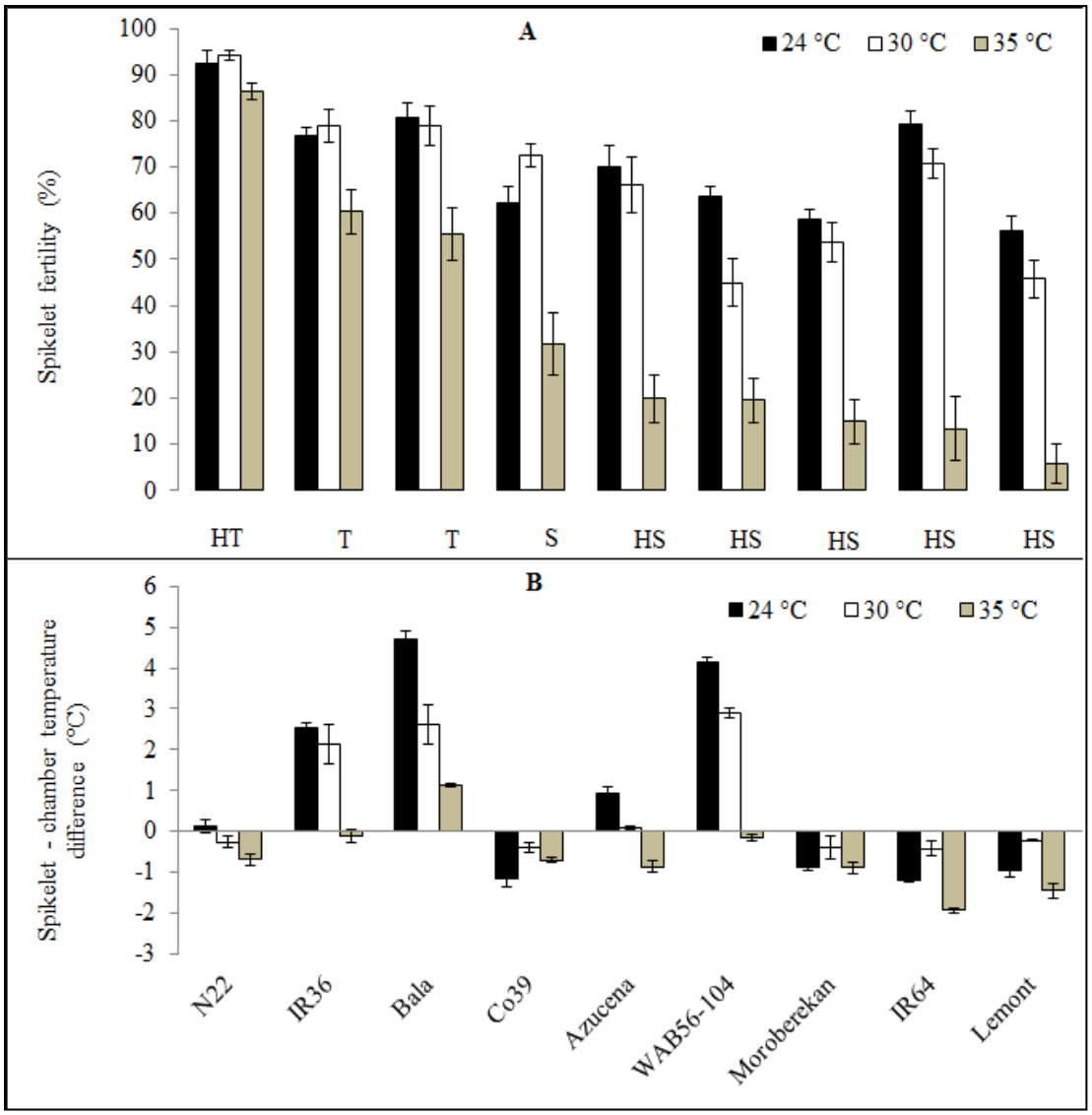


Fig. 2. Variation in spikelet fertility (A) and difference between spikelet and chamber temperature (B) for nine rice cultivars at night temperatures of 24, 30, or 35 °C. In (A) cultivars are classified for HNT tolerance on the basis of spikelet fertility at 35 °C: HT = highly tolerant (spikelet fertility>80%); T = tolerant (spikelet fertility>50 to ≤80%); S = susceptible (spikelet fertility >20 to ≤ 50%); HS = highly susceptible (spikelet fertility ≤20%). Bars represent ±standard error of means (Experiment 1).

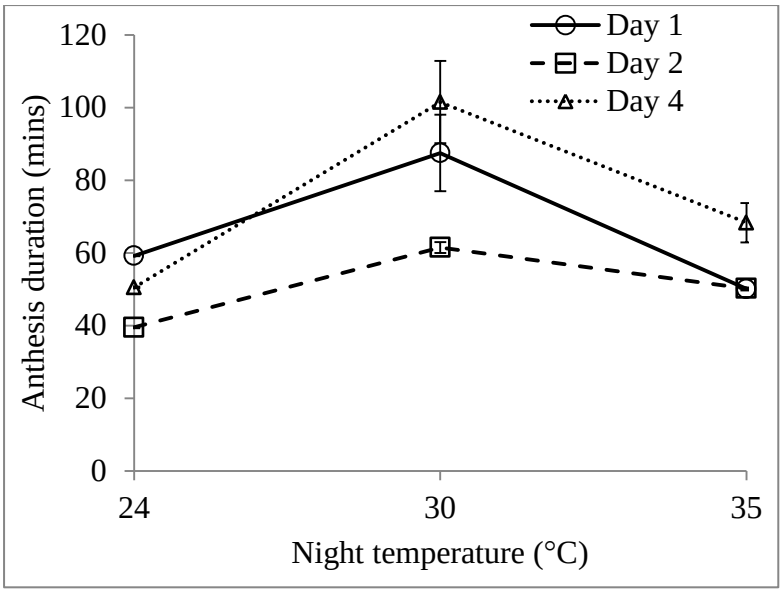


Fig. 3. The effect of night temperature on spikelet anthesis duration (main panicle) of rice cultivar IR64. Responses for cvs CG14 was comparatively slight and N22 not significant, however linear regression models are provided where significant (Suppl. Table 1). Symbols represent observations on different days after night temperature treatment: day 1 (solid lines, circles); day 2 (broken lines, squares); day 4 (dotted lines, triangles). Vertical bars represent ±s.e. about the mean (*n*=8) where larger than symbols (Experiment 2).

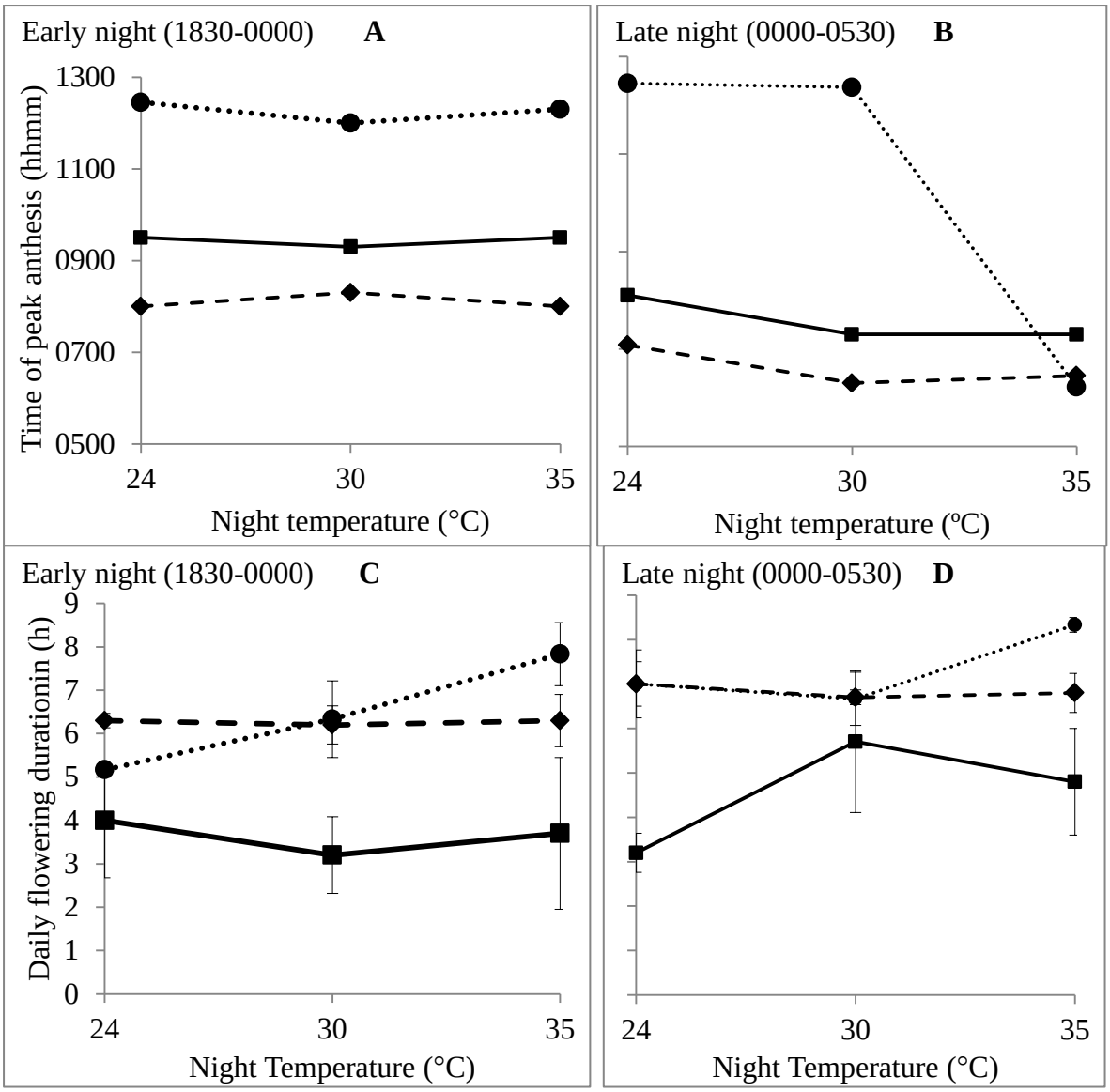


Fig. 4. Times of peak anthesis (A, B) and daily flowering duration per panicle (C,D) of three rice cultivars (CG14 = broken lines, diamonds; N22 = solid lines, squares; WAB56-104 = dotted lines, circles) following different night temperatures provided from 1830 to 0000 or 0000 to 0530 for four consecutive nights at anthesis. Vertical bars represent ±s.e. about the mean (*n*=8) where larger than symbols (C and D) (Experiment 3).

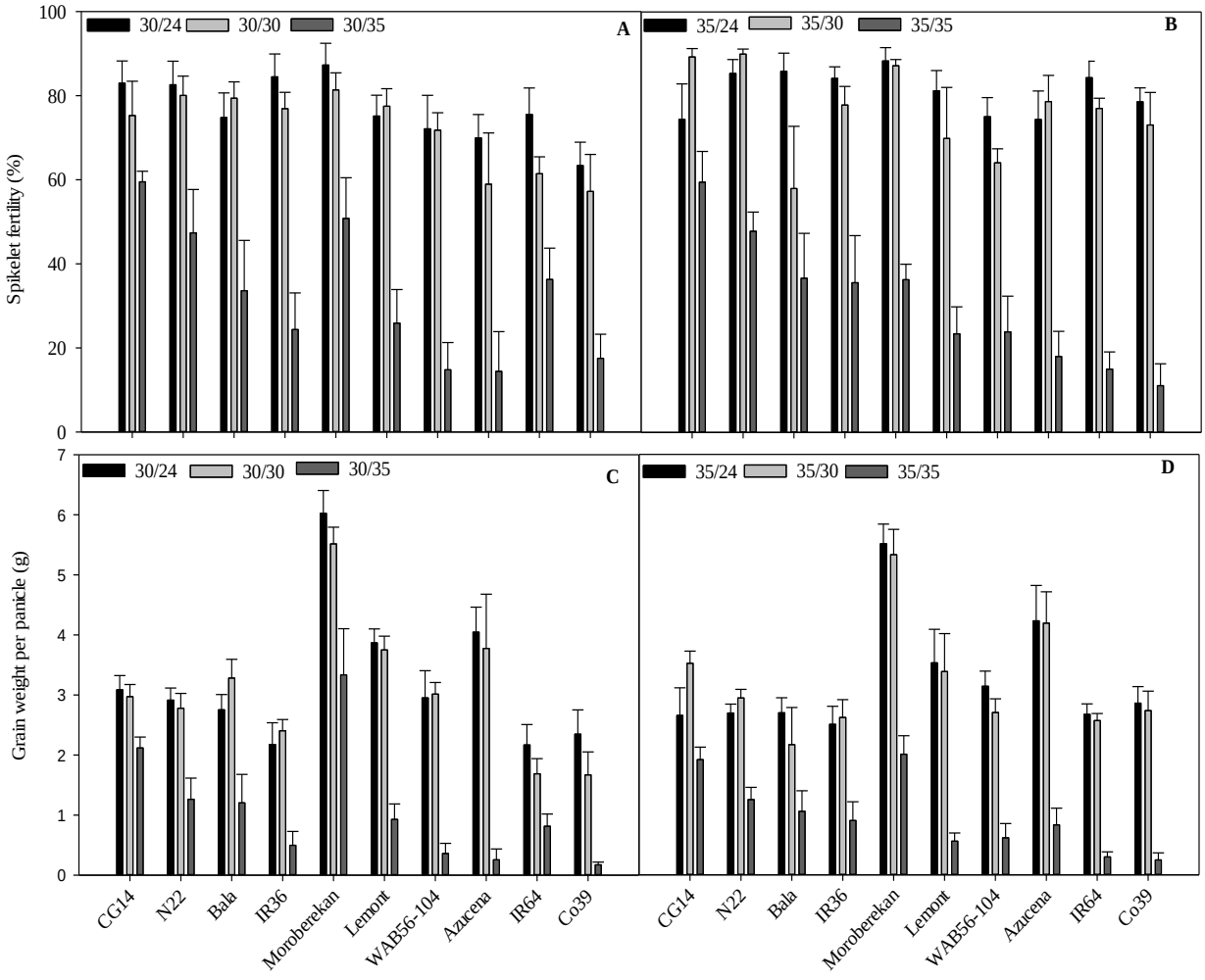


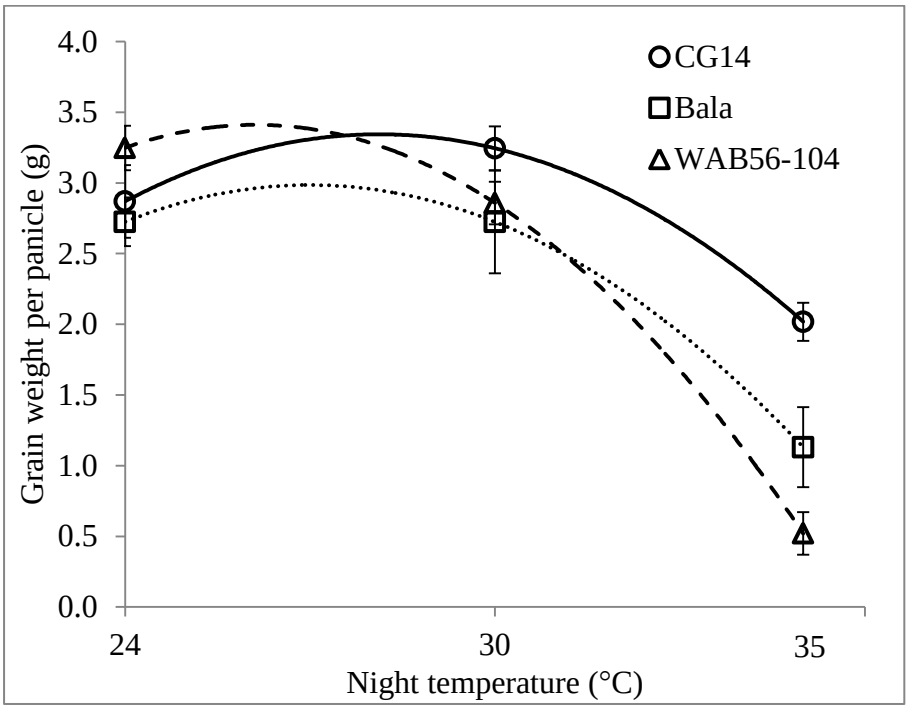
Fig. 5. Spikelet fertility (A, B) and grain weight per panicle (g; C, D) of ten rice cultivars treated to combinations of day/night temperatures. Temperatures were day of 30 ºC (control temperature) or 35 ºC (high day temperature) at anthesis and nights of 24 (black), 30 ºC (light grey) or 35 ºC (dark grey) from microsporogenesis to anthesis. Bars are standard error of means (Experiment 4).

Supplementary information

Suppl. Fig. 1. Effect of night temperature (from microsporogenesis to three days after anthesis) on grain weight per panicle (g) of three contrasting rice cultivars showing no response to DT (Table 3). Symbols combine results for 30 and 35 ºC DT at anthesis for each of CG14 (solid line, circles); Bala (dotted line, squares); WAB56-104 (broken line, triangles). Vertical bars represent ±s.e. about the mean (*n*=16) where larger than symbols (Experiment 4).

Suppl. Fig. 2. Effect of night temperature (from microsporogenesis to three days after anthesis) on grain weight per panicle (g) of cv. IR64 at either 30 (solid squares) or 35 ºC (open squares) DT (5-6 h at anthesis). The responses are quantified in Table 3. Vertical bars represent ±s.e. about the mean (*n*=7) where larger than symbols (Experiment 4).

Suppl. Fig. 1.



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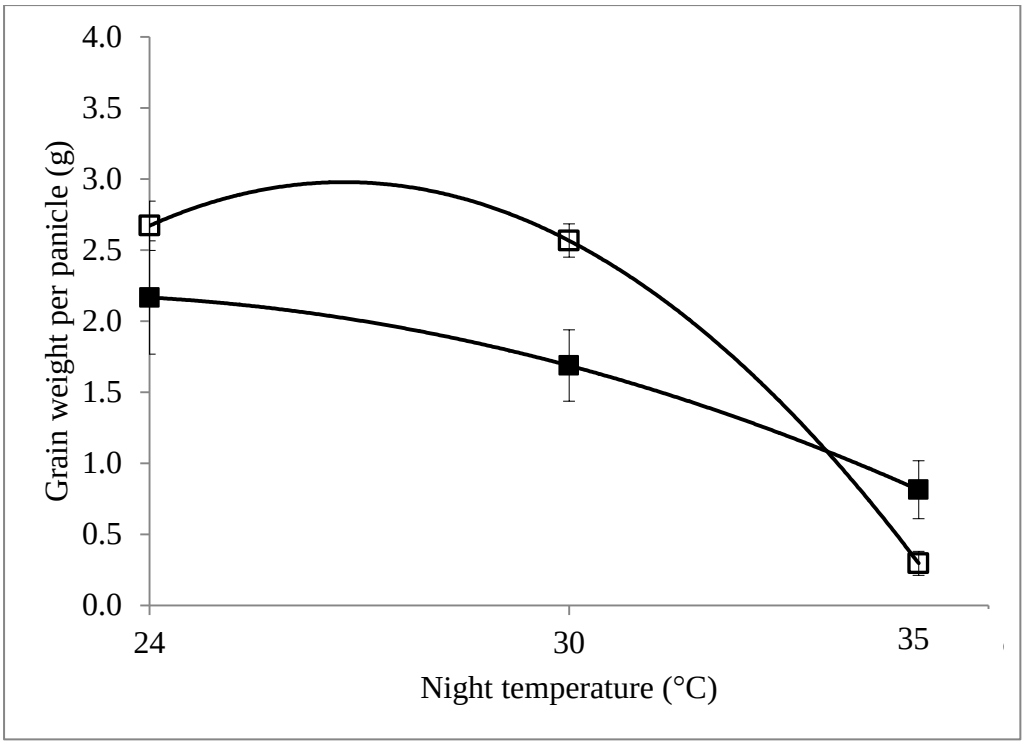
Suppl. Fig. 2.



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Supplementary Table 1. Parameter estimates of separate line regression models for response of spikelet anthesis duration of CG14 and N22 to night temperature (NT). *n* = 24 per cultivar (Experiment 2).

	CG14			N22		
Parameters	Estimate	s.e.	*P*	Estimate	s.e.	*P*
Day 1 (intercept)	31.43	7.99	<0.001	55.99	3.79	<0.001
Day 2 (intercept)	5.43	7.99	0.502	44.73	3.79	<0.001
Day 4 (intercept)	51.58	7.99	<0.001	23.37	3.79	<0.001
NT x Day 1 (slope)	0.46	0.27	0.096	-0.52	0.13	<0.001
NT x Day 2 (slope)	1.33	0.27	<0.001	-0.10	0.13	0.446
NT x Day 4 (slope)	-0.07	0.27	0.794	0.67	0.13	<0.001
Adjusted R^{2*}	0.48	0.04		0.60	0.02	

*The single R^2 value for each cultivar is of the full regression model – (individual factors and their interactions).

Supplementary Table 2. Time of start of anthesis and cumulative percentage of anthesed spikelets at time of peak anthesis of three rice cultivars. Rice cultivars were treated to two different periods of exposure (early, 1830 to 0000; or late, 0000 to 0530) to three night temperatures from the first to fourth day of anthesis (Experiment 3).

Time of start of anthesis	CG14		N22		WAB56-104	
Night temperature (°C)	Early	Late	Early	Late	Early	Late
24	0530	0530	0800	0630	0800	0530
30	0530	0530	0800	0600	0600	0600
35	0530	0530	0800	0600	0530	0500

Cumulative anthesed spikelets at time of peak anthesis (%)[A]			
Night temperature (°C)	CG14	N22	WAB56-104
24	59 ±2	71 ±12	84 ±6
30	61 ±5	77 ± 7	69 ±6
35	53 ±7	66 ±12	58 ±9
LSD (5%)	16	30	20
Time of night (EST)			
1830–0000	57 ±4	71 ±7	75 ±4
0000–0530	59 ±5	72 ±9	66 ±8
LSD (5%)	13	23	17

[A] Values are means ± standard error of means of six main panicles observed for three days from the time of start of anthesis.

Supplementary Table 3. Associations (Spearman's rank correlation) for spikelet fertility amongst different high day and night temperatures regimes (Experiment 4).

Day/night temperature (°C) combination		Correlation	*P*	Explanation
High day (35/24)				
	30/30 HNT[A]	0.745	0.004	Associated
	30/35 vHNT[B]	0.515	0.031	Associated
	35/30 HDT[C]+HNT	-0.006	0.243	Not associated
	35/35 HDT+vHNT	0.285	0.102	Not associated
High night (30/35)				
	30/30 HNT	0.612	0.015	Associated
	35/30 HDT+HNT	0.515	0.031	Associated
	35/35 HDT+vHNT	0.661	0.010	Associated

[A]HNT = high night temperature (30 °C)

[B]vHNT = very high night temperature (35 °C)

[C]HDT = high day temperature (35 °C)

Supplementary Table 4. Estimates of regression parameters for the response of (square root [√+0.5] transformed) grain weight per panicle (g) to night temperature (Experiment 4).

Cultivar	Constant ±s.e.	Linear term ±s.e.	Quadratic term ±s.e.	NT_c[A] (°C)
Equations for single curve for both DT				
CG14	-5.42±1.99	0.516±0.138	-0.00905±0.00234	28.5
IR36	-12.26±2.96	1.004±0.204	-0.01782±0.00346	28.2
Bala	-8.46±3.39	0.756±0.235	-0.01370±0.00399	27.6
Lemont	-13.01±2.81	1.109±0.195	-0.02017±0.00330	27.5
N22	-6.37±2.01	0.610±0.139	-0.01122±0.00236	27.2
Azucena	-13.29±3.82	1.151±0.265	-0.02123±0.00449	27.1
WAB56-104	-9.10±2.34	0.825±0.162	-0.01537±0.00275	26.8
LSD	7.06^{NS}	0.479*	0.00800*	1.8^{NS}
Equations for parallel and separate curves				
DT = 30 °C				
Moroberekan[B]	-5.71±2.84	0.629±0.196	-0.01180±0.00333	26.7
Co39[B]	-8.24±2.51	0.754±0.174	-0.01422±0.00295	26.5
IR64[C]	-2.64±2.98	0.327±0.206	-0.00627±0.0035	26.1
LSD	7.86^{NS}	0.568^{NS}	0.01010^{NS}	3.8^{NS}
DT = 35 °C				
IR64	-9.23±2.98	0.830±0.206	-0.01544±0.00350	26.9
Moroberekan	-5.91±2.84	0.629±0.196	-0.01180±0.00333	26.7
Co39	-8.06±2.51	0.754±0.174	-0.01422±0.00295	26.5
LSD	6.44^{NS}	0.453^{NS}	0.00782^{NS}	1.0^{NS}

[A]NT_c = critical night temperature beyond which grain weight per panicle (g) is reduced with further increase in night temperature at both day temperatures was 27 °C (±0.2)

[B]= responses of these cultivars at different DT were best fitted by two parallel curves

[C]= response best fitted by separate curves at different DT

[NS]= not significant at $P \leq 0.05$; * = $P \leq 0.05$