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Systematic determination of the reproductive growth stage most sensitive to high night temperature stress in rice (*Oryza sativa*)

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Abstract

High night-temperature (HNT) stress during the reproductive stage of rice (*Oryza sativa* L.) reduces spikelet fertility and yield by inhibiting important physiological processes. However, specifics such as the period of time that is most sensitive to HNT, is unknown. To investigate this, we conducted four controlled-environment experiments with two rice cultivars, N22 (HNT tolerant) and WAB56–104 (HNT susceptible). These cultivars were exposed to varying durations and intensities of night temperatures (control, 24°C; HNT, 30 and 35°C) during the reproductive stage. The effect of HNT on spikelet fertility and grain weight varied with duration: spikelet fertility reduced by 47–77% when exposed to HNT for 15 nights, 6–29% when exposed for four nights, and 9–15% when exposed for 5.5 h (pre-midnight, 1830–0000 h or post-midnight, 0000–0530 h) for four nights. Spikelet fertility and grain weight were most sensitive to HNT during the first 4 d of anthesis, compared with 1–4, 5–8, and 9–12 d before anthesis. At anthesis, reduction in spikelet fertility did not differ significantly between pre- and post-midnight high-temperature treatments. Our results suggest that greatest sensitivity to HNT during the reproductive stage occurs during the first 4 d of anthesis, providing a reference for future studies involving HNT tolerance in rice.

Abbreviations: DAA, days after start of anthesis; DBA, days before anthesis; ETR, electron transport rate; F_0, minimum fluorescence intensity at 20 µs or O-step; F_M, maximal fluorescence; F_V/F_M, maximum quantum yield of photosystem II; GLMM, Generalized Linear Mixed Model; HDT, high day temperature; HNT, high night temperature; IRRI, International Rice Research Institute, Philippines; MINCER, Micrometeorological Instrument for the Near-Canopy Environment of Rice; PAR, photosynthetically active radiation; PI, photosystem II performance index; PI_{ABS}, overall performance index based on equal absorption; PSII, photosystem II; UoR, University of Reading, UK.

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1 | INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of approximately 3.5 billion people, roughly 50% of the world's population. It is grown in over 100 countries on more than 163 million ha (~11.5% of the world's arable land), with an annual paddy rice harvest of more than 980 million tonnes (Food and Agriculture Organization [FAO], 2017; Laborte et al., 2017). Most rice is produced and consumed in Asia, where it provides up to 50% of the dietary caloric requirements of the population. Consumption in other parts of the world is increasing (FAO, 2013). For example, in sub-Saharan Africa, per capita consumption has doubled since 1970. It is estimated that rice production will have to increase by 40% to meet demands in 2030 (Khush, 2005). Meeting future demand could be hindered by predicted changing climates, as higher day and night temperatures have been shown to negatively affect rice yields (Coast, Ellis, Murdoch, Quinones, & Jagadish, 2015; Jagadish, Craufurd, & Wheeler, 2007; Mohammed & Tarpley, 2009a, 2009b; Satake & Yoshida, 1978). Even under current climatic conditions, rice-producing areas are experiencing increasingly higher day and night temperatures. In some of these areas, including the two highest producers, China and India, night (or mean minimum) temperature has been rising faster than day (or mean maximum) temperature (Padma Kumari, Londhe, Daniel, & Jadhav, 2007; Sillmann, Kharin, Zhang, Zwiers, & Bronaugh, 2013; Zhou et al., 2004). In addition, Peng et al. (2004) reported a closer relationship of rice grain yield with mean-minimum temperature than mean-maximum temperature, which was further supported by Nagarajan et al. (2010). Thus, improving our understanding of the effect of high night temperature (HNT) on rice production would enhance our ability to minimize anticipated rice yield losses and help meet future demand.

The sensitivity of rice to high day or night temperature varies with growth stage, duration and intensity of stress, as well as cultivar. High day temperature (HDT) at the vegetative stage limits yield by delaying emergence, inhibiting seedling growth, and reducing tillering (Akman, 2009; Yoshida, 1978, 1981). During the reproductive stage (booting to anthesis) HDT limits pollen development, anther dehiscence, pollen germination and tube growth, and spikelet fertility (Coast et al., 2015; Jagadish et al., 2007; Matsui, Omasa, & Horie, 2000; Satake & Yoshida, 1978). During the seed development and maturation stage, HDT reduces grain weight, grain-filling duration, and grain quality (Bahuguna, Solis, Shi, & Jagadish, 2017; Fitzgerald & Resurreccion, 2009; Madan et al., 2012; Resurreccion, Hara, Juliano, & Yoshida, 1977; Tashiro & Wardlaw, 1989). Of these three stages, rice is most sensitive to HDT during the reproductive stage, especially at anthesis and gametogenesis (Jagadish, Craufurd, & Wheeler, 2008; Martínez-Eixarch & Ellis, 2015; Yoshida, 1981). An hour of high air temperature (> 35–38.5°C) exposure or even less than 1 h of high spikelet temperature (> 33.7°C) at anthesis is sufficient to significantly reduce spikelet fertility (Jagadish et al., 2007; Satake & Yoshida, 1978; Yoshida, 1981). Additionally, HNT at the reproductive, seed development, and seed maturation stages reduces rice spikelet fertility, grain weight, and grain quality (Bahuguna et al., 2017; Coast et al., 2015; Martínez-Eixarch & Ellis, 2015), with the reproductive stage being more sensitive to HNT than the vegetative stage (Laza et al., 2015). However, while the most HDT-sensitive time has been pinpointed to within an hour of anthesis, the same for HNT is not clearly defined. To help focus research efforts aimed at enhancing tolerance to HNT, there is a need to identify the time of night during the reproductive stage that, when temperatures increase, has the greatest effect on spikelet fertility and yield.

High temperatures affect almost every biological process in plants. Such an effect on a fundamental process like photosynthesis will become increasingly important under future climate-change scenarios. The effect of high temperature on photosynthesis can be investigated by measuring chlorophyll-a fluorescence, the light emitted from chlorophyll molecules, which changes in relation to processes occurring within and around photosystem II (PSII). Photosystem II was considered the component of the photosynthetic apparatus most sensitive to high temperature (Berry & Bjorkman, 1980; Havaux, 1996). Although high temperature does not cause serious damage to PSII per se, it does inhibit its repair (Sharkey, 2005). Chlorophyll-a fluorescence is a multiphasic phenomenon comprising fast and slow kinetics (Papageorgiou, Tsimilli-Michael, & Stamatakis, 2007). The slow kinetics can be used to extract information about electron flow, activities of the Calvin–Benson cycle, and changes in photorespiration; however, it is time-consuming. By contrast, the fast transient response induced by a short-saturating pulse of actinic light can provide detailed information about the structural and functional attributes of components involved in the photosynthetic electron transport chain (JIP-test parameters; Strasser, Tsimilli-Michael, & Srivastava, 2004). Examples of JIP-test parameters include the maximum quantum yield of PSII (Fv/F_M), PSII performance index (PI), and overall performance index based on equal absorption (PI_{ABS}). Extensive descriptions of all JIP-test parameters can be found elsewhere (Strasser, Srivastava, & Tsimilli-Michael, 2000, 2004). Over the past two decades, there have been numerous reports of high-temperature effects on the primary photochemistry in rice via studies involving different chlorophyll-a fluorescence parameters (Supplemental Table S1), but only two have recorded the effect of HNT on chlorophyll-a fluorescence during the most sensitive reproductive stage (Alvarado-Sanabria, Garcés-Varón, & Restrepo-Díaz, 2017b; Šebela, Quiñones, Olejnickova, & Jagadish, 2015). A third study covered the reproductive, seed development, and seed maturation stages (Mohammed, Cothren, Chen, & Tarpley,

2015). Thus, our knowledge of the effects of HNT during the reproductive stage on rice primary photochemistry is limited. To this end, we conducted four controlled-environment experiments during which two rice cultivars, contrasting in tolerance to HNT, were treated to varying durations of three different night-temperature regimes during the reproductive growth stage (i.e., between gametogenesis and anthesis), to achieve the following objectives: (i) identify the most HNT-sensitive period during the reproductive growth stage; (ii) quantify the effect of HNT on primary photochemistry during the most HNT-sensitive period of the reproductive stage using selected chlorophyll-a fluorescence parameters; and (iii) investigate the effect of HNT during the first and the second half of the night on spikelet fertility in rice.

2 | MATERIALS AND METHODS

Four experiments were conducted using controlled environment facilities; two in 2010 at the Plant Environment Laboratory, University of Reading (UoR), United Kingdom (51° 27′ N, 00° 56′ W) and two in 2011 at the International Rice Research Institute (IRRI), Philippines (14° 11′ N, 121° 15′ E). Seeds of N22 (*Oryza sativa* L. ssp. aus) and WAB56–104 (*O. sativa* L. ssp. japonica), obtained from IRRI, were grown in a glasshouse (UoR) and a greenhouse (IRRI) under optimum temperature and photoperiod. These cultivars were chosen based on their contrasting tolerance of HNT at anthesis (Coast et al., 2015), with N22 being tolerant and WAB56–104 being susceptible. Seeds were obtained from the IRRI gene bank.

2.1 | Controlled environment facilities

The glasshouse and growth cabinets used for growing plants and imposing night temperature treatments at UoR, and the walk-in growth chambers used at IRRI have previously been described in Jagadish et al. (2007, 2008) and Coast et al. (2015), respectively. However, brief descriptions are given below.

At UoR, a naturally lit glasshouse, with day- and light-proof night compartments, was used for plant growth. Plants in pots were placed on automated mobile trolleys (2.85 by 0.96 m), which were drawn into and out of night compartments at 0800 and 1900 h, respectively. The glasshouse was maintained at day/night temperatures of 30:24°C, by a combination of heating (by a 16 kW heater) and venting during the day (0800–1900 h), and by air-conditioning units at night (2000–0700 h). Relative humidity was not controlled, and thus varied, with day/night values of 46:53%. A linear change in temperature from day to night and vice versa was programmed between 1900–2000 h and 0700–0800 h, respectively.

Modified Sacxil growth cabinets (1.4 by 1.4 by 1.5 m) were used to impose night temperatures of 24, 30, and 35°C and day temperature of 30°C for specified durations during the reproductive growth stage. Day/night thermo- and photo-period regimes of 12 h were maintained from 0700–2000:2000–0700 h. To simulate natural conditions, gradual changes in temperature and lighting were programmed from day to night and vice versa, between 0700–0800 h and 1900–2000 h, respectively. Relative humidity was maintained between 60–70% by adding moisture to air passing through glycol or by removing the excess by condensation. Photosynthetically active radiation (PAR) was 700 μmol photons m^2 s^{-1} at the base of the cabinets and CO_2 concentration varied between 360–380 μmol mol^{-1} CO_2. Aspirated-air temperature and relative humidity were recorded every 30 min with data loggers (Delta T Devices, Burwell, Cambridge, UK), using copper-constantan thermocouples placed at canopy height.

At IRRI, a naturally lit and well-aerated greenhouse was used to grow the plants until the reproductive growth stage. The greenhouse had a roof system that could be partially opened to increase airflow and regulate temperature and relative humidity within. This was in addition to two wall-mounted industrial fans located at the ends of the glasshouse, used to circulate air. The greenhouse day/night temperature was 30:26°C and relative humidity was 81:90%. To impose the night temperatures of 24, 30, and 35°C and day temperature of 30°C, bespoke walk-in growth rooms (3.3 by 3.2 by 2.7 m) were used. In these growth rooms, the temperature was controlled by air-conditioning units. Day/night thermo- and photo-period regimes of 13 h were maintained from 0530–1830:1830–0530 h in the chambers. The change in temperature from day to night and vice versa from 1730–1830 h and 0530–0630 h, respectively, was programmed to be linear. Light supplied by six 1-kW-high-intensity-discharge lamps provided PAR at plant height of 650 μmol photons m^{-2} s^{-1} for 11 h d^{-1}, and 215 μmol photons m^{-2} s^{-1} during the temperature change periods; totaling a photoperiod of 13 h d^{-1}. Aspirated air temperature and relative humidity in the glasshouse and chambers were measured and recorded every 10 min using MINCER (Micrometeorological Instrument for the Near-Canopy Environment of Rice; Fukuoka, Yoshimoto, & Hasegawa, 2012).

2.2 | Experimental design and crop husbandry

2.2.1 | Experiment 1 and Experiment 2: Effect of HNT throughout the reproductive growth stage on spikelet fertility and grain weight of main, primary, and other panicles

A pair of 2 by 3 factorial experiments was conducted at UoR in 2010 (Experiment 1) and IRRI in 2011 (Experiment 2). Rice

cultivars N22 and WAB56–104 were exposed to night temperatures of 24, 30, and 35°C and a common day temperature of 30°C for 15 consecutive nights, spanning gametogenesis (12 d before anthesis, DBA) to anthesis (3 d after start of anthesis, DAA).

At UoR, four or five seeds were sown into 12.5-cm (diameter) pots 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, respectively (Coast et al., 2015). Pots were soaked overnight prior to sowing. To supplement nutrition, 2 kg of controlled-release fertilizer (Osmocote Pro 3–4 mo feed, The Scotts Company, UK) was added per cubic metre of substrate. At the three-leaf stage, seedlings were thinned to one per pot. In both the glasshouse and growth cabinets, an automated irrigation system supplied approximately 65 ml of acidified water (pH 4.5–5.0) at 20°C to each pot six times daily. Plants were sprayed with SAVONA (potassium salts of fatty acids 49% w/w) from 2 wk after anthesis to harvest in order to control red spider mites (*Tetranychus urticae* Koch.) as needed. There was no major pest or disease problem.

At IRRI, pre-germinated seeds were sown in seed trays filled with natural clay loam soil. Seedlings were kept well-watered in the greenhouse for 3 wk and transplanted into 17- by 19.5-cm porcelain pots, each with 3.5 kg of natural clay loam soil, thoroughly mixed with 2.32 g of urea, 1.16 g of phosphorous (P_2O_5) and 1.16 g of potassium chloride. Soil and fertilizer mixtures were soaked for 2 d prior to transplanting. Thirty days after transplanting, 2.32 g of urea was applied to each pot. Plants were kept well-watered at all times. Weeds were removed manually from pots.

In the glasshouses and greenhouse, plants were grown until the main panicle was at microsporogenesis (Jagadish, Craufurd, Shi, & Oane, 2014), then moved to growth cabinets at UoR or growth chambers at IRRI to impose HNT treatments. Thereafter, plant growth was observed daily and the first three panicles to emerge were tagged as they emerged. The first to emerge was considered the main panicle, the second and third as the primary panicles, and the rest as other panicles. Plants were kept under controlled environmental conditions for 15 consecutive nights (from 12 DBA–3 DAA inclusive). At the end of this period, plants were returned to glasshouses and greenhouse and grown till harvest maturity.

2.2.2 | Experiment 3: Effect of short episodes of HNT between gametogenesis and anthesis on spikelet fertility and grain weight

A second experiment was conducted at UoR in 2010 (Experiment 3). The experiment was a 2 by 3 by 4 factorial, with two cultivars, three night temperatures (24, 30, and 35°C, with a common day temperature of 30°C), and four stress periods, spanning gametogenesis to anthesis. The stress periods were gametogenesis (12–9 DBA), 8–5 DBA, 4–1 DBA, and 0 (at anthesis)–3 DAA. Plants were assigned to two blocks with each block containing three biological replicates (thus, $n = 6$). Crop husbandry is the same as described for UoR Experiment 1 above; however, only the main panicles were tagged for data collection.

2.2.3 | Experiment 4: Effect of pre- or post-midnight HNT on spikelet fertility, and chlorophyll-a fluorescence parameters

Experiment 4 was also conducted at IRRI in 2011. The experiment was a 2 by 2 by 3 factorial, with the same cultivars (N22 and WAB56–104), two night periods (pre-midnight, 1830–0000 h; post-midnight, 0000–0530 h), and three night temperatures (24, 30, and 35°C) imposed at anthesis (0–3 DAA). There were eight biological replicates ($n = 8$) for each treatment combination. Plants for the pre-midnight treatment were transferred in and out of growth chambers at 1830 and 0000 h, respectively, for four consecutive nights. Similarly, those in the post-midnight treatment were transferred into growth chambers at 0000 h and removed at 0530 h for four consecutive nights. Transfers began on the first day of anthesis (0 DAA). Crop husbandry was the same as described for IRRI Experiment 1 above. There were cases of whitefly (*Bemisia* spp) and brown planthopper (*Nilaparvata lugens* Stål.) infestation about 2 wk after the night temperature treatments were imposed, immediately before harvest maturity. This pest pressure was managed by applying common pesticides (Cartap, Cypermethrin, and soap solutions).

2.3 | Measured traits

At harvest maturity, the main, primary, and other panicles for Experiment 1; main and primary panicles for Experiment 2; and main panicles for Experiments 3 and 4 were separately harvested. Harvested panicles were oven dried at 60°C for 4 d and grain weight was determined for Experiments 1, 2, and 3. The number of filled, partially filled, and unfilled spikelets, and grains per panicles were recorded and used to determine spikelet fertility. Spikelet fertility was taken as the ratio of filled and partially filled grains to the total number of spikelets. However, for Experiment 4, due to the insect infestation (see previous paragraph), only plants with main panicles containing at least 100 spikelets (filled and unfilled) were used to determine spikelet fertility (resulting in a smaller sample size, $n = 3$)

For Experiment 4 alone, in vivo chlorophyll-a fluorescence was measured by a portable fluorometer (Handy PEA, Hansatech Instruments, King's Lynn, Norfolk, UK). Measurements were taken after 20 min of dark adaptation, halfway between the base and tip of flag leaves of main panicles, avoiding the midrib. This occurred between 1000–1300 h on the last day following the night temperature treatment (i.e., on Day 4 of anthesis). Chlorophyll-a fluorescence was induced by strong-saturating pulse (1 s; intensity ∼3000 μmol photons m^{-2} s^{-1}), which was sufficient to generate maximal fluorescence (F_P or F_M). Fluorescence intensity at each of O-J-I-P steps (relative units) and their amplitudes ($A_{O–J}$, $A_{J–I}$, and $A_{I–P}$ representing O–J, J–I, and I–P phases, respectively) were investigated. Amplitudes of the chlorophyll-a fluorescence steps were calculated as the difference between the higher- and lower-chlorophyll-a fluorescence steps, and expressed as a percentage relative to F_V (i.e., the difference between maximum and/or peak fluorescence intensity at P-step [F_M] and minimum fluorescence intensity at 20 μs or O-step, F_0). Other calculated and analysed chlorophyll-a fluorescence parameters were F_V/F_M, PI, and PI_{ABS}. These analyses were based on measurements taken from four biological replicates.

2.4 | Statistical analysis

All statistical analyses were conducted with GenStat (GenStat 18th Edition, VSN International, UK). For Experiments 1, 2 and 3, spikelet fertility was treated as a binomial and analysed as logits, $\log_{10}(P/100 - P)$ of percentages of P where $0 < P < 100\%$, using the Generalised Linear Mixed Model (GLMM). The models included fixed terms of night temperature, cultivar, panicle (for Experiment 1 and Experiment 2), and stress period (for Experiment 3), together with their interactions. Replicate and block were included as random terms in the models. Grain weight was similarly analysed by GLMM, but with an identity link function and assuming a normal distribution. Data for Experiment 1 and Experiment 2 were combined for analysis, as they were considered similar. Although replicating cabinets or growth chambers was not feasible, consistent responses observed across multiple (3–6) plants within a temperature treatment indicated that the responses were representative of the temperature effect.

Experiment 4 spikelet fertility and chlorophyll-a fluorescence parameters were analysed by ANOVA using the General Treatment Structure (no blocking) function of GenStat. We initially included main and interaction effects of cultivar, temperature, and time-of-night terms in the treatment structure. However, because the main effect of cultivar differed significantly ($P < .001$) for chlorophyll-a fluorescence parameters, we re-analysed the parameters separately for each cultivar.

3 | RESULTS

3.1 | Experiment 1 and Experiment 2: Spikelet fertility and grain weight responses to a long period (15 nights) of HNT during the reproductive growth stage

Fifteen consecutive nights of HNT during the reproductive growth stage of the main panicle reduced spikelet fertility of main and primary panicles, but not the other panicles. The degree of HNT-induced reduction, however, varied with cultivar (Figure 1). Reduction of spikelet fertility was less in HNT-tolerant N22 (46–57% reduction), than the HNT-susceptible WAB56–104 (63–77%). The effect of HNT on grain weight per panicle was similar to that of spikelet fertility. First, HNT reduced grain weight of main and primary panicles, but had no effect on other panicles of either cultivar (Figure 1c, 1d). Second, the effect of HNT on grain weight was less for N22 (50–76% reduction), compared with WAB56–104 (55–86%). However, reduction in primary-panicle-grain weight was higher for N22 (76%) than WAB56–104 (55%; Figure 1c, 1d).

3.2 | Experiment 3: Spikelet fertility and grain weight responses to short episodes (four nights) of HNT between microsporogenesis (12–9 DBA) and anthesis (0–3 DAA)

Short episodes (four consecutive nights) of HNT resulted in less than 10% reduction in spikelet fertility for N22, regardless of stress period ($P = .218$). By contrast, reduction in spikelet fertility of WAB56–104 following HNT differed between the stress periods ($P < .001$). WAB56–104 plants exposed to 35°C night temperature had a 5–12% reduction in spikelet fertility during periods prior to anthesis (12–9 and 8–5 DBA) and a 29% reduction at anthesis (0–3 DAA; Figure 2a, 2b). Although spikelet fertility of N22 plants at 24°C was higher (91%) than WAB56–104 (86%), the relative difference in HNT-induced reduction of spikelet fertility at 35°C was less in N22 than WAB56–104 (compare Figure 2a vs. 2b).

There was a significant second-order interaction of cultivar, temperature, and stress period on main-panicle-grain weight ($P < .001$; Supplemental Table S4). For N22, main-panicle-grain weight did not reduce in response to HNT at anthesis (Figure 2c), but there were significant reductions at 35°C, relative to 24°C at 8–5 DBA and 4–1 DBA. By contrast, main-panicle-grain weight of WAB56–104 consistently reduced with increase in night temperature from 24–30 or 35°C. The sensitivity of WAB56–104 grain weight to HNT increased as the plants developed from microsporogenesis

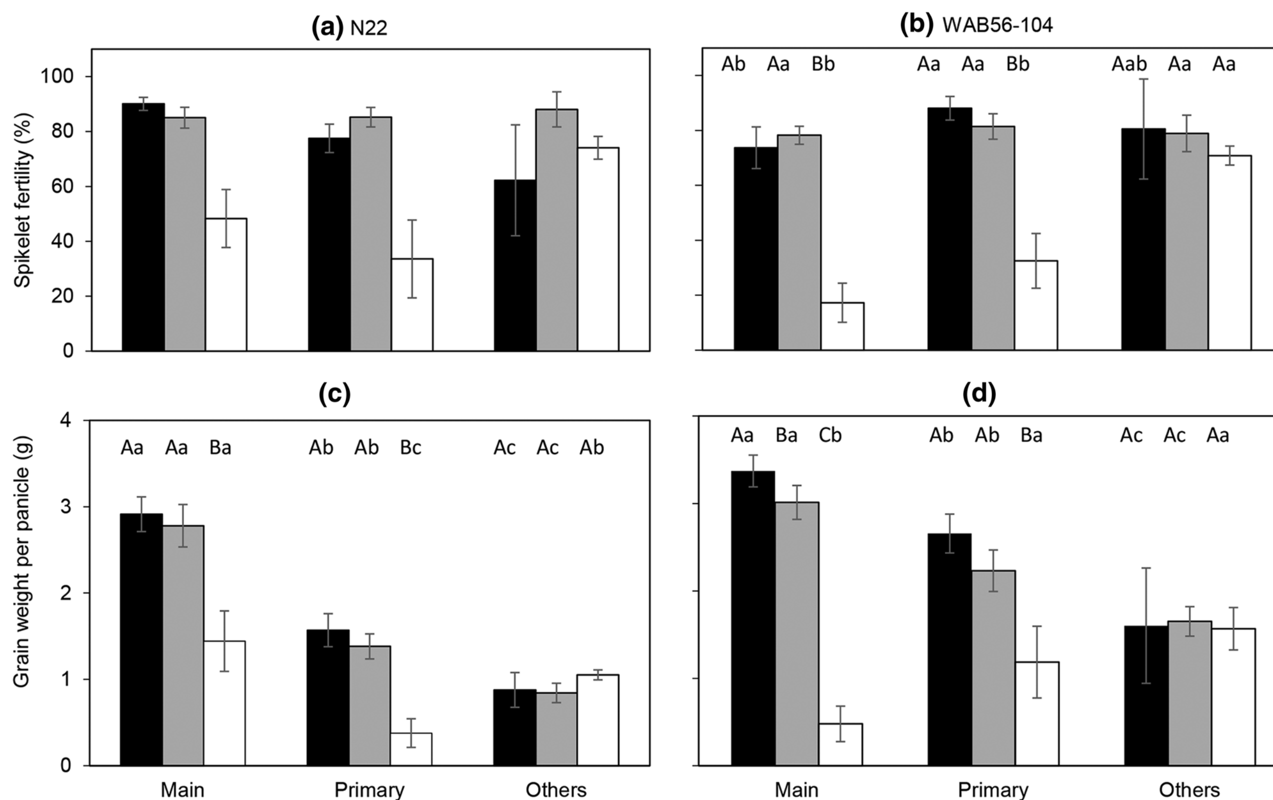

FIGURE 1 Experiment 1 and Experiment 2: spikelet fertility (a, b) and grain weight per panicle (c, d) of main, primary, and other panicles of N22 (a, c) and WAB56–104 (b, d) treated to 15 consecutive nights (spanning microsporogenesis through anthesis) at night temperatures of 24 (black bars), 30 (grey bars), or 35°C (white bars). $n = 9$ except for other panicles with $n = 6$. Error bars are ± standard error. Wald tests for fixed effects of all fixed terms (cultivar, night temperature, panicle, and all their interactions) were significant ($P \leq .05$) for spikelet fertility and grain weight per panicle. The analysis of variance tables for spikelet fertility and grain weight per panicle are presented in Supplemental Table S2 and S3, respectively. Within a panel, different uppercase letters for a cluster of bars indicate differences between night temperature treatments, while differences in lowercase letters indicate differences between panicles of plants that received the same temperature treatment

(12–9 DBA, 16% reduction) through anthesis (36% reduction; Figure 2d).

3.3 | Experiment 4: Spikelet fertility response to pre- and post-midnight high temperature at anthesis (0–3 DAA)

There were significant differences in spikelet fertility ($P < .001$), a significant effect of temperature ($P = .016$), and borderline significance for the time of night effect ($P = .059$) between cultivars. The interaction terms were not significant ($P > .05$). Spikelet fertility across temperatures or time of night was higher for N22 than WAB56–104 (Figure 3). Night temperature of 35°C, relative to 24°C, reduced spikelet fertility of N22 by 9% (Figure 3a). Similarly, spikelet fertility of WAB56–104 at night temperature of 35°C, relative to 24°C, was reduced by 15%, albeit only for post-midnight treatment (Figure 3b). Regardless of the time of night treatment, increase in temperature from 24 to 30°C did not affect spikelet fertility of either N22 or WAB56–104.

3.4 | Experiment 4: Chlorophyll-a fluorescence responses to pre- and post-midnight high temperature at anthesis (0–3 DAA)

Dark-adapted leaves of both cultivars (N22 and WAB56–104) were exposed to short-saturating light which induced in vivo chlorophyll-a fluorescence transients (see Materials and Methods). For N22, the intensity of particular intermediate steps of these chlorophyll-a fluorescence transients was altered by HNT and the time of exposure to HNT (Figure 4). Chlorophyll-a fluorescence at O-step (20 µs), 50, 100, and 300 µs increased with temperature ($P \leq .001$), and there were differences ($P \leq .001$) between pre- and post-midnight responses for chlorophyll-a fluorescence at O-step (20 µs), 50, and 100 µs. For WAB56–104, the only significant effect was of temperature at the P-step (Figure 4d). There was no significant temperature by time-of-night interaction at any of the steps for N22 and WAB56–104.

Across temperature and time-of-night, the O–J phase had the highest relative amplitude ($A_{O\text{–}J}$; 53–56% for N22 and 45–48% for WAB56–104), compared with the amplitude of the

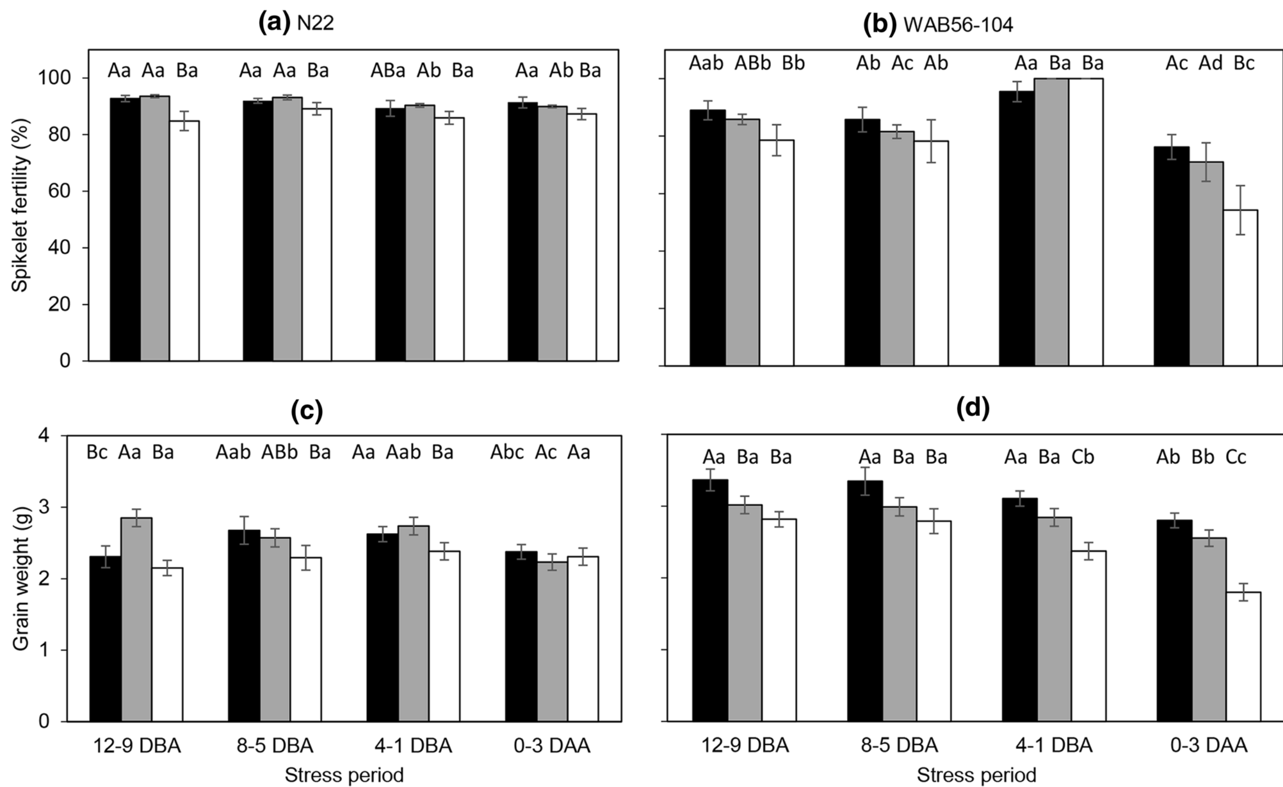

FIGURE 2 Experiment 3: main panicle spikelet fertility (a, b) and grain weight (c, d) of N22 (a, c) and WAB56–104 (b, d) treated to four consecutive nights of 24 (black bars), 30 (grey bars), or 35°C (white bars) between microsporogenesis (12–1 d before anthesis) and anthesis (0–3 d at anthesis). $n = 6$. Error bars are ± standard error. Wald tests for fixed effects of all fixed terms (cultivar, night temperature, stress period, and all their interactions) were significant ($P \leq .05$) for spikelet fertility, and grain weight per panicle (except the cultivar and stress period interaction term with $P = .397$). The analysis of variance tables for spikelet fertility and grain weight per panicle are presented in Supplemental Table S4 and S5, respectively. Within a panel, different uppercase letters for a cluster of bars indicate differences between night temperature treatments, while differences in lowercase letters indicate differences between stress periods of plants that received the same temperature treatment

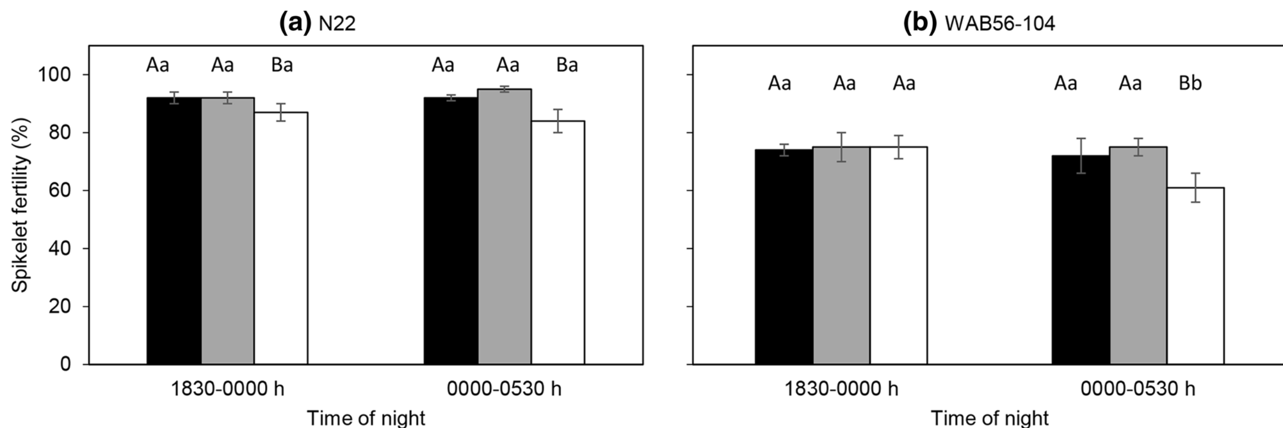

FIGURE 3 Experiment 4: main panicle spikelet fertility of N22 (a) and WAB56–104 (b) treated to pre-midnight (1830–0000 h) and post-midnight (0000–0530 h) temperatures of 24 (black bars), 30 (grey bars), or 35°C (white bars) at anthesis (0–3 d at anthesis). $n = 4$–8. Error bars are ± standard error. There were significant ($P < .05$) main effects of genotype and temperature, and borderline ($P = .059$) effect of time of night, but no significant ($P > .05$) effects of their interaction terms. Within a panel, different uppercase letters for a cluster of bars indicate differences between night temperature treatments, while differences in lowercase letters indicate differences between time of night treatments between plants that received the same temperature treatment

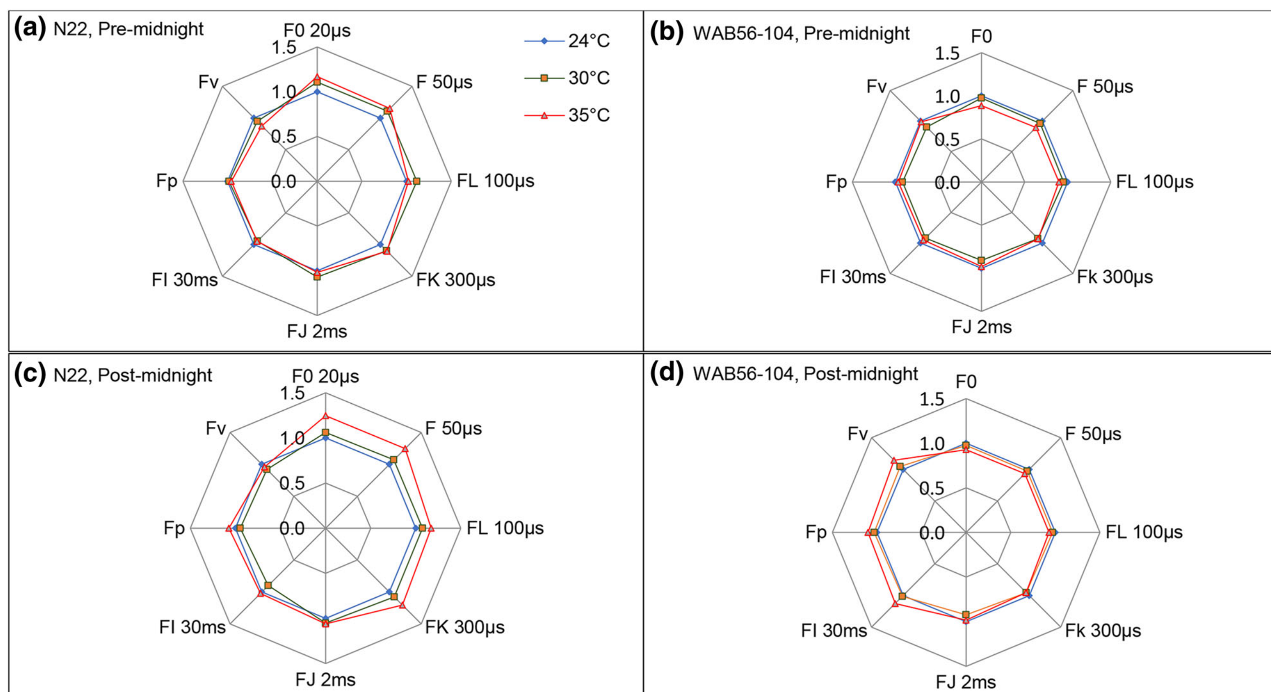

FIGURE 4 Experiment 4. Selected chlorophyll-a fluorescence parameters measured on leaves of rice cultivars N22 (a, c) and WAB56–104 (b, d) treated to pre-midnight (1830–0000 h; a, b) or post-midnight (0000–0530 h; c, d) night temperatures of 24, 30, and 35°C at anthesis (0–3 d at anthesis). Parameters are plotted (radarplot center = 0.0, maximum = 2.5) relative to night temperature of 24°C (blue lines and diamonds, set = 1.0). Descriptions of parameters plotted are adapted from Strasser et al. (2000, 2004). Beginning from F_0 and proceeding in a clockwise direction, the parameters are minimum fluorescence intensity at 20µs or O-step (F_0 20µs), fluorescence intensity at 50µs (F 50µs), at 100µs or L-step (F_L 100µs), at 300µs or K-step (F_K 300µs), at 2 ms or J-step (F_J 2 ms), at 30 ms or I-step (F_I 30 ms), maximum or peak fluorescence intensity or P-step (F_M), and variable fluorescence ($Fv = F_M – F_0$), $n = 4$

J–I phase ($A_{J–I}$; 16–22% for N22 and 22–37% for WAB56–104) and I–P phase ($A_{I–P}$; 22–31% for N22 and 29–30% for WAB56–104; Table 1). N22 consistently had a higher O–J amplitude than WAB56–104 across temperature and time-of-night. Within a phase, significant temperature or time-of-night treatment effects were observed for the J–I and I–P phases, but not the O–J phase for N22. By contrast, for WAB56–104 there was a siginificant temperature by time-of-night interaction for the O–J phase, borderline interaction ($P = .062$) for the J–I phase, and no significant difference for the I–P phase (Table 1).

Chlorophyll-a fluorescence parameters, Fv/F_M, and both performance indices (PI and PI_{ABS}) of N22 were reduced with an increase in night temperature; the extent of this reduction varied with time-of-night. Reductions in F_V/F_M of N22 were due to elevation in F_0, rather than reduction of F_M. Increase in pre- and post-midnight temperature from 24 to 35°C significantly reduced Fv/F_M (by 6–8%), but this effect was not observed when increasing temperature from 24 to 30°C (Table 2). Performance index and PI_{ABS} values were reduced by 46–50% and 50–58%, respectively, with increased night temperature in N22 (Table 2), with the greatest decrease occurring post-midnight (50–58% compared with 46–50% for pre-midnight). Across temperature, PI and PI_{ABS} values were significantly lower for pre-midnight than post-midnight treatment in N22. For WAB56–104, increase in night temperature did not significantly affect Fv/F_M, PI, or PI_{ABS} (Table 2), but time-of-night did ($P < .05$). Mean pre-midnight Fv/F_M, PI, and PI_{ABS} values of 0.80, 1.98 and 4.34 were lower than corresponding post-midnight means of 0.83, 2.76, and 6.45 (Table 2), respectively.

4 | DISCUSSION

One hour during anthesis has been previously identified as the peak of sensitivity to HDT in rice (Jagadish et al., 2008; Yoshida, Satake, & Mackill, 1981). In this study, we show that the most sensitive period of rice spikelet fertility to HNT is at anthesis (0–3 DAA), with no consistent differential sensitivity to pre- or post-midnight high temperature.

4.1 | Anthesis is the most sensitive period of rice spikelet fertility and grain weight to HNT

Using two rice cultivars contrasting in tolerance to HNT, we started by confirming in Experiments 1 and 2 that HNT

TABLE 1 Experiment 4. Amplitudes of O–J, J–I, and I–P phases of N22 and WAB56–104 treated to high temperature at anthesis for four half nights (pre-midnight, 1830–0000 h, and post-midnight, 0000–0530 h)

Amplitude	N22		WAB56–104	
%	Pre-midnight	Post-midnight	Pre-midnight	Post-midnight
O–J phase				
24	47.9	52.0	50.0	48.1
30	53.3	59.3	44.9	45.3
35	57.8	57.1	51.2	40.6
Mean	**53.0**	**56.2**	**47.7**	**44.7**
LSD (*P* = .05)[a]				
Temperature (T)	7.7 ns		5.1 ns	
Time of night (ToN)	6.3 ns		4.2 ns	
T × ToN	10.9 ns		7.3*	
J–I phase				
24	22.7	28.4	25.6	22.7
30	12.3	15.2	25.1	25.1
35	12.6	21.9	17.8	32.0
Mean	**15.8**	**21.8**	**22.3**	**36.6**
LSD (*P* = .05)				
Temperature (T)	7.2**		7.8 ns	
Time of night (ToN)	5.9*		6.4 ns	
T × ToN	10.2 ns		11.1 (*P* = .062)	
I–P phase				
24	29.5	19.6	28.5	29.3
30	34.5	25.5	30.1	29.7
35	29.7	21.0	31.0	27.4
Mean	**31.2**	**22.0**	**30.0**	**28.8**
LSD (*P* = .05)				
Temperature (T)	11.0 ns		3.8 ns	
Time of night (ToN)	9.0*		3.1 ns	
T x ToN	15.6 ns		5.4 ns	

*Significant at the .05 probability level.
**Significant at the .01 probability level.
[a]LSD, least significant difference; ns, nonsignificant.

spanning the reproductive development stage (from microsporogenesis to anthesis, 15 consecutive nights) reduces rice spikelet fertility and grain weight. Our results were in agreement with Mohammed and Tarpley (2009a, 2009b, 2010), and our earlier study (Coast et al., 2015). Subsequently, in Experiment 3 we quantified the effect of a shorter duration of HNT (four nights) over the same developmental stage as in Experiments 1 and 2, identifying anthesis as the most sensitive period to HNT. This is similar to the response of rice to HDT. While microsporogenesis has been identified as the second most sensitive reproductive stage to HDT for spikelet fertility, after anthesis (Jagadish et al., 2008, Yoshida et al., 1981), we found no evidence for that with regard to HNT. This suggests processes that determine spikelet fertility are similarly affected by both HNT and HDT at anthesis, but not at microsporogenesis.

Microsporogenesis of most spikelets (> 50–90%) in a rice panicle completes within the initial ∼4–5 d (Jagadish et al., 2014), and anthesis in about ∼4–5 d (Cao et al., 2015). The greater sensitivity of spikelet fertility and grain weight to HNT at anthesis, relative to other reproductive growth stages, might be due to similar reasons as for HDT sensitivity, including arrested pollen germination and pollen tube growth rate (Hedhly, 2011) via altered carbohydrate supply to pollen and reproductive structures. It may also be due to inadequate time to repair or recover from damage during the morning hours preceeding HNTs at anthesis. High temperature applied during the earlier stages of the reproductive phase is known to

TABLE 2 Experiment 4. Select chlorophyll-a fluorescence parameters of flag leaves of N22 and WAB56–104 treated to different pre-midnight and post-midnight temperatures at anthesis. Measurements were taken on the fourth day of treatment

Temperature	Select chlorophyll-a fluorescence parameters[a]					
°C	N22			WAB56–104		
Pre-midnight	Fv/F_M	PI	PI_{ABS}	Fv/F_M	PI	PI_{ABS}
24	0.76	1.07	2.28	0.81	2.15	4.75
30	0.73	0.76	1.64	0.78	1.92	4.27
35	0.70	0.54	0.96	0.81	1.86	4.00
Mean	**0.73**	**0.79**	**1.63**	**0.80**	**1.98**	**4.34**
Post-midnight						
24	0.80	1.51	2.99	0.82	2.20	4.79
30	0.78	0.91	1.56	0.82	2.50	5.70
35	0.75	0.81	1.47	0.84	3.59	8.87
Mean	**0.78**	**1.08**	**2.01**	**0.83**	**2.76**	**6.45**
LSD (P = .05)[b]						
Temperature (T)	0.05 ns	0.46*	1.06*	0.02 ns	0.92 ns	2.53 ns
Time of night (ToN)	0.04*	0.37 ns	0.87 ns	0.02*	0.75*	2.06*
T × ToN	0.07 ns	0.64 ns	1.50 ns	0.03 ns	1.30 ns	3.57 ns

*Significant at the .05 probability level.

**Significant at the .01 probability level.

[a]The parameters are the maximum quantum yield of Photosystem II (Fv/F_M), total performance index per absorption basis (PI), and performance index based on equal absorption (PI_{ABS}), with n = 16.

[b]LSD, least significant difference; ns, not significant.

cause abnormal cell proliferation, arrest and premature degradation in the developing anther cells of cereals (Abiko et al., 2005; Oshino et al., 2007).

4.2 | Spikelet fertility and grain weight of different panicles respond differently to HNT

Our study agrees with other reports on the tolerance of N22 and susceptibility of WAB56–104 to high temperature stress, based on spikelet fertility and grain weight data of the main panicles. However, there were interesting differences at the panicle level. For example, spikelet fertility and grain weight of primary panicles of N22 were either as sensitive (with spikelet fertility reduced by 57% compared with 63% for WAB56–104 at 35°C) or more sensitive (with grain weight reduced by 76% compared with 55% for WAB56–104 at 35°C) than those of WAB56–104 (Figure 1). These differential panicle responses have not been reported previously in the literature, perhaps because other studies have focused on main panicles alone, or combined data from all panicles, obscuring this response.

We note that differences in spikelet fertility and grain weight responses to HNT at the panicle level are probably tightly linked with variations in phenological timing among panicles. Spikelet fertility and grain weight of panicles, which develop earlier, are directly affected by HNT via increased spikelet sterility and reduced grain weight (in the absence of compensation by increasing individual grain weight). By contrast, spikelet fertility and grain weight of later-developing panicles during earlier stages of spikelet development experiencing similar HNT would probably be affected by spikelet degeneration, resulting in a reduced number of spikelets and grains.

4.3 | No consistent difference in sensitivity of spikelet fertility to pre- or post-midnight high temperature at anthesis

To narrow down the period of greatest sensitivity to HNT, we further reduced the duration of temperature treatments from four consecutive full nights to half nights. As the duration of HNT reduced, so did the effect of HNT on spikelet fertility and grain weight, with spikelet fertility reducing from 47–77% for 15 nights to 6–29% for four nights at anthesis, and then by 9–15% for four half nights at anthesis. We did not observe a consistent difference in sensitivity of spikelet fertility to pre- or post-midnight high temperature at anthesis (Figure 3). Our study does not support the differential response to HNT as was observed in cowpea [*Vigna unguiculata* (L.) Walp.], in terms of pod set and pollen viability (Mutters & Hall, 1992). Mutters and Hall (1992) reported that high temperature during the later 6-h period of the night resulted in much lower pod set (7–20%) and pollen viability (2–35%) than high temperature during the earlier 6-h period of the night (51–76% for pod set and 65–69% for pollen viability).

4.4 | High night temperature alters the chlorophyll-a fluorescence parameters of rice flag leaves at anthesis (0–3 DAA)

The rise of chlorophyll-a fluorescence intensity from F_0 to F_M exhibited a polyphasic shape with distinct O-J-I-P steps (Figure 4). All steps, including the K-step, which indicates high temperature induced changes of PSII (Srivastava, Guisse, Greppin, & Strasser, 1997), were visible for both N22 and WAB56–104 (Figure 4). Analysis of the amplitude between the steps revealed the most important phase to be the O–J phase. This could indicate a slower reduction of primary quinone-type electron acceptor, Q_A.

The maximum quantum yield of PSII (F_V/F_M), which is the most frequently used chlorophyll-a fluorescence parameter to assess high temperature stress in rice (over 80% of reported studies, see Supplemental Table S1), was significantly reduced in N22 by pre- and post-midnight high temperature at anthesis, but not in WAB56–104. The reduction in F_V/F_M probably reflects changes at the level of PSII reaction centres or disconnection of light harvesting complex antennae (Krause & Weis, 1984). Reduced F_V/F_M indicates damage to PSII proteins, such as D1, or impairments of the repair system of PSII proteins (Gururani, Venkatesh, & Tran, 2015; Song, Yue, Zhao, & Hou, 2013). Reductions in F_V/F_M, due to HNT, align with previous studies of HNT and rice (Alvarado-Sanabria, Garcés-Varón, & Restrepo-Díaz, 2017a, 2017b). In this study, we observed that even short periods of HNT (5.5 h of pre- or post-midnight stress) can reduce F_V/F_M.

Performance index on absorption basis integrates three main functional characteristics of primary photochemistry: (i) density of fully active reaction centers; (ii) maximum quantum yield of primary photochemistry at time 0 (φ_{P0}); and (iii) the efficiency that trapped exciton move further along the electron transport chain (ψ_{E0}). Change in PI_{ABS} at post-midnight HNT treatment was found to be more pronounced compared to F_V/F_M (Table 2). Reductions in PI and PI_{ABS} in response to HNT stress are in agreement with studies of other abiotic stresses in cereals, including water stress (Živčák, Brestič, Olšovská, & Slamka, 2008), high temperature (Feng et al., 2014), or a combination of the two (Jedmowski, Ashoub, Momtaz, & Bruggemann, 2015). Differences in sensitivity of PSII activity to HNT between N22 and WAB56–104, suggest differences in fundamental features of the photosystems that contribute to temperature tolerance. Al-Khatib and Paulsen (1999) suggest such features could include loss of water-splitting activity, uncoupling of noncyclicphotophosphorylation, and dissociation of light-harvesting pigments.

In conclusion, pinpointing the period of greatest sensitivity to HNT provides a reference on which future studies on HNT tolerance in rice can be built. Spikelet fertility and grain weight responses to HNT varied with cultivar, panicle, duration and intensity of HNT, reproductive growth stage, and time of night. We showed that spikelet fertility and grain weight of rice is most sensitive to HNT at anthesis, with no consistent differential response to pre- and post-midnight high temperature. More studies should be conducted to explore the mechanisms underpinning HNT-induced spikelet fertility reduction at anthesis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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