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Quantifying rice spikelet sterility in potential heat-vulnerable regions: Field surveys in Laos and southern India

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Abstract (256 words)

Global warming is projected to cause yield losses due to heat-induced spikelet sterility (HISS) in hot, vulnerable rice-growing regions, but reports documenting HISS at a regional level in the tropics are very limited. As a case study, HISS at flowering was surveyed under field conditions using local popular varieties in Savannakhet, Lao PDR, and Tamil Nadu, India, during the dry season. At the field site in Savannakhet, sterility in variety Thadokkham1 (TDK1) was 10.8% when the maximum average temperature at heading was 32.9 °C, which increased to 23.3% at 37.3 °C. When plots were covered with a shading net, solar radiation was reduced by 60% at heading, which significantly reduced sterility to 10.0%. Panicle temperature estimates produced with a micrometeorology model revealed that shading during the hours when flowering occurs decreased panicle temperature by 2.2–2.6 °C. At the field sites in Tamil Nadu, sterility of variety Coimbatore 51 (CO51) was highly correlated with the maximum temperature at heading; sterility was around 5–10% when maximum average temperature was below 36.5 °C, which increased to 33.4% at 38.0 °C. Marking of opened spikelets at hourly intervals allowed us to detect increases in sterility as temperature increased from early morning until noon. We conclude that HISS at flowering can occur in local popular varieties grown in Savannakhet and Tamil Nadu during the hot, dry season. Effective protocols such as using a shading net and hourly marking of open spikelets are useful approaches to quantifying HISS during flowering across hot and potential heat-vulnerable rice-growing regions.

Keywords: Heat stress, Spikelet sterility, Flowering, Rice, Solar radiation

1. Introduction (4366-words until Conclusion)

Rice (*Oryza sativa* L.) is very susceptible to heat stress at the flowering stage. Previous growth chamber experiments have recorded increased spikelet sterility when rice plants are exposed to temperatures above 32–36 °C at anthesis (Jagadish et al., 2010; Kim et al., 1996; Matsui et al., 1997; Satake and Yoshida, 1978; Weerakoon et al., 2008). Also, it was found that there is wide genetic variation in heat tolerance response among Indica-type and Japonica-type varieties (Jagadish et al., 2008; Maruyama et al., 2013; Matsui et al., 2001; Prasad et al., 2006; Shi et al., 2014; Weerakoon et al., 2008). According to the 5th Assessment Report of the Intergovernmental Panel on Climate Change (IPCC), warmer and/or more frequent hot days and warm nights over most land areas are virtually certain, and the frequency of heat waves is very likely to increase in the late 21st century (IPCC, 2013). Current cultivation practices have been developed to minimize the incidence of heat-induced sterility in the tropics and subtropics (Wassmann et al., 2009), however, growing-season temperatures are predicted to exceed the most extreme seasonal temperatures recorded in the past (Battisti and Naylor, 2009). In addition, climate is expected to become more variable, with frequent episodes of stressful temperatures coinciding with critical crop development stages like flowering. Heat-induced spikelet sterility (HISS) at flowering is a serious threat that could cause significant reductions in rice production (Jagadish et al., 2014)

Rice grown in the tropics and subtropics is potentially at high risk of exposure to

heat stress in the dry season as found by many researchers. Osada et al. (1973) reported a drastic increase in empty grains of Indica-type varieties in the dry season in Thailand. Matsushima et al. (1982) observed low percentages of grain set under the extremely hot and dry conditions in Sudan. Other studies identified heat-vulnerable regions which were geographically mapped based on cropping calendar and on temperatures at the flowering and early grain-filling stages. These studies found that regions where rice is exposed to temperatures of over 33 °C during flowering are widely distributed across continental South and Southeast Asia (Wassmann et al., 2009). In particular, Laos and southern India have been identified as very heat-vulnerable regions during the dry summer season (Jagadish et al., 2014; Laborte et al., 2012; Wassmann et al., 2009).

Recently, field surveys were conducted to determine the occurrence of HISS under open field conditions in temperate regions such as Australia (Matsui et al., 2014), China (Tian et al., 2010), and Japan (Hasegawa et al., 2011). In these surveys, significant reductions in seed set were observed in Chinese hybrid rice at around 35 °C under humid and low-wind conditions at the flowering stage (Tian et al., 2010). In Japan, spikelet sterility was considerably higher in the record hot summer of 2007, around a maximum temperature of 38 °C, than in the normal summer of 2008 (Hasegawa et al., 2011). On the other hand, no serious sterility problems were observed under hot and dry conditions in the fields of New South Wales, Australia, although the maximum air temperature went up to 40 °C (Matsui et al., 2014). The difference in HISS between China and Australia can be explained by the difference in panicle temperature. In China, panicle temperature was 4.0 °C higher than ambient air temperature under humid conditions accompanied by very

low wind speed (Tian et al., 2010), whereas in Australia it was 6.8 °C lower than ambient air temperature owing to high evapotranspiration under dry and windy conditions (Matsui et al., 2007). In addition, other factors that determine the microclimate such as solar radiation, wind, and humidity strongly influence the induction of spikelet sterility at flowering under field conditions by influencing panicle temperatures (Yoshimoto et al., 2011). Also, the flower opening time affects the level of HISS. Under high-temperature conditions, spikelets that flower later in the day tend to be sterile (Hirabayashi et al., 2015; Ishimaru et al., 2010).

Current information on HISS under field conditions is limited to the temperate regions mentioned above. Although some field surveys were conducted in the tropics a few decades ago (Matsushima et al., 1982; Osada et al., 1973), no systematic field surveys on spikelet sterility have been conducted using recent local popular varieties in potentially heat-vulnerable regions such as Laos and India. Thus, this study aimed to determine HISS in the local popular Indica-type rice variety Thadokkham1 (TDK1) in Savannakhet (Laos), and in the variety Coimbatore 51 (CO51) in Tamil Nadu (southern India), in the hot, dry season under field conditions.

The effect of heat stress on spikelet sterility in open fields is often difficult to determine because temperatures are difficult to control and other factors are involved in the fertilization process. General observations on spikelet sterility *per se* are often insufficient to quantify the effect of heat stress alone on spikelet sterility. In this study, we tested two possible methods to estimate HISS under field conditions based on two working hypotheses. The first hypothesis was that HISS is caused or exacerbated by high panicle temperatures as

they are directly exposed to the sun. This effect may be determined by artificially reducing solar radiation at flowering, the critical stage for spikelet fertility. To this end, we tested whether reducing solar radiation by installing a shading net above the canopy at the flowering stage could reduce sterility by reducing panicle temperatures. The second hypothesis was that spikelets that flower at hotter times of the day have higher rates of sterility under field conditions. We tested this hypothesis by recording the flowering (opening) times of spikelets and measuring spikelet sterility at harvest corresponding to each flower opening time. The first and second hypotheses were tested in the potentially heat-vulnerable regions of Laos and southern India, respectively.

2. Materials and methods

2.1 Locations and crop establishment

2.1.1 Savannakhet, Laos

The experiments were conducted during the dry season of 2013 at the Thasano Rice Research and Seed Multiplication Center in Savannakhet Province, Lao PDR (16°33'N, 104°45'E, 144.5 m above sea level [asl]). The popular local glutinous Indica-type variety TDK1 was planted in two staggered sowings to produce crops with heading dates during early March (1st crop) and early April (2nd crop). Seeds were sown on 15 November and 15 December in 2012 for the 1st and 2nd crops, respectively. Fifteen-day-old seedlings were transplanted with a planting density of 25 hills/m² (20-cm row spacing and 20-cm intra-row spacing). Basal fertilizer (N-P₂O₅-K₂O, each 30 kg/ha) was applied 1 day before transplanting. Additional nitrogen was applied three times as topdressing at 20, 40, and 60 days after transplanting at the rate of 20 kg/ha. The rice plants in the 1st and 2nd crops

reached 10% heading on 1 March and 28 March, 50% heading on 5 March and 1 April, and full heading on 8 March and 5 April in 2013, respectively. The size of each plot was 6 m² (3 m × 2 m) with four replications for unshaded (control) and three replications for shaded conditions, respectively. The experimental plots were arranged in a complete randomized design. Plants were grown under flooded conditions throughout the experiment.

2.1.2 Tamil Nadu, southern India

The experiments were conducted in two locations during the dry season of 2014 at the experimental fields of the Tamil Nadu Agricultural University in Coimbatore (11°00'N, 76°54'E, 426 m asl) and Madurai (9°54'N, 78°80'E, 147 m asl) both in Tamil Nadu, India. Coimbatore and Madurai are geographically close but are categorized under different agroclimatic zones. Because of their differing altitudes, air temperature in the dry season is generally lower in Coimbatore than in Madurai. Thus, Madurai was expected to be a potentially heat-vulnerable region with its higher HISS. The popular local variety CO51 was used in both locations. CO51 was released in 2013 as an early maturity variety and its cultivation area in southern India has been expanding since then owing to its water-saving ability and resistance to local diseases (Directorate of Research, Tamil Nadu Agricultural University, 2013).

Two staggered sowings were carried out at each location: 28-day-old seedlings were sown on 10 January and 7 February for the 1st and 2nd crops, respectively, at the Madurai site and approximately 20-day-old seedlings were sown on 20 January and 2 February for the 1st and 2nd crops, respectively, at the Coimbatore site. For the two sowings in both sites,

the seeds were transplanted into a paddy field with a planting density of 25 hills/m² (20-cm row spacing and 20-cm intra-row spacing). In total, 150 kg/ha of nitrogen and 50 kg/ha each of phosphorus (P₂O₅) and potassium (K₂O) were applied. For the Madurai site, nitrogen was split into four equal applications at two-week intervals from transplanting to the start of heading stage while for the Coimbatore site, nitrogen was split into four and five applications at intervals of two weeks from transplanting to the start of heading in the 1st and 2nd crops, respectively. The sizes of each plot were 18.7 m² (8.5 m by 2.2 m) at Madurai and 500 m² and 280 m² for the 1st and 2nd crops, respectively, at Coimbatore. In both sites, phosphorus and potassium were applied only at transplanting, and plants were grown under flooded conditions throughout the experiment.

2.2 Shading treatment

In Savannakhet in 2013, shading treatment was started in the 2nd crop by erecting 60% shading nets (SL-60, silver color; Taiyou net, Osaka, Japan) at 50% heading (1 April). This was done to ensure that no flowering panicles escaped the treatment and that the flowering period of all the plants in the plot was completed. The differences in the timing to reach 50% flowering among different plants was minimized by collecting a total of 116 and 81 panicles from the unshaded and shaded conditions, respectively, at maturity. The shading nets were placed 50 cm above and parallel to the crop canopy. The sides of the canopy were open (Fig. 1). Three plots were covered with the shading net for five days until full heading on 5 April while four plots were left unshaded.

2.3 Hourly marking of opened spikelets

The spikelet-marking exercise was conducted on the clear bright days of 4 April 2013 in the 2nd crop at Savannakhet, 2 May 2014 in the 2nd crop at Madurai, and 5 May 2014 in the 2nd crop at Coimbatore. At Savannakhet, panicles for investigation were obtained from only the 1st and 2nd rows (counting from the edge of the plot) because heading was partial or complete on the plants closer to the center of the plots. In contrast, at Madurai and Coimbatore, panicles were chosen from plants four or more rows from the edge of the plots, leaving the first three rows as border. Opened spikelets were marked using fine-tipped pens (0.5-mm diameter; Zebra Co. Ltd, Tokyo, Japan) every hour from the start to end of flowering during the day at the two locations. At each of the three field sites, a different color of ink was used to represent each marking hour to efficiently determine the effect of flower opening time on spikelet sterility at maturity. It should be noted, however, that it was difficult to figure out the color of the ink on the spikelets of TDK1 at maturity because the hulls of TDK1 change into dark color during grain filling. Thus, hourly changes in spikelet sterility were only examined in CO51.

2.4 Panicle sampling and spikelet sterility

At all three sites, panicles three to four weeks after heading were harvested from plants four or more rows from the edge of the field, leaving the first three rows as border.

One or two panicles were sampled from the two tallest tillers per hill, i.e., the two with the highest flag leaf tips. The large difference in plot area between Madurai and Coimbatore was taken into consideration when choosing sampling positions at the sites in Tamil Nadu. At Coimbatore, where the plots were much larger, panicle samples were obtained from the fourth to sixth rows from the edge of the plot to maintain uniformity in sampling position. Spikelet sterility per panicle was manually tested by pressing each spikelet in a panicle to determine whether it was filled or unfilled.

2.5 Daily meteorological data

Daily maximum and minimum temperatures were obtained from the meteorological station at the Savannakhet airport in Laos and from meteorological stations installed in the fields at Madurai (IMDAWS 01; Astra Microwave Products Ltd., Hyderabad, India) and Coimbatore (Vantage ProTM wireless-type weather station, Davis Instruments, Hayward, CA) in India. The experimental field at the Thasano Rice Research and Seed Multiplication Center is located at approximately 12.5 km north of the Savannakhet airport.

2.6 Estimation of panicle temperatures

Panicle temperatures were estimated with IM²PACT (Yoshimoto et al., 2011), which is a micrometeorology model to estimate panicle temperature by calculating heat and

radiation balance between the atmosphere and vegetation. The IM²PACT was first validated by using it to continuously measure panicle temperature in a paddy field in Tsukuba, Japan (36°01'N, 140°06'E, 23 m asl), where very thin thermocouples (copper-constantan, 0.08 mm diameter) were installed on the surface of flowering panicles. We confirmed that the estimated panicle temperature almost corresponded to the actual panicle temperature in the continuous measurement, and that the differences between estimated values and actual values were less than 1°C (Yoshimoto et al. 2011). IM²PACT was secondly validated by the panicle temperature measurement using spot-type infrared thermometer (TA-0510bF, Konica Minolta Holdings, Inc.) in the paddy fields under the hot and dry climate in New South Wales, Australia, and the hot and humid climate in Jiangnan Basin, China (Yoshimoto et al. 2007). Thus, IM²PACT was verified to be applicable across a wide range of climatic conditions.

For the estimation of panicle temperature in the 2nd crop of TDK1 in Savannakhet, a Vantage ProTM wireless-type weather station (Davis Instruments, Hayward, CA) was installed at the experimental field at the Thasano Rice Research and Seed Multiplication Center, and short-term meteorological data during heading stages were obtained at 15-min intervals. We chose to conduct the estimation of panicle temperatures on 4 April 2013 during the hours when flowering occurred because the weather was stable and the day was perfectly bright and sunny. The anemometer was positioned 1.5 m above the ground. The air temperature and relative humidity sensors were housed in a fan-aspirated radiation shield at 1.3 m above the ground. The input parameters for plant traits were based on the actual conditions in the rice paddy and were set as follows: leaf area index, 3.5; canopy

height, 100 cm; and panicle position, 80 cm above the ground. For both the unshaded and shaded treatments, the panicle transpiration conductance was set as the average of values reported for other rice cultivars (Fukuoka et al., 2012) since we did not measure the conductance of TDK1. This may have caused some error in the estimation of the absolute values of panicle temperature if the conductance of TDK1 was far from the average value; however, the calculated difference in panicle temperature after shading treatment should be reliable even if the estimated absolute temperatures are not. The 60% shading net treatment altered the radiation input to the canopy, hence, the solar radiation in the shaded condition was set to 40% of the measured data in the unshaded treatment. The downward long-wave radiation (Ld) in the unshaded (control) treatment was estimated from the measurements of solar radiation, air temperature, and relative humidity and from the geographical information by theoretical and empirical equations for atmospheric radiation (Kuwagata et al., 1990). On the other hand, the Ld in the shaded condition was estimated as the summation of 40% of the Ld estimate for the control (representing Ld from the sky) and 60% of the Ld estimate from the shading net treatment. As a first approximation, the shading net temperature was assumed to be the same as the air temperature because of the net's airy structure and high reflection of sunlight with silver color. We could not estimate the panicle temperatures for Madurai because of the absence of humidity data.

2.7 Statistical analysis

Differences in spikelet sterility among the means under the unshaded and shaded

treatments and hourly changes in spikelet sterility were analyzed using Tukey's test.

3. Results

3.1 Experiments in Savannakhet, Laos

3.1.1. Temperatures at heading

The 1st and 2nd crops headed in early March and in late March to early April, respectively (Fig. 2). The temperature conditions differed markedly between the two crops. In the 1st crop, the daily maximum temperature was over 34 °C in three of eight days during heading (Table 1; Fig. 2) and the average maximum temperature during heading was 32.9 °C. In contrast, daily maximum temperatures exceeded 34 °C on all nine days during heading in the 2nd crop (Table 1; Fig. 2) and the average maximum temperature was 37.3 °C.

3.1.2 Spikelet sterility

Under unshaded conditions, spikelet sterility of TDK1 was 10.8% in the 1st crop and 23.3% in the 2nd crop (Table 1). Shading was applied from 50% heading until the end of heading in the 2nd crop. Spikelet sterility in the shaded treatment was 10.0%, which was significantly lower than in the unshaded treatment in the 2nd crop (Table 1).

3.1.3 Flower opening time and estimation of panicle temperature by IM²PACT

The flowering pattern of TDK1 was hourly checked in the shaded and unshaded treatments (Fig. S1) and it was found that over 60% of the spikelets opened after 1030 h when air temperatures exceeded 34 °C (Fig. S1). During flower opening time of TDK1, panicle temperature was estimated in the shaded and unshaded conditions for every hour from 1000 h until 1200 h (Table 2). Under the unshaded condition, panicle temperature was estimated to be at 33.3 °C at 1000 h, 34.2 °C at 1100 h, and 34.8 °C at 1200 h. Under the shaded condition, temperatures were reduced by 2.2–2.6 °C compared to the unshaded condition (Table 2).

3.2 Experiments in Madurai and Coimbatore, Tamil Nadu, southern India

3.2.1 Correlation between spikelet sterility and temperatures during heading

At Madurai, the period from the start to the end of heading in each crop was approximately two weeks; thus, the panicles were divided into two groups based on heading date (Table 3). As a result, there were six groups, four at Madurai and two at Coimbatore, with heading dates from 1 April to 13 May. The groups also varied in maximum and minimum temperatures at the heading stage (Table 3), ranging from 35.2 °C to 38.6 °C and from 22.7 °C to 25.4 °C, respectively, across the two sites. Both maximum and minimum temperatures tended to be higher at Madurai than at Coimbatore. A high percentage of spikelet sterility (over 20%) was observed at Madurai, except for Group 4, whereas the percentage of sterility was normal (5–10%) at Coimbatore (Fig. 3A). Spikelet

sterility was around 5–10% when maximum temperatures were at or below 36.4 °C, whereas it was 21.3% at 37.1 °C and 33.4% at 38.0 °C (Fig. 3A). The percentage of spikelet sterility was highly correlated with the maximum temperature at heading ($R^2=0.85$). On the other hand, less correlation was found between spikelet sterility and minimum temperature during heading (Fig. 3B, $R^2=0.27$).

3.2.2 Flower opening time and hourly changes in spikelet sterility

Flowering pattern was investigated on an hourly basis on 2 and 5 May in the 2nd crop at Madurai and Coimbatore, respectively. At Madurai, flower opening occurred from 0900 to 1200 h (Fig. 4A). Air temperature was 31.7 °C at 1000 h, 33.2 °C at 1100 h, and 33.9 °C at 1200 h (Fig. 4A). Spikelet sterility steadily increased toward noon (Fig. 4B) as evidenced by the low sterility (8.4%) of the spikelets that flowered by 1000 h but which became significantly higher at 1000–1100 h (27.0%) and at 1100–1200 h (38.3%) (Fig. 4B). At Coimbatore, most flower opening occurred from 0900 to 1100 h (Fig. S2A). The percentage of sterility was normal (7–8%) regardless of flower opening time (Fig. S2B), and air temperature was around 30 °C during flower opening time.

4. Discussion

Although it is expected that heat stress would seriously aggravate spikelet sterility when it coincides with flowering, few assessments of actual damage have been conducted in potentially heat-vulnerable regions of the tropics and subtropics. For this study, we hypothesized that the increasing intensity of heat stress would induce higher levels of spikelet sterility at flowering in Laos (Savannakhet) and southern India (Madurai). These regions have been identified by some reports as potentially heat-vulnerable areas based on GIS mapping using weather data coinciding with the critical flowering and early grain-filling stages (Jagadish et al., 2014; Laborte et al., 2012; Wassmann et al., 2009). This is the first study to investigate HISS in the local popular Indica-type rice varieties in Laos and southern India in the hot, dry season under field conditions.

TDK1 is normally sown during late November to early December and heading occurs during mid-March. This practice is done to avoid the heat stress in April and May when maximum temperature is highest with very few rainfalls. To understand the effect of high temperatures at the flowering stage on spikelet sterility of TDK1, staggered sowing was performed to expose the flowering panicles to different temperatures, i.e., those in early March (1st crop) and those in late March to early April (2nd crop) (Fig. 2). Maximum temperatures during the heading period were 32.9 °C in the 1st crop and 37.3 °C in the 2nd crop (Table 1). The 2nd crop provided excellent conditions for a study (shaded vs unshaded) to elucidate whether higher temperatures coinciding with flowering could increase rice spikelet sterility under field conditions in Savannakhet.

During the latter half of the heading period in the 2nd crop at the Savannakhet site, the crop canopy was covered with a shading net that allowed only 40% of sunlight to pass through (Fig. 1). Air temperature, relative humidity, wind speed, and solar radiation, all of which are components of the microclimate, were used to estimate panicle temperature at various times of day at the flowering stage with IM²PACT (Yoshimoto et al., 2011). We tested our hypothesis that reducing solar radiation by 60% using the shading net would reduce HISS by reducing panicle temperature by 2.2–2.6 ° at flowering (Table 2; Fig. S1). We found that TDK1 flowers that open after 1100 h were exposed to high panicle temperatures estimated over 34.0 °C under unshaded condition (Table 2) and spikelet sterility was significantly reduced (Table 1). These data indicate that the combined stress of heat and solar radiation during daytime at Savannakhet aggravated spikelet sterility through the increase in panicle temperature. It should be noted, however, that shading treatment must be applied only during the heading period, as shown in this study. Longer shading would not reveal the occurrence of HISS because of the negative effect of shading on grain set through reduced crucial photosynthetic activity at the ripening stage.

In the field survey in Tamil Nadu, southern India, staggered sowing of CO51 was performed twice at both Madurai and Coimbatore. Madurai was expected to be a heat-vulnerable region because it generally has higher temperatures than Coimbatore, which is at a higher altitude. The field locations had a wide range of maximum and minimum temperatures, depending on the timing of the heading period and the site location (Table 3). Spikelet sterility was highly correlated with maximum temperature during heading (Fig. 3A) and not with minimum temperature (Fig. 3B), suggesting that heat stress

during daytime is the primary cause of spikelet sterility. This finding, i.e., that high night temperatures do not lead to increased spikelet sterility under field conditions, has been reported by other studies using unique field-based tents (Jagadish et al., 2014; Shi et al., 2013), and our findings support these reports.

Furthermore, we closely observed the flower opening time of CO51 by marking the opened spikelets with a different color of ink for each hour. At Madurai, spikelet sterility was normal (below 10%) for flowers that opened before 1000 h, when air temperatures were at or below 31.7 °C, but was significantly higher for flowers that opened at or above 33.2 °C after 1100 h (Fig. 4). This difference in spikelet sterility between times of day suggests that there might be a temperature threshold for CO51 in this range. In a high-temperature greenhouse experiment, spikelets that flowered at later hours tended to have higher sterility than those that flowered at earlier hours in the morning (Ishimaru et al., 2010). A similar result was obtained in this field study, implying that a shift in flower opening time to the cooler hours of early morning (i.e., 0900–1000 h) could be effective in reducing HISS of CO51 at Madurai. Recently, novel breeding material for early-morning flowering was developed using wild rice (*Oryza officinalis*) as a donor parent (Hirabayashi et al., 2015). The protocol of hourly marking the opened spikelets would help breeders determine the best time of the morning for flowering to mitigate HISS in local rice varieties.

5. Conclusions

We determined that HISS at flowering occurred in two popular local varieties, TDK1 in Laos and CO51 in southern India, during the hot dry season. Heat stress during the daytime is considered to be the primary cause of spikelet sterility. Effective protocols such as using a shading net and hourly marking of opened spikelets were developed to assess the actual damage by HISS at the flowering stage in the paddy fields. In Laos, shading treatment applied only at flowering reduced HISS through the reduction of panicle temperature under high-temperature conditions, proving true the first hypothesis that solar radiation needs to be considered as a factor inducing spikelet sterility while dealing with heat stress damage under field conditions. In southern India, spikelet sterility was highly correlated with maximum temperature during flowering, indicating that high daytime temperature is the primary cause of HISS. These results were further supported by the increasing gradient in spikelet sterility from morning toward noon, with a steady increase in air temperatures, as mentioned in the second hypothesis. The time of day at which flowering produces normal sterility (less than 10%), in the cooler early morning, should be the target time for breeding to enable flowering spikelets to escape from heat stress.

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Figure legends

Fig. 1. Field view of shading experiment at the Savannakhet site. The top of the crop canopy was fully covered with a shading net, but the sides were left open to keep the canopy airy.

Fig. 2. Maximum and minimum air temperatures in the dry season of 2013 in Savannakhet, Laos. Boxes with broken lines indicate the heading periods of the 1st crop (left) and the 2nd crop (right). Data was taken at the Savannakhet airport.

Fig. 3. Correlation between temperatures during heading and spikelet sterility at field sites in Madurai and Coimbatore (variety CO51). The *x*-axis represents maximum temperature in (A) and minimum temperature in (B). Vertical and horizontal error bars indicate SE for spikelet sterility and temperature, respectively. Numbers within each graph correspond to the groups in Table 3.

Fig. 4. Hourly changes in the percentage of opened spikelets (A), and spikelet sterility at peak flower opening time (B) at the Madurai site (variety CO51). (A) In total, 390 spikelets from 9 panicles flowered on the day. (B) 179 spikelets opened at 0900–1000 h, 76 spikelets opened at 1000–1100 h, and 134 spikelets opened at 1100–1200 h from 9 panicles. Error

bars indicate SE. Different letters indicate significant differences at the 5% level by Tukey's test.

Table 1

Daily maximum air temperature during heading and spikelet sterility of TDK1 in 1st and 2nd crop under unshaded (control) and shaded condition at the Savannakhet site.

	1st crop	2nd crop	
	Unshaded	Unshaded	Shaded
Average maximum air temperature during heading (°C) ^a	32.9	37.3	
Days of maximum daily air temperature over 34 °C during heading ^a	3 of 8 days	9 of 9 days	
Spikelet sterility (%) ^b	10.8 ± 0.5 a	23.3 ± 1.6 b	10.0 ± 1.0 a

^a Maximum temperature was determined based on the temperature data in Fig. 1.

^b Values indicate mean ± SD. Different letters indicate significance at the 1% level by Tukey's test. In total, 48 panicles from four plots, 116 panicles from four plots and 81 panicles from three plots were used for counting sterility in the 1st crop (unshaded), 2nd crop under unshaded condition, and 2nd crop under shaded condition, respectively.

Table 2

Estimated panicle temperatures during flowering of TDK1 under unshaded (control) and shaded conditions at the Savannakhet site.

Time of day (h)	Treatment	Air temperature (°C)	Relative humidity (%)	Wind speed (m/s)	Solar radiation (W/m ²) ^a	Panicle temperature (°C) ^b
1000	Unshaded	33.8	65	2.2	670	33.3
	Shaded				268	31.1
1100	Unshaded	35.8	54	2.2	795	34.2
	Shaded				318	31.8
1200	Unshaded	37.2	47	2.2	844	34.8
	Shaded				338	32.2

^a Solar radiation under shaded condition was calculated 60% reduction of sunlight by the shading net.

^b Panicle temperature was estimated by IM²PACT (Yoshimoto et al., 2011).

Table 3. Heading period of CO51 in each crop season at the Coimbatore and Madurai sites.

Location	Crop	Group	Heading period		Average air temperature (°C)		Number of panicles used
			Start	End	Maximum	Minimum	
Madurai	1 st	1	1 April	6 April	38.0	23.8	52
		2	8 April	13 April	38.6	25.4	15
	2 nd	3	30 April	4 May	37.1	24.2	15
		4	9 May	13 May	36.4	24.5	16
Coimbatore	1 st	5	7 April	12 April	35.4	22.7	26
	2 nd	6	1 May	6 May	35.2	23.9	28

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Ishimaru et al. Fig. 2