High temperature stress and spikelet fertility in rice (Oryza sativa L.)

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Abstract

In future climates, greater heat tolerance at anthesis will be required in rice. The effect of high temperature at anthesis on spikelet fertility was studied on IR64 (lowland indica) and Azucena (upland japonica) at 29.6 °C (control), 33.7 °C, and 36.2 °C tissue temperatures. The objectives of the study were to: (i) determine the effect of temperature on flowering pattern; (ii) examine the effect of time of day of spikelet anthesis relative to a high temperature episode on spikelet fertility; and (iii) study the interactions between duration of exposure and temperature on spikelet fertility. Plants were grown at 30/24 °C day/night temperature in a greenhouse and transferred to growth cabinets for the temperature treatments. Individual spikelets were marked with paint to relate fertility to the time of exposure to different temperatures and durations. In both genotypes the pattern of flowering was similar, and peak anthesis occurred between 10.30 h and 11.30 h at 29.2 °C, and about 45 min earlier at 36.2 °C. In IR64, high temperature increased the number of spikelets reaching anthesis, whereas in Azucena numbers were reduced. In both genotypes ≤1 h exposure to ≥33.7 °C at anthesis caused sterility. In IR64, there was no interaction between temperature and duration of exposure, and spikelet fertility was reduced by about 7% per °C > 29.6 °C. In Azucena there was a significant interaction and spikelet fertility was reduced by 2.4% °Cd⁻¹ above a threshold of 33 °C. Marking individual spikelets is an effective method to phenotype genotypes and lines for heat tolerance that removes any apparent tolerance due to temporal escape.

Key words: Anthesis, heat tolerance, seed-set, spikelet fertility, rice, temperature.

Introduction

Rice (O. sativa and O. glaberrima) is one of the world’s most important crops, particularly in Asia, but increasingly so in Africa and Latin America as well. Rice is extensively grown in irrigated cropping systems, allowing production in the warmer, high radiation post-monsoon and summer months. Rice production has also intensified in rainfed-lowland and dryland (upland) cropping systems, many of which are prone to drought and high temperature (Coffman, 1977). Furthermore, global climate change is likely to exacerbate the current vulnerability of the crop to climate, with a projected global average surface temperature increase of 1.4–5.8 °C by 2100 and the possibility of increased variability about this mean (IPCC, 2001). Simulations by Horie et al. (1996), for example, have suggested that the yield of current varieties in southern Japan would be reduced by up to 40% in future climates.

Flowering (anthesis and fertilization), and to a lesser extent booting (microsporogenesis), are the most susceptible stages of development to temperature in rice (Satake and Yoshida, 1978; Farrell et al., 2006). Previous studies, summarized in Satake and Yoshida (1978), have shown that spikelets at anthesis that are exposed to temperatures >35°C for about 5 d during the flowering period are sterile and set no seed. Sterility is caused by poor anther dehiscence and low pollen production, and hence low numbers of germinating pollen grains on the stigma (Matsui et al., 2000, 2001; Prasad et al., 2006). There is genotypic variation in spikelet sterility at high temperature (Matsui et al., 2001; Satake and Yoshida, 1978; Prasad et al., 2006) that can be defined by different temperature thresholds (Matthews et al., 1995; Nakagawa et al., 2002). It has been suggested that indica spp are more tolerant to higher temperatures than japonica spp (Satake and Yoshida, 1978; Matsui et al., 2000), although heat-tolerant genotypes have been found in both subspecies.
(Prasad et al., 2006; Matsui et al., 2001). Genotypes N22 (Yoshida et al., 1981; Prasad et al., 2006) and Akitakomachi (Matsui et al., 2001) are the most tolerant genotypes found to date among indica and japonica spp., respectively.

The response to duration of exposure to temperature >35 °C appears to be quantitative, with shorter durations at higher temperatures having the same effect as longer durations at cooler temperatures (Satake, 1995). However, interactions between temperature and duration have not been quantified. Where responses to high temperature have been modelled, spikelet sterility increases in response to daily maximum temperature (Matthews et al., 1995; Horie et al., 1996; Nakagawa et al., 2002). If there is an interaction between temperature and duration, then the response of spikelet fertility to temperature may be better modelled by a cumulative temperature response above a threshold temperature (Vara Prasad et al., 1999, for peanut). Furthermore, if only a short period of high temperature causes sterility, then the timing of this episode in relation to peak flowering will be critical, both for phenotyping (i.e. to differentiate between escape and absolute tolerance) and modelling the impact of high temperature (Wheeler et al., 2000). It follows that effects of temperature on flowering pattern, which have not been studied, are also likely to be important with respect to escape and the total number of spikelets.

The overall objective of this work was to quantify the effects of duration of exposure and temperature on spikelet fertility in order to develop protocols to phenotype and map this important trait in rice. Accordingly, a widely grown lowland indica, IR64, and an upland japonica, Azucena, were subjected to a range of high temperature treatments during anthesis. Specifically, (i) the effect of temperature on flowering patterns; (ii) the effect of time of spikelet opening (anthesis) relative to a high temperature episode on spikelet fertility; and (iii) the interactions between duration of exposure and temperature on spikelet fertility were studied.

Materials and methods

Two experiments were undertaken between May and October in 2003 and in 2004, using controlled environment facilities at the Plant Environment Laboratory, Department of Agriculture, The University of Reading, UK (51°27’ N, 0°05’6 W). Plants were grown in a greenhouse under optimum temperature and photoperiod conditions, and transferred at anthesis to growth cabinets to impose high temperature treatments.

Greenhouse

Plants were grown in a naturally-lit greenhouse with day and light-proof night compartments. Pots were placed on automated mobile trolleys (2.85 m x 0.96 m) that were drawn out and in to night compartments (3.57 m x 1.74 m) at 08.00 h and 19.00 h BST, respectively, giving a short, inductive photoperiod of 11 h. Temperature in the greenhouse was maintained at 30/24 °C day/night through a combination of heating (16 kW) and venting during the day, and heating at night. Fans in both the day and night compartments circulated air continuously. Aspirated air temperature was measured by copper-constantan thermocouples at canopy height and recorded every 30 min using data loggers (Delta T Devices, Burwell, Cambridge, UK).

Crop husbandry

Plants were grown in a soil-less medium. Steam-sterilized sand and gravel were mixed with peat compost and vermiculite in the proportion of 2:4:1:4 by vol., respectively. Pots of 12.5 cm diameter were filled with the potting medium and placed on the mobile trolleys in the greenhouse. Pots were soaked overnight and four to six seeds of IR64 and Azucena were sown per pot at a depth of 2–2.5 cm and then thinned down to one per pot at the three leaf stage. All the plants were de-tillered to leave three shoots to reduce overcrowding. After thinning, plants were irrigated automatically through a drip-irrigation system (greenhouse), or watered by hand (cabinet), with a complete nutrient solution containing 100 mg l⁻¹ inorganic nitrogen. The nutrient solution was acidified to pH 5 to avoid Fe-deficiency (Yoshida et al., 1976). Plants were also sprayed with a foliar feed (Miracle-Gro®, The Scotts Company UK Ltd) at 3.75 g l⁻¹ at panicle emergence. Azucena plants were sprayed with Torque (50 w/w fenbutatin oxide) at 0.5 g l⁻¹ of water to control red spider mites 82 d after sowing (DAS). There were no other major pest or disease problems.

Growth cabinets and temperature treatments

At anthesis, plants were transferred to modified Saxcill growth cabinets (internal size 1.4×1.4×1.5 m). Cabinets were maintained at 360 mmol mol⁻¹ CO₂ of air. The photosynthetic photon flux density (PPFD) at the base of the cabinet was maintained at 650 mmol m⁻² s⁻¹ by a combination of cool white fluorescent tubes and incandescent lamps. A centrally placed fan circulated heat and air uniformly. Screened and aspirated air temperature and RH were measured every 10 s using copper constantan thermocouples and recorded in a data logger (Delta T Devices, Burwell, Cambridge, UK) and averaged over 10 min for the entire period of high temperature exposure.

In 2003 (Experiment 1), growth cabinets were maintained at air temperatures of 30°C (control), 35, and 38°C between 08.00 h and 19.00 h BST. Growth cabinets were not replicated. Vapour pressure deficit (VPD) was maintained at 1.2 kPa in all temperature regimes and therefore RH ranged from 70% to 85%. VPD/RH was controlled either by adding moisture to air passing through glycol maintained at a set temperature or by removing the excess humidity by condensation.

In 2004 (Experiments 2 and 3), growth cabinets were again maintained at 30, 35, and 38°C during the day. In contrast to Experiment 1, growth cabinets were replicated in Experiment 2. RH was held constant at 60% during the day in 2004 to maximize anther dehiscence (Matsui et al., 1999, a, b, 2001; Matsui and Omasa, 2002) and therefore VPD ranged from 1.7 to 2.3 kPa. In addition, spikelet tissue temperature was measured by placing copper constantan thermocouples in spikelets of four different plants per cabinet. Temperatures were recorded by a data logger (Delta T Devices, Burwell, Cambridge, UK) every 10 s and averaged over 10 min for the entire period of high temperature exposure.

Effect of high temperature on spikelet fertility

The effect of temperature and the duration of temperature on spikelet fertility in IR64 and Azucena was examined in Experiments 1 and 2.
In Experiment 1 (2003), on the day after the first anthers were observed, individual plants of IR64 and Azucena were transferred at 08.00 h from the greenhouse to a growth cabinet kept at 30 °C. As IR64 and Azucena flowered at different times, transfers therefore occurred on different days. Transfers were at 10.00 h, to coincide with the period of peak flowering, plants were then transferred to adjacent cabinets at air temperatures of 30 (control), 35, and 38 °C for a 2 h period. A square wave heat treatment was applied to overcome the potentially confounding effects of gradually increasing temperature. At the end of this period plants were returned to the greenhouse. The previous day (after midday) anthers from spikelets at anthesis were carefully removed.

To identify spikelets exposed to high temperature, spikelets that opened (i.e. with a visible anther) during the 2 h temperature treatment were marked with red acrylic paint at the end of the temperature treatment. Most opened spikelets were towards the top of the panicle. Ten to 12 d after the temperature treatments, marked spikelets were scored for spikelet fertility. There were 10 replicate pots in each temperature (growth cabinet) treatment.

In Experiment 2 (2004), plants were again subjected to temperature treatments of 30, 35, and 38 °C in growth cabinets, using the same transfer and spikelet marking system as in Experiment 1. On the day that anthesis was first observed, plants were moved to the control cabinet (30/24 °C). On the following day, plants were subjected to 30, 35, and 38 °C for 1, 2, 4, or 6 h, centred on the peak flowering time of 11.00 to 11.30 h, i.e. plants were transferred at different times of the morning. At the end of the respective temperature treatments, plants were transferred back to the control cabinet, and from there to the greenhouse the following morning. Spikelets reaching anthesis during the high temperature treatments were marked with different coloured acrylic paints for different durations, either at the end of the treatment (1 and 2 h) or in the middle and at the end (4 and 6 h). Plants were removed from the growth cabinets for marking to ensure temperature and RH conditions were maintained at specified levels during the temperature treatments.

Spikelet fertility (seed-set) of the painted spikelets was scored 10–12 d after anthesis. In this experiment temperature treatments (growth cabinets) were replicated twice, and there were five replicate plants per cabinet.

The total numbers of spikelets marked at each temperature by duration treatment for each genotype in Experiments 1 and 2 are given in Table 1.

### Time of spikelet anthesis

The effect of the time of spikelet anthesis relative to the imposition of a high temperature stress was also examined in Experiments 1 and 2. In Experiment 1, spikelets that reached anthesis prior to the 2 h temperature treatment, i.e. between 08.00 h and 10.00 h, were marked with blue paint to distinguish them from those that reached anthesis during the temperature treatment (see above). In Experiment 2, spikelets opening 1 h before and 1 h after the temperature treatment were marked with blue and yellow paint, respectively. Seed-set of these spikelets was scored 10–12 d after anthesis.

### Flowering patterns

The effect of high temperature on flowering patterns was examined in Experiment 3 in 2004. Three replicate plants of each of IR64 and Azucena were transferred from the greenhouse immediately before anthesis to a growth cabinet at either 30/24 °C or 38/24 °C. For three consecutive days, from the start of anthesis, the number of spikelets at anthesis (i.e. anthers extruding or spikelets gaping and anthers visible), were counted every 30 min between 09.00 and 15.00 h BST.

### Statistical analysis

Spikelet fertility (Experiments 1 and 2) was treated as binomial data, there being only two states possible: fertile/seed-set (1) and infertile/no seed-set (0). These binomial data were analysed as logits [log( p/(1−p) )] of the percentages p(0 < p < 100%) by Generalized Linear Mixed Models (GLMM, Genstat version 7.1) using plant as a random factor and the spikelet as the unit of treatment. Hence, the results are expressed taking into account each individual spikelet marked as a data point from all the treatments. The logit results (A) can be back-transformed to the original scale using 1/(1+EXP(−A)), giving predicted probabilities or Odds Ratio of success (OR).

To examine the interaction between temperature and duration of exposure, a threshold temperature response (Vara Prasad et al., 1999) was used, wherein spikelet fertility was reduced in proportion to accumulated temperature above a threshold value. The threshold value was set at 33 °C based on visual observation of the data and following Nakagawa et al. (2002). Accumulated temperature or thermal time (TT) above this threshold was then calculated from day temperature (T) and duration of treatment (t) by:

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TT = (T - 33°C) \times t
\]

using values from 2003 and 2004. Logit spikelet fertility was then regressed against TT.

The total number of anthesing spikelets per day (Experiment 3) was analysed by ANOVA as a completely randomized experiment with three replicates.

### Results

#### Air and spikelet temperature

Mean daily air temperature in the greenhouse during the experimental period was maintained very close to the target temperature of 30/24 °C day/night in 2003 [29.2 (SD 1.23)/24.4 (0.42) °C] and in 2004 [29.3 (1.21)/24.0 (0.40) °C]. However, in 2003 ambient temperatures in the greenhouse reached 38 °C mid-afternoon at 65 and 66 DAS.
Although IR64 flowered <7 d after this peak, there was no spikelet sterility observed in control plants as anthesis occurred during mid-morning, while temperatures were cooler. Azucena, which flowered at 93 DAS, was unaffected by these high ambient temperatures.

Air temperature in the growth cabinets was maintained at 30 (SD 0.13), 35 (0.16), and 38 °C (0.09) during the day in both years. Spikelet temperatures recorded in 2004 were 29.6, 33.7, and 36.2 °C, i.e. 0.4, 1.3, and 1.8 °C below ambient temperatures, similar to differences observed elsewhere in rice (Satake, 1995) and in peanut flowers in the same growth cabinets (Vara Prasad et al., 2001). It was assumed that the difference between spikelet and air temperature was the same in the growth cabinets in 2003 and therefore spikelet (tissue) temperatures are used throughout this paper.

**Effect of temperature on flowering pattern**

The pattern of flowering at 30 °C was similar on the first, second, or third day of flowering in IR64 and Azucena (Fig. 1) although the total number of spikelets at anthesis over the 3 d period was lower, on average, in Azucena (109) than in IR64 (164). Flowering in IR64 at 30 °C started between 09.30 h and 10.00 h, reached at peak at 1100 h and ended by about 1300 h. Azucena started flowering slightly earlier and ended later, but with peak flowering also at 11.00 h.

The pattern of flowering and hence the total number of spikelets that reached anthesis per day (Table 2) differed significantly \( (P <0.01) \) between genotypes and in response to temperature \( (P <0.05) \). In IR64, high temperature resulted in the start, peak, and end of flowering occurring about 30 min earlier in the day, as well as increasing the mean number of spikelets at anthesis per day from 49.7 to 59.3. In Azucena, the timing of flowering was similarly affected, but, in contrast to IR64, the number of spikelets reaching anthesis was significantly reduced, from 44.3 to 28.3 spikelets per day. In IR64, spikelet production at 36.2 °C increased over the 3 d period and hence had a positive effect on the number of potential seed sites; by contrast, spikelet number in Azucena declined over the 3 d period, reducing the potential number of seed sites.

**Effect of time of spikelet anthesis relative to a high temperature episode on spikelet fertility**

In Experiments 1 and 2, spikelets of Azucena and IR64 that had reached anthesis prior to (Experiment 1 and 2), and after (Experiment 2), exposure to temperatures of...
29.6, 33.7, and 36.2 °C for varying durations were marked as well as spikelets that reached anthesis during the treatment. In both experiments and genotypes the spikelet fertility of spikelets that reached anthesis prior to the temperature treatments was affected by the subsequent temperature, and in 2004 by previous temperature, and this is illustrated by IR64 exposed to a 2 h temperature treatment in Experiment 2 (Fig. 2).

In IR64 there were significant effects of temperature ($P<0.001$), time of spikelet anthesis ($P<0.001$) and their interaction ($P<0.05$) on spikelet fertility (logit %). Spikelet fertility in spikelets reaching anthesis during the 2 h temperature treatment decreased linearly from 0.90 to 0.30 OR (i.e. slope $-0.60$ logit % fertility $°C^{-1}$) as temperature increased from 29.6 °C to 36.2 °C. Spikelet fertility in spikelets that reached anthesis 1 h before the temperature treatments were imposed was also affected by subsequent temperature in a similar manner. A comparison of regressions showed these two ‘timings’ had a common slope but different intercepts ($P=0.0018$). Spikelets that reached anthesis after the temperature treatment, however, were not significantly affected by the previous temperature and this regression was significantly different ($P=0.034$) from the other two.

**Interaction between duration of exposure and temperature on spikelet fertility**

In IR64, the logit analysis showed that temperature had a significant ($P<0.001$) effect of spikelet fertility, but there was no effect of duration or a temperature×duration interaction (Fig. 3). By contrast, in Azucena there was a significant effect of temperature ($P<0.001$) and a temperature×duration interaction ($P<0.05$).

In IR64, average spikelet fertility declined from 0.88 to 0.27 OR as temperature increased from 29.6 °C to 36.2 °C, but only from 0.66 to 0.56 OR as duration increased from 1 h to 6 h. A comparison of regressions of logit spikelet fertility against duration confirmed that there was no interaction with duration ($P>0.05$), the sensitivity to duration being $-0.095$ (±0.0266) logit % spikelet fertility $h^{-1}$ at each temperature. A comparison of regressions of logit % spikelet fertility on temperature (not presented) also revealed no effect of duration ($P>0.35$) and a common regression line describing the response to temperature could be fitted, where $y=14.70$ (±1.20)–0.42 (±0.036) logit % spikelet fertility $°C^{-1}$. Thus, in IR64, spikelet sterility increased by 0.09 OR per °C from 29.6 °C to 36.2 °C and there was no interaction with duration of exposure.

Although duration effects were not significant in IR64, it is apparent that fertility did decline with duration and this may be confounded by the number of spikelets exposed to temperature, and hence with escape from the stress. For example, with a 1 h treatment, spikelets that open towards the end of this period will be exposed to less cumulative temperature than those opening at the start, giving some scope for escape. With a 6 h exposure, by contrast, most spikelets will be exposed for at least 1 h and the chance of escape is much less. The relation between logit % spikelet fertility and the proportion of spikelets opening during the temperature treatments was therefore plotted (Fig. 4). In IR64 at 29.6 °C, 76% of spikelets reached anthesis during the 1 h treatment and there was, as expected, no effect of proportion and hence duration on fertility at this non-stress temperature. At 36.2 °C, where only 31% of spikelets had reached anthesis in the 1 h treatment compared with $>90%$ in the 4 h treatment, there was a small but non-significant ($P=0.06$) effect of proportion.

Spikelet fertility in Azucena at 29.6 °C averaged 0.64 OR, similar to 2003 (not presented) and lower than in

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**Table 2. The total number of anthesing spikelets per day, averaged over the first 3 d of flowering, in IR64 and Azucena subjected to day temperatures of 29.6 °C and 36.2 °C; SED: genotype 5.68**; genotype×temperature 8.03**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>IR64</th>
<th>Azucena</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day No. spikelets nearing anthesis</td>
<td>Day No. spikelets nearing anthesis</td>
</tr>
<tr>
<td>29.6</td>
<td>1: 46</td>
<td>1: 37</td>
</tr>
<tr>
<td></td>
<td>2: 47</td>
<td>2: 51</td>
</tr>
<tr>
<td></td>
<td>3: 56</td>
<td>3: 45</td>
</tr>
<tr>
<td>Mean</td>
<td>49.7</td>
<td>44.3</td>
</tr>
<tr>
<td>36.2</td>
<td>1: 38</td>
<td>1: 34</td>
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<tr>
<td></td>
<td>2: 60</td>
<td>2: 30</td>
</tr>
<tr>
<td></td>
<td>3: 80</td>
<td>3: 21</td>
</tr>
<tr>
<td>Mean</td>
<td>59.3</td>
<td>28.3</td>
</tr>
<tr>
<td>Genotype mean</td>
<td>54.5</td>
<td>36.3</td>
</tr>
</tbody>
</table>

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*Fig. 2. Spikelet fertility of spikelets of IR64 exposed to temperatures of 29.6, 33.7, and 36.2 °C for 2 h (solid circles and line), and seed-set of spikelets that opened at 29.6 °C either 1 h before (open circles and hashed line) or 1 h after (solid squares and dotted line) the 2 h treatment. Fitted lines: (open circles) $y=15.43-0.408x$; (filled circles) $y=14.30-0.408x$; (filled squares) $y=3.56-0.062x$.**
IR64. On average, spikelet fertility decreased from 0.64 to 0.08 OR as temperature increased from 29.6 °C to 36.2 °C. At 29.6 °C and 33.7 °C, spikelet fertility in Azucena, like IR64, exhibited little or no response to duration. However, at 36.2 °C, there was a marked effect of duration longer than 2 h and spikelet fertility was reduced to <0.03 OR after 6 h exposure (Fig. 3). A comparison of regressions of logit % spikelet fertility against duration confirmed that the response to duration at 36.2 °C was significantly different \((P < 0.001)\) from that at 29.6 °C and 33.7 °C, which had parallel slopes.

Regressions of IR64 and Azucena were also compared at each temperature to test whether Azucena was more sensitive to higher temperatures than IR64. At 29.6 °C and 33.7 °C there was no difference in the sensitivity of IR64 and Azucena, slopes being 0.008 and -0.052 logit % spikelet fertility h\(^{-1}\), respectively. However, at 36.2 °C slopes were significantly \((P < 0.05)\) different. Azucena was therefore more sensitive to high temperature than IR64.

While the response to temperature in IR64 can be described by a quantitative relation between fertility and spikelet temperature, without interaction with duration, that of Azucena cannot be modelled in this way. It was therefore examined whether the response of Azucena in 2003 and 2004 could be described by a cumulative temperature response above a threshold value, a common form of stress response used in crop models (Fig. 5). There was a strong negative relation \((r^2 = 0.86)\) between logit % spikelet fertility and accumulated hourly spikelet temperature above a threshold of 33 °C, suggesting that the interaction between temperature and duration can be modelled in this way.

**Discussion**

Increasing the heat tolerance of rice at flowering, one of the most sensitive stages of development to stress, is a vital adaptation strategy for variable and warmer climates (Horie *et al.*, 1996). Tolerance classically comprises elements of escape, i.e. the timing of panicle emergence and spikelet/floret opening relative to the occurrence of the stress, and the absolute tolerance to stress of key processes, such as anther dehiscence. It is essential for phenotyping and modelling that these two mechanisms, as well as the effects of temperature on the rate of spikelet anthesis, are clearly differentiated.

Flowering and anthesis in most *O. sativa* genotypes of rice occurs over a 5 d period, with most spikelets reaching anthesis between 10.00 h and 12.00 h (Fig. 1; Nishiyama and Blanco, 1980; Prasad *et al.*, 2006). Although *indica* spp and *japonica* spp have a similar pattern of flowering, it is worth noting that *O. glaberrima* genotypes flower much earlier in the day, with >90% spikelets nearing anthesis by 09.00 h (Nishiyama and Blanco, 1980; Prasad *et al.*, 2006). This is a potentially useful escape mechanism that should be incorporated into *O. sativa* now that...
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Fig. 5. Relationship between spikelet fertility and accumulated hourly temperature >33 °C in Azucena. Key: open symbols=33.7 °C; closed symbols=36.2 °C; 2003 (closed diamonds, open diamonds); 2004–duration: (open circles), 1 h; (open squares), 2 h; (open triangles), 4 h; (open inverted triangles), 6 h. Fitted line: \( y = \frac{-6.50}{1.67x} \), \( r^2=0.88 \).

interspecific crosses can be made (Jones et al., 1997). For phenotyping for heat tolerance, it is clearly essential that high temperature (or other stresses) are timed to coincide with this peak and that escape is taken into account, as discussed later.

In both IR64 and Azucena, the peak of flowering occurred about 30–45 min earlier at 36.2 °C, compared with 29.6 °C, presumably reflecting a simple thermal response of overall rate of development (progress towards anthesis) to spikelet (nb. not ambient) temperature whose optimum is apparently \( \geq 36.2 \) °C. In IR64, rates of spikelet opening were apparently also increased by temperature, and over 3 d about 20% more spikelets reached anthesis at 36.2 °C. By contrast, high temperature reduced the number of spikelets opening by 36% in Azucena, the effect increasing in severity over the three days. So although the overall pattern or timing of flowering was not adversely affected by temperature in Azucena, the number of potential seed sites was greatly reduced. Matsui et al. (2000) have shown that the sterility of japonica spp increases with increasing duration (days) of exposure during flowering, in contrast to indica spp where fertility was similar throughout the flowering period. These results agree with this, but suggest this is due as much to effects of temperature on the number of spikelets reaching anthesis as fertility, with increased rates of spikelet anthesis in the indica IR64 compensating for reduced fertility. The effects of high temperature on spikelet anthesis, both pattern and rate, are clearly important factors to be considered in phenotyping for heat tolerance, and not just fertility per se.

One hour at a spikelet tissue temperature of \( \geq 33.7 \) °C was sufficient to cause sterility if this coincided with anthesis in both IR64 and Azucena, in agreement with previous studies (Satake and Yoshida, 1978). Sterility in rice is invariably associated with low numbers of pollen or germinated pollen on the stigma (Matsui et al., 2000, 2001; Prasad et al., 2006; Farrell et al., 2006). Anther dehiscence is acutely sensitive to high temperature, both prior to and during anthesis (Matsui et al., 2001). Rice florets open for about 30 min (Ekanyake et al., 1989), exposing anthers and pollen to ambient temperature and humidity. Pollen is also acutely sensitive and loses its viability within 10 min (Song et al., 2001). After pollination it takes about 30 min for the pollen tube to reach the embryo sac (Cho, 1956) and it is very likely that very high temperatures will also influence pollen germination and pollen tube growth (cf Kakani et al., 2002, in peanut). Nonetheless, poor anther dehiscence is apparently the main cause of sterility at high temperature in rice.

Although 1 h at \( \geq 33.7 \) °C was sufficient to induce sterility, it is apparent that shorter periods than this may also affect spikelet fertility. From the marking studies, spikelets that opened in the hour before, and to a lesser extent the hour after, exposure to high temperature were affected as well. This probably reflects the fact that spikelets opening 30 min before high temperature, for example, will also experience 30 min at high temperature, seemingly enough to reduce spikelet fertility to some extent. The timing of anthesis is clearly important for phenotyping for heat tolerance in terms of spikelet fertility or sterility; even with spikelet marking, short periods of high temperature will still include an element of escape. The small effect of duration on spikelet fertility in IR64 (Fig. 3) was interpreted to be due to more spikelets escaping heat stress in shorter than longer duration treatments (Fig. 4), giving a higher apparent fertility.

In these experiments, plants were transferred to growth cabinets at the target temperature and spikelets were therefore given no opportunity to acclimate. However, given the very short period of exposure required to induce sterility, it is unlikely that acclimation would occur. Furthermore, under natural conditions, where temperature increases gradually during the morning, escape rather than acclimation is likely to be more significant.

IR64 and Azucena responded differently to varying temperatures and durations of temperature. In Azucena, which was clearly very susceptible to temperatures >29.6 °C, there was a significant temperature\( \times \)duration interaction, which could be quantified by a cumulative temperature response above a threshold temperature of 33 °C. Vara Prasad et al. (1999) reported a similar response in ground-nut. Although the relationship was strong (\( r^2=0.86 \)), the two temperature cohorts (33.7 °C and 36.2 °C) only overlap slightly and further data that create a wider range of accumulated temperatures are needed to confirm this response. By contrast, there was no interaction in IR64 such that a temperature stress of \( \geq 1 \) h reduced spikelet fertility by \( 9\% \ \text{°C}^{-1} \). Yoshida et al. (1981) previously described the effect of varying duration and temperature on seed-set in several genotypes, including N22 and
IR747B, both indica spp. These data were re-analysed and no significant temperature x duration interactions were found (data not presented). However, given that Matsui and Omasa (2002) have shown that a japonica sp., Akitokomachi is also highly tolerant, it is unlikely that the difference observed between IR64 and Azucena is subspecies-dependent. Although heat stress primarily affects anther dehiscence (Matsui et al., 2000, 2001; Prasad et al., 2006), it is possible that, in susceptible genotypes such as Azucena, that a number of other processes before fertilization are affected. For example, pollen swelling, anther pore size (Matsui and Kagata, 2003), and pollen stickiness may all be affected, as might pollen germination and the rate of pollen tube growth (Kakani et al., 2002). Farrell et al. (2006) have also shown that cold tolerance in rice is related to stigma size, as well as anther size. It is unlikely that processes after fertilization are affected as spikelets exposed to high temperature 1 h after opening are fertile and set seed in Azucena as well as IR64. Some of these factors are being investigated.

In conclusion, this study has shown that ≤1 h of exposure to high temperature is sufficient to induce sterility in rice (O. sativa). IR64 and Azucena exhibited different sensitivities and responses to temperature of spikelet fertility. Nonetheless, phenotyping of RILs and breeding lines of tolerant and susceptible types can be effectively carried out by exposing plants to high temperature (>33.7 °C) for ≥4 h, centred around the hours of maximum anthesis. This will ensure that >90% of spikelets opening experience high temperature, effectively removing ‘escape’ as a potential confounding factor. This study has also shown that high temperature affects the pattern of flowering and the number of spikelets that reach anthesis, and phenotyping needs to include these factors as well.

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