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Temporal Sensitivities of Rice Seed Development from Spikelet Fertility to Viable Mature Seed to Extreme-Temperature

Maite Martínez-Eixarch[★] and Richard H. Ellis

ABSTRACT

Extreme temperature during reproductive development affects rice (*Oryza sativa* L.) yield and seed quality. A controlled-environment reciprocal-transfer experiment was designed where plants from two japonica cultivars were grown at 28 and 24°C (day and night temperatures) and moved to 18 and 14°C and vice versa or from 28 and 24° to 38 and 34°C and vice versa, for 7-d periods to determine the respective temporal pattern of sensitivity of spikelet fertility, yield, and seed viability to each temperature extreme. Spikelet fertility and seed yield per panicle were severely reduced by extreme temperature in the 14-d period before anthesis; both cultivars were affected at 38 and 34°C while only Gleva was affected at 18 and 14°C. The damage was greater the earlier the panicles were stressed within this period. Later-exserted panicles compensated only partly for yield loss. Seed viability was significantly reduced by 7-d exposure to 38 and 34°C or 18 and 14°C at 1 to 7 d and 1 to 14 d after anthesis, respectively, in Gleva. Taipei 309 was not affected by 7-d exposure at 18 and 14°C; no consistent temporal pattern of sensitivity was evident at 38 and 34°C. Hence, *brief* exposure to low or high temperature was most damaging to spikelet fertility and yield 14 to 7 d before anthesis, coinciding with microsporogenesis; it was almost as damaging around anthesis. Seed viability was most vulnerable to low or high temperature in the 7 or 14 d after anthesis, when histodifferentiation occurs.

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Abbreviations: DAA, days after anthesis; DAS, days after sowing.

RICE, the world's most important staple food crop feeding some 2.7 billion people (Fairhurst and Dobermann, 2002), is grown in diverse agroclimatic zones from temperate through subtropical to tropical regions, temperature being the limiting factor to cultivation (DeDatta, 1981), with critical limits ranging from 12 to 20°C to 34 to 38°C (Yoshida, 1981). Productivity is vulnerable to warming temperatures in tropical (Wassmann et al., 2009) and perhaps also subtropical and temperate (Teixeira et al., 2013) rice-growing regions. Short periods of high temperature around anthesis reduce spikelet fertility in rice and hence subsequent grain yield, with high temperature thresholds of around 35 to 38.5°C depending on cultivar (Jagadish et al., 2008; Yoshida, 1981). Low temperatures constrain rice production in temperate areas and cause annual yield losses of 1 to 3.9 t ha⁻¹ (Jena and Hardy, 2012). Rice is vulnerable to low temperature during the reproductive stage, with critical temperatures for spikelet fertility ranging from 12 to 20°C (Yoshida, 1981). Rice seed quality is also sensitive to high temperature and, in japonica rice at least, this sensitivity to temperature is greater than that for grain filling (Ellis and Hong, 1994; Ellis et al., 1993).

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Climate change is projected to increase mean global surface temperature by 0.6 to 4°C by 2100 (Intergovernmental Panel on Climate Change, 2007) and increase temperature variability (Tebaldi et al., 2006), with more frequent extreme events (Intergovernmental Panel on Climate Change, 2012). Moreover, other environmental pressures and population growth are likely to extend rice production into a wider range of environments than today. Cereal grain yields are particularly sensitive to even brief periods of extreme temperature if they coincide with vulnerable stages of early reproductive development (Wheeler et al., 2000). Short-duration exposures to high temperature have identified the periods about 10 d before anthesis, i.e., at microsporogenesis, and at anthesis as being the most sensitive for spikelet fertility in rice (Satake and Yoshida, 1978). Subsequent research has confirmed the susceptibility of spikelet fertility in rice to either extreme low temperature during panicle development and anthesis (Farrell et al., 2006; Gunawardena et al., 2003; Heenan and Lewin, 1982; Oliver et al., 2005; Pereira da Cruz et al., 2006; Shimono et al., 2007) or high temperatures around anthesis (Jagadish et al., 2007; Matsui et al., 2001). High temperature later in reproductive development can also affect seed production (Tashiro and Wardlaw, 1991b), but there are few studies on the effects of brief exposure to low temperature.

High quality seeds are required to establish rice crops with high yield potential. The impact of extreme temperature on rice seed quality has received less attention than that on yield. Studies at high temperature throughout seed development and maturation detected poor seed quality in japonica rice which did not become evident until seed filling had ended (Ellis et al., 1993; Ellis and Hong 1994), but a subsequent study with high temperature applied during maturation only (i.e., after seed filling had ended) suggested that developing seeds may be more vulnerable than maturing seeds to high-temperature stress affecting seed quality (Ellis, 2011).

Improved understanding of the temporal pattern of sensitivity of the development of grain yield and of seed quality to extreme temperature is of high relevance to crop modeling in future climates and in assessing the various options to mitigate the effect of stress on crop production through improved agronomy and by plant breeding. Indeed, Wheeler and von Braun (2013) have highlighted the importance of investment in “climate-smart agriculture” as one component of improved agricultural resilience to aid progress towards global food security.

We report the results from a study of the effects of low- and high-temperature stress applied at different stages of rice seed development on fertility, yield, and seed viability in two contrasting japonica cultivars (one early and one late maturity from Europe and Asia, respectively). We test the null hypothesis that in both cultivars

all three factors are equally sensitive to either extreme low or extreme high temperature throughout flowering and seed development by employing the reciprocal transfer of plants among different temperature regimes.

MATERIALS AND METHODS

Cultivars and Temperature Treatments

Two cultivars of japonica rice with different maturity and geographic origin were selected for these experiments. Gleva is an early-maturing cultivar from Europe (seeds provided by the Institute for Food and Agricultural Research and Technology, Catalonia) and Taipei 309 is a late-maturing cultivar from Asia (seeds provided by the International Rice Research Institute, the Philippines).

Before the experiment began until emergence of last leaf, plants were grown from seed in a greenhouse at 28 and 20°C day and night (11 and 13h) with an 11 h d⁻¹ photoperiod. The plants were then moved to controlled-environment growth cabinets to begin the experiment in one of three regimes: 18 and 14°C, 28 and 24°C, or 38 and 34°C, all with a photoperiod of 11 h d⁻¹ and synchronous 11 and 13h thermoperiod. The experiment comprised a reciprocal-transfer design (e.g., Ellis et al., 1992). In this design, plants are moved for a set period (in this case 7 d) between two regimes, returned, and left at the end of this period to their respective original regimes and then a different set of plants is transferred for the next (7-d) period and so on, providing a set of serial transfers. Here, 28 and 24°C provided a “normal” regime with reciprocal transfers (of different plants) to and from a cooler regime of 18 and 14°C or to and from a warmer regime of 38 and 34°C. The serial, reciprocal transfers occurred at 7-d intervals from around 14 d before anthesis to 28 d after anthesis (DAA) (i.e., a total of six different transfer periods). Transfer from 28 and 24°C to either 38 and 34°C or 18 and 14°C for 7 d represented a short-term temperature stress, while a 7-d transfer from either 38 and 34°C or 18 and 14°C to 28 and 24°C represented a brief respite from long-term temperature stress. Control plants were held throughout the experimental period, from 14 d before anthesis onward, in the same temperature: 28 and 24°C represented control for short-term temperature stress, whereas 38 and 34°C and 18 and 14°C represented control for long-term high- and low-temperature stress, respectively. Each reciprocal-transfer treatment combination was represented by four pots, whereas each set of control plants, at either 28 and 24°C, 18 and 14°C, or 38 and 34°C, comprised eight pots.

Plants were monitored three times a week during the experiment and anthesis date recorded separately for each panicle. Panicle anthesis was designated here as first florets in the panicle. Panicles in a single pot reached anthesis on different dates. Hence, the results shown are calculated from individual panicle results averaged in terms of the timing of transfer treatment relative to the date of panicle anthesis. That is (unless otherwise stated), the results were not averaged within pot level because this would have led to considerable imprecision with regard to the timing of anthesis.

Extreme temperatures were selected according to temperature-stress events currently occurring in some rice production areas such as Taiwan, Japan (extreme low temperature), and

Spain (extreme high temperature) to provide realistic 7-d exposure to temperature stress.

The six serial 7-d treatments were provided to enable temporal variation in sensitivity to be detected, while nonetheless enabling plants to survive. The extreme regimes were also selected to investigate the effects of future—and potentially more variable—climates. These include the special problems for rice yield of disproportionately warmer night temperatures consequent to climate change (Peng et al., 2004). Continuous extreme temperature regimes provided throughout the experiment, however, are unrealistic. In such climates, farmers would select other ecogeographic rice types or indeed other crops. They do, however, serve a comparative role to evaluate treatment effects whereby the effects of a 7-d escape from temperature stress can be investigated.

Plant Growth

Plants were grown in pots at the Plant Environment Laboratory, University of Reading, UK (51°27' N, 00°56' W) until emergence of last leaf in a controlled-environment, naturally-lit greenhouse with dark compartments for simulating night conditions. The pots were placed on trolleys which were drawn out from and into night (dark) compartments to provide a short, inductive photoperiod of 11 h d⁻¹. Temperature was maintained at 28 and 20°C day and night (11 and 13h).

Seven seeds were sown into a soilless medium comprising steam-sterilized sand and gravel mixed with peat compost and vermiculite (2:4:1:4) within each 18-cm diameter, 3-L pot on 21 April 2011. Slow-release fertilizer (NPK 17–11–10) was added to the medium at 3 kg m⁻³ and pots were irrigated 3 times a day for 3 min and maintained at pH 4.8 to avoid iron deficiency. Plants were sprayed weekly with a foliar feed (Miracle-Gro, The Scotts Company UK Ltd, Godalming, Surrey, UK) at 3.75 g L⁻¹ and boron (H₃BO₃) at 0.8 g L⁻¹. Red spider mites were controlled by applications of Savona (an insecticidal soap containing 50% w/w potassium salts of fatty acids) and also by biological control (*Phytoseiulus persimilis*).

Seedlings were thinned to four per pot 20 d after sowing (DAS). Plants were moved to four modified Saxil growth cabinets (internal dimensions 1.4 by 1.4 by 1.5 m) for the reciprocal-transfer experiment which began close to the end of the vegetative phase. Carbon dioxide was maintained at 385 μmol mol⁻¹ of air and relative humidity at 60 ± 5% by day and 80 ± 5% at night. A combination of cool white fluorescent tubes and incandescent lamps provided a photosynthetic photon flux density of 650 μmol m⁻² s⁻¹.

Measurements and Data Collection

Leaf stage was monitored weekly (Haun, 1973) to estimate progress towards anthesis and phenology monitored throughout the reproductive stage until maturity: date of anthesis and start and end of seed filling were recorded for each panicle individually by labeling each tiller, including the sequence of panicle exertion within each plant. The start and end of seed filling were estimated nondestructively by recording when the expansion of spikelet(s) in each panicle was first seen and 90% of the seeds in the panicle became yellow, respectively. After the last transfer treatment, emergence of new tillers was observed. These tillers were allowed to grow and their yield was

determined to evaluate the plant's capacity to compensate for possible yield loss due to stress. Panicles were cut individually from plants after the end of seed filling close to harvest maturity and total number of florets per panicle were recorded and then air dried enclosed in a paper envelope with further drying at laboratory temperature (about 7 d at 20°C). Each panicle was gently threshed and empty seed separated and discarded. The filled seed in each panicle were counted, weighed, and all seeds from the panicle tested for germination between moist rolled paper towels in an alternating-temperature regime of 34 and 11°C (16 and 8h) for 28 d to maximize the germination of dormant and nondormant seeds (Ellis et al., 1983). The progress of radicle emergence was monitored weekly and nongerminated fresh seeds were pricked with a needle at 21 d to aid loss of dormancy. Seedlings were classified (International Seed Testing Association, 2005) at the end of the test and ungerminated seeds classified as fresh (firm) or dead.

Statistical Analyses

The experiment was a split-plot design where the main factor was temperature regime and the subfactor was timing of transfer to either extreme (18 and 14°C or 38 and 34°C) or to optimum (28 and 24°C) temperatures for short- or long-term temperature stress, respectively. The timing of transfer was for pots, with four plants and many more panicles which anthesed at different times. Hence, the transfer time relative to anthesis for each panicle (the unit of observation) was determined individually after monitoring date of anthesis of each panicle and pots comprised a population of tillers representing several different treatment timings.

The data were therefore unbalanced and the experimental units (pots) contained different observational units (panicles) so that the condition of independence of errors could not be assumed. Hence, a mixed model approach (Piepho et al., 2003) was used, with the repeated measures procedure to analyze the significance of fixed effects because of adjacent observations within one experimental unit and the possibility of correlation among them (Piepho et al., 2004). Restricted maximum likelihood was applied to estimate the variance of the components and provide LSD and the Adjusted Tukey-Kramer tests for means and pairwise-means comparison, respectively. SAS 9.2 (SAS Institute, 2008) was used for all analyses.

RESULTS

The treatments affected phenology. Anthesis occurred at 75 to 90 DAS in Gleva and peaked at 75 to 80 DAS for plants continuously grown at 28 and 24°C or for those exposed for a 7 d period to 18 and 14°C within the reproductive phase. Plants exposed for a 7-d period to 38 and 34°C within the reproductive phase peaked at 75 to 90 DAS. Fewer tillers anthesed with long exposures to extreme temperatures; peak anthesis occurred in a limited number of these tillers at about 90 or 70 DAS at 18 and 14°C or 38 and 34°C, respectively. Taipei 309, which has longer plant-growth duration, showed greater variability when exposed to extreme temperatures. Anthesis occurred from 145 to 175 DAS when plants were grown at 28 and 24°C with or without 7 d at 18 and 14°C (peak 165

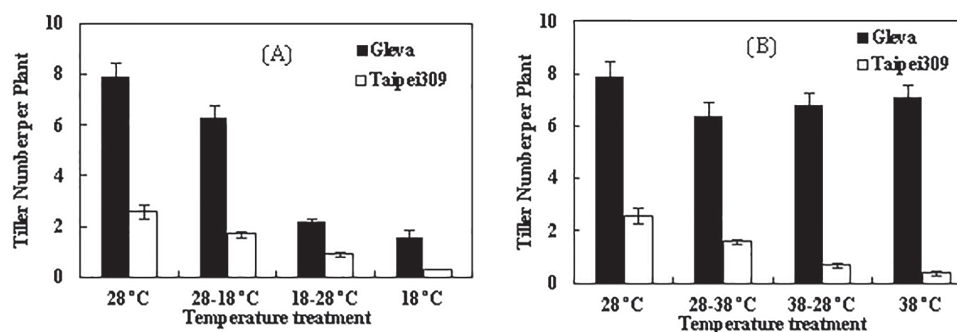


Figure 1. Mean number of tillers per rice plant (fertile and nonfertile) at harvest for rice plants of cultivars Gleva and Taipei 309 cultivated in growth chambers during 2011 at the University of Reading, UK. Plants were exposed to extremes of (A) 18 and 14°C (A) or (B) 38 and 34°C for different periods during reproductive development: 18 and 14°C throughout (18°C), 28 and 24°C throughout (28°C), 38 and 34°C throughout (38°C), 18 and 14°C interrupted by 7-d periods at 28 and 24°C (18–28°C), 28 and 24°C interrupted by 7-d periods at 18 and 14°C (28–18°C), 38 and 34°C interrupted by 7-d periods at 28 and 24°C (38–28°C), or 28 and 24°C interrupted by 7-d periods at 38 and 34°C (28–38°C). Results for 18 to 28°C, 28 to 18°C, 38 to 28°C, and 28 to 38°C are means of the six different timings of transfers.

DAS), but this range increased to 125 to 185 DAS when plants were exposed to 7 d at 38 and 34°C. Long exposures (i.e., only 7 d at 28 and 24°C) to 18 and 14°C extended this period (145 to 205 DAS), whereas long exposures to 38 and 34°C advanced anthesis (125 to 165 DAS).

The total number of tillers per plant at maturity differed considerably among treatments ($P < 0.0001$) in both cultivars in response to exposure to extreme temperatures (Fig. 1). These numbers were reduced greatly in both cultivars the longer plants were exposed to 18 and 14°C compared with the control at 28 and 24°C. Similar reductions occurred when plants were exposed to 38 and 34°C in Taipei 309, whereas in Gleva the reduction was not significant.

Fertility

Seed set was reduced by certain treatments at 18 and 14°C or 38 and 34°C (Fig. 2). The mean spikelet fertility in panicles of Gleva and Taipei 309 maintained at 28 and 24°C was $51.8 \pm 4.8\%$ and $88.1 \pm 4.8\%$, respectively (Fig. 2A, B). Panicles exposed continuously to 18 and 14 or 38 and 34°C from 14 d before until 28 d after anthesis produced few or no fertile spikelets (Fig. 2C, D).

The sensitivity of plants to temperature stress varied with their stage of development; timing of exposure to short-term (7d) temperature stress affected spikelet fertility ($P < 0.0001$). Seven days at low (18 and 14°C) or high temperature (38 and 34°C) from 7 and 14 d before anthesis reduced spikelet fertility considerably in both cultivars (Fig. 2A, B). Plants from both cultivars were more susceptible to high than to low temperature stress and Gleva plants exposed to 18 and 14°C before anthesis had lower spikelet fertility than Taipei 309. Seven-day exposures to 18 and 14°C or 38 and 34°C at a later developmental stage did not reduce spikelet fertility in panicles from either cultivar.

Limited spikelet fertility was detected when long-term exposure to 18 and 14°C and to 38 and 34°C was interrupted by 7 d at 28 and 24°C (Fig. 2C, D). Surprisingly, spikelet fertility increased in a limited number of

panicles of Taipei 309 when plants grown in long-term high temperature were exposed to 28 and 24°C for 7 d at 15 to 21 and 22 to 28 DAA. In Gleva, more fertile spikelets were detected when long exposure to 18 and 14 or 38°C and 34°C was interrupted by 7 d at 28 and 24°C immediately before anthesis than other transfers (Fig. 2C, D). This timing of a positive effect (i.e., release from temperature stress) coincided with that for the negative effect in the opposite treatments (i.e., imposition of temperature stress) where the same timing of 7-d exposure to 18 and 14°C or 38 and 34°C resulted in low fertility (Fig. 2A, B).

A brief exposure to 18 and 14°C reduced spikelet fertility in Gleva plants while Taipei 309 was unaffected (Fig. 2A). The effect of plant exposure to temperature stress between the two cultivars was complex. The proportion of panicles which reached anthesis (anther dehiscence detected) at 18 and 14°C was much greater in Gleva than Taipei 309 (data not shown). However, Taipei 309 plants produced a greater number of fertile spikelets.

Panicles were monitored individually within pots. Figure 3 shows the relationship between percentage spikelet fertility and date of anthesis of individual panicles relative to the date when the 7-d stress treatments began. In both cultivars, there were few fertile spikelets if the high temperature treatment of 38 and 34°C began 7 to 14 d before the anthesis. However, a trend of increasing spikelet fertility was detected the later the exposure to 38 and 34°C; no reduction in fertility was observed with exposures beginning 5 d after anthesis or later (Fig. 3B). At low temperature stress of 18 and 14°C, the results were more variable (Fig. 3A). The response to low temperature treatment in Gleva was similar to that at high temperature treatment, while spikelet fertility in Taipei 309 fertility was less affected at 18 and 14°C than at 38 and 34°C.

Seed Yield per Panicle

Seed yield per panicle (Fig. 4A, B) was affected by 7-d periods at 18 and 14°C or 38 and 34°C, following the same

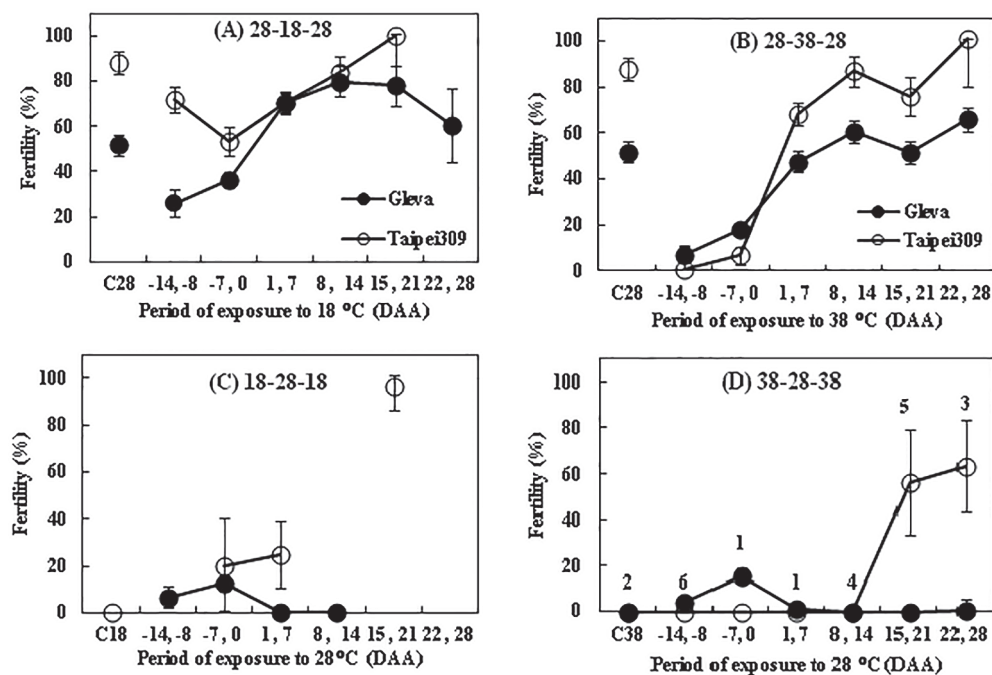


Figure 2. Spikelet fertility of panicles of rice plants cultivated in growth chambers during 2011 at the University of Reading, UK under a brief (A) low- or (B) high-temperature stress or a long (C) low- or (D) high-temperature stress during reproductive development in cultivars Gleva and Taipei 309. Note that -14, -8 days after anthesis (DAA) indicates that anthesis of individual panicles exposed to treatment occurred 14 to 8 d after treatment, C18, C28, and C38 are control treatments maintained at 18 and 14, 28 and 24, or 38 and 34°C throughout plant growth. Observations (mean \pm S.E.) are for all panicles reaching anthesis in the appropriate 7-d period (cf. Fig. 3). Observations were not limited to the first panicle to appear on a plant: results are reported for panicles that were the first, second, and occasionally the third to exsert within a plant. The number of fertile panicles under long-term temperature stress is shown for Taipei 309 (C, D) observations because numbers were low. Many more fertile panicles were produced in Gleva (>25 in each treatment combination).

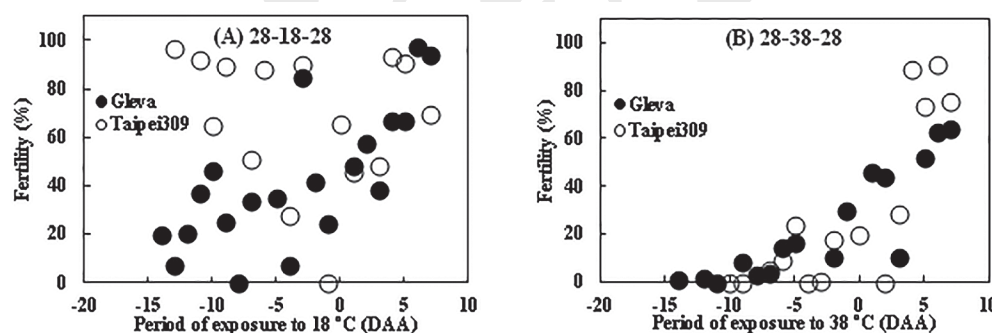


Figure 3. Spikelet fertility of rice panicles cultivated in growth chambers during 2011 at the University of Reading, UK under short-term (7d) (A) low- or (B) high-temperature stress in cultivars Gleva and Taipei 309 relative to the date of anthesis of individual panicles from the date when the treatment began. Points on the graph represent spikelet fertility of individual rice panicles. DAA, days after anthesis.

pattern as spikelet fertility (Fig. 2A, B). Short-term low-temperature stress had little effect on seed yield per panicle in Taipei 309, save for the unusually high value for 15 to 21 DAA. In contrast, reduced or increased yield ($P < 0.05$) resulted in Gleva from exposure to 18 and 14°C before or after anthesis, respectively, relative to control treatment (28 and 24°C) (Fig. 4A). The results for 7 d at 38 and 34°C showed a more consistent temporal pattern of sensitivity in both cultivars (Fig. 4B): 7 d at 38 and 34°C before anthesis reduced yield ($P < 0.05$) substantially, by 69 and 79% in Gleva and Taipei 309, respectively, whereas later

treatments provided similar yield to those from controls. The 7-d treatments at 38 and 34°C during the seed development phase increased seed filling durations ($P < 0.05$) in both cultivars by 4 to 6 d: from 20.6 ± 1.2 and 19.2 ± 1.6 d in the 28 and 24°C controls for Gleva and Taipei 309, respectively, to 24.6 ± 0.7 and 24.9 ± 1.0 d.

Negligible seed yields were obtained when long-duration low- or high-temperature stress was applied (Fig. 4C, D). However, 2 or 3 panicles among all panicles exposed to long-term temperature stress did provide higher seed yields in Taipei 309 for the later transfers to 28 and 24°C

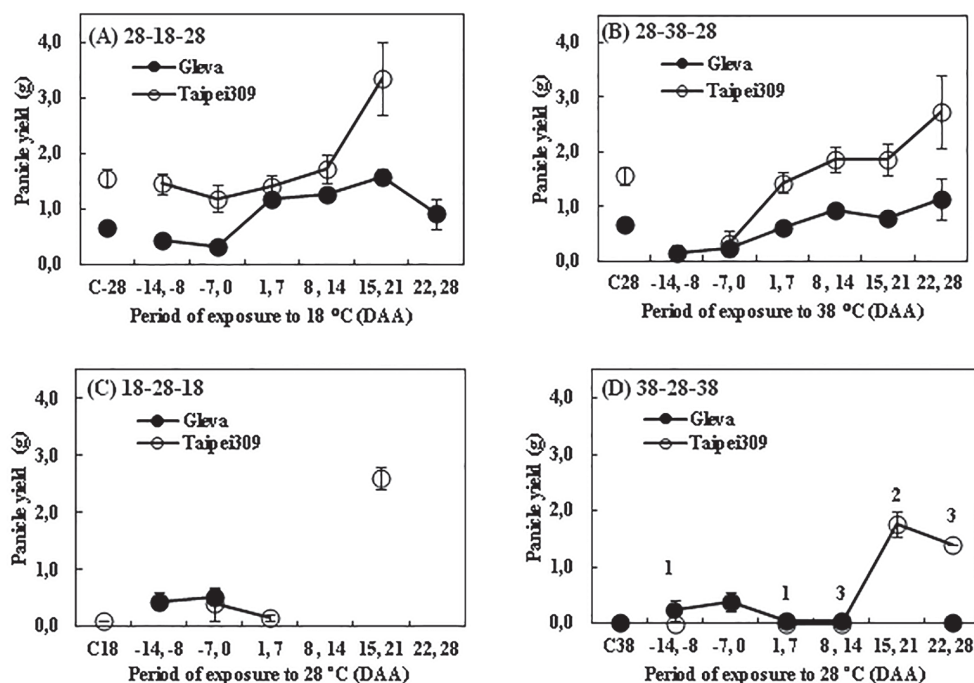


Figure 4. Seed yield per panicle (g at about 15% moisture content) of rice cultivated in growth chambers during 2011 at the University of Reading, UK under short-term (A) low- or (B) high-temperature stress or long-term (C) low- or (D) high-temperature stress at different times relative to anthesis in cultivars Gleva and Taipei 309. Note that -14, -8 days after anthesis (DAA) indicates that anthesis of individual panicles exposed to a treatment occurred 14 to 8 d after treatment. C18, C28, and C38 are control treatments maintained at 18 and 14, 28 and 24, or 38 and 34°C throughout plant growth. Observations (mean \pm S.E.) are for all panicles reaching anthesis in the appropriate 7-d period. The absence of a symbol represents no panicle, no fertile spikelet, or panicles failed to produce a mature seed. Observations were not limited to the first panicle to appear on a plant: results are reported for panicles that were the first, second, and occasionally the third to exert within a plant.

(15–21 DAA from 18 and 14°C; 15–21 and 22–28 DAA from 38 and 34°C) following the same temporal patterns of sensitivity observed for fertility (Fig. 2C, D).

Seed Yield per Pot

Many tillers emerged and reached anthesis from the plants after the 7-d stress treatments. Hence, pots also provided grains produced by panicles exerted after the temperature stress treatment. Gleva showed reduced seed yield per pot following 7-d temperature stress, except for the late exposures (8–14 and 15–21 DAA) to 18 and 14°C (Fig. 5A), whereas the yield reduction was more consistent across all dates when plants were transferred to 38 and 34°C (Fig. 5B). Tillers emerged after the temperature-stress treatments contributed in high proportion of seed yield per pot in those treatments where the earlier panicles had been exposed to extreme temperatures before anthesis (Fig. 5), especially under heat stress in which relative yield reduction was less per pot (45%) than for stressed panicles alone (69%). Despite this contribution from late tillers, seed yield per pot was substantially reduced when the majority of panicles were exposed for 7 d to 18 and 14°C up to 7 DAA or exposed to 38 and 34°C at any time up to 21 DAA.

Seed Viability

Within Gleva, time of transfer from 28 and 24 to 18 and 14 (Fig. 6A) or 38 and 34°C (Fig. 6C) affected seed viability ($P < 0.01$): panicles exposed for 7 d to temperature stress at 1 to 7 or 8 to 14 DAA showed a greater proportion of dead seeds ($P < 0.001$ or $P = 0.051$, respectively) than the 28 and 24°C control. A clear, smooth temporal pattern of sensitivity was apparent for 7 d at 38 and 34°C: the proportion of dead seeds was greater the later the transfer occurred until 1 to 7 DAA, with successively lower proportions of dead seed thereafter (Fig. 6C). Overall, fewer dead seeds resulted when plants were transferred to 18 and 14°C than to 38 and 34°C ($P < 0.05$). The temporal pattern of sensitivity at 18 and 14°C was more abrupt: a greater proportion of dead seeds were observed when plants were transferred to the low temperature in the 14 d immediately after anthesis. The number of dead seeds observed for the remaining transfer dates was broadly similar to the continuous 28 and 24°C control (Fig. 6A).

There were fewer dead seeds in Taipei 309 and there were no significant effects when plants were transferred to 18 and 14°C for 7 d (Fig. 6B). However, seeds were sensitive to high-temperature stress ($P < 0.05$) throughout the seed development stage (Fig. 6D), showing progressive decline of seed mortality from 7-d preanthesis to 14 DAA, followed by a large increase for 15 to 21 DAA. The latter

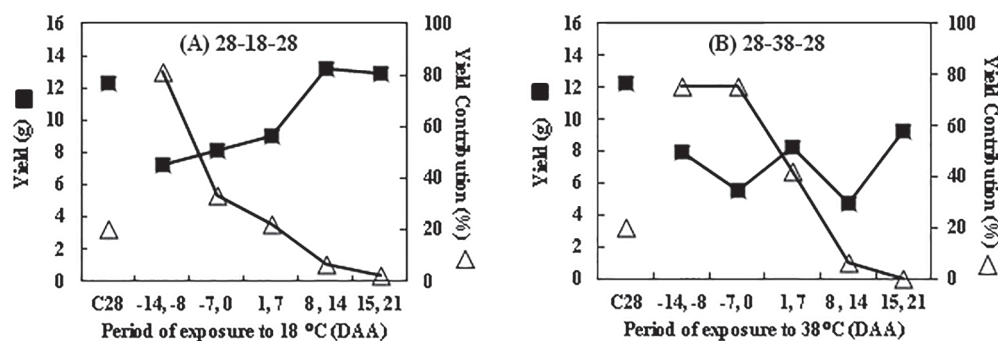


Figure 5. Seed yield per pot (g at about 15% moisture content; 4 plants pot⁻¹) of rice (cultivar Gleva) cultivated in growth chambers during 2011 at the University of Reading, UK for plants in which some panicles were subject to short-term (A) low- or (B) high-temperature stress at different times relative to anthesis and others emerged later (those which reached anthesis 10 d after the end of the last transfer treatment) and the relative contribution of late tillers to yield. C28 represents control treatments maintained at 28 and 24°C throughout plant growth. DAA, days after anthesis

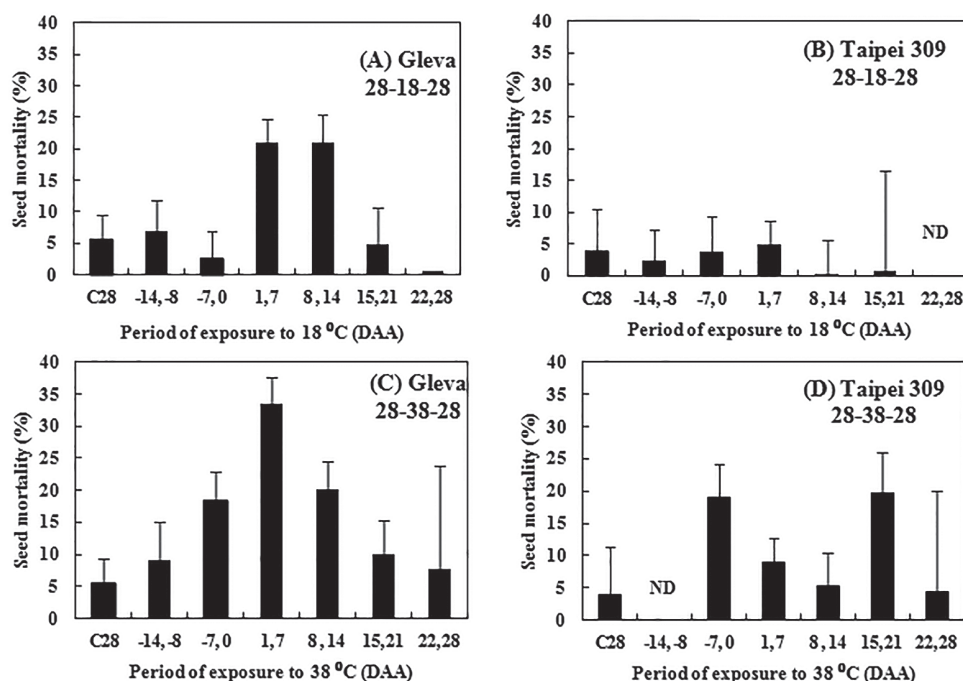


Figure 6. Seed mortality (percentage of dead seeds identified in germination tests of mature seeds) of rice cultivated in growth chambers during 2011 at the University of Reading, UK following short-term (A, B) low- or (C, D) high-temperature stress at different times relative to anthesis in rice cultivars (A, C) Gleva and (B, D) Taipei 309. Observations (mean \pm S.E.) are for all panicles reaching anthesis in the appropriate 7-d period. ND, no data (panicles did not produce mature seed). Observations were not limited to the first panicle to appear on a plant: results are reported for panicles that were the first, second, and occasionally the third to exert within a plant. C28 represents control treatments maintained at 28 and 24°C. DAA, days after anthesis.

may be due to experimental error given the small sample size (seven panicles).

Long exposure to either 18 and 14°C or 38 and 34°C resulted in few fertile panicles with seeds in both cultivars and, consequently, it was difficult to detect any trends. However, on the few occasions when seeds were produced, the percentage of dead seeds was large (Fig. 7). Nevertheless, we observed that plants exposed to long-term temperature stress during reproductive development produced some viable seeds, especially when plants from Taipei at 38 and 34°C were exposed to 28 and 24°C for 7 d between 1 and 28 DAA (Fig. 7D).

DISCUSSION

While 28°C is representative of historical, long-term mean maximum in traditional japonica rice cultivation (e.g., Tsukuba, Japan; Ellis et al., 1993), the high- and low-temperature regimes selected in this study represented the occasional extreme temperatures reached or exceeded for short periods in current japonica rice production environments. For example, mean maximum temperatures in July and August in rice fields in south and southwest Spain range from 34.0 to 36.5°C and brief peaks above 40°C can occur, whilst night temperatures may on occasion reach 34°C (Junta de Andalucía, 2014).

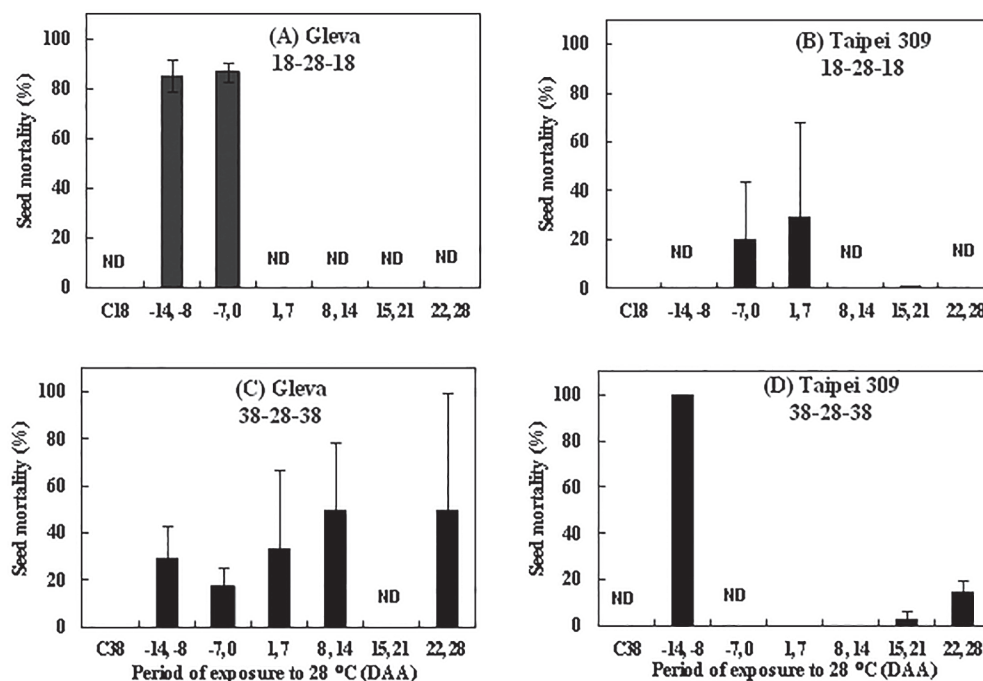


Figure 7. Seed mortality (percentage of dead seeds identified in germination tests of mature seeds; viability is 100 minus the value shown) of rice cultivated in growth chambers during 2011 at the University of Reading, UK following long-term (A, B) low-or (C, D) high-temperature (C, D) stress at different times relative to anthesis in rice cultivars (A, C) Gleva and (B, D) Taipei 309. Observations (mean \pm S.E.M.) are for all panicles reaching anthesis in the appropriate 7-d period. Note that some treatments produced very few seeds (Fig. 4), which nevertheless were all viable (e.g., C38, Gleva). Controls at 18 and 14°C produced no fertile panicles. Observations were not limited to the first panicle to appear on a plant: results are reported for panicles that were the first, second, and occasionally the third to exert within a plant. C18 and C38 are controls at 18 and 24 and 38 and 34°C, respectively; DAA, days after anthesis; ND, no data (no panicles produced mature seed).

Reduced seed yield in the current season was observed in plants exposed to extreme temperature stress for the 14-d period before anthesis due to low spikelet fertility. In addition, plants exposed to extreme temperature stress for the period immediately after anthesis showed poor seed viability; hence reduced seed yield in the subsequent season is likely through poor crop establishment. Both low (18 and 14°C) or high (38 and 34°C) temperature stress negatively impacted yield and seed viability of japonica rice, although the impact of low-temperature stress was less severe for Taipei 309, from Taiwan, than Gleva, bred in Catalonia. This result could be explained by differences in genotypic tolerance to low temperature (Farrell et al., 2006; Nakamura et al., 2000). The average air temperature in Taiwan in the second crop season is 18°C (Lur et al., 2009), which is lower than the average of 22°C in Catalonia. Short-duration transfers from a control to an extreme environment have been used in rice to study the effect of high-temperature stress on a particular developmental stage, the critical high temperature, or changes in sensitivity across phenological stages (e.g., Jagadish et al., 2007; Tashiro and Wardlaw, 1991a, 1991b). We used reciprocal transfers to consider both long- and short-term exposure to low and high extreme temperatures.

The reciprocal-transfer design (28 and 24°C to 18 and 14°C and vice versa, or 28 and 24°C to 38 and 34°C

and vice versa) enabled the temporal pattern of sensitivity of reproductive development to extreme temperature to be determined. The treatments providing plants 7-d relief from temperature stress and the opposite treatments providing only 7-d temperature stress independently confirmed the most sensitive plant developmental periods to damage from such stress, as did the concurrent exposures of different plants to either low or high extreme temperatures. For example, in both cultivars the peak in spikelet fertility in Fig. 2C, D (7-d relief at 28 and 24°C from extreme temperature) before anthesis coincided temporally with severely reduced spikelet fertility in Fig. 2A, B (7-d temperature stress at either 18 and 14°C or 38 and 34°C).

Spikelet fertility and seed yield were most negatively affected when plants were exposed to extreme temperature in the 14-d period up to and including anthesis, particularly the earlier part of this period. Microsporogenesis, or the process of microspore and pollen grain formation through meiosis and mitosis, typically occurs 10 to 12 d before anthesis in rice. Short periods of high-temperature stress at this stage reduced spikelet fertility in a heat-sensitive indica rice (Satake and Yoshida, 1978). The authors showed two separate peak periods of maximum damage, where anthesis (the greater peak) was more sensitive to high-temperature stress than microsporogenesis (the lesser peak). In contrast, our research, which employed a slightly longer period of

exposure as well as different cultivars, demonstrated that the damage at 38 and 34°C was severe throughout pre-anthesis, as opposed to bimodal peaks, and the negative effect was slightly less at anthesis than at microsporogenesis (Fig. 3B). Note also that, in Gleva at least, the temporal pattern of sensitivity at low temperature was similar (Fig. 3A). Sensitivity at microsporogenesis (Farrell et al., 2006; Gunawardena et al., 2003; Imin et al., 2006; Nakamura et al., 2000; Shimono et al., 2007), with increasing cold tolerance over the period towards anthesis (Gunawardena et al., 2003) and at anthesis with high (Jagadish et al., 2007; Matsui et al., 2001; Satake and Yoshida, 1978; Yoshida, 1981) or low temperatures (Julia and Dingkuhn, 2012), has been reported previously. Given the different durations of temperature stress applied amongst the several studies, the difference in temperature regimes (including extreme night temperatures in our research), and the different genotypes, we suggest assuming high sensitivity to extreme temperature throughout the 14-d period up to and including anthesis instead of pointing out microsporogenesis as a more-susceptible stage to extreme temperatures. Although both cultivars we investigated showed a similar temporal pattern of sensitivity, it is worth noting the greater sensitivity of Taipei 309 to cold stress before anthesis (Fig. 2A). Hence, one might speculate whether all cultivars necessarily show the same relative temporal patterns of sensitivity. Some cultivars may be more sensitive to extreme temperature at microsporogenesis, others equally sensitive at microsporogenesis and at anthesis, and others more sensitive at anthesis.

The temporal pattern of sensitivity of seed yield per panicle in plants exposed to extreme low or to extreme high temperature was similar to that for spikelet fertility (i.e., greatest when exposure was in the first 14 d until anthesis). However, a few of the 7-d extreme-temperature treatments yielded more than the controls (e.g., 15–21 DAA at 18 and 14°C or 22–28 DAA at 38 and 34°C in Taipei 309; Fig. 4A, B). These particular treatments had greater durations of seed filling, which may well explain these results. Clearly, 7-d exposures to extreme temperatures during the seed-filling phase did not reduce final seed yield. Hence the seed-filling phase in rice is less sensitive to a particular extreme temperature than the preceding plant developmental phases are for seed set. Partial yield compensation was detected within the rice plants. If spikelet fertility, and thus seed yield, were reduced severely in a panicle by extreme temperature, then panicles exerted later contributed substantially more to seed yield per plant (Fig. 5). It is also possible that the apparent higher tolerance to extreme temperature at anthesis than at microsporogenesis might result from some spikelets escaping temperature stress due to within-panicle flowering variability (Jagadish et al., 2007; Julia and Dingkuhn, 2012). Hence, we suggest that developing new rice cultivars with greater flowering diversity, as exhibited here by Taipei 309, is a

useful adaptive strategy to extreme temperature events. Similar results were found in wheat (*Triticum aestivum* L.) (Lukac et al., 2012). The greater variability within Taipei 309 might indicate that it was less suited for this study than Gleva, but we suggest that this characteristic might also indicate the greater resilience to stress of Taipei 309. This diversity in temporal sensitivity to extreme temperatures among cultivars could be exploited in breeding programs to release cultivars adapted to specific regional risk of extreme temperature occurrence.

Seed quality in terms of capacity to germinate normally is important in establishing subsequent crops. In Gleva exposed to low or high temperature for 7 d, the greatest proportions of dead seeds at harvest maturity resulted from low temperature in the first or second (Fig. 6A) or high temperature in the first (Fig. 6C) 7-d period after anthesis. Histodifferentiation is the first developmental phase to occur in the seed after fertilization. This phase is characterized by rapid cell division and differentiation to form the tissues of the seed and precedes reserve accumulation (seed filling). Hence, we conclude that seed quality development is most sensitive to low and high temperature damage soon after anthesis and that histodifferentiation is the most sensitive phase for effects on seed quality. Determining the proportion of dead seeds at maturity is less sensitive than the experimental procedure of Ellis et al. (1993) and Ellis (2011) to estimate seed quality, where the development of seed longevity was assessed from serial harvests. Nevertheless, our results are not only compatible with earlier studies (Ellis, 2011; Ellis and Hong, 1994; Ellis et al., 1993) but we now provide the first direct evidence to support the hypothesis (Ellis, 2011) that the damaging effect of high-, and we can now also add low-, temperature stress for seed quality development is greater the earlier it is imposed after anthesis, even though the consequences may not be manifest until much later (i.e., approaching harvest maturity).

Crop modeling is a powerful tool to estimate the impact of climate change on yields in different agroecological systems under different scenarios (Nelson et al., 2010). Crop model outputs for climate-change-driven rice crop yield and/or components of yield tend to predict linear trends against mean temperature (Kim et al., 2013; Matthews et al., 1997; Peng et al., 2004; Sheehy et al., 2006) because they are based on plant development, carbon, water, nitrogen, and phosphorous capture (Craufurd et al., 2013). As a result, they underestimate the negative effects of climate variability (Nelson et al., 2010). Our results confirm that short-term exposure to extreme temperatures during particularly sensitive developmental stages caused substantial yield reduction, indicating that models with linear yield-temperature responses would be unrealistic in highly-variable temperature scenarios.

In conclusion, 7-d exposures to extreme low or high temperatures were at least as damaging to spikelet fertility at the time of microsporogenesis as they were at anthesis in rice, while successively later exposures were harmless. The negative effects on final seed yield were through effects on seed set, with little evidence of any direct effect of 7-d exposure to low or high extreme temperature during the seed-filling phase (other than extended seed-filling periods). The viability of seeds was reduced by extreme-temperature treatments applied in the 7 or 14 d immediately after anthesis, which coincides with histodifferentiation.

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