Temporal sensitivity of rice seed development from spikelet fertility to viable mature seed to extreme-temperature

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Relative temporal sensitivity of rice seed development from spikelet fertility to viable mature seed to low- or to high-temperature stress

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Abbreviations: cv, cultivar; DAA, days after anthesis; DAS, days after sowing; ISTA, International Seed Testing Association; LSD, least square difference; REML, Restricted maximum likelihood; S.E, standard error; S.E.M, standard error of the mean

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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 28/24 ^{0}C and moved to 18/14 ^{0}C and *vice versa*, or from 28/24 to 38/34 ^{0}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 prior to anthesis; and both cultivars were affected at 38/34 ^{0}C while only cv. Gleva was affected at 18/14 ºC. 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 38/34 ^{0}C or 18/14 ^{0}C at 1 to 7 and 1 to 14 d after anthesis, respectively, in cv. Gleva. Cultivar Taipei 309 was not affected by 7 d exposure at 18/14 ^{0}C; and no consistent temporal pattern of sensitivity was evident at 38/34 ^{0}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; and it was almost as damaging around anthesis. Seed viability was most vulnerable to low or high temperature in the 7 or 14 dafter anthesis, when histodifferentiation occurs.

Key words: anthesis, climate change, *Oryza sativa* L. subsp. Japonica, panicle sterility, reproductive development, rice, seed development, seed viability, temperature stress, yield

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INTRODUCTION

Rice (*Oryza sativa* L.), the world's most important staple food crop feeding some 2.7 billion people (Fairhurst and Dobermann, 2002), is grown in diverse agro-climatic zones from temperate through sub-tropical to tropical regions, temperature being the limiting factor to cultivation (DeDatta, 1981) with critical limits ranging from 12-20 ^{0}C to 34-38 ^{0}C (Yoshida, 1981). Productivity is vulnerable to warming temperatures in tropical (Wassmann *et al.*, 2009) and perhaps also sub-tropical 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 ^{0}C to 38.5 ^{0}C depending on cultivar (Jagadish *et al.*, 2008; Yoshida, 1981). Low temperatures constrain rice production in temperate areas and cause annual yield losses of 1-3.9 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 ^{0}C to 20 ^{0}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 *et al*., 1993; Ellis and Hong, 1994).

Climate change is projected to increase mean global surface temperature by 0.6 ^{0}C to 4 ^{0}C by 2100 (IPCC, 2007), increase temperature variability (Tebaldi *et al.*, 2006), with more frequent extreme events (IPCC, 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 (Heenan and Lewin, 1982; Gunawardena *et al.*, 2003; Oliver *et al.*, 2005; Farrell *et al.*, 2006; Pereira da Cruz *et al.*, 2006; Shimono *et al*., 2007) or high temperatures around anthesis (Matsui *et al*., 2001; Jagadish *et al*., 2007). High temperature later in reproductive development can also affect seed production (Tashiro and Wardlaw, 1991), 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 of 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

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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. Cultivar 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 28/20 ^{0}C day/night (11h/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: 18/14, 28/24, or 38/34 ^{0}C, all with a photoperiod of 11 h d^{-1} and synchronous 11h/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 transferred for the next (7-d) period; and so on, providing a set of serial transfers. Here, 28/24 ^{0}C provided a "normal" regime with reciprocal transfers (of different plants) *to and from* a cooler regime of 18/14 ^{0}C or *to and from* a warmer regime of 38/34 ^{0}C. The serial, reciprocal transfers occurred at 7-d intervals from around 14 d prior to anthesis to 28 d after anthesis (DAA), i.e., a total of six different transfer periods. Transfer from 28/24 to either 38/34 or 18/14

^{0}C for 7 d represented a short-term temperature stress, while a 7-d transfer from either 38/34 or 18/14 to 28/24 ^{0}C represented a brief respite from long-term temperature stress. Control plants were held throughout the experimental period, from 14 d before anthesis onwards, in the same temperature: 28/24 ºC represented control for short-term temperature stress, whereas 38/34 ºC and 18/14 ºC did so for long-term high and low temperature stress, respectively. Each reciprocal-transfer treatment combination was represented by 4 pots whereas each set of control plants, at either 28/24, 18/14 or 38/34°C comprised 8 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 upon climate change (Peng *et al*., 2004). Continuous extreme temperature regimes provided throughout the experiment, however, are unrealistic. In such climates, farmers would select other

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eco-geographic 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 28/20°C day/night (11h/13h).

Seven seeds were sown into a soil-less 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 minutes 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 (H_3BO_3) 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 ds after sowing. Plants were moved to four modified Saxil growth cabinets (internal dimensions 1.4 x 1.4 x 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, start and end of seed filling were recorded for each panicle individually by labeling each tiller, including the sequence of panicle exsertion within each plant. The start and end of seed filling were estimated non-destructively 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 recorded, and then air-dried enclosed in a paper envelope with further drying at laboratory temperature (c. 7 d at 20 ^{0}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 34/11 ^{0}C (16h/8h) for 28 d to maximize the germination of dormant and non-dormant seeds (Ellis *et al*., 1983). The progress of radicle emergence was monitored weekly and non-germinated fresh seeds were pricked with a needle at 21 d to aid loss of dormancy. Seedlings were classified (ISTA, 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 (18/14ºC or 38/34 ºC) or to optimum (28/24ºC) temperatures, for short or long-term temperature stress, respectively. The timing of transfer was

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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 (REML) was applied to estimate the variance of the components and provide least square difference (LSD) and the Adjusted Tukey-Kramer tests for means and pairwise-means comparison, respectively. SAS 9.2 (SAS Institute Inc. 2008) was used for all analyses.

RESULTS

The treatments affected phenology. Anthesis occurred at 75 to 90 d after sowing (DAS) in cv. Gleva, and peaked at 75-80 DAS for plants continuously grown at 28/24 ^{0}C or for those exposed for a 7 d period to 18/14 ºC within the reproductive phase. Plants exposed for a 7 d period to 38/34 ºC within the reproductive phase peaked at 75-90 DAS. Fewer tillers anthesed with long exposures to extreme temperatures; peak anthesis occurred in a limited number of these tillers at c. 90 or 70 DAS at 18/14 ^{0}C or 38/34 ^{0}C, respectively. Cultivar 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 28/24 ^{0}C with or without 7 d at 18/14 ^{0}C (peak 165 DAS), but this range increased to 125-185 DAS when plants were exposed to 7 d at 38/34 ^{0}C. Long exposures (i.e., only 7 days at 28/24 ^{0}C) to 18/14 ^{0}C extended this period (145 to 205 DAS) whereas long exposures to 38/34 ^{0}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 18/14 ºC compared to the control at 28/24 ºC. Similar reductions occurred when plants were exposed to 38/34 ºC in cv. Taipei 309, whereas in cv. Gleva the reduction was not significant.

Fertility

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

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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 (18/14°C) or high temperature (38/34°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 plants from cv. Gleva exposed to 18/14°C before anthesis had lower spikelet fertility than those from cv. Taipei 309. Seven-day exposures to 18/14ºC or 38/34°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 18/14°C and to 38/34°C was interrupted by 7d at 28/24°C (Fig. 2C,D). Surprisingly spikelet fertility increased in a limited number of panicles of cv. Taipei 309 when plants grown in long-term high temperature were exposed to 28/24°C for 7 d at 15-21 and 22-28 DAA. In cv. Gleva, more fertile spikelets were detected when long exposure to 18/14 or 38/34 ^{0}C was interrupted by 7 d at 28/24^{0}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 days' exposure to 18/14 or 38/34 ºC resulted in low fertility (Fig. 2A,B).

A brief exposure to 18/14 ^{0}C reduced spikelet fertility in plants from cv. Gleva while cv. 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 18/14 ºC was much greater in cv. Gleva than cv. Taipei 309 (data not shown). However, plants from cv. Taipei 309 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-dstress treatments began. In both cultivars, there were few fertile spikelets if the high temperature treatment of 38/34°C began 7 to 14 d before the anthesis. However, a trend of increasing spikelet fertility was detected the later the exposure to 38/34^{0}C; no reduction in fertility was observed with exposures beginning 5 d after anthesis or later (Fig. 3B). At low temperature stress of18/14°C, the results were more variable (Fig. 3A). The response to low temperature treatment in cv. Gleva was similar to that at high temperature treatment, while spikelet fertility in cv. Taipei 309 fertility was less affected at 18/14°C than at 38/34 ºC.

Seed yield per panicle

Seed yield per panicle (Fig. 4A,B) was affected by 7-d periods at 18/14 or 38/34 ^{0}C, following the same pattern as spikelet fertility (Fig. 2A,B). Short-term low-temperature stress had little effect on seed yield per panicle in cv. Taipei 309, save for the unusually high value for 15-21 DAA. In contrast, reduced or increased yield ($P<0.05$) resulted in cv. Gleva from exposure to 18/14°C before or after anthesis, respectively, relative to control treatment (28/24 ºC) (Fig. 4A). The results for 7 d at 38/34°C showed a more consistent temporal pattern of sensitivity in both cultivars (Fig. 4B): 7 d at 38/34°C before anthesis reduced yield ($P<0.05$) substantially, by 69 and 79% in cvs Gleva and Taipei 309, respectively, whereas later treatments provided similar yield to those from controls. The 7d treatments at 38/34°C during the seed development phase increased seed filling durations ($P<0.05$) in both cultivars by 4-6 d: from 20.6±1.2 and 19.2±1.6 d in the 28/24°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

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temperature stress did provide higher seed yields in cv. Taipei 309 for the later transfers to 28/24°C (15-21 DAA from 18/14°C; 15-21 and 22-28 DAA from 38/34°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 exserted after the temperature stress treatment. Cultivar Gleva showed reduced seed yield per pot following 7-d temperature stress, except for the late exposures (8-14 and 15-21 DAA) to 18/14 ^{0}C (Fig. 5A), whereas the yield reduction was more consistent across all dates when plants were transferred to 38/34°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 18/14°C up to 7 DAA, or exposed to 38/34°C at any time up to 21 DAA.

Seed viability

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

sensitivity at 18/14°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 were broadly similar to the continuous 28/24°C control (Fig. 6A).

There were fewer dead seeds in cv. Taipei 309, and there were no significant effects when plants were transferred to 18/14°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 7d pre-anthesis to 14 DAA followed by a large increase for 15 to 21 DAA. The latter may be due to experimental error given the small sample size (7 panicles).

Long exposure to either 18/14°C or 38/34°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 where 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 cv. Taipei at 38/34ºC were exposed to 28/24 ºC for 7 d between 1 and 28 DAA (Fig. 7D).

Crop Science Accepted paper, posted 08/18/2014. doi:10.2135/cropsci2014.01.0042

DISCUSSION

While 28 ^{0}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 South-west Spain range from 34.0 ºC to 36.5 ºC and brief peaks above 40 ºC can occur, whilst night temperatures may on occasion reach 34 ºC (Junta de Andalucía, 2014).

Reduced seed yield in the current season was observed in plants exposed to extreme temperature stress for the 14 d period prior to anthesis due to low spikelet fertility. In addition, plants exposed to extreme temperature stress for the period immediately after anthesis showed poor seed viability and hence reduced seed yield in the subsequent season is likely through poor crop establishment. Both low (18/14 ºC) or high (38/34 ^{0}C) temperature stress negatively impacted yield and seed viability of japonica rice, although the impact of low-temperature stress was less severe for cv. Taipei 309 ,from Taiwan, than cv. Gleva, bred in Catalonia. This result could be explained by differences in genotypic tolerance to low temperature (Nakamura *et al.*, 2000; Farrell *et al.*, 2006). The average air temperature in Taiwan in the second crop season is 18 ºC (Lur et al., 2009), which lower than the average in Catalonia is 22 ºC 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., Tashiro and Wardlaw, 1991a; Tashiro and Wardlaw, 1991b; Jagadish et al., 2007). We used reciprocal transfers in order to consider both long- and short-term exposure to low and high extreme temperatures.

The reciprocal-transfer design (28/24 to 18/14 ºC and *vice versa,* or 28/24 to 38/34 ºC 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 Figure 2C,D (7 d relief at 28/24 ºC from extreme temperature) before anthesis coincided temporally with severely reduced spikelet fertility in Figure 2A,B (7 d temperature stress at either 18/14 ºC or 38/34 ºC).

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 10-12 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 38/34 ^{0}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 cv. Gleva at least, the temporal pattern of sensitivity at low temperature was similar (Fig. 3A). Sensitivity at microsporogenesis (Nakamura *et al.*, 2000; Gunawardena *et al.*, 2003; Farrell *et al.*, 2006; Imin *et al.*, 2006; Shimono *et al*., 2007), with increasing cold tolerance over the period towards

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anthesis (Gunawardena *et al.,* 2003), and at anthesis with high (Satake and Yoshida, 1978; Yoshida, 1981; Matsui *et al.*, 2001; Jagadish *et al.*, 2007) 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 to assume 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 cv. 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 18/14 ^{0}C or 22-28 DAA at 38/34 ^{0}C in cv. 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 exserted 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 cv. Taipei 309, is a useful adaptive strategy to extreme temperature events. Similar results were found in wheat (Lukac *et al.*, 2012). The greater variability within cv. Taipei 309 might indicate that it was less suited for this study than cv. Gleva; but we suggest that this characteristic might also indicate the greater resilience to stress of the former. 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 cv. 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 periods 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

Crop Science Accepted paper, posted 08/18/2014. doi:10.2135/cropsci2014.01.0042

studies (Ellis *et al*., 1993; Ellis and Hong, 1994; Ellis, 2011), 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 agro-ecological 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 (Matthews *et al.*, 1997; Peng *et al.*, 2004; Sheehy *et al.*, 2006; Kim *et al.*, 2013) 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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LEGENDS TO FIGURES

Figure 1. Mean number of tillers per rice plant (fertile and non-fertile) at harvest for rice plants of cvs Gleva and Taipei 309 cultivated in growth chambers during 2011 in the University of Reading (UK). Plants were exposed to extremes of 18/14 ^{0}C (A) or 38/34 ^{0}C (B) for different periods during reproductive development: 18/14 ^{0}C throughout (18ºC), 28/24 ^{0}C throughout (28ºC), 38/34 ^{0}C throughout (38ºC), 18/14 ^{0}C interrupted by 7-d periods at 28/24 ^{0}C (18-28ºC), 28/24 ^{0}C interrupted by 7-d periods at 18/14 ^{0}C (28-18ºC), 38/34 ^{0}C interrupted by 7-d periods at 28/24 ^{0}C (38-28ºC), or 28/24 ^{0}C interrupted by 7-d periods at 38/34 ^{0}C (28-38ºC). Results for 18-28ºC, 28-18 ºC, 38-28 ºC, and 28-38 ºC are means of the six different timings of transfers.

Figure 2. Spikelet fertility of panicles of rice plants cultivated in growth chambers during 2011 in the University of Reading (UK) under a brief low- (A) or high-temperature (B) stress or a long low- (C) or high-temperature (D) stress during reproductive development in cvs Gleva and Taipei 309. Note that -14,-8 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 18/14, 28/24, or 38/34 ^{0}C throughout plant growth. Observations (mean ± 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 cv. Taipei 309 (C,D) observations because numbers were low Many more fertile panicles were produced in cv. Gleva (>25 in each treatment combination). DAA= days after anthesis.

Figure 3. Spikelet fertility of rice panicles, cultivated in growth chambers during 2011 in the University of Reading (UK), under short-term (7d) low- (A) or high-temperature (B) stress in cvs 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.

Figure 4. Seed yield per panicle (g at c. 15% moisture content) of rice cultivated in growth chambers during 2011 in the University of Reading (UK) under short-term low- (A) or high-temperature (B) stress or long-term low- (C) or high-temperature (D) stress at different times relative to anthesis in cvs Gleva and Taipei 309. Note that -14,-8 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 18/14, 28/24, or 38/34 ^{0}C throughout plant growth. Observations (mean ± S.E.) are for all panicles reaching anthesis in the appropriate 7-d period. The absence of a symbol represents no panicle, or 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 exsert within a plant. DAA=days after anthesis

Figure 5. Seed yield per pot (g at c. 15% moisture content; 4 plants pot^{-1}) of rice (cv. Gleva) cultivated in growth chambers during 2011 in the University of Reading (UK) for plants in which some panicles were subject to short-term low- (A) or high-temperature (B) stress at different times relative to anthesis and others emerged later (those which reached anthesis 10 d

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after the end of the last transfer treatment) and the relative contribution of late tillers to yield. C28 are control treatments maintained at 28/24 ^{0}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 in the University of Reading (UK) following short-term low- (A,B) or high-temperature (C,D) stress at different times relative to anthesis in rice cvs Gleva (A,C) and Taipei 309 (B,D). Observations (mean ± 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 exsert within a plant. C28 = control at 28/24 ^{0}C; DAA=days after anthesis.

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 in the University of Reading (UK) following long-term low (A,B) or high temperature (C,D) stress at different times relative to anthesis in rice cvs Gleva (A,C) and Taipei 309 (B,D). Data presented are mean ±S.E.M since for cv. Taipei 309 and cv. Gleva at low and high temperatures, respectively, could not be estimated because of lack of degrees of freedom. Observations 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, cv. Gleva). Controls at 18/14^{0}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

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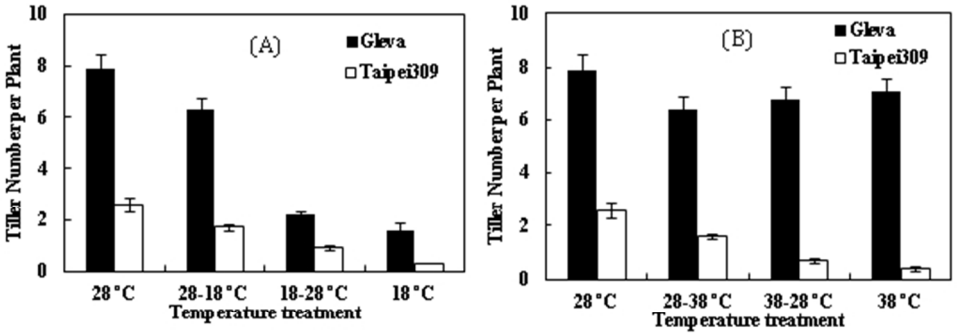

Figure 1

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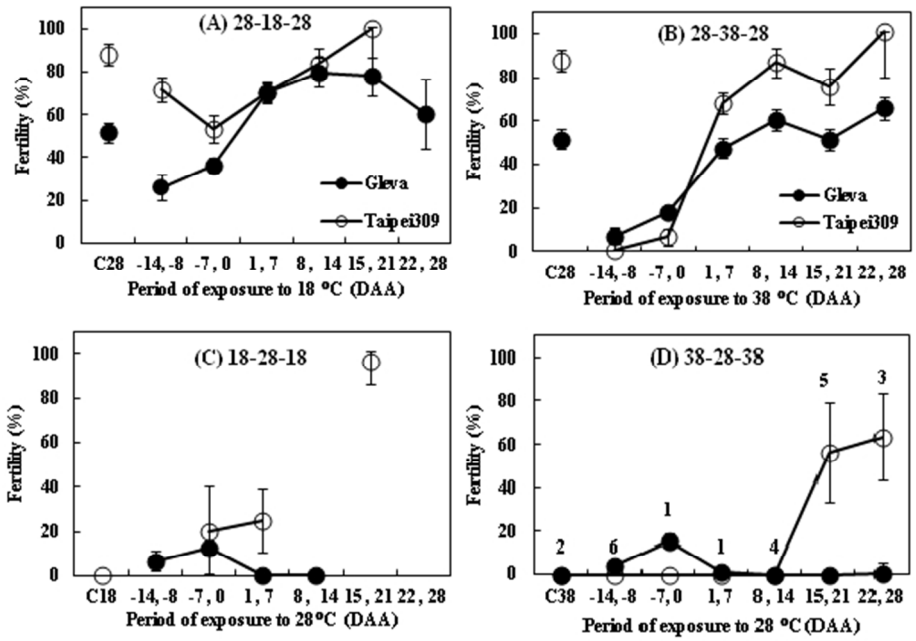

Figure 2

Figure 2. Spikelet fertility of panicles of rice plants cultivated in growth chambers during 2011 in the University of Reading (UK) under a brief low- (A) or high-temperature (B) stress or a long low- (C) or high-temperature (D) stress during reproductive development in cvs Gleva and Taipei 309. Note that -14,-8 DAA indicates that anthesis of individual panicles exposed to treatment occurred 14 to 8 days after treatment. C18, C28 and C38 are control treatments maintained at 18/14, 28/24, or 38/34 ^{0}C throughout plant growth. Observations (mean ± S.E.) are for all panicles anthesing in the appropriate 7-day 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 cv. Taipei 309 (C,D) observations because numbers were low Many more fertile panicles were produced in cv. Gleva (>25 in each treatment combination). DAA= days after anthesis.
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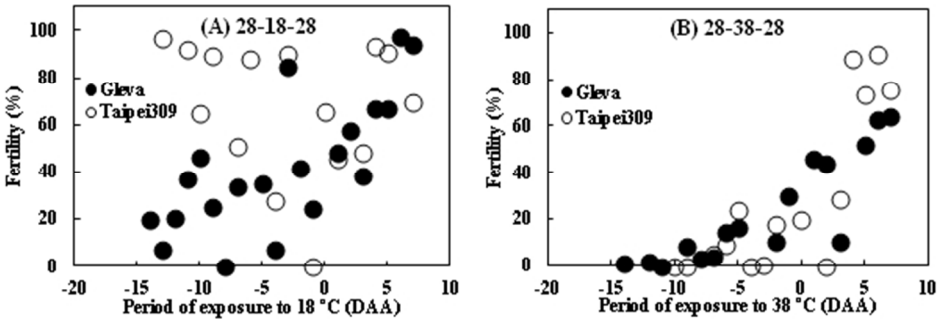

Figure 3

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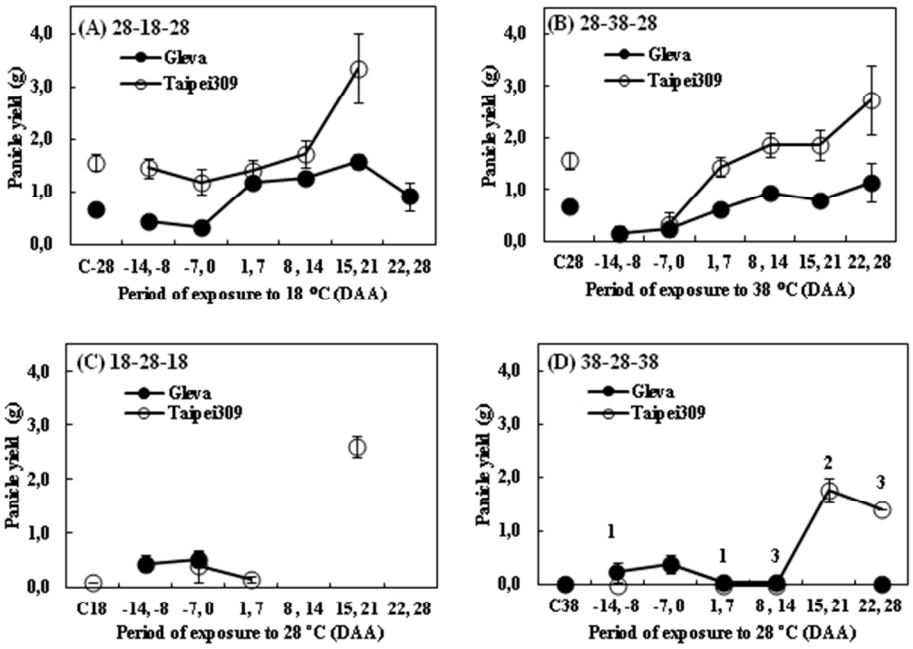

Figure 4

Figure 4. Grain yield per panicle (g at c. 15% moisture content) of rice cultivated in growth chambers during 2011 in the University of Reading (UK) under short-term low- (A) or high-temperature (B) stress or long-term low- (C) or high-temperature (D) stress at different times relative to anthesis in cvs Gleva and Taipei 309. Note that -14,-8 DAA indicates that anthesis of individual panicles exposed to a treatment occurred 14 to 8 days after treatment. C18, C28 and C38 are control treatments maintained at 18/14, 28/24, or 38/34 ^{0}C throughout plant growth. Observations (mean ± S.E.) are for all panicles reaching anthesis in the appropriate 7-day period. The absence of a symbol represents no panicle, or 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 exsert within a plant. DAA=days after anthesis

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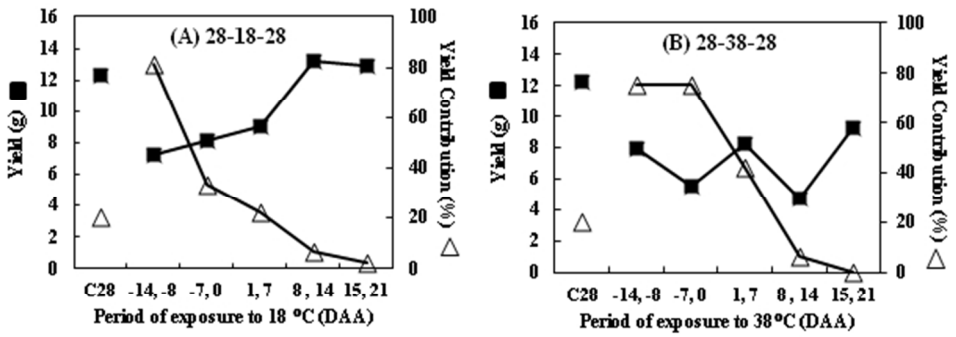

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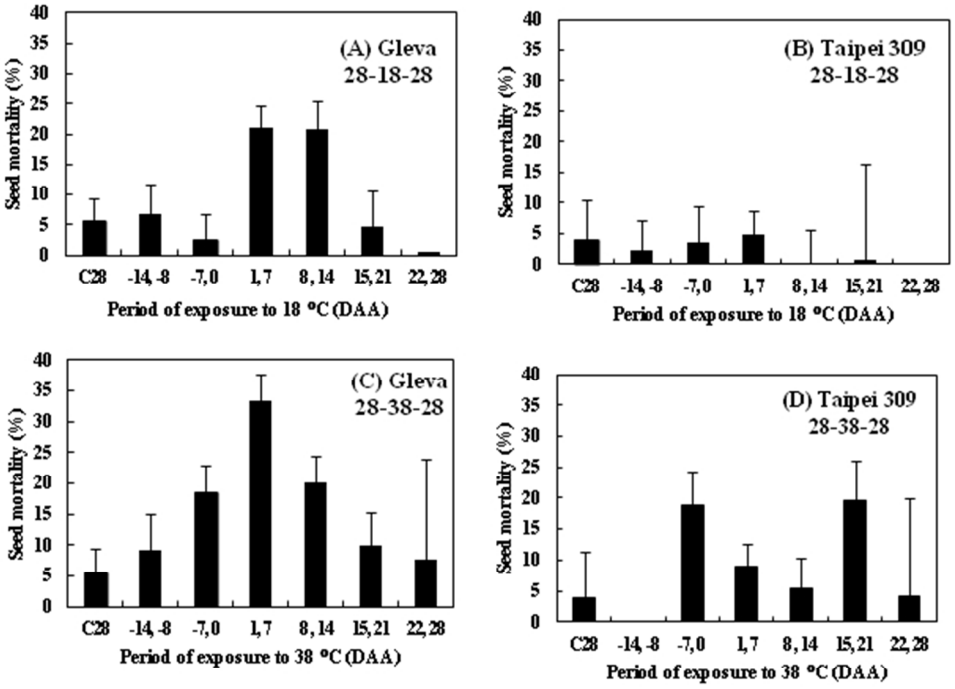

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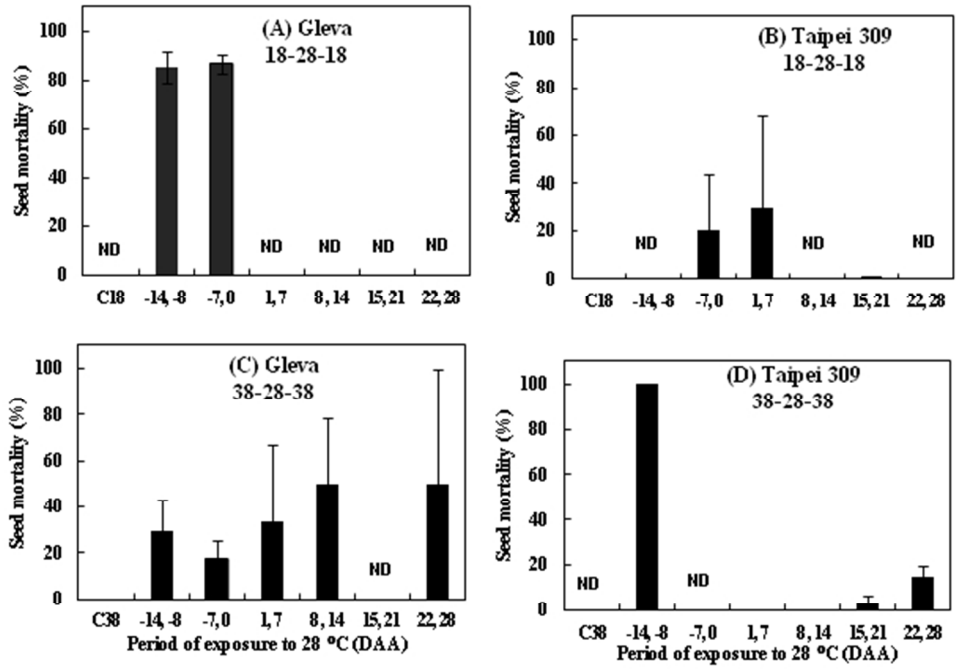

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