Marker-assisted pyramiding of QTLs for heat tolerance and escape upgrades heat resilience in rice (Oryza sativa L.)

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Abstract

Key message This study demonstrated that pyramiding of early morning flowering and heat tolerance QTLs (qEMF3 and qHTSF4.1) in rice is an efficient approach to maintain high spikelet fertility under high-temperature stress at flowering stage.

Abstract High temperature at flowering stage of rice causes low spikelet fertility and low yield. To cope with high-temperature stress brought by climate change, two strategies were proposed to develop heat-resilient rice varieties. One is to escape the high temperature by flowering early in the morning, another is to enhance tolerance to high-temperature stress per se. Two promising QTLs for early morning flowering (qEMF3) and heat tolerance (qHTSF4.1) were introgressed into IR64 background, and Near isogenic lines (NILs) IR64 + qEMF3 (IR64EMF3) and IR64 + qHTSF4.1 (IR64HT4) were developed in previous studies. In this study, a QTL pyramiding line IR64 + qHTSF4.1 + qEMF3 (IR64HT4EMF3) was developed by marker-assisted selection of the progenies of previous NILs. The NILs were subjected to different high-temperature regimes in the indoor growth chambers and different locations in the field. In the indoor growth chambers, when high temperature starts early (before 11:00 am), IR64HT4 and IR64HT4EMF3 had higher spikelet fertility than IR64EMF3; when high temperature comes later (after 11:00 am), IR64EMF3 and IR64HT4EMF3 had higher spikelet fertility than IR64HT4. The flowering pattern of the IR64HT4EMF3 was earlier than IR64HT4, but similar to IR64EMF3 in the glasshouse, field and indoor growth chambers. IR64HT4EMF3 showed higher spikelet fertility than IR64EMF3 and IR64HT4 in the field in the Philippines. Thus, combination of early morning flowering and heat tolerance QTLs is an elegant breeding strategy to cope with future extreme climate.

Introduction

Following continuous global warming in the recent decades, high temperature has become a major constraint for agriculture and food security of the world, especially for major crops like rice (Battisti and Naylor 2009). Yield losses of rice production caused by high temperature have been reported in many countries (Hasegawa et al. 2009; Ishimaru et al. 2016b; Li et al. 2004; Matsui et al. 2007; Matsushima et al. 1982; Osada et al. 1973; Tian et al. 2010; Yang et al. 2004). Developing heat resilient rice cultivars is considered as an efficient way to stabilize rice
production under high-temperature conditions (Ishimaru et al. 2016a).

Rice plant is susceptible to high temperature, especially at flowering stage (Matsui and Omasa 2002; Nishiyama and Satake 1981; Satake and Yoshida 1978). Spikelet sterility caused by high temperature at flowering stage directly leads to low grain yield (Jagadish et al. 2007; Matsui 2009; Prasad et al. 2006; Wassmann et al. 2009). To maintain high spikelet fertility under high temperature at flowering stage, two strategies were proposed. One is to develop cultivars with enhanced heat tolerance, and another is to develop cultivars that flower early in the morning before heat stress starts (Satake and Yoshida 1978).

Flowering stage is the most susceptible phase for high-temperature stress, and many studies have been carried out to identify QTLs associated with heat tolerance of rice at flowering stage (Cao et al. 2003; Chen et al. 2008; Cheng et al. 2012; Jagadish et al. 2010a; Xiao et al. 2011b; Ye et al. 2012, 2015a; Zhang et al. 2008, 2009). Among the identified QTLs, qHTSF4.1 on chromosome 4 was identified in different populations derived from rice varieties 996, N22, Milyang23, Giza178, Takanari, and a diversity panel of indica and aux accessions (Lafarge et al. 2017; Takai et al. 2020; Xiao et al. 2011b; Ye et al. 2012, 2015a). It is a promising QTL for improving heat tolerance of rice at flowering stage. The spikelet fertility of the NIL (IR64HT4) was about 15% higher than the recurrent parent IR64 after high-temperature treatment (Ye et al. 2015b).

On the other way, a major QTL qEMF3 controlling Flower open time (FOT) was identified on chromosome 3 by using a population derived from a wild rice species Oryza officinalis (Hirabayashi et al. 2015; Ishimaru et al. 2010). This QTL promotes rice plant to flowering early in the morning when the temperature is still cool, and it was introgressed into IR64 background by marker-assisted backcrossing, a Near-isogenic line (NIL) IR64EMF3 was developed (Hirabayashi et al. 2015). The IR64EMF3 plants flowering about 1.5–2.0 h earlier than IR64 (Hirabayashi et al. 2015).

This study demonstrates whether pyramiding of QTLs for heat tolerance (qHTSF4.1) and heat escape (qEMF3) into a single cultivar, IR64, has potential to upgrade heat resilience at flowering stage. The overall results suggest that pyramiding of qHTSF4.1 and qEMF3 showed positive effect on spikelet fertility under high-temperature conditions resulting from the advanced Flower open time (FOT) and heat tolerance. Changes in plant phenotype due to the introgression of qHTSF4.1 and qEMF3 were also investigated in the field. Our study formulated a useful breeding strategy to cope with future extreme high temperature and to ensure the global food security.

### Materials and methods

#### Plant materials

By using a rice 6 K SNP chip, a BC5F2 plant with minimal N22 introgression harboring qHTSF4.1 in the genetic background of IR64 was selected as a heat-tolerant NIL (Ye et al. 2015b). This NIL is designated as IR64HT4. As a heat escape NIL, the BC3F4-derived IR64EMF3 were used (Hirabayashi et al. 2015). These two NILs were crossed, and the F1 plants were self-pollinated to generate F2 seeds. Markers linked to qHTSF4.1 and qEMF3 (Table S1) were used to select the plants with homozygotes at both loci, and then the selected plants and their parental NILs were genotyped using the rice 6 K SNP chip (Thomson et al. 2017). The F2 plant with minimal donor introgression was selected for seeds multiplying, and the F3 seeds were used for the following experiments (Fig. 1). The pyramided NIL carrying both qHTSF4.1 and qEMF3 in the IR64 background was designated as IR64HT4EMF3. N22, the donor of qHTSF4.1, and a heat-tolerant rice variety GIZA178 were used as heat-tolerant check varieties.

#### Evaluation of flower open time and spikelet fertility in the glasshouse and indoor growth chamber (IGC)

Seeds of IR64 and the NILs were germinated and sown in soil-filled plastic trays, and the 21-day-old seedlings were transplanted into plastic pots filled with 1 kg natural clay loam soil and 1 g of complete fertilizer (14 N-14P-14 K). Since the heading date of N22 is earlier than IR64, the seeds of N22 were sown and transplanted 10 days later than IR64 and the NILs. A total of 50 pots were transplanted for each genotype. Only one seedling was maintained in each pot, and the plants were grown inside a net-house under natural temperature and sunlight till booting stage.

At the booting stage, all plants were moved into a glasshouse under controlled temperature condition (30 °C/24 °C Day/Night, ± 2 °C). When the first panicle started heading, the heading date was recorded, the earliest 3–5 panicles of each plant were marked, and then the pot was moved into an indoor growth chamber for high-temperature treatment.

The temperature inside the IGC was programmed to simulate diurnal temperature changes, and to make sure air temperature reaches up to 38 °C during the heat stress period. Four temperature regimes varying in the start time and duration of high temperature were used in subjecting treatments (Table 1). The high-temperature treatment at 38 °C started (i) from 08:00 to 14:00 for 6 h in the first
Fig. 1 Breeding scheme to develop the NIL IR64 + qHTSF4.1 + qEMF3 (IR64HT4EMF3). Marker-assisted selection (MAS) was used for foreground and background selection. The graphical genotype showed the introgression of the QTLs (arrowheads for qEMF3 and qHTSF4.1) and few fragments (blue) in IR64 background (red).

Table 1 Environmental setting for the indoor growth chambers (IGC)

| Chamber      | Time duration | Temperature (°C) | Relative humidity (%) | Light intensity (µmol m⁻² s⁻¹) |
|--------------|---------------|-----------------|-----------------------|-------------------------------|
| IGC1 (Treatment 08) | 6:00–8:00     | 24–38           | 75                    | 0–580                         |
|              | 8:00–14:00    | 38              | 70                    | 580                           |
|              | 14:00–18:00   | 38–24           | 75                    | 580–0                         |
|              | 18:00–6:00    | 24              | 75                    | 0                             |
| IGC2 (Treatment 09) | 6:00–9:00     | 24–38           | 75                    | 0–580                         |
|              | 9:00–14:00    | 38              | 70                    | 580                           |
|              | 14:00–18:00   | 38–24           | 75                    | 580–0                         |
|              | 18:00–6:00    | 24              | 75                    | 0                             |
| IGC3 (Treatment 10) | 6:00–10:00    | 24–38           | 75                    | 0–580                         |
|              | 10:00–14:00   | 38              | 70                    | 580                           |
|              | 14:00–18:00   | 38–24           | 75                    | 580–0                         |
|              | 18:00–6:00    | 24              | 75                    | 0                             |
| IGC4 (Treatment 11) | 6:00–11:00    | 24–38           | 75                    | 0–580                         |
|              | 11:00–14:00   | 38              | 70                    | 580                           |
|              | 14:00–18:00   | 38–24           | 75                    | 580–0                         |
|              | 18:00–6:00    | 24              | 75                    | 0                             |
IGC, (ii) from 9:00 in the second IGC for 5 h, (iii) from 10:00 in the third IGC for 4 h, and (iv) from 11:00 in the fourth IGC for 3 h. Relative humidity and number of lights turned on inside the chamber were also programmed to simulate actual conditions. Relative humidity was kept at 70% when the temperature is 38 °C, and at 75% when temperature is below 38 °C. The night (dark) time was from 18:00 to 6:00.

Ten plants of each genotype were subjected to each treatment chamber. Beginning of Flower opening time (FOT) was recorded and observed by “plot” basis (or for one treatment group as a whole, not individual plants), and observed only from the glass window of the IGC to minimize micro-environment variations that may affect flowering pattern and spikelet fertility. FOT was observed for 3–5 consecutive days. After about 10 days of high-temperature treatment, when the marked 3–5 panicles have completed flowering, the plants were moved back to the glasshouse and maintained until physiological maturity.

The plants of control group were remained inside the glasshouse (30 °C/24 °C Day/Night, ± 2 °C). The heading date of each plant was recorded when it started heading, and one panicle per plant was selected and tagged for FOT observation based on previous method (Hirabayashi et al. 2015). The number of spikelets that flowered (spikelet was open and anthers protruded from the glume) was recorded at 30-min intervals, starting from 6:00 for the NILs carrying qEMF3 and 8:00 for other genotypes, until the end of anthesis. Observation was conducted for 3 consecutive sunny days.

At physiological maturity, three uniform panicles were collected from each plant for measuring spikelet fertility. The number of filled (full and partially filled) spikelets and empty spikelets were counted for each individual panicle. Spikelet fertility, the ratio of filled grains to the total number of spikelets on the panicle expressed as percentage, was computed for each panicle. The average spikelet fertility of the three panicles was computed.

Evaluation of spikelet fertility and agronomic traits in the field

The five genotypes (IR64, IR64HT4, IR64EMF3, and IR64HT4EMF3, and N22) were grown in the experimental field of Yezin Agricultural University, Nay Pyi Taw, Myanmar (19° 49′ 59.6″N, 96° 16′ 30.4″E) in the dry season of 2015. Seeds were sown on 22 December 2014, and 21-day-old seedlings were transplanted in the field at a density of 20 cm × 20 cm. The size of each plot was 1.44 m² (0.6 m × 2.4 m) with randomized block design for four replications per genotype. A total amount of fertilizer application is 21-day-old seedlings were transplanted in the field at a density of 20 cm × 20 cm. The size of each plot was 1.44 m² (0.6 m × 2.4 m) with randomized block design for four replications per genotype. A total amount of fertilizer application is 12 m² (2 m × 6 m). The plots were arranged randomly with three replications. Heading date of each genotype was recorded when 50% of the panicles in the plot emerged from the flag leaf sheath.

At maturity, fifteen plants were randomly selected from the inner rows of each plot for measuring the plant height, flag leaf length, panicle length, panicle exertion, number of tillers, and three uniform panicles of each plant were harvested for counting the filled and empty grains. The spikelet fertility was calculated as the ratio of filled grains on each panicle. For grain yield computation, all panicles from an 1 m × 1 m (25 hills) area were harvested from the inner rows of each plot. The panicles were threshed, and the grains were dried. The moisture content of the grains was measured, and the potential yield of each plot was computed and converted to kilogram per hectare.

The maximum and minimum temperature data at IRRI and CSU were abstained from local weather station. The high-temperature stress (maximum temperature over 35 °C lasted for at least 3 days) at IRRI started from 19 April till 4 June, while the high temperature at CSU started from 2 May till 3 July (Supplemental Fig. 1).

Evaluation of flower open time in the field

The five genotypes (IR64, IR64HT4, IR64EMF3, and IR64HT4EMF3, and GIZA178) were grown in the experimental field of Yezin Agricultural University, Nay Pyi Taw, Myanmar (19° 49′ 59.6″N, 96° 16′ 30.4″E) in the dry season of 2015. Seeds were sown on 22 December 2014, and 21-day-old seedlings were transplanted in the field at a density of 20 cm × 20 cm. The size of each plot was 1.44 m² (0.6 m × 2.4 m) with randomized block design for four replications per genotype. A total amount of fertilizer application is 74–20–20 kg 10a⁻¹ for N-P2O5-K2O, respectively. Flowering pattern was observed for four days (3–6 April 2015) during heading period using four panicles per plot every day.

Statistical analysis

The means of the phenotypic data were calculated by using Excel 2013. Differences among genotypes were compared by Tukey Honestly significant difference (HSD) test and One-way ANOVA using Minntab17. Genotypic data were sorted by using Excel 2013, and graphs of introgression in IR64 background were drawn by using GGT32.
Results

Background of the QTL pyramiding NILs for heat tolerance and escape

The background of the NILs was evaluated by using the 6 K SNP chip. There were only few fragments introgressed from the donors. The size of the donor fragments ranged from 0.31 to 4.79 Mb, and the total length of the donor fragments ranged from 5.04 to 15.53 Mb in the NILs. The backgrounds of the NILs were highly similar to IR64, ranging from 95.8 to 98.64% (Table 2). The donor fragment for QTL qEMF3 was 4.57 Mb, and the donor fragment for QTL qHTSF4.1 was 2.33 Mb in IR64HT4EMF3.

Flower opening time of the NILs

NILs IR64EMF3 and IR64HT4EMF3 started flowering earlier than IR64HT4 and the check varieties both in the glasshouse and in the field. IR64EMF3 and IR64HT4EMF3 finished flowering at 9:30 in the glasshouse and 10:30 in the field. NILs IR64HT4 and IR64 reached peak flowering at 10:00 in the glasshouse and 11:00 in the field. The heat-tolerant check varieties N22 and Giza178 reached the peak approximately 30 min later than IR64HT4 and IR64. Across the glasshouse and field conditions, FOT of each NIL showed the similar tendency (Fig. 2), while in the indoor growth chambers, start and end of the flower opening by visual monitoring was 6:30–8:00 (range in 4 chambers) for IR64EMF3, 6:30–9:00 for IR64HT4EMF3, 8:30–10:00 for IR64HT4, 8:30–10:30 for IR64, and 9:30–13:00 for N22, respectively.

Spikelet sterility under elevated temperature regimes in IGC

Spikelet sterility of the three NILs and check varieties were evaluated at physiological maturity. For the control treatment, no high-temperature stress was applied in the glasshouse, all the genotypes showed high spikelet fertility (86.1–94.7%). When high-temperature stresses were applied as four temperature regimes, the spikelet fertility of all the genotypes decreased. In all the treatments, IR64 showed the lowest spikelet fertility, N22 showed the highest spikelet fertility, and the average spikelet fertility of the NILs for four temperature regimes increased as IR64EMF3 < IR64HT4 < IR64HT4EMF3.

When high-temperature treatments started from 8:00 (Treatment 08) and 9:00 (Treatment 09), some of the spikelets of IR64EMF3 already flowered, and the spikelet fertility of IR64EMF3 (25.5% and 24.8%) was higher but not significantly different from IR64 (16.0% and 13.3%). The spikelet fertility of IR64HT4 (48.8% and 39.7%) and IR64HT4EMF3 (48.7% and 43.5%) was significantly higher than that of IR64 (Fig. 3a, b).

Table 2 The number and size of fragments from the donors in the near isogenic lines (NILs)

| NILs            | Number of donor fragments | Size of donor fragments (Mb) | Total length of donor fragments (Mb) | Size of IR64 genome (Mb) | Similarity to IR64 (%) |
|-----------------|---------------------------|-------------------------------|--------------------------------------|-------------------------|------------------------|
| IR64EMF3        | 13                        | 0.31–4.79                     | 15.53                                | 369.37                  | 95.80                  |
| IR64HT4         | 4                         | 0.24–2.43                     | 5.04                                 | 369.37                  | 98.64                  |
| IR64HT4EMF3     | 6                         | 0.32–4.57                     | 10.37                                | 369.37                  | 97.19                  |

The lengths of the fragments and IR64 genome were calculated by using the positions of the SNP markers based on Nipponbare reference genome.

![Fig. 2 Flower opening time of the NILs and check varieties in the glasshouse (a) and in the field in Myanmar (b)]
When high-temperature treatments started from 10:00 (Treatment 10), all the spikelets of IR64EMF3 and IR64HT4EMF3 already flowered, and the spikelet fertility of IR64EMF3 (27.7%) and IR64HT4EMF3 (40.3%) were significantly higher than IR64 (14.2%). The spikelet fertility of IR64HT4EMF3 (50.1%) was higher than IR64EMF3 and IR64HT4 (Fig. 3c).

When high-temperature treatments started from 11:00 (Treatment 11), the spikelet fertility of all NILs was significantly higher than that of IR64 (Fig. 3d). Over two hours already passed since the fertilization process of IR64EMF3 and IR64HT4EMF3 was completed, the spikelet fertility of IR64EMF3 (53.5%) and IR64HT4EMF3 (70.4%) was higher than IR64HT4 (41.1%).

**Fig. 3** Spikelet fertility of the NILs and the check varieties treated in the indoor growth chambers. High temperature of 38 °C started from 8:00 for Treatment 08 (a), 9:00 for Treatment 09 (b), 10:00 for Treatment 10 (c), and 11:00 for Treatment 11 (d). Once temperature reached 38 °C, it lasted till 14:00 for all the treatments. The bars indicate the 95% confidence interval of the means. The letters on the bars indicate the differences among the genotypes compared by Tukey honestly significant difference test.

**Agronomic traits and spikelet fertility of the NILs and the check varieties in the field**

The agronomic traits of the NILs and the check varieties were examined at IRRI and CSU in the Philippines. At IRRI, the date of 50% heading was May-21 for IR64, May-24 for IR64EMF3, IR64HT4 and IR64HT4EMF3, and May-26 for N22, while at CSU, the date of 50% heading was June-13 for IR64, June-14 for IR64EMF3, IR64HT4 and IR64HT4EMF3, and June-15 for N22. Slight differences of some agronomic traits, including plant height, panicle length, flag leaf length, number of tillers and number of spikelets per panicle, were observed among the...
NILs at IRRI or at CSU. The yield of the NILs was not significantly different at IRRI or at CSU (Table 3).

The maximum temperature during flowering period was higher at CSU than at IRRI. The maximum temperature did not exceed 36 °C at IRRI, whereas it sometimes reached 38 °C at CSU (Supplemental Fig. 1). The average maximum temperature during flowering period was 35.2–35.3 °C for all the genotypes at IRRI, and it was 36.0–36.3 °C for
all the genotypes at CSU. There is no significant difference on average maximum temperature during flowering period among genotypes at IRRI or CSU, but the average maximum temperature during flowering period for all the genotypes at IRRI (35.3 ± 0.4) was significantly lower than that at CSU (36.1 ± 0.9). The spikelet fertility of all genotypes was high at IRRI (89.8–93.2%), whereas large difference in spikelet fertility among genotypes was found at CSU (73.5–93.9%). The spikelet fertility of IR64, IR64EMF3 and IR64HT4 was lower than IR64HT4EMF3 and N22 at CSU (Table 3).

Discussions

Flower opening time critically affects spikelet fertility under heat stress. One of the objectives in the present study is to elucidate the FOT of each NILs carrying the single QTL, either qHTSF4.1 or qEMF3, and developed NIL carrying two QTLs (qHTSF4.1 + qEMF3) in the genetic background of IR64. Flowering pattern observation in the greenhouse and field under the heat stress conditions revealed the significant advancement of FOT in the NILs carrying qEMF3 (Fig. 2). Flowering pattern of IR64HT4 was very similar with that of IR64, confirming that qHTSF4.1 confers the true heat tolerance inherited from the donor parents (N22) to IR64 (Ye et al. 2012, 2015a, b). Significant advancement of FOT in IR64HT4EMF3 indicates that heat tolerance and heat escape attributed from qHTSF4.1 and qEMF3, respectively, works under different mechanisms.

The QTL qEMF3 is a major QTL controlling flower opening time (Hirabayashi et al. 2015). qEMF3, however, just confer the heat escape but not heat tolerance (Hirabayashi et al. 2015). This study clarified that effectiveness of EMF trait was high temperature time-dependent; if the high temperature comes early time of day up to 10:00, EMF trait is not so effective in mitigating heat-induced spikelet sterility while heat tolerance is effective. By contrast, when high temperature started from 11:00, spikelet fertility was slightly higher in IR64EMF3 than IR64HT4 (Fig. 3). These results clearly indicated that EMF trait was very effective to escape from heat damage when heat stress commenced approximately 2–3 h after completion of flower opening under the given high temperature (38 °C) and relative humidity (70%) conditions. Previous report showed that heat stress over 38 °C only one hour after completion of flower opening was sufficient to escape the damage on fertility under the relative humidity conditions of 50–60% (Hirabayashi et al. 2015; Ishimaru et al. 2010), but the result obtained in this study was not the case. Humidity has a critical negative effect on spikelet fertility under high-temperature condition through the decrease in viable pollen grains shed onto stigma (Weerakoon et al. 2008). One example using IR36 showed that the difference in spikelet fertility between control humidity (60%) and high humidity (85%) was only 13.7% at 34 °C, while the difference became much larger by 49.6% at 36 °C due to the great increase in panicle surface temperature under highly humid condition (Weerakoon et al. 2008). Even 10–20% difference in relative humidity in the previous studies (Hirabayashi et al. 2015; Ishimaru et al. 2010) and the present study would result in the different effectiveness of EMF trait in heat escape.

The QTL qHTSF4.1 was identified in different rice populations and conferred heat tolerance in different backgrounds (Lafarge et al. 2017; Takai et al. 2020; Xiao et al. 2011a; Ye et al. 2012, 2015a). It was considered as a promising candidate for improving heat tolerance of rice (Ye et al. 2015b). The heat-tolerance QTLs qHTSF1.1 and qHTSF4.1 (Ye et al. 2012) were introgressed from N22 into a rice variety named Improved white ponni (IWP) through marker-assisted selection. The progenies with both qHTSF1.1 and qHTSF4.1 also showed higher spikelet fertility under high-temperature stress at the flowering stage (Vivitha et al. 2017). However, with only qHTSF4.1, the spikelet fertility of IR64HT4 and IR64HT4EMF3 was lower than that of N22 under high-temperature stress (Fig. 3), because there are qHTSF1.1 and possibly other QTLs exist in N22. Higher spikelet fertility in IR64HT4 than IR64 and IR64EMF3 under high-temperature stress was possibly due to the higher number of viable pollen grains shed onto stigma similar to its donor N22 (Jagadish et al. 2010b) and higher germination of the pollen grains (Shi et al. 2018). After high-temperature (38/23 °C, day/night) treatment, the percentage of spikelets with normal fertilization of HT NIL (IR64HT4) was significantly higher than the recurrent parent IR64 (Shi et al. 2018).

To mitigate heat-induced spikelet sterility, two strategies have been proposed. One is to develop heat-tolerant cultivars that maintain more vital pollen grains with high germination under heat stress. Another strategy is to breed cultivars that escape high temperature by flowering in the early-morning (Satake and Yoshida 1978). By pyramiding qEMF3 and qHTSF4.1 into the genetic background of IR64, the spikelet fertility of IR64HT4EMF3 showed the highest value among the NILs in all temperature regimes in the indoor growth chambers. While in the field, the high-temperature stress at IRRI is not so severe, all the lines showed high spikelet fertility (89.8–93.2%); the high-temperature stress at CSU was much severe during the flowering stage, some of the lines (IR64, IR64EMF3 and IR64HT4) showed lower spikelet fertility (73.5–77.7%), but the QTL pyramiding line IR64HT4EMF3 showed high spikelet fertility (89.3%). Notably, the spikelet fertility of IR64HT4EMF3 reached the similar level as that of N22 under late high-temperature stress (Treatment 11) in the indoor growth chamber and in the field at CSU. N22 is known as one of the most heat-tolerant varieties (Satake and Yoshida 1978), thereby it is widely used as heat-tolerant check variety (Jagadish et al.
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