Marker Assisted Pyramiding of QTLs for Heat Tolerance and Escape Upgrades Heat Resilience in Rice (*Oryza Sativa* L.)

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Research Article

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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.

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.

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; Ye et al. 2015a; Zhang et al. 2009; Zhang et al. 2008). 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 *aus* accessions (Lafarge et al. 2017; Takai et al. 2020; Xiao et al. 2011b; Ye et al. 2012; Ye et al. 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 hours 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 6K 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 BC3-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* were used to select the plants with homozygotes at both loci, and then the selected plants were genotyped using the rice 6K 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 (Figure 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 1g of complete fertilizer (14N-14P-14K). 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, $\pm 2^\circ\text{C}$). When the first panicle started heading, the heading date was recorded, the earliest 3-5 panicles of each plant were marked, 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 hours in the first IGC, (ii) from 9:00 in the second IGC for 5 hours, (iii) from 10:00 in the third IGC for 4 hours, and (iv) from 11:00 in the fourth IGC for 3 hours. 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.

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

Chamber	Time duration	Temperature (°C)	Relative humidity (%)	Light intensity ($\mu\text{mol m}^{-2}\text{s}^{-1}$)
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
	6: 00-9:00	24-38	75	0-580
IGC2 (Treatment 09)	9: 00-14:00	38	70	580
	14:00-18:00	38-24	75	580-0
	18: 00-6: 00	24	75	0
	6: 00-10:00	24-38	75	0-580
	10: 00-14:00	38	70	580
IGC3 (Treatment 10)	14:00-18:00	38-24	75	580-0
	18: 00-6: 00	24	75	0
	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
IGC4 (Treatment 11)	18: 00-6: 00	24	75	0

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 microenvironment 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, $\pm 2^\circ\text{C}$). 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 a 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-minute 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 in 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 under field conditions in two sites in the Philippines in 2015: the International Rice Research Institute (IRRI) at Los Baños, Laguna (14° 10' 7.8"N, 121° 15' 18.8" E); and the Cagayan State University (CSU) at Piat, Cagayan (17° 47' 30.7"N, 121° 30' 58.2"E). Based on the past 30 years local temperature records, the seeding dates of the genotypes were adjusted to ensure the plants flowering during the hottest period. Seeds were germinated and sown in the seeding bed, and transplanted at a density of 20 cm x 20 cm, one seedling per hill. The size of each plot was 12 m² (2 m x 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 x 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 were 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 Figure 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 x 20 cm. The size of each plot was 1.44 m² (0.6 m x 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 Minitab17. Genotypic data were sorted by using Excel 2013, and graphs of introgression in IR64 background were drawn by using GGT32.

Results

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 minutes later than IR64HT4 and IR64. Across the glasshouse and field conditions, FOT of each NIL showed the similar tendency (**Figure 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 four 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 (Figure 3a, 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 (Figure 3c).

When high temperature treatments started from 11:00 (Treatment 11), the spikelet fertility of all NILs was significantly higher than that of IR64 (Figure 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%) were higher than IR64HT4 (41.1%).

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 2).

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 Figure 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 were lower than IR64HT4EMF3 and N22 at CSU (Table2).

Table 2
Agronomic traits of the NILs and the check varieties in the field at IRRI and CSU.

		IRRI			CSU		
Traits	Genotype	Count	Mean	StDev	Count	Mean	StDev
Plant	IR64	15	103.1b	2.6	45	104.3bc	6.8
height	IR64EMF3	15	93.1d	2.4	45	100.5c	5.6
(cm)	IR64HT4	15	97.9c	2.9	45	106.2b	9.2
	IR64HT4EMF3	15	98.8c	3.5	45	102.2bc	6.3
	N22	15	115.3a	3.7	45	129.1a	8.3
Panicle	IR64	15	25.9a	1.7	45	25.8ab	1.5
length	IR64EMF3	15	24.2b	1.7	45	24.3b	1.8
(cm)	IR64HT4	15	25.8a	1.1	45	26.7a	2.3
	IR64HT4EMF3	15	25.2ab	0.9	45	26.5a	1.5
	N22	15	19.5c	1.4	45	19.3c	4.7
Flag	IR64	15	30.8b	4.6	45	32.8a	3.1
leaf	IR64EMF3	15	26.5c	3.9	45	28.3b	4
length	IR64HT4	15	36.0a	3.4	45	33.5a	5
(cm)	IR64HT4EMF3	15	28.8bc	3.6	45	30.2b	5.5
	N22	15	30.8b	2.7	45	30.4b	2.9
Number	IR64	15	14.5b	1.8	45	18.1b	3.1
of	IR64EMF3	15	10.9c	1.8	45	20.7a	3.5
tillers	IR64HT4	15	14.5b	2.2	45	18.3b	5.5
	IR64HT4EMF3	15	13.5b	1.6	45	17.1bc	3.4
	N22	15	17.2a	3.3	45	15.6c	3.9
Spikelets	IR64	15	130.2a	11.9	45	101.7b	16.4
per	IR64EMF3	15	113.1b	18.6	45	116.3a	18.3
panicle	IR64HT4	15	132.6a	9.6	45	116.7a	17.3
	IR64HT4EMF3	15	123.7ab	19.9	45	119.8a	16.9
	N22	15	96.1c	15.7	45	111.5a	12.8

		IRRI			CSU		
Spikelet	IR64	15	89.8b	1.6	45	73.5b	11.7
fertility	IR64EMF3	15	89.8b	2.4	45	77.8b	9.7
(%)	IR64HT4	15	93.2a	2.5	45	77.7b	12.3
	IR64HT4EMF3	15	90.6b	3.1	45	89.3a	3.4
	N22	15	90.5b	2.3	45	93.9a	2.4
Yield	IR64	3	4697.8a	167.1	3	4678.0a	281
(kg/ha)	IR64EMF3	3	4351.5a	132.5	3	4241.4a	170.3
	IR64HT4	3	4437.0a	223	3	4847.8a	119.6
	IR64HT4EMF3	3	4569.0a	374	3	4892.0a	340
	N22	3	2180.0b	344	3	2951.0b	278
Maximum	IR64	11	35.3a	0.4	11	36.0a	0.9
temperature	IR64EMF3	11	35.3a	0.4	11	36.1a	0.8
during	IR64HT4	11	35.3a	0.4	11	36.1a	0.8
flowering	IR64HT4EMF3	11	35.3a	0.4	11	36.1a	0.8
(°C)	N22	11	35.2a	0.4	11	36.3a	1.0

The maximum temperature during flowering included 5 days before 50% heading, 50% heading and 5 days after 50% heading. The letters after the means indicate the differences among the genotypes compared by Tukey honestly significant difference test.

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* (**Figure 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; Ye et al. 2015a; Ye et al. 2015b). 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 open 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 (Figure 3). These results clearly indicated that EMF trait was very effective to escape from heat damage when heat stress commenced approximately 2-3 hours 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; Ye et al. 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). 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. At the same time, the percentage of spikelets with arrested fertilization (fertilization stopped) of HT NIL (IR64HT4) was significantly lower 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. 2010a; Mackill et al. 1982; Manigbas et al. 2014; Poli et al. 2013; Shi et al. 2015; Ye et al. 2012). High spikelet fertility of IR64HT4EMF3 under high temperature stress clearly indicates that the pyramiding of *qHTSF4.1* and *qEMF3* is very effective strategy to mitigate heat-induced spikelet sterility at flowering stage resulting from enhanced heat tolerance and heat escape. Pyramiding of QTLs for heat tolerant and early morning flowering would be able to finish pollination processes without environmental disturbance under the severe high temperature stress.

Declarations

Author contribution C Ye, T Ishimaru and Z Su designed the experiments. C Ye, T Ishimaru, L Lambio and TM Htun conducted the experiments. C Ye, L Li, Y Long and Z He analyzed the data. C Ye and T Ishimaru wrote the paper. S Tang supervised the writing and editing.

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Conflict of interest The authors declare that they have no conflict of interest.

Data availability The data supporting the findings of this study are available within the article and its supplementary materials. Raw data of this study are available from the corresponding author [C. Ye] on request.

References

1. Battisti D, Naylor R (2009) Historical warning of future food insecurity with unprecedented seasonal heat. *Science* 323:240-244
2. Cao L, Zhao J, Zhan X, Li D, He L, Cheng S (2003) Mapping QTLs for heat tolerance and correlation between heat tolerance and photosynthetic rate in rice. *Chinese Journal of Rice Science* 17:223-227
3. Chen Q, Yu S, Li C, Mou T (2008) Identification of QTLs for heat tolerance at flowering stage in rice. *Scientia Agricultura Sinica* 41:315-321
4. Cheng L, Wang J, Uzokwe V, Meng L, Wang Y, Sun Y, Zhu L, Xu J, Li Z (2012) Genetic analysis of cold tolerance at seedling stage and heat tolerance at anthesis in rice. *Journal of Integrative Agriculture* 11:359-367

5. Hasegawa T, Yoshimoto M, Kuwagata T, Ishigooka Y, Kondo M, Ishimaru T (2009) The impact of global warming on rice production: lessons from spikelet sterility observed under the record hot summer of 2007. NIAES Annual Report 2008:23-25
6. Hirabayashi H, Sasaki K, Kambe T, Gannaban RB, Miras MA, Mendioro MS, Simon EV, Lumanglas PD, Fujita D, Takemoto-Kuno Y, Takeuchi Y, Kaji R, Kondo M, Kobayashi N, Ogawa T, Ando I, Jagadish KS, Ishimaru T (2015) qEMF3, a novel QTL for the early-morning flowering trait from wild rice, *Oryza officinalis*, to mitigate heat stress damage at flowering in rice, *O. sativa*. J Exp Bot 66:1227-1236
7. Ishimaru T, Hirabayashi H, Ida M, Takai T, San-Oh Y, Yoshinaga S, Ando I, Ogawa T, Kondo M (2010) A genetic resource for early-morning flowering trait of wild rice *Oryza officinalis* to mitigate high temperature-induced spikelet sterility at anthesis. Annals of Botany 106:515-520
8. Ishimaru T, Hirabayashi H, Sasaki K, Ye C, Kobayashi A (2016a) Breeding efforts to mitigate damage by heat stress to spikelet sterility and grain quality. Plant Production Science 19:12-21
9. Ishimaru T, Seefong X, Nallathambi J, Rajendran S, Yoshimoto M, Phoudalay L, Benjamin S, Hasegawa T, Hayashi K, Gurusamy A, Muthurajan R, Jagadish S (2016b) Quantifying rice spikelet sterility in potential heat-vulnerable regions: field surveys in southern Laos and southern India. Field Crops Research 190:3-9
10. Jagadish S, Cairns J, Lafitte R, Wheeler T, Price A, Craufurd P (2010a) Genetic analysis of heat tolerance at anthesis in rice. Crop Science 50:1633-1641
11. Jagadish S, Craufurd P, Wheeler T (2007) High temperature stress and spikelet fertility in rice. Journal of Experimental Botany 58:1627-1635
12. Jagadish S, Muthurajan R, Oane R, Wheeler T, Heuer S, Bennett J, Craufurd Q (2010b) Physiological and proteomic approaches to address heat tolerance during anthesis in rice. Journal of Experimental Botany 61:143-156
13. Lafarge T, Bueno C, Frouin J, Jacquin L, Courtois B, Ahmadi N (2017) Genome-wide association analysis for heat tolerance at flowering detected a large set of genes involved in adaptation to thermal and other stresses. PLoS ONE 12:e0171254
14. Li C, Peng C, Zhao Q, Xie P, Chen W (2004) Characteristic analysis of the abnormal high temperature in 2003 midsummer in Wuhan City. Journal of Central China Normal University 38:379-381
15. Mackill D, Coffman W, Rutger J (1982) Pollen shedding and combining ability for high temperature tolerance in rice. Crop Science 22:730-733
16. Manigbas N, Lambio L, Madrid L, Cardenas C (2014) Germplasm Innovation of Heat Tolerance in Rice for Irrigated Lowland Conditions in the Philippines. Rice Science 21:162-169
17. Matsui T (2009) Floret sterility induced by high temperatures at the flowering stage in rice. Japanese Journal of Crop Science 78:303-311
18. Matsui T, Kobayasi K, Yoshimoto M, Hasegawa T (2007) Stability of rice pollination in the field under hot and dry conditions in the Riverina Region of New South Wales, Australia. Plant Production Science 10:57-63

19. Matsui T, Omasa K (2002) Rice cultivars tolerant to high temperature at flowering: Anther characteristics. *Annals of Botany* 89:683-687
20. Matsushima S, Ikewada H, Maeda A, Honma S, Niki H (1982) Studies on rice cultivation in the tropics. 1. Yielding and ripening responses of the rice plant to the extremely hot and dry climate in Sudan. *Japanese Journal of Tropical Agriculture* 26:19-25
21. Nishiyama I, Satake T (1981) High temperature damage in the rice plant. *Japanese Journal of Tropical Agriculture* 25:14-19
22. Osada A, Sasiprapa V, Rahong M, Dhammanuvong S, Chakrabandho H (1973) Abnormal occurrence of empty grains of indica rice plants in the dry hot season in Thailand. *Proceedings of Crop Science Society of Japan* 42:103-109
23. Poli Y, Basava R, Panigrahy M, Vinukonda V, Dokula N, Voleti S, Desiraju S, Neelamraju S (2013) Characterization of a Nagina22 rice mutant for heat tolerance and mapping of yield traits. *Rice (New York, NY)* 6:36
24. Prasad P, Boote K, Allen L, Sheehy J, Thomas J (2006) Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Research* 95:398-411
25. Satake T, Yoshida S (1978) High temperature induced sterility in Indica rice at flowering. *Japanese Journal of Crop Science* 47:6-17
26. Shi W, Ishimaru T, Gannaban R, Oane W, Jagadish S (2015) Popular rice cultivars show contrasting responses to heat stress at gametogenesis and anthesis. *Crop Science* 55:589-596
27. Shi W, Li X, Schmidt R, Struik P, Yin X, Jagadish S (2018) Pollen germination and in vivo fertilization in response to high-temperature during flowering in hybrid and inbred rice. *Plant Cell & Environment* 41:1287-1297
28. Takai T, Lumanglas P, Simon E (2020) Genetic mechanism of heat stress tolerance at anthesis among three different rice varieties with different fertilities under heat stress. *Plant Production Science* 23:529-538
29. Thomson M, Singh N, Dwiyantri M, Wang D, Wright M, Perez F, DeClerck G, Chin J, Malitic-Layaoen G, Juanillas V, Dilla-Ermita C, Mauleon R, Kretzschmar T, McCouch S (2017) Large-scale deployment of a rice 6 K SNP array for genetics and breeding applications. *Rice (New York, NY)* 10:40
30. Tian X, Matsui T, Li S, Yoshimoto M, Kobayasi K, Hasegawa T (2010) Heat-induced floret sterility of hybrid rice (*Oryza sativa* L.) cultivars under humid and low wind conditions in the field of Jiangnan basin, China. *Plant Production Science* 13:243-251
31. Vivitha P, Raveendran M, Vijayalakshmi D (2017) Introgression of QTLs Controlling Spikelet Fertility Maintains Membrane Integrity and Grain Yield in Improved White Ponni Derived Progenies Exposed to Heat Stress. *Rice Science* 24:32-40
32. Wassmann R, Jagadish S, Heuer S, Ismail A, Redona E, Serraj R, Singh R, Howell G, Pathak H, Sumfleth K (2009) Climate change affecting rice production: The physiological and agronomic basis for possible adaptation strategies. *Advances in Agronomy* 101:59-122

33. Weerakoon W, Maruyama A, Ohba K (2008) Impact of humidity on temperature-induced grain sterility in rice. *Journal of Agronomy and Crop Science* 194:135-140
34. Xiao Y, Pan Y, Luo L, Deng H, Zhang G, Tang W, Chen L (2011a) Quantitative trait loci associated with pollen fertility under high temperature stress at flowering stage in rice. *Rice Science* 18:204-209
35. Xiao Y, Pan Y, Luo L, Zhang G, Deng H, Dai L, Liu X, Tang W, Chen L, Wang G (2011b) Quantitative trait loci associated with seed set under high temperature stress at the flowering stage in rice. *Euphytica* 178:331-338
36. Yang H, Huang Z, Jiang Z, Wang X (2004) High temperature damage and its protective technologies of early and middle season rice in Anhui province. *Journal of Anhui Agriculture Science* 32:3-4
37. Ye C, Argayoso M, Redoña E, Sierra S, Laza M, Dilla C, Mo Y, Thomson M, Chin J, Delaviña C, Diaz G, Hernandez J (2012) Mapping QTL for heat tolerance at flowering stage in rice using SNP markers. *Plant Breeding* 131:33-41
38. Ye C, Tenorio F, Argayoso M, Laza M, Koh H, Redona E, Jagadish K, Gregorio G (2015a) Identifying and confirming quantitative trait loci associated with heat tolerance at flowering stage in different rice populations. *BMC Genetics* 16:41
39. Ye C, Tenorio F, Redona E, Morales-Cortezano P, Cabrega G, Jagadish K, Gregorio G (2015b) Fine-mapping and validating qHTSF4.1 to increase spikelet fertility under heat stress at flowering in rice. *TAG Theoretical and applied genetics Theoretische und angewandte Genetik* 128:1507-15017
40. Zhang G, Chen L, Xiao G, Xiao Y, Chen X, Zhang S (2009) Bulk segregant analysis to detect QTL related to heat tolerance in rice using SSR markers. *Agricultural Sciences in China* 8:482-487
41. Zhang T, Yang L, Jiang K, Huang M, Sun Q, Chen W, Zheng J (2008) QTL mapping for heat tolerance of the tassel period of rice. *Molecular Plant Breeding* 6:867-873

Figures

Figure 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).

Figure 2

Flower opening time of the NILs and check varieties in the glasshouse (a) and in the field in Myanmar (b).

(a) Treatment 08

(b) Treatment 09

(c) Treatment 10

(d) Treatment 11

Figure 3

Spikelet fertility of the NILs and the check varieties treated in the indoor growth chambers. High temperature of 38 oC started from 8:00 for (a) Treatment 08, 9:00 for (b) Treatment 09, 10:00 for (c) Treatment 10, and 11:00 for (d) Treatment 11. Once temperature reached at 38 oC, 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.

Supplementary Files

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- [FigS1Themaximumtemperatureduringthecropseason.pdf](#)