1 Research paper

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3 An early-morning flowering trait in rice helps retain grain yield under heat stress field
4 conditions at flowering stage

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1 Abstract (199-words)

2

3 Early-morning flowering (EMF) trait is supposed to be effective in retaining grain yield

4 due to mitigation of heat-induced spikelet sterility at flowering in rice. This study

5 evaluated (i) phenotypic differences between a near-isogenic line carrying a QTL for

6 EMF trait, designated as IR64+*qEMF3*, and a recurrent parent, IR64, under wide

7 variation in climates and (ii) whether an EMF trait can retain grain yield under heat

8 stress at flowering. IR64+*qEMF3* had significant earlier flower opening time (FOT) in

9 diverse environmental conditions including temperate, subtropical, and tropical regions.

10 Under normal temperatures at flowering, IR64+*qEMF3* had similar grain yield to IR64

11 with some significant changes in agronomic traits and yield components. Field trials in

12 heat-vulnerable regions of central Myanmar for seven crop seasons showed that higher

13 percentage of filled grains contributed to the significantly higher grain yield in

14 IR64+*qEMF3* among yield components when plants were exposed to daily maximum

15 air temperatures around 36.5 °C or higher. Lower spikelet sterility in IR64+*qEMF3* was

16 attributed to the earlier FOT during cooler early morning hours. This is the first field

17 study that clearly demonstrates the advantage of the EMF trait for retaining grain yield

18 by stabilizing percentage of fertile grains under heat stress at flowering.

1

2 Keywords: early-morning flowering; flower opening time; heat stress; percentage of
3 filled grains; rice; yield-related traits

4

5 Abbreviations:

6 CARC/NARO; Central Region Agricultural Research Center, National Agriculture and
7 Food Research Organization

8 DAR; Department of Agricultural Research

9 EMF; early-morning flowering

10 FOT; flower opening time

11 ICRR; Indonesian Center for Rice Research

12 JIRCAS; Japan International Research Center for Agricultural Sciences

13 NIL; near-isogenic line

14 PFG; percentage of filled grains

15 PN; panicle number per hill

16 SN; spikelet number per panicle

17 TNAU; Tamil Nadu Agricultural University

18 YAU; Yezin Agricultural University

1 1000GW; one thousand grain weight

1 **Introduction**

2

3 Rice (*Oryza sativa* L.) is one of the most important staples feeding half the
4 world's population (Carriger and Vallee, 2007). While there is a need to produce more
5 rice in order to feed the increasing population, challenges for increasing rice production
6 have been rising due to progressive global warming (IPCC, 2013). Consequently,
7 development of climate-smart rice cultivars is a pressing issue today. Genetic
8 improvement of heat-resilience is one of the key requirements for enhancing global food
9 security to tackle the increasing episodes of heat stress damage on crop production.

10 Major cereal crops show extreme sensitivity to heat during anthesis (Prasad et
11 al., 2017). Chamber experiments conducted previously revealed that high proportion of
12 sterility occurs in rice when spikelets are exposed to temperatures around 35 °C (Matsui
13 et al. 1997; Satake and Yoshida, 1978) even for a short period of time (Jagadish et al.,
14 2007). Actual increase in spikelet sterility by heat stress at flowering was reported in hot
15 dry seasons of tropical and subtropical regions (Ishimaru et al., 2016; Matsushima et al.
16 1982; Osada et al., 1973). Rice crop is considered to be already at critical limits of heat
17 stress vulnerability in central Thailand and Myanmar in dry season (Wassmann et al.,
18 2009). Prediction models have identified high risk areas for reduction in rice grain yield

1 by global warming in many rice-growing regions of Asia, mostly due to heat-induced
2 spikelet sterility (See review, Horie, 2019). Central Myanmar has been projected as one
3 among the heat-vulnerable regions for spikelet sterility and yield reduction in the future
4 (Horie, 2019; Wassmann et al., 2009).

5 The EMF trait is proposed to be effective in escaping heat-induced spikelet
6 sterility at flowering by shifting FOT to the cooler early morning times (Satake and
7 Yoshida, 1978). Flower opening of modern cultivars predominantly occurs between
8 3.5–5.5 hours after dawn in the natural conditions of tropics (Bheemanahalli et al.,
9 2017; Hirabayashi et al., 2015). Under elevated high temperature greenhouse conditions,
10 spikelets that flower at later hours tend to be sterile with higher proportion; hence,
11 shifting flower opening time to the cooler early morning, even a one-hour advancement,
12 is effective in mitigating heat-induced spikelet sterility at flowering (Ishimaru et al.,
13 2010). We previously developed a near-isogenic line (NIL) carrying a QTL for EMF
14 (*qEMF3*) derived from a wild rice, *O. officinalis*, in the genetic backgrounds of IR64
15 (Hirabayashi et al., 2015). The NIL was designated as IR64+*qEMF3*. *qEMF3* advanced
16 FOT by approximately 1.5–2.0 h in the tropical Philippines during both wet and dry
17 seasons (Hirabayashi et al., 2015). Another field experiment also showed earlier FOT
18 and lower sterility in IR64+*qEMF3* compared to a genetically diverse panel of

1 germplasm in the dry rice growing season in Tamil Nadu, India (Bheemanahalli et al.,
2 2017). However, whether the EMF trait has yield advantage under different heat stress
3 levels on the field during flowering is yet to be ascertained. Whether *qEMF3* itself
4 influences grain yield and other phenotypes of IR64 under normal temperatures during
5 flowering stage is still not clear.

6 This study consists of two major objectives: the first is to evaluate
7 advancement of FOT, agronomic traits, and yield performance of IR64+*qEMF3* in a
8 wide range of field environments, including temperate, subtropical, and tropical regions,
9 under normal rice-growing temperatures at flowering, and the second objective is to
10 investigate if the EMF trait in the field helps in retaining rice grain yield in the
11 heat-vulnerable region of central Myanmar.

1 **Materials and Methods**

2

3 **Crop establishment**

4

5 IR64, an Indica group cultivar, and a BC₃-derived NIL with IR64 genetic
6 background, IR64+*qEMF3* (Hirabayashi et al., 2015), were used for all six experimental
7 sites, which were located in the temperate (Joetsu, Niigata, Japan), subtropical (Ishigaki,
8 Okinawa, Japan), and tropical (Nay Pyi Taw, Myanmar; Los Baños, Laguna,
9 Philippines; Coimbatore, Tamil Nadu, India; Subang, West Java, Indonesia) regions
10 across five countries (Table 1), following the local rice cultivation practices
11 (Supplementary Table S1). Plants were grown in fields during the summer/dry season
12 (Table 1). Note that two staggered sowings were employed in Nay Pyi Taw for 2017DS,
13 2018DS, and 2019DS to expose rice plants to different temperatures at heading. 50%
14 heading date was recorded for each genotype in each trial to determine the
15 days-to-heading. Plant materials were kept healthy by spraying agrochemicals, when
16 required.

17

18 Flower opening time, spikelet sterility, and monitoring of local air temperatures

1

2 Flowering pattern observation and data analysis for the time to reach the peak

3 and end of flower opening (FOT50 and FOT90, respectively) was conducted following

4 the method of Hirabayashi et al., (2015). Using four heading panicles per plot per day,

5 opened spikelets were marked every 30 min by fine-tipped pens, and this flowering

6 observation was continued for more than three days in all sites. FOT50 and FOT90 were

7 calculated using the Koyomi Station software

8 (<http://eco.mtk.nao.ac.jp/koyomi/index.html.en>) and R program (ver. 3.6.2). Note that

9 FOT50 and FOT 90 were presented as time after dawn. On 22 April of 2018, on Crop 1

10 in Nay Pyi Taw, opened spikelets were marked with different colored pens following the

11 protocol of Ishimaru et al. (2010); spikelets opened before 0700H were marked with

12 blue pens, those between 0700H-0830H with green pens, those between 0830H-1000H

13 with purple pens, and those after 1000H with black pens. At maturity, marked spikelets

14 marked with different colors were collected and sterility was physically checked. Air

15 temperature during flowering was monitored by the meteorological station installed in

16 each experimental site.

17

18 Agronomic traits, grain yield, and yield components

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2 At maturity, agronomic traits such as plant height (from ground level to the tip
3 of panicles), panicle length (from panicle neck to the tip of panicle), flag leaf length,
4 and flag leaf width were measured using the longest tiller in a hill. Grain yield and yield
5 components per plant were determined in Joetsu, Nay Pyi Taw, Los Baños, Coimbatore,
6 and Subang. Note that only one replicate was designed in Subang, but 3–5 replicates in
7 other sites (Supplementary Table S1). Subsamples were prepared for selecting filled
8 grains by an air-blower or water method and for subsequent determination of one
9 thousand grain weight (1000GW). Selection of filled grains for seven yield trials in Nay
10 Pyi Taw was consistently conducted by water method. Grain yield and 1000 GW were
11 expressed based on 14% moisture content. Grain length, width, and thickness were
12 measured with a digital caliper (SINWA Sokutei, Sanjo, Niigata, Japan) using the grains
13 harvested in Joetsu, Ishigaki, and Nay Pyi Taw.

14

15 Statistical analysis

16

17 Analysis of variance was tested by R program (ver. 3.6.2). Multiple regression
18 analysis and *t*-test between genotypes were conducted by Microsoft Excel 2016.

1 **Results**

2

3 Flower opening time

4

5 Flowering pattern observation was conducted in Joetsu, Ishigaki, Nay Pyi Taw,
6 Los Baños, and Subang under the condition that there was great variation in average
7 daily maximum temperature during heading (Table 1; Supplementary Table 2). In Joetsu,
8 Ishigaki, Los Baños, and Subang, FOT50 was significantly earlier in IR64+*qEMF3* than
9 IR64 in each of the tested sites, ranging from 1.9 h to 3.5 h in IR64+*qEMF3* and 3.7 h to
10 4.6 h in IR64 on the basis of time after dawn (Table 2). FOT90 was also significantly
11 earlier in IR64+*qEMF3* than in IR64, ranging from 2.5 h to 4.0 h in IR64+*qEMF3* and
12 4.2 h to 5.0 h in IR64 (Table 2). The genotype difference in the average of FOT50 and
13 of FOT90 across locations was −1.5 h and −1.2 h, respectively (Table 2). In Nay Pyi
14 Taw, flowering pattern observation conducted under a wide range of average daily
15 maximum temperatures for seven crop seasons also showed significantly earlier FOT in
16 IR64+*qEMF3* than IR64 (Supplementary Table 2).

17

18 Agronomic traits, grain yield, and yield components under normal temperatures at

1 flowering

2

3 Days-to-heading in IR64+*qEMF3* was the same as IR64 or up to five days
4 longer than in IR64 (Tables 3, 4; Supplementary Table S3). Panicle length were not
5 significantly different, while flag leaf length and flag leaf width were slightly larger in
6 IR64+*qEMF3* (Table 3; Supplementary Table S3). Plant height was significantly higher
7 in IR64+*qEMF3* than IR64 in Subang (Supplementary Table S3).

8

9 Grain yield and yield components under normal temperatures at flowering

10

11 Grain yield and yield components were further examined in Joetsu, Los Baños,
12 Coimbatore, and Subang (Table 4; Supplementary Table S3), where average daily
13 maximum temperatures at flowering was in the normal ranges below 35.5 °C (Table 1).
14 Grain yield and panicle number per plant (PN) was not significantly different between
15 IR64 and IR64+*qEMF3*, but spikelet number per panicle (SN) was significantly larger
16 and 1000GW was significantly smaller in IR64+*qEMF3* (Table 4; Supplementary Table
17 S3). Smaller 1000GW in IR64+*qEMF3* was mainly due to the smaller length and width
18 of grains compared to IR64 grains (Supplementary Table S4). Percentages of filled

1 grains (PFG) was similar or slightly higher in IR64+*qEMF3* on an average, but the trend
2 was not the same on all locations (Table 4; Supplementary Table S3).
3
4 Grain yield and yield components in heat-vulnerable regions of central Myanmar
5
6 Grain yield and yield components were evaluated in heat-vulnerable regions of
7 central Myanmar for seven crop seasons across four years (Table 5). Average daily
8 maximum temperatures during heading ranged from 35.0 °C to 38.3 °C (Table 5). Grain
9 yield per plant was significantly higher in IR64+*qEMF3* than IR64, resulting in yield
10 advantage of IR64+*qEMF3* by 22.4% over IR64 on an average. PN was similar between
11 genotypes, while SN and 1000GW was significantly lower in IR64+*qEMF3* than in
12 IR64. Such a trend in PN, SN, and 1000GW characters was similar in other locations as
13 well, as shown in Table 4. Significant interaction between the genotype and
14 environment was observed in PFG; difference in PFG between genotypes in each season
15 ranged from 0.8% to 17.4%, and PFG was 9.3% higher in IR64+*qEMF3* than in IR64
16 on an average (Table 5). Multiple regression analysis revealed a significant positive
17 effect of PFG on yield advantage of IR64+*qEMF3*, while other parameters such as PN,
18 SN and 1000GW did not have a significant effect (Figure 1). The relationship between

1 PFG and average daily maximum temperatures during heading is plotted in Figure 2.

2 Difference in PFG between genotypes was small when average daily maximum

3 temperatures during heading was below 36.5 °C. PFG steadily decreased in IR64 when

4 average daily maximum temperatures during heading was greater than 36.5 °C, and it

5 sharply dropped to 65.6% at 37.9 °C (Figure 2). On the contrary, PFG was stable at the

6 high level of around 90% until 37 °C and gradually decreased to 83.0% at 38.3 °C in

7 IR64+*qEMF3* (Figure 2).

8 Spikelet sterility was examined in different time periods of flower opening (Figure 3).

9 During flowering pattern observation (recorded with different colored pens), air

10 temperature steadily increased from 25.8 °C at 0700H to 30.0 °C at 0930H, and up to

11 34.0 °C at noon (Figure 3A). Under such air temperature condition, flower opening in

12 IR64+*qEMF3* started after 0730H, peaked at 0900H, and almost ended by 0930H

13 (Figure 3A). On the other hand, flower opening in IR64 started after 0830H, peaked at

14 0930H, and ended by 1030H (Figure 3A). Spikelet sterility in each time period was

15 3.2% and 8.6% for spikelets that flowered during 0700-0830H (green-lined bar) and

16 during 0830-1000H (purple-lined bar), respectively, in IR64+*qEMF3* (Figure 3B). On

17 the other hand, spikelet sterility was 10.2% and 47.2% for spikelets flowered during

18 0830-1000H (purple bar) and after 1000H (black bar), respectively, in IR64 (Figure 3B).

- 1 As a consequence, spikelet sterility was 17.0% and 8.9% in IR64 and IR64+*qEMF3*,
- 2 respectively, on that single day (Figure 3B).

1 Discussion

2

3 *Significant advancement of FOT by qEMF3 in a wide range of environmental*
4 *conditions*

5

6 Hirabayashi et al., (2015) and Bheemanahalli et al., (2017) previously reported
7 an earlier FOT in IR64+*qEMF3* than IR64 in Philippines (dry and wet seasons) and
8 southern India (dry season), under field conditions. This study validated the genetic gain
9 in advancing FOT by *qEMF3* under diverse environments including temperate to
10 tropical climates in the genetic background of IR64 (Table 2; Supplementary Table S2).

11 It should be noted, however, that the difference in FOT between IR64 and IR64+*qEMF3*
12 varies depending on the experimental site or seasons tested (Table 2; Supplementary
13 Table S2). It is possible that this difference is because FOT is determined in the fields
14 by microclimates such as solar radiation and vapor pressure deficit in addition to air
15 temperature (Julia and Dingkuhn, 2012; Kobayasi et al., 2010). Development of a
16 prediction model for FOT that takes into consideration various such meteorological
17 parameters will help us in identifying the locations where *qEMF3* can make a
18 significant advancement in FOT to escape heat stress at flowering. The nature of

1 interactions between genetic and environmental factors on FOT in IR64+*qEMF3* is still
2 not very clear.

3

4 *qEMF3 helps retain grain yield by stabilizing percentage of filled grains under heat*
5 *stress at flowering*

6

7 Central Myanmar is projected as one of the heat-vulnerable regions for spikelet
8 sterility and yield reduction in the future (Horie, 2019; Wassmann et al., 2009). By
9 employing staggered sowings in different dry seasons, wide range of average daily
10 maximum temperatures during heading was obtained (Table 5). IR64+*qEMF3* was
11 tested under such field conditions to examine if an EMF trait could mitigate the yield
12 loss under heat stress at flowering.

13 PFG was similar between genotypes or slightly higher in IR64+*qEMF3* under
14 normal temperature at flowering (Table 4; Supplementary Table S3), whereas a total of
15 seven trials in the central Myanmar revealed that PFG greatly varied depending on the
16 seasons (Table 5). Genetic and environmental interaction were not observed in other
17 yield components such as PN, SN, and 1000GW (Table 5). A clear difference in
18 response of PFG to average daily maximum temperature at heading between genotypes

1 was observed (Figure 2). PFG steadily dropped in IR64 as average daily maximum
2 temperature at heading increased above 36.5 °C (Figure 2). On the other hand, high PFG
3 of greater than 80% was maintained in IR64+*qEMF3* even when average daily
4 maximum temperature at heading was around 38 °C (Figure 2). Among yield
5 components, PFG was the only parameter that significantly contributed to the yield
6 advantage of IR64+*qEMF3* (Figure 1). Flowering pattern observation revealed less
7 frequency of spikelet sterility in IR64+*qEMF3* than in IR64 on a single day due to the
8 earlier FOT in IR64+*qEMF3* (Figure 3A, 3B). These results indicate the yield advantage
9 that *qEMF3* conferred under high temperature conditions at heading by significant
10 advancement of FOT in IR64 genetic background. Notably, an approximately one-hour
11 difference in FOT50 and FOT 90 in Crop 1 of 2018 (Supplementary Table S2) made a
12 difference in spikelet sterility between IR64 and IR64+*qEMF3* (Figure 3B), supporting
13 that one-hour advancement of FOT is sufficient to avoid heat-induced spikelet sterility
14 as shown in previous chamber experiments (Ishimaru et al. 2010; Satake and Yoshida
15 1978).

16 Horie (2019) documented that EMF is one of the key traits that could retain rice grain
17 yield by mitigating heat-induced spikelet sterility during flowering under future hotter
18 temperatures in inland of continental South East Asia. Our study clearly demonstrated

1 that EMF trait could diminish heat stress damage at flowering on grain yield through
2 stabilization of PFG in the hot dry season of central Myanmar. IR64 is a moderately
3 heat-tolerant cultivar (Shi et al., 2015). Some of Myanmar cultivars could be reaching
4 critical limits of heat tolerance as described by Wassmann et al., (2009). Assessment of
5 heat tolerance among Myanmar local cultivars helps to identify the target cultivars to be
6 conferred with EMF trait. Heat-vulnerable regions, in terms of heat-induced spikelet
7 sterility, are estimated to be located in different geographic locations including China,
8 South-East Asia, South Asia, and West Africa (Laborte et al., 2012). Since
9 IR64+*qEMF3* can significantly advance FOT in diverse environmental conditions
10 (Table 2; Supplementary Table S2), *qEMF3* may potentially minimize reduction in
11 heat-induced spikelet sterility across regions. It should be noted, however, microclimate
12 including humidity (Matsui et al., 2014; Tian et al., 2011), wind velocity (Ishimaru et al.,
13 2012; Matsui et al., 1997), and solar radiation (Ishimaru et al., 2016), which are not
14 inclusive in analysis in the present study, have a complex interaction effect on spikelet
15 sterility under field heat stress through changes in panicle temperature (Yoshimoto et al.,
16 2011). Whether IR64+*qEMF3* can mitigate yield loss in other heat-vulnerable regions
17 with different microclimate needs to be further tested.

18

1 *Further breeding efforts for the genetic improvement of IR64+qEMF3 and other*
2 *backgrounds*

3
4 Some yield components were significantly different between genotypes. SN
5 tended to be higher while 1000GW was lower in IR64+*qEMF3* than in IR64 (Table 4, 5;
6 Supplementary Table S3). However, PN was not different between genotypes (Table 4;
7 Supplementary Table S3). IR64+*qEMF3* had larger flag leaf size compared to IR64
8 (Table 3; Supplementary Table S3). Smaller 1000GW in IR64+*qEMF3* was mainly due
9 to the smaller length of the grains (Supplementary Table S4). These results suggest that
10 physical constraints due to small husk size, and not insufficient source supply, would be
11 responsible for the smaller 1000GW observed. Ohsumi et al., (2011) demonstrated that
12 there is a negative correlation between spikelet number per panicles and 1000GW using
13 NILs for increased spikelet number. Whether *qEMF3* has pleiotropic effects on SN and
14 1000GW as well as the EMF trait must be further investigated. Introgression of QTL for
15 grain size detected in genetic background of Indica group cultivars (Kato et al., 2011;
16 Qing et al., 2018) may retrieve 1000 GW of IR64+*qEMF3* to similar levels as those of
17 IR64. Regarding other agronomic traits, minor change in days-to-heading were
18 observed (Table 3, 5; Supplementary Table S3). IR64 is a popular Indica group cultivar

1 that is most widely grown in Asia (Brennan and Malabayabas, 2011) and its progeny
 2 continues to be grown quite widely. Our study provides breeders some fundamental
 3 information on the changes in plant phenotypes that can occur due to the introgression
 4 of *qEMF3* to IR64 genetic background. Transfer of *qEMF3* to popular cultivars in the
 5 temperate, subtropical, and tropical regions by marker-assisted breeding is an ongoing
 6 project. The effects of *qEMF3* on agronomic traits and grain yield on other genetic
 7 backgrounds need to be further investigated.

1 **Conclusion**

2

3 This is the first field study to clearly demonstrate that the EMF trait can retain
4 rice grain yield through stabilization of PFG under stressful conditions in
5 heat-vulnerable regions. Significant advancement of FOT due to *qEMF3* was observed
6 in wide range of environments including temperate, subtropical, and tropical regions,
7 while the changes in agronomic traits due to the introgression of *qEMF3* into genetic
8 background of IR64 were minimal. This result indicates the possibility that *qEMF3* can
9 also be effective in retaining rice grain yield in other heat-vulnerable regions and
10 genetic backgrounds. A breeding program to transfer *qEMF3* to local cultivars grown in
11 hot dry seasons through marker-assisted selection is an appropriate strategy that is
12 recommended.

Supplementary data

Supplementary Table S1 Experimental design and fertilizer conditions in each experimental site.

^aThe experimental plots were arranged in a randomized complete block design in Joetsu, Los Baños, Nay Pyi Taw, and Coimbatore.

^b 60 panicles that were randomly chosen from the bulk of panicles were used for the determination of yield and yield components.

Supplementary Table S2 Time to reach the peak (FOT50) and end (FOT90) of flower opening in Nay Pyi Taw.

***Significant at 0.1% level by ANOVA.

Dates of observation were 3–6 April in Crop 1 of 2015, 28–30 April and 1 May in Crop 1 of 2017, 16–19 May in Crop 2 of 2017, 21–24 April in Crop 1 of 2018, 16–19 May 12–14 in Crop 2 of 2018, 4–7 April for IR64 and 8–11 April for IR64+*qEMF3* in Crop 1 of 2019, and 3–6 May for IR64 and 8–11 May for IR64+*qEMF3* in Crop 2 of 2019.

FOT50 and FOT90 are expressed as hours after dawn. Average of maximum temperature during heading is shown in Table 5.

Supplementary Table S3 Agronomic traits (upper), yield, and yield components (lower) of IR64 and IR64+*qEMF3* in Subang.

Measurement was conducted by individual 14 plants except for Days to heading.

†, **, *** Significant at the 10%, 1%, and 0.1% level, respectively, by *t*-test.

Supplementary Table S4 Physical dimensions of the grain in IR64 and IR64+*qEMF3*.

^a Measurement in Nay Pyi Taw was done using grains harvested in Crop 1 of 2019.

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Table 1 Geographical location of experimental sites and growth season(s).

Experimental site	Geographical location	Above sea level (m)	Season(s) of experiment (summer/dry season)	Average daily maximum temperature during heading (°C) ^b	
				IR64	IR64+ <i>qEMF3</i>
CARC/NARO, Joetsu, Niigata, Japan	37°1'N, 138°2'E	10	2016	29.5	30.8
JIRCAS, Ishigaki, Okinawa, Japan	24°4'N, 124°2'E	27	2015	32.8	33.1
YAU, Nay Pyi Taw, Myanmar	19°8'N, 96°3'E	98	2015, 2017 ^a , 2018 ^a , 2019 ^a	35.0-37.9 ^c	35.0-38.3 ^c
IRRI, Los Baños, Laguna, Philippines	14°1'N, 121°2'E	22	2014	29.6	27.7
TNAU, Coimbatore, Tamil Nadu, India	11°0'N, 76°5'E	426	2020	34.4	35.5
ICRR, Subang, West Java, Indonesia	6°4'S, 107°6'E	26	2015	32.2	32.2

^aTwo staggered sowings were employed in each dry season.

^bAverage daily maximum temperature during heading was calculated for six days around 50% heading date. 50% heading date was positioned as third day of six days.

^cAverage daily maximum temperature during heading in each season and genotype is indicated in Table 5.

Table 2 Time to reach the peak (FOT50) and end (FOT90) of flower opening in Joetsu, Ishigaki, Los Baños, and Subang.

Experimental site	FOT50		FOT90	
	IR64	IR64+ <i>qEMF3</i>	IR64	IR64+ <i>qEMF3</i>
Joetsu	4.6	3.2	5.0	4.0
Ishigaki	3.7	2.5	4.2	3.3
Los Baños	4.4	1.9	4.8	2.5
Subang	4.3	3.5	4.8	4.0
ANOVA	Genotype (G)	***	***	***
	Location (E)	***	***	***
	G × E	**	**	**
	Average of genotype	4.3	2.8	4.7

, * Significant at the 1%, and 0.1% level by ANOVA, respectively.

Dates of observation were 12–14 August 2016 in Joetsu, 23–26 June 2015 in Ishigaki, 19, 20, 23 February 2014 in Los Baños, 19–23 August 2015 in Subang.

FOT and FOT90 are expressed as hours after dawn.

Table 3 Agronomic traits of IR64 and IR64+*qEMF3* in Joetsu, Nay Pyi Taw, Los Baños, and Coimbatore.

Experimental site		Days to heading		Plant height (cm)		Panicle length (cm)		Flag leaf length (cm)		Flag leaf width (cm)	
		IR64	IR64 + <i>qEMF3</i>	IR64	IR64 + <i>qEMF3</i>	IR64	IR64 + <i>qEMF3</i>	IR64	IR64 + <i>qEMF3</i>	IR64	IR64 + <i>qEMF3</i>
Joetsu		109	111 ^b	99.3	99.9	23.9	23.2	21.7	21.0	1.25	1.31
Nay Pyi Taw ^a		101	101 ^b	84.8	83.8	23.5	22.8	20.5	21.1	1.25	1.28
Los Baños		82.6	86.4	83.3	82.4	22.8	22.1	29.4	30.8	1.24	1.28
Coimbatore		88.3	88.7	85.2	85.7	23.3	24.1	23.1	24.4	1.06	1.11
ANOVA	Genotype (G)	-		ns		ns		†		**	
	Location (E)	-		***		***		***		***	
	G × E	-		ns		†		ns		ns	
	Average of genotype	95.2	96.8	88.2	88.0	23.4	23.1	23.7	24.3	1.20	1.25

†, **, *** Significant at the 10%, 1%, and 0.1% level, respectively, by ANOVA; ns: not significant.

^aMeasurement in Nay Pyi Taw was conducted in 2015DS.

^bDays-to-heading data was taken only from one plot with intermediate days-to-heading for each genotype.

Table 4 Grain yield and yield components in IR64 and IR64+*qEMF3* at normal temperatures during heading.

Experimental site	Grain yield		Panicle Number		Spikelet number		Filled grains		1000GW	
	(g plant ⁻¹)		(hill ⁻¹)		(panicle ⁻¹)		(%)		(g)	
	IR64	IR64 <i>+qEMF3</i>	IR64	IR64 <i>+qEMF3</i>	IR64	IR64 <i>+qEMF3</i>	IR64	IR64 <i>+qEMF3</i>	IR64	IR64 <i>+qEMF3</i>
Joetsu	38.6	39.6	18.0	18.5	95.5	99.9	75.6	82.8	29.7	25.9
Los Baños	25.3	25.8	13.8	12.7	73.0	102.4	91.5	88.9	27.5	22.7
Coimbatore	46.6	48.9	19.3	20.0	111.8	117.8	86.7	91.8	24.2	22.2
ANOVA	Genotype (G)	ns	ns	ns	***	***	**	**	***	***
	Location (E)	***	***	***	***	***	***	***	***	***
	G × E	ns	ns	ns	*	*	***	***	**	**
	Average of genotype	36.8	38.1	17.0	17.1	93.4	106.7	84.6	87.8	27.1

*, **, *** Significant at the 5%, 1% and 0.1% level by ANOVA, respectively.

Yield and yield components in Nay Pyi Taw are shown in Table 5.

Table 5 Heading date, average of daily maximum temperature during heading, grain yield and yield components in IR64 and IR64+*qEMF3* in Nay Pyi Taw.

Year	Crop	50% heading date		Average daily maximum temperature during heading (°C)		Grain yield (g plant ⁻¹)		Panicle number (plant ⁻¹)		Spikelet number (panicle ⁻¹)		Filled grains (%)		1000GW (g)	
		IR64	IR64 + <i>qEMF3</i>	IR64	IR64 + <i>qEMF3</i>	IR64	IR64 + <i>qEMF3</i>	IR64	IR64 + <i>qEMF3</i>	IR64	IR64 + <i>qEMF3</i>	IR64	IR64 + <i>qEMF3</i>	IR64	IR64 + <i>qEMF3</i>
2015	Crop 1	3 April	3 April	35.5	35.5	24.9	28.5	16.6	16.9	67.3	84.5	90.8	91.6	24.7	21.8
2017	Crop 1	1 May	2 May	37.9	38.3	17.6	25.0	14.0	14.3	79.1	96.8	65.6	83.0	24.7	21.1
2017	Crop 2	18 May	19 May	37.1	37.6	25.0	29.7	16.4	14.5	104.2	116.0	75.0	85.8	23.6	20.8
2018	Crop 1	21 April	23 April	36.6	36.5	21.8	26.5	13.8	14.0	78.3	89.0	83.4	94.1	24.2	22.6
2018	Crop 2	18 May	18 May	35.0	35.0	22.8	24.7	13.1	12.5	81.3	85.7	86.1	91.3	25.1	25.3
2019	Crop 1	5 April	9 April	36.4	36.9	25.4	31.5	18.0	19.6	62.9	77.9	85.0	92.9	27.2	22.9
2019	Crop 2	4 May	9 May	36.9	37.9	37.6	48.2	28.2	30.2	76.7	83.0	76.2	88.2	23.5	22.2
ANOVA		Genotype (G)		-	-	***		ns		***		***		***	
		Season (E)		-	-	***		***		***		***		***	
		G × E		-	-	ns		ns		ns		**		ns	
		Average of genotype		36.5	36.8	25.0	30.6	17.2	17.4	78.5	90.4	80.3	89.6	24.7	22.4

, * Significant at 1% and 0.1% level by ANOVA, respectively.

Figure legends

Figure 1 A diagram showing multiple regression of yield advantage in IR64+*qEMF3* on yield components, along with the correlation between explanatory variables for the data obtained in seven seasons of Nay Pyi Taw. Values with the single head arrows are standardized multiple regression coefficients. *, $P < 0.05$; ns, not significant.

Figure 2 Relationship between average daily maximum temperatures during heading and percentages of filled grains. Bars indicate means of percentages of filled grains $\pm$ standard deviation.

Figure 3 Daily flowering pattern and change in air temperature (A), and changes in percentages of spikelet sterility in each time period and on a single day (B) on 22 April 2018. Bars shown in blue, green, purple, and black colors indicate that the spikelets opened before 0700H, between 0700H-0830H, 0830H-1000H, and after 1000H, respectively. In B, color bars for IR64 and white bars with color outline for IR64+*qEMF3* are shown.

Ishimaru et al. Figure 1

Ishimaru et al. Figure 2

Ishimaru et al. Figure 3