Effect of drought stress on flowering characteristics in rice (*Oryza sativa* L.): a study using genotypes contrasting in drought tolerance and flower opening time

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**ABSTRACT**

Rice is most susceptible to heat and drought stress at flowering stage, but flowering characteristics under drought stress are not well characterized. This study investigated flowering characteristics of rice genotypes contrasting in their flower opening time (FOT) and level of drought tolerance. Near-isogenic lines for the early-morning flowering trait (IR64+ qEMF3) and for drought tolerance (IR87707-445-B-B-B), and their recurrent parent cultivar (IR64) were used. IR64+ qEMF3 had stable earlier FOT than IR64 and IR87707-445-B-B-B under drought stress conditions. Drought stress occasionally affects FOT depending on genotype. The number of open spikelets was higher in IR87707-445-B-B-B than in IR64 and IR64 + qEMF3, and the difference among genotypes increased as the rice plants were subjected to more severe stress levels. Panicle temperature increased under drought stress conditions and was similar among genotypes when it was measured at the same time of day, demonstrating that earlier FOT in IR64+ qEMF3 must be beneficial to avoid heat stress at flowering under drought stress conditions. However, IR64+ qEMF3 did not exhibit drought avoidance, as evidenced by the root mass at depth. To assess the potential for the EMF trait to complement ongoing drought breeding efforts, heat tolerance among 13 advanced drought breeding lines and released cultivars was tested. Wide variation in heat tolerance at flowering was observed and, notably, none of the 13 lines possessed the EMF trait. This study therefore proposes that a breeding strategy that transfers the EMF trait into drought tolerant lines could enhance the resilience of rice spikelets to the combined stresses of heat and drought at flowering.

**Abbreviations:** Analysis of variance (ANOVA), dry season (DS), early-morning flowering (EMF), flower opening time (FOT), near-isogenic line (NIL), panicle water potential (PWP), quantitative trait locus (QTL), wet season (WS)

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Introduction

Rice is most vulnerable to heat stress at flowering (Satake & Yoshida, 1978). A general threshold of high temperatures around 35°C induces spikelet sterility under flooded conditions (Satake & Yoshida, 1978; Kim et al., 1996; Mastui et al., 1997). It was demonstrated that the early-morning flowering (EMF) trait is effective in mitigating heat-induced spikelet sterility at flowering by shifting flower opening time (FOT) to the cooler temperatures in the morning (Ishimaru et al., 2010). A near-isogenic line (NIL) carrying the QTL qEMF3 for the EMF trait in the IR64 genetic background, designated as IR64+ qEMF3 (Hirabayashi et al., 2015), reduced heat-induced spikelet sterility in the field of southern India as evidenced by lower spikelet sterility compared to a range of genotypes tested (Bheemanahalli et al., 2017). Trials in the heat-vulnerable region of central Myanmar revealed that the EMF trait can enhance grain yield under heat stress under flooded field conditions at flowering stage (Ishimaru et al., 2022).

Like heat stress, rice is most susceptible to drought stress at flowering (O’Toole, 1982). Under water-limited conditions such as alternative wet and dry (AWD) and drought stress, the canopy temperature is higher than that under flooded conditions (Alberto et al., 2009; Garrity & O’Toole, 1995; Lawas et al., 2018). The canopy temperature increases as drought stress becomes more severe together with decreasing leaf water potential, resulting in a high percentage of sterile spikelets (Garrity & O’Toole, 1995). Hence, heat and drought stress simultaneously occur in rice growing fields through the aggravated water status and increased tissue temperature in the panicles, when water availability is limited.

Recent climate change has increased the risk of yield loss in crops due to extreme events, such as heat and drought (Ciais et al., 2005; Lesk et al., 2016; Lobell et al., 2013). Due to the high sensitivity of rice plants to heat and drought stress at flowering, the EMF trait is hypothesized to minimize the damage to grain fertility under combined stresses of heat and drought since both water and temperature factors are more favorable to rice plants in the early morning (O’Toole, 1982; Prasad et al., 2017). IR64+ qEMF3 is, however, not previously tested under drought stress conditions. Rice spikelets require ample water for opening by swelling the lodicule and anthers and by elongating the filament at flower opening (Hoshikawa, 1993). Therefore, the first step in considering utilization of the EMF trait to develop rice genotypes to mitigate the combined stresses of heat and drought is to ask whether or not the EMF trait is retained under drought stress conditions. While major effects of drought-yield QTLs (qDTY) have been demonstrated to improve grain yield under drought stress conditions (Kumar et al., 2014), the flowering characteristics and heat tolerance levels of drought-tolerant genotypes carrying qDTY have not yet been characterized.

This study was therefore designed with two objectives. The first objective was to clarify the flowering characteristics of IR64, IR64+ qEMF3, and a drought-tolerant genotype with the IR64 genetic background (IR87707-445-B-B-B; Swamy et al., 2013) under drought stress conditions. The aim was to determine if IR64 + qEMF3 can flower early in the morning when panicle temperature is still cooler under drought stress conditions. The second objective was to classify the heat tolerance and FOT of drought tolerant genotypes recently developed at the International Rice Research Institute (IRRI), to assess the potential of the EMF trait and to improve the existing drought breeding pool. As an outcome of this study, we propose a potential application of the EMF trait attributed to qEMF3 in the breeding program to enhance drought-aggravated heat stress at flowering.

Materials and methods

Experiment 1: Flowering characteristics of rice genotypes under drought stressed field conditions

Plant materials

IR64, an Indica group cultivar, and NILs for early-morning flowering (IR64+ qEMF3; Hirabayashi et al., 2015) and drought tolerance (IR87707-445-B-B-B; Swamy et al., 2013) with the IR64 genetic background, were grown in two fields of the International Rice Research Institute (IRRI, 14°11’N, 121°15’E). IR64 and IR64+ qEMF3 were grown in the dry seasons (DS) of 2014 and 2015 and in the wet season (WS) of 2014, and IR 87707-445-B-B-B was included in WS2014 and DS2015 trials. Seeds were sown in a wet seedbed and transplanted to puddled lowland fields at 17–18 days after sowing. 360 kg ha−1 complete basal fertilizer (14–14-14 N-P-K) was applied at 12 days after transplanting and 250 kg ha−1 ammonium sulfate was applied as top dressing at 39 days after transplanting. Each genotype was planted in two individual paddy fields with five (DS2014) and four (WS2014 and DS2015) replicate plots arranged in a randomized complete block design, with six 3-m rows per plot and 0.25 m x 0.2 m plant spacing. Days to heading were recorded at the time when 50% of the hills in the plot reached heading.
**Drought stress treatment**

One field was used for the well-watered treatment that was maintained flooded throughout the growing season, and another field was used for drought stress treatment that was planted within an automated rainout shelter. In the drought stress treatment field, water was drained at 12 days, 20 days, and 9 days before heading in DS2014, WS2014, and DS2015, respectively. In DS2015, a light shower was provided from a sprinkler for 10-min every 2–3 days until heading to avoid a very severe drought stress treatment before flowering. The difference in heading date among genotypes was up to 6.0 days across seasons (Supplementary Table S1). Among genotypes, panicles that had exerted from the tallest culm on a hill to approximately half the entire panicle length from the leaf sheath on the same day were used in both the flooded and drought stress treatments for measurement each day. This precise selection of panicles was to avoid differences in the level of drought stress experienced before heading to ensure unbiased evaluation of flowering characteristics for all genotypes.

**Panicle water potential**

Panicle water potential (PWP) was measured from two panicles per plot starting at 0830 H and 1030 H for 2 days in the early and late periods of the flower opening time observation. Water potential was measured using a 3000HGL Plant Water Status Console (Soilmoisture Equipment Corp., CA, USA) after quickly transporting the panicle in a moist, dark folder to the pressure chamber. Each set of measurements required approximately 20 minutes to complete both treatments. Four plots per genotype were selected for this measurement.

**Flower opening time observation**

Open spikelets were marked for every 30-min with fine-tipped pens based on the protocol of Hirabayashi et al. (2015). Flower opening time observation was conducted three times every 2 days during heading in DS2014 (25th and 27th February, and 1st March) and DS2015 (13th, 15th, and 17th March) and twice in the early and late periods of flower opening time observation in WS2014 (6th and 10th September). In the flooded treatment, one panicle per plot was used per day of flower opening time observation in DS2014 (five plots), whereas 2–3 panicles per plot were used per day of flower opening time observation in WS2014 and DS2015 (four plots). In the drought stress treatment, 2–4 panicles per plot were used per day of flower opening time observation both in DS2014 (three plots), and WS2014 and DS2015 (three to four plots). The time (hours) after sunrise (HAS) to reach 50% and 90% of the total number of open spikelets on the day of flower opening time observation was calculated as FOT50 and FOT90, respectively, in R ver. 3.6.2 as described by Hirabayashi et al. (2015).

**Panicle temperature**

Panicle temperature was manually measured at 0830–0900 H and 1030–1100 H on 28 February 2014 (DS2014), and 7 September 2014 (WS2014) during the flowering period. Four panicles were measured per plot using an infrared sensor (Apogee Instruments, North Logan, UT). Three plots were selected for manual measurement. The time of sunrise was 0615 H (DS2014) and 0543 H (WS2014) on the panicle measurement days.

**Root dry weight at different soil depths**

Root dry weight was determined at the late grain filling stage. Three subreplicate cores per plot were sampled using a steel tube of 4-cm diameter, 60-cm length. Each core was separated into 15-cm depth segments, and the roots were carefully washed from the soil cores before drying at 80°C for over 3 days, and then root dry weight was measured.

**Meteorological parameters**

Meteorological parameters of the field were monitored by a Davis weather station (Davis Instruments Corporation, CA, USA).

Experiment 2: Heat tolerance test of 13 advanced drought breeding lines and released varieties of environmentally controlled chambers

**Plant materials**

A total of 13 advanced breeding lines and released varieties developed at the International Rice Research Institute (IRRI) were grown in pots. These lines and varieties had single, double or triple QTLs for drought tolerance (qDTY; Kumar et al., 2014) in the genetic background of Sabitri, Swarna+Sub1, IR64, and TDK1. In addition to IR64+ qEMF3, N22, IR64, and Moroberekan were also used as heat-tolerant, moderately heat-tolerant, and highly heat-susceptible check cultivars, respectively (Shi et al., 2015). Two rice seedlings were transplanted per 10-L plastic pot, and plants were grown in a greenhouse until the first day of panicle emergence.
Plants were grown under flooded conditions throughout the experiment to understand the heat tolerance without the effect of drought stress.

**Heat tolerance test in environmentally controlled growth chambers**

On the day of the heat stress treatment, the start of flower opening was visually checked outdoors every 10 min. Once the first spikelet of a genotype was about to start opening, the time was recorded, and pots were immediately transferred to a growth chamber set at 30°C (control treatment) and 37°C (heat stress treatment) with 60% relative humidity under light levels of 1400 μmol m⁻² s⁻¹ for 6 hours. After the temperature treatment was completed, the pots were returned to the greenhouse, and spikelets that had flowered during the temperature treatment were marked with a fine-tipped pen. At maturity, the marked spikelets were collected, and the number of sterile spikelets was manually counted.

**Statistical analysis**

Analysis of variance (ANOVA) and Tukey-HSD tests were conducted using the R ver. 3.6.2 and JMP ver. 14.0.0.

**Results**

**Changes in maximum air temperature during heading period**

Average of mean maximum air temperature during heading period was highest in WS2014 (31.1°C) followed by DS2015 (29.8°C) and DS2014 (28.1°C; Figure 1). The flower opening time observations were conducted for all genotypes during the period from -1 to +5 days after 50% heading for IR64, using headed panicles on the same day for the measurement in each day.

**Stress level measured by panicle water potential during flower opening time observation**

Panicle water potential (PWP) of each genotype ranged from -0.3 to -0.9 MPa under flooded conditions across seasons (Figure 2). Under drought stress conditions, PWP reached as low as approximately -2.0 MPa across days when flower opening time was observed in DS2014 (Figure 2A), whereas PWP was at a similar level as that in the flooded condition on 6th September then it declined to -2.0 MPa on 10th September in WS2014 (Figure 2B). PWP steadily decreased from -0.5 to -1.6 MPa under drought stress conditions across the days when flower opening time was observed in DS2015 (Figure 2C). Such differential changes in stress level among seasons were reflected in a combined ANOVA; water treatment significantly affected PWP in all seasons, whereas time (day of flower opening time observation) did not affect PWP in DS2014 (Supplementary Table S2). The combined effect of water treatment and time was different between seasons (Supplementary Table S2). The effect of genotype on PWP was not significant in DS2014 and WS2014, whereas it was significant in DS2015 (Supplementary Table S2).

**Number of open spikelets per panicle**

Changes in the number of open spikelets during days of flower opening time observation between flooded and drought stress conditions are shown in Figure 3. In DS2014, the number of open spikelets steadily decreased as panicles headed on later dates under drought stress conditions. Only 4–5 open spikelets flowered on the last day of observation on 1st March under drought stress condition in DS2014 (Figure 3A). In WS2014, the number of open spikelets was similar between flooded and drought stress conditions on the first day of observation on 6th September, while it decreased to only 1.2 in IR64 and IR64+ qEMF3 on average under drought stress conditions on the last day of observation on 10th September (Table 1; Figure 3B). The number of open spikelets was significantly greater in IR 87707–445-B-B-B (13.1) than IR64 and IR64+ qEMF3 (1.2) under drought stress condition on 10th September (Table 1). In DS2015, the number of open spikelets was relatively maintained across the days of flower opening time observation in all genotypes except for a reduction on the last day of observation on 17th March in IR64+ qEMF3 (Figure 3C). The number of open spikelets was greatest in IR 87707–445-B-B-B (20.7),
followed by IR64 (15.6) and IR64+ qEMF3 (7.3) under drought stress condition on 17th March (Table 1). Changes in the ratio of drought stress to flooded conditions in terms of the number of open spikelets per panicle followed by changes in the number of open spikelets between flooded and drought stress conditions in each genotype (Figure 3D-F).

**Flower opening time (FOT)**

FOT50 and FOT90 were calculated on 25th February 2014 (DS2014), 6th September 2014 (WS2014), and 15th March 2015 (DS2015). These days of observation were selected because of the different PWP levels including −2.0 MPa (25th February 2014), higher than −1.0 MPa (6th September 2014; Figure 2A, 2B). PWP was not

Figure 2. Changes in panicle water potential (PWP, MPa basis) under flooded and drought stress conditions in (A) DS2014, (B) WS2014, and (C) DS2015. Values are the mean ± standard deviation (n = 4 plots). Results of ANOVA for PWP is indicated in Supplementary Table S2.
measured on 15 March 2015, but the PWP level can be estimated to be between −1.0 and −1.5 MPa, according to the decrease in PWP from 13th to 17th March 2015 (Figure 2C). On 25th February 2014 (DS2014), there was a significant difference in FOT between IR64 and IR64 + qEMF3, whereas water treatment did not affect either FOT50 or FOT90 in each genotype (Supplementary Table S3). On 6th September 2014 (WS2014) and 15th March 2015 (DS2015), FOT50 and FOT90 were significantly earlier in IR64+ qEMF3 than IR64 and IR 87707–445-B-B-B under drought stress conditions (Table 2). The water treatment did not affect either FOT50 or FOT90 in IR64 and IR 87707–445-B-B-B. In IR64+ qEMF3, the water treatment did not affect either FOT50 or FOT90 in DS2014, whereas both FOT50 and FOT90 were significantly earlier under drought stress conditions than flooded conditions in DS2015.
Table 1. Results of ANOVA for number of open spikelets per panicle under flooded and drought stress conditions in DS2014 (top table), WS2014 (middle table), and DS2015 (bottom table).

|                | DS2014          | WS2014          | DS2015          |
|----------------|-----------------|-----------------|-----------------|
|                | Genotype        | Day             | Treatment       |
| ANOVA          | ns              | ns              | ***             |
| Genotype × Day | ns              | ns              | ***             |
| Genotype × Treatment | ns            | ns              | ***             |
| Day × Treatment | ***            | ***             | ***             |
| Genotype × Day × Treatment | ns        | ns              | ns              |
| Tukey-HSD      | IR64 Drought    | IR64+ qEMF3 Drought | IR87707-445-B-B-B Drought |
| (10th September)| 1.2b            | 1.2b            | 13.1*           |

Table 2. Time (hours) after sunrise to reach peak (FOT50) and end (FOT90) of open spikelets under flooded and drought stress conditions in WS2014 and DS2015.

| Season       | Genotype      | Treatment       | FOT50 | FOT90 |
|--------------|---------------|-----------------|-------|-------|
| WS2014       | IR64          | Flooded         | 4.0 ab| 4.8 a |
|              | IR64+ qEMF3   |                 | 2.7 c | 3.7 ce|
|              | IR 87707–445-B-B-B |               | 3.8 ab| 4.3 ac|
|              | IR64          | Drought stress  | 4.3 a | 4.8 a |
|              | IR64+ qEMF3   |                 | 2.7 c | 3.2 e |
|              | IR 87707–445-B-B-B |               | 4.2 a | 5.0 a |
| DS2015       | IR64          | Flooded         | 3.7 ab| 4.0 bcd|
|              | IR64+ qEMF3   |                 | 2.4 c | 3.4 de |
|              | IR 87707–445-B-B-B |               | 4.0 ab| 4.6 ab|
|              | IR64          | Drought stress  | 3.5 b | 4.4 ac|
|              | IR64+ qEMF3   |                 | 1.1 d | 1.8 f |
|              | IR 87707–445-B-B-B |               | 3.8 ab| 4.9 a |
| Average      | Genotype      |                 |       |       |
|              | IR64          |                 | 3.9   | 4.5   |
|              | IR64+ qEMF3   |                 | 2.2   | 3.0   |
|              | IR 87707–445-B-B-B |               | 3.9   | 4.7   |
| Season       |                |                 |       |       |
|              | WS2014        |                 | 3.6   | 4.3   |
|              | DS2015        |                 | 3.1   | 3.8   |
| Treatment    | Flooded       |                 | 3.4   | 4.1   |
|              | Drought Stress|                 | 3.3   | 4.0   |

Table 2. Time (hours) after sunrise to reach peak (FOT50) and end (FOT90) of open spikelets under flooded and drought stress conditions in WS2014 and DS2015.

| Season | Genotype | Treatment | FOT50 | FOT90 |
|--------|----------|-----------|-------|-------|
| WS2014 | IR64     | Flooded   | 4.0 ab| 4.8 a |
|        | IR64+ qEMF3 |         | 2.7 c | 3.7 ce|
|        | IR 87707–445-B-B-B |   | 3.8 ab| 4.3 ac|
|        | IR64     | Drought stress | 4.3 a| 4.8 a |
|        | IR64+ qEMF3 |         | 2.7 c | 3.2 e |
|        | IR 87707–445-B-B-B |   | 4.2 a | 5.0 a |
| DS2015 | IR64     | Flooded   | 3.7 ab| 4.0 bcd|
|        | IR64+ qEMF3 |         | 2.4 c | 3.4 de |
|        | IR 87707–445-B-B-B |   | 4.0 ab| 4.6 ab|
|        | IR64     | Drought stress | 3.5 b| 4.4 ac|
|        | IR64+ qEMF3 |         | 1.1 d | 1.8 f |
|        | IR 87707–445-B-B-B |   | 3.8 ab| 4.9 a |

Values are the mean of one day in WS2014 (6 September 2014) in DS2015 (15 March 2015) of flowering pattern observation with 3–4 replicates (plots).

Significant at 5%, 1%, and 0.1% level by ANOVA, respectively. ns; not significant at 5% level.

Different letters following the values indicate significance at 5% level by Tukey-HSD in each column.
Figure 4. Manual measurement of panicle temperature (°C) by an infrared sensor under flooded and drought stress conditions on (A) 28 February 2014 (DS2014) and (B) 7 September 2014 (WS2014). Air temperature and relative humidity was 26.3°C and 72.5%, respectively, at 0830–0900 H, and 29.3°C and 58.5%, respectively, at 1030–1100 H on 28 February 2014. Air temperature and relative humidity was 30.4°C and 75.5%, respectively, at 0830–0900 H, and 32.4°C and 68.0%, respectively, at 1030–1100 H on 7 September 2014. Values in each bar indicate the mean ± standard deviation (n = 3 plots). Different letters for each value indicate significant difference at the 5% level by Tukey-HSD.
Table 3. Heat tolerance and start of flower opening time (sFOT) among check cultivars and lines for heat escape and drought tolerance.

| Genotype  | Drought-tolerant QTLs introgressed | sFOT (hour) | Spikelet sterility (%) | Total spikelets observed | Remarks                        | Remarks* |
|-----------|-----------------------------------|------------|------------------------|--------------------------|--------------------------------|----------|
|           | Control | Heat | Control | Heat |                      |          |                                        |          |
| Moroberekan | -      |      | 0900   | 4.1 ± 1.4 | 78.6 ± 3.3 a | 195 (6) | 222 (8) | Heat susceptible check cultivar          |          |
| IR 102777-5-83-1-2-2 | qDTY<sub>6</sub><sup>+</sup> + qDTY<sub>6</sub> | 0900   | 21.3 ± 7.8 | 75.7 ± 4.2 a | 108 (4) | 315 (8) | Drought-tolerant QTL(s) was not different from that of IR64 and N22, the heat-tolerant check. |
| IR 87707–445-B-B-B | qDTY<sub>2</sub><sup>+</sup> + qDTY<sub>4</sub><sup>+</sup> | 0900   | 25.1 ± 4.2 | 65.1 ± 5.6 ab | 568 (12) | 375 (8) | DRR dhain 42 (India) with IR64 background; Swamy et al. (2013) |
| IR 106523–24-6-1 | qDTY<sub>2</sub> | 0820   | 12.1 ± 4.2 | 49.8 ± 7.9 bc | 70 (3) | 268 (8) | Sabriti background |
| IR 106522–37-36-1 | qDTY<sub>12</sub><sup>+</sup> + qDTY<sub>12</sub><sup>+</sup> | 0900   | 2.7 ± 1.2 | 36.0 ± 3.1 cd | 201 (6) | 302 (9) | Sabriti background |
| IR64+ qEMF3 | -      |      | 0700   | 12.1 ± 1.5 | 24.9 ± 3.2 de | 158 (6) | 467 (12) | Hirabayashi et al. (2015) |
| IR64 | -      |      | 0830   | 5.5 ± 1.6 | 20.9 ± 2.4 def | 384 (11) | 349 (10) | Moderately heat tolerant check cultivar |
| IR 96322–34-127- B-2-1-3 | qDTY<sub>11</sub><sup>+</sup> + qDTY<sub>3</sub><sup>+</sup> | 0840   | 12.0 ± 2.4 | 20.9 ± 2.5 def | 100 (4) | 407 (11) | Swarna+Sub1 background |
| IR 106523–23- B-1-2-B | qDTY<sub>3</sub><sup>+</sup> | 0840   | 1.0 ± 0.9 | 19.5 ± 3.3 def | 137 (3) | 201 (4) | Dixit et al. (2017b); Sabriti background |
| IR 96321–558-257- B-4-1-2 | qDTY<sub>11</sub><sup>+</sup> + qDTY<sub>12</sub><sup>+</sup> + qDTY<sub>11</sub><sup>+</sup> | 0840   | 15.1 ± 3.5 | 18.4 ± 6.8 def | 115 (4) | 115 (4) | Swarna+Sub1 background |

Donor parent is N22 for qDTY<sub>11</sub>, Apo for qDTY<sub>12</sub> and qDTY<sub>3</sub>; Aday sel for qDTY<sub>2</sub>, qDTY<sub>12</sub>, and qDTY<sub>3</sub>; Way Rarem for qDTY<sub>3</sub> (Kumar et al. 2014).

Values represented are mean ± SE. Different letters following the values indicate the significant difference at 5% level by Tukey–HSD test under heat stress condition.

Numbers in the parenthesis indicate the numbers of panicles used for determining spikelet sterility across control and heat stress treatments.

*Heat tolerance level in check cultivars is based on Shi et al. (2015). For Sub1, see Xu et al. (2006).

(Table 2). There was a significant difference in FOT50 and FOT90 between seasons; FOT50 and FOT90 were earlier in DS2015 than WS2014, except for FOT90 in IR 87707–445-B-B-B under drought stress conditions (Table 2).

Panicle temperature

In both DS2014 and WS2014, there were no significant differences in panicle temperature among genotypes in each water treatment, whereas there was a significant difference between water treatments at the same time of day in each genotype (Figure 4). Under drought stress conditions, panicle temperature was significantly higher at the later time of day (1030–1100 H) than at the earlier time of the day (0830–0900 H) in each genotype, while a difference in panicle temperature between times of day was not clear under flooded conditions (Figure 4).

Root dry weight at different soil depth

Root dry weight was very similar between IR64 and IR64 + qEMF3 (Supplementary Figure S1). Root dry weight at the 30 cm depth tended to be higher in IR 87707–445-B-B-B than other genotypes both under flooded and drought stress conditions, although there was no significant difference among genotypes in each water treatment (Supplementary Figure S1).

Assessment of heat susceptibility and flower opening time in drought-tolerant lines

The start of flower opening time (sFOT) for IR64+ qEMF3 was at 0700 h, whereas sFOT for all other genotypes in Experiment 2 was later than 0800 h (Table 3). The level of heat susceptibility of IR 102777-5-83-1-2-2 was as high as the heat-susceptible check Moroberekan (Table 3). The level of heat tolerance of three genotypes (IR 87707–445-B-B-B, IR106523-24-6-1, and IR 106522–37-36-1) was classified as between Moroberekan and the moderately heat tolerant check cultivar IR64. As reported by Hirabayashi et al. (2015), the level of heat tolerance of IR64+ qEMF3 was similar to that of IR64. The level of heat tolerance of other genotypes with drought tolerant QTL(s) was not different from that of IR64 or was between that of IR64 and N22, the heat-tolerant check.
Discussion

Both high air temperature and reduced transpiration due to drought stress can cause plant reproductive structures to experience heat stress. This study investigated flowering characteristics of rice genotypes contrasting in their level of drought tolerance and their flower opening time, in order to explore a potential strategy for improving rice productivity under heat and drought stress by utilizing NILs carrying individual QTLs for drought tolerance and the EMF trait.

Flowering characteristics were different among genotypes under drought stress conditions

Across seasons, IR64+ qEMF3 had significantly earlier FOT than IR64 and IR 87707–445-B-B-B under drought stress conditions (Table 2; Supplementary Table S3) irrespective of the wide variation in temperature conditions at flowering stage among seasons (Figure 1). This study clearly demonstrated that the FOT in IR64+ qEMF3 was stably advanced under drought stress conditions, which had previously been reported only under flooded conditions (Bheemanahalli et al., 2017; Hirabayashi et al., 2015; Ishimaru et al., 2022). On the other hand, FOT under drought stress conditions was not different from that under flooded condition, except for IR64+ qEMF3 in DS2015 (Table 2), indicating that the drought stress treatment occasionally affected FOT depending on the genotype. FOT was significantly earlier in DS2015 than in WS2014 (Table 2), supporting the result of Bheemanahalli et al. (2017) that FOT is earlier in the dry season compared to the wet season.

The number of open spikelets per panicle changed in a different manner among seasons (Figure 3). A steady decrease in the ratio of drought stress to flooded conditions was observed in DS2014 and WS2014 (Figure 3A, B, D, E), whereas the ratio was relatively maintained during the period of flower opening time observation in DS2015, except for a reduction on the last day of observation on 17th March in IR64+ qEMF3 (Figure 3C, F). This result indicates that the level of drought stress was more severe in DS2014 and WS2014 than DS2015, as supported by a panicle water potential value (Figure 2). Root dry weight was not different between IR64 and IR64+ qEMF3 (Supplementary Figure S1), indicating a similar level of belowground response to drought (i.e. avoidance) between the two genotypes. The lower ratio of drought stress to flooded conditions in the number of open spikelets at the late heading period (Figure 3D-F) suggests that the lack of water supply to the spikelets prohibits normal flower opening because rice spikelets require ample water for opening (Hoshikawa, 1993). It should be noted that the advantage of early FOT in IR64 + qEMF3 decreased as drought stress levels became more severe as very few numbers of spikelets opened. On the other hand, the higher number of open spikelets in IR 87707–445-B-B-B even at the late heading period of WS2014 and DS2015 (Table 1) supports its drought tolerance inherited by drought tolerant QTLs (qDTY2.2 + qDTY4.1; Swamy et al., 2013). Based on the evidence of flowering characteristics in IR64+ qEMF3, the EMF trait attributed to qEMF3 is retained under drought stress conditions. However, the contribution of qEMF3 to productivity under drought stress conditions remains to be tested, as discussed below.

Early-morning flowering is beneficial to avoid hotter environments at flowering under drought stress conditions

Canopy temperature under water-limited conditions such as alternative wet and dry (AWD) and drought stress conditions, is higher than under flooded conditions (Alberto et al., 2009; Garrity & O’Toole, 1995; Lawas et al., 2018). As in previous studies, panicle temperature in this study was significantly higher under drought stress conditions than under flooded conditions in all genotypes (Figure 4). Furthermore, we emphasize that the increase in panicle temperature due to the later time of day (1030–1100 H vs 0830–0900 H) was much more prominent under drought stress conditions than under flooded conditions (Figure 4). Since flower opening time as estimated by the range of FOT50–FOT90 corresponded to approximately 0930 H-1110 H in IR64, to 0710 H-0920 H in IR64+ qEMF3, and to 0930 H-1100 H in IR 87707–445-B-B-B under drought stress conditions (Table 2; Supplementary Table S3), this study provides solid evidence that earlier FOT attributed to qEMF3 benefits rice plants to reach anthesis at much cooler panicle temperatures under drought stress conditions, as hypothesized by O’Toole (1982) and Prasad et al. (2017). The EMF trait helps rice plants at flowering to escape from hotter environments under drought stress conditions.

Potential of an early-morning flowering trait to develop promising rice genotypes to mitigate the combined stresses of heat and drought

Although our study demonstrated the advantage of an EMF trait to avoid hotter environments at flowering under drought stress conditions, the flowering characteristics of IR64+ qEMF3 under drought stress conditions also revealed the necessity of introducing additional drought tolerance mechanisms to enhance the response to the combined stresses of heat and drought. IR64 has shallow root architecture (Uga et al., 2011) and is very susceptible to drought stress at reproductive stage (Kumar et al., 2009). To maximize the benefits of the EMF trait for opening spikelets at much cooler temperatures under drought stress conditions, the EMF trait
should be conferred to drought tolerant cultivars. The levels of heat tolerance of drought tolerant varieties and lines were previously uncharacterized.

In this regard, heat tolerance and the start of FOT (sFOT) of 13 advanced breeding lines and released varieties for drought tolerance were assessed in a pot study under flooded conditions (Table 3). Our observations indicated that none of the drought tolerant lines tested exhibited the EMF trait (Table 3), suggesting that the heat-escape strategy under drought stress conditions is applicable to the drought breeding program. In addition, our high-temperature chamber experiment showed wide variation in heat tolerance among advanced drought breeding lines and released varieties; heat susceptibility of IR 102777-5-83-1-2-2 was as high as Moroberekan, and IR 87707–445-B-B-B, IR106523-24-6-1, and IR 106522–37-36-1 were identified as moderately-heat susceptible genotypes (Table 3). The remaining nine genotypes showed moderately to high heat tolerance (Table 3). Although our pot experiment restricted the root growth of drought-tolerant genotypes tested, this result could determine a breeding priority to confer the EMF trait to drought tolerant lines with high or moderately high heat susceptibility. Jagadish et al. (2011) pointed out that drought tolerant varieties do not always exhibit heat resilience, possibly because tolerance to heat and drought stress may be conferred by separate mechanisms. In fact, collective evidence showed an example that rice variety KDML-105 is known as a relatively drought tolerant cultivar (Kameoka et al., 2016) but is heat susceptible (Shi et al., 2015) without the EMF trait (Hirabayashi et al., 2015). Interestingly, one genotype carrying two drought tolerant QTLs (qDTY12.2 + qDTY21.2) with the Sabitri genetic background (IR106522-35-5-3-1-B) had high heat tolerance with less than 10% spikelet sterility (Table 3). Although IR106522-37-36-1 carries the same drought tolerant QTLs as IR106522-35-5-3-1-B, also with the Sabitri genetic background, it was assessed as moderately heat-susceptible genotype. The development of a mapping population of IR106522-37-36-1 crossed with IR106522-35-5-3-1-B could find QTL(s) that confer a difference in heat tolerance between the two genotypes.

**Conclusion**

This study revealed flowering characteristics of rice genotypes contrasting in their degree of drought tolerance and their flower opening time under drought stress conditions. The earlier FOT of the NIL for the EMF trait (IR64+ qEMF3) was observed to be retained under drought stress conditions in this study. Significant advancements in FOT in IR64+ qEMF3 can benefit rice plants by allowing them to reach anthesis at much cooler panicle temperatures under drought stress conditions. Although IR64+ qEMF3 was affected by drought stress as evidenced by the low number of spikelets that opened under the most severe drought conditions (PWP around –2.0 MPa), the EMF trait is promising to complement existing drought tolerance mechanisms in the breeding pool since none of the drought-tolerant genotypes tested exhibited the EMF trait, and some of them showed high or moderately high heat susceptibility. This study proposes a potential application of the EMF trait to develop promising lines for combined stresses of heat and drought at flowering. Marker-assisted breeding of pyramid QTLs for heat escape (qEMF3) and drought tolerance (qDTY) would enhance the resilience of rice spikelets to combined heat and drought stress at flowering, which is projected to be more severe and more frequent in the era of global warming.

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**Disclosure statement**

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