



RESEARCH PAPER

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Field validation of heat tolerance and early morning flowering QTLs (*qHTSF4.1* and *qEMF3*) and combination of the two QTLs introduced into IR64 (*Oryza sativa* L.) background at CSU Piat, Philippines

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Abstract

Heat stress reduces rice yield by 10% for every degree Celsius increase beyond optimum temperature. Field testing of IR64-derived near-isogenic lines with heat tolerance and early morning flowering QTLs was conducted at CSU Piat during hottest months of 2016-2017. To evaluate how well IR64 NILs tolerated heat, morpho-agronomic data were collected and analyzed when they were subjected to high temperature at field conditions. Flower opening time (FOT), the peak flowering time (PFT), and the time when all of the flowers are closed (FCT) were also determined for early morning flowering traits (EMF). Results showed that morpho-agronomic features of IR64-derived NILs such panicle length, number of tillers per hill, spikelet fertility, spikelet/panicle, plant height, days to 50% flowering and maturity were similar when compared to its recurrent parent. Moreover, EMF traits results revealed that IR64HT+EMF and IR64EMF NILs exhibited the earliest FOT, PFT, and CFT. This research under high temperature field condition clearly validated the heat tolerance performance of IR64-derived NILs had similar morpho-agronomic traits compared to its recurrent parent indicating recovery of recurrent parent genome. Furthermore, IR64HT+EMF and IR64EMF NILs exhibited the earliest FOT, PFT, and CFT indicating that the presence of *qEMF3* and its combination with *qHTSF4.1* strongly confers EMF traits as an escape mechanism from heat stress. The researchers recommend the use of genetic materials with combined genes of heat tolerance (*qHTSF4.1*) and early morning flowering (*qEMF3*) for these are useful germplasm for future and expected global warming.

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Introduction

In rice, temperature above optimum levels affects all growth stages. Among these, the flowering stage is considered the most sensitive stage to high temperature (Satake and Yoshida, 1978; Yoshida *et al.*, 1981). Heat-induced spikelet sterility results if the sensitive physiological processes of anther dehiscence, pollination, pollen germination on the stigma, and pollen tube growth are aggravated (Wassmann *et al.*, 2009a). In the study of Jagadish *et al.* (2007), sterility was induced for less than 30 minutes of exposure to 35°C ambient temperature and 33.7°C spikelet tissue temperature during anthesis. However when spikelets opened either before or for more than an hour after the onset and exposure of high temperature, they were unaffected by the heat treatment (Jagadish *et al.*, 2007).

Flowering is the most sensitive stage to high temperature in the rice life cycle. High temperature of over 35°C at flowering stage increases pollen and spikelet sterility, which leads to significant yield losses, low grain quality, and low harvest index. Large cultivar variation exists in the spikelet sensitivity to high temperature damage, and the primary cause of this cultivar variation in high temperature (heat) tolerance at flowering is the number of viable pollen grains shed on the stigma, resulting from the changes in the extent of anther dehiscence, which directly affect the spikelet fertility and grain yield. Thus, spikelet fertility under high temperature has been widely used as a screening index for heat tolerance at reproductive stage.

Heat tolerance is the ability of the plant to grow, develop, and produce an economic yield under high temperature stress (Wahid *et al.*, 2007; Paupiere *et al.*, 2014). There are three basic mechanisms of heat tolerance in plants: (1) true heat tolerance, where plants can shed a large amount of pollen or viable pollen able to germinate under heat stress and (2) heat avoidance, where the plant performs its sensitive functions (ie. fertilization) before the onset of the stress (Yoshida *et al.*, 1981; Ishimaru *et al.*, 2010). The latter can be done by several ways: macroscape (heading during the cooler parts of the season), and microescape (anther dehiscence occurring during the

cooler parts of the morning) (Wassmann *et al.*, 2009a); and (3) heat escape, by changing leaf orientation, efficient transpirational cooling of the canopy, reduction in non-photosynthetic energy intercepted by the canopy, and reflection of solar radiation (Bahuguna *et al.*, 2014).

Significant genotypic variation had also been found for time of day of flowering (TDF) and early morning flowering (EMF) or peak anthesis in rice germplasm. It has been reported that *O. glaberrima* is an EMF wild rice species with the ability to flower immediately after dawn, and with more than 90% of spikelets nearing anthesis by 0900H (Prasad *et al.*, 2006). Interspecific crosses were made between *O. glaberrima* and *O. sativa*, which produced lines that had significantly earlier peak anthesis hours than the original parent (Yoshida *et al.*, 1981). Ishimaru *et al.* (2010) successfully introgressed the EMF trait from another wild rice, *O. officinalis* into Koshihikari (*O. sativa*), and the produced Koshihikari + EMF line can open its spikelets 2 hours earlier than the Koshihikari wild type. This adaptation allowed the line to garner higher spikelet fertility than others popular varieties lacking the EMF trait. The produced EMF introgression line was used to develop near-isogenic lines of Nanjing 11 (temperate cultivar) and IR64 (tropical/subtropical cultivar) and successfully and stably exhibited the EMF trait (Hirabayashi *et al.*, 2014).

Redoña *et al.* (2009) expressed that identifying the quantitative trait loci (QTL) for heat tolerance and employing marker-assisted selection (MAS) could compensate for the difficulty of field screening and significantly improve the overall efficiency of the breeding process. Genomic techniques and tools like MAS can ease selection of target traits, that can be used to (1) identify, quantify, and characterize genetic variation; (2) tag, clone, and introgress genes and/or QTL; and (3) manipulate (eg. pyramid, integrate) genetic variation in breeding populations (Xu and Crouch, 2008). Genetic mapping studies for EMF and heat-tolerant QTLs during the reproductive stage of different rice populations have been undertaken (Jagadish *et al.*, 2008; Ishimaru *et al.*, 2010; Jagadish *et al.*, 2010a; Xiao *et al.*, 2011; Ye *et al.*, 2012;

Hirabayashi *et al.*, 2014; Ye *et al.*, 2016). In the mapping study conducted by Ye *et al.* (2012), four major heat-tolerance QTLs were identified from the progeny of IR64 x N22 cross, to which N22 is the heat-tolerant variety. Of the four chromosomal locations identified, QTLs *qHTSF1.1* (on chromosome 1 of IR 64) and *qHTSF4.1* (on chromosome 4 of N22) were confirmed to have the most significant role for increasing spikelet fertility under high temperature (Ye *et al.*, 2012) and were found to be very close to major QTLs identified in the studies of Jagadish *et al.* (2010a) and Xiao *et al.* (2011). Between the two QTLs, plants with the *qHTSF4.1* exhibited higher spikelet fertility than other genotypes, and was also detected and confirmed by Ye, *et al.* (2016) in an IR64/Giza 178 bi-parental cross and IR64/Milyang/Giza 178 three-way cross, suggesting its potential significance in enhancing heat tolerance of rice during the flowering stage.

Some species of wild rice were found to flower early in the morning, such as *O. glaberrima* (A genome), *O. rufipogon* (A genome), and *O. officinalis* (C genome) (Yoshida *et al.*, 1981; Ishimaru *et al.*, 2010; Thanh *et al.*, 2010). The group of Ishimaru *et al.* (2010) transferred the EMF trait from *O. officinalis* into the genetic background of *O. sativa* cv. Koshihikari, producing EMF20, an introgression line. The EMF20 was crossed with Nanjing 11. Using SSR markers, significant QTLs were identified on chromosome 3 (*qEMF3*) and chromosome 8 (*qEMF8*). Comparison of the recurrent parent and near-isogenic lines with the *qEMF3* showed that the EMF20 allele of the QTL significantly advanced the flowering opening time (FOT) by 1.5-2.0 hours.

Developing near-isogenic lines (NILs) are advantageous in evaluating the effect of the QTLs on the phenotype (marker-trait association). Gene expression can change during morpho-physiological and reproductive development as well as when subjected to biotic and abiotic stresses. Validation of the function of the introgressed QTLs in NILs will allow breeders to optimize phenotypic selection procedures (Xu and Crouch, 2008).

This study aimed to determine the effect of QTLs for heat tolerance (*qHTSF4.1*), EMF trait (*qEMF3*) and a combination of the two QTLs, introduced into the background of IR64 (*O. sativa*) on (1) the floret morpho-physiological responses when flowering is exposed to elevated temperatures; (2) agronomic characters, and (3) spikelet fertility and grain yield of the lines.

Materials and methods

I. Plant Materials

Three IR64-derived near-isogenic lines (NILs) developed at the International Rice Research Institute (IRRI) was used in the experiment. The NILs differ in the QTL present, designated as IR64 HT (*qHTSF4.1*), IR64 EMF (*qEMF3*), and IR64 HT + EMF (*qHTSF4.1* + *qEMF3*) (Table 1).

Table 1. IR64-derived near-isogenic lines with corresponding introgressed QTLs.

Designation	QTL Present	Filial Generation
IR64 HT	<i>qHTSF4.1</i>	BC ₅ F ₅
IR64 EMF	<i>qEMF3</i>	BC ₃ F ₄
IR64 HT + EMF	<i>qHTSF4.1</i> + <i>qEMF3</i>	F ₄
IR64		Recurrent parent
N22	<i>qHTSF4.1</i>	Donor/ Tolerant check

The IR64 HT NIL (BC₅F₅) was developed by introgressing the heat tolerance allele *qHTSF4.1* (QTL on chromosome 4), which is a possible significant genetic factor for the trait, into the mega-variety IR64. This QTL was first found in N22 (formerly Nagina 22) in an IR64 x N22 mapping population (Ye *et al.*, 2012). N22 is an *Indica*-type *O. sativa* Aus variety which is both heat and drought tolerant (Yoshida *et al.*, 1981; Prasad *et al.*, 2006; Jagadish *et al.*, 2010; Rang *et al.*, 2011) as well as tolerant to combined heat and drought stress (Jagadish *et al.*, 2011). Marker assisted foreground and background selection has allowed the group of Ye *et al.* (2016) to generate NILs which has more than 99% of the IR64 genome background.

The IR64 + EMF NIL (BC₃F₄) were developed by the group of Ishimaru *et al.* (2010). The EMF trait was obtained from wild rice *O. officinalis* (C genome),

transferred into *O. sativa* cv. Koshihikari to create an introgression line, EMF20. The EMF20 was used to transfer the EMF locus *qEMF3* into Nanjing 11. Subsequently, the Nanjing 11 + EMF was used to transfer the QTL into IR64 (Hirabayashi *et al.*, 2014). The IR64 HT NIL was then crossed with the IR64 EMF NIL that generated IR64 HT + EMF F₁, and was subsequently selfed until the F₄ generation. Development of the NILs is presented in Fig. 1.

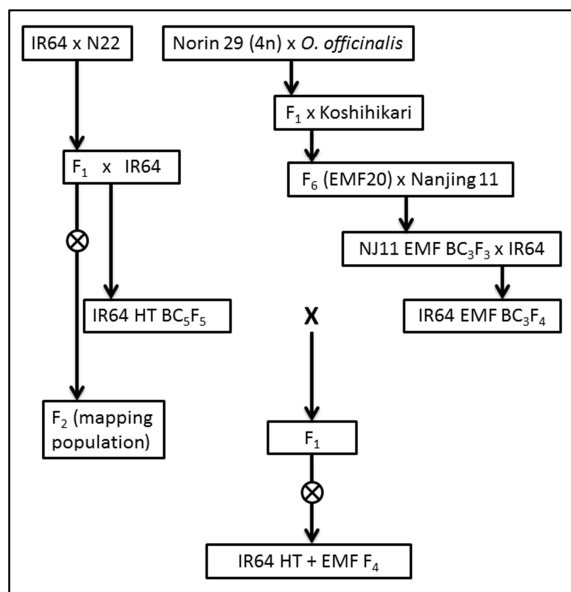


Fig. 1. Development of IR64 NILs used in the experiment.

Data collected at flowering stage

Date of heading (when 50% of the inflorescence emerge from the leaf sheath) of each plant was recorded. Data on flower opening time was collected based on the protocol of Hirabayashi *et al.* (2014), with minor modification on the time frame.

When all plants from the control group of a genotype are heading, one panicle per plant was selected and tagged for flower opening time (FOT) observation. Panicles selected must be uniform or at the same stage with each other. The number of spikelets that flowered (spikelet is open and anthers protrude from the glume) was recorded at 30-min interval, starting from 0600H (for those with the EMF allele) and 0800H (parentals, IR64 HT), until the end of anthesis or around 1400H. Observations were made for 3 consecutive sunny days. In the event of a cloudy/overcast day, an additional observation day was added.

Flower opening time will also be recorded for the treated plants inside the IGCs, following a modified methodology. Beginning of FOT will only be recorded and observed by “plot” basis (or for one treatment group as a whole, not individual), and observed only from the glass window of the IGC to minimize microenvironment variations that may affect flowering pattern and ultimately, the reliability of the experiment. Observations were in when majority of the genotype have been entered into the IGCs for treatment, and FOT was recorded for 3-5 consecutive days.

Data collected at harvest

When plants reach physiological maturity, agronomic parameters were measured, specifically: plant height, flag leaf length, panicle length, and panicle exertion of one panicle. Plant height was measured from soil surface to tip of tallest panicle, excluding awns. Flag leaf length was measured from leaf collar to tip of the leaf. Panicle length was measured from panicle base to tip. Panicle exertion was measured from the flagleaf collar up to the panicle base.

After data collection, three uniform panicles were collected from each plant for measuring spikelet fertility. The number of filled (full and partially filled) spikelets and empty spikelets was counted for each individual panicle. Spikelet fertility, the ratio of number 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 will then be obtained.

Yield was determined by harvesting all panicles from a 1 m x 1 m (25 hills) plot from the inner rows/middle of each plot, with 3 replications within each plot (3 x 25 hills). The grains were dried to 14% moisture content and weighed and computed for plot yield and converted to kilogram per hectare.

Field Trial and Evaluation of IR64 Near-Isogenic Lines

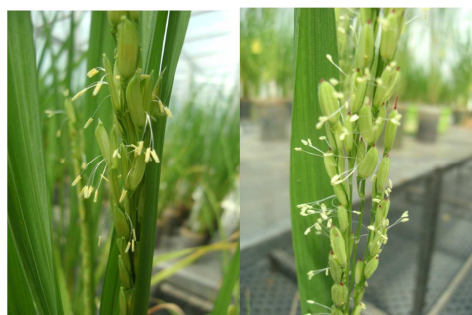
The five rice genotypes were grown under field condition in a replicated manner at the Rice Research and production area of Cagayan State University, Piat, Cagayan.

Seeds were pregerminated and transplanted at 20 cm x 20 cm plant spacing, with one seedling per hill. Each plot measured 12 m² (10 rows by 25 hills). The experiment was laid out in RCBD with three replications. Each genotype was evaluated for agronomic parameters, namely: days to 50% heading, beginning of flower opening time, plant height, panicle length, number of productive tillers, spikelet fertility of three panicles, grain yield, and grain quality. Date of 50% heading was recorded when 50% of the plants in the plot are at heading stage. Data on FOT was recorded when majority of the plants are at heading. Plots were monitored at 30 min interval within 0700 to 1200H. The time when spikelets begin to open (BFOT) and the peak of flower opening (PFOT) was recorded. Observation was made from panicles of all plants from the inner rows of the plot.

Fifteen plants were randomly selected from the inner rows of each plot for the measurement of plant height, flag leaf length, panicle length, panicle exertion, number of tillers, and harvesting of three uniform panicles for the determination of measuring spikelet sterility. For grain yield determination, all panicles from a 1 m x 1 m (25 hills) crop cut was harvested from the inner rows of each plot (3 x 25 hills). Panicles were threshed, and grains were dried to 14% moisture content (MC). Plot yield was adjusted to 14% MC and converted to kg ha⁻¹. After measuring yield, grain samples (3 replications per line) was submitted to IRRI grain quality laboratory for quality analysis, including grain weight, grain size, chalkiness, amylose content, and gel temperature.

Flower Opening Time (FOT)

Florets are considered open (flowering) when anthers protrude from the glume, as shown below.



(L-R) Example of beginning of flowering and peak of flowering.

At 50% heading, record the time when spikelets begin to open and the peak of flowering (when many spikelets are open simultaneously). Take the observation from panicles of all plants from the inner rows of the plot. Monitor in 30-minute intervals, beginning from 6:00 AM until 12:00 NN, for three consecutive sunny/warm days. As initially observed under controlled conditions, N22, IR64, and IR64 HT begin flowering within the range of 8:30 to 9:30 AM and peak around 10:00 to 10:30 AM. While IR64 EMF and IR 64 HT + EMF begin flowering at 6:00 AM. It is possible that flowering will be earlier under field conditions. In addition, if one observation day is cloudy or overcast, add another observation day.

Statistical Analysis

All floret characteristics data were analyzed using randomized complete block design with 4 temperature treatment at time of flowering (38°C starting from 0800H, 0900H, 1000H, and 1100H), five genotypes, with 81 data points for each genotype per replicate for the following traits: anther width/length, pollen number before and after flowering; and 18 data points per genotype per replicate for pollen number on stigma, number of germinated pollen, and pollen tube length. Agronomic data and spikelet sterility was analyzed by one-way ANOVA. Analysis of variance was determined using appropriate statistical analysis program.

Results and discussion

Phenotyping IR64-derived near isogenic lines (NILs) and their parents,

IR64 and N22

Plant height (cm)

Significant differences in plant height were observed between IR64-derived near-isogenic lines (NILs), recurrent parent IR64, and heat tolerant donor parent N22 under high temperature field condition at CSU Piat (Table 1). Fig. 1 showed that the heat tolerant donor N22 significantly differed ($p=0.0011$) in height (128.61cm) compared to IR64-derived NILs screened or evaluated. Interestingly, IR64-derived NILs designated as IR64EMF, IR64HT, and IR64HT+EMF have similar plant heights of 100.52cm, 106.24cm, and 102.18cm respectively,

compared to its recurrent parent. The plant height of IR64 NILs and its recurrent parent IR64 ranged from 100.52cm to 106.24cm with an average of 109.72cm. This is a good indication that IR64-derived NILs performed the same with its recurrent parent in terms of plant height which is the primary goal of backcrossing to restore the recurrent parent genome. Similar result was obtained by Amasidha *et al.*, 2016, indicating that most of the DUS traits, both BC2F3 and BC1F4 lines were having closer values to the recurrent parent attributing to the recovery of the recipient parental genome. In addition, it concurs with the study of Ye *et al.*, 2012 using a BC1F1 population derived from the cross IR64//IR64/N22.

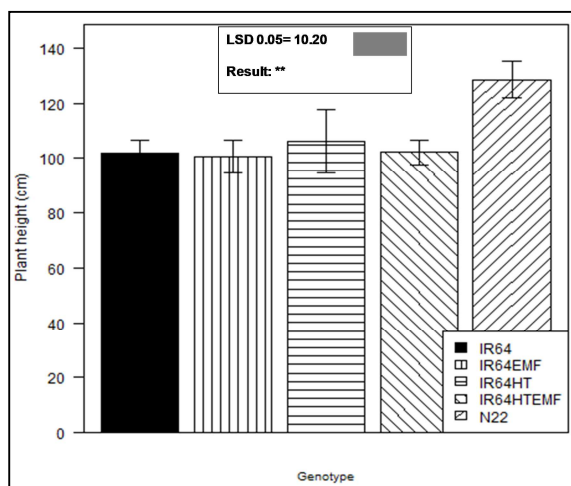


Fig. 1. Plant height (cm) of IR64-derived Near-Isogenic Lines (NILs) compared to the parents IR64 and N22, CSU-Piat.

Panicle Length (cm)

Differences in panicle length (cm) were observed between IR64-derived near-isogenic lines (NILs), recurrent parent IR64, and heat tolerant donor parent N22 under high temperature field condition at CSU Piat. Fig. 1 showed that the heat tolerant donor N22 was the shortest in panicle length (19.54cm) that is significantly different ($p=0.0170$) when compared to IR64-derived NILs screened or evaluated under high temperature field condition at CSU Piat. However, the recurrent parent IR64 has similar panicle length (30.78cm) or no significant difference when compared to IR64-derived near isogenic lines: IR64EMF, IR64HT, and IR64HT+EMF with 24.64cm, 26.73cm, and 26.45cm respectively. This is

attributed to the restoration of IR64 genome into the background of its derived lines being evaluated in the field. This concurs with the study of Amasidha *et al.*, 2016, that reported most of the DUS traits, both BC2F3 and BC1F4 lines were having closer values to the recurrent parent attributing to the recovery of the recipient parental genome.

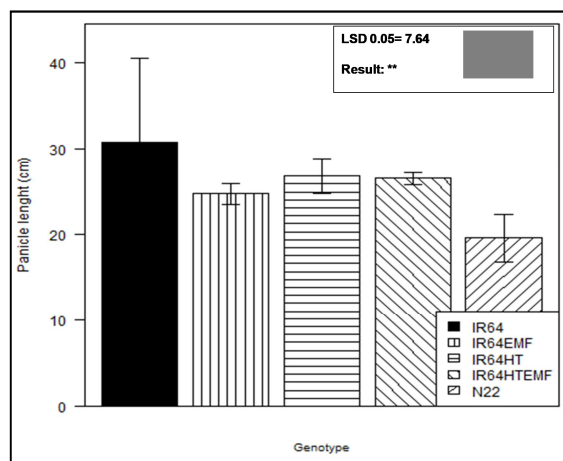


Fig. 2. Panicle length (cm) of IR64-derived Near-Isogenic Lines (NILs) compared to the parents IR64 and N22, CSU Piat.

Number of Tillers/Hill

Significant differences in number of tillers per panicle were observed among the genotypes evaluated under high temperature field condition at CSU-Piat (Fig. 3). Statistical analysis further revealed highly significant differences ($p=0.0000$) in the number of tillers per hill among IR64-derived near isogenic lines and their parents.

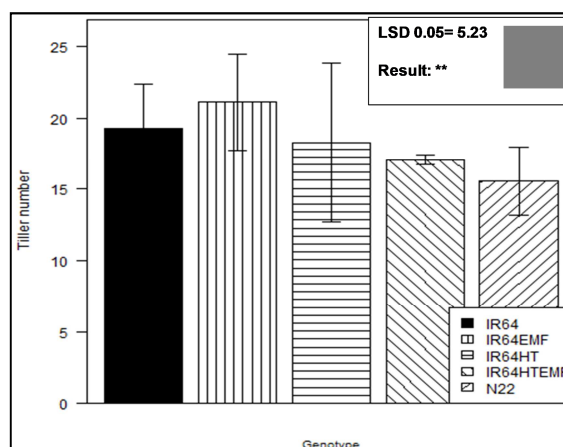


Fig. 3. Number of tillers per hill data of IR64-derived Near-Isogenic Lines (NILs) compared to the parents IR64 and N22, CSU Piat.

IR64EMF exhibited the highest number of tillers per hill (21.13), followed by the recurrent parent IR64 with 19.31 tillers, IR64HT with 18.27 tillers, and IR64HT+EMF with 17.07 tillers. While the heat tolerant donor obtained the lowest number of tillers per hill (15.58) when compared among the genotypes evaluated. This is similar with the study of Amasidha *et al.*, 2016 indicating that the overall performance of BC2F3 lines was slightly better than the BC1F4 lines for morpho-physiologically important characters like tiller number.

Number of Spikelets/Panicle

Differences in the number of spikelets per panicle of IR64-derived near-isogenic lines and their parents IR64 and N22 were significant following high temperature field condition at CSU Piat. Fig. 4 shows the number of spikelets per panicle of the IR64-derived NILs and their parent IR64 and N22 ranged from 101.73 to 119.90 with a mean of 113.12. The combination of the two QTLs in IR64HT+EMF conferred the highest number of spikelets per panicle with 119.90 than the single QTL in IR64EMF, and IR64HT with 116.25, and 116.72 spikelets respectively. The recurrent parent IR64 obtained the lowest number of spikelets per panicle (101.73) while the heat tolerant donor N22 having 110.97 spikelets/panicle.

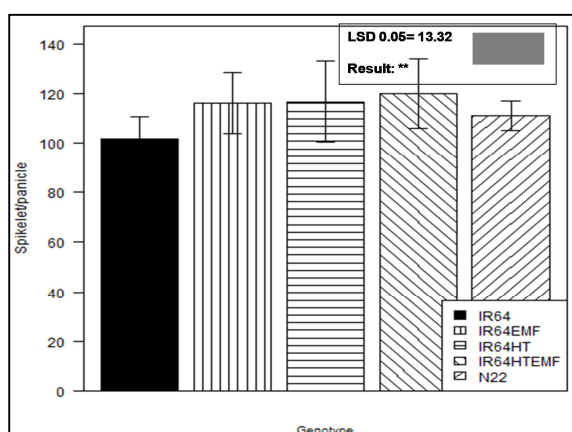


Fig. 4. Number spikelets per panicle of IR64-derived Near-Isogenic Lines (NILs) compared to the parents IR64 and N22, CSU Piat.

This concur with the results of Ye *et al.*, 2016 that the more QTLs introduced into the material, the higher

tolerance the plant has using the population IR64//Milyang23/Giza178. Similar results were reported by Baliuag *et al.*, 2015 indicating that the HT and EMF trait QTLs introduced into PSB Rc82/WAB56-125 showed relative number of spikelets per panicle.

Spikelet Fertility (%)

Spikelet fertility has been used by several scientists as screening or selection criteria for heat tolerance at reproductive stage in rice (Baliuag *et al.*, 2015). Significant differences in spikelet fertility ($p=0.0000$) were observed among IR64-derived near-isogenic lines (NILs), recurrent parent IR64, and heat tolerant donor parent N22 under high temperature field condition at CSU Piat. The recurrent parent IR64 obtained the lowest spikelet fertility of 73.48% and similarly followed by IR64HT and IR64EMF having spikelet fertilities of 76.82 and 77.39% respectively. The highest spikelet fertility was obtained by the heat tolerant donor N22 (93.81%). The combination of the two QTLs in IR64HT+EMF confers higher spikelet fertility than the single QTL in IR64EMF and IR64HT near-isogenic lines. This result strongly indicated that there is an additive effect of the two QTLs introduced into the background of IR64 conferring the high fertility (%) in IR64HT+EMF compared to other IR64-derived NILs and recurrent parent IR64. This coincides with the result of Ye *et al.*, 2016 and Baliuag *et al.*, 2015 that the more QTLs introduced into the material, the higher tolerance the plant has using the population IR64//Milyang23/Giza178 and PSB Rc82/WAB-56-125 respectively.

Baliuag *et al.*, 2015 further reported that the 11 extreme heat-tolerant (best tail) BC₁F₁ progenies have relatively higher percent seed set ranging from 32.8% to 56.1% compared to its parents having only 20.86% (PSB Rc82) and 2.5% (WAB56-125). This means that some BC₁F₁ progenies outperformed their parents in terms of seed set or spikelet fertility. The phenomenon is termed transgressive segregation where recombinant lines exhibit phenotypes better or lower than either of the two parents (Rieseberg *et al.*, 1999; De Vicente & Tanksley, 1993; Rieseberg and Ellstrand, 1993; Cosse *et al.*, 1995).

The number of viable pollen grains shed on the stigma as a result of changes in the extent of anther dehiscence, which have a direct impact on spikelet fertility and grain yield, is the primary cause of genetic variation in high temperature (heat) tolerance at flowering stage in rice. Thus, spikelet fertility under high temperature has been widely used as a screening index for heat tolerance at reproductive stage (Jagadish *et al.*, 2007).

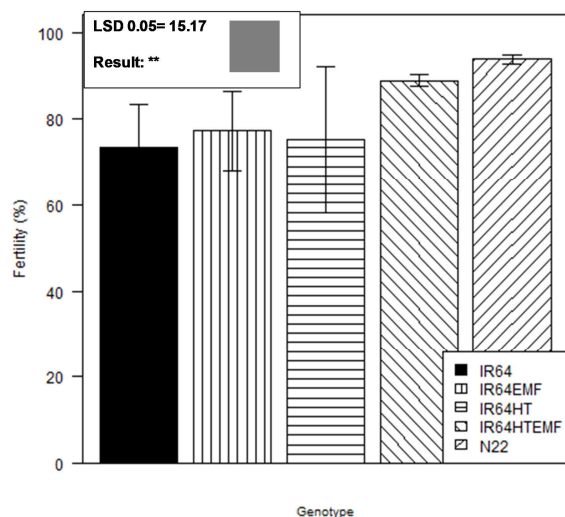


Fig. 5. Fertility (%) of IR64-derived Near-Isogenic Lines (NILs) compared to the parents IR64 and N22, CSU Piat.

II. Early Morning Flowering Traits

Early morning flowering traits of IR64-derived NILs were field evaluated at CSU Piat that coincided during the hottest month of the year (June 2016-June 2017) with maximum temperature of 39.6 °C (Fig. 6).

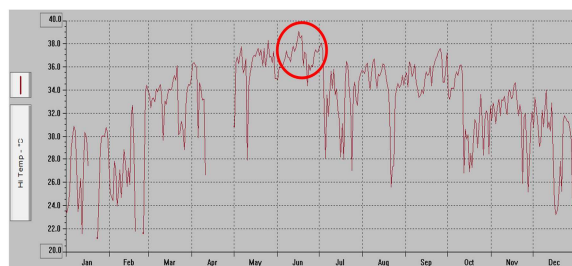


Fig. 6. Temperature data during the conduct of the study. CSU Piat, Cagayan, 2016-2017.

Start of Flower Opening

The start of flower opening of IR64-derived near-isogenic lines (NILs) compared to their parents is

shown in Fig. 7. Results revealed that IR64 NILs containing *qEMF3* and combination of *qHTSF4.1* and *qEMF3* had the earliest to start opening its flowers with IR64HT+EMF at 6:36AM (396 hr) and IR64EMF at 7:21AM (441hr) (Fig. 7). While, spikelets of N22 was the last to open at 9:04AM with air temperature of 32.6 °C and relative humidity (RH) of 68% (Fig. 7). While the spikelets of IR64 recurrent parent started to open flowers at 7:50AM at cooler temperature of 30.2 °C. In addition, statistical analysis further revealed that start of flower opening showed highly significant differences ($p=0.0000$) among genotypes evaluated. This is attributed to the QTL effect of *qEMF3* and combination of *qEMF3* and *qHTSF4.1* that advanced flower opening time by 1.5h compared from the recurrent parent IR64. Same result was obtained by Hirabayashi *et al.*, 2014, who found that *qEMF3* contributes to heat escape at flowering by advancing FOT to early morning.

Similar results were obtained by Baliuag *et al.*, 2015, that reported that majority of the BC₁F₂ progenies started opening spikelets before 540 minutes or <9:00 AM. While the parents, WAB56-125 (donor parent) was earlier to start opening flowers at 431.67 min. (8:11 A.M.) than PSB Rc82 (recurrent parent) at 495 minutes or 9:15 AM.

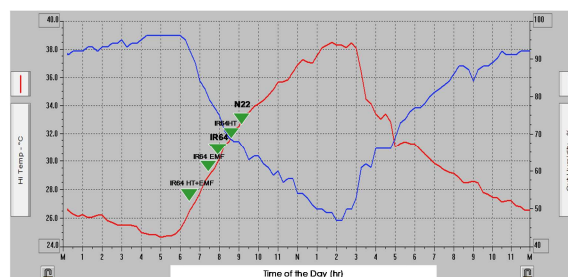


Fig. 7. Start of flower opening (FOT) of IR64-derived near-isogenic lines (NILs) and their parents, N22 and IR64 during flowering stage. Source: AWDL, Cagayan State University (CSU) Piat, Cagayan, June 1-30, 2017.

Relatively, WAB56-125 is earlier to start opening spikelets in the morning than most of the 684 BC₁F₂ populations. This could be attributed to its genetic make-up since WAB56-125 is derived from a cross between *Oryza sativa* L. and *Oryza glaberrima* Steud. Generally, *Oryza glaberrima* Steud and its hybrids

derived from interspecific crosses to *O. sativa* open flowers earlier than those of *Oryza sativa* L. (Nishiyama and Blanco, 1980; Jagadish *et al.*, 2008; Nishiyama and Satake, 1981).

Peak Flowering Time

Fig. 8 illustrates the time of peak flowering among IR64-derived near isogenic lines and their parents, IR64 and N22. The IR64HT+EMF exhibited the earliest peak flowering at 7:33AM (454 hr) followed by IR64EMF which at 8:08AM (530 hr). The last to peak flowering was the heat tolerant donor N22 at 9:47AM (588 hr). Results revealed that IR64 NILs containing *qEMF3* and combination of *qHTSF4.1* and *qEMF3* (IR64EMF and IR64HT+EMF respectively) reached peak flowering earlier than the two parents, IR64 and N22. This result concurs with the study of Hirabiyashi *et al.*, 2014 using Nagina 11 and wild rice *Oryza officinalis* (CC genome) as source of QTL for early morning flowering at chromosome 3. In addition, Baliuag *et al.*, 2015 reported earliest peak flowering time of the BC1F2 populations derived from the cross PSB Rc82/WAB56-125, than the recurrent parent PSB Rc82 which flowers peaked at later times. Ishamaru *et al.*, 2010 further reported that, the early-morning flowering (EMF) trait mitigates heat-induced spikelet sterility at the flowering stage by escaping heat stress during the daytime.

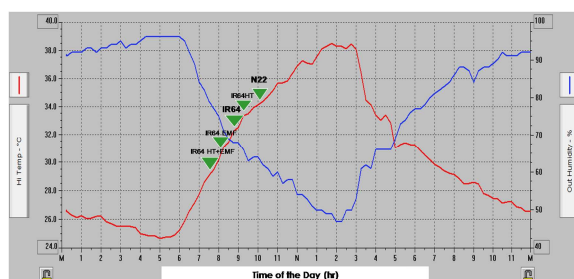


Fig. 8. Peak flowering time (PFT) of IR64-derived near-isogenic lines (NILs) and their parents, N22 and IR64 during flowering stage. Source: AWDL, Cagayan State University (CSU) Piat, Cagayan, June 1-30, 2017.

Closed Flowering Time (CFT)

Fig. 9 shows the trend of flower closing time among the breeding lines evaluated. Fig. 9 further shows that IR64HT+EMF closed all of its florets at 10:30AM (630 hr) followed by IR64EMF which closed florets at

11:01AM (660 hr). The last to closed all its florets was the heat tolerant donor N22 at 12:58PM (778 hr). Generally, for the early morning flowering traits (EMF) traits evaluated, IR64HT+EMF and IR64EMF NILs exhibited earliest to open its flower, peak of flowering, and closing of flowers during the time of the day compared to the recurrent parent IR64. This result is attributed to the *qEMF3* conferring the EMF locus that was derived from its wild rice parent, *Oryza officinalis* (CC genome) to mitigate heat stress damage during the flowering stage of the crop (Hirabiyashi *et al.*, 2014 and Ishamaru *et al.*, 2010). It was reported further by Hirabiyashi *et al.* (2014), that a stable quantitative trait locus (QTL) for flower opening time was detected on chromosome 3. This QTL was named as *qEMF3* conferring earlier flower opening time by 1.5-2.0 hours than Nanjing 11 in temperate Japan and IR64 in the Philippine tropics.

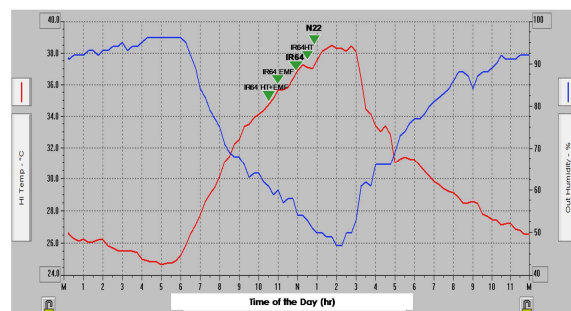


Fig. 9. Close flowering time (CFT) of IR64-derived near-isogenic lines (NILs) and their parents, N22 and IR64 during flowering stage. Source: AWDL, Cagayan State University (CSU) Piat, Cagayan, June 1-30, 2017.

Days to 50% Flowering and Maturity

Results revealed showed highly significant differences ($p=0.0000$) in days to 50% flowering and maturity between N22 and IR64 NILs and recurrent parent. Fig. 10 indicates that the earliest to flower and mature was the heat tolerant donor N22 relative to the IR64 NILs. While the last to flower and mature was IR64 EMF. However, IR64 NILs evaluated have similar days to 50% flowering and maturity. This result is attributed to recovery of the recurrent parental genome used while the genetic variations of genotypes evaluated under high temperature field conditions are attributed to the genetic backgrounds of the breeding lines used.

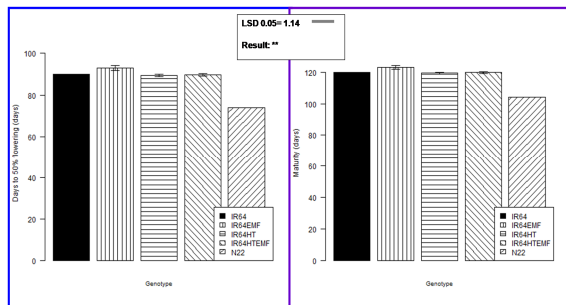


Fig. 10. Days to 50% flowering and maturity of the breeding materials IR64-derived near-isogenic lines (NILs) and their parents, IR64 and N22. CSU Piat, March to July 2016.

Yield (kg ha^{-1})

Highly significant differences ($p=0.0000$) in yield (kg ha^{-1}) were observed among IR64-derived NILs and their parents N22 and IR64 evaluated under high temperature field condition at CSU Piat. Results revealed that IR64HT obtained the highest yield of 5,850.44 kg/ha , followed by IR64EMF and IR64HT+EMF with mean yields of 4,974.19 and 4,891.88 kg/ha respectively (Fig. 11). The lowest yield was obtained by N22 (2,951.43 kg/ha) (Fig. 11). These indicate that introgressing HT and EMF QTLs into mega-variety IR64 will relatively increase yield even under high temperature condition.

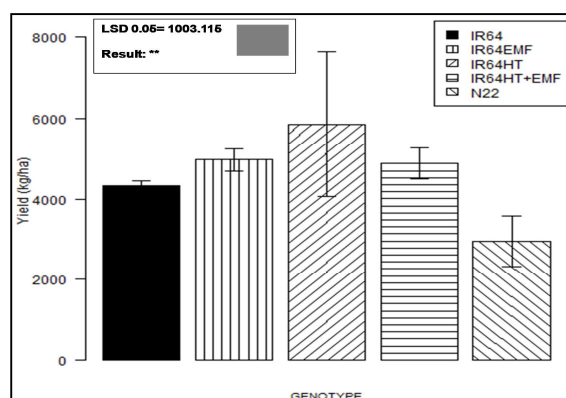


Fig. 11. Yield (kg ha^{-1}) of IR64-derived near-isogenic lines compared to the parents IR64 and N22. CSU Piat.

A stronger tolerance can be achieved by combining and assembling QTLs that confer the trait into a single genotype because heat tolerance at flowering stage is controlled by many QTLs with small, minor effects (Ye *et al.*, 2016). Pyramiding the identified *qHTSF4.1* for true heat tolerance and *qEMF3* for early

morning flowering into a single genotype has the potential to create a cultivar superior in terms heat tolerance during the flowering stage.

Conclusion and recommendation

Based from the results of the study, it is concluded that IR64-derived NILs have similar morpho-agronomic traits compared to its recurrent parent indicating restoration of recurrent parent genome. For early morning flowering traits (EMF) evaluated, IR64HT+EMF and IR64EMF NILs exhibited the earliest to start flower opening, peak of flower opening, and closing of flowers during the day. It is further concluded that the presence of *qEMF3* and its combination with *qHTSF4.1* strongly indicate early morning flowering traits.

Based from the results of the study, the researchers recommend the use of combined genes of heat tolerance (*qHTSF4.1*) and genes for early morning flowering (*qEMF3*) as these are useful genetic materials for future breeding programs of rice adapted in warmer environment.

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