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Quantitative Trait Loci for High-Temperature Tolerance in Rice (*Oryza sativa* L.)

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Dular, a rice variety that is tolerant to high temperature, usually has low yield potential and undesirable traits. Due to its diverse genetic background, however, Dular in combination with a high-yielding and improved rice variety such as NSIC Rc150, (Tubigan 9) which yields 8.5 t ha^{-1}, can produce new high-temperature tolerant rice genotypes with high yield potential and suitable for quantitative trait loci (QTL) analysis. To identify QTL for high-temperature tolerance in rice, the BC_2F_4 lines derived from NSIC Rc150 x Dular were grown in a glasshouse during the 2014 dry season. The temperature reached 39.88 °C ± 0.17 (min. 29.84 ± 0.14) during heading and maturity stage. Pearson correlation and multiple linear regression analysis were used to evaluate phenotypic traits associated with fertility. Heading days, time of flowering, and dehiscent high-temperature were found to be associated with spikelet fertility. A total of 126 simple sequence repeat (SSR) DNA markers, spanning the 12 chromosomes in 3mb Bin system, were used in genotyping 246 progenies. QTLs were identified through inclusive composite interval mapping (ICIM) method using IciMapping 4.0 software. The six major QTLs detected for high-temperature tolerance were *q*HTfert1 with phenotypic variance explained (PVE) of 18.6% (logarithm of odds, LOD 8.99), *q*HTfert3 with PVE of 57.4% (LOD 9.18), *q*HTfert4 with PVE of 10.2% (LOD 8.46), *q*HTtof10 with PVE of 16.3% (LOD 9.95), *q*HTdht3 with PVE of 31.7% (LOD 7.83) and *q*HThd3 with PVE of 30.9% (LOD 6.54). Three minor QTLs were identified namely, *q*HTdht4 with PVE of 9.4% (LOD 8.15), *q*HTdht5 with PVE of 9.2% (LOD 7.18) and *q*HTdht10 with PVE of 7.1% (LOD 6.69). Nine QTLs identified for high-temperature tolerance were located in chromosomes 1, 3, 4, 5 and 10. These findings can be used in rice breeding using marker-assisted selection (MAS) and fine mapping of novel genes for high-temperature tolerance.

Key Words: quantitative trait loci, high-temperature tolerance, inclusive composite interval mapping, associated traits

Abbreviations: LOD – logarithm of odds, MAS – marker-assisted selection, PCR – polymerase chain reaction, PVE – phenotypic variance explained, QTL – quantitative trait loci, *q*HTfert – QTL for fertility, *q*HTtof – QTL for time of flowering, *q*HTdht – QTL for dehiscent high-temperature, *q*HThd – QTL for heading days, RH – relative humidity, SSR – simple sequence repeat

INTRODUCTION

The world is inevitably facing climate change. Global warming has a significant impact in sustaining and improving agricultural productivity (Ye et al. 2011). Chilling, drought, salinity, submergence and high-temperature stress have become increasingly important as yield-limiting factors coupled with increased frequency of short-term but extremely high temperatures. Global warming is particularly damaging to vulnerable regions in South and Southeast Asia, including the Philippines (Ye et al. 2011; Jagadish et al. 2012; Buu et al. 2014). Global temperatures are estimated to rise by 1.1 to 6.4 °C during the next century (IPCC 2012) due to both anthropogenic and natural factors (Eitzinger et al. 2010). Analysis of temperature and rice yield in 1992–2003 at the International Rice Research Institute (IRRI) showed that rice grain yield declined by 10% for each 1 °C increase in temperature, indicating vulnerability of rice to heat stress (Peng et al. 2004). Ceccarelli et al. (2010) also predicted reduction of rice yields by 41% due to high temperatures by the end of the 21st century. Thus, predicted increment in temperature poses a threat to rice production (Shah et al. 2011).

Many reports confirmed that high temperature affects all rice growth stages, from emergence to ripening. The flowering stage and, to a lesser extent, the booting stage are the most sensitive to high temperature (Imaki et al. 1982; Shah et al. 2011). Temperatures over 35 °C at flowering stage cause high pollen and spikelet sterility, which leads to serious yield losses, low grain quality and low harvest index (Osada et al. 1973; Matsushima et al. 1982; Matsui et al. 1997a,b; Zhong et al. 2005; Prasad et al. 2006). The main cause of spikelet sterility induced by high temperature at the flowering stage is anther indehiscence. In high temperature conditions, the anthers of heat-tolerant cultivars dehisce more easily compared with those of susceptible cultivars (Satake and Yoshida 1978; Mackill et al. 1982; Matsui et al. 1997a,b 2001) due to tight closure of the locules by the cell layers,

which delays locule opening and decreases spikelet fertility at high temperature (Matsui and Omasa 2002).

Plant responses to high temperatures clearly depend on genotypic parameters, as certain genotypes are more tolerant (Prasad et al. 2006; Challinor et al. 2007). Interactions of numerous biochemical and metabolic traits are involved in the development and maintenance of thermo-tolerance such as gene expression and translation, and protein stability (Kaya et al. 2001). These interactions respond to a wide range of temperatures by reprogramming the transcriptome, protein and metabolome and even activating the cell death mechanism, apoptosis, leading to organ abortion or entire plant death (Qi et al. 2011; Bita and Gerats 2013).

As rice production in the hot regions expands and the global temperature continuously rises, incorporation of high-temperature tolerance in rice is becoming a key breeding objective (Manigbas and Sebastian 2007; Ye et al. 2011). Development of new crop cultivars that are tolerant to high temperature is a major challenge to mitigate the effect of high-temperature stress. Scientists in rice research are studying plant responses that could lead to high-temperature tolerance, and how rice plants can be managed in high-temperature environments (Hasanuzzaman et al. 2013).

Germplasm innovation and identification of high-temperature-tolerant inbred rice varieties are initiatives for the development of susceptible high-yielding Philippine rice varieties (Manigbas et al. 2014). Identification and mapping of genes and quantitative trait loci (QTL) analysis for high-temperature tolerance will not only facilitate marker-assisted breeding (MAS) but also direct the way for cloning and characterization of underlying factors that could be useful for genetic engineering. This technology is made possible by exploitation of new genes and alleles to predict gene function, isolate homologues, and conduct transgenic experiments to develop new varieties under high-temperature stress conditions.

This study was conducted to identify QTL associated with high-temperature tolerance and simple sequence repeat (SSR) markers that could potentially be applied in molecular-aided breeding as a prerequisite for fine mapping of novel genes for high-temperature tolerance in rice.

MATERIALS AND METHODS

Plant Materials

A total of 246 progenies of BC_2F_4, a backcrossed inbred line (BIL) of NSIC Rc150 (*recurrent*) x Dular (*donor*), were used as mapping population. The seeds of parental varieties NSIC Rc150 (*susceptible*) and Dular (*tolerant*) and of check varieties Nagina22 (*tolerant*), Gayabyeo (*susceptible*) and IR64 (*intermediate tolerant*) were obtained from the Philippine Rice Research Institute and the International Rice Research Institute. The seeds were pre-germinated and sown in seed beds and transplanted in plastic pots (L x W x H = 40 x 30 x 12 cm) filled with natural clay loam soil.

Plants were grown in the glasshouse during the dry season from January to May 2014. The temperature (°C), relative humidity (RH) and dew point (°C) in the glasshouse were automatically recorded using MINCER (Micrometeorological Instrument for Near Canopy Environment of Rice) set to gather data every 2 min for 24 h. Fertilizer rate at 120-60-60 (N-P_2O_4-K_2O) was applied 12, 30 and 50 d after transplanting. Plants were watered from 5:00 PM to 6:00 PM to avoid drought during the time of flowering.

Twenty-one days after transplanting, five young leaves (~10 cm long) of each tagged plant were collected. Leaf samples were placed in a designated glassine bag and placed in an icebox during transport. Collected leaf samples were temporarily stored in a freezer at −20 °C prior to DNA extraction.

DNA Preparation and Amplification

Purified genomic DNA leaf samples were extracted using the Modified Cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson 1980; Perez et al. 2012), and dissolved in TE buffer with RNAse. DNA quality and quantity were estimated spectrometrically. Polymerase chain reaction (PCR) amplification was carried out using 6.82 µL for each reaction well containing sdH_2O; 5x PCR buffer; 10 mM $MgCl_2$; 1 mM dNTP; 10 µM forward and reverse primers; 5 U Taq polymerase; DNA sample (1:5, ~20 ng) and over laid with a drop of mineral oil. The PCR regime was: 94 °C for 5 min, followed by 30 cycles of 1 min denaturing at 94 °C, 1 min primer annealing at 55 to 67 °C, and 2 min primer extension at 72 °C; a final extension of 5 min at 72 °C completed the PCR cycle in a programmable thermal cycler (PTC® 100, MJ Research Inc.).

Polyacrylamide Gel Electrophoresis (PAGE)

PCR-amplified products were loaded into 8% non-denaturing polyacrylamide gel with 1x TBE buffer. The samples were prepared by adding 6 µL of loading dye (3x STR dye), and 3.5 µL samples were loaded on the gel. A 100 bp molecular weight ladder was loaded and run side by side with the sample. The gels were electrophoresed at 100 V for 1 h and stained with 5% SYBR® Safe DNA staining solution for 10 min. The products were viewed in Molecular Imager® Gel Doc™ XR System with Image Lab™ Software for gel imaging and analysis.

Polymorphism Survey

For SSR assay, 396 SSR DNA primer pairs derived from the Cornell University SSR Linkage map (McCouch et al. 2002) were surveyed between parental DNAs (Fig. 1 A-B). SSR DNA markers showing different banding patterns on varieties Dular and NSIC Rc150 were selected. The location of the chromosome in 3mb Bin system, the repeat motif, the annealing temperature and the expected product were also considered in the selection of polymorphic markers. A total of 126 SSR primer pairs were selected and utilized in genotyping the BC_2F_4 lines. Figure 2 shows the graphical distribution of polymorphic markers used in genotyping the mapping

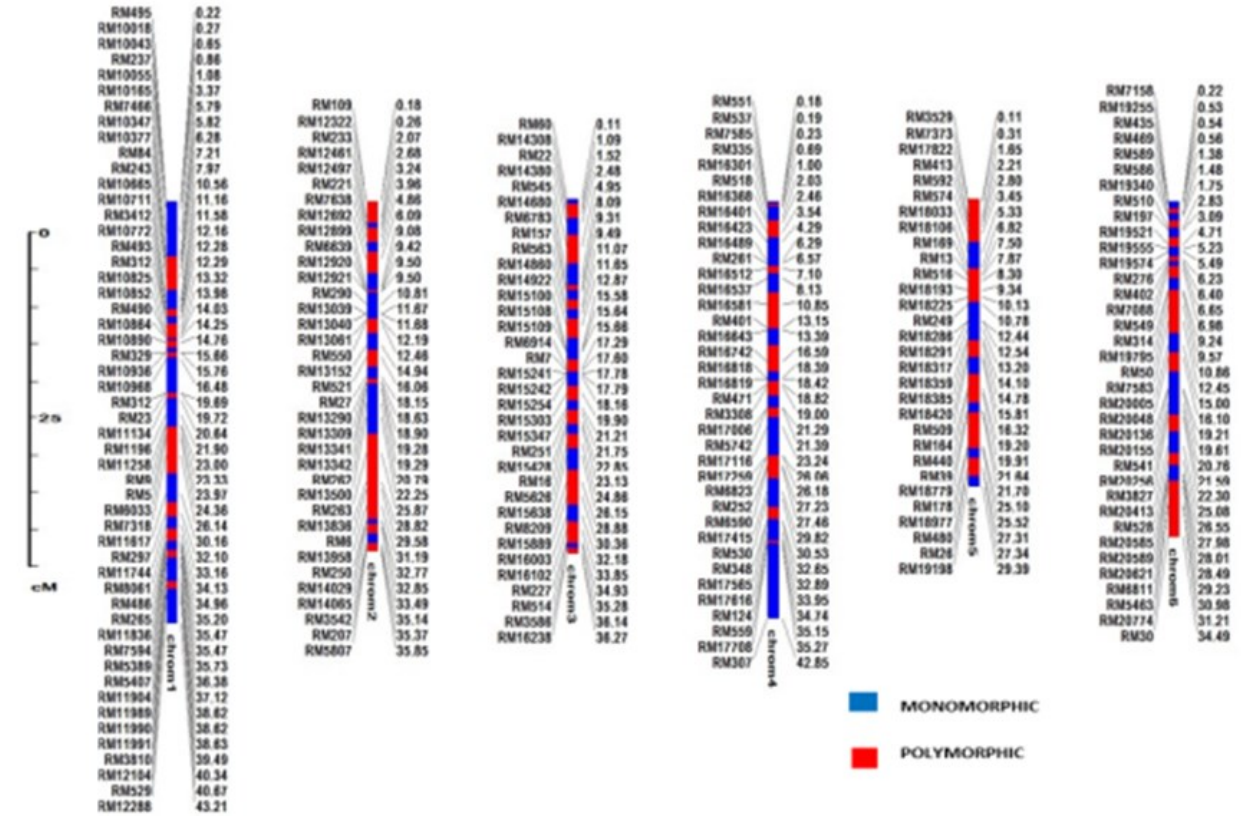


Fig. 1A. Graphical distribution of simple sequence repeat (SSR) markers used in polymorphism survey (Chr. 1–6).

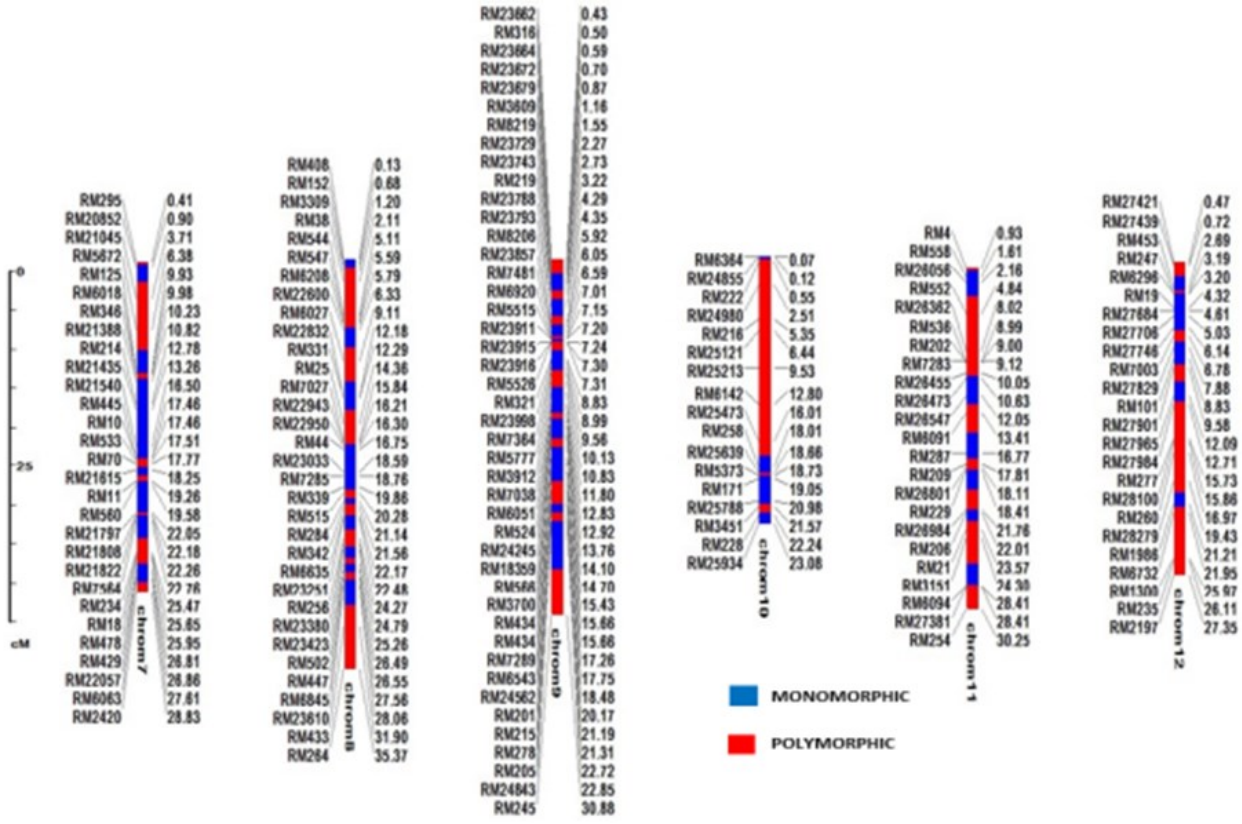
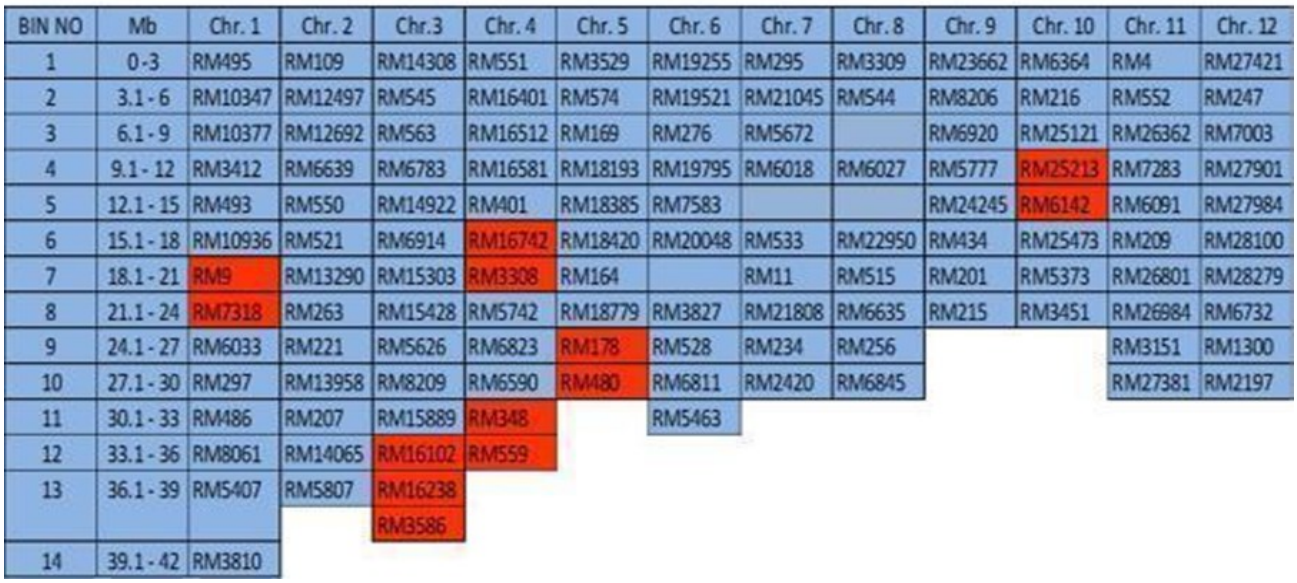


Fig. 1B. Graphical distribution of simple sequence repeat (SSR) markers used in polymorphism survey (Chr. 7–12).



BIN NO	Mb	Chr. 1	Chr. 2	Chr.3	Chr. 4	Chr. 5	Chr. 6	Chr. 7	Chr. 8	Chr. 9	Chr. 10	Chr. 11	Chr. 12
1	0-3	RM495	RM109	RM14308	RM551	RM3529	RM19255	RM295	RM3309	RM23662	RM6364	RM4	RM27421
2	3.1 - 6	RM10347	RM12497	RM545	RM16401	RM574	RM19521	RM21045	RM544	RM8206	RM216	RM552	RM247
3	6.1 - 9	RM10377	RM12692	RM563	RM16512	RM169	RM276	RM5672		RM6920	RM25121	RM26362	RM7003
4	9.1 - 12	RM3412	RM6639	RM6783	RM16581	RM18193	RM19795	RM6018	RM6027	RM5777	RM25213	RM7283	RM27901
5	12.1 - 15	RM493	RM550	RM14922	RM401	RM18385	RM7583			RM24245	RM6142	RM6091	RM27984
6	15.1 - 18	RM10936	RM521	RM6914	RM16742	RM18420	RM20048	RM533	RM22950	RM434	RM25473	RM209	RM28100
7	18.1 - 21	RM9	RM13290	RM15303	RM3308	RM164		RM11	RM515	RM201	RM5373	RM26801	RM28279
8	21.1 - 24	RM7318	RM263	RM15428	RM5742	RM18779	RM3827	RM21808	RM6635	RM215	RM3451	RM26984	RM6732
9	24.1 - 27	RM6033	RM221	RM5626	RM6823	RM178	RM528	RM234	RM256			RM3151	RM1300
10	27.1 - 30	RM297	RM13958	RM8209	RM6590	RM480	RM6811	RM2420	RM6845			RM27381	RM2197
11	30.1 - 33	RM486	RM207	RM15889	RM348		RM5463						
12	33.1 - 36	RM8061	RM14065	RM16102	RM559								
13	36.1 - 39	RM5407	RM5807	RM16238									
				RM3586									
14	39.1 - 42	RM3810											

Fig. 2. Graphical distribution (3mb BIN system) of selected 126 polymorphic markers utilized in genotyping 246 lines.

population. SSR primer markers were arranged in 3 mb Bin system to facilitate the selection and even distribution of DNA markers throughout the genome covering 1308.5205 centiMorgan (cM). Markers' position, map start and map stop were based on the Cornell University SSR Linkage map (retrieved from www.gramene.org).

Phenotyping of BC_2F_4 Population

Mapping population was screened under high temperature condition from heading to maturity stage. When plants started heading, the first three panicles were marked. Heading date was used as basis for the number of days to maturity. Duration of heading (HD) was determined from the day of start to the end of heading. The time and date on MINCER and improvised digital clock were synchronized to facilitate phenotype marking on time of flowering (TOF), dehiscent high-temperature (temp. at TOF), relative humidity (RH at TOF) and dehiscent dew point (dew point at TOF). At physiological maturity, data on plant height, number of productive tillers, panicle length, and number of filled and unfilled grains were also recorded. The mean spikelet fertility of the three panicles was used to evaluate high-temperature tolerance of the progenies.

Phenotypic Correlation and Regression

The phenotypic data were analyzed using Pearson correlation and multiple linear regression. The statistical software used was SPSS version 13.0. Association of traits for high-temperature tolerance was conducted with the theoretical assumption that spikelet fertility was a significant direct indicator of high-temperature tolerance. Pearson correlation was a preliminary analysis for multiple linear regression to test whether or not the traits correlated with fertility would fit the regression model. Spikelet fertility was used as the dependent variable and correlated traits were used as independent variables. The null hypothesis (Ho) for regression analysis inferred that there was no significant correlation between fertility and other observed traits while the alternative hypothesis (Ha) inferred that at least one of the independent variables was correlated with fertility. Phenotypes that gave significant information at 5% level of significance on spikelet fertility were used in the QTL association analysis.

QTL Analysis

Inclusive composite interval mapping (ICIM) through IciMapping 4.0 software was used to identify the genetic loci responsible for the variation of study traits**.** Linkage groups were identified using command group with logarithm of odds (LOD). The LOD value was used to check linkage among the SSR markers. LOD profile was used to identify the most likely position for a QTL in relation to the linkage map. Permutation test was done at 1,000 iterations to increase the precision of putative QTLs or to reduce the probability of finding false marker-QTL association. Detected QTL, which could meet the new threshold set by permutation, was selected based on significance level at $p>0.05$ and a LOD score of 3.5. Epistasis mapping was also conducted to determine QTL interaction.

RESULTS

Environmental Evaluation for High-temperature Screening

Daily mean temperature, relative humidity and dew point in glasshouse from heading to maturity stage are shown in Table 1. The mean temperature from heading to maturity was 37.04 °C ± 0.15 (39.88–35.10 °C) with mean relative humidity of 67.60% ± 1.82 (89.00–35.03%), and mean dew point of 27.55 °C ± 0.37 (31.98–18.7 °C) (Fig. 3). The highest daytime mean temperature of 35.96 °C was at 01:00 AM to 03:00 PM (Table 2). There was a mean difference of 3.45 °C at 07:00 to 09:00 AM, 1.92 °C at 09:00 to 11:00 AM, 0.75 °C at 11:00 AM

Table 1. Daily mean temperature, relative humidity and dew point in glasshouse from heading to maturity stage of rice recorded in 2014 dry season by MINCER.

Time Segment	Temperature (°C)	Relative Humidity (%)	Dew Point (°C)
07:00–09:00 AM	29.84 ± 0.14 (29.95 – 36.53)	84.42 ± 0.82 (69.56 – 92.28)	24.59 ± 0.16 (22.47 – 26.82)
09:00–11:00 AM	33.29 ± 0.14 (33.02 – 38.37)	75.01 ± 1.19 (53.22 – 87.88)	25.88 ± 0.18 (21.19 – 28.23)
11:00 AM–01:00 PM	35.21 ± 0.15 (31.84 – 38.75)	70.11 ± 1.37 (42.72 – 81.04)	26.50 ± 0.24 (20.10 – 28.84)
01:00–03:00 PM	35.96 ± 0.17 (31.85 – 39.88)	69.20 ± 1.56 (38.29 – 86.05)	26.93 ± 0.29 (19.40 – 29.89)
03:00–05:00 PM	35.16 ± 0.17 (31.32 – 37.17)	71.24 ± 1.52 (38.66 – 84.39)	26.69 ± 0.28 (19.50 – 29.56)

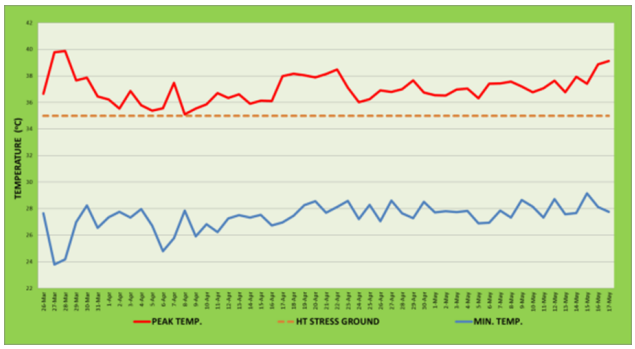


Fig. 3. Temperature in glasshouse from heading to maturity stage of rice during 2014 dry season.

Table 2. Phenotypic correlation and regression analysis for traits associated with fertility.

Traits	Fertility		Regression
	Pearson r	P-value	P-value
Fertility (constant)	-	-	0.00*
Plant height	0.36	0.00*	0.49ns
No. of productive tillers	0.02	0.70ns	0.82ns
Panicle length	0.34	0.00*	0.05ns
No. of spikelets	0.39	0.00*	0.91ns
Heading day	0.46	0.00*	0.00*
Time of flowering	0.57	0.00*	0.01*
Dehiscent high-temperature	0.65	0.00*	0.00*
Dehiscent relative humidity	−0.33	0.00*	0.27ns
Dehiscent dew point	0.30	0.00*	0.68ns

*Correlation is significant at the 5% level (2-tailed) in LSD.
nsCorrelation is not significant at the 5% level in LSD.

to 01:00 PM, and 0.80 °C at 01:00 to 03:00 PM. The observed temperature was higher than the optimum temperature threshold for rice (27.0–32 °C). The temperature 35.0 to 43.0 °C was high enough to cause spikelet fertility. This observation shows high-temperance stress in the glasshouse, conferring a suitable environment for screening high-temperature-tolerant progenies.

Relative humidity from heading to maturity stage suggested that there was a very dry (38.29% ± 1.56) to humid (92.28% ± 0.82) environment in the glasshouse. Although fluctuations in RH were observed from heading to maturity stage, these were enough to modify the effect of high temperature on spikelet fertility, as higher relative humidity at the flowering stage under increased temperature affected spikelet fertility negatively.

Phenotypic Correlation of BC_2F_4

Pearson correlation showed that plant height, panicle length, number of spikelet, heading days, time of flowering, dehiscent temperature, dehiscent relative humidity and dehiscent dew point were significantly correlated with spikelet fertility but insignificant in terms of the number of productive tillers (Table 2).

Regression analysis indicated that heading days, time of flowering, and dehiscent high-temperature were significantly related to spikelet fertility (Table 2). The results showed that 57.1% of the variation could be explained.

The mapping population had a mean fertility of 76.34% ± 0.83 with maximum fertility of up to 98.51% and the minimum at 13.07%. A total of 124 lines which account for 50% of the total lines had the highest frequency of 70–84% fertility. Promising lines (67 plants) were identified which account for 27% of the total lines with fertility greater than 85% (Fig. 4-A). There were only two lines with fertility lower than 39%. BC_2F_4 population had lower fertility (76.3%, min. 13.07 – 95.81, SE = 0.83) compared with Dular (91.9%, min. 91.86 – max. 91.88, SE = 0.04), N22 (94.4%, min. 93.21 – max 95.73, SE = 0.72) and IR64 (89.5%, min. 86.73 – max. 94.85, SE = 2.69) but higher than NSIC Rc150 (72.6%, min. 68.1614 – max. 76.52) and Gayabyeo (73.3%, min. 67.52 – max. 77.31, SE = 2.95) (Fig. 5-A). The results indicated that there are high-temperature tolerant lines and susceptible lines in the mapping population.

The mapping population had a mean time of flowering at 11:53 AM (11.89 ± 0.06) with the earliest time at 09:22 AM (9.37 ± 0.06) and late flowering at 02:46 AM (14.77 ± 0.06). The 102 BC_2F_4 lines, which accounted for 41% of the total lines, dehisced from 11:00 AM to 11:59 PM (Fig. 4-B), followed by 80 lines (32% of the total lines) which dehisced from 12:00 PM to 12:59 PM. Twenty-one lines which accounted for 8% of the total population dehisced from 01:00 PM to 01:59 PM. Eight and 29 lines dehisced at 09:00 to 09:59 AM and at 10:00 to 10:59 AM, respectively.

Eight flowering lines (09:00 to 09:59 AM) had lower fertility (61.47% ± 7.62) compared with the lines dehisced at 10:00 to 10:59 AM (66.66% ± 2.40) and at 11:00 to 11:59 AM (72.49% ± 1.14). No lines dehisced earlier than 09:00 AM but there were six lines which dehisced from 02:00 to 03:00 PM. A total of 182 lines dehisced at 35 to 36 °C from 11:00 AM to 01:00 PM.

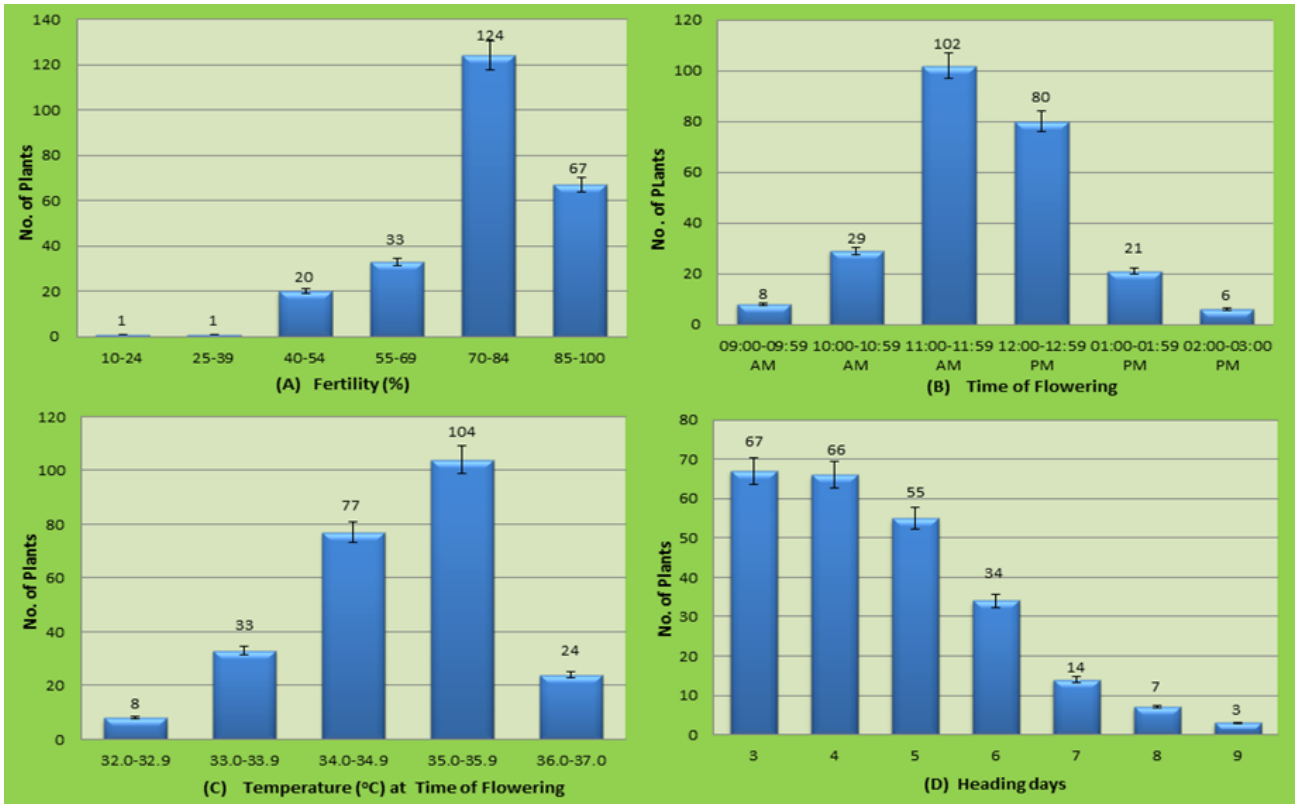


Fig. 4. Frequency distribution of significant correlated traits, spikelet fertility, time of flowering, temperature at time of flowering and duration of heading.

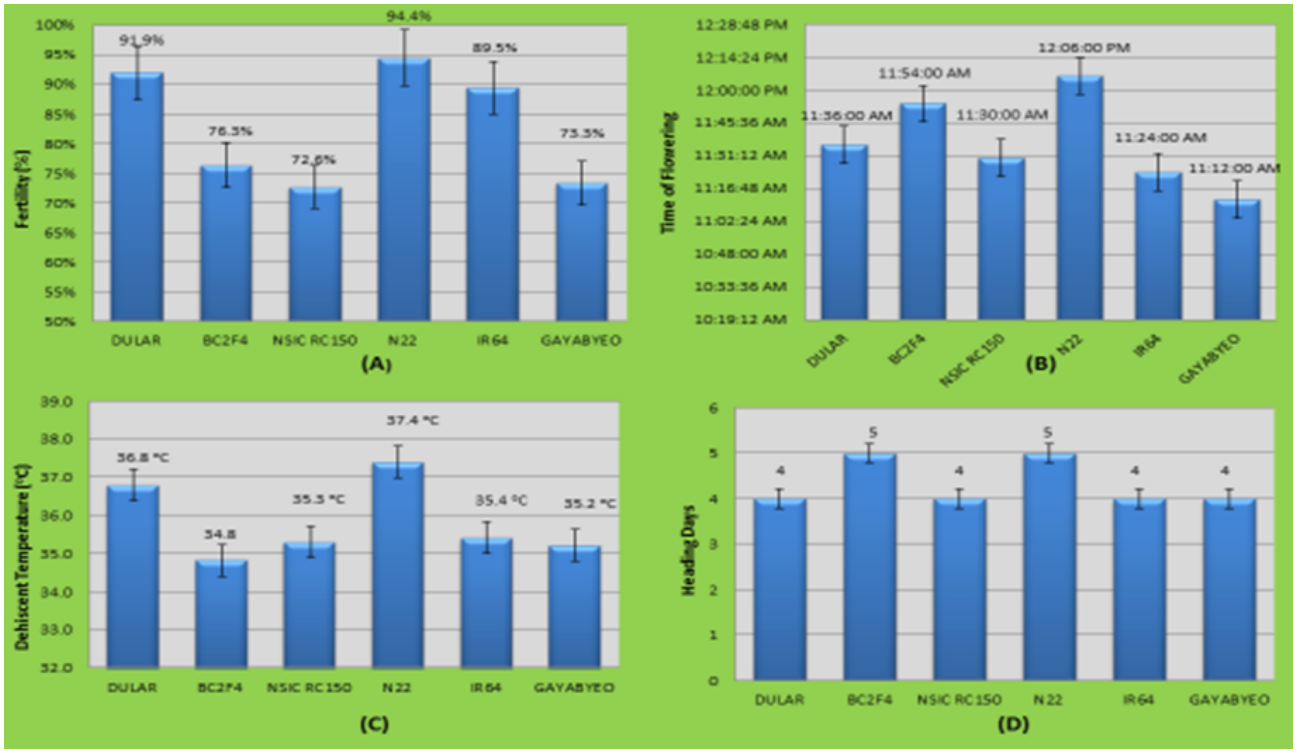


Fig. 5. Comparison of phenotypic mean of parentals, BC_2F_4 and check varieties.

Twenty-one lines dehisced at 01:00 to 01:59 PM with fertility of 88.71% ± 1.08, and 6 lines dehisced at 02:00 to 02:59 PM with 91.46% ± 1.25 fertility. This result indicates the number of tolerant lines in the mapping population showing anther dehiscence at high-temperature stress. The time of flowering was from 11:00 AM to 12:00 noon for Dular, BC_2F_4, NSIC Rc150, IR64 and Gayabyeo. N22 dehisced from 12:00 noon to 01:00 pm (Fig. 5-B). The mean time of flowering of the population conformed to the mean temperature in the glasshouse from 11:00 AM to 01:00 PM with 35.21 °C and from 01:00 PM to 03:00 PM with 35.96 °C, indicating the presence of high-temperature stress during flowering of tolerant lines.

The mean dehiscent temperature of BC_2F_4 lines was at 34.8 °C ± 0.06, with the highest dehiscent threshold at 36.6 °C and the minimum at 32.4 °C. Dehiscent temperature is the temperature at which pollens dehisce at a given temperature. The highest frequency was observed at 35.0 to 35.9 °C in 104 lines, which constituted 43% of the population (Fig. 4-C). Twenty-four lines, constituting 10% of the total lines, dehisced below 36 °C. The second largest frequency was observed at 34.0 to 34.9 °C in 77 lines, constituting 31% of the population. Thirty-three lines (13% of the total lines) dehisced at 33.0 to 33.9 °C while only eight lines (3% of the population) dehisced at 32.0 to 32.9 °C. The results showed that 128 BC_2F_4 lines (52% of the total mapping population) dehisced at temperatures greater than 35 °C. Dular had a dehiscent temperature of 36.8 °C; N22, 37.4 °C; BC_2F_4, 34.8 °C; NSIC Rc150, 35.3 °C; IR64, 35.4 °C and Gayabyeo, 35.25 °C (Fig. 5-C). N22 showed higher dehiscent temperature than Dular; however, Dular had higher dehiscent temperature than IR64, NSIC Rc150 and Gayabyeo.

The BC_2F_4 lines had mean heading days of 4.57 ± 0.09, with the longest at 9 d and the shortest at 3 d (Fig. 4-D). The highest frequency had 67 lines followed by 66 BC_2F_4 lines which constituted 54% of the total population. There were 113 BC_2F_4 lines which headed longer than 6 d, representing 46% of the total lines. N22 and BC_2F_4 lines headed longer than Dular, NSIC Rc150, IR64 and Gayabyeo (Fig. 5-D).

Quantitative Trait Loci for High-Temperature Tolerance

There were 3 QTLs for spikelet fertility, 1 QTL for time of flowering, 4 QTLs for dehiscent high-temperature, and 1 QTL for heading days (Table 3). The profile of the likely sites of QTL between adjacent linked markers is shown in Figure 6 and the putative location is shown in Figure 7. Six QTLs were major and three were declared minor QTLs. The *q*HTfert1 explained 18.6% of the phenotypic variance showing partial dominance on NSIC Rc150. Similarly, *q*HTfert3 explained 57.4% of the phenotypic variance indicating partial dominance on NSIC Rc150. In contrast, *q*HTfert4 had 10.2% phenotypic variance, indicating partial dominance on Dular. On the other hand, *q*HTtof10 explained 16.3% of the phenotypic variance which showed partial dominance. The *q*HTdht3 with 31.7% phenotypic variance showed partial dominance with Dular. Partial dominance was also identified in *q*HTdht4 with 9.4% phenotypic variance explained. The *q*HTdht5 with PVE of 9.2% showed overdominance in the direction of the donor parent. In addition, *q*HTdht10 explained 7.1% of the phenotypic variance and showed partial dominance with Dular. Overdominance was identified in qHThd3 as directed by Dular.

This study revealed the interaction of QTLs with other QTLs in the different chromosomes (Fig. 8). The QTLs included were *q*HTfert1 - *q*HTfert3 with 14.0% interaction variation, *q*HTfert4 - *qtl*HTfert3 with 14.3%, *q*HTtof10 - *q*HTfert3 with 13.9%, *q*HTtof10 - *q*HTdht3 with 5.0% and *q*HTdht5 – *q*HTdht3 with 13.2%. It was notable that there was one QTL interacting on two QTLs. These QTLs included *q*HTfert3 (*q*HTfert1, 14.05%; *q*HTfert4, 14.3%) and *q*HTdht3 (*q*HTtof10, 13.6%; *q*HTdht5, 13.2%). There were also QTLs, *q*HThd3 - *q*HTfert3, located on the same chromosome interacting with one another.

The *q*HTfert3 - *q*HTfert4 had an interaction variation of 14.3% which indicates suggestive epistasis. Similarly, *q*HTfert3 - qHTfert1 had suggestive epistasis explaining 14.0% interaction variation. In addition, *q*HTfert3 - *q*HTtof10 explained 13.0% interaction variation which showed suggestive epistasis. Moreover, *q*HTfert3 - *q*HThd3, located on the same chromosome but different marker interval, had 5.0% PVE, and showed supplemental interaction. Epistasis was also identified in *q*HTfert4 and *q*HTdht4, having 13.2% interaction

Table 3. Quantitative trait loci (QTL) identified for high-temperature tolerance in rice.

QTL Name	QTL Position (cM)	Left Marker	Right Marker	PVE (%)	LOD	DPE	Action
*q*HTfert1	332.8	RM9	RM7318	18.6	8.99	B	PD
*q*HTfert3	987.9	RM16238	RM3586	57.4	9.18	B	PD
*q*HTfert4	757.6	RM348	RM559	10.2	8.46	A	PD
*q*HTtof10	81.2	RM25213	RM6142	16.3	8.95	A	PD
*q*HTdht3	963.9	RM16238	RM3586	31.7	7.83	A	PD
*q*HTdht4	201.6	RM16742	RM3308	9.4	8.15	A	PD
*q*HTdht5	458.4	RM178	RM480	9.2	7.18	A	OD
*q*HTdht10	78.2	RM25213	RM6142	7.1	6.69	A	PD
*q*HThd3	846.9	RM16102	RM16238	30.9	6.54	A	OD

PVE – phenotypic variance explained, DPE – direction of phenotypic effect, A – NSIC Rc150, B – Dular, PD – partial dominance, OD – overdominance, LOD – logarithm of odds

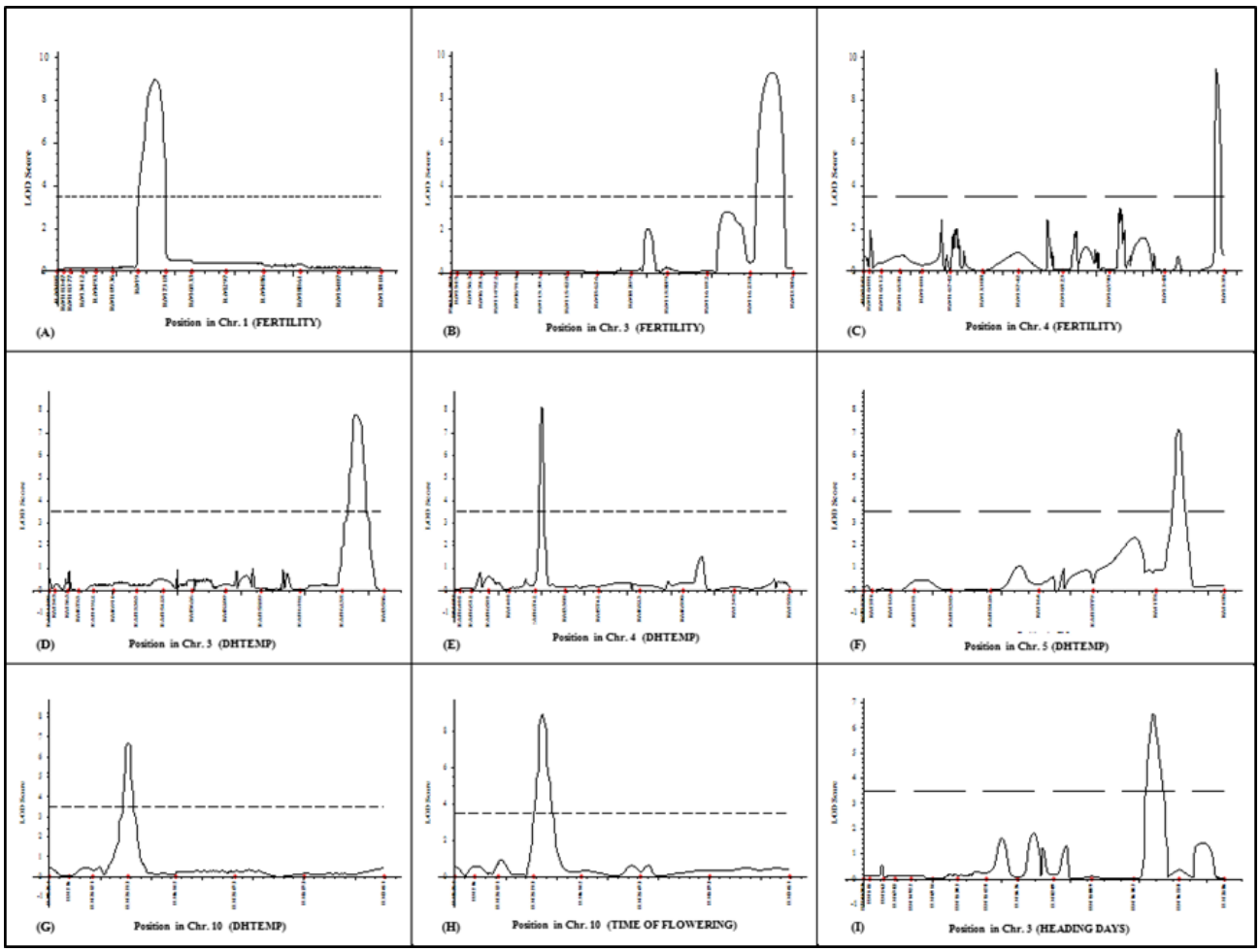


Fig. 6. Logarithm of odds (LOD) score threshold for identified quantitative trait loci (QTL) determined by permutation tests.

variance, indicating suggestive epistasis. This result inferred that *q*HTfert3 interacts in the different QTLs located in chromosome 1, 3, 4, and 10.

The *q*HTdht5 - *q*HTdht3 had 13.2% interaction variance implying suggestive epistasis. Moreover, *q*HTdht3 - *q*HTtof10 had 13.8% interaction variance, commencing suggestive epistasis and which implied that *q*HTdht3 interacts with QTL for dehiscent high-temperature (*q*HTdht5) and QTL for time of flowering (*q*HTtof10). These results showed polygenic interaction of QTL related/ associated for high-temperature tolerance. It also depicted multifactorial inheritance which includes interaction with high-temperature stress environment.

DISCUSSION

Spikelet fertility is the key factor in rice grain maturation and this can be maintained by reducing spikelet temperature under decreasing RH in a high-temperature environment. Decrease in the fertility of spikelets at high air temperatures with increased humidity modifies the impact of high temperature on spikelet fertility (Matsui et al. 1997b). An RH of 85 to 90% at the heading stage induced almost complete grain sterility in rice at a day/ night temperature of 35/30 °C (Abeysiriwardena et al. 2002). An increase in mean temperatures or of short episodes (SD) of high temperature especially during the reproductive stage is supra-optimal and reduces grain yield (Yan et al. 2010).

The tolerant cultivars such as Dular with 91.9% fertility and Nagina22 with 94.4% fertility showed high-temperature tolerance in this study. Both the intolerant cultivars NSIC Rc150 (72.7%) and Gayabyeo (73.3%) showed high sensitivity to high temperature unlike BC_2F_4 (76.3%) and IR64 (89.5%) which showed intermediate tolerance. These results concur with those of a previous study that N22 and Dular are excellent sources of genes for high-temperature tolerance and can be effective only in particular cross combinations (Manigbas et al. 2014). The greater high-temperature tolerance of N22 and Dular could be due to accumulation of heat shock proteins in the anthers.

Flowering and anthesis in most *O. sativa* genotypes of rice occur over a 5-d period, with most spikelets reaching anthesis between 1000 h and 1200 h (Nishiyama and Blanco 1980; Prasad et al. 2006). These results were also observed in the present study. In cultivated rice,

flowering time depends on the climatic conditions, but flowering predominantly occurs between 1000 and 1200 h (Nishiyama and Blanco 1980). Exposure to high-temperature stress for 1 h at anthesis is sufficient to induce spikelet sterility (Jagadish et al. 2007), but sterility does not occur when spikelets flower 1 h prior to the high-temperature treatment, suggesting that spikelets have achieved a considerably high tolerance after the completion of fertilization. Exposure at 41 °C for 4 h during flowering causes irreversible damage and plants became completely sterile (Satake and Yoshida 1978).

In the high-temperature tolerant *japonica* cultivar Akitakomachi, half of the spikelets become sterile at 40.8 °C at anthesis (Matsui et al. 2001). The response of duration to temperature >35 °C is quantitative, with shorter durations at higher temperatures having the same effect as longer durations at cooler temperature (Satake et al. 1995). Spikelet sterility increases in response to daily maximum temperature (Jagadish et al. 2007; Nakagawa et al. 2002; Horie et al. 1996; Matthews et al. 1995). If there is an interaction between temperature and duration, then the response of spikelet fertility to temperature has a

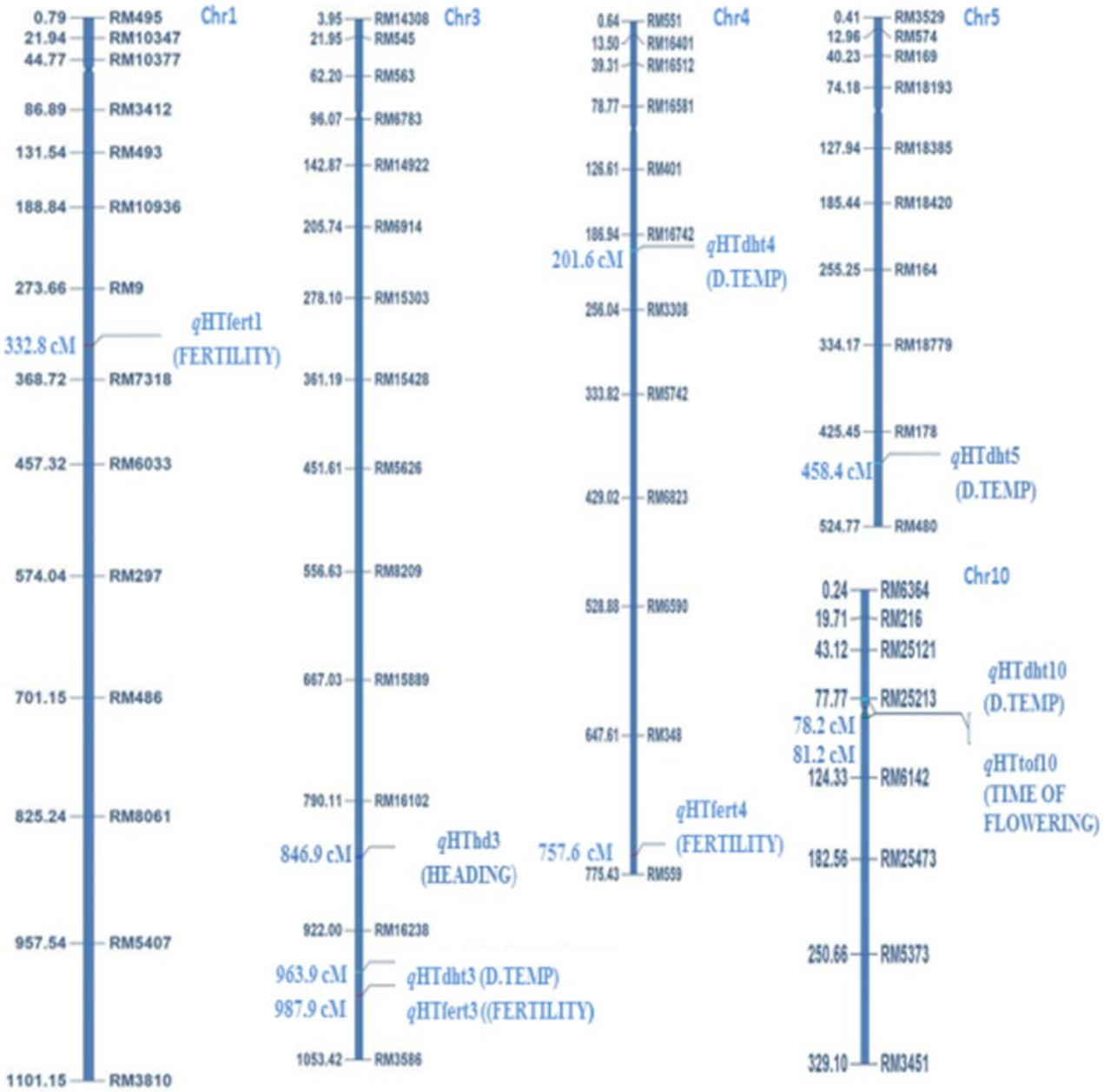


Fig. 7. Quantitative trait loci (QTL) linkage map for high-temperature tolerance showing the putative location of genes.

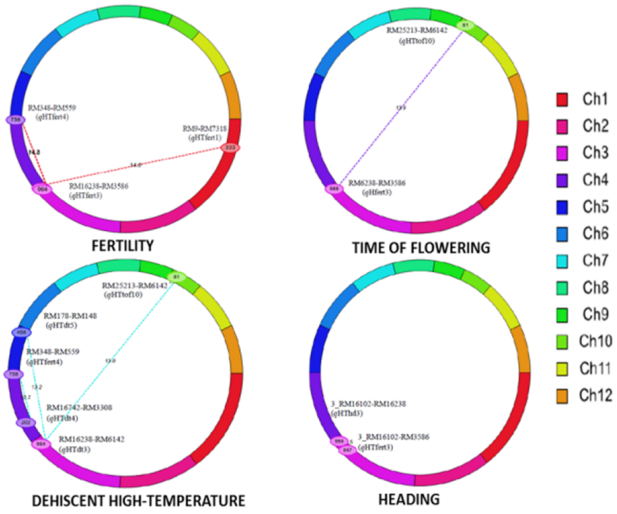


Fig. 8. Cyclic graph showing epistasis of different identified quantitative trait loci (QTL) for high-temperature tolerance in rice.

cumulative temperature response above the threshold temperature (Vara Prasad et al. 2001). Field surveys revealed that sterility increased up to 15% when the heading stage of the rice plants coincided with such high-temperature days (Hasegawa et al. 2009, unpublished). Growth chamber and greenhouse studies suggest that high temperature is most deleterious when flowers are visible and sensitivity continues for 10 to 15 d. Reproductive phases most sensitive to high temperature are gametogenesis (8 to 9 d before anthesis) and fertilization (1 to 3 d after anthesis) (Foolad 2005). Both male and female gametophytes are sensitive to high temperature and the response varies with genotype; ovules are generally less heat-sensitive than pollen (Peet and Willits 1998; Wahid et al. 2007).

The result of this study conferred 3 QTLs on high temperature located in chromosome 3 (Fig. 7). The *q*HTfert3 (RM16238-RM3586) on fertility and *q*HTdt3 (RM16238-RM3586) on dehiscent high-temperature had the same flanking markers and similar interval map position. Both *q*HTfert3 and *q*HTdt3 were positioned at 131.42cM. This result suggests that fertility and dehiscent temperature were controlled by QTL located on flanking markers RM16238-RM3586 and could be considered as hotspot for high-temperature tolerance. The detected QTLs on chromosome 3 conformed to the previously reported QTL for high-temperature tolerance. The reported QTLs were *qhr3-1* PVE 2.9% (Cao et al. 2003), *q*Ht3 PVE 11.4% (Chen et al. 2008), *q*hts-3 (Zhang et al. 2008), *qtl_3.4* PVE 11.4% (Jagadish et al. 2010), and *q*hts-3 PVE 36.2% (Buu et al. 2014). The present study suggests higher deliverance of chromosome 3 on QTL for high-temperature tolerance.

The QTLs detected on chromosome 4, *q*HTfert4 (RM348-RM559) and *q*HTdt4 (RM16742-RM3308), had shown conformity in chromosome location with the other findings. The reported QTLs on chromosome 4 were *qhr4-3* (Cao et al. 2003), *q*hts-4 (Zhang et al. 2008), *SSf4* (Xiao et al. 2011) and *qtl_4.1* (Jagadish et al. 2010). This comparison with other studies also suggests a higher deliverance of chromosome 4 for high-temperature tolerance.

The QTL detected on chromosome 10, *q*HTtof10 (RM348-RM559) and *q*HTdt10 (RM25213-RM6142), also manifested conformity on chromosome location with other findings. The *q*HTtof10 (131.42cM) and *q*HTdt10 (131.89cM) were both closely positioned on interval map. The reported QTLs on chromosome 4 for high-temperature tolerance were SSPf10 (Xiao et al. 2011) and *qtl_10.1* (Jagadish et al. 2010).

The QTL detected on chromosome 5, *q*HTdt5 (RM178-RM480), showed no conformity on chromosome location with other findings. This result could be due to the different phenotypic screening tool and DNA marker used. Parents that provide adequate polymorphism are selected on the basis of the level of genetic diversity between parents (Anderson et al. 1993; Joshi and Nguyen 1993; Yu and Nguyen 1994; Collard et al. 2005) and it implies that QTL from different bi-parental populations having diverse genetic background could result in different QTL findings. The choice of DNA markers used for mapping may depend on the availability of characterized markers or the appropriateness of particular markers for a particular species (Collard et al. 2005).

CONCLUSION

This study was able to identify the QTLs for high-temperature tolerance using backcrossed population of NSIC Rc150 x Dular. The six major QTLs identified for high-temperature tolerance were *q*HTfert1 (RM9-RM7318), *q*HTfert3 (RM16238-RM3586), *q*HTfert4 (RM348-RM559), *q*HTtof10 (RM25213-RM6142), *q*HTdht3 (RM16238-RM3586), and *q*HThd3 (RM16102-RM16238). The three minor QTLs were *q*HTdht4 (RM16742-RM3308), *q*HTdht5 (RM178-RM480), and *q*HTdht10 (RM25213-RM6142). Polygenic interaction among the identified QTL associated traits for high-temperature tolerance described putative polygenic mechanism and provides an indicator for a wider spectrum of gene-gene interactions.

The heading days, time of flowering, fertility and dehiscent temperature were associated with high-temperature tolerance. These morpho-physiological traits could be used as candidate screening tools for high-temperature tolerance in rice. Screening of phenotypic data by conducting phenotypic correlation and multiple linear regression analysis is a promising emerging method for selection of phenotypes for QTL association mapping, specifically in finding traits associated with fertility.

A linkage map provided information that can be used in fine-mapping of the actual genes via map-based cloning. These results can be used as baseline information for the selection of QTLs for marker-assisted selection (MAS), marker-assisted breeding (MAB), and marker-assisted backcrossing.

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