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Identification of QTLs associated with heat tolerance at the heading and flowering stage in rice (*Oryza sativa* L.)

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Abstract The ongoing rise in temperatures caused by global climate change is a critical climatic risk factor for rice production, and enhancing rice heat tolerance is an area of particular research interest. A recombinant inbred line (RIL) mapping population was developed from heat sensitive, rice cultivar IAPAR-9 crossed with heat tolerant, Liaoyan241. RIL and parental lines were exposed to high temperature at the heating and flowering stage in experiments in 2014 and 2015. As indicators of heat tolerance, the seed setting rate under natural (NS) and heat stress (HTS) conditions were measured, and the reduction rate of seed set (RRS) was calculated. Quantitative trait loci (QTL) analysis revealed eleven heat tolerance QTLs located on chromosomes 1, 3, 4, 5, and 6. Single QTL contribution rates were 4.75–13.81% and effect values were − 5.98 to 5.00. Four major QTLs (*qNS1*, *qNS4*, *qNS6,* and *qRRS1*) were stable detected in different environments in both years. Thirteen QTLs with epistatic interactions and nine QTLs with environmental interactions were also detected. Major QTLs were all involved in epistatic and environmental interactions. Three QTLs from the SSR marker interval RM471 to RM177 region of chromosome 4 (*qNS4*, *qHTS4*, and *qRRS4*) were all involved in epistatic and environmental interactions and contributed to phenotypic variation, indicating that this region constituted a major QTL hotspot. The major QTL for heat tolerance identified in this study will aid in breeding tolerant cultivars and facilitating investigation of the molecular underpinnings of heat tolerance in rice.

Keywords Rice (*Oryza sativa* L.) · Heat stress · Flowering stage · Heat tolerance · QTL

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Introduction

The ongoing rise in temperatures caused by global climate change is a critical climatic risk factor for rice production. Rice, one of the most important food crops

worldwide, is a stable part of the diet of > 65% of the population of China. And securing rice yields is thus critical for national food security. The average global temperature has increased in recent years as a result of ongoing climate change. The fifth IPCC climate change assessment report estimates that the global average surface temperature will rise by 0.3–4.8 °C by the end of the 21st century (IPCC 2013). Rice yields are adversely affected by elevated temperatures, and Peng et al. (2004) estimate that for every 1 °C rise in global temperature, rice yield decreases by 10%.

In rice the early reproductive growth stages are particularly sensitive to high temperature. High temperature stress results in abnormal anther dehiscence, lower pollen production, and low pollen germination rate, followed by a decrease in the seed setting rate (Chen et al. 2008; Das et al. 2014; Jagadish et al. 2007; Rang et al. 2011). Rang et al. (2011) showed that daily maximum temperatures exceeding 35 °C for 1 h resulted in high levels of sterility in rice. High temperature stress occurs particularly frequent in the Yangtze River Valley due to the influence of the subtropical high. During the critical period from mid-July to early August, when rice is flowering, daily maximum temperatures often exceed 35 °C for > 3 days and can reach 40 °C, causing serious yield losses (Peng et al. 2005; Wang et al. 2016; Xie et al. 2010). Li et al. (2014) found that heat tolerance varies among different rice varieties and conventional varieties are more heat-tolerant than hybrid rice varieties. The development of heat-tolerant rice varieties producing stable yields is of critical importance.

Rice heat tolerance during the heading and flowering stage is a quantitative trait controlled by multiple micro-quantitative trait loci (QTLs) (He 2011; Kui et al. 2008; Liu et al. 2015; Xiao et al. 2011a, b; Ye et al. 2012; Zhang et al. 2008; Zhao et al. 2006). Xiao et al. (2011a) used a recombinant inbred line (RIL) population to identify two major QTLs mapped to chromosomes 4 and 6 and associated with pollen fertility under high temperature stress. Xiao et al. (2011b) used RIL populations to detect two major QTLs, mapped to chromosomes 4 and 10, and associated with seed setting rate under high temperature stress. Ye et al. (2012) used RIL and F_2 populations to detect two major QTLs, mapped to chromosomes 1 and 4, and associated with spikelet fertility under high temperature stress. Finally, Liu et al. (2015) used RIL populations to detect twelve major QTLs associated with seed setting rate under high temperature stress that mapped to chromosomes 1, 2, 4, 5, 6, 7, 9, 10, and 12. Reports of heat tolerance QTL have increased in recent years, and suggest that QTLs are widely distributed across the 12 rice chromosomes. However, most of the identified QTLs cannot be detected in different environments and have a contribution rate of < 10%. QTL loci that had been fine-mapped or cloned are less (Cao et al. 2003, 2015; Chen et al. 2008; He 2011; Jagadish et al. 2010; Kui et al. 2008; Liu et al. 2015; Sun 2015; Xiao et al. 2011a, b; Ye et al. 2012; Zhang et al. 2008; Zhao et al. 2006). In additions, global climate models predict an increase in global mean temperature and a higher frequency of intense heat spikes during past 20 years, indicating a critical risk in rice production. Rice is more susceptible to heat stress, seed setting rate and grain filling often go through serious damage under high temperature, leading to significant losses. Recently, cloning and fine mapping significant affect QTL responses to high temperature stress, and enhancing heat tolerance is one of the most hot spot method for molecular and breeding biology. In this study, to unravel the genetic basis of the heat-tolerant characters and identify novel QTLs, the major heat tolerance QTLs were determined by using a RIL population derived from a cross between the heat-sensitive upland rice cultivar IAPAR-9 and the heat-tolerant irrigated rice cultivar Liaoyan241. QTL analyses were performed at the heading and flowering stage in natural and high temperature stress conditions in 2014 and 2015. The effects of epistatic and environmental interactions on rice heat tolerance were also examined. The results of this study will be used in breeding heat-tolerant rice varieties to reduce rice production damages caused by high temperature stress.

Materials and methods

Experimental materials

The F_1 hybrid was produced by crossing upland rice cultivar IAPAR-9 which is heat sensitive, with heat tolerant, irrigated rice cultivar Liaoyan241. One F_1 plant was self-pollinated to produce F_2 seed. A stable F_8 RIL population containing 200 lines was derived from the F_2 by the single seed descent method.

Field experiment

Field experiments were conducted at the Nanchang Experimental Base, Rice Research Institute, Jiangxi Academy of Agricultural Sciences, China, in 2014 and 2015. RIL and parental seed were sown on May 10th, 15th, and 20th. This ensured that plants from each line could be selected at the flowering stage for exposure to the same heat stress conditions despite their different growth rates. Thirty-two plants from each line were transplanted with 16.6 cm (between rows) × 20 cm (within rows) of the planting density, one seedling per hill, at 25 days. The crop was managed according to standard rice agronomic procedures.

High temperature treatment

Plants were exposed to high temperatures in an artificial greenhouse covering an approximate area of 40 m^2 to a height of 2.5 m. The top of the greenhouse was closed, and with the exception of the lower 20 cm, the structure was enclosed with plastic film. Natural irradiation of the greenhouse produced the warming effect. During the day, temperatures within the artificial greenhouse were generally 3–5 °C higher than in the external field environment. Night-time temperatures were similar within and outside the greenhouse. Irradiation duration did not differ between the greenhouse and field conditions. Temperature and humidity in the greenhouse were recorded every 30 min with an automatic temperature and humidity recorder (Table 1).

The local weather forecast was used to select sunny periods of at least 5 days suitable for the high temperature treatment. Two days before the high temperature treatment, nine uniform plants that were predicted to undergo heading during the treatment were selected per line in the afternoon. Five panicles per plant were selected and marked with a fiber rope. Three plants were left in the field as controls, while the remaining six plants were transferred to plastic pots (two replicate pots, each containing three plants). Plants received conventional fertilizer and water management. Potted plants were moved to the greenhouse on the 3rd day, 36 h after selection. After 5 days in the greenhouse, the potted rice plants were transferred to natural conditions until seeds matured in the pots. Seeds from the five marked panicles were harvested upon reaching grain maturity. Filled and empty seeds were counted to calculate the seed setting rate under natural and high temperature conditions.

Data analysis

Three panicles with similar seed setting rates were selected from the five marked panicles for statistical analysis. The seed setting rate under high temperature stress and reduction rate of seed setting rate were selected as heat tolerance indices. Seed setting rate and reduction rate of seed setting rate were calculated as follows:

$$\text{Seed setting rate } (\%) = \left(\frac{\text{Filled seeds per panicle}}{\text{Total florets per panicle}}\right) \times 100\%.$$

$$\text{Reduced seed setting rate } (\%) = \left[\frac{(\text{Seed setting rate in natural conditions} - \text{Seed setting rate in high temperature stress})}{\text{Seed setting rate in natural conditions}}\right] \times 100\%.$$

Table 1 Greenhouse temperature and humidity during high temperature treatment

Year	Maximum temperature (°C)		Minimum temperature (°C)		Daily average temperature (°C)		Humidity (%)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
2014	37.7–42.5	41.3	22.1–26.1	24.5	28.3–31.4	29.8	86.2–89.7	88.7
2015	41.7–48.5	45.9	25.8–28.1	27.2	31.9–35.2	33.7	64.3–76.5	70.3

DNA extraction and molecular genotyping

Leaves from each F_8 RIL and parents were sampled and genomic DNA was extracted using an improved CTAB method (Edwards et al. 1991). PCRs were conducted in a 10 μL reaction volume containing 10 × PCR buffer (Mg^{2+}), 1.0 μL; 2.5 mmol/L dNTPs, 0.2 μL; 5 U/μL Taq enzyme, 0.25 μL; 10 μmol/L SSR marker primers, 0.25 μL; 20 ng/μL DNA, 1.0 μL; and 7.25 μL ddH_2O. The PCR thermal cycle was as follows: initial denaturation at 94 °C for 4 min; followed by 36 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 55 °C, and extension for 30 s at 72 °C; and final extension for 7 min at 72 °C, before cooling to 4 °C. PCR products were separated on 6% (m/v) polyacrylamide gels and detected by silver staining.

Linkage map construction and QTL analysis

A total of 1000 SSR primer pairs were used to measure polymorphisms between the two parental rice cultivars and 210 of them showing polymorphism between the parents and evenly distributed across the 12 rice chromosomes were selected for QTL analysis. Relative genetic distances for each marker are given according to the Gramene (http://www.gramene.org/archive and http://www.ricedata.cn). In total, 175 evenly distributed polymorphic SSR markers were used to construct a genetic map for the RIL population. The genetic map was 2092.83 cM in length, with an average of 14.58 SSR markers per chromosome and an average distance of 11.96 cM between adjacent markers. The QTL analysis was conducted by the inclusive composite interval mapping (ICIM) method of ICIMapping 3.0 (http://www.isbreeding.net), the software and method were used to analyze the QTL additive, epistatic and environmental interaction effects, and the genetic linkage map was drawn through Mapchart software (Voorrips 2002).

Results

Heat tolerance heredity variations in the RIL population

The seed setting rate was assessed for RILs and rice parents under normal and high temperature conditions. For both of the parent rice varieties, the high temperature seed setting rate was higher in 2014 than in 2015, and in both years the high temperature seed setting rate was higher in Liaoyan241 than in IAPAR-9 (Table 2). Liaoyan241 also presented a correspondingly lower reduction rate of seed setting rate than IAPAR-9. Thus, Liaoyan241 showed higher heat tolerance, while IAPAR-9 showed higher heat sensitivity.

Seed setting rates of the RILs varied considerably under normal and high temperature conditions (Table 2). Seed setting rate under natural and high temperature conditions varied by 40–50%, with maximum seed setting rate values that exceeded those of the parents. Seed setting rate under both the natural and high temperature conditions exhibited an approximate continuous normal distribution (Fig. 1), as indicated by skewness and kurtosis values < 1.0 (Table 2). This confirmed that the heat tolerance trait was suitable for QTL analysis.

Analysis of correlation between seed setting rate, environment, and year

Pollination and seeding of rice under normal conditions were impacted somewhat by the relatively high summer temperature in Nanchang during the rice flowering and seeding period. Nevertheless, there were significant positive correlations for seed setting rate characteristics between years, as shown by correlation coefficients of 0.48, 0.40, and 0.27 for natural conditions, high temperature, and seed set reduction rate, respectively (Table 3). Seed setting rate were significantly positively correlated between natural and high temperature conditions, as shown by correlation coefficients of 0.36 (2014) and 0.21 (2015), indicating that the seed setting rate under natural conditions somewhat reflected heat tolerance. Correlations between seed setting rate and reduction rate of seed setting rate under natural conditions were minimal. However, there were significant negative correlations between seed setting rate and reduction rate of seed setting rate under high temperature conditions (correlation coefficients of -0.95 and -0.93), indicating that lower reduction rate of seed setting rate reflect high seed setting rate under high temperature stress. The correlation analysis demonstrated that seed setting rate and reduction rate of seed

Table 2 Seed setting rates in IAPAR-9/Liaoyan241 RILs and their parents

Years	Traits	Parents		RILs			
		Liaoyan241	IAPAR-9	Mean ± SD	Range	Kurtosis	Skewness
2014	NS	89.18	79.48	78.72 ± 7.96	57.30–96.17	− 0.32	− 0.19
	HTS	52.02	35.61	38.90 ± 12.59	13.13–66.61	0.11	− 0.58
	RRS	41.67	55.19	50.66 ± 14.19	17.50–81.81	0.04	− 0.59
2015	NS	84.44	74.34	76.89 ± 8.74	55.89–97.05	− 0.37	− 0.47
	HTS	24.68	18.92	33.49 ± 11.29	14.00–60.96	0.29	− 0.66
	RRS	70.77	74.55	56.16 ± 14.83	17.22–80.72	− 0.39	− 0.52

SR seed setting rate (%), *NS* SR in natural condition, *HTS* SR in high temperature stress, *RRS* reduction rate of seed setting rate

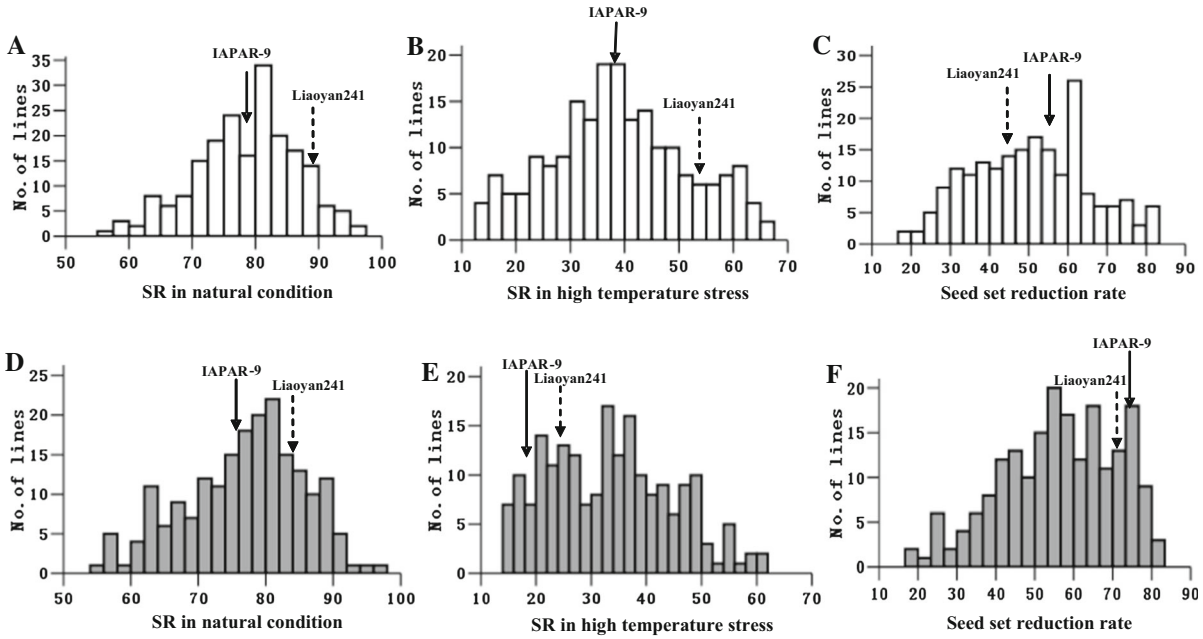

Fig. 1 Distribution of seed setting rates in RIL populations derived from IAPAR-9/Liaoyan241. Seed setting rates (SR) were assessed in parental and RIL populations in 2014 (**a–c**) and 2015 (**d–f**)

Table 3 Correlation analysis for the seed setting rate traits

Traits	NS	HTS	RRS
NS	0.48**	0.21**	0.15*
HTS	0.36**	0.40**	− 0.93**
RRS	− 0.05	− 0.95**	0.27**

SR seed setting rate (%), *NS* SR in natural condition, *HTS* SR in high temperature stress, *RRS* reduction rate of seed setting rate

*, **Significant difference at the 5 and 1% levels. The lower correlations between the three traits are for 2014, the upper correlations are for 2015 and the correlations on the diagonal (0.48, 0.40, 0.27) are between the 2 years

setting rate under natural and high temperature were inherited stably with high heritability.

QTL detection and association with seed setting traits

QTL associated with seed setting characteristics were detected and characterized (Table 4). Four QTL were associated with seed setting rate under natural conditions (NS), *qNS1*, *qNS4*, *qNS5*, and *qNS6*, located on chromosomes 1, 4, 5, and 6, respectively (Table 4, Fig. 2). Single year contribution rates were 33.04 and

Table 4 QTL detection and genetic effects in RIL populations of IAPAR-9/Liaoyan241

Traits	QTL locus	Chromosome	Peak position	Interval markers	LOD values		Additive effect[a]		PVE (%)	
					2014	2015	2014	2015	2014	2015
NS	*qNS1*	1	192.5	RM6840–RM220	2.89	2.74	2.46	2.75	7.57	7.68
	qNS4	4	18.0	RM16575–RM177	4.73	5.47	− 2.66	− 3.33	13.41	13.37
	qNS5	5	38.5	RM164–RM440	2.74		− 1.76		4.75	
	qNS6	6	41.0	RM3330–RM20224	3.12	3.76	− 2.03	− 2.59	7.31	7.78
HTS	*qHTS1a*	1	58.0	RM11054–RM5646	3.61		3.87		5.47	
	qHTS1b	1	90.0	RM1231–RM302		3.70		− 3.22		8.02
	qHTS3	3	70.0	RM411–RM341		3.85		4.01		11.40
	qHTS4	4	22.0	RM471–RM177	10.17		− 5.98		13.81	
RRS	*qRRS1*	1	95.0	RM1231–RM302	3.01	3.00	− 4.14	− 3.92	4.95	6.81
	qRRS3	3	66.5	RM411–RM341		2.72		− 3.75		5.62
	qRRS4	4	21.5	RM471–RM177		4.99		5.00		7.38

Peak position represents for the QTL of LOD value peak loci (cM); underline font SSR marker represents for peak SSR marker, respectively

SR seed setting rate (%), *NS* SR in natural condition, *HTS* SR in high temperature stress, *RRS* reduction rate of seed setting rate, *PVE* percentage of explained phenotypic variation

[a]Negative values attribute the increase to the Liaoyan241 allele and positive values to the IAPAR-9 allele

28.83% for 2014 and 2015, respectively. *qNS5* was detected only in 2014, and the contribution rate and additive effect values were both small, indicating that *qNS5* was a micro-QTL. *qNS1*, *qNS4*, and *qNS6*, the three QTLs were detected in both years. The highest LOD and phenotypic variation scores were associated with *qNS4*, which contributed to 13.41 and 13.37% of phenotypic variation for 2014 and 2015, respectively. Additive effect values were negative for *qNS4* and *qNS6*, indicating an association of Liaoyan241 alleles. Conversely, additive effects for *qNS1* were positive, indicating an association of the IAPAR-9 allele.

QTL related to seed setting rate under high temperature conditions were found in 2014 or 2015, but not both. Two high temperature (HTS) QTLs were detected in 2014 (Table 4), *qHTS1a* on chromosome 1 and *qHTS4* on chromosome 4 (Table 4, Fig. 2). Two additional seed setting rate QTLs were detected in 2015, *qHTS1b* and *qHTS3*, on chromosomes 1 and 3, respectively. Among these QTLs, *qHTS3* had the greatest effect on phenotypic variation at 11.40% with an increased tolerance for *qHTS3*, *qHTS1b* and *qHTS4* attributed to the Liaoyan241 alleles and the increased tolerance for *qHTS1a* and *qHTS3* attributed to IAPAR-9 alleles.

Three QTLs related to reduction rate of seed setting rate (RRS) were found: *qRRS1*, on chromosome 1, detected in 2014 and 2015, and *qRRS3* and *qRRS4*, on chromosomes 3 and 4, respectively (Table 4, Fig. 2). Among these QTLs, *qRRS4* had the greatest effect on phenotypic variation at 7.38%. The increased seed setting rate was attributed to the Liaoyan241 alleles for *qRRS1* and *qRRS3* and to the IAPAR-9 allele for *qRRS4*.

Analysis of epistatic and environmental interaction QTL for seed setting rate related traits

Thirteen additive × additive epistatic interactions were detected between QTL on chromosomes 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 (Table 5). LOD values ranged from 3.29 to 4.18, contribution values (H^2) from 6.97 to 25.14%, and epistasis scores from − 4.57 to 5.87. Several QTLs were involved in more than one epistatic interaction. For example, there were seven epistatic interactions involving chromosome 4, five of which involved interval RM3367–RM3288 and the other two involved interval RM471–RM177. The epistasis results showed that epistatic QTL played an important role in phenotypic interaction rates. In

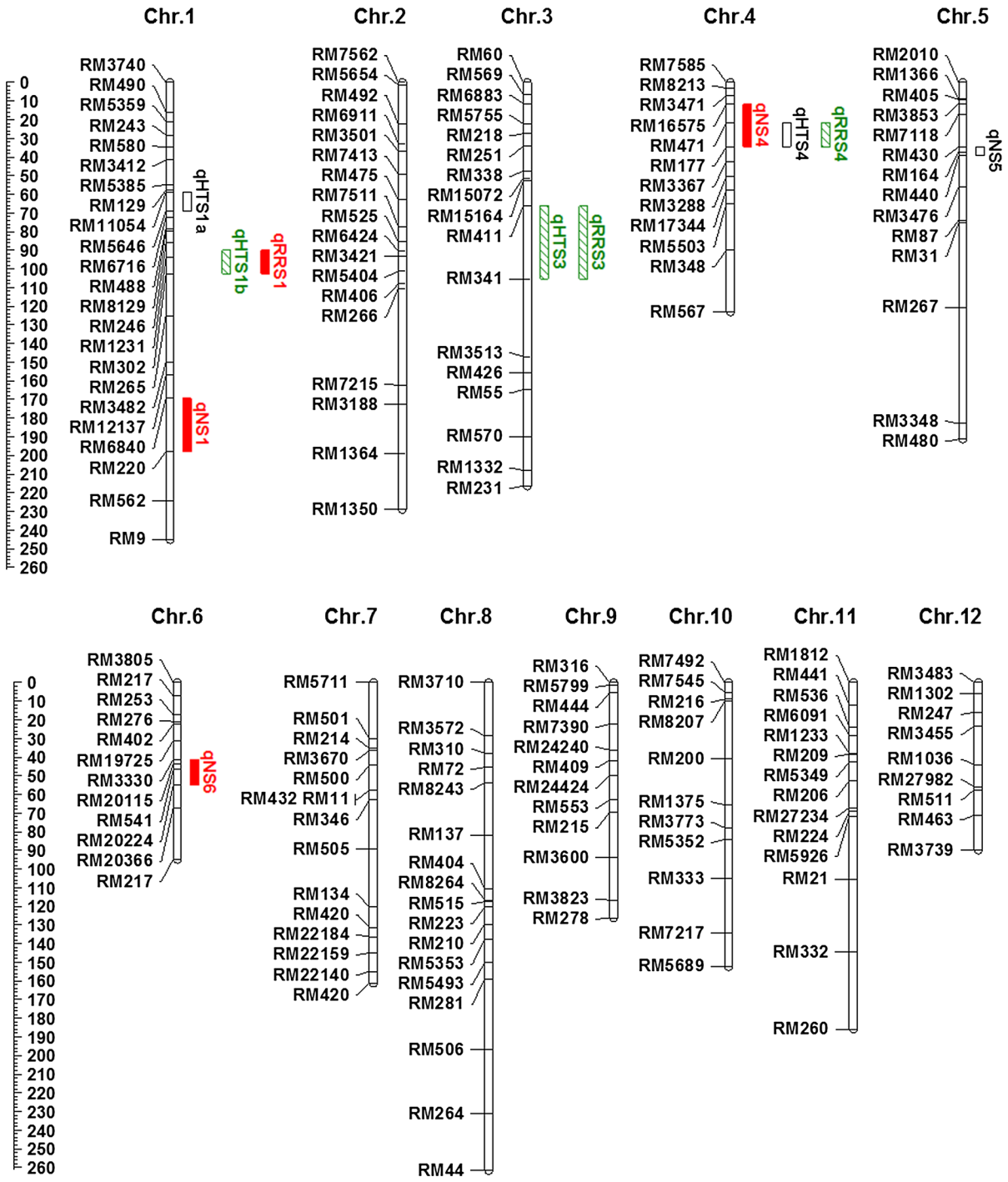

Fig. 2 Chromosomal distribution of QTLs associated with seed setting rate traits. QTL associated with seed setting rate in normal conditions (NS) and high temperature conditions (HTS) and with reduction rate of seed setting rate (RRS) were determined in 2014 (no shading), 2015 (green shading), or both 2014 and 2015 (solid red). (Color figure online)

Table 5 Epistatic interaction QTL for seed setting rate related traits in RIL populations of IAPAR-9/Liaoyan241

Trait	QTLi	Chr.i	Interval marker	QTLj	Chr.j	Interval marker	LOD	H^2 (%)	Epistasis effect
NS	*qNS2-1i*	2	RM5654–RM492	*qNS6j*	6	RM253–RM276	3.45	7.24	2.38
(2014)	*qNS2-2i*	2	RM475–RM7511	*qNS2j*	2	RM1364–RM1350	3.71	9.59	− 2.64
	qNS5i	5	RM405–RM3853	*qNS9j*	9	RM215–RM3600	3.60	16.35	3.32
HTS	*qHTS4i*	4	RM471–RM177	*qHTS7j*	7	RM500–RM432	3.29	9.27	− 4.57
(2014)									
NS	*qNS4i*	4	RM3367-RM3288	*qNS7j*	7	RM3670-RM500	3.71	7.36	− 2.62
(2015)	*qNS8i*	8	RM210–RM5353	*qNS11j*	11	RM1812–RM441	3.80	8.03	− 2.70
HTS	*qHTS2i*	2	RM7413–RM475	*qHTS10j*	10	RM333–RM7217	3.53	9.63	3.80
(2015)	*qHTS4i*	4	RM3367–RM3288	*qHTS3j*	3	RM411–RM341	3.91	21.25	5.58
	qHTS4i	4	RM3367–RM3288	*qHTS7j*	7	RM5711–RM501	3.81	7.74	3.32
	qHTS4i	4	RM3367–RM3288	*qHTS8j*	8	RM210–RM5353	3.72	6.97	3.20
	qHTS4i	4	RM3367–RM3288	*qHTS10j*	10	RM8207–RM200	3.41	25.14	5.87
	qHTS5i	5	RM3348–RM480	*qHTS7j*	7	RM346–RM505	4.18	16.48	5.39
RRS	*qRRS4i*	4	RM471–RM177	*qRRS10j*	10	RM333–RM7217	3.60	12.22	5.44
(2015)									

i and j mean epistatic interaction QTL from the different chromosome locus, respectively

SR seed setting rate (%), *NS* SR in natural condition, *HTS* SR in high temperature stress, *RRS* reduction rate of seed setting rate, *Chr.* represents chromosome, *LOD value* likelihood of oddvalue, H^2 percentage of phenotypic variation explained by additive effect

Table 6 Environmental interaction QTL for seed setting rate related traits in RIL populations of IAPAR-9/Liaoyan241

Traits	QTL	Marker	Peak position	PVE	ADD	PVE(AbyE)	AE1	AE2
NS	*qNS1**	RM6840–RM220	190	7.62	− 1.66	0.50	0.42	− 0.42
	qNS4	RM16575–RM471	15	5.41	− 1.30	3.43	− 1.04	1.04
	*qNS4a**	RM3288–RM17344	55	7.61	− 1.57	3.22	1.02	− 1.02
	qNS6	RM3330–RM20115	45	4.76	− 1.27	2.62	− 0.94	0.94
HTS	*qHTS1*	RM3412–RM5385	55	4.23	2.61	0.27	0.66	− 0.66
	qHTS4	RM471–RM177	20	10.40	− 3.94	2.37	− 1.88	1.88
RRS	*qRRS1*	RM1231–RM302	95	3.09	− 2.47	0.46	0.95	− 0.95
	*qRRS1a**	RM3412–RM5385	55	3.18	− 2.59	0.85	− 1.34	1.34
	qRRS4	RM471–RM177	20	6.40	3.52	1.69	1.81	− 1.81

Peak position represents for the QTL of LOD value peak loci (cM); underline font SSR marker represents for peak SSR marker

SR seed setting rate (%), *NS* SR in natural condition, *HTS* SR in high temperature stress, *RRS* reduction rate of seed setting rate

*QTL detected only in environmental effects; PVE represents percentage of explained phenotypic variation; ADD represent additive effect expressed in terms of estimated change in the phenotype; PVE(AbyE) represent the heritability of PVE by environment interaction effect; AE represent the predicted additive by environment interaction effect, E1 and E2 represent 2014 and 2015 year, respectively

addition, three major QTLs that were involved in these interactions (*qNS4*, *qHTS4*, and *qRRS4*) corresponded to interval RM471–RM177 (Fig. 2). This indicated that epistatic effects play an important role in the control of rice seed setting rate related traits.

Nine environmental interaction QTL were detected on chromosomes 1, 4, and 6 (Table 6). Of these, the

major QTLs *qHTS1*, *qHTS4*, *qNS4*, *qNS6, qRRS1*, and *qRRS4* exhibited clear environmental interaction effects, but the environmental effect values and contribution rates were relatively small. This suggested that environmental interaction QTL played only a minor role in explaining phenotypic contribution rates and control of rice seed setting rate traits.

Discussion

Identification method of heat tolerance in rice

Exposure of rice plants to elevated temperatures can have serious impacts on yield. A rare period of elevated temperatures in southern China in 2003 led to the loss of more than two billion kilograms in rice grain yield (Zhang and Chen 2005). A similar period of high temperature and drought in Sichuan and Chongqing in 2006 also had considerably adverse effects on rice production. The accurate identification, development, and breeding of heat tolerant traits is thus an area of ongoing interest in rice germplasm research. Existing studies of heat tolerance primarily used artificial climate chambers for high temperature treatment. Temperature and humidity can be accurately and stably controlled by climate chambers, but operation costs are high and the amount of material that can be screened is limited compared to a closed greenhouse system (Chen et al. 2008; Ye et al. 2012; Zhao et al. 2006). In this study, a high temperature treatment greenhouse was constructed with plastic film. This allowed internal temperatures to be regulated by adjusting the height of the plastic film from the ground. In this system, rice plants were exposed to daytime temperatures 3–5 °C higher than the surrounding natural environment, whereas night-time temperatures were the same inside and outside the system. Another advantage of this system was that daytime temperatures in the greenhouse rose and fell in a similar manner to external natural temperatures. To avoid fluctuations in greenhouse temperatures during treatment, local weather forecasts were consulted to ensure that high temperature treatments occurred over a period of five sunny days. Rice plants were sown successively to ensure that heading plants were available from each line during the experimental period. Experiments were performed in 2014 and 2015, and proved to be reliable and accurate.

Seed setting traits were scored for RIL population and the parents, IAPAR-9 and Liaoyan241 whose seed setting was shown to decrease significantly under high temperature stress. The Liaoyan241 parent had significantly higher seed setting rate in natural and high temperature conditions compared to IAPAR-9 parent. Overall, the RIL seed setting rate decreased after high temperature exposure but several RILs exhibited high seed setting rates. This suggests that Liaoyan241 has strong heat tolerance and some RILs inherit these heat tolerance genes. Molecular markers should be developed for marker-assisted selection and used to introduce these heat tolerance-related genes into elite breeding lines, creating heat tolerance germplasm resources for future cultivar development.

Heat tolerance QTL at the early reproductive stage

High temperature stress can lead to decreased rice yield and poor quality (Asako et al. 2013; Murata et al. 2014; Wada et al. 2015; Ye et al. 2015; Zhao et al. 2016). Heat tolerance research has increased in recent years (Cao et al. 2003, 2015; Liu et al. 2015; Zhang et al. 2008), and several studies showed rice heat tolerance to be a quantitative trait controlled by multiple genes. These studies identified 120 heat tolerance QTLs in total with uneven distribution across 12 rice chromosomes (Cao et al. 2015; Liu et al. 2015; He 2011; Xiao et al. 2011a, b; Ye et al. 2012; Zhang and Chen 2005).

Cao et al. (2003) detected six heat tolerance QTLs with additive effects and epistatic interactions on eight chromosomes in a doubled haploid (DH) population treated in a greenhouse. Zhao et al. (2006) found three heat tolerance QTLs in a population exposed to high temperature in an artificial climate chamber at the booting stage. Four QTLs interactions on eight chromosomes were detected. In current research, 11 heat tolerance QTLs were identified on chromosomes 1, 3, 4, 5, and 6. Single QTL contribution rates were 4.75–13.81% and effect values were − 5.98 to 5.00. *qNS1*, *qNS4*, *qNS6*, and *qRRS1*, were four stable major QTLs detected in different environments over 2 years. Thirteen QTLs with epistatic interactions and nine QTLs with environmental interactions were detected, with epistatic interactions being more pronounced. The major QTLs were all involved in epistatic and environmental interactions. Epistatic and interactional

effects thus have important effects on regulation of rice seed setting rate and heat tolerance.

Heat tolerance QTL from http://www.gramene.org/archive, http://www.ricebase.org, http://qtaro.abr.affrc.go.jp and recent published papers were identified and their chromosomal distributions and locations compared to the QTL identified in this study (Cao et al. 2003, 2015; Chen et al. 2008; Liu et al. 2015; Chen et al. 2008; He 2011; Xiao et al. 2011a, b; Ye et al. 2012; Zhang et al. 2008; Zhang and Chen 2005; Zhao et al. 2006). The location of *qHTS1a* is similar to that of an epistasis heat tolerance QTL on chromosome 1 identified at the heading stage by Chen et al. (2008). The location of *qNS4* was similar to that of a heat tolerance QTL identified on chromosome 4 at the flowering stage by Xiao et al. (2011a, b). Two QTLs identified in this study, namely, *qRRS1* on chromosome 1 (RM1231–RM302) and *qNS6* on chromosome 6 (RM3330–RM20224), were not identified previously. These QTLs were detected in both 2014 and 2015, and may thus constitute novel, stably expressed, major QTLs. Three QTLs from the same region of chromosome 4 (RM471–RM177), namely, *qNS4*, *qHTS4*, and *qRRS4*, were all involved in epistatic and environmental interactions and contributed to phenotypic variation, indicating that *qSSR4* marks a major QTL hotspot stably expressed in different environments. Near-isogenic lines are currently under construction to allow further study to fine map and clone the gene on chromosome 4 between RM471–RM177 and on chromosome 1 between RM1231–RM302.

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