Mapping of QTL Associated With Heat Tolerance At The Reproductive Stage In Rice(*Oryza Sativa* L.)

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Abstract

Climate change has a negative effect on rice production and food security. High temperature stress is a major obstacle and can significantly reduce yield. A set of recombinant inbred lines (RILs) derived from the cross between Longdao5 (heat-sensitive) and Zhongyouzao8 (heat-tolerant) was used in the identification of heat tolerant QTL. Spikelet fertility (SF) and heat tolerance (HT) indexes showed a significant difference among the parents and RILs population, and SF and HT have different of effect under natural and artificial high temperature conditions. Sixty-one QTLs were detected on chromosomes 1-8, 10 and 12, while 25, 27 and 14 additive QTLs were identified under the control, natural and artificial high temperature conditions, respectively. Pleiotropic effects and QTL hotspots are the key factors affecting these traits, three key major QTL clusters *qHTSF1*, *qHTSF4*, and *qHTSF12* can be stably expressed. In addition, epistatic effect is an important component in the regulation of heat tolerance. A total of 17 pairs of epistatic interaction loci were detected, and these additive QTL clusters have a significant epistatic effect. Bulk segregant analysis (BSA) method was proved to be a convenient method to detect major QTLs. Three QTLs, namely *qSF1*, *qSF2* and *qSF12* were detected under high temperature environment, and there is a highly significant correlation among these addictive QTLs. These results will lay the foundation for the further fine mapping of these major QTLs and enrich the molecular marker-assisted selection of heat-tolerant gene resources in rice breeding.

Introduction

Due to the global warming caused by greenhouse gases the global average surface temperature has increased by0.85°C over the past century, which has a huge effect on sustainable agricultural development (Teixeira et al. 2013; Lawas et al. 2019). Heat stress is defined as the rise in temperature beyond a critical threshold for a period of time sufficient to cause irreversible damage to plant growth and development (Wahid et al., 2007; Cheabu et al., 2019). Extremely high temperature leads to severe crop yield losses and reduced milling quality, which is predicted to cause food crises and result in widespread risk of food insecurity and social problems in the future (Mba et al. 2012; Zhang et al., 2014).

Rice (*Oryza sativa* L.) is one of the most important grain crops in the world, and it i a stable food source for more than half of the world's population (Wang et al. 2014). Rice is highly susceptible to heat stress, and spikelet fertility and grain filling often go through serious damage under high-temperature stress, leading to significant yield losses (Ye et al., 2015). High temperature of over 35℃ at the flowering stage reduces pollen viability and spikelet sterility in rice, which can lead to a significant decrease of the yield, quality, and harvest indexes (Matsui et al. 2001; Wang et al., 2019). Rice yield losses due to high temperature have been reported in many countries across continents (Mba et al., 2012; Trnka et al., 2014). Through climate modeling analysis, an increase in global mean temperature and a higher frequency of intense heat spikes have been predicted, and then 16% of rice growing areas will be continuously subjected to five days of heat stress during the reproductive stage in the coming decades (Jagadish et al. 2015; Huang et al. 2017). Reducing the occurrence of heat-related damages, such as rice chalkiness and spikelet sterility, is the major goal in global rice production under climate change. Breeding approaches for improving heat tolerance and developing new heat-tolerant rice varieties are of essential and critical importance to the sustenance of rice production in future global warming (Huang et al., 2017; Karwa et al., 2020).

Climate change-driven heat stress during the reproductive stage of the crop affects spikelet fertility and yield. Heat tolerance during the heading and flowering stage is a quantitative trait controlled by multiple QTLs, and eight QTLs for spikelet fertility under high temperature conditions were identified (Jagadish et al., 2010; Zhao et al., 2016). Ye et al (2015) identified 8 QTLs controlling spikelet fertility under high temperature, while the stable major QTL *qHTSF4.1* was an important source for enhancing heat tolerance. Cloning and fine mapping major QTL responses to high temperature stress will help breeders produce rice varieties adapted to future climates, and enhancing heat tolerance is one of the most hot-spot method for molecular and breeding biology (Jagadish et al., 2010). Several QTLs associated with heat stress tolerance have been reported in rice. However, several major QTLs have been fine-mapped and cloned, while the molecular genetic mechanisms of spikelet fertility and yield under different high temperatures have not been comprehensively studied. This study dissected the genetic basis and identified novel QTLs of heat tolerance in a set of RIL populations derived from a cross between the heat-sensitive Longdao5 and the heat-tolerant Zhongyouzao8. QTL analyses were performed under the control, natural and artificial high temperature environments at the heading and flowering stage in two years. The research goal of QTL comparison analysis is to identify the stable QTL expression which affects important heat tolerant traits under different high temperature stress, and to provide the basis for future fine mapping and molecular breeding of rice.

Materials And Methods

Plant materials and field trials

A set of RILs (F_8) populations consisting of 187 lines were developed from a cross between Longdao5 (LD5, japonica) and Zhongyouzao8 (ZYZ8, indica) by the single seed descent method. In particular, ZYZ8 was a heat-tolerant and high-yielding cultivar, and LD5 was a heat-sensitive cultivar. The RILs along with the parents were evaluated under natural field and artificial greenhouse conditions. At all locations, plants were sown in seedling nurseries, and 25-day-old seedlings were transplanted into paddies. Each line was planted in three rows of 8 hills, spaced at 16.5cm ×20cm, with one seedling per hill. The fifield management (including irrigation, fertilizer application and pest contro) was conducted according to the normal procedures for rice

Two staggered sowings were made during the middle season of 2019 and 2020, with the first sowing timed to coincide the normal growing season and then the second sowing to coincide with the natural and artificial heat stress during the heading and flowering stage. The first sown set did not experience any stress, namely control environment, while the second set experienced the heat stress, natural and artificial high temperature environments. The parents and RILs populations used in this experiment were provided with adequate exposure to different temperature treatments during the heading and flowering period (Table 1).

Table 1
The temperature recorded from different temperature treatments during heading and flowering stage

Years	Treatments	Maximum temperature (℃)		Minimum temperature (℃)		Daily average temperature (℃)	
		Range	Mean	Range	Mean	Range	Mean
2019	E1	25.0–36.0	32.4	20.0–29.0	24.3	23.0-32.5	28.3
	E2	34.0–38.0	36.2	27.0–29.0	28.3	30.0-34.5	32.5
	E3	40.5–46.0	43.2	26.5–30.0	28.5	34.0-36.5	34.8
2020	E4	21.0–34.0	27.0	18.0–26.0	21.4	19.5–30.0	24.2
	E5	35.0–39.0	37.5	26.0–30.0	27.9	31.0–35.0	32.7
	E6	38.1–44.0	41.0	27.0–31.0	28.5	33.2–36.3	34.5

E1-3 represents for control, natural high temperature and artificial high temperature in 2019; E4-6 represent for control, natural high temperature and artificial high temperature in 2020, respectively.

Phenotypic evaluation

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, ten 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. When each plant started heading, six plants were left in the field as controls, three plants was transferred into artificial greenhouse, two replicate pots. After 6 days of high temperature treatment, the plants were moved back to the natural conditions until seeds matured.

For estimating spikelet fertility, seeds from the five marked panicles in each plant were sampled. Spikelet fertility was calculated as the ratio of filled spikelets to total number of spikelets per panicle and expressed as percentage. The observations averaged over three plants were used for statistical analyses and evaluation of the heat tolerance of the lines. Meanwhile, HT index was calculated for spikelet fertility to assess the relative performance of RILs under heat stress through the following formula.

HT = Spikelet fertility under heat stress /Spikelet fertility in control condition, HT range 0–1.

DNA extraction and linkage map construction

Genomic DNA was extracted from fresh frozen leaves according to the CTAB method. Polymerase chain reaction (PCR) reaction mixture (10 μL) contained 1.5 μL 20 ng/μL genomic DNA, 1.0 μL each primer, 1.5 μL 10·PCR buffer, 2.0 μL 2.5 mmol/L dNTP, 0.2 μL 5 U/μL Taq DNA polymerase (Tiangen Biotech, Beijing, China), and 2.8 μL ddH_2O. The PCR program consisted of an initial denaturation for 5 min at 94°C, followed by 35 cycles of 94°C denaturation for 45 s, 55–60°C annealing for 45 s, and 72°C extension for 45 s, with a final extension at 72°C for 10 min and then the products holding at 12°C. PCR products were separated by electrophoresis on 4%-5% agarose gels, mixed with 0.1 mg/ml of ethidium bromide, and the results were observed and recorded using a gel imaging system. A total of 762 SSR, Indel and ILP primer pairs were used to survey the polymorphism between the two parents. Evenly distributed on chromosomes 1–12 of polymorphic 223 SSR, Indel and ILP markers were selected for polymorphism survey between the parents, to construct a genetic map for the RIL population. The genetic map was 1,514 cM in length, with an average of 18.58 markers per chromosome and an average distance of 6.79 cM between adjacent markers.

QTL analysis and bulked segregant analysis

Additive effect QTL analysis was performed based on inclusive composite interval mapping implemented with the QTL IciMapping4.2 program (Wang et al. 2019). In additive effect QTLs analysis, the walking speed along chromosomes was 1.0 cM, when their logarithm of odds (LOD) scores exceeded 2.50 (when QTLs were detected in more than two environments LOD ≥ 2.40). The relative contribution of a genetic component was calculated as the PVE (%) and additive effect in the selected model. The QTLs were named according to the guidelines described by McCouch (2008).

In the initial approach to run a bulked segregant analysis (BSA), only those markers which were polymorphic between the parents were utilized. To construct the bulks, ten genotypes (5% of RILs) that were found to show extreme phenotypes for SF and HT were selected to construct two DNA pools, one for heat tolerant and the other for heat sensitive. The polymorphic SSR markers were then surveyed over the bulks and the parents.

Results

Phenotypic variation in the RIL population

Grain number (GN), setting grain number (SN) and SF showed a significant difference among the parents (Table 1). There is a significant difference of heat tolerance index(HT) between LD5 and ZYZ8 under high temperature environments. GN, SN, SF and HT of the RILs varied exhibited an approximate continuous normal distribution under different conditions. Meanwhile, the transgressive segregation that fell beyond the parents was observed, suggesting that the set of RILs was suitable for QTL analysis (Table 2, Fig. 1). In addition, natural and artificial high temperature have different degrees of effect on SF and HT. The latter has strong heat stress, and then the heat tolerance mechanism from different treatments maybe different.

Table 2
Heat tolerance-related traits from the parents and RILs populations

Year	Treatments	Trait	Parents		RILs populations			
			LD5	ZYZ	Mean ± Sd	Range	Skewness	Kurtosis
2019	Control	GN	78.40 ± 5.60	256.20 ± 15.60**	143.29 ± 44.80	50.80-290.20	0.58	0.65
		SN	65.40 ± 4.00	166.22 ± 10.34**	85.54 ± 34.38	24.80-223.20	0.58	0.65
		SF	83.42 ± 5.65	64.88 ± 10.45**	60.22 ± 16.71	36.50–100.00	-0.47	0.05
	Nature high temperature	SN	50.40 ± 5.65	127.07 ± 10.32**	111.83 ± 34.81	28.60–218.00	0.42	0.03
		SF	63.47 ± 6.40	58.47 ± 12.30*	70.59 ± 14.91	27.68–96.32	-0.52	-0.37
	Artificial high temperature	SN	24.25 ± 5.20	54.14 ± 8.30**	35.11 ± 16.85	4.53–86.54	0.42	0.19
		SF	26.23 ± 5.00	31.24 ± 10.20*	28.44 ± 12.66	1.44–65.05	0.32	-0.04
		HTN	0.76 ± 0.09	0.90 ± 0.07*	0.36 ± 0.39	0.08–0.87	-0.40	1.24
		HTA	0.31 ± 0.06	0.48 ± 0.04*	0.52 ± 0.20	0.03–0.91	-0.29	-0.70
2020	Control	GN	112.40 ± 10.20	211.00 ± 20.50**	160.74 ± 45.20	82.20-284.20	0.45	-0.18
		SN	88.80 ± 8.50	146.10 ± 15.00**	111.83 ± 34.81	28.60–218.00	0.42	0.03
		SF	79.00 ± 8.20	69.24 ± 10.25**	74.10 ± 11.75	34.78–96.32	-0.44	-0.12
	Nature high temperature	SN	71.81 ± 12.00	101.67 ± 15.00**	106.47 ± 34.26	10.00-210.57	0.28	0.18
		SF	61.71 ± 5.50	54.95 ± 10.40*	61.69 ± 13.84	11.32–87.93	-0.51	0.46
	Artificial high temperature	SN	44.75 ± 8.50	63.60 ± 10.50**	54.22 ± 30.83	7.80-193.60	1.45	3.59
		SF	28.87 ± 5.50	35.90 ± 3.00*	36.52 ± 13.20	5.01–68.72	0.09	-0.37
		HTN	0.78 ± 0.08	0.80 ± 0.05*	0.84 ± 0.18	0.18–1.19	-0.41	0.53
		HTA	0.37 ± 0.04	0.51 ± 0.06*	0.50 ± 0.18	0.07–1.03	0.13	-0.23

Grain number, GN; Setting grain number, SN; Spikelet fertility, SF; Heat tolerance under nature high temperature, HTN; Heat tolerance under artificial high temperature, HTA; *and ** represent significant differences at the 5% and 1% level, respectively.

There were significantly positive correlations for all traits between two years, as shown by correlation coefficients range from 0.146–0.417(Table 3). As expected, SF was significant positively correlated with SN, and negatively correlated with GN under different environments. There were significantly positive correlations between SF and HT under high temperature environments. In addition, strong and significant correlations were found in GN, SN, SF and HT under different temperature environments in two years. The correlation analysis demonstrated that these traits were stable and had high heritability from the control, natural and artificial high temperature environments.

Table 3. Correlation coefficients among SF in RILs populations under different environments

Treatment	Traits	Control			Natural high temperature		Artificial high temperature		HTN	HTA	
		GN	SN	SF	SN	SF	SN	SF			HTN
Control	GN	**0.310****	0.715**	-0.244**	0.155*	-0.172*	0.171*	-0.098	-	-	
	SN	0.685**	**0.245****	0.335**	0.208**	0.251**	0.215**	0.001	-	-	
	SF	-0.085	0.307**	**0.146***	0.116	***0.445****	0.058	***0.213****	-0.310**	-0.229**	
Natural high temperature	SN	0.017	0.159*	0.238**	**0.189****	0.355**	0.387**	0.059	-	-	
	SF	-0.040	0.452**	***0.282****	0.245**	**0.194****	0.147*	***0.273****	0.702**	0.282**	
Artificial high temperature	SN	0.131	0.210**	0.132	0.169*	0.138	**0.173***	0.509**	-	-	
	SF	-0.195**	0.141	***0.270****	0.027	***0.280****	0.671**	**0.330****	0.485**	0.888**	
HTN		-	-	**-0.426****	-	**0.730****	-	**0.061**	**0.417****	0.268**	
HTA		-	-	**-0.149***	-	**0.132**	-	**0.874****	0.223**	**0.197****	

Lower and upper triangle data represent for 2019 and 2020; *and ** represent significant differences at the 5% and 1% level, respectively.

QTL mapping within the RIL population

A total of sixty-one QTLs were detected using the ICIM-ADD method, 25, 27 and 14 additive QTLs were identified under the control, natural and artificial high temperature conditions, respectively(Table 4, Fig. 2). These QTLs were located on chromosomes 1–8, 10 and 12, explained around 3.18–42.31% of the phenotype variation. Twenty-five QTL were associated with the SF-related traits under control environments, 13 and 12 QTLs for 2019 and 2020, respectively. Among these QTLs, six QTLs were commonly detected in both years, the other QTLs only can be found in one environment. Ten QTLs associated with GN were identified on chromosomes 1, 4, 6, 8 and 10, these QTLs individually accounted for 4.82–15.84% of the phenotypic variance. Among these QTLs, only *qGN1a*, *qGN4b* and *qGN6* were identified in different years, while the major QTL *qGN1a* has the highest phenotypic variation and additive effect. Nine putative QTLs associated with SN were detected on chromosomes 1, 4, 5, 6 and 8; among these, *qSN1a*, *qSN1b* and *qSN4* were identified across two years. Six QTLs for SF were identified, and all of these QTLs only can be found in one environment; *qSF2* and *qSF4* have a higher phenotypic variation score, which contributed to 13.80% and 37.92% of phenotypic variation, respectively.

Table 4
Putative QTLs for spikelet fertility related traits were detected in RILs population

Treatment	Trait	Locus	Marker	LOD value		PVE (%)		Additive effect	
				2019	2020	2019	2020	2019	2020
Control	GN	*qGN1a*	RM428-RM323	6.13	5.80	11.48	15.84	20.07	18.13
		qGN1b	RM1198-RM1361.1		2.85		7.75		-16.91
		qGN4a	RM471-RM1359		5.86		14.99		-19.47
		qGN4b	RM2441-R4M50	3.50	2.48	8.44	4.59	-13.55	-9.80
		qGN6	RM111-STS6.2	2.49	4.12	7.97	13.26	-16.61	18.08
		qGN8	RM3754-RM3496		3.80		10.83		-14.85
		qGN10	R10M40-STS10.3	3.03		4.82		13.19	
	SN	*qSN1a*	RM6902-RM428	6.89	2.91	9.41	6.88	18.39	9.13
		qSN1b	R1M37-RM3738	3.94	3.22	7.39	9.25	15.33	-13.92
		qSN4	RM1359-R4M43	2.82	5.82	3.18	18.84	-10.35	-16.90
		qSN5	R5M13-RM3476	4.45		10.86		18.29	
		qSN6	RM217-RM111		2.66		5.85		9.39
		qSN8	RM3754-RM3496		2.70		7.79		-9.70
	SF	*qSF1*	RM8097-STS1.4	3.14		7.19		-5.05	
		qSF2	STS2.4-RM13603	6.58		13.80		-9.00	
		qSF3	MM3720-MM3778		3.07		9.52		4.67
		qSF4	RM5688-RM471		2.59		37.92		-9.97
		qSF10	RM3451-RM590	3.05		4.37		5.00	
		qSF12	RM247-RM7003	4.18		5.78		5.66	
Nature high temperature	SN	*qSN1*	RM7180-RM6703	2.68	3.45	4.27	9.89	-9.82	-14.08
		qSN2a	RM3732-RM1361.2		3.06		6.88		12.07
		qSN2b	STS2.3-STS2.4		2.60		6.04		8.55
		qSN3	STS3.6-STS3.7		4.20		11.42		13.41
		qSN6	RM111-STS6.2	2.51		4.28		9.47	
		qSN12	RM101-STS12.1	2.59		8.42		-12.03	
	SF	*qSF2*	STS2.4-RM13603	2.74		5.98		7.55	
		qSF3	RM1350-RM3199	3.26		9.39		4.98	
		qSF7	R7M37-RM1132	2.90		7.43		-4.16	
		qSF12	RM7003-RM101	2.89	2.50	6.40	11.20	-7.57	-9.32
	HTN	*qHTN2*	RM13603-ID2	2.66		7.06		0.11	
		qHTN12	RM247-RM7003	3.34	2.60	7.13	11.45	-0.10	-0.15
Artificial high temperature	SN	*qSN4*	R4M43-RM3288	2.90	3.73	10.54	9.88	-9.50	-8.83
		qSN7	RM5055-RM5711		2.67		8.20		9.57
		qSN10	RM3451-RM590	2.74		6.68		4.72	
	SF	*qSF2*	STS2.4-RM13603		2.92		42.31		-10.04

Treatment	Trait	Locus	Marker	LOD value		PVE (%)		Additive effect	
				2019	2020	2019	2020	2019	2020
		qSF3	RM3199-RM1352		2.53		6.61		3.95
		qSF6	RM1340-R6M44	2.79		6.55		3.80	
		qSF7	RM8261-RM1209		2.53		6.17		-3.84
		qSF8	RM3754-RM3496		2.88		10.11		-4.87
		qSF12	RM7003-RM1337	3.01	2.64	9.50	10.23	4.75	4.91
	HTA	*qHTA1*	R1M30-RM3240	2.70	2.65	7.54	5.07	0.10	0.07
		qHTA3	MM3720-MM3778		2.81		6.73		-0.08
		qHTA6	RM111-STS6.2	2.63		5.31		-0.09	
		qHTA12	RM7003-RM1337		2.59		12.64		-0.20

Twenty-seven QTLs were associated with heat tolerance-related traits under natural high temperature environments. Seven additive QTLs identified for SN were detected on chromosomes 1, 2, 3, 6 and 12, except for *qSN1* which was found in both years and explaining − 9.82 and − 14.08 of the phenotypic variation, all other QTLs were only detected in one environment. Four QTLs associated with SF, namely *qSF2*, *qSF3*, *qSF7* and *qSF12*, were detected on chromosomes 2, 3, 7, and 12 respectively. Among these QTLs, the stable expressed major QTL *qSF12* has a higher LOD value and phenotypic variation, the other QTLs can be detected only in one environment. Two QTLs associated with HTN were detected, among which *qHTN12* with the greatest effect on phenotypic variation was collocated with the QTL *qSF12*.

Sixteen QTLs associated with the heat tolerance-related traits were identified under artificial high temperature environments, 6 and 10 QTL in 2019 and 2020, respectively. Three QTLs associated with SN were detected on chromosomes 4, 7 and 10, and these QTLs can be identified in one environment, which explained 6.68–9.88% of phenotypic variance. Seven putative QTLs associated with SF were detected on chromosomes 2, 3, 6, 7, 8, and 12. Among these QTLs, only *qSF12* was located in two years. Four QTLs associated with HTA were detected, that is *qHTA1*, *qHTA3*, *qHTA6* and *qHTA12* located on chromosomes 1, 3, 6 and 12, respectively. Among these QTLs, only *qHTA1* can be found in two years.

Our results reveal that pleiotropic effects and QTL hotspots are the key factors affecting GN and SF in rice, and then these traits may have a similar genetic regulation. We could assign the 64 QTLs to 15 chromosome regions, each corresponding region contains about 4 QTLs. Seven major QTL clusters were assembled on the chromosomes 1, 2, 3, 4, 6 and 12, which contain 38 QTLs, accounting for 59.38% of the whole locus. These related QTLs were identified in previous studies. Among these QTLs, clusters *qHTSF1a*, *qHTSF1b*, qHTSF4 and *qHTSF12* were stably detected (Table 5, Fig. 2).

Table 5
Heat tolerance-related traits of QTL hotspot and pleiotropic region

Major QTL cluster	Chr.	Interval	Pleiotropic QTL	Related reports
qHTSF1a	1S	RM3740-RM323	*qGNC1a,qSNC1a,qGNN1,qGNA1*	*Gn1a* (Ashikari et al.,2005)
qHTSF1b	1L	RM3240-RM1198	*qGNC1b,qSNC1b,qSFC1,qSNN1,qHTA1*	*qHBT1-1* (Cao et al., 2020)
qHTSF2	2	STS2.4-RM13603	*qSNC2,qSFC2,qSNN2,qSFN2,qHTN2,qSFA2*	-
qHTSF3	3	R3M30-RM1350	*qSFC3,qSFN3,qSFA3,qHTA3*	*qSF3.2* (Nubankoh et al., 2020)
qHTSF4	4	RM471-RM3288	*qGNC4,qSNC4,qSFC4,qGNN4,qGNA4,qSNA4*	*qHTS4.1* (Ye et al., 2015b)
qHTSF6	6	RM111-STS6.2	*qGNC6,qSNC6,qGNN6,qSNN6,qHTA6*	*qHTSF6.1* (Raza et al., 2020)
qHTSF12	12	RM247-RM101	*qSFC12,qSNN12,qSFN12,qHTN12,qSFA12*	-
The bold represent the stable QTL under different environments, respectively.				

In this study, 17 pairs of epistatic interaction QTLs for heat tolerance-related traits were detected (Table 6). All these QTLs are located on chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9 and 12, and LOD value of these QTLs range from 6.7 to 12.9, accounting for a wide phenotypic variation ranging from 8.32–28.09%. 13 out of 17 epistatic QTL pairs were nearby the additive QTL. For example, the genomic region RM5688-RM471 on the chromosome 4 is interacted with the chromosomes 2, 3, 5,7, 8,11 and 12; the genomic region RM1361-RM6321 on chromosome 1 epistatic effection on the chromosomes 2,4,8 and 12. There were 8 pairs of epistatic interaction QTL for HT (HTN and HTA), no epistatic QTL of epistatic interaction for SF under natural and artificial high temperature stress. Therefore, epistatic QTLs played an essential role in controlling the genetic expression of the heat tolerance gene.

Table 6
Identification and analysis of the epistatic interaction QTL under different temperature environments

Environments	Trait	Chr.*i*	Marker1	Chr.*j*	Marker2	LOD	PVE(%)	AA
Control	GN	1	STS1.4-RM7180	9	RM7048-STS9.1	5.91	12.39	17.90
	SF	1	RM1361-RM6321#	4	R4M43-RM3288	7.29	23.35	-9.70
Nature high temperature	SN	12	RM247-RM7003#	6	RM587-RM217	5.95	16.62	-30.98
	HTN	1	RM1198-RM1361#	2	STS2.4-RM13603	9.64	15.09	-0.43
		1	RM1198-RM1361#	12	STS12.2-RM1226	12.19	13.69	0.43
		4#	RM5688-RM471	2	RM5699-RM1358	11.15	8.32	-0.43
		4#	RM5688-RM471	11	RM21-STS11.4	10.21	18.23	0.39
Artificial high temperature	SN	1	STS1.3-RM306	2	RM7451-STS2.1	6.33	13.36	-19.89
		1	R1M30-RM3240	3	RM3199-RM1352	6.38	15.69	-23.34
		4#	RM5688-RM471	5	R5M13-RM3476	7.09	19.92	26.81
		4#	RM5688-RM471	7	RM1132-RM8261	6.85	23.04	-31.10
		4#	RM5688-RM471	12#	RM19-RM247	7.34	25.68	-31.36
		7	RM5711-STS7.1	8	RM5556-RM6208	6.45	21.45	-24.61
	HTA	1	RM1361-RM6321#	8	RM1376-RM4085	20.71	26.72	-0.72
		4#	RM5688-RM471	7	RM3826-R7M37	22.56	25.12	-0.70
		4#	RM5688-RM471	8	RM6208-RM3395	21.26	22.85	-0.69
		8	RM1376-RM4085	12#	RM19-RM247	18.55	28.09	-0.71

represents that the additive QTL chromosome region have an epistatic interaction.

Identify the major QTLs through BSA

BSA method was proved to be a convenient method to the detection of major QTLs, in particular to identification of QTLs for adversity stresses such as drought, salinity, heat and cold (Zhang et al., 2009; Sandhu et al.,2014; Sun et al. 2018; Wu et al., 2019). In this study, SF and HT were used to evaluate the heat tolerance, two extreme bulks, including the heat-tolerant bulk with higher SF and HTA, and the heat-sensitive bulk with lower SF and HT under artificial high temperature conditions. 10 lines with heat tolerance and sensitivity were selected in the $F_{5:6}$ populations (510 lines), respectively. The SF and HTA were significantly lower in the S-bulk than in the T-bulk, indicating that the overall phenotypic of the S-bulk was greatly affected by high temperature (Fig. 3). 24 markers have polymorphism from two bulks and the parents, which have clear correspondence with phenotype. Out of these markers, 11 markers were together located on the chromosomes 1, 2 and 12, namely three putative QTLs, *qSF1*, *qSF2* and *qSF12*, these QTLs exhibited the contrasting genotype between the T-bulk and S-bulk, respectively (Fig. 4A). Through BSA, it was found that these QTLs revealed the highly significant correlation among these addictive QTLs through ICMI mapping methods, which further confirmed the reliability of the detected QTLs (Fig. 4B).

Discussion

Identification method of heat tolerance in rice

Rice is one of the most important food crops in the world. Therefore, maintaining high yield of rice plays an important role in food security (Khush, 2005). However, global warming threatens many aspects of human life. It also becomes a big concern in sustaining and improving rice yields. Breeding heat-tolerant rice is a fundamental way to help address this challenge (Yan et al. 2020; Zhao et al., 2016). High temperature was already a major environmental limiting factor affecting yield and rice is highly susceptible to heat stress at the reproductive stage (Tian et al. 2009; Prasad et al. 2017; Fang et al., 2020). High temperature causes irreversible damage by retarding anther dehiscence, pollen fertility, pollen tube elongation in pollinated pistils, spikelet fertility and grain filling (Jagadish et al. 2007; Zhang et al. 2018), and different cultivars respond differently to high temperature stress, while these differences are largely controlled by genetic factors (Prasad et al. 2006; Yan et al., 2020; Ye et al., 2015). One of the best strategies for maintaining rice productivity under increasing temperature is to develop heat-tolerant varieties (Kilasi et al. 2018). In this study, ZYZ8 was a heat-tolerant and high-yielding cultivar, and LD5 was a heat-sensitive cultivar under both natural and artificial high temperature. High temperature has different degrees of effection SF and HT, the latter has strong heat stress, and then heat tolerance of mechanism from different treatments methods maybe different. In the RIL population, the SN, SF and HT values have a normal frequency distribution, have a large variation and transgressive segregations in response to high temperature. There were significant positive correlations between SF and HT under the natural and artificial high temperature environments. Heat-tolerant lines contain some major QTL with higher SF and HTA, while the heat-sensitive lines have a few or no related QTLs with lower SF and HT under high temperature conditions. Therefore, the main step of breeding heat tolerant cultivars is selecting and identifying extremely heat resistant germplasm resources or genes.

To clone and apply the major QTL of heat tolerance, the most critical step is quickly and accurately identifying heat resistance phenotype. A standardized temperature treatment for phenotyping is very useful for accurately evaluating the heat tolerance of promising populations and breeding lines, and making the results more comparable. In the past twenty years, the natural, greenhouse and chamber high temperature conditions have been used to screening penotype and identify QTL for heat tolerance (Chen et al. 2008, Zhang et al. 2009, Jagadish et al. 2010a, Xiao et al. 2011). The high temperature in the growth chamber is more accurate and stable, the results from different research groups become more comparable when different mapping populations. However, more plants could be treated with limited space (Ye et al. 2010). Compared with the natural and greenhouse high temperature methods, the chamber method used to evaluate heat tolerance for phenotyping the genetic and nature populations is very efficient. However, this method cannot be used for evaluating the heat tolerance of big germplasm resources populations and advanced breeding lines of rice (Li et al., 2018). In most of the earlier studies, SF under the high temperature environments was taken as the main criterion for selecting heat-tolerant genotypes, but in our study, the GN, SN, SF and HT under the natural and greenhouse high temperature were used to evaluate heat resistance of each line. The GN, SF and TGW were correlated with yield under both normal and heat stress conditions. Overall results showed positive correlation between SF and yield per plant only in heat stress conditions but not in normal conditions. HT have been regard as the heat tolerance evaluation index under the natural and greenhouse high temperature, which can eliminate the effects of the SF under the normal environment, so that the HT can be used to truly and accurately reflect the heat resistance. Hence, the major QTL cluster control with these traits can be used for the key chromosome regions of yield under heat stress.

Heat tolerance QTLs at the reproductive stage

High temperature stress, as an increasingly important problem in crop production, can significantly reduce crop yield (Xu et al. 2017; Aleem et al., 2020; Karwa et al. 2020). Rice is known to be sensitive to heat stress particularly at the heading and flowering stage, SF and thousand-grain weight have a significant decreased under high temperature Rice heat tolerance during different development stages is a quantitative trait controlled by multiple micro-QTL (Jagadish et al., 2010; Li et al. 2018; Ravikiran et al., 2020). QTLs for rice heat tolerance at the flowering stage have been mapped on all chromosomes by using various rice populations and high temperature treatment methods (Cheng et al. 2012; Ye et al., 2015a; Li et al. 2018). 11 QTLs were identified in three populations, and then the distribution of these QTLs in chromosomes 1, 2, 3, 4, 6 and 11, *qHTSF4.1* was consistently detected across different genetic backgrounds and could be an important source for enhancing heat tolerance in rice at flowering stage (Ye et al., 2015b). According to Cheng et al(2012) introgression lines derived from a cross between Xiushui 09 and IR2061were put into greenhouse and subjected to heat treatment, four QTLs for SF were identified on chromosomes 4, 5, 6 and 11. Among these QTLs, *qSF4* and qSF6 reduced SF under heat stress and normal conditions. Li et al (2018) used the RIL population exposed to high temperature at the heating and flowering stage. QTL analysis revealed 11 QTLs associated with heat tolerance, which located on chromosomes 1, 3, 4, 5 and 6, and found 13 QTLs with epistatic interactions and nine QTLs with environmental interactions. In our study, 61 QTLs were detected on chromosomes 1–8, 10 and 12 under three different temperature treatment environments; among these QTLs, 12 QTLs were stably expressed in different years, the other QTLs only can be found in one year. These major QTL clusters distributed on chromosomes 1, 2, 3, 4, 6 and 12, namely *qHTSF1a qHTSF1b qHTSF2 qHTSF3 qHTSF4 qHTSF6* and *qHTSF12*. In addition, 17 QTLs with epistatic interactions were detected, and the major QTLs were all involved in epistatic interactions, which showed that the epistatic interactions have important effects on regulation of rice SF and HT. Recently, more than 200 heat tolerance-

related QTLs have already identified from the previous published papers and http://www.gramene.org/, compared these QTL identified with our research, we found that chromosomal distributions and locations of the QTL clusters *qHTSF1a*, *qHTSF1b*, *qHTSF3*, *HTSF4* and *qHTSF6* are similar to that of *Gn1a*, *qHBT1-1*, *qSF3.2*, *qHTSF4.1* and *qHTSF6.1* (Ashikari et al., 2005; Cao et al., 2020; Nubankoh et al., 2020; Ye et al., 2015b; Raza et al., 2020). The major QTL *qHTSF2* and *qHTSF12* are two novelly identified major QTLs, and *qHTSF12* can be found in different environments.

BSA method was proved to be a convenient technology in detecting major QTLs, in particular for identify resistance QTLs (Illa-Berenguer et al., 2015; Yao et al., 2016; Nubankoh et al.,2020). Recently, an effective approach QTL-seq has been developed to rapidly identify QTLs in plants. It combines the potential of BSA method and the power of high-throughput whole-genome resequencing to locate genomic regions in the two bulk populations that contain 10–20 individual plants with extreme phenotypes in each bulk (Takagi et al. 2013; Illa-Berenguer et al., 2015). Nubankoh et al.(2020) applied the QTL-seq method to rapidly identify QTLs for SF under high-temperature stress based on an F_2 population, three QTLs were fine mapped on chromosomes 1, 2 and 3, and then candidate genes for each QTL were proposed and were supported. In our study, the BSA method was used to check the heat tolerance QTL for its authenticity and correctness. First, a total of 750 markers from the rice molecular map were subjected to polymorphism surveys involving, of which 24 pairs exhibited polymorphism from the parents and two bulks, and then these markers were together located on chromosomes 1, 2 and 12. Through BSA, three QTLs *qSF1*, *qSF2* and *qSF12* revealed the highly significant correlation among these addictive QTL clusters *qHTSF1*, *qHTSF2* and *qHTSF12*, which further confirmed the reliability of the detected QTL. Hence, the BSA method without the laborious and time-consuming step of genotyping of a whole population to construct a genetic linkage map and with the feasibility to be applied to any segregating population, has been successfully used to rapidly identify the major QTL in rice. *qHTSF1*, *qHTSF2* and *qHTSF12* was three important QTLs for control the heat tolerance, so that we will developbackcross populations to introduce the QTLs into LD5 background for further validation and fine mapping these QTLs.

Declarations

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Data Availability All data included in this study are available upon request by contact with the corresponding author.

Animal Research (Ethics) Not applicable

Consent to Participate (Ethics) Not applicable

Consent to Publish (Ethics) Not applicable

Plant Reproducibility Not applicable

Clinical Trials Registration Not applicable

Author Contribution Jin Liu conducted the field work, generated phenotypic data, preformed data analysis and wrote the manuscript; Huiying Zhou, JiaxiaoHu and Shuhui Li helped for field work and generated phenotypic data; Xiaoding Ma, Di Cui and Bing Han helped for field work and preformed data analysis; Maomao Li and Longzhi Han designed the research, guided the experiments and manuscript revision. All authors read and approved the final version.

Conflict of Interest The authors declare that they have no competing interests.

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Figures

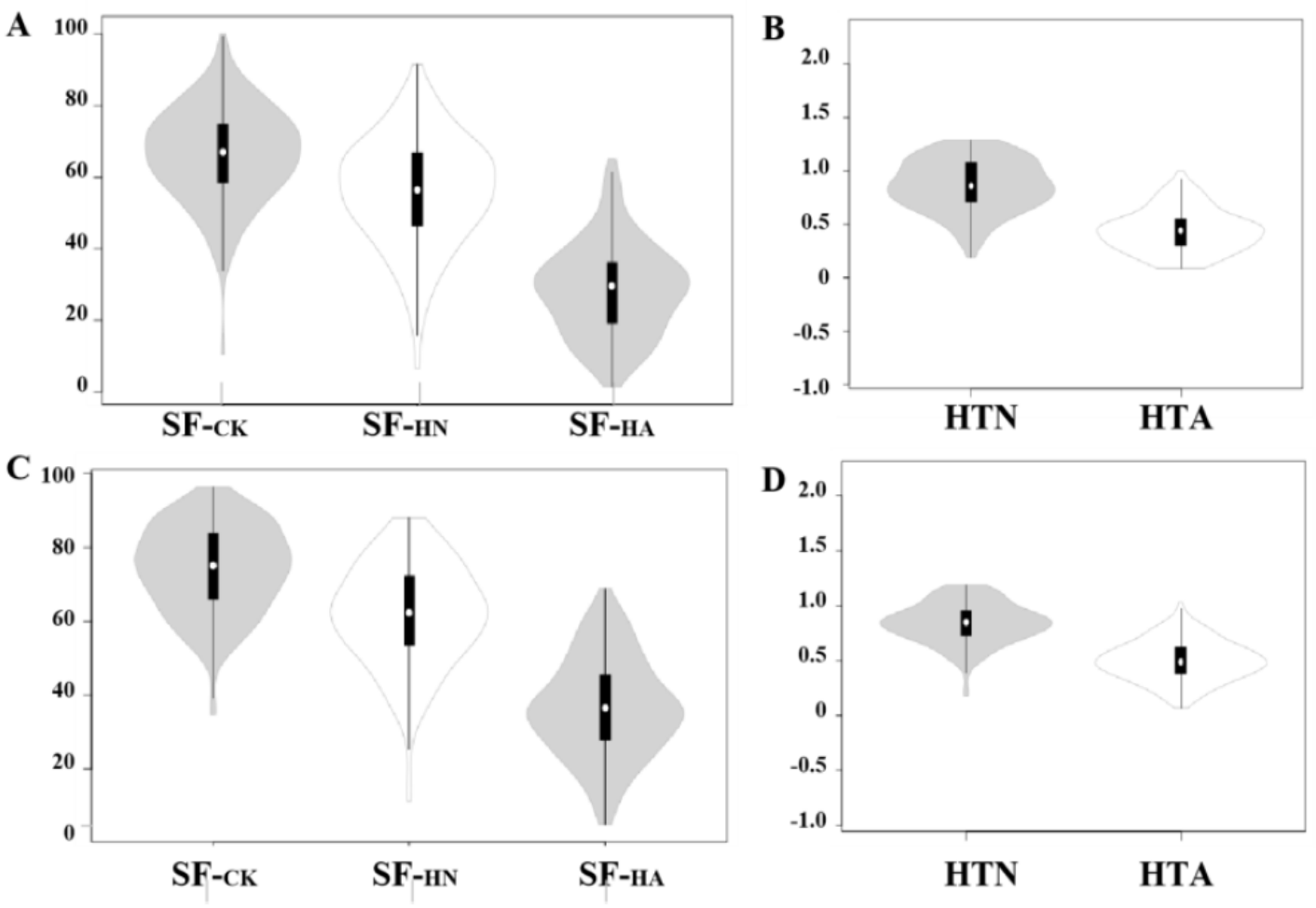


Figure 1

Violin plots of frequency distributions for SF and HT under different environments in the RIL populations. Note: A and B represent for SF and HT under the control, natural and artificial high temperature environments in 2019; C and D represent for SF and HT under the control, natural and artificial high temperature environments in 2020, respectively.

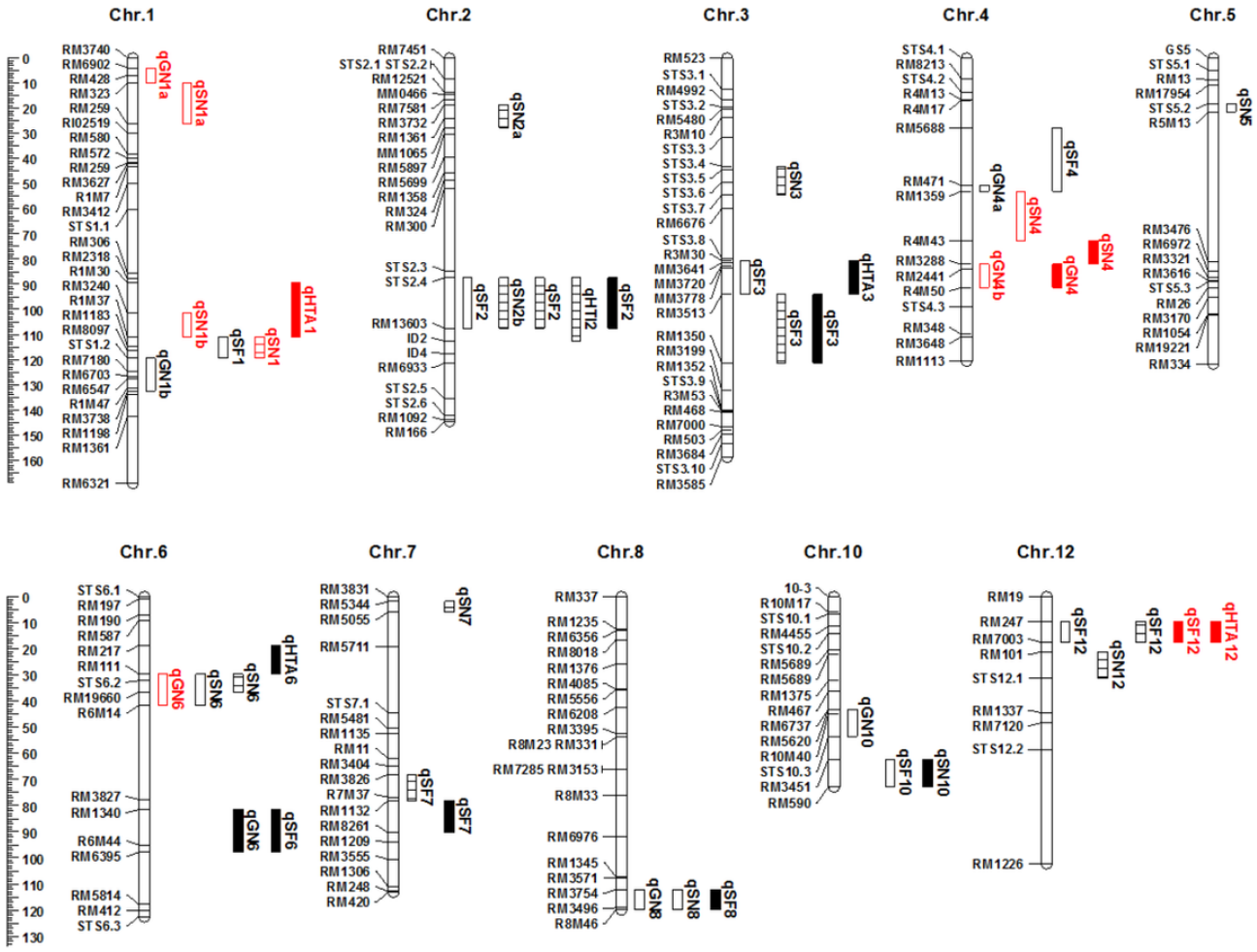


Figure 2. Location of QTL detected for heat tolerance-related traits on the RILs populations genetic map

, and QTL were detected on control, natural and artificial high temperature treatments, the black and red color represent for QTLs detected in one and two year, respectively.

Figure 2

See image above for figure legend.

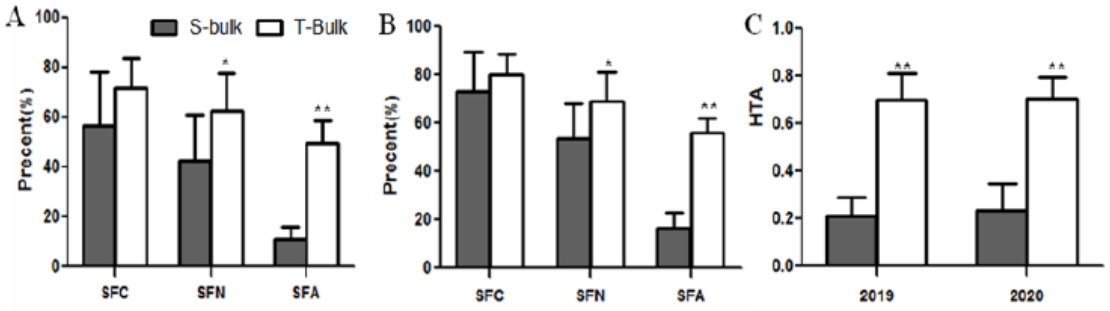
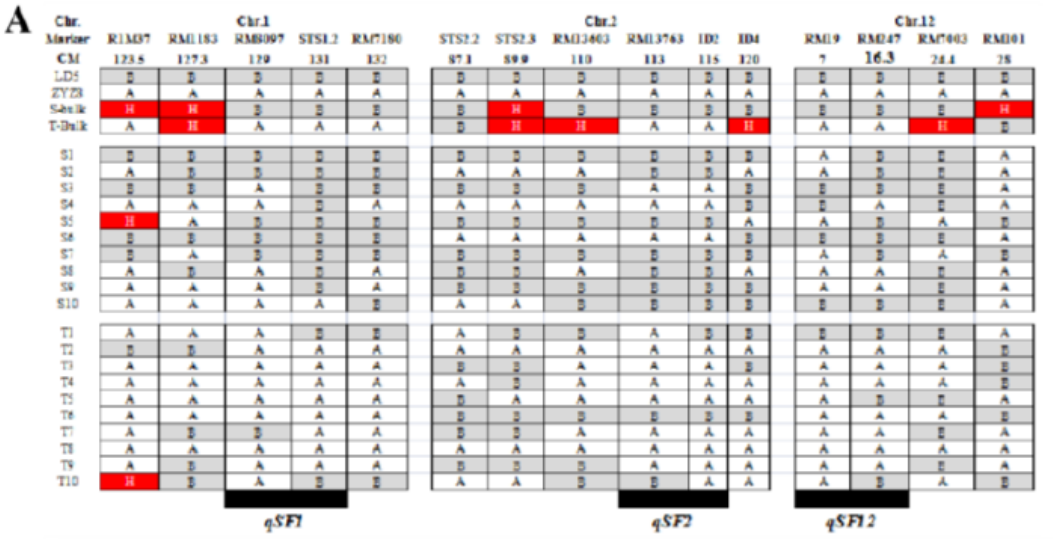


Figure 3

Comparative analysis of the SF-related traits between the HS-bulk and HT-bulk A and B represent SF under control, natural and artificial high temperature in 2019 and 2020, respectively; C represents HTA in 2019 and 2020.



B

Chr.1	Chr.2	Chr.12	2019 SFA	2019 HTA	2020 SFA	2020 HTA	
-	-	-	8.77	0.16	15.82	0.23	n=6
qSF1	-	-	20.81	0.39	32.20	0.42	n=4
-	qSF2	-	18.20	0.25	15.64	0.20	n=2
-	-	qSF12	12.16	0.25	7.86	0.09	n=1
qSF1	qSF2	-	47.77	0.65	50.79	0.66	n=3
qSF1	-	qSF12	50.45	0.69	54.93	0.75	n=3
-	qSF2	qSF12	65.05	0.74	57.00	0.64	n=2
qSF1	qSF2	qSF12	47.36	0.77	57.07	0.68	n=4

Figure 4

Bulked segregant analysis and three QTLs affected the SF in extremely performing lines