QTL Mapping and Identification of Candidate Genes for Heat Tolerance at the Flowering Stage in Rice

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Abstract

Background: High-temperature stress can cause serious abiotic damage that limits the yield and quality of rice. Heat tolerance during the flowering stage of rice is a key trait that can guarantee a high and stable yield under heat stress. Heat tolerance is a complex trait that is regulated by numerous quantitative trait loci (QTLs); of which, however, few underlying genes have been fine mapped and cloned.

Results: In this study, the $F_{2:3}$ population derived from a cross between Huanghuazhan (HHZ), a heat-tolerant *indica* cultivar, and 9311, a heat-sensitive *indica* rice variety, were used to map the QTLs for heat tolerance during the flowering stage of rice. The $F_{2:3}$ lines were treated under 38 °C from 9:30 am to 3:30 pm continuously for 3 days in a phytotron, and spikelet fertility was assessed. A new major QTL, *qHTT8*, controlling heat tolerance was located on chromosome 8 using the bulked-segregant analysis (BSA)-seq method. The QTL *qHTT8* was determined to be located in the 355500–4520000 bp region, with a size of 0.965 Mb. The candidate region of *qHTT8* on chromosome 8 contained 65 predicted genes. Ten putative predicted genes were found to be associated with abiotic stress tolerance. In future studies, the backcross population will be constructed to fine map and validate the effect of *qHTT8*.

Conclusion: The information obtained in this study is useful for the fine mapping and cloning of *qHTT8* and breeding heat-tolerant varieties of rice using marker-assisted selection.

Background

Rice (*Oryza sativa* L.) is a major staple food crop for nearly half of the world's population. As global temperatures have increased in recent years, extreme, high temperatures have led to serious losses in yield, decreased grain quality and a lowered harvest index, especially during the flowering stage, which has a net negative impact on the normal seed setting of rice [1]. Average global temperatures are expected to increase by 2–3 ºC over the next 30–50 years [2]. However, rice yields are expected to decrease by 10% for every increase in daily maximum and minimum temperature of 1 ºC [3]. In addition, the average daily temperature is expected to exceed 35 ºC for several consecutive days, which will lead to spikelet sterility and abnormal pollination, seriously reducing the seed-setting rate [4]. Current strategies to deal with high-temperature stress via alterations to technical and management systems are insufficient for sustaining yields [5]. For these reasons, there is an urgent need to breed heat-tolerant rice varieties.

The study of heat tolerance (HT) at the flowering stage has become a major focus of rice breeding. Understanding the genetic mechanisms of heat tolerance and developing heat-tolerant varieties are essential for the ability of rice to cope with future global warming [6]. Germplasm resources are the material basis for the breeding of new rice varieties. The most effective method is to select different types of heat-tolerant materials to identify different rice germplasm resources, characterize HT and build a robust population, which can provide a foundation for the breeding of stress-tolerant varieties, a reference for the identification of heat-tolerant genes and a means for the exploration of heat-tolerant mechanisms. Effective measures for dealing with high-temperature stress in rice include the identification of heat-tolerant genes, the acquisition of intermediate materials and the cultivation of heat-tolerant varieties [7].

Much research over the past decades has focused on the mining of heat-tolerant genes in rice, primarily through the construction of different genetic populations, and yield or quality traits related to HT have been used as the evaluation indexes for rice heat-tolerant QTL analysis. This work has resulted in the detection of heat-tolerant QTLs in different regions of multiple chromosomes. Recent work has focused on the physical and chemical properties and agronomic characters of rice during each sensitive period, resulting in breakthroughs in the study of heat-tolerance mechanisms. Specifically, numerous achievements have been made in research on rice HT molecular genetics, including the mapping of several rice HT QTLs. However, several QTLs related to rice HT have still not been detected; thus, there is an urgent need to conduct more research examining the correlations associated with the inheritance of rice HT.

Genetic analysis has revealed that HT at the flowering stage in rice is a complex quantitative trait controlled by multiple genes. The resistance of rice to high temperatures shows variety specificity, which indicates that genetic factors contribute the most to explaining variation in HT among rice varieties. With the development and wide application of molecular biology and genomic tools in recent years, there has been an increasing number of QTL-mapping studies of rice HT using molecular markers. QTLs/genes for rice HT have been mapped across all 12 chromosomes (Supplemental Table 1) using different types of molecular markers, such as RFLP, SSR and SNP, which has facilitated the identification of chromosomal regions associated with tolerance of high temperatures [8-36]. In addition, different parents and types of mapping populations (e.g., F_2, $F_{2:3}$ lines, BC, NILs, RILs, CSSLs and DH) have been used to analyze QTLs/genes with different yields (e.g., seed setting rate, spikelet fertility, pollen fertility, grain weight, flowering time and heading days) or quality traits (e.g., white-back kernels, basal-white grain, chalky grain rate, amylose content and gel consistency) related to rice heat-stress tolerance at different stages, such as the seeding and reproductive stages[8-36].

The presence of similar heat-tolerant QTLs in rice indicates that the heat-tolerant metabolic pathways might be conserved among different rice varieties and that some QTLs with greater effects could be stably expressed. However, some heat-tolerant QTLs have not been consistently detected, which may be related to the different genetic backgrounds of varieties, or because there are greater differences in test environment conditions. Rice HT is characterized by quantitative trait inheritance, and its molecular mechanism is relatively complex. An important line of research on the molecular mechanism of rice HT is the determination of genes involved in the regulation of the response of rice to heat stress. Although QTLs for rice HT at the flowering stage have been mapped on all 12 chromosomes using various rice populations, the additive effect of each QTL is low. As a result, introducing one or a few QTLs into a variety may not sufficiently increase its heat tolerance [6]. Therefore, the fine mapping, validation and characterization of more QTLs and the design of functional SNP chips with QTL-linked markers are necessary for accelerating the selection and incorporation of multiple QTLs and, in turn, improving the efficiency of rice heat-tolerant breeding.

Here, the heat-tolerant variety HHZ and the heat-sensitive variety 9311 selected by our research group in a previous study were hybridized (F_1) and then continuously self-crossed to develop source materials (F_2 and $F_{2:3}$) for HT identification and QTL mapping. A total of 365 $F_{2:3}$ populations were selected for HT evaluation at the flowering stage. The QTLs for spikelet fertility under high-temperature stress were rapidly identified using the BSA-seq method combined with whole-genome resequencing (WGS) technology [37]. To provide a foundation for molecular marker-assisted selection breeding, cloning and functional analysis of HT QTLs, as well as the entire genome association analysis, were conducted to determine the location of HT QTLs.

Results

Phenotypic characterization of HT of $F_{2:3}$

Spikelet fertility has been previously used to screen and select for HT during the reproductive stage. To analyze the genetic basis of HT during the flowering stage in rice, we constructed an $F_{2:3}$ population derived from crosses between HHZ and 9311. Significant differences in spikelet fertility were observed among 365 $F_{2:3}$ plants under high-temperature conditions (38 ºC; Fig. 1). Spikelet fertility ranged from 1.04 to 85.24%, with an average value of 50.00%, standard deviation of 0.1847 and coefficient of variation of 36.95%, indicating that the phenotype of HT in the $F_{2:3}$ population was normally distributed (Fig. 1). Meanwhile, the spikelet fertilities of their parents HHZ and 9311 were 54.5% and 14.3%, respectively, under high-temperature stress (38 ºC) for 3 consecutive days. The $F_{2:3}$ population showed a large degree of segregation and a large number of super-parent lines, suggesting that the HT of rice at anthesis was a quantitative trait controlled by multiple QTLs/genes. To construct the heat-sensitive (S) and heat-tolerant (T) pools, 50 heat-sensitive and 50 heat-tolerant $F_{2:3}$ plants were selected. The percentage of spikelet fertility of the 50 $F_{2:3}$ plants in the HS-bulk ranged from 1.04 to 26.73% and that of the 50 $F_{2:3}$ plants of the HT-bulk ranged from 69.90 to 85.24%.

BSA-seq analysis

Genomic DNA samples of two parents (HHZ and 9311) and the two pools (T-pool and S-pool) were sequenced by an Illumina HiSeq™ sequencer, and 85.7 Gb of clean data were generated after being filtered (Table 1).

Using Nipponbare as the reference genome, the parents and mixed-pool sequencing data were compared, and mutations were detected (Table 2). The effective reads of HHZ accounted for 98.01% of the entire genome, with an average sequencing depth of 35.93×, while the effective reads of 9311 covered 98.14% of the entire genome at an average read depth of 35.30×. Both varieties showed good coverage and sequencing depth. BSA association analysis is a gene-mapping method based on mixed pool sequencing, which primarily analyzes regions with significant differences in the frequency of mixed pool genotypes to determine the QTL positions related to target traits. In this study, BSA was used to analyze the associated SNPs. Before association analysis, the SNPs were filtered to obtain 627717 high-quality SNP loci. After filtering, 33205 effective SNP loci with differences between the two pools were identified.

QTL mapping

Based on resequencing and association analysis, the SNP-index of the T-pool and S-pool were compared. At a 99.9% confidence level, the window above the threshold was considered the candidate interval. There was an imbalanced SNP between 355500–4520000 bp on chromosome 8 (Fig. 2-C in the red-dotted box). In this region, the SNP-index value of the T-pool (heat-tolerant type) was greater than or equal to 0.7, while that of the S-pool (heat-sensitive type) was less than or equal to 0.3, indicating that the single plant in the heat-tolerant pool had the same fragment as HHZ in this region and that the single plant in the heat-sensitive pool had the same fragment as 9311 in this region. With a 99.9% confidence level as the screening threshold, the value of △(SNP-index) in this region was greater than the

screening threshold. Therefore, the region of 3555000–4520000 bp on chromosome 8, which was named *qHTT8*, may be the putative locus controlling the HT of rice at the flowering stage. The size of this region was 0.965 Mb; the number of effective SNPs and indels were 258 and 29, respectively (Table 3).

Candidate genes for HT located in the QTL intervals

Gene ontology (GO) analysis was used to classify all of the genes expressed into different functional categories, including biological processes, cellular components and molecular functions. Genes located in the genomic regions for the identified QTL were extracted (Supplementary Table 2). The *qHTT8* QTL harbored 53 genes that were annotated in the GO database. A sum of 119 GO terms were grouped to the three categories, within which genes corresponding to molecular function (39) and biological process (46) were the most abundant. Significantly enriched GO terms were associated with all of the three categories, including proteolysis involved in cellular protein catabolic process (GO:0051603), serine-type carboxypeptidase activity (GO:0004185), molecular_function (GO:0003674) and integral component of membrane (GO: 0016021) (Fig. 3).

To further analyze the candidate genes in the chromosome region containing *qHTT8*, we predicted 65 putative genes in the Nipponbare genome using the Rice Genome Annotation Project database (RGAP http://rice.plantbiology.msu.edu/) (Supplementary Table 3). Among these genes, 10 genes that are located within the mapped region have been reported to be involved in abiotic stress tolerance, such as high night temperature, drought, cold, salinity and saline-alkaline (Table 4) [38-47].

Discussion

With the rapid development of molecular biology and genomics in recent years, research on rice molecular marker-assisted selection breeding has increased extensively. As a consequence of this increased interest, there has been a substantial improvement in the identification of candidate genes and a shortening of the breeding cycle, which has facilitated the breeding of new rice varieties. With the threat of climate change, the development of heat-tolerant rice cultivars is critically important for future rice production. Over the past decades, researchers have identified dozens of QTLs controlling heat stress tolerance (Supplementary Table 1). However, as HT is a quantitative trait, its underlying genetic mechanism is relatively complex. To date, the use of these candidate genes to breed high-yielding, heat-tolerant rice varieties has been rare.

In this study, we evaluated the HT phenotype of $F_{2:3}$ families developed from HHZ crossed with 9311. The HT of $F_{2:3}$ populations at the flowering stage was a quantitative genetic trait controlled by multiple QTLs/genes. BSA-seq combined with the conventional gene mapping method can significantly accelerate the fine mapping of genes [48]. The extreme expression of HT was selected via phenotypic identification to locate the heat-tolerant QTL at the anthesis of rice using BSA combined with WGS. The heat-tolerant QTL was located between 355500 and 4520000 bp on chromosome 8.

Several QTLs/genes for HT have been identified on chromosome 8. For example, Tabata detected a QTL for the occurrence of white-back kernels associated with high temperatures during the ripening period of rice at 0.15 Mb (RM2680) [14]. The QTL *qhr8-1* of HT at the flowering stage around 17.43 (RG978)–21.65 Mb (RG1) was mapped by Cao, and *qtl_8.2* for absolute spikelet fertility near 20.53 Mb was detected by Jagadish, which overlapped the QTL *qHTGC8* for the thermo-tolerance of gel consistency in the 19.31 (R727)–20.66 Mb (G1073) region located by Zhu [8, 9, 11, 18]. The QTL *qht8* located by Chen in the interval of 5.59 (RM547)–39.4 Mb (MRG2181) contained the *qhr8-1* located by Cao et al, the *qtl_8.3* (27.60 Mb) located by Jagadish, the heat-tolerant QTL ranging from 2355534 (RM6976) to 37615523 bp (RM264) mapped by Zheng and the QTL of spikelet and pollen fertility (24.72 Mb) and the early morning flowering QTL of heat escape (22.34 Mb) mapped by Baliuag [8, 9, 15, 18, 49, 50]. The QTL *qHT8* for HT at the flowering stage located at 4.59–17.95 Mb by Kui and *HD8* of days-to-heading in the range of 3.02 (RM3819)–4.38 Mb (RM25) detected by Thanh were located close to the QTL *qHTT8* detected in this study [51, 52]. As previously mentioned, researchers have identified multiple heat-tolerant QTLs on chromosome 8 using different population materials with different timing, duration and intensity of high-temperature stress treatment and evaluation traits related to HT. However, these QTLs/genes reported to be associated with HT in rice are different loci from *qHTT8*, which represents a new QTL.

The survival of plants under heat stress depends on their ability to perceive the heat stress stimulus, generate and transmit the signal and initiate the appropriate physiological and biochemical changes. The induction of gene expression and metabolite synthesis via heat stress substantially improves heat tolerance in rice [53]. Several proteins have been considered to be involved in the formation of HT mechanisms in rice and play an important role in HT regulation. Although many studies have documented HT QTLs, there are relatively few studies that have fine mapped, cloned and functionally verified HT QTLs/genes at the flowering stage in rice. For example, Ye mapped the HT QTL *qHTSF4.1* at the flowering stage, and the interval of the QTL was determined to be approximately 1.2 Mb [6]. However, the

sequence in the region was highly conservative, and a large number of genes in the same gene family were observed to be clustered. Further verification has shown that rice varieties with *qHTSF4.1* exhibited a certain degree of HT under high-temperature conditions. He used advanced backcross progeny to fine map the HT gene and located the heat-tolerant QTL on chromosome 4 between 17.48 (RM16770) and 18.19 Mb (RM16792) [54]. A total of 59 candidate genes were found through preliminary screening, and most of these candidates were primarily related to the protein synthesis underlying the rice physiological response and heat-tolerant regulation under high-temperature stress. Liao found two heat-tolerant target genes, *OsHP29* and *OsHP40*, that participate in the regulation of growth and development, energy metabolism and protein binding [55-57]. The relationship between these related proteins and the genetic regulation mechanism of heat-tolerant parents requires further confirmation. In addition, the mechanism underlying the action of these related proteins needs to be analyzed, and associated heat-tolerant genes should be screened.

According to the RGAP database, 65 predicted genes were located in the target region containing *qHTT8*. Transcripts annotated as “hypothetical protein,” “expressed protein” or “retrotransposon protein” were not included. The genes that were annotated to abiotic stress in rice, which are listed in Supplementary Table 3, were identified based on previous research. This analysis identified 10 annotated genes that were potential candidate genes for heat stress tolerance during the flowering stage in rice. Out of 10, six genes are related to drought tolerance in rice, namely *LOC_Os08g06430*, *LOC_Os08g06500*, *LOC_Os08g06630*, *LOC_Os08g07060*, *LOC_Os08g07440* and *LOC_Os08g07760* [38, 39, 41- 43, 46]. It has been reported that gene expression might play the same role under different stress conditions. For example, *MYB*, a member of transcription factors (TFs), were up-regulated when plants were exposed to a combination of drought and heat stress [58]. *OsbHLH148*, a basic helix-loop-helix TFs, was responsive to heat stress, salt, dehydration, low temperature stress, and cold [59]. *OsHCI1* which is a rice gene encoding the RING finger protein was specifically induced by heat and cold stress treatments but not by salinity or dehydration and over-expressed during the heat and cold stress to enhance the acquired thermo-tolerance [60]. Water deficit and high-temperature stresses frequently occur simultaneously in field conditions [61]. The expression of a trait could result from the contribution of many genes with similar or complementary functions [6]. Therefore, these candidate genes which played a role in other abiotic stress conditions may have the similar effects on HT of rice at the flowering stage. Of course, to further fine map and validate the effect of *qHTT8*, PCR-based SNP markers will be developed and used to mark the genotypes of backcrossed populations from the HHZ/9311 population.

Conclusion

In conclusion, 6 h (9:30 am–3:30 pm) of exposure to high temperature (38 ºC) for 3 continuous days is sufficient for identifying HT at the flowering stage in rice (*Oryza sativa* L.) [62]. Ensuring that opened spikelets were removed before the plants moved into the phytotron ensured the accuracy of the data. A new QTL for HT at the flowering stage in rice, *qHTT8*, was rapidly identified through BSA-seq within the region of 3555000–4520000 bp on chromosome 8. Several putative genes controlling rice abiotic stress tolerance also were proposed in this target region. Maybe these genes were related to the HT of rice at the flowering stage. These findings enhance our understanding of the genetic basis and the breeding of heat-tolerant cultivars in rice. However, how *qHTT8* affects the agronomic traits of rice and its molecular functions is still elusive. Thus, additional study is needed to elucidate its role in the molecular mechanisms underlying heat tolerance at the flowering stage. In future studies, the expression and functional analysis of *qHTT8* will be performed to validate candidate genes using transgenic studies.

Methods

Rice materials

HHZ and 9311 are both conventional *indica* rice varieties, which were kindly provided by Guangdong Academy of Agricultural Sciences and Huazhong Agricultural University, respectively. A set of 365 $F_{2:3}$ lines derived from a cross between HHZ, a heat-tolerant cultivar [63-64] and 9311, a heat-susceptible cultivar (our previous work), was used to evaluate rice HT at the flowering stage. In 2018, $F_{2:3}$ lines and their parents were planted in the net-house of Guangxi Academy of Agricultural Sciences, Nanning, Guangxi, China.

Evaluation of HT in $F_{2:3}$ at the flowering stage

An $F_{2:3}$ population of 365 individuals was planted in plastic pots under natural conditions until heading. At the start of heading, 3–5 uniform panicles, in which the opened florets were carefully removed, were marked with PVC tags [65]. The plant was then moved into a phytotron. During this period, the spikelets in the panicle were exposed to 38/24 °C day/night temperatures with 6 h (from 09:30 am to 3:30 pm) of 38 °C during the day. After 3 days of exposure to high temperature, the plants were moved back to the net house and grown to maturity [62]. After harvest, the labeled panicles were tested for seed set, and the number of filled grains (NFG), unfilled grains (NUFG) and

empty grains (NEG) were counted. For unfilled grain, grains were scored for fertility by checking whether a kernel was present or not. Next, the absolute spikelet fertility percentage was calculated using the formula below, and HT was assessed based on absolute fertility at high temperature [21, 28].

Spikelet fertility (%) = (NFG + NUFG)/(NFG + NUFG + NEG)×100

Whole genome re-sequencing and BSA-seq analysis

According to the phenotypic characterization HT of derived $F_{2:3}$ lines at anthesis under heat stress, pools of tolerant and sensitive bulk samples (n = 50, each group) were constructed from 365 F_2 individuals, and the same amount of DNA of fresh leaves was extracted from each plant and evenly mixed.

After the sample DNA was quantified by a Nanodrop 2000, the DNA in each mixing pool was mixed equally. The parents and the mixing pool DNA sequence were segmented into random fragments using ultrasound. The segmented DNA was successively repaired at the end, A was added at the 3´ end and the sequencing connector was connected. Next, the magnetic beads were used to absorb and enrich the fragments with lengths of approximately 400 bp, which were amplified by PCR to form a sequencing library. After inspection of the constructed library, the qualified library was sequenced using Illumina HiSeq™ platform. The sequencing approach used was Illumina PE150, and the total sequencing read length was 300 bp.

After the sequencing data (raw data) of the Illumina HiSeq™ platform were removed from the instrument, the quality of the data was controlled by filtering out low-quality data and keeping high-quality data (clean data). BWA software was used to compare the clean reads to the *Oryza sativa L. ssp. japonica cv.* Nipponbare reference genome (http://rice.plantbiology.msu.edu/) sequence, and the location of the sequence (i.e., the BAM file) was obtained. The best practices pipeline in GATK software was used to correct BAM files and detect SNPs and small Indels.

SnpEff software and gene prediction information of the reference genome were used to annotate the variation function, and the function annotation information for SNPs and indels was obtained. Based on the characteristics of the data for parents and mutation pools, the SNP-index (the SNP frequency) value was calculated for BSA association analysis to locate the target loci. Specifically, for the SNP and indel loci among the samples obtained by filtering and screening, the SNP-index values of each locus in the heat-tolerant mixed pool (or the sensitive mixed pool) were calculated. The average SNP-index values of all SNPs in the window were then counted as the SNP-index of that window. The window was sliding, with a size of 500 kb and 5-kb steps. The SNP-index of the T-pool and S-pool was defined as the ratio between the HHZ SNP and the total number of reads corresponding to the SNP. The Δ (SNP-index) was calculated according to the formula Δ(SNP-index) = [(SNP-index of T-pool) − (SNP-index of S-pool)]. Because of the linkage between the heat-tolerant loci and surrounding markers, the SNP-index in the T-pool was closer to 1 (while the SNP-index in the S-pool was closer to 0). Because of the weak linkage or lack of linkage, the loci were randomly distributed, and the SNP-index of the other normal locus was 0.5. The region with the most differences in SNP-index values between the two pools (Δ) (using the 99.9% confidence level as the threshold for screening) was the candidate region of the target trait correlation.

To eliminate false-positive loci, we used the location of the markers in the genome, adopted the method of the sliding window, fit the index value, eliminated the similar difference loci caused by random amplification, visualized the distribution of the index of the heat-tolerant locus across the entire genome and annotated the genes around the heat-tolerant loci. After obtaining the sequence structure of the gene, the highly homologous sequence was located in the published gene function database (GO) to obtain functional information. The gene sequence and protein sequence were compared by Blast software to determine the function of the gene, and the number of genes annotated to the database was counted in the candidate region.

Abbreviations

QTLs: Quantitative trait loci; HHZ: Huanghuazhan; BSA: Bulked-segregant analysis; HT: Heat tolerance; RFLP: Restriction fragment length polymorphism; SSR: Simple sequence repeats; SNP: Single nucleotide polymorphism; BC: Backcross; NILs: Near isogenic lines; RILs: Recombinant inbred lines; CSSLs: Chromosome segment substitution lines; DH: Doubled haploid; WGS: Whole-genome resequencing; GO: Gene ontology; RGAP: Rice genome annotation project; NFG: Number of filled grains; NUFG: Number of unfilled grains; NEG: Number of empty grains.

Declarations

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Author's contributions

LGJ and TFL conceived and supervised the research. LC and QW designed the experiments. LC, QW, XLZ, MYT, YHP, GQG, RHL and WT performed the experiments and analyzed the results. LC and QW wrote the paper. All authors have read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Table and Fig Information

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Tables

Table 1. The quality of sequencing data				
Sample	Clean Reads (bp)	Clean Base (bp)	GC content (%)	Q30 (%)
HHZ	55685598	16765143297	43.77	94.13
9311	53801932	16198479368	44.14	92.54
T-pool	86378436	26006038749	44.14	93.85
S-pool	88929269	26772575482	44.67	94.09

Table 2 Statistical analysis of sequencing depth and coverage								
Sample	Mapped Ratio (%)	Properly Mapped (%)	Duplication Ratio (%)	Average Insert Size	Average Depth (×)	Real Depth (×)	Genome Coverage (1×) (%)	Genome Coverage (5×) (%)
HHZ	98.01	90.51	18.44	378.7	35.93	38.52	93.26	89.5
9311	98.14	90.47	17.12	383.5	35.3	37.84	93.3	89.51
T-pool	97.8	90.23	17.87	384.5	56.01	58.5	95.75	92.63
S-pool	97.89	90.27	18.21	377	57.47	59.94	95.88	92.58

Table 3 Statistical table of related region variation sites						
Chr	Location (bp)	Interval size (Mb)	No. of SNP	No. of Indel	No. of effective SNP	No. of effective Indel
Chr8	3555000–4520000	0.965	6821	1155	258	29

Table 4 Putative genes associated with abiotic stress tolerance in the *qHTT8* region

No.	Gene	Type and putative protein function	Physical location (bp)
1	*LOC_Os08g06430*	Mitochondrial NADH-ubiquinone oxidoreductase, putative, expressed	3613350–3615878
2	*LOC_Os08g06500*	PPR repeat domain containing protein, putative, expressed	3680925–3688728
3	*LOC_Os08g06550*	Acyl CoA binding protein, putative, expressed	3698312–3700553
4	*LOC_Os08g06630*	RNA polymerase sigma factor, putative, expressed	3732441–3736521
5	*LOC_Os08g07010*	ABC-2 type transporter domain containing protein, expressed	3928462–3933577
6	*LOC_Os08g07060*	CRR6, putative, expressed	3949017–3951136
7	*LOC_Os08g07100*	Terpene synthase, putative, expressed	3972216–3977334
8	*LOC_Os08g07440*	AP2 domain containing protein, expressed	4178549–4175872
9	*LOC_Os08g07760*	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor, putative, expressed	4344171–4350502
10	*LOC_Os08g07970*	Transcription factor, putative, expressed	4508263–4505738

Supplementary Information

Supplementary Table 1. Reported QTLs related to heat stress tolerance in rice.

Supplementary Table 2. List of genes annotated into GO database in the *qHTT8*.

Supplementary Table 3. List of putative genes in RGAP database containing the *qHTT8*.

Figures

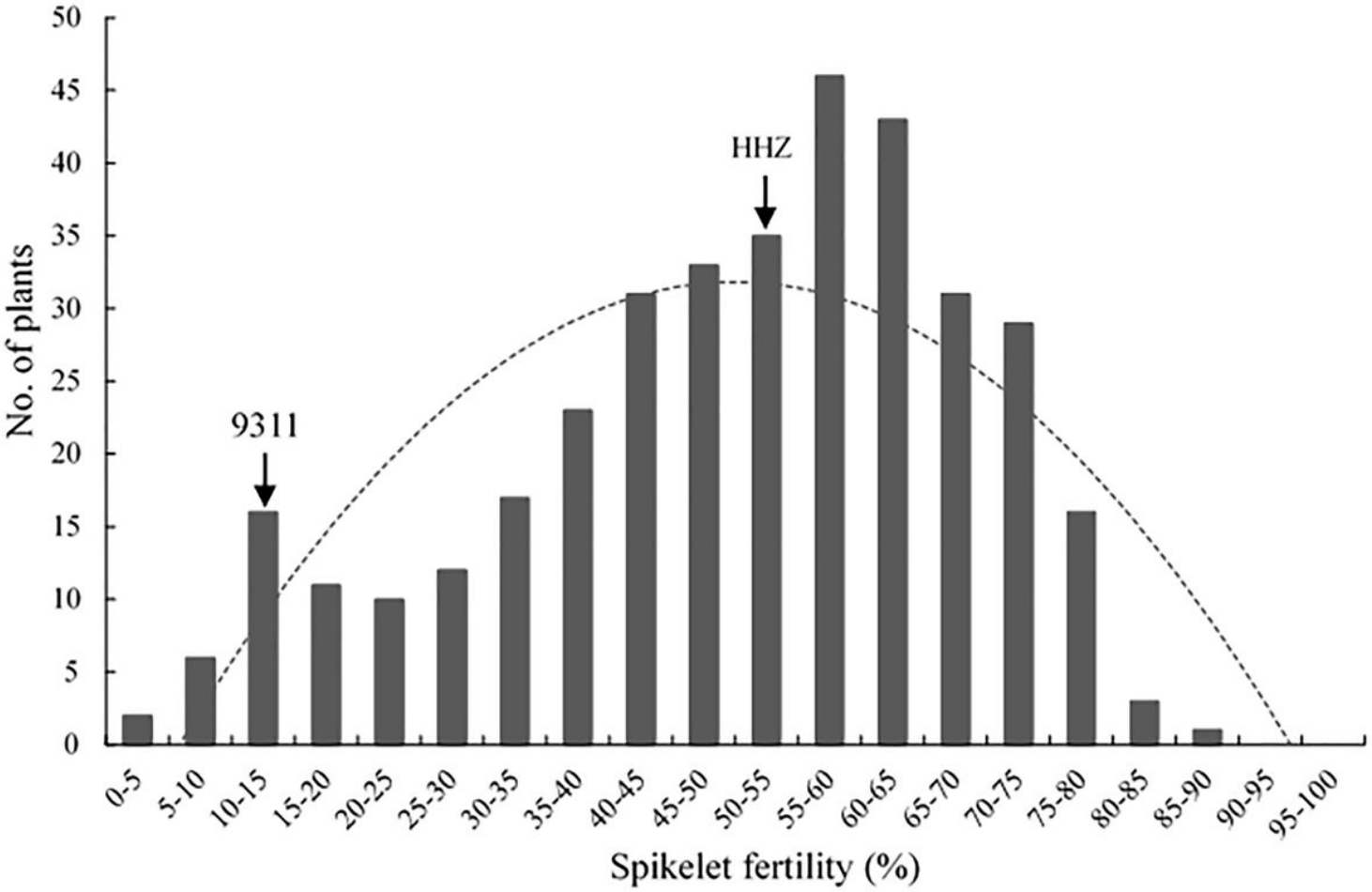

Figure 1

Frequency distribution of spikelet fertility in the F2:3 population containing 365 plants.

Figure 2

SNP-index graphs of T-pool (A), S-pool (B), and Δ(SNP-index) graph (C) from BSA-seq analysis. X-axis represents the position of the 12 chromosomes in rice; Y-axis represents the SNP-index. The black dotted line shows the association threshold at a 99.9% confidence level. HT major QTL is located to chromosome 8 (red dotted box).

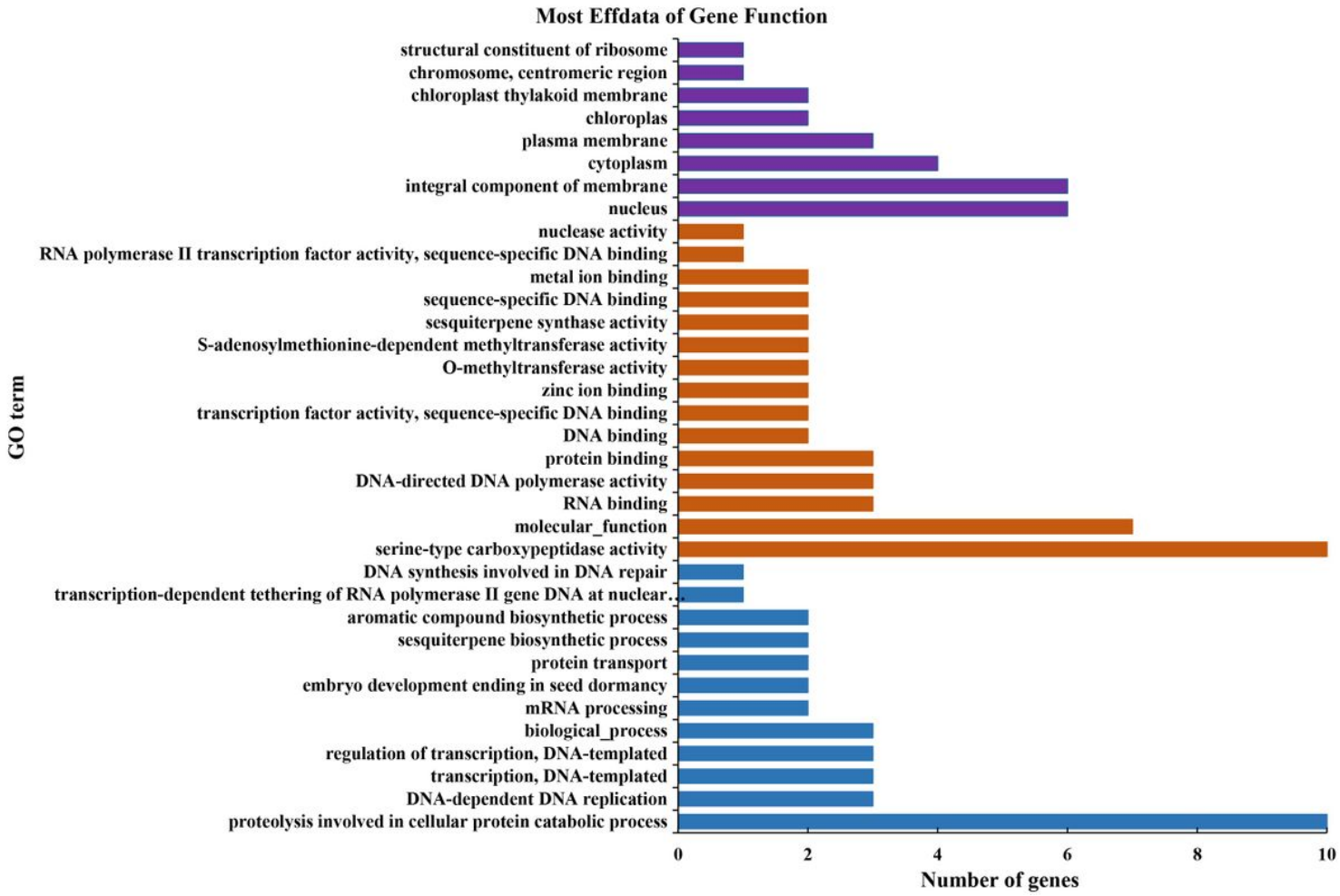

Figure 3

Significantly enriched GO terms of the genes located in qHTT8. The blue box represents biological processes, the red box represents molecular function and the purple box represents cellular components.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable3.xlsx
- SupplementaryTable2.xlsx
- SupplementaryTable1.xlsx