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Full Length Article

Identification of Heat-Tolerance Genes using an Introgression Line from Yuanjiang Common Wild Rice (*Oryza rufipogon*)

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Abstract

Heat stress severely affects rice (*Oryza sativa* L.) growth, yield and milling quality. It is very important to breed heat-tolerant rice varieties to adapt to global warming year by year. However, heat tolerance is a very complex quantitative trait, and only a few related genes were cloned in the past few decades. Previously, we generated introgression lines with Teqing (*Oryza sativa* L. sspp. *indica*) as the background and Yuanjiang common wild rice (*Oryza rufipogon* Griff.) as the donor, and subsequently found that the line YIL106 carried three heat-sensitive QTLs. Here, we integrated microarray and QTL mapping methods to uncover candidate genes conferring heat-sensitivity at the seedling stage of YIL106. 33 genes with response to abiotic stress were chosen for further investigation. After annotation analysis of the Rice Gene Ontology Database, three candidate heat stress responsive genes, *LOC_Os01g19020*, *LOC_Os01g23630*, and *LOC_Os12g43440*, were selected. Moreover, overexpression of *LOC_Os12g43440* greatly increased rice heat tolerance at the seedling stage. This work uncovers a novel gene for heat tolerance with potential value in breeding. © 2020 Friends Science Publishers

Keywords: Rice; Microarray; Expression profile; Introgression line; Heat-tolerance genes

Introduction

Rice (*Oryza sativa* L.) is one of the most important crucial cereals for over half of the world's population. As the world population keeps growing, the improvement of rice yield and quality has become a major task. Heat stress becomes a critical factor in rice production as a result of global warming (Patindol *et al.* 2015). Rice originated in tropical and subtropical regions has a certain ability to tolerate high temperature. The optimum temperature range for rice is 28–32°C at the seedling stage, and 25–35°C at the heading stage. Temperature higher than the threshold affects rice growth and development (Welch *et al.* 2010; Arshad *et al.* 2017). For instance, temperature over 35°C at the heading stage leads to pollen inactivation and infertility (Jagadish *et al.* 2010b). Human activities have caused extreme temperature changes, and these changes have resulted in devastating damage to rice production (Lobell *et al.* 2011; Lesk *et al.* 2016). Grain yield declines by 10% for each 1°C increase when exceeding the maximum growth temperature (Peng *et al.* 2004). Therefore, screening and identification of heat-tolerant rice resources, together with research on related genes and mechanisms, is an effective way to cope with high temperature stress caused by global warming (Sreenivasulu *et al.* 2015).

Rice heat tolerance is a complicated quantitative trait controlled by multiple genes. The first quantitative trait locus (QTL) conferring rice heat tolerance was mapped from double haploid populations (Cao *et al.* 2003). For decades, F_2, backcross inbred lines (BILs), and recombinant inbred lines (RILs) have been used to map a considerable number of heat-tolerance related QTLs at the seedlings, grouting, flowering and heading stages (Zhu *et al.* 2005; Jagadish *et al.* 2010a; Xiao *et al.* 2011; Ye *et al.* 2012; Poli *et al.* 2013; Ye *et al.* 2015; Shanmugavadivel *et al.* 2017). Theoretically, these QTLs are of great potential in breeding to improve rice heat tolerance. However, it is difficult to use them effectively because of different genetic backgrounds. So far, only a few genes related to rice heat tolerance have been cloned and functionally analyzed. For example, *OgTT1* encoding the proteasome α2 subunit, is identified to protect cells from heat stress through efficiently eliminating cytotoxic denatured proteins and maintaining heat-response processes (Li *et al.* 2015). *TOGR1* encoding a DEAD-box RNA helicase is demonstrated to bean essential component in coordinating primary metabolism to support thermotolerant growth (Wang *et al.* 2016). *OsHTAS* encoding a ubiquitin ligase promotes heat

tolerance through modulating hydrogen peroxide accumulation, altering the stomatal aperture status in leaves, and promoting ABA biosynthesis at the seedling stage (Liu *et al.* 2016). AET1, a $tRNA^{His}$ guanylyl transferase, regulates high temperature response through dual function in tRNA modification and transcriptional control of auxin signaling (Chen *et al.* 2019).

Introgression lines (ILs), constructed through multiple backcrossing and self-crossing, are of great advantage in the fine mapping due to the single genetic background. IL carrying a single target QTL provides an efficient device to access the genetic possibility of promising alleles. There are quite a few precedents for QTLs introgression from wild rice varieties to improve key agricultural traits such as yield, heterotic loci, abscisic acid sensitivity, and salt tolerance (Fridman *et al.* 2004; Qiu *et al.* 2015; Cai *et al.* 2017; Nassirou *et al.* 2017; Xu *et al.* 2018). Unlikely other populations, ILs with phenotypic variation have high efficiency in QTL identification and more reliable mapping results due to the clean genetic background. For instance, two ILs were used for mapping QTLs conferring salt tolerance, and eventually a candidate gene *qDSS11* was validated. It is showed that introgression of the favorable alleles could facilitate the development of superior lines (Cai *et al.* 2017). A set of 79 ILs are used to identify QTLs and heterotic loci, and 11 QTLs in 5 marker loci exhibit pleiotropic phenotypes (Nassirou *et al.* 2017). Moreover, a drought-tolerant IL is used for QTL mapping, and a marker useful for drought-tolerant breeding is identified recently (Xu *et al.* 2018).

Although many QTLs have been identified through map-based cloning method, it is still difficult to identify genes with major effects (Yano *et al.* 2012; Liu *et al.* 2013). Whole genome microarray-based analysis has offered a strategy that could explicate the candidate genes related to morphological traits, but the microarray results include many differentially expressed genes (Tan *et al.* 2007). Previously, transcriptome profiling studies based on microarray are applied to identify QTL genes for regulating seedling vigor, showing a helpful manner for efficiently searching candidate QTL genes. Using this approach, a gene is identified to play an essential role in regulating plant height and leaf sheath length at the initial stage of rice seedling growth (Tan *et al.* 2007). In 2013, a report combining microarray analysis with QTL mapping identified one candidate gene related to cold tolerance in rice (Yano *et al.* 2012).

To uncover novel genes conferring heat tolerance in rice, we carried out further microarray analysis to identify candidate genes for regulating heat tolerance at the seedling stage, based on our previous work that a IL line YIL106 exhibited less heat tolerance than the parent Teqing and include the putative QTLs. A target gene *LOC_Os12g43440* was cloned and functional analysis demonstrated that *LOC_Os12g43440* plays a positive role in the regulation of heat tolerance.

Materials and Methods

Plant materials and RNA preparation

Previously published heat-sensitive line YIL106 is a heat-sensitive introgression line, which was evaluated under heat stress treatment at the seedling stage (Lei *et al.* 2013). YIL106 is constructed by recurrent backcrossing between heat-sensitive variety Yuanjiang common wild rice (*Oryza rufipogon* Griff.) as the donor parent and heat-tolerant variety Teqing (*Oryza sativa* L. ssp. *indica*) as the recurrent parent (Lei *et al.* 2013; Cai *et al.* 2017). In this study, YIL106 and Teqing seedlings were grown in an artificial climate incubator with 28°C/25°C (day/night) for normal growth. After growing to 2.5 leaves, the seedlings were moved to the exposure of 42°C continuous heat stress for 1 h, and the collected leaves were immediately frozen in liquid nitrogen. One of each of the non-treatment leaves sampled from the above two groups were also collected for total RNA extraction. Total RNA isolation from the 4 leaf samples were carried out using TRIzol reagent (Invitrogen) and purified with RNeasy Plant Mini Kit (Qiagen) according to manufacturer's instructions. Total RNA quantification was tested by a Nanodrop spectrophotometer (Thermoscientific) and the purity of RNA was analyzed by referring to A260/280 and A260/230 ratio. The purified RNA was dissolved by RNase-free water and stored at -80°C.

Preparation of the cDNA library and expression profiling

An oligo $dT_{(24)}$ primer including 5′-T7 RNA polymerase promoter sequence and SuperScript II reverse transcriptase (Invitrogen) were used to generate the double-stranded cDNA from total RNA according to manufacturer's instructions. Double-stranded cDNA was filtered successively through phenol/chloroform extraction and ethanol precipitation following second strand synthesis. Biotinylated complementary RNAs (cRNAs) were transcribed from synthesized cDNA by T7 RNA polymerase (ENZO Biochem) in vitro. Hybridizations with fragmented cRNA were performed with rice Gene Chip micro array (Affymetrix). The GeneChip contained probe sets for 57,194 rice genes. Microarray hybridization, washing, staining, and scanning were done according to the Gene Chip® Standard Protocol. Probe normalization procedures were conducted as previously described (Zhu *et al.* 2003). The chip images were scanned and extracted by default settings. The CEL files were generated with the help of the Affymetrix Gene Chip Operating Software and were subsequently imported into the Bioconductor (version 2.0) (Gentleman *et al.* 2004) in R 2.5.1 statistical environment. GC-RMA method was used to adjust the background, normalize the raw data, and estimate the probe sets signal intensities (Wu *et al.* 2004). Fold changes and *P*-values of probe sets were figured up by limma nested F-test (Smyth 2004). The p-values for multiple testing were corrected by

the false discovery rate (FDR) (Benjamini and Hochberg 1995). Each probe was given a *P*-value, the $\log_2$-transformed signal ratios of genes were calculated. Only change was two-fold above with the adjusted p-value of FDR below 0.05, then was designated as a significant change.

Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

The reverse transcription was carried out using the Omniscript reverse transcription kit (Qiagen). As template, the cDNA was diluted 10-fold with sterile distilled water after being quantified by RT-PCR analyses. Quantitative real-time PCR using the SYBR Green PCR kit (Qiagen) was performed on a LightCycler system (Roche). The rice *ACTIN1* gene was used as an internal control to analyze the cDNA concentration variations. Quantification of the gene expression was done by the comparative $2^{-\Delta\Delta C}$T routine (Livak and Schmittgen 2001). Three independent biological replicates were set to verify the gene expression level. The gene specific primers were designed using Primer Premier 5.0 (http://www.PremierBiosoft.com).

Cloning and sequence analysis

DNA was extracted from fresh leaves of Teqing and YIL106 plants, by the CTAB (Hexadecyltrimethyl Ammonium Bromide) method (Doyle and Doyle 1990). DNA concentration was measured using a Beckman spectrophotometer. Primers for gene sequencing were designed using Primer Premier5.0 (http://www.PremierBiosoft.com) (Table 1). PCR products were sequenced by an ABI 3730 automatic sequencer (Sangon). Sequence analysis was conducted using DNAssist 2.0 (http://www.dnassist.com/).

Plasmid construction and transformation

To produce overexpression in rice, the coding domain of candidate genes from Teqing and YIL106 were amplified using the gene specific primers (Supplemental Table 1). The genomic fragments of three candidate genes, *LOC_ Os01g19020* from YIL 106, *LOC_Os01g23630* from YIL 106 and *LOC_Os12g43440* from Teqing, were amplified. Each coding domain of candidate genes was inserted into pCAMBIA1301 vector with the *CaMV 35S* promoter to generate the overexpression plasmid. All the constructs in this study were confirmed by sequencing and induced into the rice cultivar Zhonghua17, a *japonica* variety, by particle gun transformation as previous report (Dai *et al.* 2001).

Results

General changes in gene expression in response to heat

To investigate the genes differentially expressing in YIL106 with its parent Teqing, we analyzed the expression profiles of heat-treated lines and control lines of both Teqing and YIL106 through microarrays. There was a noticeable difference in overall transcriptional responses between the heat-tolerant line Teqing and the heat-sensitive line YIL106 upon heat stress treatments. All the 57,194 identified genes were commonly expressed in the four lines. A total of 3,543 transcripts in heat-treated Teqing showed significant difference compared with the untreated control lines, representing heat responsive genes in Teqing. Among them, 1,340 of these differentially expressed transcripts were up-regulated while the rest 2,203 transcripts were down-regulated. Among the 3298 differentially expressed transcripts in heat-treated YIL106 compared with the untreated control, 1,389 genes were increased in abundance and 1,909 genes were decreased significantly. As shown by a Venn diagram, the heat responsive genes in Teqing overlapped partially with those in YIL16. 1,853 genes were regulated by heat stress in both Teqing and YIL16. Meanwhile, 1,690 genes responded to heat stress only in Teqing while 1,445 genes responded to the stress only in YIL106 (Fig. 1).

A cluster analysis was constructed to cluster the total differentially expressed genes using normalized values (Fig. 2). Cluster analysis revealed that the expression patterns of most genes in Teqing and YIL106 were similar. Between the two heat-treated lines YIL106 and Teqing, a total of 421 transcripts showed significant differential expression. Among them, 147 were up-regulated and 274 were down-regulated. Differentially expressed transcripts were classified referring to the rice Gene Ontology (GO) database (Fig. 3; http://rice.plantbiology.msu.edu/). Although similar representation of up-and down-regulated genes were seen in many categories, there were more genes down-regulated rather than up-regulated. The top 20 categories of biological processes showed that the dominant GO termed "metabolic process" and "responses to stress and abiotic stimulus". The results suggested that these differentially expressed genes respond to heat stress and stimulus during the seedling stage.

Expression profiles of the genes in four introgressed segments of YIL106

The genome of YIL106 was similar with that of Teqing, except for four introgressed segments from Yuanjiang common wild rice, the heat-sensitive QTL *qHTS1-1* and *qHTS3* were identified to be located within two of the four introgressed segments (Lei *et al.* 2013). The size of the introgressed segment on chromosome 1 was hardly to be determined because only one marker RM1320 was anchored. For the other three introgressed segments, significant differentially expressed genes were extracted from the probe sets (Supplemental Table 2). In total, 33 significant differentially expressed genes were located in the three intervals. Among these genes, 17 genes were expressing at higher level while 16 genes were expressing at lower level in YIL106 than in Teqing. Among the 17 up-

Table 1: Primers for qRT-PCR and putative functions of the top seven genes

Locus	Forward primer	Reverse primer	Description, Putative function
LOC_Os01g19020	AGTCCTGCTGGACAAGTCGT	TAGATGAGGATGTCGGAGCA	Peroxidase family protein
LOC_Os01g23630	GAAAGACCTGCAGCACATGA	TCTTTGATGCAACGTTCTGG	Transcription initiation factor
LOC_Os03g48710	GCTACGTCTACCTCGACATC	CTGGGCAAAGAAGTTCATC	expressed protein
LOC_Os03g48750	TGATCTGACGATCCGAGAGT	GATGGCGACGACAAGTTTAT	Oxalate oxidase GF-2.8 precursor
LOC_Os12g43380	GCAGCCAGGACTTCTACGAC	GGCAGAAGACGACTTGGTAG	Thaumatin-like protein precursor
LOC_Os12g43430	TGCCCATAGATCTTCTCACC	TCTATAGCAGCCAGCTAATGC	Thaumatin-like protein precursor
LOC_Os12g43440	CAGACGTGGACCATCAAC	ACCGACAGGTTGTAGAAGTC	Thaumatin-like protein precursor

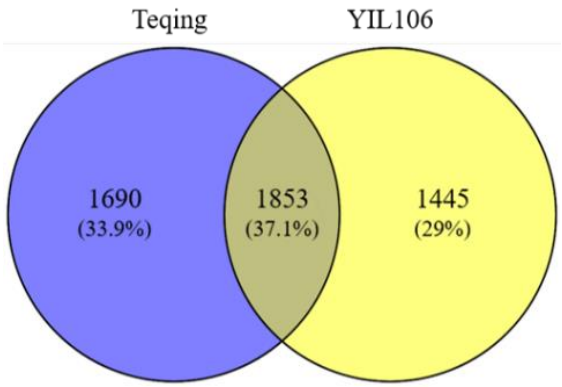

Fig. 1: Venn diagram analysis of differentially expressed genes between Teqing and YIL106

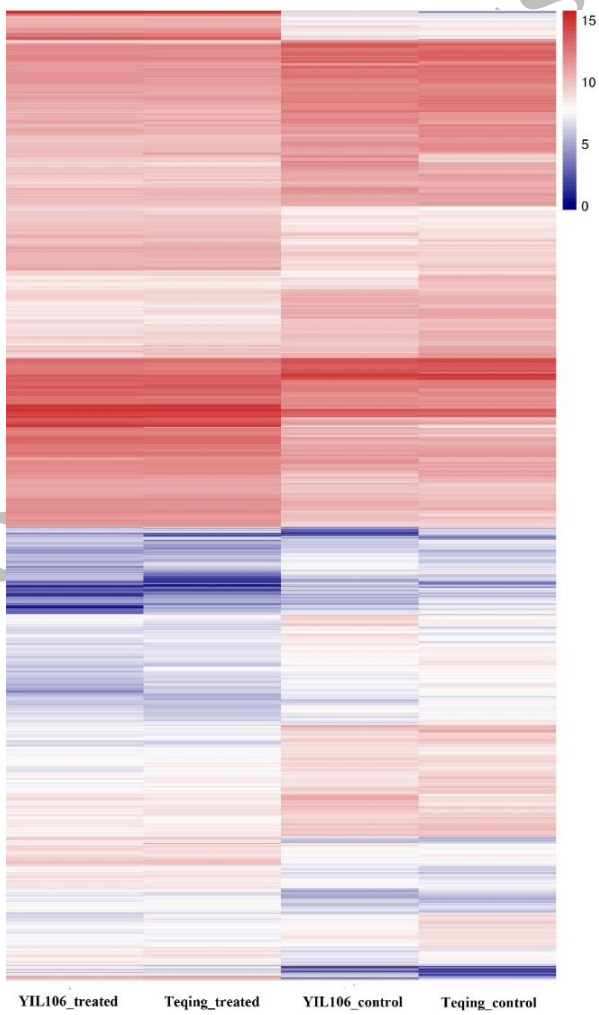

Fig. 2: Hierarchical cluster analysis of differentially expressed genes between Teqing and YIL106. The branch length indicates the degree of variance, red represents high-expression genes, and blue indicates low-expression genes. The normalized values of genes were made following a log transformation

regulated genes, 14 genes were located on chromosome 1, 1 gene on chromosome 3, and 2 genes on chromosome 12. Half of the down-regulated genes were located on chromosome 1, and there were 3 and 5 down-regulated genes located on chromosomes 3 and 12, respectively. All of these 33 genes were functionally categorized in the GO database (Supplemental Table 2). Among the biological processes represented, 7 of the up-regulated genes and 4 of the down-regulated genes with large-fold changes were categorized in genes with response to stress and abiotic stimulus.

Verification of the microarray data with qRT-PCR

To verifythe significantly differentially expressed genes identified by the microarray, the 7 genes with top fold changes in expression levels were further confirmed by qRT-PCR (Table 1). All the gene expression patterns demonstrated by qRT-PCR were in accordance with those obtained from the original microarray results, except for *LOC_Os03g48710* (Fig. 4). The expression patterns of these selected genes between microarray and qRT-PCR analyses displayed high similarity, thus confirming the reliability of our microarray data.

Genome sequencing for the seven top differentially expressed genes of YIL106 and Teqing

To analyze sequence variations of the 7 differentially expressed genes between Teqing and YIL106, primers were designed to amplify the full length of these genes using genomic DNA isolated from Teqing and YIL106. The results illustrated no large insertion or deletion in the genomic DNA of these genes. We predicted CDS (coding sequence) of the 7 genes were predicted through the BLAST tool. The amino acid sequences of predicted proteins encoded by *LOC_Os03g48710*,

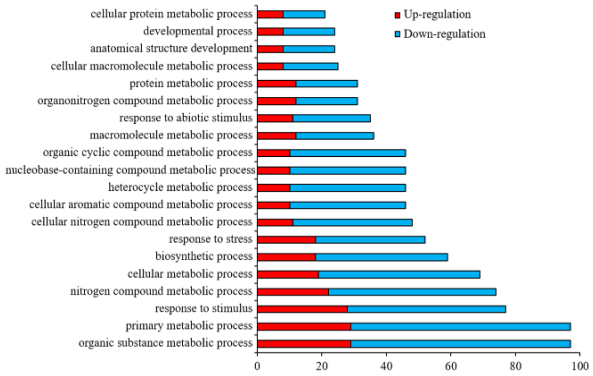

Fig. 3: Biological process categorization. Top 20 categories of transcripts with significant changes in gene expression in YIL106 and Teqing in response to heat stress

LOC_Os12g43380, and *LOC_Os12g43430* in Teqing are the same as those in YIL106. However, the other four gene (*LOC_Os01g19020*, *LOC_Os01g23630*, *LOC_Os03g48750* and *LOC_Os12g43440*)-encoding proteins in YIL16 showed differences in 1–2 amino acids compared with its parent Teqing, demonstrated by the multiple sequence alignment analysis (Fig. 5). According to the GO annotation (Supplemental Table 2), three genes (*LOC_Os01g19020*, *LOC_Os01g23630* and *LOC_Os12g43440*) were categorized into genes with response to stress. Therefore, these 3 genes were selected as the candidate genes for further study.

Identification of the thermotolerance locus in rice

To reveal the roles of the 3 putative genes in rice heat tolerance, overexpressing lines of *LOC_Os01g19020*, *LOC_Os01g23630* and *LOC_Os12g43440* were generated. Transgenic plants of the three genes were validated by PCR amplification and hygromycin selection. The T_1 progenies of the transgenic lines of the three genes and wild type seedlings of Zhonghua17 were grown to the 2.5-leaf stage, and then exposed to high temperature (45°C). After 1 h of high temperature treatment, they were subsequently recovered under normal growth temperature for 2 more days. The putative genes showed higher expression levels in transgenic plants than in wild type, shown by qRT-PCR analysis. However, *LOC_Os01g19020*- and *LOC_Os01g23630*- overexpressing plants showed no significant phenotypic differences upon high temperature treatment compared with wild type. Notably, the transgenic lines of *LOC_Os12g43440*-overexpressing plants exhibited significantly enhanced heat tolerance compared with wild type (Fig. 6). These results suggested a positive role of *LOC_Os12g43440* in the regulation of rice heat tolerance at the seedling stage.

Discussion

Heat tolerance is a key agricultural trait for rice yield, which is controlled by multiple genes intricately. So far, more than

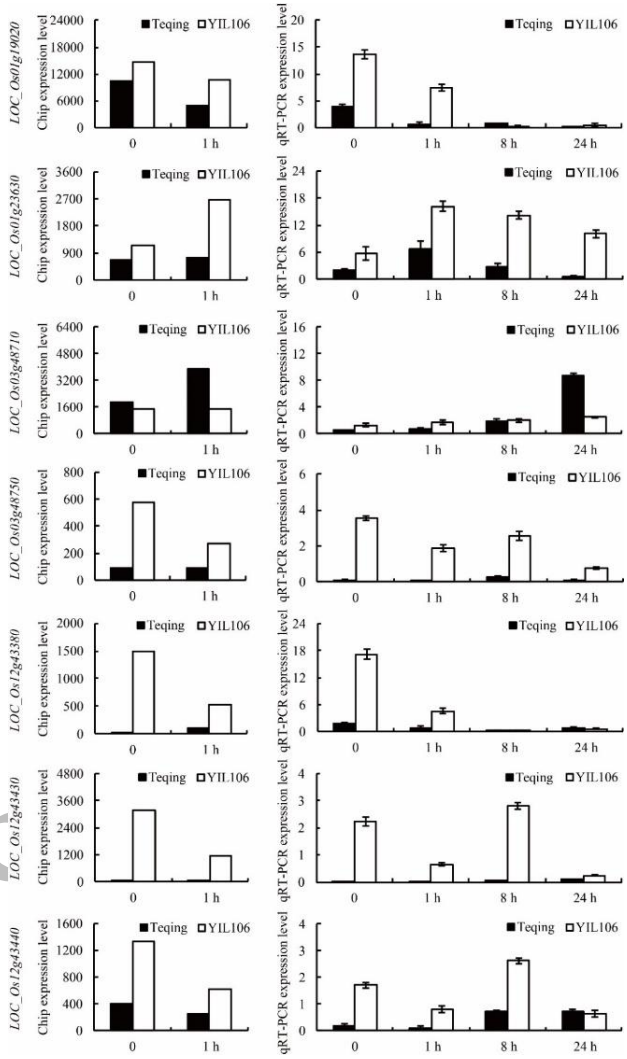

Fig. 4: Validation of 7 top differentially expressed genes by qRT-PCR. The first column displays the microarray expression patterns, the second column displays the qRT-PCR results and the gene names are shown on the left of each row. The x-axis represents the treatment hour, and the y-axis indicates the expression level of the gene in two lines. Error bars represent SD ($n = 3$)

100 heat tolerance related QTLs have been identified on all 12 chromosomes (Lei *et al.* 2013; Shanmugavadivel *et al.* 2017; Zhang *et al.* 2017; Li *et al.* 2018). We assigned the three inserted heat tolerant regions in this study to the rice genome (IRGSP v. 1.0, http://rice.plantbiology.msu.edu/) through BLAST analysis of markers linked to the three genome regions and compared these genome regions with previously reported studies. The introgressed segment of chromosome 1 was co-localized with *qHTS1*, which was reported to improve seed setting rate under heat stress previously (Li *et al.* 2018). The introgressed segment of chromosome 12 shows similarity with *qHT12*, which was identified as a heat tolerance regulator at the heading stage (Chen *et al.* 2008). We analyzed the expression profiles of genes on the introgressed segments between Teqing and

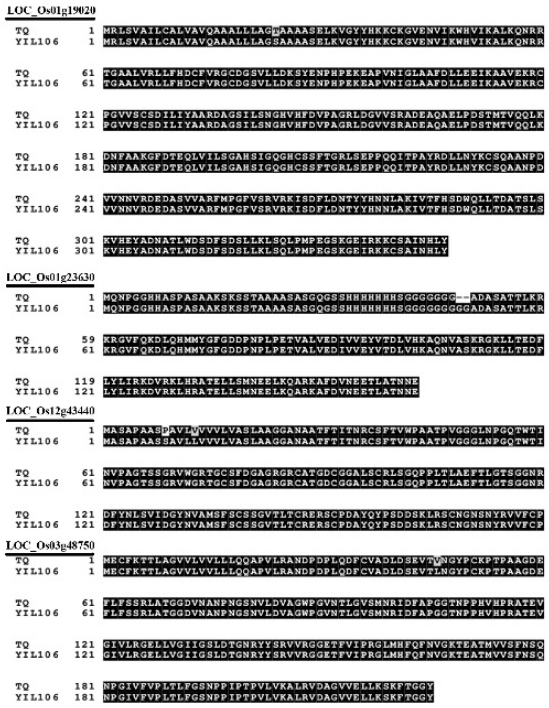

Fig. 5: Sequence alignment of the four candidate genes between Teqing and YIL106. Gene locus names are underlined. TQ, Teqing

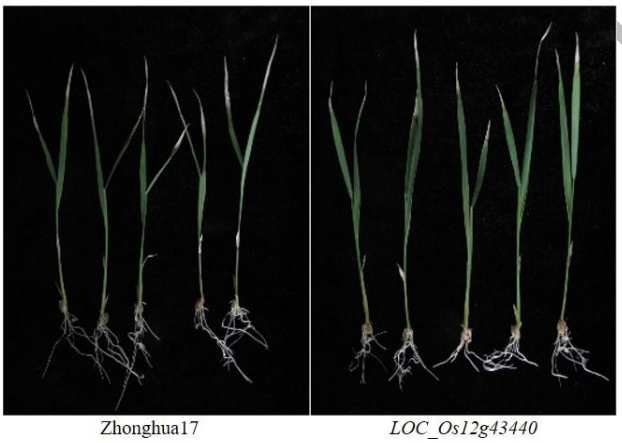

Fig. 6: Effect of high temperature on overexpression lines of *LOC_Os12g4344* and wild type Zhonghua17

YIL106, and then further narrowed the range of candidate genes. These results will widen our understanding of the complex mechanisms underlying thermotolerance regulation. It is suggested that genes controlling seed setting rate are involved in heat stress tolerance.

The introgressed segments in five other introgressed lines (YIL20, YIL21, YIL27, YIL42, and YIL79) were also investigated (Lei *et al.* 2013). Among all five introgressed lines carrying one or two of the introgressed segments from YIL106 on chromosomes 1 and 3, only YIL106 carried the introgressed segment on chromosome 12 and the YIL106 was much more sensitive to heat stress. These results demonstrated that the candidate gene located on the chromosome 12 insertion region could be the major gene conferring for the hypersensitivity to heat stress in YIL106, compared with its parent Teqing (Lei *et al.* 2013). *LOC_Os12g43440* is very likely to contribute to the effect of increased sensitivity of YIL106, compared with the five other introgressed lines. The QTL *qHTS1-1* in the introgressed segments of chromosome 1 mainly confers the heat-sensitivity of YIL106, compared with Teqing (Lei *et al.* 2013). *LOC_Os01g19020* and *LOC_Os01g23630* located on the region of *qHTS1-1* were overexpressed; however, no significant phenotypic difference was saw between the transformed plant and wild type plant, suggesting that the two genes may not be the target genes responsible for *qHTS1-1*. As microarray analyses used in this study can only screen out the genes with different response pattern to heat between YIL106 and Teqing, a lot of genes with difference in coding region but no differences in expression pattern between the two materials were missed out. Other gene mapping methods, such as map-based cloning will be adopted to clone the gene of *qHTS1-1* in our future study. Similar case is also for *qHTS3*, another major QTL detected for the contribution of heat-sensitivity in YIL106 (Lei *et al.* 2013).

LOC_Os12g43440 encodes a thaumatin-like protein (TLP) according to the annotation from IRGSP. TLPs, also named PR5 family proteins, can respond to both biotic and abiotic stresses. For instance, *TmTLP1* and *TmTLP2* are strongly induced by the injectionof *E. coli* and *L. monocytogenes* (Kim *et al*. 2017). *AsPR5* is induced by *Fusarium oxysporum* f. spp. *cepae* (FOC) infection in garlic (*Allium sativum*), and ectopically expressing it in Arabidopsis confers enhanced resistance to fungal pathogen (Rout *et al.* 2016). *LePR5* can be induced by *A. alternata* and *C. laurentii*, and plays a role in the defense system of cherry tomatoes (Guo *et al.* 2016). Several *TLPs* respond to *Pectobacterium carotovorum* subspp. *carotovorum* infection or show responses to abscisic acid (ABA), salt, cold and drought in Chinese cabbage (Ahmed *et al.* 2013). *AdTLP* is induced by the treatment of the late leaf spot pathogen of peanut, *P. personata* and multiple hormone treatments, suggesting its role in response to biotic and abiotic stresses (Singh *et al.* 2013). Thaumatin-like protein 1 precursor is suggested to be involved in protecting peach against chilling injury (Degar *et al.* 2010). Above all, the role of *LOC_Os12g43440* in heat tolerance provides a new perspective to study the function of TLPs. Interestingly, the previous study cloned the gene, also named *Tolerance of Nitrogen Deficiency 1* (*TOND1*), and found that the gene confers tolerance to N deficiency in Teqing (Zhang *et al.* 2015). More work is needed to study the details of the dual roles of *LOC_Os12g43440* in regulating heat tolerance and nitrogen deficiency tolerance in the future.

Conclusion

In this study, *LOC_Os12g43440* was revealed as a novel gene to enhance heat tolerance at the seedling stage, which could be helpful for uncovering the genetic mechanisms and improvement of rice heat tolerance.

Acknowledgments

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References

Arshad MS, M Farooq, F Asch, JSV Krishna, PVV Prasad, KHM Siddique (2017) Thermal stress impacts reproductive development and grain yield in rice. *Plant Physiol Biochem* 115:57–72

Ahmed NU, JI Park, HJ Jung, KK Kang, YP Lim, Y Hur, IS Nou (2013). Molacular characterization of thaumatin family genes related to stresses in *Brassica rapa*. *Sci Hortic* 152:26–34

Benjamini Y, Y Hochberg (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J Roy Stat Soc Ser B* 57:289–300

Cai WY, LB Tan, FX Liu, CQ Sun (2017). Identification of quantitative trait loci and candidate genes associated with ABA sensitivity in common wild rice (*Oryza rufipogon* Griff.). *J Integr Agric* 16:2375–2385

Cao LY, JG Zhao, XD Zhan, DL Li, LB He, SH Cheng (2003). Mapping QTLs for heat tolerance and correlation between heat tolerance and photosynthetic rate in rice. *Chin J Rice Sci* 17:223–227

Chen K, T Guo, XM Li, YM Zhang, YB Yang, WW Ye, NQ Dong, CL Shi, Y Kan, YH Xiang, H Zhang, YC Li, JP Gao, XH Huang, Q Zhao, B Han, JX Shan, HX Lin (2019). Translational regulation of plant response to high temperature by a dual function tRNAHis guanylyltransferase in rice. *Mol Plant* 12:1123–1142

Chen QQ, SB Yu, CH Li, TM Mou (2008). Identification of QTLs for heat tolerance at flowering stage in rice. *Sci Agric Sin* 41:315–321

Dai SH, P Zheng, P Marmey, SP Zhang, WZ Tian, SY Chen, RN Beachy, C Fauquet (2001). Comparative analysis of transgenic rice plants obtained by Agrobacterium-mediated transformation and particle bombardment. *Mol Breed* 7:25–33

Degar A, H Friedman, S Lure (2010). Thaumatin-like proteins and their possible role in protection against chilling injury in peach fruit. *Postharv Biol Technol* 57:77–85

Doyle JJ, JL Doyle (1990). Isolation of plant DNA from fresh tissue. *Focus* 12:13–15

Fridman E, F Carrari, YS Liu, AR Fernie, D Zamir (2004). Zooming in on a quantitative trait for tomato yield using interspecific introgressions. *Science* 305:1786–1789

Gentleman RC, VJ Carey, DM Bates, B Bolstad, M Dettling, S Dudoit, B Ellis, L Gautier, Y Ge, J Gentry, K Hornik, T Hothorn, W Huber, S Iacus, R Irizarry, F Leisch, C Li, M Maechler, AJ Rossini, G Sawitzki, C Smith, G Smyth, L Tierney, JYH Yang, J Zhang (2004). Bioconductor: Open software development for computational biology and bioinformatics. *Genome Biol* 5:1–16

Guo J, X Zhao, HL Wang, T Yu, Y Mao, XD Zheng (2016). Expression of the LePR5 gene from cherry tomato fruit induced by *Crytococcus laurentii* and the analysis of LePR5 protein antifungal activity. *Postharv Biol Technol* 111:337–344

Jagadish SVK, J Cairns, R Lafitte, TR Wheeler, AH Price, PQ Craufurd (2010a). Genetic analysis of heat tolerance at anthesis in rice. *Crop Sci* 50:1633–1641

Jagadish SVK, R Muthurajan, R Oane, TR Wheeler, S Heuer, J Bennett, PQ Craufurd (2010b). Physiological and proteomic approaches to address heat tolerance during anthesis in rice (*Oryza sativa* L.). *J Exp Bot* 61:143–156

Kim DH, MY Noh, KB Park, YH Jo (2017). Expression profiles of two thaumatin-like protein (TmTLP) genes in responses to various micro-organisms from *Tenebrio molitor*. *Entomol Res* 47:35–40

Lei DY, LB Tan, FX Liu, LY Chen, CQ Sun (2013). Identification of heat-sensitive QTL derived from common wild rice (*Oryza rufipogon* Griff.). *Plant Sci* 201:121–127

Lesk C, P Rowhani, N Ramankutty (2016). Influence of extreme weather disasters on global crop production. *Nature* 529:84–87

Li MM, X Li, QL Yu, JW Wu, H Li, J Liu, XD Ma, SM Jo, DS Park, Y Song, D Shin, LZ Han (2018). Identification of QTLs associated with heat tolerance at the heading and flowering stage in rice (*Oryza sativa* L.). *Euphytica* 214; Article 70

Li XM, DY Chao, Y Wu, XH Huang, K Chen, LG Cui, L Su, WW Ye, H Chen, HC Chen, NQ Dong, T Guo, M Shi, Q Feng, P Zhang, B Han, JX Shan, JP Gao, HX Lin (2015). Natural alleles of a proteasome α2 subunit gene contribute to thermotolerance and adaptation of African rice. *Nat Genet* 47:827–833

Liu FX, WY Xu, Q Song, LB Tan, JY Liu, ZF Zhu, YC Fu, Z Su, CQ Sun (2013). Microarray-assisted fine-mapping of quantitative trait loci for cold tolerance in rice. *Mol Plant* 6:757–767

Liu JP, CC Zhang, CC Wei, X Liu, MG Wang, FF Yu, Q Xie, JM Tu (2016). The RING finger ubiquitin E3 ligase OsHTAS enhances heat tolerance by promoting H_2O_2-induced stomatal closure in rice. *Plant Physiol* 170:429–443

Livak K, T Schmittgen (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods* 25:402–408

Lobell DB, W Schlenker, J Costa-Roberts (2011). Climate trends and global crop production since 1980. *Science* 333:616–620

Nassirou TY, WC He, CJ Chen, AYM Nevame, A Nsabiyumva, XL Dong, YL Yin, QQ Rao, W Zhou, H Shi, WB Zhao, DM Jin (2017). Identification of interspecific heterotic loci associated with agronomic traits in rice introgression lines carrying genomic fragments of *Oryza glaberrima*. *Euphytica* 213:176

Patindol JA, TJ Siebenmorgen, YJ Wang (2015). Impact of environmental factors on rice starch structure: A review. *Starch-Stärke* 67:42–54

Peng S, J Huang, JE Sheehy, RC Laza, RM Visperas, X Zhong, GS Centeno, GS Khush, KG Cassman (2004). Rice yields decline with higher night temperature from global warming. *Proc Natl Acad Sci USA* 101:9971–9975

Poli Y, RK Basava, M Panigrahy, VP Vinukonda, NR Dokula, SR Voleti, S Desiraju, S Neelamraju (2013). Characterization of a Nagina22 rice mutant for heat tolerance and mapping of yield traits. *Rice* 6:36–44

Qiu XJ, ZH Yuan, H Liu, XJ Xiang, LW Yang, WJ He, B Du, GY Ye, JL Xu, DY Xing (2015). Identification of salt tolerance-improving quantitative trait loci alleles from a salt-susceptible rice breeding line by introgression breeding. *Plant Breed* 134:653–660

Rout E, S Nanda, RK Joshi (2016). Molecular characterization and heterologous expression of a pathogen induces PR5 gene from garlic (*Allium sativum* L.) conferring enhanced resistance to necrotrophic fungi. *Eur J Plant Pathol* 144:345–360

Shanmugavadivel PS, AM Sv, C Prakash, MK Ramkumar, R Tiwari, T Mohapatra, NK Singh (2017). High resolution mapping of QTLs for heat tolerance in rice using a 5K SNP array. *Rice* 10:28–39

Singh NK, KRR Kumar, D Kunar, P Shukla, PB Kirti (2013). Characterization of a pathogen induced thaumatin-like protein gene *AdTLP* from *Arachis diogoi*, a wild peanut. *PLoS One* 8; Article e83963

Smyth GK (2004). Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* 3; Article 3

Sreenivasulu N, VM Butardo, G Misra, RP Cuevas, R Anacleto, PBK Kishor (2015). Designing climate-resilient rice with ideal grain quality suited for high-temperature stress. *J Exp Bot* 66:1737–1748

Tan LB, FX Liu, W Xue, GJ Wang, S Ye, ZF Zhu, YC Fu, XK Wang, CQ Sun (2007). Development of *Oryza rufipogon* and *O. sativa* introgression lines and assessment for yield-related quantitative trait loci. *J Integr Plant Biol* 49:871–884

Wang D, B Qin, X Li, D Tang, YE Zhang, Z Cheng, Y Xue (2016). Nucleolar DEAD-box RNA helicase TOGR1 regulates thermotolerant growth as a pre-rRNA chaperone in rice. *PLoS Genet* 12; Article e1005844

Welch JR, JR Vincent, M Auffhammer, PF Moya, A Dobermann, D Dawe (2010). Rice yields in tropical/subtropical Asia exhibit large but opposing sensitivities to minimum and maximum temperatures. *Proc Natl Acad Sci USA* 107:14562–14567

Wu ZJ, RAIrizarry, RGentleman, F Martinez-Murillo, F Spencer (2004). Amodel-based background adjustment for oligonucleotide expression arrays. *J Amer Stat Assoc* 99:909–917

Xiao YH, Y Pan, LH Luo, GL Zhang, HB Deng, LY Dai, XL Liu, WB Tang, LY Chen, GL Wang (2011). Quantitative trait loci associated with seed set under high temperature stress at the flowering stage in rice (*Oryza sativa* L.). *Euphytica* 178:331–338

Xu YH, HK Zhang, JQ Hu, XD Wang, M Huang, HQ Wang (2018). Further QTL mapping for yield component traits using introgression lines in rice (*Oryza sativa* L.) under drought field environments. *Euphytica* 214; Article 33

Yano K, T Takashi, S Nagamatsu, M Kojima, H Sakakibara, H Kitano, M Matsuoka, K Aya (2012). Efficacy of microarray profiling data combined with QTL mapping for the identification of a QTL gene controlling the initial growth rate in rice. *Plant Cell Physiol* 53:729–739

Ye CR, FA Tenorio, MA Argayoso, MA Laza, HJ Koh, ED Redona, KSV Jagadish, GB Gregorio (2015). Identifying and confirming quantitative trait loci associated with heat tolerance at flowering stage in different rice populations. *BMC Genet* 16; Article 41

Ye CR, MA Argayoso, ED Redona, SN Sierra, MA Laza, CJ Dilla, Y Mo, MJ Thomson, J Chin, CB Delavina, GQ Diaz, JE Hernandez (2012). Mapping QTL for heat tolerance at flowering stage in rice using SNP markers. *Plant Breed* 131:33–41

Zhang C, F Chen, R Hong, QF Li, MH Gu, QQ Liu (2017). Mapping QTLs for heat tolerance in rice (*Oryza sativa* L.) at heading stage using chromosome segment substitution lines. *Agric Biotechnol* 2:15–18

Zhang YJ, LB Tan, ZF Zhu, LX Yuan, DX Xie, CQ Sun (2015). *TOND1* confers tolerance to nitrogen deficiency in rice. *Plant J* 81:367–376

Zhu CL, YH Xiao, CM Wang, L Jiang, HQ Zhai, JM Wan (2005). Mapping QTLs for heat tolerance during grain filling in rice. *Chin J Rice Sci* 19:117–121

Zhu T, P Budworth, W Chen, N Provart, HS Chang, S Guimil, W Su, B Estes, G Zou, X Wang (2003). Transcriptional control of nutrient partitioning during rice grain filling. *Plant Biotechnol J* 1:59–70