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META-QTL MAPPING REVEALED POTENTIAL GENES FOR REPRODUCTIVE HEAT STRESS TOLERANCE IN RICE (*ORYZA SATIVA* L.)

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SUMMARY

Heat stress during the reproductive stage, especially during flowering, can considerably diminish grain yield of rice (*Oryza sativa* L.). A meta-QTL analysis provides a powerful and stable approach to identify QTLs, regardless of the genetic background of the mapping population and the environmental conditions. In the presented research, 95 previously published QTLs underwent MQTL analysis, recognizing 37 most consistent MQTLs across the different genetic backgrounds and panels. The 30 MQTLs identified had narrow confidence intervals (<5 cM), with 20 having attributed over 10% of phenotypic variance, ranging from 2.4% to 40.55%. In 37 MQTLs, 10 main candidate genes representing 10 gene families were successful for selection based on high R^2 values. Two genes, *LOC_Os02g04710* (*OsOSC2*) and *LOC_Os06g05550* (*OsGELP74*), distinctly appeared with elevated transcript levels in the panicle tissue. The haplotype analysis revealed two rare haplotypes (H017 and H018) for *OsOSC2*, distinguishing *indica* and *japonica*. At the same time, *OsGELP74* was evident across several accessions, with only one rare haplotype (H006) representing *indica, tropical japonica, temperate japonica,* and admixed accessions. The identification of such novel haplotypes associated with key heat-stress-related traits can help accelerate the development of heat-stress-tolerant rice genotypes in Vietnam.

Keywords: Rice (*O. sativa* L.), accessions, 3K RG, haplotype analysis, MQTL, rice heat tolerance, genetic backgrounds, phenotypic variance

Key findings: In rice (*O. sativa* L.), heat stress at the flowering stage severely reduces the grain yield, demanding targeted breeding for stable and heat-tolerant germplasm. Meta-QTL analysis identified the key genes and validated haplotype-based breeding for the development of heat-stress-tolerant rice genotypes in Vietnam.

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INTRODUCTION

With climate change and global warming, rice (*Oryza sativa* L.) grain yields will tend to gradually drop due to its high susceptibility to heat stress, especially during the reproductive stage. The study, carried out at the International Rice Research Institute (IRRI), revealed that during the growing season, a 1 °C increase in temperature can reduce rice grain yields by 10% (Peng *et al.,* 2004). In Vietnam, rice cultivation dominates the agricultural sector, and the use of 82% of the country's arable land ensued in 2023. The Mekong Delta River produces over half of the national rice output, and it is also facing challenges of increased temperature due to climate change (Statista, 2024).

Rice crops are highly sensitive to heat stress and thrive well in temperatures ranging from 22 °C to 28 °C during the reproductive phase. Grain yield experiences adverse effects from a temperature exceeding 35 °C during the critical phenological stages of anthesis and booting, as these high temperatures surpass the tolerance threshold (Prasad *et al.,* 2006). However, the rice crop's sensitivity to heat stress varies across the plant's different developmental stages, from seedling to mature stages, particularly at the gametogenesis and flowering stages, which are the most vulnerable (Jagadish *et al.,* 2014). Rice's ability to withstand high temperatures at varying growth stages, including its effect on spikelet fertility, grain quality during grain filling, and seedling growth, defines heat tolerance (Ishimaru *et al.,* 2009; Lanning *et al.,* 2011). Spikelet fertility is a crucial factor in determining rice grain yield, especially in warmer conditions, and that may be a reliable indicator of heat tolerance during the reproductive stage (Prasad *et al.,* 2006).

Plant heat tolerance is a complex trait influenced by the genetic and existing environmental factors. More than 60 quantitative trait loci (QTLs) are known to have an association with the flowering stage. For example, the identification of two large QTLs (*qHTSF1.1* and *qHTSF4.1*) explained 12.6% and 17.6% of the variation in spikelet fertility under high temperatures, respectively (Ye *et al.,* 2012). The MQTL approach has successfully identified QTLs/genes related to plant drought stress conditions (Swamy *et al.,* 2011; Selamat and Nadarajah*,* 2021), salinity tolerance (Satasiya *et al.,* 2024), and heat tolerance (Raza *et al.,* 2020). Specifically, a meta-analysis focused on heat tolerance identified 35 highly consistent MQTLs across the diverse rice cultivars and environments. Hence, the meta-QTL (MQTL) approach will help identify more precise and stable genomic regions by integrating the data obtained from various relevant studies (Goffinet and Gerber, 2000; Arcade *et al.,* 2004). Therefore, the following research aimed to study the existing literature on various QTLs associated with heat-stress tolerance during the rice's reproductive stage and identify the precise and consistent early flowering-related QTL regions through MQTL analysis. Likewise, this study sought to detect the candidate genes associated with early morning flowering (EMF) and understand their expression pattern and co-expression network and determine the superior haplotypes in the selected candidate genes from the 3K rice genome.

MATERIALS AND METHODS

Collection of QTL mapping literature

A comprehensive Google Scholar search proceeded to find published QTL/GWAS (genome-wide association studies) mapping studies on rice heat tolerance during the reproductive stage (Table 1). The collection of information on population size, markers, traits, the number of QTLs, phenotypic variance explained (PVE), and logarithm of odds (LOD) scores was successful. However, the study did not include the QTL/GWAS with LOD scores below 3.0.

Database, MQTL, and candidate gene

A set of 13K markers comprised this high-density reference map, based on mapping data for 1K-RiCA SNPs (Arbelaez *et al.,* 2019), 10K SNP markers (Islam *et al.,* 2019), and 2.1K SSRs (http://www.ricediversity.org/). The QTL

Table 1. Summary of QTL studies used for reproductive-related traits under heat stress.

No.	Pop. type	Marker type	Traits	No. of QTLs	LOD (>3.0)	R^2	References
1	BC	SSR	Booting stage	*2*	7.3-8.8	16.9-17.8	Cao *et al.,* 2020
2	NIL	SSR	Booting stage	*1*	13.1	37.3	Cao *et al.,* 2022
3	BC	SSR	Anthesis	*2*	4.92	10.3-14.5	Cheng *et al.,* 2012
4	RIL	SSR	Begin flower opening time	*5*	3.3-8.4	12.3-19.7	Hirabayashi *et al.,* 2015
5		GBS	Anthesis	*2*	4	11.4-12.3	Hu *et al.,* 2022
6	RIL	SSR, AFLP, RFLP	Anthesis	*4*	3.3-5.4	7.4-14.7	Jagadish *et al.,* 2010
7	RIL	SSR	Spikelet fertility-related traits	*6*	3-3.45	7.13-9.8	Jin *et al.,* 2021
8		GBS	Heading stage	*2*	4.2-4.6	18.5-20.4	Kwon *et al.,* 2021
9		GBS	Spikelet fertility	*12*	5.0-9.0	8-12	Lafarge *et al.,* 2017
10	RIL	SSR	Heading and flowering stage	*8*	3-10.2	5.4-13.8	Li *et al.,* 2018
11	BC	SSR	Flowering stage	*3*	3.6-10.8	7.6-26.3	Nguyen *et al.,* 2022
12		GBS	Flowering stage	*14*	4	2.5-33.6	Pan *et al.,* 2023
13		GBS	Reproductive stage	*7*	5-6	8-10	Ravikiran *et al.,* 2022
14	RIL	SNP	Spikelet sterility	*2*	3.9-4.2	6.3-16.1	Ps *et al.,* 2017
15	RIL	SSR/SFP	Flowering stage	*2*	6.6-13.6	11.5-21.3	Xiao *et al.,* 2011a
16	RIL	SSR/SFP	Flowering stage	*2*	4.4-7.5	9.3-15.1	Xiao *et al.,* 2011b
17		GBS	Heat stress responsive	*9*	6-7	1.6-78.6	Yang *et al.,* 2022
18	3-way F_1	SNP	Spikelet fertility	*10*	3.5-4.6	11.6	Ye *et al.,* 2015
19	RIL	SNP	Spikelet fertility	*2*	4.6-6.7	12.6-17.6	Ye *et al.,* 2012

and map input files aided the analysis using Biomercator v4.2 software (https://mybiosoftware.com/biomercator-genetic-maps-qtl-integration.html). All the MQTLs underwent a candidate gene analysis. The candidate genes' identification was within or near the MQTL regions (±100 kb) to avoid redundancy. All the genes within this physical interval, as determined by the Rice Genome Annotation Project (RGAP; https://rice.uga.edu), deemed as potential candidate genes, particularly those with functions related to heat-related elements.

Co-expression, promoter, and haplotype analysis

Ten candidate genes relevant to all the heat tolerance traits succeeded their retrieval from the CoNekT database (http://conekt.mpimp-golm.mpg.de/). The RGAP (resistance gene analog polymorphism) provided the two kb upstream DNA sequence of chosen candidate genes, which then attained analysis using the PlantPAN4.0 to identify the GC islands in the promoter regions (https://plantpan.itps.ncku.edu.tw/). Haplotype analysis of the selected candidate genes continued using the SNP search database's built-in tool (https://snp-seek.irri.org/). The 3k filtered SNP set, encompassing all subpopulations (3K-RGP), was beneficial for the analysis. Only the functional SNPs located within the UTR, promoter, and gene variant regions entailed considerations. The geneHapR package offered a user-friendly interface for haplotype identification, statistics, and visualization (https://github.com/ZhangRenL/geneHapR).

Table 2. Summary of the integrated consensus map using 13K markers.

Chr.	No. of markers	Length (cM)	Mean distance of two markers (cM)
1	1,716	199.82	0.12
2	1,382	182.94	0.13
3	1,456	191.23	0.13
4	1,178	148.06	0.13
5	1,067	135.57	0.13
6	1,165	148.61	0.13
7	1036	127.36	0.12
8	773	120.4	0.16
9	813	93.5	0.12
10	684	95.17	0.14
11	861	121.77	0.14
12	868	109.2	0.13

RESULTS AND DISCUSSION

Mapping heat-tolerant traits

Ninety-five published QTLs reached identification to ascertain consensus genomic regions linked to attributes and significantly correlated with heat tolerance in rice at the reproductive stage (Table 1). The QTLs were also notable for multiple reproductive stage characteristics, including blooming, heading, anthesis, booting, and spikelet fertility. However, the flowering time enunciated the highest quantity of QTLs (36) among the assessed factors for heat tolerance, followed by spikelet fertility (32), heat stress responsiveness (8), reproductive stage (2), and days to heading (2). The single-use QTL exhibited phenotypic variations between 1.6% and 13.1%, with LOD values ranging from 3.01 to 37.3.

Consensus map

The consensus map, constructed from reference and genetic maps of various studies, had a marker density of 13K, with an average of 1083.2 markers per chromosome and a total genetic map length of 1673.6 cM, averaging 139.5 cM per chromosome (Table 2). However, the number of markers per chromosome ranged from 684 to 1716. Chromosome 1 emerged with 1716 markers and seemed to be the longest at 199.82 cM, while chromosome 10, with 684 markers, appeared to be the shortest one at 93.5 cM.

Predicting MQTLs for reproductive heat tolerance

Given the climate change, rising temperature considerably affects rice production, especially in warmer regions. Heat stress during the flowering stage can severely reduce the rice genotypes' grain yield potential. In most rice cultivars, flowering occurs from mid-morning to early afternoon, with the peak activity around noon. However, spikelets that open after 11:00 AM proved to be more vulnerable to high temperature, leading to sterility (Ayyenar *et al.,* 2023). Numerous studies have used QTLs/GWAS to identify the genomic regions linked to heat tolerance, and their obtained findings vary widely. The identified QTLs often have broad confidence intervals (CIs) and frequently overlap to the same genomic areas across the different studies. This complexity highlighted the further need for more precise and consistent genetic mapping to identify the crucial genes for heat tolerance. Thus, the MQTL analysis occurred as the suitable approach to achieve these objectives and has been successfully effective in several crop species with diverse traits, including rice (Goffinet and Gerber, 2000).

In the MQTL analysis, the Veyrieras method defined 37 MQTLs distributed across all rice chromosomes, except for chromosomes 8

and 9 and those chromosomes containing 1-14 QTLs (Table 3). In the presented study, the identified MQTLs totaled 30 with a narrow confidence interval of less than 5 cM. Each MQTL explained a mean R^2 between 2.4% and 40.55%, with 20 MQTLs explaining more than 10% of the phenotypic variance. The MQTLs appear to have the highest mean R^2 on chromosome 1 (MQTL1.2), while the lowest was on chromosome 10 (MQTL10.2).

Under the investigation, the confidence intervals of the QTLs ranged from 0.12 to 39.85 cM. With the C6AIR, 221 useful SNP markers were evident on chromosome 1, focusing the research on a 0.88 Mb (~3 cM) stretch of DNA between positions 41.04 and 41.91 Mb (Thomson *et al.,* 2017). A high-density consensus genetic map comprised ~13K markers with an average two-marker interval of 1.58 cM (Table 2). Raza *et al.* (2020) conducted a meta-QTL analysis of 163 individual QTLs and identified 35 MQTLs across the 12 rice chromosomes. These MQTLs had 95% Cis, ranging from 0.30 to 13.77 cM, with a median of 3.03 cM. Notably, 23 out of the 35 MQTLs had CIs less than 5 cM. These MQTLs have the potential to be the most effective candidate targets for further exploration in marker-assisted selection.

Identification of candidate genes and *in-silico* analysis

In the RGAP, a total of 10 potential genes achieved their identification within the 37 MQTL regions, attaining authentication for further analysis and exploration (Table 3). Based on the highest mean R^2 values, the 10 candidate genes succeeded in their selection from the 37 MQTLs. These genes showed association with 10 gene families: *PK, CAS, NAS, Malectin, DUF1618, GELP, ADH, AGAL, NB-LRR,* and *CRK* (Table 4). The differentially expressed genes, or DEGs, entailed further filtering based on their highest expression in rice panicle datasets to identify a core group of heat-associated genes. The selected 10 genes had expression data available for root, leaf, and panicle tissues in the CoNekT database. The heat map displayed the degree of gene expression in various parts during the reproductive stage and development (Figure 1). Two differentially expressed candidate genes, *LOC_Os02g04710* (*OsOSC2*) and *LOC_Os06g05550* (*OsGELP74*), emerged with the highest transcript levels in the panicle tissues. Moreover, these genes' predominant expression prevailed in the leaf tissue during the reproductive stage, with peak expression levels of ~3.03 and ~3.47, respectively. The results suggested these two candidate genes could be pivotal for heat tolerance and may influence the rice grain yield and quality. However, other genes exhibited low and uniform expression across all the plant tissues, suggesting broader and less tissue-specific roles, respectively.

It is well-known that plant OSC enzymes use several cyclization mechanisms to develop triterpenoids in the *OsOSC* gene group (Thimmappa *et al.,* 2014). The pollen coat, composed of stearic, linolenic, and palmitic acids, protects pollen from excessive desiccation. For instance, the gene *OsOSC12* initiates a novel triterpene pathway that produces poaceatapetol and its esters. Rice genotypes lacking the conserved grass triterpene synthase *OsOSC12/OsPTS1* exhibited defective pollen coat development. The rice *GDSL* esterase/lipase, *LOC_Os06g05550*, could contribute to various enzymatic activities, potentially including plant cell wall modification and stress responses (Watkins *et al.,* 2019). With its homology to *Xanthophylls* (*Xat*), reports have been suggesting it as a candidate gene. These processes might indirectly influence the flowering by considerably affecting stress signaling pathways and plant architecture, and both play a crucial role in successful reproduction. Besides, the clustering also revealed relationships among the genes and their grouping with similar expression profiles. The gene's differential expression pattern underscored the functional diversity of these genes and their contribution to the specific physiological processes. These results were vital for prioritizing candidate genes for further functional validation and leveraging them in breeding programs aimed at improving the heat tissue-specific traits.

Table 3. Details of identified MQTLs for reproductive-related traits.

No.	MQTLs	AIC	Model 4	Left marker	Right marker	Marker interval (cM)	CI (95%)	Mean R^2 (%)
1	MQTL1.1	229.3	2.4	S1_1059696	20215	1.9-2.9	1	16.9
2	MQTL1.2		11.9	chr01_2726468	chr01_3529691	10.5-13.5	2.9	40.5
3	MQTL1.3		109.9	S1_24093685	S1_29532660	108.1-134.4	26.3	8.5
4	MQTL1.4		133.7	S1_29127258	S1_29532660	132.9-134.4	1.57	9.5
5	MQTL2.1	36.5	3.4	chr02_890901		3.4	-	12
6	MQTL2.2		12.3	S2_1103758	1492736	4.8-19.8	15	16.9
7	MQTL2.3		52.9	id2005033	chr02_16224316	43-63	20	17.1
8	MQTL2.4		83.6	RM7624	S2_20257225	81.8-85.5	3.7	19.4
9	MQTL3.1	696.2	10.8	S3_1356326	S3_1883483	8.9-12.7	3.8	15.1
10	MQTL3.2		41.7	RM1338	S3_7066823	40.3-43.1	2.7	21.1
11	MQTL3.3		115.8	S3_22657507	chr03_30247826	113.9-117.7	3.7	26
12	MQTL4.1	257.8	23.5	id4002166	chr04_6596219	21.6-25.5	3.9	9.6
13	MQTL4.2		66.9	S4_20622937	S4_28765287	65.6-105.5	39.8	13.2
14	MQTL4.3		104.6	S4_28403708	4603923	103.7-123.3	19.6	9.8
15	MQTL4.4		121.9	S4_31520309	4603923	120.6-123.3	2.7	18.1
16	MQTL5.1	44.9	11.6	4892211	S5_1808980	11.1-12.2	1.1	19.4
17	MQTL5.2		25.3	chr05_6129755	chr05_6833061	23.9-26.7	2.7	20.7
18	MQTL5.3		79.6	S5_18694973	RM305	78.5-80.8	2.2	8.6
19	MQTL5.4		88.8	RM3575	chr05_23143060	87.7-9	2.2	9
20	MQTL6.1	33.3	10	chr06_1765761	chr06_3431721	6.8-13.3	6.5	14.5
21	MQTL6.2		12.6	5904398	chr06_3431721	11.9-13.3	1.3	9.3
22	MQTL6.3		51.1	chr06_10006906	S6_10885140	38.8-63.4	24.6	16.1
23	MQTL6.4		108.6	6844435		108.6	-	7.5
24	MQTL7.1	11.6	0.9	S7_425945		0.97	-	2.7
25	MQTL7.2		58.2	S7_15190947		58.2	-	4
26	MQTL7.3		94.8	S7_23686607		94.8	-	3.2
27	MQTL7.4		113.7	S7_28192334		113.7	-	17.2
28	MQTL10.1	160.6	27.5	S10_11251070	chr10_7397104	26.2-2	2.7	11.4
29	MQTL10.2		48.4	chr10_12145295	S10_16819763	47-49.8	2.7	2.4
30	MQTL10.3		72.8	RM1374	chr10_18852690	72.8-72.9	0.1	11.5
31	MQTL10.4		81.6	10744000	S10_21050429	81.1-82.1	0.9	8.5
32	MQTL11.1	112.6	8.8	S11_2071390	S11_2431868	7.7-10.1	2.3	10.9
33	MQTL11.2		35.4	11095170	S11_5902556	33.5-37.4	3.8	11.6
34	MQTL11.3		78	RM457		78	-	8
35	MQTL12.1	22.1	9	chr12_2324105		9	-	6.4
36	MQTL12.2		19.2	12086489	S12_3323965	12.3-26.2	13.9	7.1
37	MQTL12.3		96.6	13009053		93.7-99.5	5.7	17.5

Noted: Akaike Information Criterion (AIC); Confidence interval (CI).

Table 4. List of 10 potential genes identified in the 37 MQTLs' regions.

MQTL	Chr.	Gene Family	Gene IDs	P-value	FDR
MQTL1.1	1	*PK*	*LOC_Os01g02290*	4.59	0.00
MQTL2.2	2	*CAS*	*LOC_Os02g04710*	5.10	0.00
MQTL3.2	3	*NAS*	*LOC_Os03g19427*	4.53	0.00
MQTL4.3	4	*Malectin*	*LOC_Os04g52590*	3.74	0.02
MQTL5.4	5	*DUF1618*	*LOC_Os05g39150*	9.70	0.00
MQTL6.1	6	*GELP*	*LOC_Os06g05550*	4.27	0.00
MQTL7.4	7	*ADH*	*LOC_Os07g48920*	3.37	0.01
MQTL10.3	10	*AGAL*	*LOC_Os10g35070*	5.04	0.00
MQTL11.2	11	*Clp*	*LOC_Os11g16590*	4.42	0.00
MQTL12.3	12	*CRK*	*LOC_Os12g41490*	5.33	0.00

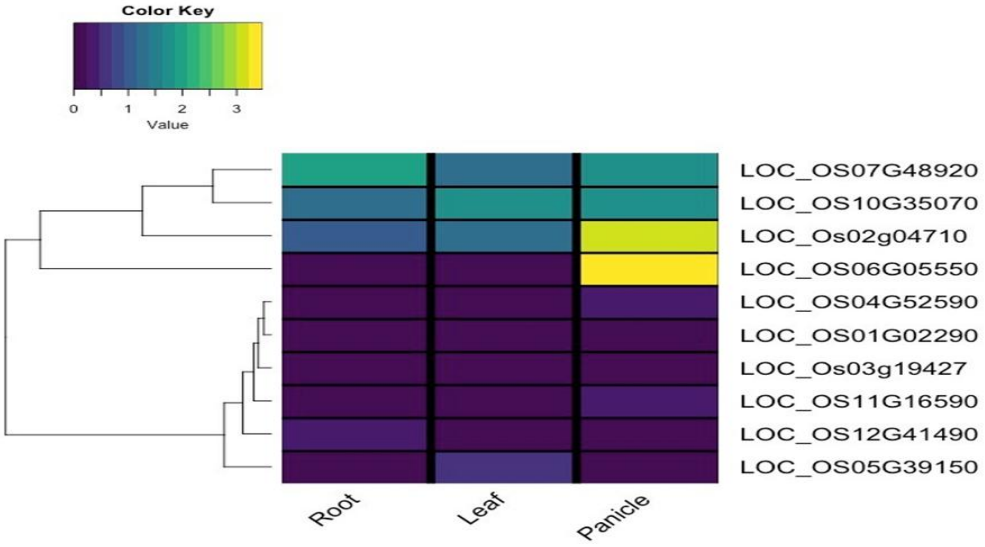

Figure 1. A heatmap showing gene expression levels in different tissues for 10 selected genes.

A CpG island in the promoter region

The 2 kb upstream regions of these two genes, which contain enriched CpG islands, sustained analysis using PlantPAN4.0 (Table 5). The analysis identified GC islands within the promoter regions and provided insights into their structural and functional characteristics. Gene regulation was evident to be linked to GC-rich regions known as CpG islands. These regions with a G+C content ranging from 50% to 60% had elevated CpG dinucleotide frequencies. The observed-to-expect (o/e) CpG ratios ranged from 1.12 to 1.17, suggesting that these regions can be hypomethylated, a characteristic frequently associated with active gene promoters. The GC islands varied in length, ranging from 972 to 507 bp and 512 to 1,343 bp, respectively, signifying differences in the composition of the promoter region. However, some loci showed minor variations that could indicate specific structural features; the AT and CG skew values were nearly neutral, implying a balanced base distribution. Both the start and strand probabilities were generally moderate to high, revealing that the identified regions were reliable and have a favorable strand orientation. Two specific loci, characterized by the highest G + C content and CpG o/e ratios, were particularly noteworthy as potential hotspots for gene regulation and for further investigations into their biological functions.

Table 5. The GC's island in promoter region of two key genes.

Loc name	Begin site	End site	Length	G+C frequency	CpG o/e ratio	AT Skew	CG Skew	Start-p	Strand	Strand-p
LOC_Os02g04710	1	972	957	0.6	1.17	-0.24	0.05	0.79	+	0.97
	8507	9021	507	0.5	1.12	-0.02	0.09	0.62	+	0.8
LOC_Os06g05550	1	520	512	0.51	0.61	-0.06	-0.05	0.12	+	0.5
	737	2101	1343	0.5	0.97	-0.02	-0.03	0.56	+	0.53

Superior haplotypes for heat responsiveness

Haplotype analysis succeeded for the two selected key genes using the 3K Rice Genome Project (3K RGP) dataset. The number of haplotypes ranged from eight to 25 across the two genes. *LOC_Os02g04710* showed 25 haplotypes (Figures 2A, 2B, and 2C), while *LOC_Os06g05550* had nine haplotypes (Figures 3A, 3B, and 3C). For the two selected genes, the estimated haplotype frequencies were *LOC_Os02g04710* (1 to 341) and *LOC_Os06g05550* (1 to 1332). The results suggested the major haplotypes were likely common in the population and might considerably influence the genetic makeup of the individuals. In contrast, the minor haplotypes appeared less frequent and could play a crucial role in determining the heat stress tolerance at the plant's reproductive stage. The identified SNPs totaled 38 within the *LOC_Os02g04710* genomic region across 3K RG accessions.

Among these, 12 SNPs were in the promoter region, approximately 2 kb upstream of the start codon. The remaining 26 SNPs manifested a distribution across the remaining genomic region. For *LOC_Os06g05550*, three SNPs resulted in the promoter region, and six SNPs were in the remaining regions. Two rare haplotypes (H017 and H018) for *LOC_Os02g04710* emerged using the haplotype network analysis, with each having fewer than 10 accessions. Between the *indica* and *japonica* groupings, these two uncommon haplotypes showed the most considerable variance. *LOC_Os06g05550*, which belongs to *indica, tropical japonica, temperate japonica*, and the admixed accessions, only has the rare haplotype H006.

The N22 (heat tolerant) exhibited the haplotype GGAGTGCAGCACGCGGTGTAATG CTGCCTTCCGCAGCC for *LOC_Os02g04710* and GTCCATCAC for *LOC_Os06g05550*. In contrast, IR64 (heat sensitive) showed different haplotypes: GGAGTGCAGTTAGCGATGTG TTGCTGTCCTTCGCGGCC for *LOC_Os02g04710* and TACCGAAAC for *LOC_Os06g05550* (Table 6). With a focus on heat responsiveness, haplotype analysis of indica (IR64) and aus (N22) subgroups revealed 10 superior haplotypes of *OsOSC2* and five superior haplotypes of *OsGELP74*. The contrasting haplotypes between these subgroups suggested that the genetic variation proved to be linked to their heat responsiveness, with N22 potentially harboring alleles contributing to tolerance and IR64 showing alleles associated with sensitivity. This comparison provided valuable insights into the genetic basis for heat tolerance at the reproductive stage in rice plants.

CONCLUSIONS

In the preceding study, 37 MQTLs were evident to harbor reliable genomic regions essential for heat tolerance in rice reproductive drought stress. Among the genes identified, two important candidate genes, *LOC_Os02g04710* (*OsOSC2*) and *LOC_Os06g05550* (*OsGELP74*), appeared along with superior haplotypes that will be useful later in accelerating the development of reproductive heat-tolerant varieties for Vietnam through marker-aided breeding.

Table 6. Depicting the number of haplotypes of two predicted genes between *aus* and IR64 subgroups in the 3K rice panel.

Genotype	*LOC_Os02g04710*	*LOC_Os06g 05550*	Group	References
N22	GGAGTGCAGCACGCGGTGTAATGCTGCCTTCCGCAGCC	GTCCATCAC	*aus*	Ye *et al.,*2012
IR64	GGAGTGCAGTTAGCGATGTGTTGCTGTCCTTCGCGGCC	TACCGAAAC	*Indica*	

N22: Tolerance; IR64: Sensitive.

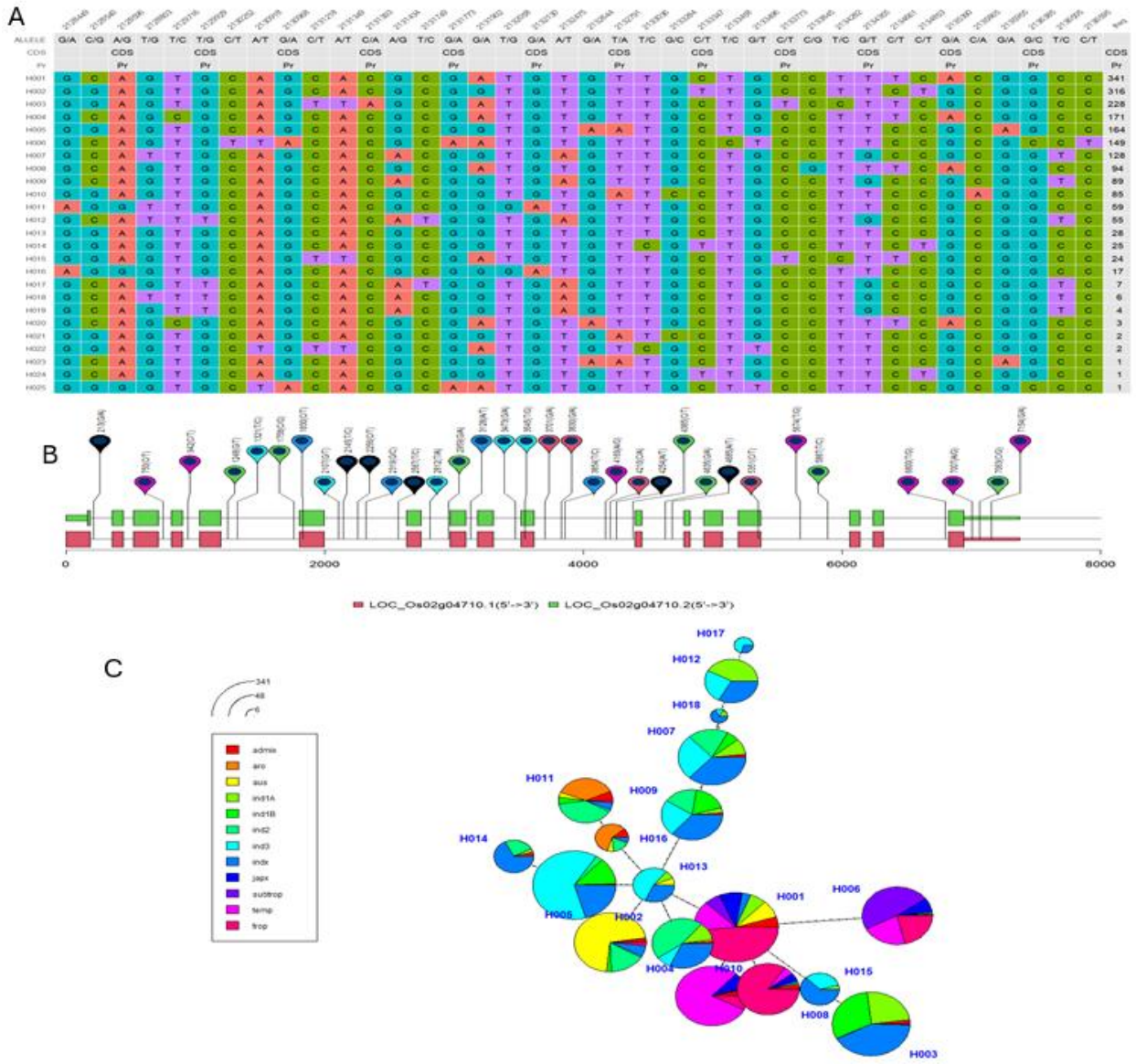

Figure 2. Gene structure and haplotype analysis of *LOC_Os02g04710*. A. Haplotype analysis of the gene associated with the trait across the 3K RGP. B. Gene structure. C. Haplotype networks of heat-related genes across the 12 subgroups.

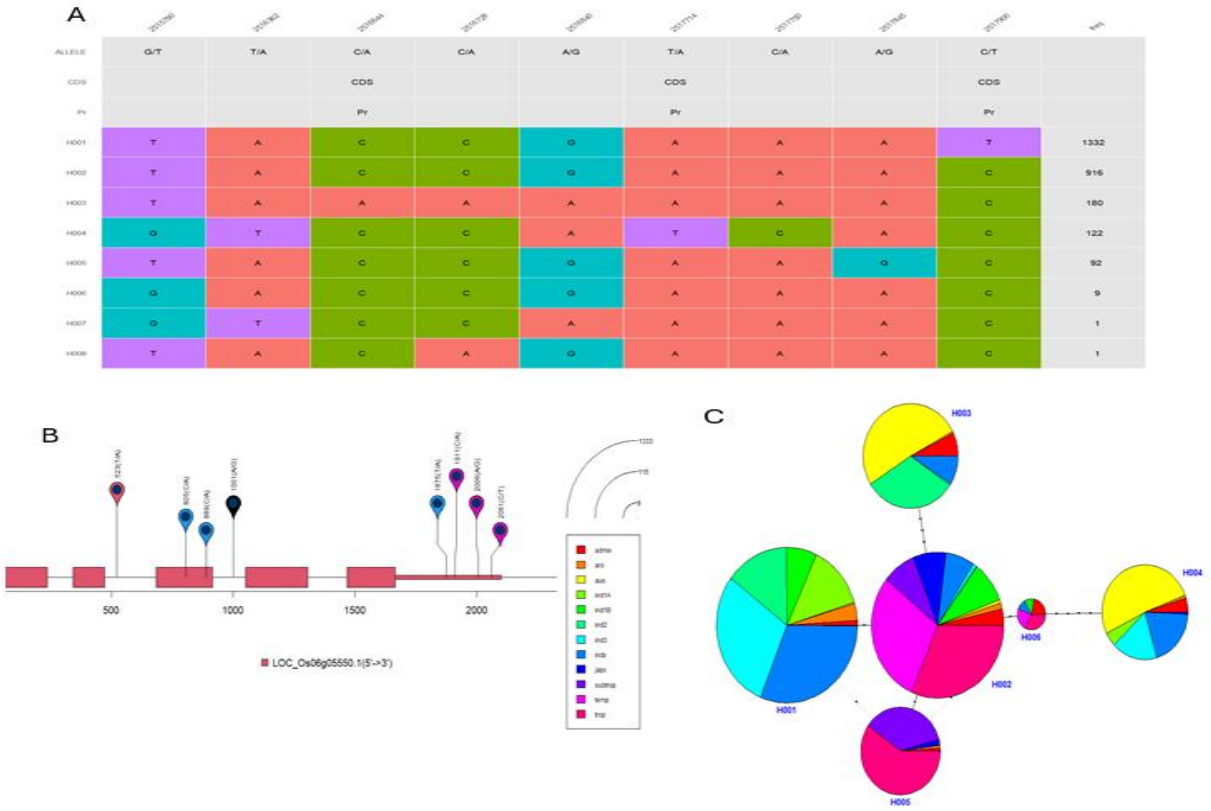

Figure 3. Gene structure and haplotype analysis of *LOC_Os06g05550*. A. Haplotype analysis of the gene associated with the trait across the 3K RGP. B. Gene structure. C. Haplotype networks of heat-related genes across the 12 subgroups.

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