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OsHTR, an AP2-Type Transcription Factor, Regulates Disease Resistance in Rice

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Abstract

Rice (*Oryza sativa* L.) production is constantly threatened by devastating diseases such as rice blast, bacterial blight, and brown planthopper infestation. The AP2-type transcription factor *OsHTR* (also known as *SMOS1/SHB/RAL1/NGR5/GR5*) has been previously implicated in hormonal signaling networks and nitrogen use efficiency; however, its role in disease resistance remains largely unexplored. In this study, we functionally characterized *OsHTR* in disease resistance using knockout (KO) and overexpression (OE) transgenic lines in the ZH11 background. Transcriptome analysis revealed that differentially expressed genes in the *htr* mutant were significantly enriched in plant–pathogen interaction pathways, with multiple NBS-LRR and NB-ARC resistance-related genes upregulated. Real-time PCR validation confirmed the upregulation of 15 candidate resistance genes in the *htr* mutant. Comprehensive resistance evaluations suggested that *HTR-KO* lines exhibited enhanced resistance to rice blast and bacterial blight compared to wild-type ZH11 and *HTR-OE* lines, which displayed moderate susceptibility. In contrast, all lines remained highly susceptible to brown planthopper, indicating a disease-specific regulatory function of *OsHTR*. Furthermore, targeted knockout of individual upregulated resistance-related genes (*LOC_Os10g04090*, *LOC_Os12g29690*, *LOC_Os02g11980*, and *LOC_Os11g11770*) and *OsHTR-interacting* gene *LOC_Os06g03710* confirmed their distinct contributions to blast and bacterial blight resistance but did not establish them as direct targets of *OsHTR*. Collectively, our results indicate that *OsHTR* functions as a negative regulator of disease resistance in rice, likely acting through transcriptional repression of defense-related genes, although direct binding remains to be demonstrated. This study uncovers a novel regulatory module connecting AP2-type transcription factors to disease resistance and provides valuable genetic resources for molecular breeding of broad-spectrum-resistant rice cultivars.

Keywords: rice; *OsHTR*; AP2 transcription factor; blast resistance; bacterial blight; disease resistance

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1. Introduction

Rice is a staple food crop for more than half of the world's population, and its yield stability is crucial for global food security. Among the major constraints on rice productivity, diseases caused by fungal pathogens, bacteria, and insect pests cause substantial annual losses. Rice blast, caused by *Pyricularia oryzae*, is the most destructive fungal disease,

responsible for 10–30% yield losses globally [1]. The disease threatens rice production across Asia, Africa, and the Americas, with particularly severe outbreaks in regions where high-yielding but susceptible cultivars are widely grown. Bacterial blight, caused by *Xanthomonas oryza* pv. *aryzae* (*Xoo*), is another devastating disease, particularly in irrigated and rainfed lowland ecosystems [2]. The emergence of new *Xoo* races that overcome existing resistance genes underscores the urgent need for continued discovery of novel resistance sources and understanding of resistance mechanisms. Additionally, the brown planthopper (*Nilaparvata lugens*) poses a significant threat as a phloem-feeding insect pest that can cause direct damage and transmit viral diseases [3]. Breeding cultivars with durable and broad-spectrum resistance is therefore a major objective in rice improvement programs.

Plant disease resistance is governed by a multilayered immune system, including pattern-triggered immunity (PTI) and effector-triggered immunity (ETI), which involve complex transcriptional reprogramming and phytohormone signaling [4,5]. PTI is activated by the recognition of conserved pathogen-associated molecular patterns (PAMPs) by cell-surface pattern recognition receptors, whereas ETI is triggered when intracellular nucleotide-binding leucine-rich repeat (NLR) receptors perceive pathogen effectors, typically leading to a hypersensitive response and localized cell death. Transcription factors play pivotal roles in orchestrating defense gene expression, and members of various transcription factor families, including WRKY, NAC, MYB, and AP2/ERF, have been functionally characterized as key regulators of plant immunity [6–8]. The AP2/ERF superfamily is one of the largest plant-specific transcription factor families, and its members are involved in diverse biological processes, including development, hormone signaling, and stress responses [9]. Recent reviews have highlighted that AP2/ERF transcription factors regulate plant disease resistance by acting downstream of mitogen-activated protein kinase (MAPK) cascades and modulating the expression of genes associated with hormonal signaling pathways, secondary metabolite biosynthesis, and the formation of physical barriers [10]. However, the functional characterization of individual AP2-type transcription factors in disease resistance, particularly in rice, remains incomplete.

OsHTR (Height Tiller Reduced) was originally identified from a mutant library of the elite rice cultivar 93-11, and map-based cloning revealed that it encodes an AP2-type transcription factor [11]. The same gene has been independently studied under different names, including *SMOS1*, *SHB*, *RAL1*, *NGR5*, and *GR5*, and has been shown to be involved in brassinosteroid, gibberellin, and auxin signaling networks [12–16]. Recent studies have demonstrated that *GR5*—allelic to *SMOS1*/*SHB*/*RLA1*/*NGR5*—acts as a transcriptional activator that determines grain size by influencing cell proliferation and expansion, and physically interacts with G protein γ subunits to regulate downstream target genes in grain development [16]. *OsHTR*/*NGR5* participates in nitrogen use efficiency through interactions with key nitrogen regulatory factors, and natural variation in its expression level has been associated with agronomic performance [15]. Importantly, given its known roles in growth and development, we hypothesized that *OsHTR* might also participate in balancing growth–defense trade-offs. Despite these advances in understanding its roles in development and nutrient signaling, the potential involvement of *OsHTR* in disease resistance has not been systematically investigated. The central objective of this study was therefore to determine whether *OsHTR* plays a role in regulating rice immunity against major pathogens and, if so, to identify downstream resistance genes and evaluate the specificity of this regulation.

In this study, we present evidence that *OsHTR* functions as a negative regulator of disease resistance in rice. Through transcriptome analysis, we discovered that differentially expressed genes in the *htr* mutant are significantly enriched in plant–pathogen interaction pathways. We generated knockout and overexpression transgenic lines and conducted

comprehensive resistance evaluations against rice blast, bacterial blight, and brown planthopper. Our results demonstrate that loss of *OsHTR* function enhances resistance to blast and bacterial blight, while overexpression leads to increased susceptibility. Furthermore, we validated the functional relevance of downstream upregulated resistance genes through targeted knockout experiments. This study expands the functional repertoire of *OsHTR* and provides novel insights into the molecular mechanisms connecting development-related transcription factors with disease resistance in rice.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The wild-type rice cultivars used in this study were *Oryza sativa* L. ssp. *japonica* cv. Zhonghua 11 (ZH11) and the highly blast-susceptible cultivar CO39. The highly resistant cultivar Gumei 4 was used as a resistant control for blast evaluation. For bacterial blight evaluation, TN1 was used as a susceptible control and R498-*Xa23* as a resistant control. For brown planthopper evaluation, TN1 was used as a susceptible control and Rathu Heenati as a resistant control. All plants were grown in a greenhouse at Huazhi Biotechnology Co., Ltd. (Changsha, China) under controlled conditions with a 14 h light/10 h dark photoperiod at 28 °C/25 °C.

2.2. Generation of Transgenic Lines

CRISPR/Cas9-mediated knockout lines of *OsHTR* (*HTR-KO-1* and *HTR-KO-2*), using the binary vector BGK030 (BIOGLE GeneTech, Jiangsu, China, http://www.seedseek.cn) (accessed on 16 October 2024), and overexpression lines (*HTR-OE-1* and *HTR-OE-2*), using the binary vector pCUbi1390, were generated in the ZH11 background. For targeted gene knockout, single guide RNAs were designed to target the coding regions of *LOC_Os10g04090*, *LOC_Os12g29690*, *LOC_Os02g11980*, *LOC_Os11g11770*, and *LOC_Os06g03710* using the binary vector BGK030 (BIOGLE GeneTech). All constructs were introduced into rice calli via *Agrobacterium tumefaciens*-mediated transformation as described previously [17]. Transgenic lines were confirmed by sequencing and Real-time PCR. Primer sequences are listed in Table S8.

2.3. RNA-Seq, KEGG Pathway, and GO Analysis

Total RNA was extracted from leaves of wild-type 93-11 and the *htr* mutant at the heading stage (three biological replicates per genotype). RNA-seq libraries were prepared and sequenced on an Illumina platform generating >22 million paired-end reads (150 bp) per sample. Raw reads were trimmed and mapped to the rice reference genome (https://www.ncbi.nlm.nih.gov/assembly/GCA_000004655.2) (accessed on 15 December 2021) using HISAT2. Differentially expressed genes (DEGs) were identified using DESeq2 with a threshold of $|\log2 \text{ fold change}| \geq 1$ and adjusted p-value < 0.05. KEGG pathway enrichment and GO enrichment analysis was performed using the clusterProfiler package (version 4.20.0).

2.4. Real-Time Quantitative PCR (RT-qPCR)

Total RNA was extracted from the third leaf of two-week-old seedlings by an RNA Prep Pure Plant kit (TIANGEN Biotech, Beijing, China, http://www.tiangen.com/en/) (accessed on 15 January 2022). The first-strand cDNA was synthesized using oligo (dT) (TaKaRa, Tokyo, Japan. http://www.takara-bio.com) (accessed on 15 January 2022) for nuclear-encoded genes and reverse-transcribed using Prime scriptase (TaKaRa). qRT-PCRs were performed on an ABI PRISM 7900 device (Applied Biosystems, Foster City, CA, USA) using SYBR Green Supermix (Bio-Rad, Hercules, CA, USA). All reactions were run with

three biological replicates. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method with rice *Ubiquitin1* (NM _001056014) as the internal reference. Primer sequences are listed in Table S7.

2.5. Rice Blast Resistance Evaluation

Blast resistance was evaluated in an artificial climate chamber using a seedling spray inoculation method as described previously [18], with minor modifications. Briefly, a conidial suspension (1×10^5 conidia/mL in 0.02% Tween-20) was sprayed evenly onto 3-week-old seedlings until runoff. Three mixed-strain inocula were used: Yunchuan mixed strains (CH11391 from Yunnan, China, 12-1 and 20DT-14 from Sichuan, China), Hunan mixed strains (21TJ-23, 21DWS-2, 20CH-2), and Northeast mixed strains (22DF-18, 20CC-2, 22GZL-21, 22GZF-1). Inoculated seedlings were incubated in the dark at 26 °C with saturated humidity for 24 h, then transferred to normal growth conditions. Disease severity was scored at 7 days post-inoculation (dpi) using a 0–9 scale, where 0 = no lesions (highly resistant, HR), 1–3 = resistant (R) to moderately resistant (MR), and 5–6 = moderately susceptible (MS), and 7–9 = susceptible (S) to highly susceptible (HS). Each experiment included at least 12 seedlings per line, and the entire experiment was repeated three times independently.

2.6. Bacterial Blight Resistance Evaluation

Bacterial blight resistance was evaluated in an indoor greenhouse. Three *Xoo* strains were used: FuJ (Fujian, China), GD1358 (Guangdong, China), and HNA1-4 (Hunan, China). Plants at the booting stage were inoculated using the leaf-clipping method as previously described [19]. Briefly, a bacterial suspension (OD600 = 1.0) was prepared, and scissors dipped in the suspension were used to clip 2 cm from the leaf tip. Disease severity was scored at 21 dpi using a 0–9 scale, where 0 = no visible lesion at the inoculation cut site, 1 = lesion extends downward, length 2–3 cm, 3 = lesion length < 1/4 of the total leaf length, 5 = lesion length $\geq$ 1/4 but <1/2 of the leaf length, 7 = lesion length $\geq$ 1/2 but <3/4 of the leaf length, 9 = lesion length $\geq$ 3/4 of the leaf length. Each experiment included at least 7 leaves per line per strain, with three independent biological replicates.

2.7. Brown Planthopper Resistance Evaluation

Brown planthopper resistance was evaluated using a standard seedbox screening technique as described previously [20], with 2–3 instar nymphs derived from natural field populations in Changsha after two generations of purification and propagation. Each experimental unit consisted of 20 seedlings, and 10 nymphs were introduced per seedling. The average seedling mortality rate (%), average damage grade, and resistance level were recorded. Resistance was classified on a 1–9 scale, where 1 = seedling mortality $\leq$ 10.0%, 3 = seedling mortality $\leq$ 30.0%, 5 = seedling mortality $\leq$ 50.0%, 7 = seedling mortality $\leq$ 70.0%, 9 = seedling mortality $\geq$ 70.1%, 1 = HR and 9 = HS. Each line was tested with three replicates.

2.8. Statistical Analysis

All experiments were conducted with at least three biological replicates. Data are presented as mean $\pm$ standard deviation. Statistical significance was determined using Student's *t*-test or one-way ANOVA followed by Tukey's HSD test.

3. Results

3.1. Transcriptome Analysis Reveals Involvement of OsHTR in Plant–Pathogen Interaction Pathways

To gain insight into the molecular function of *OsHTR*, we performed RNA-seq analysis comparing the wild-type 93-11 and the *htr* mutant at the heading stage. Transcriptome

analysis identified a total of 364 differentially expressed genes (DEGs) in the *htr* mutant compared to the wild type, among which 194 genes were significantly upregulated and 170 genes were significantly downregulated (Tables S1 and S2). KEGG pathway enrichment and GO enrichment analysis of the upregulated DEGs revealed significant enrichment in plant–pathogen interaction pathways and defense responses (Figure 1), which is consistent with previous independent findings on the *smos1* mutant [12]. Among the upregulated DEGs, we identified multiple resistance-related genes, including NBS-LRR and NB-ARC family members. Based on a combination of high fold change ($|\log2FC| > 2$), annotation as known resistance-related genes (NBS-LRR, NB-ARC, or receptor-like kinases), and previous reports [12], we selected 16 upregulated candidate genes for RT-qPCR validation (Table S3). The results showed that, except for *LOC_Os11g46210*, which exhibited no significant change, the remaining 15 genes were significantly upregulated in the *htr* mutant (Figure 2). It is important to note that these data indicate correlation rather than direct regulation; whether *OsHTR* directly binds to the promoters of these genes remains to be determined. These results suggest that *OsHTR* may negatively regulate the expression of a broad set of defense-related genes in rice, thereby participating in rice defense responses.

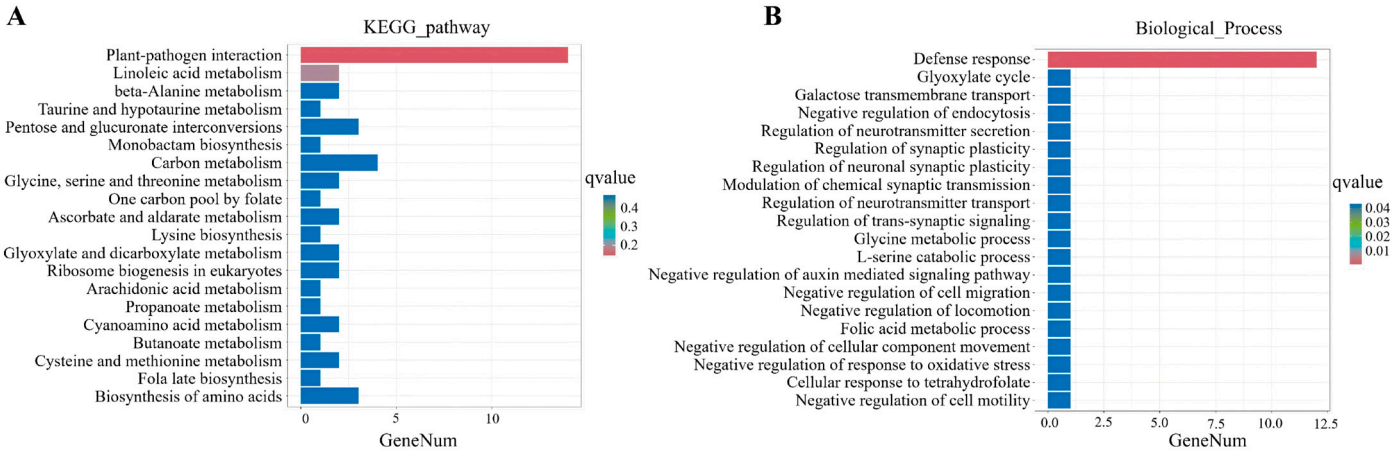


Figure 1. KEGG pathway and GO analysis of up-regulated differentially expressed genes in the transcriptome of wild-type 93-11 and mutant *htr.* (**A**) KEGG pathway enrichment analysis. (**B**) GO enrichment analysis (biological processes).

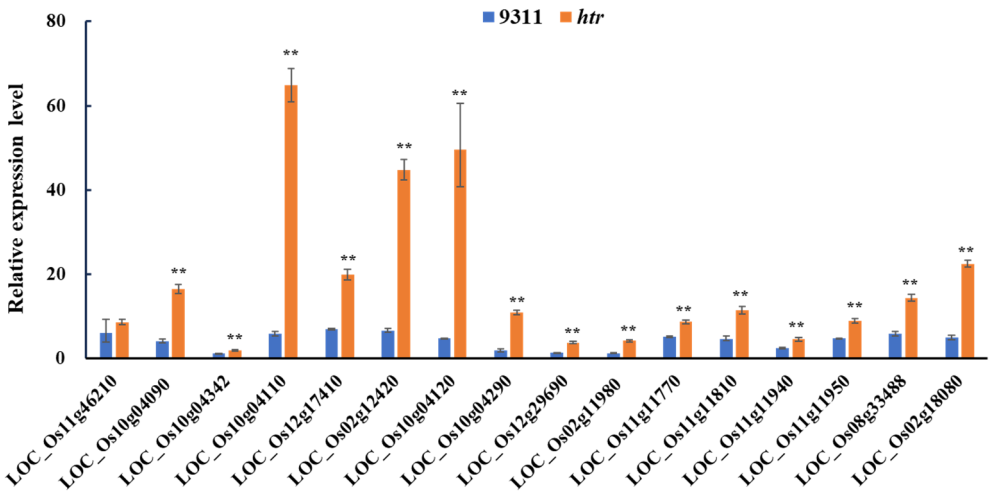


Figure 2. Wild-type 93-11 and mutant *htr* transcriptome upregulated differentially expressed genes real-time PCR validation. Values are the mean ± SD of three biological replicates. Asterisks indicate a statistically significant difference between the wild type and *htr* mutant. *t*-test, ** $p < 0.01$. Data are presented as mean ± SD ($n = 3$).

3.2. Generation and Validation of OsHTR Transgenic Lines

To further investigate the role of *OsHTR* in disease resistance, we generated knockout (*HTR-KO-1*, *HTR-KO-2*) and overexpression (*HTR-OE-1*, *HTR-OE-2*) transgenic lines in the ZH11 background. Successful gene editing and overexpression were confirmed by sequencing and RT-qPCR (Figure S1; Table S4). We then selected eight resistance-related genes that were upregulated in the htr mutant (*LOC_Os10g04090*, *LOC_Os12g17410*, *LOC_Os02g12420*, *LOC_Os02g11980*, *LOC_Os11g11810*, *LOC_Os11g11940*, *LOC_Os08g33488*, and *LOC_Os02g18080*) for expression analysis in the transgenic lines. The results confirmed that these genes were also upregulated in the knockout lines in the ZH11 background, consistent with the observations in the *htr* mutant (Figure 3). Although the transcriptome analysis was performed in the indica background 93-11, the similar upregulation patterns in the japonica background ZH11 suggest that the negative regulatory relationship between *OsHTR* and these defense genes is conserved across these two subspecies. This indicates that *OsHTR* functions similarly in both *indica* (93-11) and *japonica* (ZH11) backgrounds in regulating the expression of defense-related genes.

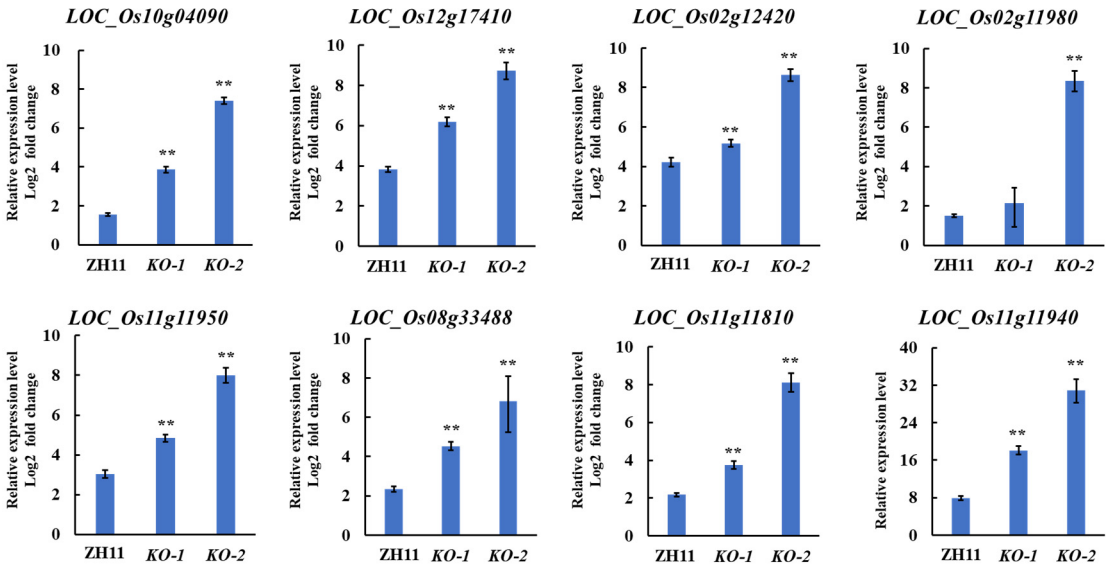


Figure 3. Real-time PCR analysis of differentially upregulated genes in ZH11 and *OsHTR* knockout mutants and overexpression plants. Values are the mean ± SD of three biological replicates. *t*-test, ** $p < 0.01$. Data are presented as mean ± SD ($n = 3$).

3.3. OsHTR Negatively Regulates Rice Blast Resistance

We evaluated the blast resistance of *OsHTR* transgenic lines alongside wild-type ZH11 and control cultivars CO39 (susceptible) and Gumei 4 (resistant) using three geographically distinct mixed strain inocula (Yunchuan, Hunan, and Northeast mixed strains) (Table 1; Figure 4). The susceptible control CO39 exhibited high susceptibility (grade 8–9) to all three mixed strains, while the resistant control Gumei 4 displayed high resistance (grade 0) across all strains. Wild-type ZH11 showed high resistance (grade 0–1) to all three mixed strains. Thus, the wild-type background already exhibits strong resistance against these strain mixtures. Notably, the *HTR-OE* lines consistently displayed moderate resistance (grade 2–3) across all three inocula, indicating that overexpression of *OsHTR* compromises blast resistance. In contrast, the *HTR-KO* lines maintained or even enhanced resistance. Specifically, *HTR-KO-2* exhibited high resistance (grade 0) to the Yunchuan and Northeast strains and resistance (grade 1) to the Hunan strain. These results demonstrate that *OsHTR* functions as a negative regulator of blast resistance and that loss of its function can enhance resistance even in an already resistant background.

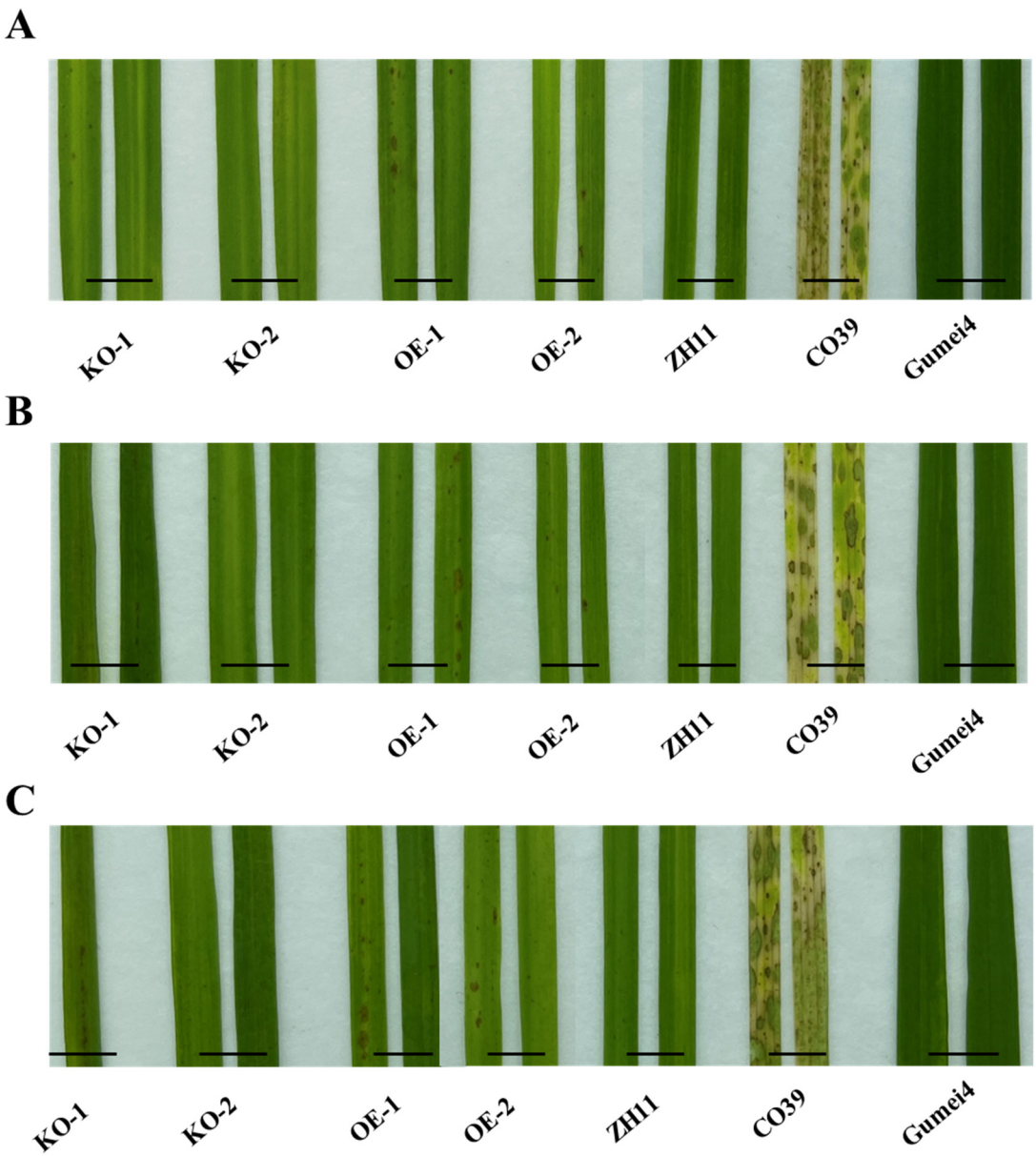


Figure 4. Identification of rice blast resistance in ZH11 and *OsHTR* knockout mutants and overexpression plants. (**A**), Yunchuan mixed strains; (**B**), Hunan mixed strains; (**C**), Northeast mixed strains. Data are representative images from one of three independent experiments, each with at least 12 seedlings per line.

Table 1. Rice blast disease resistance inoculation identification.

Material	Resistance Level (0–9)		
	Yunchuan Mixed Strains	**Hunan Mixed Strains**	**Northeast Mixed Strains**
ZH11	0 (HR)	0 (HR)	1 (R)
HTR-KO-1	0 (HR)	1 (R)	1 (R)
HTR-KO-2	0 (HR)	1 (R)	0 (HR)
HTR-OE-1	3 (MR)	3 (MR)	3 (MR)
HTR-OE-2	3 (MR)	2 (R)	3 (MR)
LOC_Os10g04090-KO	3 (MR)	3 (MR)	0 (HR)
LOC_Os12g29690-KO	3 (MR)	3 (MR)	1 (R)
LOC_Os02g11980-KO	0 (HR)	2 (R)	0 (HR)

Table 1. *Cont.*

Material	Resistance Level (0–9)		
	Yunchuan Mixed Strains	**Hunan Mixed Strains**	**Northeast Mixed Strains**
LOC_Os11g11770-KO	0 (HR)	2 (R)	3 (MR)
LOC_Os06g03710-KO	0 (HR)	0 (HR)	1 (R)
CO39	9 (HS)	9 (HS)	8 (S)
Gumei4	0 (HR)	0 (HR)	0 (HR)

Footnote: HR, highly resistant (grade 0); R, resistant (grade 1–2); MR, moderately resistant (grade 3–4); S, susceptible (grade 7–8); HS, highly susceptible (grade 9). Values represent disease scores based on the 0–9 scale described in Section 2.

3.4. Knockout of Individual Upregulated Resistance Genes Confers Blast Resistance

To validate the functional relevance of the upregulated resistance-related genes identified downstream of *OsHTR*, we generated knockout lines for five candidate genes: *LOC_Os10g04090*, *LOC_Os12g29690*, *LOC_Os02g11980*, *LOC_Os11g11770*, and *LOC_Os06g03710* (the latter being an interacting gene of *OsHTR* [21]) using CRISPR/Cas9 gene editing (Table S4). Blast resistance evaluation results are shown in Table 1 and Figure 5. The *LOC_Os10g04090-KO* and *LOC_Os12g29690-KO* lines showed significantly reduced resistance to the Yunchuan and Hunan mixed strains (grade 3) compared to wild-type ZH11, while no significant change was observed against the Northeast mixed strains. The *LOC_Os02g11980-KO* line exhibited significantly reduced resistance to the Hunan mixed strain (grade 2) but no significant change against the Yunchuan and Northeast strains. The *LOC_Os11g11770-KO* line displayed significantly reduced resistance to the Hunan and Northeast mixed strains (grade 2–3) but no significant change against the Yunchuan strain. Interestingly, the *OsHTR*-interacting gene *LOC_Os06g03710-KO* maintained high resistance (grade 0–1) consistent with wild-type ZH11 against all three mixed strains. The observation that individual KO lines generally show less pronounced phenotypes than the *HTR-KO* lines suggests functional redundancy among these downstream genes. These results confirm that the upregulated genes downstream of *OsHTR* are bona fide resistance-related genes and that their individual knockout can confer significant changes in blast resistance.

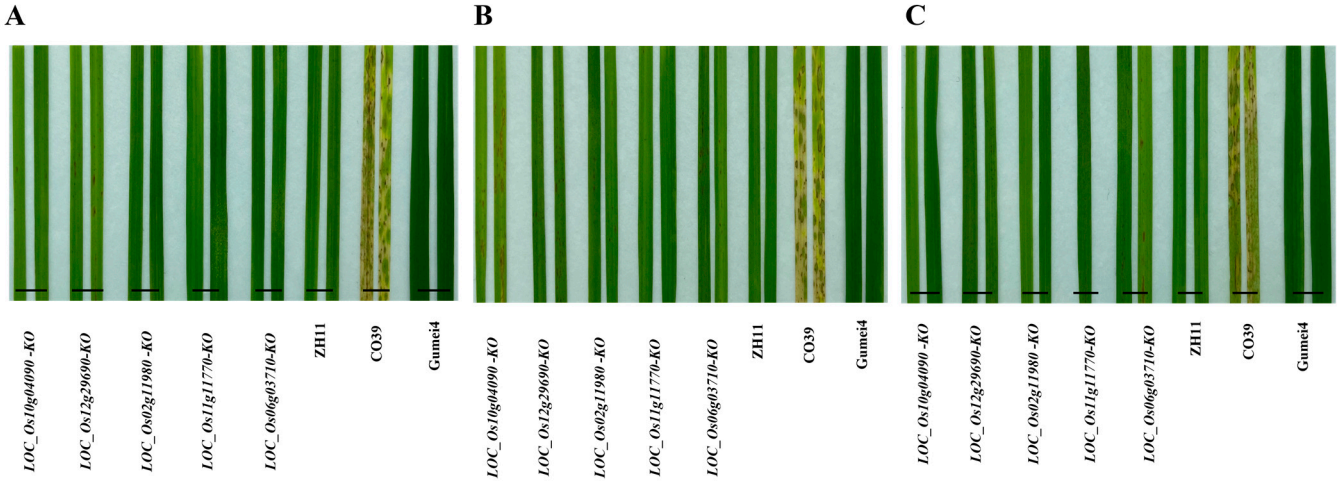


Figure 5. Identification of rice blast resistance in ZH11 and gene-edited knockout mutants. (**A**), Yunchuan mixed strains; (**B**), Hunan mixed strains; (**C**), Northeast mixed strains. Data are representative images from one of three independent experiments, each with at least 12 seedlings per line.

3.5. OsHTR Regulates Bacterial Blight Resistance

We further evaluated bacterial blight resistance using three *Xoo* strains (FuJ, GD1358, and HNA1-4). The evaluation included wild-type ZH11, *OsHTR* transgenic lines, and knockout lines of the downstream resistance genes (Table 2; Figure 6; Table S5). The susceptible control TN1 exhibited lesion lengths > 24.0 cm (grade 7) for all three strains, while the resistant control R498-*Xa23* showed lesion lengths < 1.0 cm (grade 1). Compared to ZH11, the *HTR-KO* lines exhibited significantly shorter lesion lengths, indicating enhanced resistance, particularly against the GD1358 strain (grade 1). In contrast, the *HTR-OE* lines showed variable results: *HTR-OE-1* did not differ significantly from ZH11 across all strains, while *HTR-OE-2* showed a modest increase in lesion length for the GD1358 strain (grade 5). This inconsistency may reflect position effects of the transgene or strain-specific interactions. Among the downstream gene knockout lines, *LOC_Os10g04090-KO* and *LOC_Os02g11980-KO* showed enhanced resistance (shorter lesion lengths) against the GD1358 strain compared to ZH11, while no significant changes were observed for the other knockout lines against the three tested *Xoo* strains (Table S5 and Figure S3). These results indicate that *OsHTR* acts as a negative regulator of bacterial blight resistance and that loss of its function can enhance resistance, although the downstream genes may play distinct roles against different bacterial strains.

Table 2. Bacterial blight resistance inoculation identification.

Strain	Lesion Length (cm) and Resistance Level						
	HTR-KO-1	***HTR-KO-2***	***HTR-OE-1***	***HTR-OE-2***	**ZH11**	**TN1**	**R498-Xa23**
FuJ	5.5 ± 0.3 (3)	3.4 ± 0.2 (1)	6.2 ± 0.3 (3)	8.2 ± 0.4 (3)	7.3 ± 0.4 (3)	25.1 ± 2.5 (7)	0.6 ± 0.1 (1)
GD1358	3.5 ± 0.2 (1)	4.4 ± 0.2 (1)	6.1 ± 0.2 (3)	11.0 ± 0.8 (5)	6.6 ± 0.3 (3)	24.4 ± 2.0 (7)	0.6 ± 0.1 (1)
HNA1-4	5.4 ± 0.3 (3)	4.9 ± 0.1 (1)	7.8 ± 0.5 (3)	9.6 ± 0.3 (3)	9.9 ± 1.1 (3)	26.6 ± 2.4 (7)	0.6 ± 0.1 (1)

Footnote: Values represent lesion length (cm) ± SD. Numbers in parentheses indicate resistance grades: 1 = lesion extends downward, length 2–3 cm, 3 = lesion length < 1/4 of the total leaf length, 5 = lesion length ≥ 1/4 but <1/2 of the leaf length, 7 = lesion length ≥ 1/2 but <3/4 of the leaf length. Each experiment included at least 7 leaves per line per strain, with three independent biological replicates.

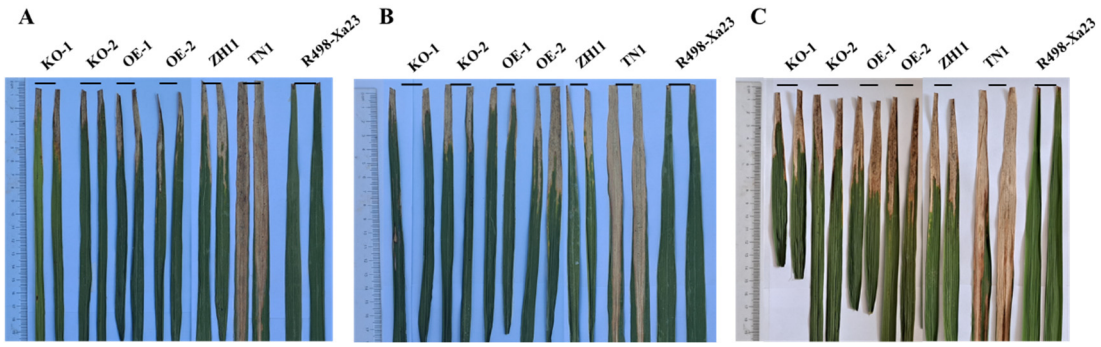


Figure 6. Identification of bacterial blight resistance in ZH11 and *OsHTR* knockout mutants and overexpression plants. (**A**), FuJ strain; (**B**), GD1358 strain; (**C**), HNA1-4 strain. Representative images from one of three independent experiments, each with at least 7 leaves per line.

3.6. OsHTR Does Not Affect Brown Planthopper Resistance

To determine whether *OsHTR*-mediated resistance extends to insect pests, we evaluated brown planthopper resistance of *OsHTR* transgenic lines and the downstream resistance gene knockout lines (Table S6; Figures S2 and S4). All tested materials, including wild-type ZH11, *HTR-KO*, *HTR-OE*, and the five downstream gene knockout lines, exhibited high susceptibility (HS, grade 9) to brown planthopper, with mortality rates ranging from 88.30% to 100.00%. In contrast, the resistant control Rathu Heenati showed high resistance (HR, grade 1) with only 2.00% mortality. These results demonstrate that neither

OsHTR nor its downstream target genes contribute to brown planthopper resistance, suggesting that the *OsHTR* regulatory module is specific to microbial pathogen defense rather than insect resistance.

4. Discussion

In this study, we demonstrate that the AP2-type transcription factor *OsHTR* functions as a negative regulator of disease resistance in rice against both fungal and bacterial pathogens. Our findings provide several novel insights into the molecular mechanisms underlying rice immunity and the functional diversification of AP2 transcription factors.

4.1. OsHTR Bridges Development and Defense

OsHTR was previously characterized as a pleiotropic regulator of plant architecture and hormone signaling, with roles in brassinosteroid, gibberellin, and auxin pathways [11–16]. Its involvement in nitrogen uses efficiency and grain size determination further highlighted its significance in agronomic trait regulation [15,16]. Our transcriptome analysis revealed that the *htr* mutant exhibits significant upregulation of defense-related genes, particularly those encoding NBS-LRR and NB-ARC resistance proteins, suggesting that *OsHTR* normally suppresses defense gene expression under non-stress conditions. This is consistent with the emerging paradigm that plants must balance growth and defense, and that certain transcription factors function as nodes for this trade-off [22,23]. Recent studies have identified analogous mechanisms in other plant species: in *Arabidopsis*, the homeobox transcription factor HB34 balances immunity and growth by differentially regulating jasmonic acid biosynthetic and growth-related genes [24]. In rice, ERF transcription factor regulons have been shown to underpin the growth–defense trade-off under acute heat stress [10]. The enhanced disease resistance observed in *HTR-KO* lines suggests that targeted modulation of *OsHTR* could potentially decouple resistance from yield penalties—a major challenge in resistance breeding. However, given the previously reported effects of *OsHTR* on grain size, tillering, and nitrogen use efficiency [11–16], any potential application in breeding must carefully assess possible pleiotropic effects on agronomic traits under field conditions.

4.2. A Regulatory Module Involving OsHTR and Downstream Resistance Genes

The identification of 15 upregulated resistance-related genes in the *htr* mutant and their validation in transgenic lines establishes a transcriptional correlation in which *OsHTR* negatively correlates with multiple defense genes. Among these, *LOC_Os10g04090*, *LOC_Os12g29690*, *LOC_Os02g11980*, and *LOC_Os11g1177* were functionally validated through targeted knockout, each contributing to blast and bacterial blight resistance to varying degrees. Our data do not distinguish between direct and indirect regulation; determining whether *OsHTR* directly binds to the promoters of these genes will require ChIP-seq or yeast one-hybrid assays. The differential resistance levels observed among the knockout lines against different pathogen strains suggest that these genes may recognize distinct pathogen effectors or function in different branches of the immune signaling network. The involvement of NBS-LRR and NB-ARC family members among these downstream genes is consistent with recent meta-QTL analyses, which identified that NBS-LRR type resistance gene analogs and NB-ARC domain-containing proteins constitute major components of the rice blast resistance gene network [25]. Additionally, novel blast resistance genes such as *OsBRW1* have recently been shown to encode NBS-LRR proteins that interact with host factors to balance resistance and growth [26].

4.3. Disease-Specific Regulation by OsHTR

Interestingly, neither *OsHTR* transgenic lines nor the downstream gene knockout lines showed any resistance to brown planthopper, with all lines exhibiting high susceptibility comparable to the susceptible control TN1. This stark contrast between pathogen and insect resistance indicates that the *OsHTR* regulatory module is specific to microbial pathogen defense. The differential effectiveness likely reflects the distinct molecular mechanisms underlying resistance to biotrophic/hemibiotrophic pathogens versus phloem-feeding insects. Plant resistance to pathogens often involves NBS-LRR-mediated ETI and hormonal signaling through salicylic acid and jasmonic acid pathways, whereas resistance to brown planthopper typically involves distinct resistance genes and signaling mechanisms. A growing body of evidence has demonstrated that AP2/ERF transcription factors are key regulators of plant disease resistance, acting downstream of MAPK cascades and modulating hormonal signaling pathways, secondary metabolite synthesis, and physical barrier formation [10]. Our results suggest that the *OsHTR*-regulated defense genes are primarily components of antimicrobial immunity rather than anti-herbivory defense, reinforcing the functional specialization of distinct AP2/ERF family members in different biotic stress contexts.

4.4. Implications for Molecular Breeding

The discovery that *OsHTR* negatively regulates disease resistance has significant implications for rice improvement. Natural variation in *OsHTR* expression levels has been reported across diverse rice germplasm [15], and our results suggest that low-expression alleles may confer enhanced disease resistance. This makes *OsHTR* a potential target for marker-assisted selection or gene editing to improve disease resistance. However, caution is warranted because OsHTR also controls grain size, tillering, and nitrogen use efficiency [15,16]; therefore, knockout or suppression of *OsHTR* may negatively impact yield under field conditions. Field evaluations under natural disease pressure and normal agronomic management are essential before any breeding application. Furthermore, the identification of downstream resistance genes provides additional genetic resources for pyramiding resistance in elite cultivars [27,28]. Given that rice blast alone can cause yield losses of 10–35% globally, stacking multiple effective resistance genes is an essential strategy for durable resistance breeding. Additionally, executor R genes such as *Xa7*, *Xa10*, *Xa23*, and *Xa27* provide broad-spectrum resistance against *Xoo* by being specifically induced by pathogen TAL effectors [27,29], and the genes identified in this study may complement existing *Xa* genes in pyramiding strategies. Future studies should investigate the natural allelic variation at these loci and their potential utility in breeding programs.

4.5. Future Perspectives

While this study establishes *OsHTR* as a negative regulator of disease resistance, several questions remain to be addressed. The direct molecular mechanism by which *OsHTR* represses downstream resistance genes—whether through direct promoter binding or indirect regulation via intermediate factors—requires further investigation through ChIP-seq and protein–protein interaction studies. Understanding whether *OsHTR* functions as a direct transcriptional repressor of defense genes or acts by modulating hormone signaling pathways is a critical next step. The recent characterization of *GR5* (the same gene) as a transcriptional activator that physically interacts with G protein γ subunits and determines grain size suggests that *OsHTR* may exert its developmental functions through distinct transcriptional activation mechanisms while simultaneously repressing defense genes via a different mode of action [20]. This bifunctional behavior would position *OsHTR* as a central regulatory hub for the growth–defense trade-off. Additionally, the

role of phytohormone signaling in mediating the *OsHTR*–defense connection needs to be elucidated, given the established involvement of *OsHTR* in multiple hormone pathways. Field evaluations of *HTR-KO* lines are necessary to assess the durability and stability of enhanced resistance under natural disease pressure and to evaluate potential yield trade-offs under agronomic conditions. Finally, exploring whether the *OsHTR*-mediated regulatory module is conserved across other cereal crops could extend the translational relevance of our findings.

5. Conclusions

This study uncovers a novel function of the AP2-type transcription factor *OsHTR* as a negative regulator of disease resistance in rice. We demonstrate that loss of *OsHTR* function leads to upregulation of multiple defense-related genes and enhanced resistance to rice blast and bacterial blight, while overexpression increases susceptibility. The downstream resistance genes identified in this study represent valuable resources for molecular breeding. Our findings expand the functional repertoire of *OsHTR* beyond development and nutrient use efficiency to include disease resistance, highlighting its potential as a target for improving multiple agronomic traits simultaneously, although potential trade-offs with yield-related traits require careful evaluation. This work provides a foundation for understanding the molecular coordination of growth, development, and immunity in rice and offers practical tools for developing disease-resistant cultivars.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy16131213/s1, Table S1. Up-regulated genes in flag leaf of *htr* mutant; Table S2. Down-regulated genes in flag leaf of *htr* mutan; Table S3. Information on 16 upregulated resistance-related genes in *OsHTR* mutants; Table S4. Gene editing mutation sites; Table S5. Bacterial blight resistance inoculation identification for downstream gene knockout lines; Table S6. Brown planthopper resistance identification for *OsHTR* transgenic lines and downstream gene knockout lines; Table S7. Information on the real-time PCR primers used in this study; Table S8. Gene editing target primer; Figure S1. Creation of *OsHTR* knockout mutants and overexpressing plants in the background of *japonica* rice ZH11 for verification; Figure S2. Identification of brown planthopper resistance in ZH11 and *OsHTR* knockout mutants and overexpression plants; Figure S3. Identification of bacterial leaf blight resistance in ZH11 and gene-edited knockout mutants; Figure S4. Identification of brown planthopper resistance in ZH11 and gene-edited knockout mutants.

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Data Availability Statement: Data are contained within the article. The raw sequence data of transcriptome sequencing reported in this paper have been deposited in the NCBI's Sequence Read Archive (SRA) of PRJNA1469631 that are publicly accessible at https://dataview.ncbi.nlm.nih.gov/object/PRJNA1469631 (accessed on 24 May 2026). The original contributions presented in the study are included in the article/Supplementary Materials, and further inquiries can be directed to the corresponding author.

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