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Fine mapping and Candidate Gene Identification of a Novel Rice Bacterial Blight Resistance Gene Xa51(t) from Yunnan Landrace Haonuoyang

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Research Article

Keywords: Xanthomonas oryzae, Recombinant inbred line (RIL), Resistant gene Xa51(t), Fine mapping, GRAS family protein

Posted Date: September 22nd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10567798/v1

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Additional Declarations: No competing interests reported.

Abstract

Bacterial blight (BB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most severe limitations on rice production. The most renewable strategy to combat this disease is the spread of host plant resistance. In a previous study, we identified an introgression line (H197) derived from the Yunnan landrace rice variety Haonuoyang that possesses extensive resistance to *Xoo*. To analyze the inheritance of resistance in Haonuoyang and to identify genomic regions Linked to BB resistance, an F_2 population stemmed from a cross between H197 and Jingang 30 was utilized to verify and fine-map the BB resistance-related gene in H197. The observation of a 3:1 phenotypic ratio (resistant: susceptible) in the F_2 population suggests that a single dominant gene controls the resistance in H197. In the F_3 generation, the constructed bulks, consisting of 47 resistant and 47 susceptible lines, were genotyped using SLAF-seq a high-throughput reduced-representation genome sequencing approach. This has enabled the discovery and analysis of thousands (40K) of single nucleotide polymorphism (SNP) markers at the genome level. By compound interval mapping, a main locus was identified between SNP markers Chr11.28863864 and Chr11.28894443, and the locus was named *Xa51(t)*. Five putatively expressed genes were identified within the genomic region between the two markers flanking *Xa51(t)*. Based on cloning and sequence comparison of these genes in the parents, *Os11g47910* (encoding a GRAS domain-containing protein) was found to be the most candidate gene for BB resistance. A 250-bp deletion at the 3′end of the gene was detected between Haonuoyang and Jingang30. The association of *Os11g47910* with BB resistance was further confirmed by sequence-based DNA marker analysis in the RIL population. A co-segregating PCR-based(Kompetitive Allele Specific PCR)KASP marker was developed for marker-assisted breeding. The cloning of this novel BB resistance gene *Xa51(t)* not only enriched the molecular mechanism study of rice disease resistance but also expanded the genetic basis of resistance breeding by tapping excellent gene resources from Yunnan local rice landraces, laying a solid foundation for efficient marker-assisted breeding of durable disease-resistant rice varieties.

1. Introduction

Rice (*Oryza sativa* L.) is the most important staple food crop for more than 3.5 billion people worldwide, underpinning food security, livelihoods, and economic development across Asia, Africa, and Latin America. By 2050, the global population is expected to reach 9.7 billion, growing rice output potential and ensuring production steadiness have become critical challenges for global agriculture (Faostat, 2023). Rice productivity is economically constrained by various biotic stresses, including plant diseases (Ashwini et al., 2023; Ullah et al., 2022). *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) the causal agent of rice bacterial blight (BB), it is one of the disease threats to rice production all over the world (Lin et al., 1996; Ou, 1985). The disease occurs globally, particularly in rice-producing countries in South and Southeast Asia, resulting in severe yield losses that can reach 50–80% in epidemic years (Ashwini et al.,2023; Chen et al., 2011). BB is widely distributed in all rice-growing regions of China (Zhang, 2005; Zhang, 2009).

The management of rice diseases such as blast (Kalboush et al., 2023) and BB mainly relies on chemical pesticides. However, this strategy often proves ineffective, costly, and environmentally harmful because of variations in the sensitivity of pathogenic races to chemical treatments (Kalboush et al., 2025; Zhang, 2009). In addition, the emergence of mutations in pathogenic races represents a major obstacle to durable disease control and normally leads to the breakdown of newly released rice cultivars within only a few years (Kalboush et al., 2023). Crossbreeding has long been the primary method for introducing disease-resistance genes into rice cultivars. The most cost-effective strategy has been the development and utilization of disease-resistance genotypes (Kalboush et al., 2023; Kumar et al., 2020). Since the first BB resistance gene *Xa1* was identified in the 1960s, substantial progress has been made in the discovery and functional characterization of *Xa* genes. To date, about 50 *Xa* genes in the rice genome have been known and mapped across, with 18 located on the long arm of chromosome 11, a well-known hotspot for BB resistance gene clustering (Deng et al., 2025; Li et al., 2025; Li, 2022). *Xa1*, *xa3 / Xa26*, *xa5, xa13, Xa21, Xa23* and *xa27* have been cloned using map-based cloning approaches (Yang et al., 2022). With the rapid advancement of high-throughput genotyping technologies, whole-genome sequencing (WGS) and RNA-seq have provided new strategies for studying resistance genes. For example, *Xa39* and *Xa40* were identified through a combination of map-based cloning and high-throughput sequencing (Kim et al., 2015; Zhang et al., 2015).

Yunnan is one of the centers of origin and diversification of cultivated rice in Asia and harbors abundant and diverse rice genetic resources. The local *indica* rice variety Haonuoyang from Gengma County, Yunnan Province, has been used as a reference (control) variety for BB resistance in the Yunnan Plateau because of its broad-spectrum resistance to BB (Peng et al., 2011). We have successively identified pathogenic types among more than 180 rice BB isolates collected from central, northwestern, and southern Yunnan Province in 2003, 2005, 2007, and 2009. Haonuoyang exhibited resistance to all tested strains (He et al., 2020; Peng et al., 2011). Remarkably, Haonuoyang has maintained stable BB resistance in the field for nearly 20 years, even in the presence of high genetic diversity and rapid virulence differentiation within *Xoo* populations in the Yunnan Plateau (Zhang, 2009). This long-term stable resistance strongly suggests that Haonuoyang carries novel BB resistance gene(s) that evolved through long-term co-evolution with local *Xoo* strains within the unique plateau ecological environment (He et al., 2020). Early allelism tests conducted in 1996 using crosses between Haonuoyang and rice lines carrying known *Xa* genes demonstrated that the dominant resistance gene in Haonuoyang is non-allelic to *Xa1, Xa2, xa3, Xa4, xa5*, and *Xa14*, providing the first evidence that Haonuoyang harbors a previously uncharacterized BB resistance gene (He et al., 2020; Zhang, 2005).

Our previous genetic analysis showed that the F_1 plants from the cross between Haonuoyang and the susceptible cultivar Jingang30 (JG30) were resistant to the Philippine *Xoo* race PXO99 (P6), and the F_2 population segregated in a 3:1 ratio of resistant to susceptible plants, indicating that BB resistance in Haonuoyang is controlled by a single dominant nuclear gene, temporarily designated *Xa51(t)*. The disease-resistance gene carried by Haonuoyang was preliminarily mapped using the bulked segregant analysis (BSA) method based on Specific-Locus Amplified Fragment simplified genome sequencing (SLAF-seq) technology, and the open reading frames (ORFs) within the chromosomal region were predicted. In this study, we developed the advanced introgression line H197 (BC_4F_2) using Haonuoyang as the donor parent and JG30 as the recurrent parent, which showed high resistance to PXO99. We evaluated the resistance spectrum of Haonuoyang and H197 against eight virulent *Xoo* strains, performed genetic analysis, and fine-mapped the *Xa51(t)* locus using F_2 to F_4 populations derived from the cross between H197 and JG30. Furthermore, we identified the candidate gene for *Xa51(t)* through gene annotation, expression analysis, sequence variation analysis, and transgenic functional validation. This study identifies a novel GRAS-type BB resistance gene from a

Yunnan rice landrace, which not only expands our understanding of the molecular basis of rice BB resistance but also provides a valuable genetic resource and molecular tool for the advancement of durable BB-resistant rice cultivars.

2. Materials and methods

2.1. Plant materials

The rice cultivar Haonuoyang, harvested from Gengma County, Yunnan Province, China, was chosen as the resistant parent based on earlier research showing broad-spectrum resistance to all tested *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strains (He et al., 2020; Yang et al., 2025). In contrast, the indica cultivar Jingang30 (JG30) and Zhenzhuai was chosen as the susceptible parent due to its high susceptibility to the same *Xoo* isolates. H197 represents an advanced introgression line (BC_4F_2) developed through four rounds of backcrossing using Haonuoyang as the resistance donor and JG30 as the recurrent parent. F_1 progeny from the cross H197 and JG30 were analyzed to characterize the inheritance mode (dominant or recessive) of the BB resistance locus. Subsequently, 2089 F_2 populations ,2191 F_3 populations,7480 F_4 populations segregants derived from selfed F_1 individuals were subjected to genetic mapping.

Twenty-four rice genotypes are shown in (Supplementary Table S1) were evaluated by eight virulent *Xoo* strains to select the aggressive strain and find effective resistance genes based on its resistance level by monogenic rice lines as well as, the F_1 and F_2 populations derived from the cross between Haonuoyang and JG30, which were used to evaluate its resistance level under artificial inoculation against *Xoo* strains. The selected *Xoo* strain used to evaluated the resistance spectra of the parental lines and examine the phenotypic reactions of F_1, F_2 and $F_{3:4}$ populations.

2.2. *Xoo* strains and Inoculum Preparation

Eight virulent *Xoo* strains were used in this study. Three strains originated from Yunnan Province, China, including CX28-3, CX30-1 (isolated from Chuxiong City) and 2001-28 (isolated from Dali City). The remaining five strains (PXO61, PXO86, PXO99, PXO112, and PXO339) were obtained from the International Rice Research Institute (IRRI), Philippines. The *Xoo* strains kept in glycerol at-80℃were used in this study. The revived and cultured on nutrient agar (NA) medium (containing beef extract 3g, peptone 5g, glucose 2.5g, agar 18g and distilled water 1000 mL, pH adjusted to 7.0-7.2) at 28℃. For inoculation the bacterial culture was purified via two rounds of streak plating and adjusted to a concentration of 3×10^8 CFU·mL^{-1} (OD_{600} = 0.5).

2.3. Field Experimental, Artificial Inoculation, assessment of resistance to *Xoo* strains

The field experiments were conducted at Songming Scientific Research Base, Yunnan Academy of Agricultural Sciences.The seeds of the different rice genotypes were surface-sterilized in a 5% sodium hypochlorite solution for 25 ± 3 min. Afterward, the seeds were thoroughly rinsed five times with distilled water and then germinated at room temperature. The germinated seeds were planted under field conditions as a single plant in five rows; 6 m long and 20cm x 20cm apart among plants and rows. They were arrayed in a randomized complete block design (RCBD) with three replications, with a common package of recommended practices. The rice genotype inoculated at the booting stage with different bacterial strains using the leaf-clipping method and perform within 1 h. (Ji et al., 2016,2022). Following inoculation, the rice plants were maintained at approximately 28 ± 2℃ (average temperature during the infection period) and disease incidence was evaluated after 21 days post-inoculation. For each plant, five inoculated leaves were measured for total length and lesion length to calculate the average lesion length percentage. According to the Standard Evaluation System (SES) for rice of the IRRI (IRRI, 2013), resistance to rice BB was classified into five grades based on lesion length and leaf disease percentage; Grade 1, Resistant (R), with extremely short lesions (< 1 cm) and a leaf disease percentage < 10%; Grade 3, Moderately Resistant (MR), with short lesions (1–5 cm) and a leaf disease percentage of 10%–25%; Grade 5, Moderately Susceptible (MS), with medium lesions (5–10 cm) and a leaf disease percentage of 26%–50%; Grade 7, Susceptible (S), with long lesions (10–15 cm) and a leaf disease percentage of 51%–75%; and Grade 9, Highly susceptible (HS), with extremely long lesions (> 15 cm) and a leaf disease percentage > 75%.

2.4. Allelism Test with Known Resistance Genes

To test the allelic relationship between the resistance gene in Haonuoyang and the known genes *Xa21* and *Xa23*, to check whether the new gene is an allele of either of the known genes. two independent crosses were performed; the first between Haonuoyang and IRBB21 (carrier of *Xa21*), and the second between Haonuoyang and IRBB23 (carrier of *Xa23*). F_1 plants were obtained, and then two F_2 communities were produced by self-pollination. The resistance of the parents, F_1 and F_2 to strain P*XO*99 was assessed. We analyzed the segregation ratios in the two F_2 communities using the χ^2 test at a significance level of 0.05, and tested the hypothesis of two independent genes (segregation ratio 15:1) versus the hypothesis of allelic genes (ratio 3:1).

2.5 Preliminary mapping of the resistance locus

Genomic DNA was extracted from rice leaf tissues following the protocol developed by Murray and Thompson (1980), with only slight modifications applied to the original procedure. From the 305 F_2 individuals derived from the H197/JG30 cross, 47 resistant and 47 susceptible plants were separately screened, and their genomic DNA was isolated and mixed to construct the resistant and susceptible DNA bulks. Genotyping information of the two parents and the contrasting bulks was acquired through SLAF-seq (reduced-representation genome sequencing) (Li et al., 2017). The SNP-index method was used to identify genomic regions associated with bacterial blight resistance by relying on Bulked Segregant Analysis (BSA). The SNP index calculates the proportion of reads carrying the resistant parent's allele in the resistant pool, and the proportion of the susceptible parent's allele in the susceptible pool. The Δ(SNP-index) value was calculated by subtracting the index value of the susceptible pool from that of the resistant pool. A sliding window approach (window size: 1 Mb, step size: 10 kb) was applied to calculate the average difference across the genome. The 95% confidence interval for the null hypothesis (no association) was determined through 1000 permutation tests. Marks with Δ values exceeding the threshold (0.1574 at $p < 0.05$) were considered significantly associated with resistance.

For the primary mapping of the resistance gene in H197, 12 SSR markers uniformly distributed on chromosome 11 were selected from the Gramene database (http://www.gramene.org) and the International Rice Genome Sequencing Project (Project, 2005), with detailed information listed in Supplementary Table S2. To validate the genetic linkage results derived from SLAF-seq technology, 12 SSR markers were selected to cover the long arm of chromosome 11 equally at intervals of approximately 2–3 Mb, based on their physical locations in the Gramene database. This group was specifically chosen for independent verification of SLAF sequencing results using conventional polymerase chain reaction (PCR) genotyping. The selected markers covered the candidate region identified by genomic sequencing (BSA) to confirm association and rule out any false-positive signals resulting from high-throughput sequencing data. These markers were subsequently used for genotyping analysis, Polymorphic variations between H197 and JG30 were identified using PCR amplification. All PCR reactions were performed following the manufacturer's guidelines of the 2×T5 Super PCR Mix (Basic) kit (Tsingke Biotechnology, Beijing, China). The amplified PCR products were separated via electrophoresis on agarose gels (4%) in 1× TAE buffer, and then visualized with GelRed staining.

2.6. Fine mapping of the bacterial blight resistance gene *Xa51*(t)

For the fine mapping of the *Xa51*(t) locus in Haonuoyang, a series of molecular markers comprising 11 SSR, 7 INDEL, 8 iHAD,10 KASP markers were designed to span the target region from 28,848,673 bp to 28,963, 768bp on rice chromosome 11 (Supplementary Tables S2, S3, S4). These markers were designed by screening insertion–deletion (InDel) polymorphisms ranging from 5 to 50 bp between japonica rice 'Nipponbare' (Kawahara et al., 2013; Project, 2005) and indica rice'93 – 11' (Wang et al., 2022), using the NCBI database (https://www.ncbi.nlm.nih.gov/) and Primer Premier 5.0 software (Premier Biosoft, CA, USA).

2.7. Genetic linkage analysis and map construction

The detection of resistance genes was conducted by means of bulked segregant analysis (BSA) as described by (Michelmore et al., 1991). Linkage analysis of the developed molecular markers was carried out using JoinMap 5.0 software, with a logarithm of odds (LOD) score threshold set at 3.0 (Ooijen et al., 2006). Genetic map distances were calculated applying the Kosambi mapping function (Kosambi, 2017). The construction and visualization of genetic linkage maps were accomplished using MapChart 2.32 software (Voorrips, 2002).

2.8. Sequence retrieval and bioinformatic analysis of candidate genes

The reference genomic sequence of japonica rice cultivar Nipponbare, covering the interval between the two flanking markers, was retrieved from the Gramene database. Conserved domain and structural annotation of the candidate gene sequences were carried out using the CDD online tool (https://www.ncbi.nlm.nih.gov/Structure/cdd/ wrpsb.cgi) and the SMART web server (http://smart.embl-heidelberg.de/).

2.9. qRT-PCR analysis of candidate gene expression patterns

Total RNA was extracted from leaves at 0, 12, 24, 48 and 72 h after inoculation with strain PXO99, together with mock-treated leaves treated with distilled water at the same time intervals. The extraction was performed using the Eastep® Super Total RNA Isolation Kit (Promega, Shanghai, China). The first strand of cDNA was prepared using 1 μg of total RNA, using the Vazyme Biotech (Nanjing, China) HiScript® III RT SuperMix kit for qPCR reaction, in compliance with the manufacturer's instructions According to the genomic sequences of the five candidate genes (LOC_Os11g47860, LOC_Os11g47870, LOC_Os11g47890, LOC_Os11g47900, LOC_Os11g47910) obtained from the Gramene database by aligning to the *Oryza sativa* Japonica Group IRGSP-1.0 reference genome, gene-specific qRT-PCR primers were designed using Primer3 Plus online software (http://www.primer3plus.com/), with the rice Actin gene set as the internal reference (Table 1). QRT-PCR amplifications were carried out using ChamQ Universal SYBR qPCR Master Mix (Vazyme Biotech, Nanjing, China) on a LightCycler® 96 real-time PCR system (Roche). The relative expression levels were calculated with the $2^{-\triangle\triangle CT}$ method (Livak and Schmittgen, 2001), and the expression patterns were graphically displayed using GraphPad Prism 8.0.

Table 1
Specific primer pairs for five candidate genes designed by Primer-BLAST based on their genomic sequences exported from Gramene

Target Gene or candidate genes	Name of primers	primer sequence		purpose
LOC_Os11g47860	G1	F	ATGAGGAGGAGTATGTTGTTGC	qPCR
		R	TTATGTTCCCAGCGCGG	
LOC_Os11g47870	G2	F	CGCCTTCCTTGAACCTGT	qPCR
		R	TACATTGCAAATGAAGATATTATATCATGTAGGAG	
LOC_Os11g47890	G3	F	ATGGCTGCCGCGCCGAAG	qPCR
		R	TCTAATGTAGCTCTAACCGTGCATCTAATTTACAGTGCAAAT	
LOC_Os11g47900	G4	F	GGGACGGTCAATAGCAGCAA	qPCR
		R	GGTAGTGGTCAGTGGTCACC	
LOC_Os11g47910	G5	F	ATGGCCAACCCAGAGGATTT	qPCR
		R	TCATCCGGAGGAATTGTCTTGTTGT	
OsActin		F	GAGTATGATGAGTCGGGTCCAG	qRT-PCR
		R	ACACCAACAATCCCAAACAGAG	

2.10 Transmission electron microscopy observation

During the booting stage, leaf samples were collected from the inoculation sites of HNY and JG30 at five time periods (0, 1–3, 5, 9, 14, and 21 d) after inoculation with strain PXO99. To ensure an appropriate size for further processing, the sampling section was restricted to no more than 2 mm × 1.5 mm × 1 mm. The collected samples were immediately fixed in 2.5% glutaraldehyde and placed in a vacuum pump for 5–8 min, then rinsed three times with phosphate-buffered saline (PBS) buffer, with each rinse lasting 15 min. The remaining rinse solution was carefully removed from the centrifuge tube. Subsequently, 1% osmium tetroxide was added, and the samples were left for 1.5–2 h to complete fixation. Following fixation, the samples were soaked in PBS buffer and rinsed three times. Dehydration was approved out through six gradients. For the fourth and fifth dehydration steps, anhydrous ethanol was used as the dehydrating agent, with each step lasting 20 min. For the final dehydration stage, anhydrous acetone was used for 20 min. After successful dehydration, the samples were infiltrated, embedded, polymerized, and used for subsequent microscopic observation. To enhance the accuracy and reliability of the electron microscopy observations, 6–9 independent leaves were excised from each target sample to ensure that each independent leaf yielded three ultrathin sections. Observation and photography were conducted using a Leica transmission electron microscope (Kim et al., 2002). The main area of focus was the vascular bundle cells at the rice inoculation site (Hassan et al., 2023).

2.11. *Xa51*(t) subcellular localization

Using the corresponding sequence of the rice gene *Os11g47910* from *Oryza sativa* in the NCBI database as a template, specific primers GFP (+) and GFP (−) for the target gene were designed (Supplementary Table S5). The full-length ORF sequence of *Os11g47910* was amplified using 2× Biorun Pfu PCR Mix (Biorun Biotechnology, Wuhan, China). The reaction system was according to the manufacturer's protocols. Gel electrophoresis was used to detect the target fragment, and the gel was used to recover and purify the PCR product (Murray and Thompson, 1980). We performed double-enzyme digestion on the recovered PCR product and ligated the target vector into the recipient cell DH5α. A total of ten positive clones from single colonies were selected and sent for sequencing. Positive clones matching the target sequence were stored at -80 ℃ for further use.

2.12. Transient expression and subcellular localization of target genes in tobacco leaves

The plasmid was transformed into *Agrobacterium* GV1301 by heat shock (incubating recipient cells pre-stored at − 80 ℃ on ice until nearly thawed, immediately freezing them in liquid nitrogen for 1 min, bathing them in 37 ℃ water, and repeating this step) (Berestovoy et al., 2018). After successful transformation, the cells were cultured in a dark environment at 30 ℃ for 2 days. A single colony was selected under sterile conditions and cultured in liquid LB medium containing dual antibiotics (kanamycin and rifampicin) until the logarithmic growth phase. The bacterial solution was centrifuged at low speed for 5 min. The milky white precipitate at the bottom of the centrifuge tube was collected and diluted with *Agrobacterium* suspension until the final concentration reached OD_{600}= 0.7–0.9. A needle-free syringe was used to aspirate the *Agrobacterium* suspension and inject it into the abaxial side of tender tobacco seedling leaves. The injected leaves were labeled and incubated at room temperature in the dark for 2 days. Laser confocal microscopy was used for observation, recording, and photography (Berestovoy et al., 2018).

2.13. Construction of expression vectors

For RNA interference (RNAi) vector construction, three gene-specific primers (G8F, G8R, and G8-loop; sequences listed in Supplementary Table 5) were designed according to the coding sequence of *Os11g47910*. PCR amplification was carried out using cDNA from the resistant genotype Haonuoyang (HNY) as the template. The amplification mixture was identical to that described in section 2.9. The amplified fragments were purified by agarose gel extraction. Both the purified PCR products and the pBWA(V)HS empty vector were digested with the restriction enzymes BsaI/Eco31I (Biorun biotechnology, Wuhan, China), and the resulting fragments were ligated into the linearized vector using T4 DNA ligase (Biorun biotechnology, Wuhan, China). The ligation products were transformed into *Escherichia coli* DH5α competent cells. Recombinant clones carrying the correct insert were verified by colony sequencing and selected for subsequent plant transformation experiments.

For construction of the overexpression vector, specific primers H1(+) and H1(−) were designed based on the *Os11g47910* sequence from the Nipponbare cultivar (NCBI database). PCR amplification was performed using Nipponbare cDNA as the template under the same reaction conditions and cycling parameters described above. The amplified fragment was inserted into the pBWA(V)HS expression vector following the same restriction digestion and ligation procedure. The recombinant plasmids were introduced into DH5α competent cells, and positive clones were confirmed by sequencing before being used for genetic transformation.

For CRISPR/Cas9-mediated gene editing, target sequences and corresponding primers (GH998-Y and GH998-B; sequences listed in Supplementary Table 5) were designed based on the *Os11g47910* sequence obtained from the Nipponbare genome in the NCBI database (Ma et al., 2015). The amplification mixture was identical to that described in section 2.9. The amplification program followed the same thermal cycling conditions described above. The amplified target fragment and the pYL-Hu-Cas9pl empty vector were digested with BsaI/Eco31I, and the resulting fragments were ligated using T4 DNA ligase to generate the recombinant vector pYL-Hu-Cas9pl-G5. The ligation mixture was introduced into *E. coli* competent cells by electroporation. Positive colonies containing the correct construct were identified by sequencing and subsequently used for rice transformation.

2.14 Generation of transgenic rice plants

The production of RNAi interference lines (HNY genetic background), gene knockout lines (HNY background), and overexpression lines (Nipponbare background) was conducted by Biorun Biotechnology Co., Ltd. (Wuhan) following established rice transformation protocols (Sunilkumar et al., 1999). Transgenic plants from the T_0 generation that tested positive for the introduced constructs were grown in the greenhouse facilities of Agricultural Sciences, the Yunnan Academy. At the booting stage, the T_1 plants were used to inoculate by *Xoo strains*, and disease severity (lesion lengths) were measured to identify lines displaying the expected phenotypes. Seeds were collected from mature plants and replanted in the following season. This selection process was repeated for two additional generations, ultimately producing stable T_2 transgenic lines. Seeds from the T_2 generation were then cultivated and used for subsequent analyses in this study.

3. Results

3.1 Evaluation of twenty-two rice genotypes and two check varieties against eight Xoo strains under field conditions

A total of twenty-two rice genotypes and two check varieties were evaluated against eight strains of *Xoo*. The tested strains were inoculated onto Haonuoyang, 21 other varieties carrying single resistance genes, and two check control varieties (Jingang30 and Zhenzhuai) (Supplementary Table S 1). Compared with the other genotypes, the average lesion lengths of Zhenzhuai and Jinggang30 were 10 cm ± 0.34 cm and 8 cm ± 0.86 cm, respectively, and both were susceptible to most of the strains (Fig. 1A, B and C). Isolate PXO99 infected the highest number of genotypes (75.0%), followed by PXO86 (54.2%). In contrast, isolate 2001-28 infected the fewest genotypes (25.0%) (Fig. 1D). The interactions between strain and genotype are shown in Fig. 1E. Haonuoyang showed 100% BB resistance, which was similar to IRBB21 and IRBB23 (Fig. 1F).

3.2. Evaluation of the selected lines in the F_1 and F_2 generations against BB caused by Xoo under field condition

After inoculation with strain PXO99 (P6), all F_1 plants from H197 × JG30 were resistant to BB disease under field conditions. In the first F_2 population ($F_{2\text{-}1}$), 84 of 330 plants were susceptible and 246 of 330 were resistant. In the second F_2 population ($F_{2\text{-}2}$) and the third F_2 population ($F_{2\text{-}3}$), 79 and 95 out of 305 and 350 plants, respectively, were susceptible to the same strain, whereas 226 and 255 plants were resistant. These results showed that the ratio of resistant to susceptible plants in the F_2 populations of H197× JG30 conformed to the Mendelian inheritance ratio of 3:1, indicating that the BB resistance of Haonuoyang is dominant (Table 2).

Table 2
Field evaluation of H197× JG30 hybrid progenies for BB resistance after PXO99 inoculation under field conditions

Name of materials	R plants	S plants	Total plant	$X^2_{(3:1)}$	*P*
HNY	60	0	60	0	1
JG30	0	60	60	0	1
(H197×JG30) F_1	53	0	53	0	1
(H197×JG30) F_{2-1}	246	84	330	0.036	0.849
(H197×JG30) F_{2-2}	226	79	305	0.132	0.716
(H197×JG30) F_{2-3}	255	95	350	0.857	0.355
Total	727	258	985	0.748	0.387

Note: S, susceptible; R, resistant

The results of inoculation with eight strains of the BB pathogen (Fig. 1E and 1F) showed that the resistance spectrum of Haonuoyang was similar to that of IRBB21 and IRBB23, which are single-gene resistant varieties. In order to explore the relationship between the resistance gene carried by Haonuoyang and the genes *Xa21* and *Xa23*, Haonuoyang was hybridized with IRBB21 (*Xa21*) and IRBB23 (*Xa23*), respectively, and the F_1 and F_2 populations (HNY×IRBB21 and HNY×IRBB23) were inoculated with PXO99 (Table 3). All F_1 plants from the combinations HNY × IRBB21 and HNY × IRBB23 were resistant. However, in the F_2 generation, the combination HNY×IRBB21 showed 22 susceptible plants and 315 resistant plants out of 337 total plants, whereas the combination HNY×IRBB23 showed 28 susceptible plants and 410 resistant plants out of 438 total plants. These results indicated that the resistance gene carried by Haonuoyang differs from *Xa21* and *Xa23*, representing a new disease-resistance gene with independent inheritance.

Table 3
Evaluation of F_1 and F_2 populations (HNY×IRBB21/23) for BB resistance after PXO99 inoculation under field conditions.

Names of plants	R plants	S plants	Total plant	$X^2_{(15:1)}$	P
HNY	36	0	36	0	0
IRBB21	43	0	43	0	0
(HNY×IRBB21)F_1	50	0	50	0	0
(HNY×IRBB21)F_2	315	22	337	0.045	0.833
IRBB23	39	0	39	0	0
(HNY×IRBB23) F_1	41	0	41	0	0
(HNY×IRBB23) F_2	410	28	438	0.015	0.902

Note: S, susceptible; R, resistant

3.3 Preliminary mapping of *Xa51*(t) based on SLAF-seq and association analysis

Genotype data for Haonuoyang and JG30, as well as the resistant pool and susceptible pool of F_2 plants, were obtained by SLAF-seq (simplified genome sequencing). The detected SNPs were analyzed for their association with the trait.

Among the 102,328 SLAF tags developed, 18,917 were polymorphic, representing a polymorphism rate of 18.49%. Polymorphic SNPs were obtained on 12 chromosomes, including 2,177 on chromosome 1, 1,666 on chromosome 21,705 on chromosome 31,186 on chromosome 41,383 on chromosome 5, 1,542 on chromosome 6, 1,330 on chromosome 71,083 on chromosome 8, 848 on chromosome 91,155 on chromosome 101,151 on chromosome 111,088 on chromosome 12, and 2,603 on others.

The SLAF tags were aligned to the 93 − 11 reference genome using SOAP software, and the polymorphic SLAF tags on different chromosomes were counted (Fig. 2A). The candidate polymorphic SLAF markers obtained were subjected to correlation analysis by the SNP_Index method. The correlation threshold of the mixed pools AA (disease resistance) and aa (disease susceptibility) was 0.1574, and a total of nine SLAF markers significantly associated with disease-resistance traits were obtained. The polymorphic SLAF markers associated with disease-resistance traits were located on chromosome Chr11. The SNP_Index association results are shown in Fig. 2B. The disease-resistance gene carried by Haonuoyang was preliminarily mapped to the long arm of chromosome 11, within a 0.12 Mb interval between 22,887,903 and 23,002,998 bp. We considered *Xa51(t)* to be a new gene. Through comparison, we found that the initial location of *Xa51(t)* on chromosome 11 was 22,887,904 − 23,002,998 bp in 93 − 11 and 28,848,673 − 28,963,768 bp in Nipponbare,subsequent analyses were performed using the Nipponbare IRGSP-1.0 genome sequence as the reference sequence. According to the positions and cloning information of 13 known BB resistance genes on chromosome 11, their physical intervals were calculated(Fig. 2C). It was found that *Xa51(t)* carried by Haonuoyang did not overlap with the positions of these 13 genes and is located between *Xa23* and *Xa3/Xa26* (Fig. 2C). By comparison with the 93 − 11 rice reference genome, 14 genes were identified in the associated region on chromosome 11 (Table 4).

Table 4
Statistical analysis of related region information for chromosome 11

Chromosome ID	Start	End	Size (Mb)	Associated Marker number	Average Depth	Aver Index	Class	Gene number
Chr11	28,848,673	28,963,768	0.12	9	152.11	0.1579	1	14

3.4 Fine mapping of *Xa51*(t) via molecular marker genotyping and recombinant analysis

For the fine-scale mapping of the *Xa51*(t) locus, a series of molecular markers comprising 11 SSR, 7 INDEL, 10 KASP, 8 iHAD markers were designed to span the target region from 28,848,673 bp to 28,963,768 bp on rice chromosome 11 (Supplementary Tables S2, S3 and S4).Nevertheless, KASP markers were ultimately selected for fine mapping based on experimental results.In 2018, Whole-genome resequencing was performed on Haonuoyang and Jingang 30, with 15 G of sequencing data per sample and an effective sequencing depth of 42.95×to56.45×, using the Nipponbare IRGSP-1.0 as the reference genome, and a total of 1383 SNP variants between the two parents were identified in the corresponding 28848673–28963768 bp region on Nipponbare chromosome 11. Based on the SNP variants, 7 sets of iHDA markers, 11 pairs of SSR markers, and 11 sets of KASP markers were designed and synthesized, and after parental polymorphism validation, 2 polymorphic iHDA marker sets, 1 polymorphic SSR marker pair, and 2 polymorphic KASP markers (28849494-1, 28934599-1) were obtained(Supplementary Fig. 1)).Banding-pattern analysis of F_2 recombinant plants was performed using polymorphic molecular markers.The banding pattern of the resistant parent was designated “A”, that of the susceptible parent was designated “B”, and the heterozygous banding pattern was designated “H”. The tightly linked molecular markers RM27051, RM27054 and Marker2-2R were all located on the left side of *Xa51*(t). *Xa51*(t) showed the shortest genetic distance of approximately 0.4 cM to Marker2-2R(Supplementary Fig. 2).

A total of 2191 F_3 segregating plants were grown, inoculated with *Xoo* strain PXO99,genotyping of the whole population with flanking KASP markers revealed a significant correlation between genotype and lesion length via analysis of variance, verifying the reliability of the preliminary mapping, and 76 recombinant crossover individuals were finally identified(**Supplementary Fig. 3)**. Among them,4 plants showed moderate resistance, 17 moderate susceptibility, 54 susceptibility, and 1 high susceptibility, Banding patterns were designated as “HY” (resistant parent), “JG30” (susceptible parent), and “H” (heterozygote). Genotype-phenotype association showed that genotypes with Haonuoyang-derived and heterozygous alleles (HY/H) had the shortest lesion length, while JG30-heterozygous (JG30/H) genotypes had the longest. Among recombinants, 12 were HHY, 7 HJG30, 36 HYH, 4 HYJG30, and 7 JG30HY. Association analysis of banding patterns and resistance phenotypes of recombinants using polymorphic marker 28849494-1 demonstrated that the Haonuoyang resistance gene *Xa51*(t) was linked to this marker and located nearby. Six newly-designed KASP markers in the target interval were used for recombinant screening, *Xa51*(t) was further mapped between KASP markers 28849494 and 28918188 bp, reducing the physical interval to ~ 68.7 kb, which contains 8 predicted genes including C_2H_2-type zinc finger protein and GRAS family protein genes.

In 2020, two consecutive rounds of validation using F_3 and $F_{3:4}$ populations in the early and late rice seasons further densified markers and completed the final fine mapping. For the first-round validation in the early rice season, 17 heterozygous recombinant lines (120 seedlings per line) and 4 extreme resistant/susceptible homozygous recombinant lines (40 seedlings per line) were selected from F_3 recombinants, totaling 2191 F_3 plants(Table 5), and 5 additional KASP markers (28863864, 28866073, 28885000, 28894443, 28905370) were developed for high-resolution genotyping of the recombinants. Genotype-phenotype association showed that genotypes of Haonuoyang-type (A) and heterozygous (H) had the shortest lesion length, while the Jingang 30-type genotype (B) produced the longest lesion length. Marker genotyping data combined with lesion-length measurements were subjected to analysis of variance. 15 recombinant plants exhibited susceptible phenotypes: they showed genotype‘A’at markers 28849494 and 28863864, and genotype ‘B’at markers 28894443, 28905370, 28918188 and 28934599. Another 47 recombinant plants were also susceptible, displaying genotype‘A’ at marker 28849494 and genotype ‘H’ at markers 28863864, 28866073, 28885000, 28894443, 28905370, 28918188 and 28934599. Association analysis of banding patterns and resistance-susceptibility phenotypes of recombinant plants demonstrated that the disease-resistance gene *Xa51*(t) from Haonuoyang was linked to these

markers, and was located within the interval between markers 28849494 and 28894443 bp, containing 6 predicted open reading frames(Supplementary Fig. 4).

Table 5
Haplotypes of the 21 F_3 recombinants in the target region

Number of recombinant individual plant	Varieties and lines	Markers								Averag lesion length(
		28849494	28863864	28866073	28885000	28894443	28905370	28918188	28934599	
1	HNY	A	A	A	A	A	A	A	A	10.6
2	JG30	B	B	B	B	B	B	B	B	15.1
3–5	F2/3/10	A	A	A	A	A	A	A	B	11.2
6	F21	A	A	A	A	A	A	B	B	14.5
7–8	F19/24	A	A	A	A	B	B	B	B	10.0
9–10	F19/24 – 1	A	A	A	A	H	H	H	H	8.7
11	F14	A	B	B	B	B	B	B	B	18.9
12	F14-1	A	H	H	H	H	H	H	H	18.4
13–16	F5/6/7/15	B	B	B	B	B	B	B	A	16.2
17	F21-1	H	H	H	H	H	H	B	B	15.5
18–20	F15/18/19	H	H	H	H	H	H	H	A	14.6
21–23	F10/22/23	H	H	H	H	H	H	H	B	12.2

For the large-population repeat validation in the late rice season, F_4 progenies of 61 key recombinant lines were planted at the Gasa Experimental Base in Xishuangbanna, with 120 seedlings per line plus 2 parental controls totaling 7480 plants, and inoculation assays were repeated to confirm phenotypic stability. 8 KASP markers (28849494, 28863864, 28866073, 28885000, 28894443, 28905370, 28918188, 28934599) were used for fine genotyping, and analysis of variance demonstrated that markers within the 28863864–28905370 interval were all significantly associated with lesion length(Table 7). Integrated analysis of recombination breakpoints and phenotypic association finally delimited *Xa51*(t) to the physical interval of 28863864–28905370 bp on rice chromosome 11 with a total length of 41.5 kb, and this interval harbors 5 predicted genes, laying a solid foundation for subsequent candidate gene screening and cloning.. This interval is flanked by KASP markers 28863864 and 28894443, with which the target gene exhibited perfect co-segregation (Fig. 3).

Table 7
Haplotypes of the 61 F_4 recombinants in the target region

Number of recombinant individual plant	Varieties and lines	Markers						Average lesion length(cm)
		28849494	28863864	28894443	28905370	28918188	28934599	
1	F14_1	A	H	H	H	H	H	14.32
2	F14_4	A	H	H	H	H	H	15.52
3	F14_5	A	H	H	H	H	H	13.22
4	F14_6	A	H	H	H	H	H	17.1
5	F14_7	A	H	H	H	H	H	18.42
6	F14_8	A	H	H	H	H	H	19.42
7	F14_9	A	H	H	H	H	H	16.7
8	F14_12	A	H	H	H	H	H	16.9
9	F14_14	A	H	H	H	H	H	16.36
10	F14_15	A	H	H	H	H	H	65.14
11	F14_16	A	H	H	H	H	H	25.84
12	F14_18	A	H	H	H	H	H	21.74
13	F14_19	A	H	H	H	H	H	24.78
14	F14_21	A	H	H	H	H	H	21.52
15	F14_22	A	H	H	H	H	H	18.48
16	F14_23	A	H	H	H	H	H	22.46
17	F14_25	A	H	H	H	H	H	25.1
18	F14_28	A	H	H	H	H	H	20.84
19	F14_30	A	H	H	H	H	H	18.52
20	F14_31	A	H	H	H	H	H	17.6
21	F14_34	A	H	H	H	H	H	16.14
22	F14_37	A	H	H	H	H	H	15.06
23	F14_38	A	H	H	H	H	H	16.96
24	F14_40	A	H	H	H	H	H	14.3
25	F14_41	A	H	H	H	H	H	14.06
26	F14_43	A	H	H	H	H	H	16.46
27	F14_45	A	H	H	H	H	H	17.4
28	F14_46	A	H	H	H	H	H	13.12
29	F14_47	A	H	H	H	H	H	14
30	F14_48	A	H	H	H	H	H	14.32
31	F14_51	A	H	H	H	H	H	13.64
32	F14_53	A	H	H	H	H	H	10.54
33	F14_54	A	H	H	H	H	H	11.02
34	F14_59	A	H	H	H	H	H	16.38
35	F14_60	A	H	H	H	H	H	15.3
36	F14_61	A	H	H	H	H	H	22.12
37	F14_64	A	H	H	H	H	H	16.86
38	F14_66	A	H	H	H	H	H	16.22
39	F14_67	A	H	H	H	H	H	17.7
40	F14_70	A	H	H	H	H	H	19

Number of recombinant individual plant	Varieties and lines	Markers						Average lesion length(cm)
		28849494	28863864	28894443	28905370	28918188	28934599	
41	F14_71	A	H	H	H	H	H	12.94
42	F14_76	A	H	H	H	H	H	14.7
43	F14_77	A	H	H	H	H	H	15.62
44	F14_85	A	H	H	H	H	H	22.38
45	F14_86	A	H	H	H	H	H	23.74
46	F14_88	A	H	H	H	H	H	15.32
47	F14_91	A	H	H	H	H	H	20.58
48	F19_11	A	A	B	B	B	B	9.48
49	F19_13	A	A	B	B	B	B	9.48
50	F19_24	A	A	B	B	B	B	10.175
51	F19_25	A	A	B	B	B	B	12.96
52	F19_26	A	A	B	B	B	B	11.04
53	F19_40	A	A	B	B	B	B	12.7
54	F19_47	A	A	B	B	B	B	4.7
55	F19_52	A	A	B	B	B	B	7.95
56	F19_54	A	A	B	B	B	B	8.08
57	F19_68	A	A	B	B	B	B	11.9
58	F19_79	A	A	B	B	B	B	10.18
59	F19_84	A	A	B	B	B	B	11.12
60	F24_1	A	A	B	B	B	B	10.06
61	F24_5	A	A	B	B	B	B	10.1
62	JG30	B	B	B	B	B	B	15.07
63	HNY	A	A	A	A	A	A	10.62

3.5 Candidate gene identification and molecular characterization of *Xa51*(t)

According to the genome annotation information from the MSU Rice Genome Annotation Project, six hypothetical open reading frames were predicted in the target interval. Among these loci, one encodes an uncharacterized expressed transposon protein (*LOC_Os11g47860*, G1), and four are predicted to encode GRAS family proteins, namely *LOC_Os11g47870* (G2), *LOC_Os11g47890* (G3), *LOC_Os11g47900* (G4) and *LOC_Os11g47910* (G5). With reference to the Nipponbare reference genome, specific amplification primers were developed for each of the five candidate genes located in the finely mapped region. The results showed that G2 and G3 could not be amplified in either parent; G4 could be amplified in Jingang30 but not in Haonuoyang. There was no difference in the DNA sequence of G1 between Haonuoyang and Jingang30, and the predicted protein functional domain was the same. In contrast, G5 showed substantial differences between Haonuoyang and Jingang30; the sequence in Haonuoyang contained a terminator at 1485 bp compared with Jingang30. The total nucleotide sequence length of the G5 gene in Haonuoyang was 1488 bp, and the translated amino acid sequence was 495 aa long, whereas the total length of the G5 gene in JG30 was 1738 bp and the translated amino acid sequence was 576 aa long (Supplementary Fig. 5). Thus, the amino acid sequence encoded by G5 in HNY was shorter than that encoded in JG30. Through SMART software analysis, it was found that these base mutations and deletions in G5 of HNY led to changes in the protein functional domain (Fig. 4A,Supplementary Fig. 6). At the same time, NCBI BLAST comparison showed that this gene shared 100% sequence similarity with OsI_37091 (encoding scarecrow-like protein 9), indicating similarity to the indica rice sequence. The similarity to XP_015616386.1 was 98.8% (Fig. 4B), corresponding to the predicted protein E2562 of *Oryza meyeriana* var. *granata*, whereas the similarity to 030786 was 82%. The similarity of scarecrow-like proteins from wild emmer wheat, switchgrass, goatgrass, two-rowed barley, *Brachypodium distachyon*, and *Eragrostis curvula* ranged from 45.82% to 54.72% (Fig. 4B). The *Xa51(t)* protein clustered closely with *Oryza sativa* japonica (protein 9), *Oryza sativa* indica (OsI_37091), and *Oryza meyeriana*. Within the genus *Oryza*, this clustering indicates strong evolutionary conservation. In grasses, proteins from *Aegilops tauschii*, *Triticum dicoccoides*, *Hordeum vulgare*, *Brachypodium distachyon*, *Panicum virgatum*, and *Eragrostis curvula* shared ancestry with GRAS proteins. We considered *LOC_Os11g47910* to be the most likely candidate gene for *Xa51(t)*.

3.6 Pathogen-induced upregulation of LOC_Os11g47910 in *Xa51*(t)-carrying rice genotypes following PXO99 inoculation

For further confirmation that LOC_Os11g47910 serves as the potential target of *Xa51*(t), we examined its expression patterns in HNY, H197, and JG30 following PXO99 inoculation. Importantly, the gene was markedly upregulated in both resistant genotypes (HNY and H197), with expression levels far exceeding those in the susceptible JG30 (Fig. 5). In detail, in HNY, the gene's expression at 48 h post-inoculation (hpi) was roughly 18 times higher than that at 0 and 12 hpi (Fig. 5B), while in H197, it was approximately 16-fold higher than the initial level (Fig. 5C) in contrast to the uninoculated controls of HNY and H197, which showed only 0.47- and 1.4-fold changes, respectively, at the same time point. By comparison, the susceptible JG30 exhibited the lowest expression of the gene, with a mere 0.93-fold change at 48 hpi relative to its uninoculated control (0.26-fold change at the same time point; Fig. 5A). These results strongly indicated that LOC_Os11g47910 was strongly induced by the bacterial blight (BB) pathogen in HNY and H197, suggesting its participation in the plant-pathogen interaction. This evidence not only shed light on the functional role of LOC_Os11g47910 but also provided a solid basis for further exploring the molecular mechanisms governing *Xa51*(t) mediated plant-pathogen interactions.

3.7. Cellular mechanism of the BB resistance mediated by gene *Xa51(t)*

The effect of inoculation with PXO99 on BB resistance in the *Xa51(t)*-mediated resistant genotype HNY and the susceptible genotype JG30 was examined by transmission electron microscopy (TEM) at different time intervals after inoculation. Remarkable differences in cellular tissue structure were observed between the resistant (HNY) and susceptible (JG30) rice genotypes after infection with *Xoo*. To investigate the ultrastructural cellular changes mediated by *Xa51(t)*, water was used as the control for leaf inoculation (Fig. 6). From day 1 to day 3 post-inoculation with the pathogen, small yellow and brown spots appeared at the inoculation sites on some leaves of the resistant material HNY (Fig. 6B). In contrast, the inoculated leaves of the susceptible material JG30 showed no significant changes (Fig. 6B). On the 5th day after inoculation with the bacterial pathogen, hypersensitive reaction spots were generally observed at the inoculation sites of resistant HNY (Fig. 6C), presenting a brown coloration. However, no hypersensitive reaction spots were observed at the inoculation sites of JG30, which instead showed yellowish and slightly green water-soaked symptoms (Fig. 6C). After 9 days of infection with the bacterial pathogen, there was a significant difference in lesion length at the inoculation site between the resistant and susceptible materials, with JG30 showing considerably longer lesions than resistant HNY (Fig. 6D). On the 14th day after inoculation, the inoculation sites of both susceptible and resistant materials exhibited obvious white leaf blight symptoms, and the leaves began to wilt, with a marked difference in lesion length (Fig. 6E). On the 21st day after inoculation, the rice materials were completely infected, and this time point was also the optimal stage for measuring white leaf blight lesion length (Fig. 6F).

Therefore, in this study, resistant and susceptible materials inoculated with *Xoo* at the above time points were selected to observe changes in cellular tissue structure at the *Xoo* infection site and to explore the possible role of *Xa51(t)* in cellular mechanisms. According to TEM sections, autophagosomes were present in both HNY and JG30 infected with BB for 1–3 days (Fig. 6B1), with independent organelles and complete, clear morphological structures (Fig. 6). The number of autophagosome-like structures increased in HNY after 5 days of inoculation with PXO99, with some organelles beginning to lyse and some cells exhibiting plasmolysis (Fig. 6C1). The organelles were relatively intact, and the morphology of each organelle could still be distinguished. When HNY was inoculated with PXO99 for 9 days (Fig. 6D1), the organelles became disorganized and were generally disintegrated, with dispersed organelles filling the cells. When HNY was inoculated with PXO99 for 14 days (Fig. 6E1), autophagosomes were widely present, and most cells showed plasmolysis, with organelles deformed and having lost their original structure. When HNY was inoculated with PXO99 for 21 days (Fig. 6F1), few intact cells remained, and severe cell and organelle disintegration was observed. However, some identifiable autophagosomes were still present (Fig. 6F1). In contrast, in JG30, after inoculation with strain PXO99 for 1–3 days (Fig. 6B2), the cells and their intracellular structures were clearly visible. At this stage, some xylem parenchyma cells had already begun to show characteristics of plasmolysis. Five days after inoculation with PXO99, cytoplasmic plasmolysis became common in JG30 (Fig. 6C2). The organelles began to disintegrate, accompanied by partial rupture of the cell membrane. By the 9th day after inoculation, the cells in JG30 had completely disintegrated (Fig. 6D2). Each organelle had lost its characteristic morphology, and autophagosomes were scarcely detectable. At 14 days after inoculation (Fig. 6E2), most xylem parenchyma cells in JG30 exhibited plasmolysis. After 21 days of inoculation (Fig. 6F2), the thin-walled xylem cells of JG30 were essentially necrotic. Nearly all organelles had disintegrated, making it extremely difficult to observe intact cell structure (Fig. 6F2). These results indicated that in the *Xa51(t)*-mediated disease-resistance response, cells were able to maintain their structure for a relatively longer period, and the rate of cell disintegration was slower. Meanwhile, xylem parenchyma cells exhibited autophagosome-like structures, and cell autophagy continued to occur during the prolonged disease-resistance response. These observations suggest that cell autophagy generally occurred in resistant HNY and may have played a role in maintaining cellular and organelle structure.

3.8 Inoculation phenotype of G5 transgenic plants

To precisely elucidate the relationship between the candidate gene G5 and resistance to BB, we used RNA interference (RNAi), gene knockout, and gene overexpression technologies to modify the target plants. The T_3 generation of G5 knockout plants with the HNY background, the T_3 progeny of RNAi interference lines, and the T_3 generation of G5-overexpressing transgenic plants with the Nipponbare background were inoculated with *Xoo* for further analysis (Fig. 7). The results showed that the lesion ratio after inoculation with the rice BB pathogen was 7.146% for 146 control HNY plants, 30.32% for 123 JG30 plants, 18.76% for 87 Nipponbare plants, and 16.77% for 21 G5 knockout plants 900-9. For 96 RNAi interference plants (G8-6), the ratio was 14.11%, whereas it was 6.45% for 71 overexpression plants (H9-7). Following knockout and interference of the candidate gene G5, the resistance of rice plants to the disease was markedly reduced. In contrast, overexpression of the candidate gene G5 substantially enhanced the resistance of rice plants to the disease. Based on these findings, this study identifies the candidate gene G5 (*Os11g47910*) as the rice BB resistance gene *Xa51(t)*.

3.9 Subcellular localization of *Xa51*(t)

Proteins encoded by plant genes rely on localization signals embedded within their amino acid sequences to be precisely targeted to specific cellular compartments, thereby enabling gene function. Therefore, subcellular localization is a crucial step in investigating the function of the protein encoded by *Xa51(t)*.

To further investigate the functional role of the protein encoded by *Xa51(t)*, we observed *Agrobacterium*-infiltrated tobacco leaves with preset markers using a laser scanning confocal microscope (Fig. 8). The green fluorescence of the empty vector control was distributed throughout various cellular components, whereas the fusion construct of the fluorescent plasmid and *Xa51(t)* showed green fluorescence specifically localized to the cell membrane.

4. Discussion

Modern rice cultivars face increasing susceptibility to BB because of the continuous evolution of pathogen races and intensified disease pressure. To tackle this challenge, advanced genomic tools and new BB resistance genes help develop rice with durable disease resistance. Exploring novel resistance sources remains a core ongoing goal in modern rice breeding practices(Kan et al., 2025; Bhatia et al., 2024). Dominant gene conferring resistance to bacterial blight, designated *Xa51(t)*, was located and characterized in the Haonuoyang-derived introgression line H197 by means of fine genetic mapping.This gene conferred resistance to eight *Xoo* strains, namely PXO61, PXO86, PXO99, PXO112, PXO339, CX28-3, CX30-1 and 2001-28, thereby demonstrating broad-spectrum resistance. This discovery has important potential for genetic improvement in breeding for BB resistance. Several alien genes from *Oryza rufipogon* have been successfully introduced into cultivated rice to develop many BB-resistant (Qi et al., 2000) and high-yielding varieties (Tian et al., 2006).

The target gene was precisely mapped to the long arm of chromosome 11, and 50 BB resistance genes had previously been identified or mapped, as documented in earlier studies (Chen et al., 2024; Kan et al., 2025; Li, 2022; Yang et al., 2025). Among them, approximately 18 previously identified BB resistance genes, including *Xa1* (Yoshimura et al., 1998), *Xa3/Xa26, Xa4* (Sun et al., 2003), *Xa10, Xa21* (Song et al., 1995), *Xa22, Xa23, xa26(t)* (Yang et al., 2003), *Xa31* (Wang et al., 2009), *Xa32, Xa35, Xa36, Xa39, Xa40* (Kim et al., 2015), *Xa43*(t)(Kim and Reinke, 2019), *Xa44*(t), *Xa47*(t), and *Xa48*(t), had also been localized on chromosome 11 (Jiang et al., 2020; Li et al., 2025; Li, 2022; Tian et al., 2006). The locus identified in this study was found to be in close proximity to the genes *Xa3/Xa26, Xa4, Xa40, Xa43*(t), *Xa47*(t), *and Xa48*(t). (Sun et al., 2003; Kim et al., 2015; Kim and Reinke, 2019; Sinha et al., 2023). It was determined that Haonuoyang carries *Xa51(t)*, which does not overlap with the positions of 16 other known genes and is located between *Xa23* and *Xa26(t)*. While these resistance genes all mapped to rice chromosome 11, they showed no allelic relationship with *Xa51*(t) (Cheema et al., 2008; Lin et al., 2026; Sinha et al., 2023).

To more effectively combat rice BB, some cloned disease-resistance genes, such as *Xa1, xa3, Xa4, xa5*, and *xa13*, have been incorporated into rice breeding programs. Nevertheless, because of the singularity and narrow resistance spectra of these genes, the resistance of the resulting varieties is vulnerable to breakdown (Zhang et al., 2009). *Xa21* has been the most widely used gene because of its broad resistance spectrum and durable resistance characteristics. Over the past two decades, *Xa23* has also progressively emerged as an important gene in breeding applications (Chen et al., 2024). Despite the identification of numerous resistance genes, several persistent problems remain. First, some resistance genes confer resistance only during specific growth stages, for example, only at the seedling or reproductive stage, thus showing stage-specific resistance. Second, most *Xa* genes have narrow resistance spectra, which limit their effectiveness in large-scale rice production. Third, given the continuous evolution and diversification of pathogen races, together with the long-term extensive cultivation of single rice varieties, resistance mediated by a single *R* gene against rice BB is prone to breakdown under production conditions. For example, *Xoo* isolates capable of overcoming the resistance conferred by *Xa21* have already been detected in nature (Pruitt et al., 2015). Therefore, the exploration of novel genes for BB resistance is of paramount importance for enriching the genetic reservoir of rice BB resistance and counteracting continuously evolving bacterial strains, thereby ensuring the long-term stability and productivity of rice cultivation.

When rice interacts with *Xoo*, it recognizes pathogen-associated molecular patterns (PAMPs) and activates a pathogen-associated molecular pattern-triggered immune (PTI) response through receptor proteins. For example, *xa3/Xa26* (Xiang et al., 2006) encodes receptor-like kinase (RLK) subfamily proteins with kinase activity spanning extracellular, membrane, and intracellular domains (Da Silva et al., 2004; Song et al., 1995)). Similarly, *Xa21* (Song et al., 1995) also encodes an RLK subfamily protein, and the resistance mechanism mediated by *Xa21* falls under PTI. To successfully infect rice, the BB pathogen secretes effector factors that suppress the rice immune response, thereby triggering effector-triggered susceptibility (ETS). For example, *xa13* belongs to the nodulin3 family and is located on the cell membrane. Its corresponding avirulence gene is *pthxo1* (Chu et al., 2006), and the interaction between *xa13* and *pthxo1* is a typical example of ETS.

The functional classifications of the other two cloned *R* genes differ. *Xa1* encodes an NBS-LRR structural protein that functions in the cytoplasm (Yoshimura et al., 1998), representing a typical disease-resistance gene type. In contrast, *Xa5* is a subunit of the TFIIA transcription factor complex, located in the nucleus (Jiang et al., 2006). The *Xa51(t)* gene identified in this study expands the repertoire of major genes imparting resistance to BB and provides a solid foundation for further investigation of the interaction mechanisms between rice plants and the BB pathogen. The qRT-PCR results of gene expression across different samples showed that *Xa51(t)* expression level was substantially higher in the resistant parent Haonuoyang and homozygous resistant lines than in the BB-susceptible parent Jingang30 and the homozygous susceptible line. It is predicted that *Xa51(t)* encodes a GRAS family protein.

Notably, GRAS family proteins, belonging to the GRAS (*GAI, RGA*, and *SCR*) family, play pivotal roles in gibberellin (GA) signaling and regulate plant growth and development. The functional annotation of this gene suggested that it encodes a Pfam domain-containing protein that has been implicated in disease resistance (El Kasmi and Nishimura, 2016; Förderer and Kourelis, 2023). Subsequent sequencing of the *Xa51(t)* alleles in the resistant and susceptible parents revealed a 250-bp deletion at the 3' end in the resistant parent (Supplementary Fig. 1). This 250-bp deletion was exclusive to the resistant parental line and was also detected in the Haonuoyang genome, suggesting that this missing motif may be crucial for BB resistance. Remarkably, this motif was predicted to contain transcription factor-binding sites/cis-elements (Supplementary Fig. 2), potentially associated with the induction of gene expression upon *Xoo* infection (Jiang et al., 2020). We generated *Xa51(t)* overexpression and knockout lines using genome editing technology, and the overexpression lines exhibited significantly enhanced resistance to *Xoo*. In summary the 250-bp missing motif represents an especially interesting target for in-depth investigation. The markers developed in this study could be used for the introgression of resistant alleles of the *Xa51(t)* gene through marker-assisted breeding.

The candidate resistance gene *Xa51(t)* was isolated from the local 'Haunoyang' variety in Yunnan Province. However, its positive impact goes beyond this geographic area and extends globally. First, the broad-spectrum resistance provided by this gene against eight different strains of *Xoo* bacteria (including isolates from the Philippines and China) makes it an effective candidate for combating globally diverse pathogen groups, offering a genetic solution to the problem of bacterial strain evolution and resistance breakdown in crops that rely on a single gene. Second, although the protein encoded by this gene belongs to the well-known GRAS family, attributing bacterial blight resistance to this family is a new discovery, opening up new perspectives for understanding the overlap between growth pathways and defense pathways in plants, moving away from familiar proteins like NLR and RLK. Third, the molecular marker (KASP) developed in this study is a precise and low-cost tool, easily integrated into marker-assisted selection (MAS) programs worldwide, allowing the transfer of this gene into high-yield varieties while maintaining the desired genetic background, as clearly demonstrated in the improved H197 line. Finally, this study highlights the critical importance of genetic exploration in traditional local varieties, which have evolved alongside local pathogens and represent a valuable genetic resource for disease management in the face of climate change challenges and strain diversity.

Declarations

Funding

This work was supported by National Key Research and Development Program of China (Grant No. 2021YFD1200505), the National Natural Science Foundation of China (32160455,31760375) and the Major Science and Technology Project of Yunnan Province(202302AE090011, 202205AR070001-07).

Author Contribution

Nie Ruimin, Amr A. Hassan, Ge Na: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Writing – review & editing. Tang Cuifeng, Ye Changrong: Methodology, Formal analysis, Data curation. Dong Chao, Zhang Feifei: Conceptualization, Data curation, Formal analysis, Investigation, Validation, Writing – review & editing. A Xin-xiang: Visualization, Validation, Investigation. Li Zhou, Yang Chunyun, Dai Luyuan: Writing – review & editing, Writing – original draft, Supervision, Data curation. Sun Yiding,Yang Yi, Huang Rongman, Zeinab, A. Kalboush: Project administration, Methodology, and Investigation. Yang Yayun, Xiaowei: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Writing and were responsible for the design, planning, and implementation of the wholeresearch also, responsible for supervising and coordinating the overall research. All authors commented and edited all versions of the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgement

The authors would like to express their sincere appreciation and gratitude to Dr. Sijun Zheng and Dr. Qinzhong Yang for their technical support and advice during this research. Our appreciation also goes to Mrs. Lihau Pu, Suxian Gan, and Tao Sun from Agriculture and Rural Areas in Xishuangbanna State, China, for hosting the visit of the senior author during the field experiments.

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Figures

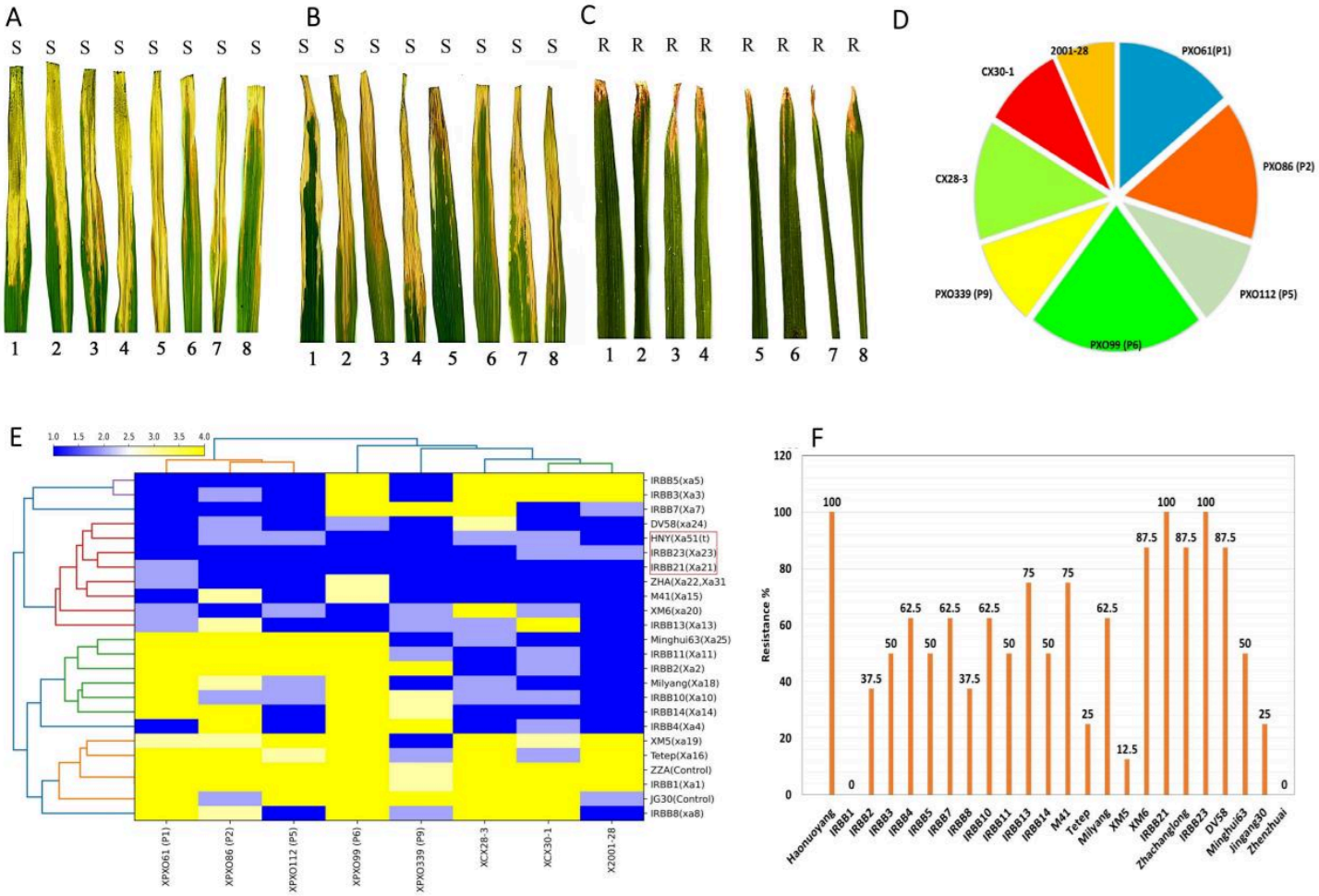


Figure 1

Bacterial blight disease reaction of twenty-four rice genotypes showing resistance and susceptibility response patterns with known single resistant gene under artificial inoculation with eight strains of *Xanthomonas oryzae pv. oryzae* under field conditions. (A) Symptoms on eight leaves of Jingang 30 (JG30) as susceptibility variety;(B) Symptoms on eight leaves of Zhenzhuai (ZZA) as susceptibility variety (C)Symptoms on eight leaves of Haonuoyang (HNY) as resistant genotype. (D) Infected genotypes/isolates, (E) Heat map analysis of correlation between twenty- four rice genotypes and eight bacterial blight strain, (F) Percentage of resistance for different rice genotypes against *Xanthomonas oryzae* pv. *oryzae*.

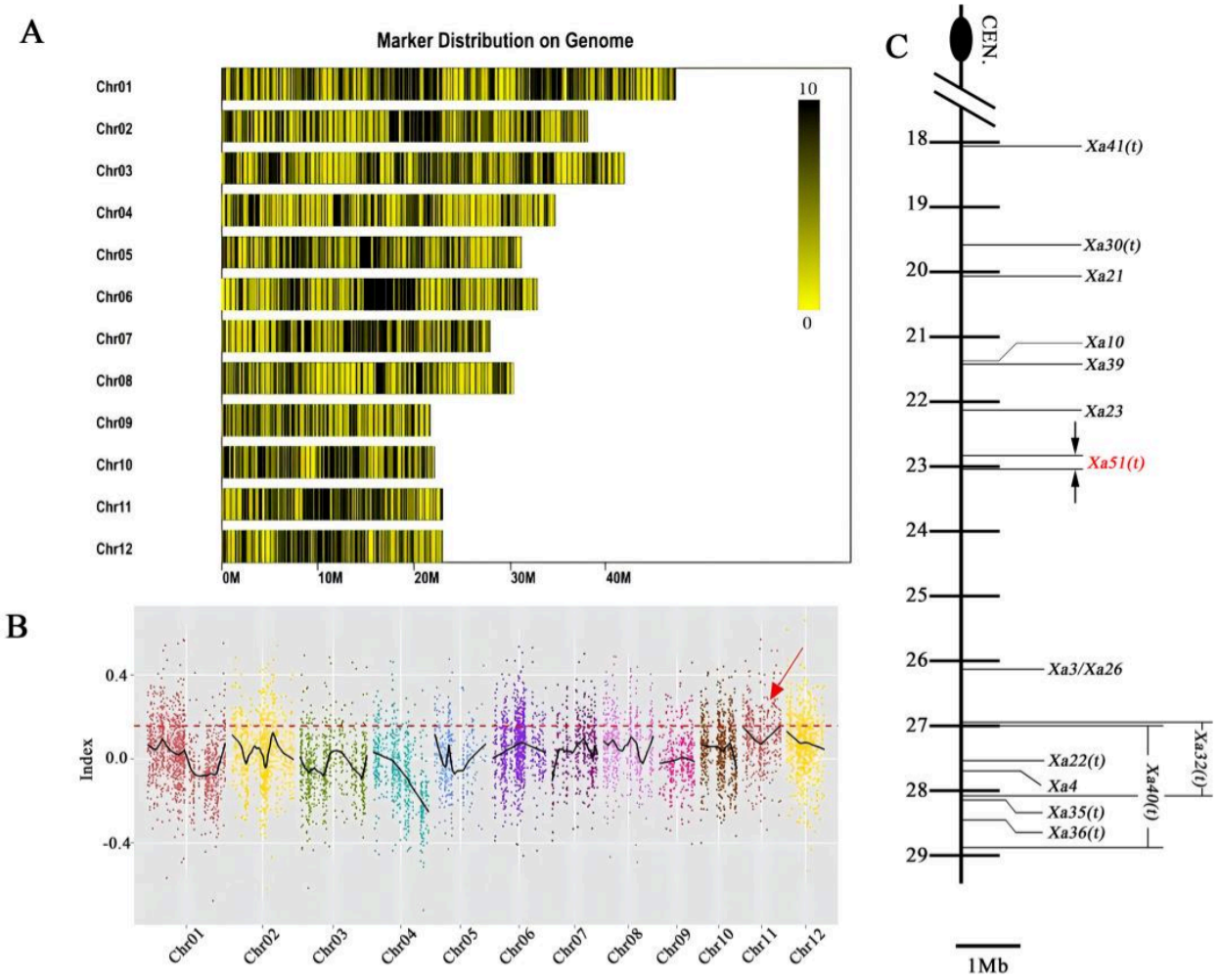


Figure 2

Mapping of the *Xa51(t)* gene related with bacterial blight resistance. (A) Genome-wide distribution of polymorphic SLAF on 12 chromosomes (Ch1-Ch12). Horizontal bar represents an individual chromosome, with the bar length corresponding to its physical length in megabases (Mb). The abscissa is the chromosome length, and each yellow band represents a chromosome. The Genome is divided according to the size of 1m. Marker density along each chromosome is visualized using a color gradient ranging from yellow (less SLAF marker) to black (the more SLAF marker), as indicated by the color scale on the right. (B) Result of marker SNP associated with disease resistance traits Index association, the horizontal axis represents the position of chromosomes, while the vertical axis represents The value of Δ (SNP-index); The black line represents all Δ(SNP-index) fitted results; Red line marking the threshold of Δ(SNP-index), the SNP-index was stronger indicated stronger correlation. (C) Location of 13 *Xa* genes and Haonuoyang carried *Xa51*(t) on the long arm of chromosome 11.

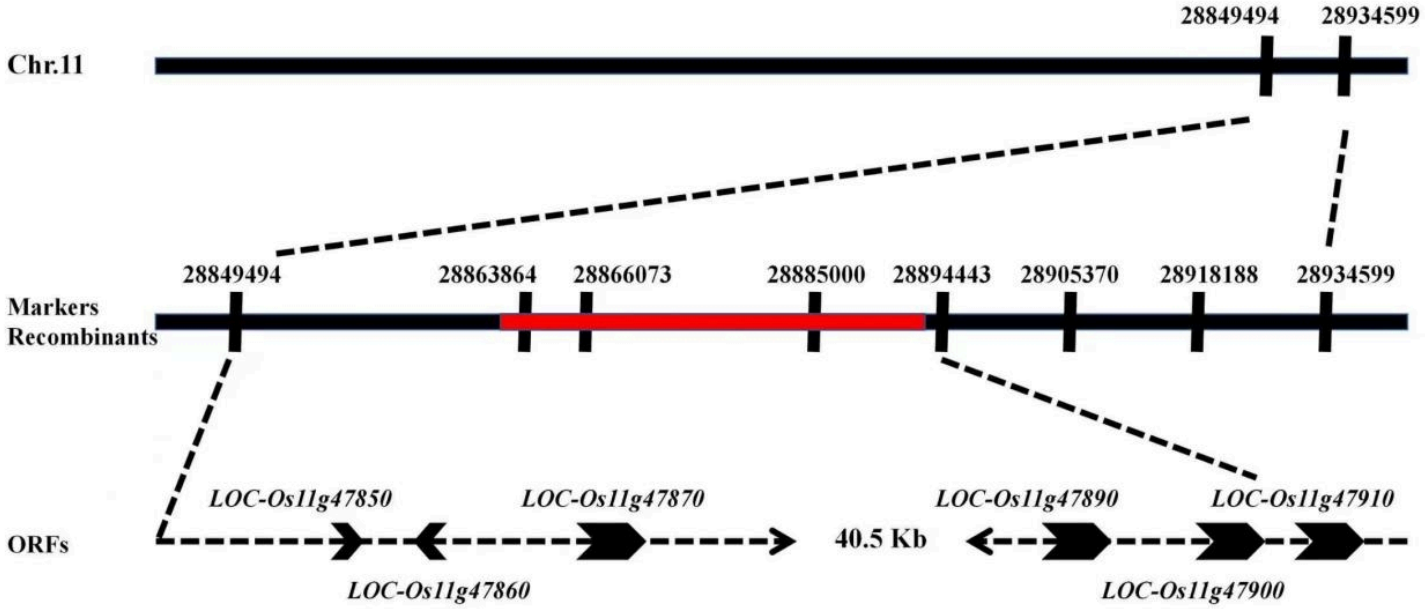


Figure 3

Fine mapping of the *Xa51(t)*containing six genes and annotations of the target region in chromosome 11 of rice. In the chromosome 11, the upper line represents a schematic overview, with a specific part determination between positions 28,849,494 bp and 28,934,599 bp. The dashed lines indicate the zoomed-in region that is further analyzed in the lower line. The middle line displays the distribution of molecular markers and recombinant breakpoints across the mapped interval. Several markers are positioned at specific physical coordinates (28849494, 28863864, 28866073, 28885000, 28894443, 28905370, 28918188, and 28934599 bp). The red-highlighted part represents the critical interval identified through recombinant analysis, narrowing the candidate region to approximately 41.5 kb. Recombination events between flanking markers were used to delimit the locus of interest. The lower panel presents the annotated open reading frames (ORFs) located within the 41.5 kb candidate region. Gene models are represented by black arrows indicating transcriptional orientation. The predicted genes within this interval incl**u**de: *LOC_Os11g47860*(468bp), *LOC_Os11g47870*(2079bp),, *LOC_Os11g47890*(1917bp), *LOC_Os11g47900*(1929bp), and *LOC_Os11g47910*(1788bp). The orientation of arrows shows both forward and reverse transcription directions, suggesting the presence of genes on both DNA strands.

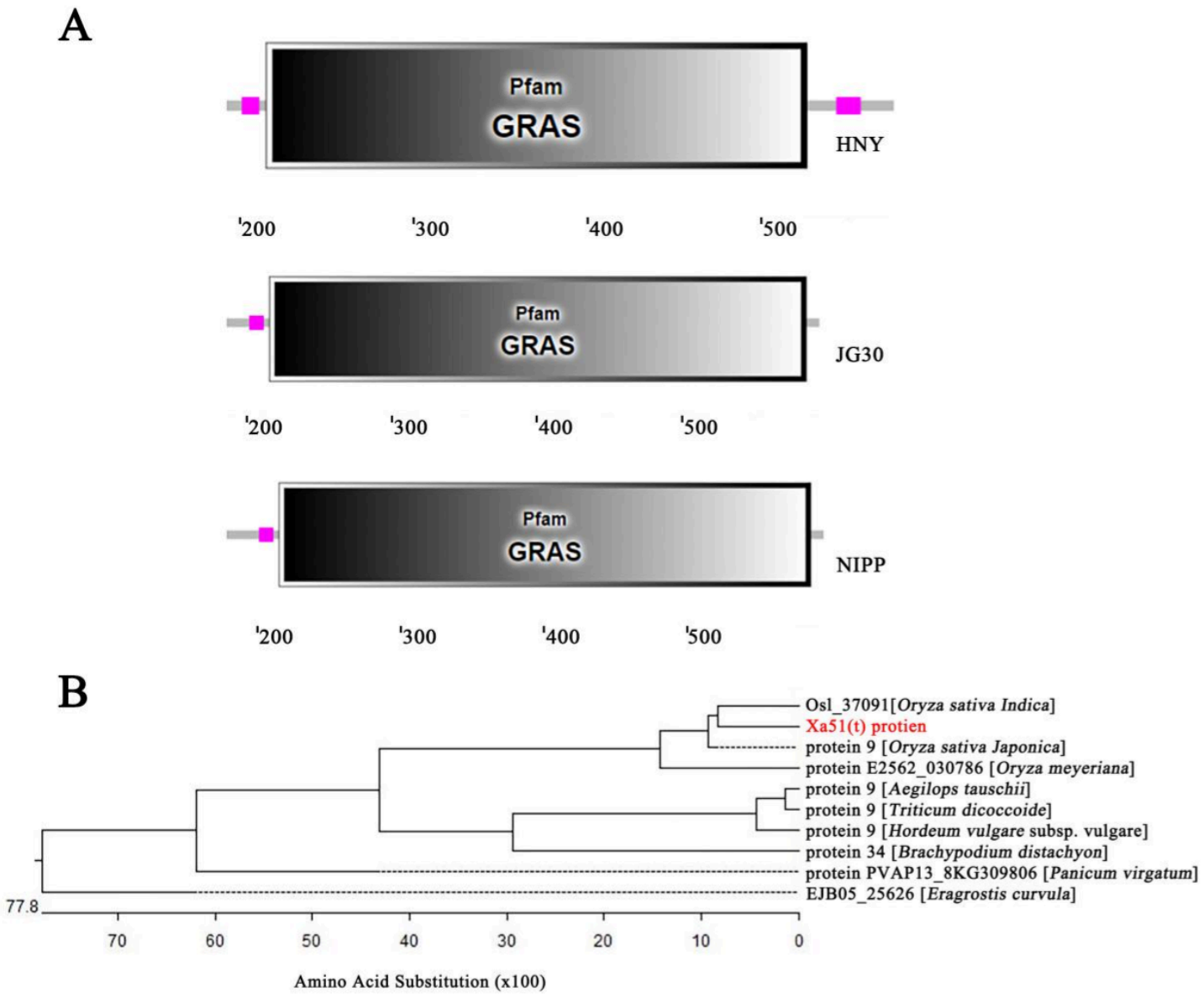


Figure 4

Structural conservation and phylogenetic relationship of the *Xa51(t)* associated GRAS protein across *Oryzae* and related grass species. (A) Conserved domain architecture analysis, presents schematic representations of the GRAS domain-containing protein identified in three rice genotypes: HNY, JG30, and NIPP. All three proteins exhibit a highly conserved GRAS domain as predicted by Pfam analysis. The GRAS domain spans the central and C-terminal regions of the protein, extending approximately from amino acid position ~200 to ~520, indicating strong structural conservation among the different rice backgrounds. Small colored boxes near the N-terminal region likely represent short conserved motifs or regulatory segments. The similar protein length and identical domain positioning among HNY, JG30, and NIPP suggest functional conservation of the *Xa51*-related protein across different rice cultivars. The absence of major insertions or deletions further supports structural stability of this transcription factor family member. (B)phylogenetic tree of *Xa51(t)* protein carried by Haonuoyang, phylogenetic tree constructed based on amino acid sequence alignment of the *Xa51(t)* protein with homologous GRAS proteins from multiple monocot species. The evolutionary distances are represented as amino acid substitutions (×100). More distant branches represent increasing evolutionary divergence. The relatively short branch lengths among *Oryza* species indicate minimal amino acid substitutions, reflecting high conservation, while longer branches toward other genera suggest gradual evolutionary divergence.

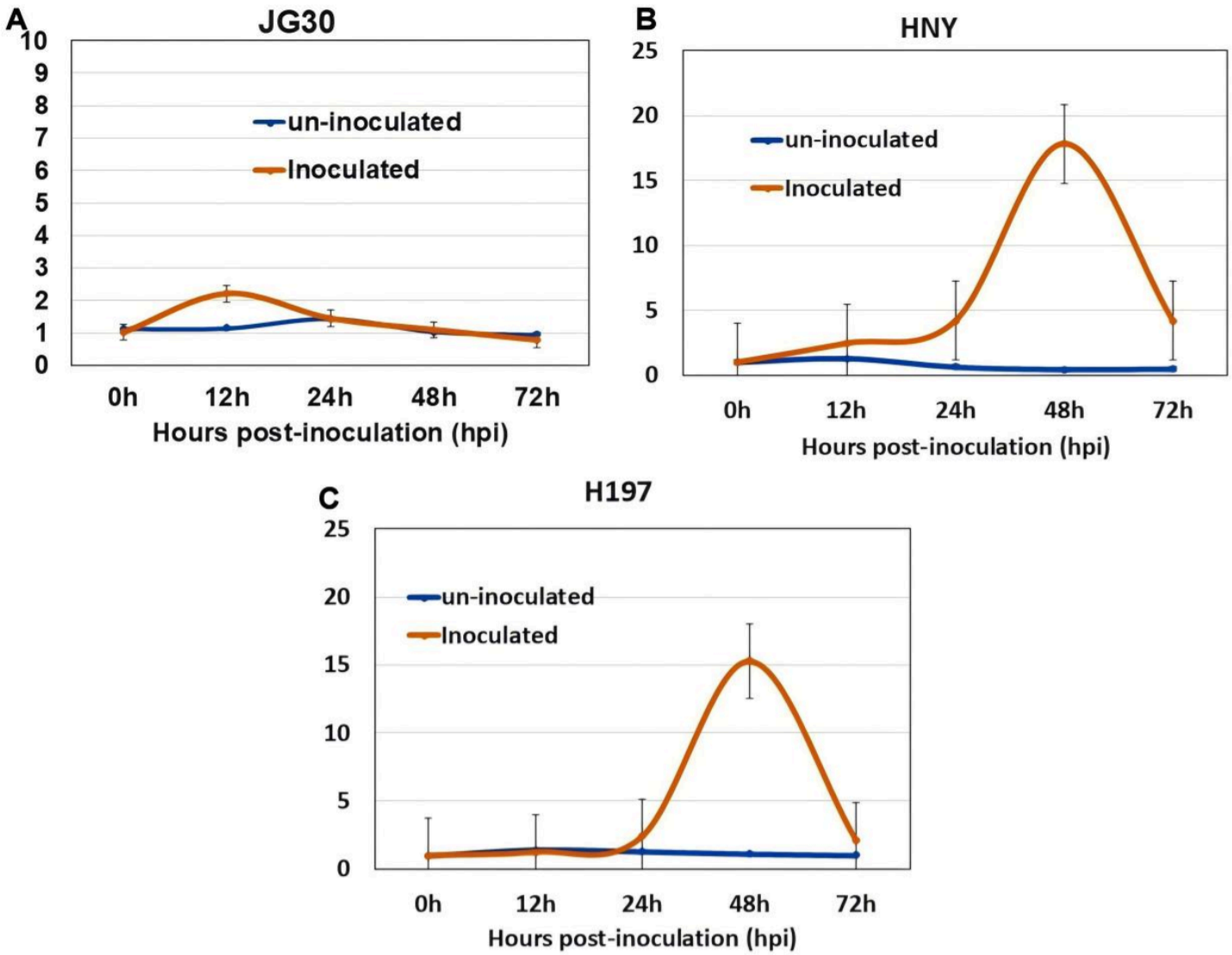


Figure 5

Expression analysis of the candidate gene, LOC-Os11g46200 of resistant and susceptible rice genotypes following *Xanthomonas oryzae*pv. *oryzae* inoculation. The expression levels of the genes in (A) Jingang 30 (JG 30), (B) Haonuoyang (HNY) and (C) H197 were measured at 0, 12, 24 and 48 hours after inoculation with strain PXO99. Bars represent the average of three biological replicates, whereas whiskers represent the standard error (means ± SE).

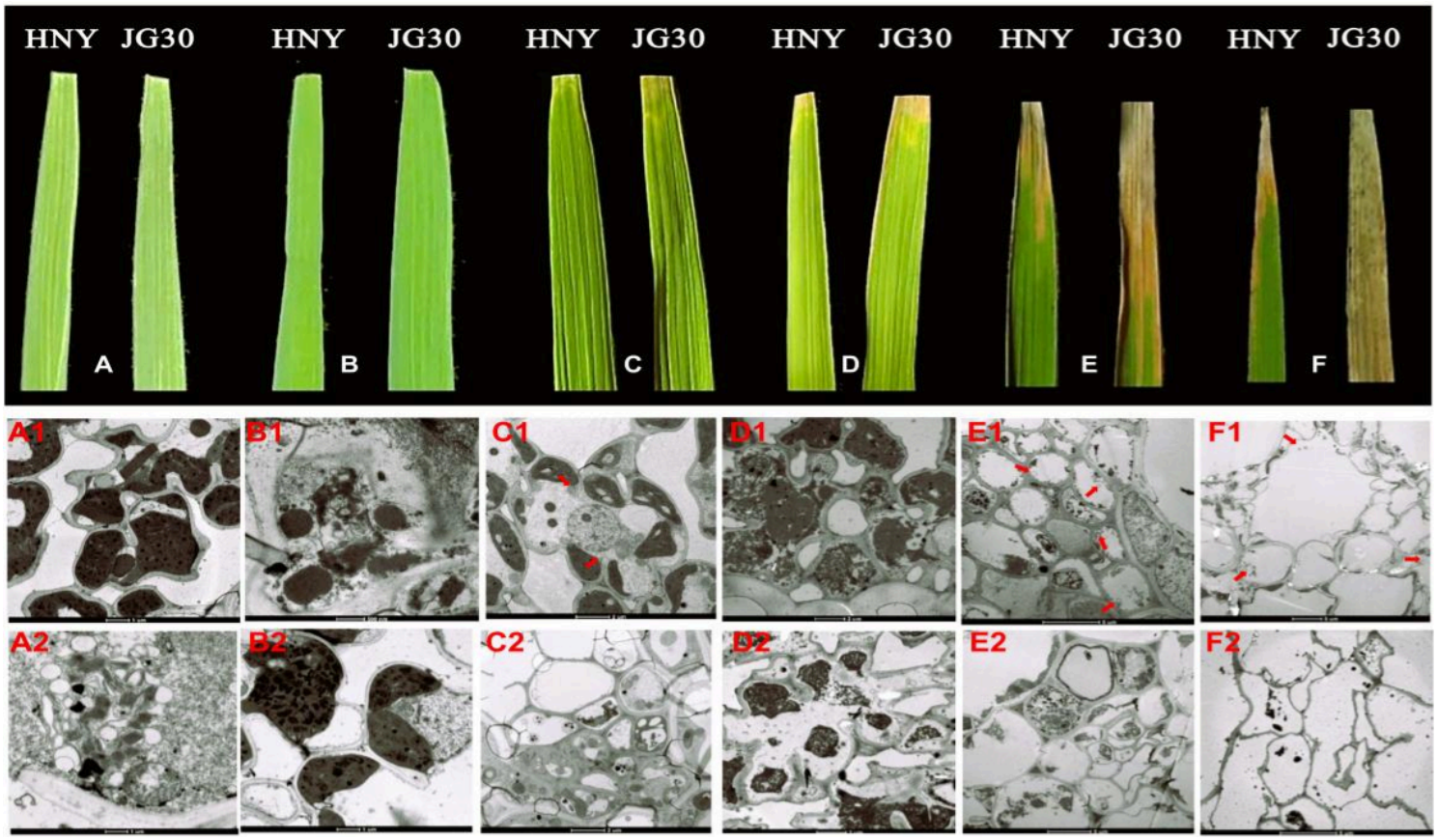


Figure 6

Symptoms and Ultrastructural changes of xylem parenchyma cells of Haonuoyang (HNY) and Jingang 30 (JG30) at five time periods (0, 1-3, 5, 9, 14, 21d) after inoculation with strain PXO99. (A-F) The symptom of the HNY and JG30 inoculated with clear water and inoculated with strain PXO99 after 1-3, 5, 9, 14, 21 days. (A1-F1) The ultrastructure of the HNY cells inoculated with clear water and inoculated with Bacterial for 1-3, 5, 9, 14, 21 days. Red arrows indicate autophagosome-like. (A2-F2) The ultrastructure of the JG30 cells inoculated with clear water and inoculated with Bacterial for 1-3, 5, 9, 14, 21 days.

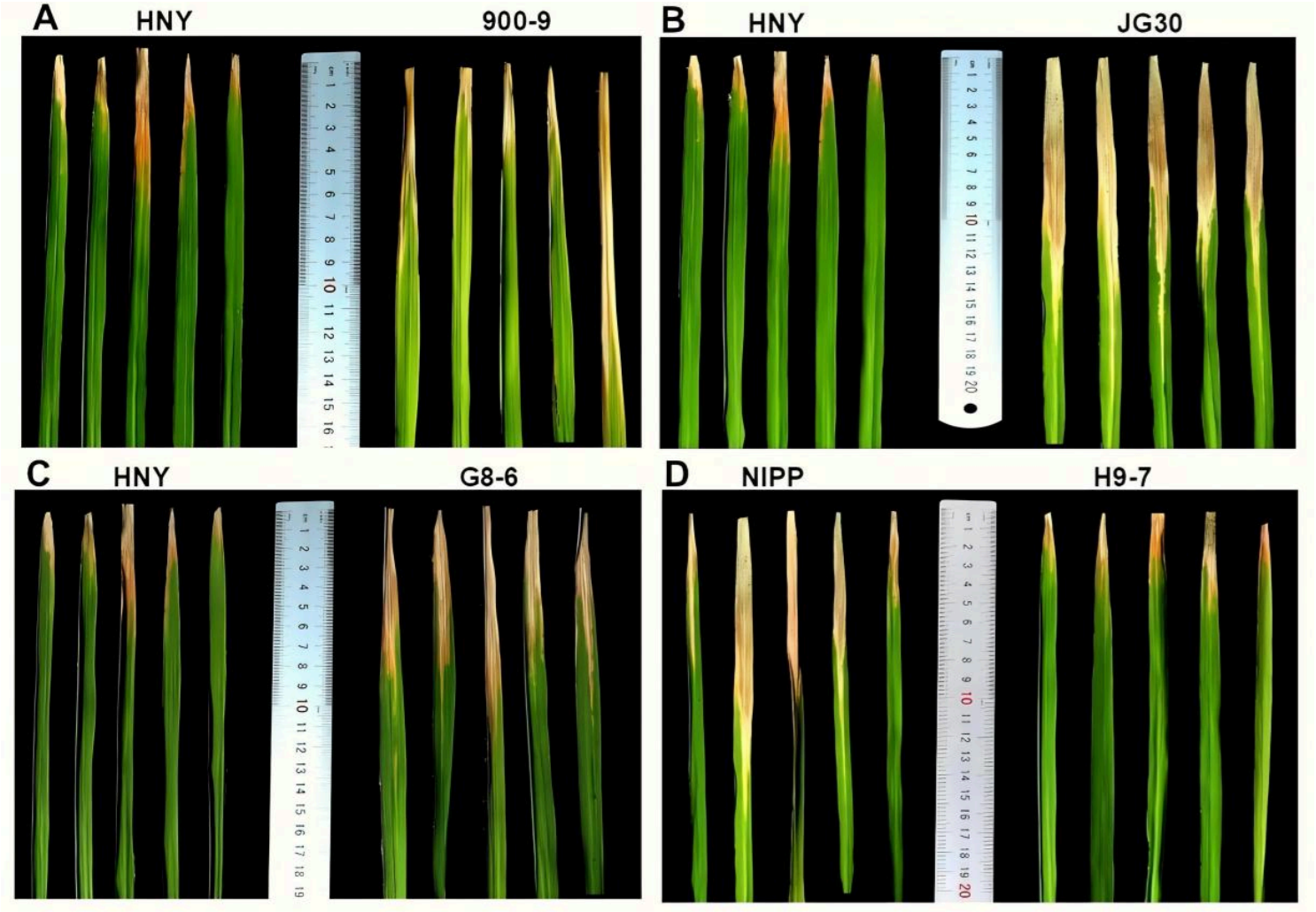


Figure 7

Phenotypic of transgenic plants with different approaches. (A) Comparison of lesion length between Haonuoyang (HNY) and G5 knockout plants (900-9). (B) Comparison of lesion length of HNY and Jingang 30 (JG30) plants. (C) Comparison of lesion length of HNY and RNAi interference plants (G8-6), (D) Comparison of lesion length of Nipponbare and G5 overexpression plant(H9-7).

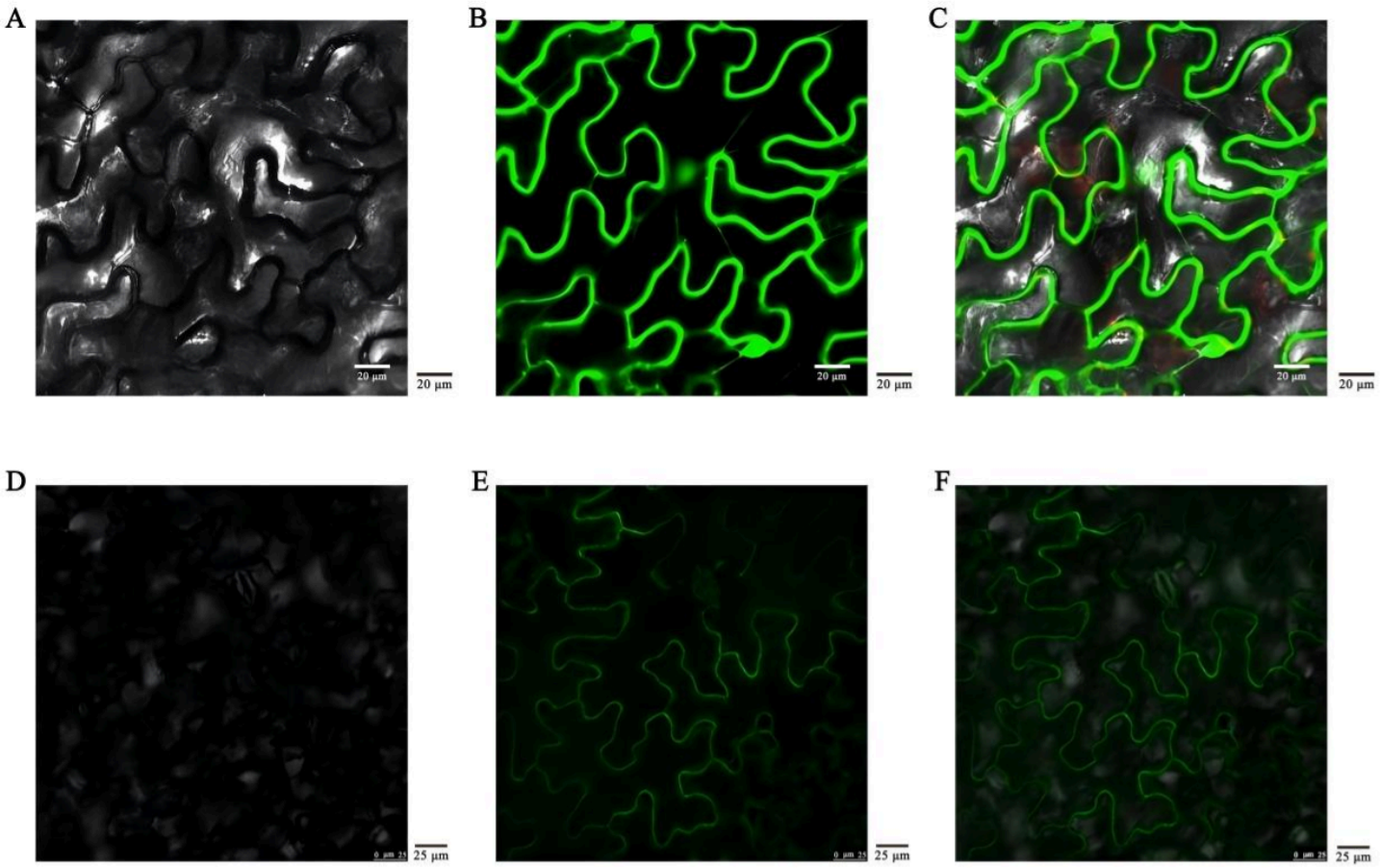


Figure 8

Subcellular localization of Empty vector control and *Xa51*(t) under confocal microscopy.(A) Empty vector control in bright..(B)Empty vector GFP control. (C)Empty vector control in bright field overlay fluorescence integration.(D)*Xa51*(t) vector in bright field. (E)*Xa51*(t) vector GFP. (F) Bright field overlay fluorescence integration of *Xa51*(t)

Supplementary Files

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

- SupplementTables.xlsx
- supplementaryfiguresrice8.6.docx