Precision mapping and expression analysis of recessive bacterial blight resistance gene xa-45(t) from Oryza glaberrima

Ankita Babbar
Punjab Agricultural University
Nidhi Rawat
University of Maryland at College Park: University of Maryland
Pavneet Kaur
Punjab Agricultural University
Navdeep Singh
Punjab Agricultural University
Jagjeet Singh Lore
Punjab Agricultural University
Yogesh vikal
Punjab Agricultural University
kumari neelam
kneelam@pau.edu

Punjab Agricultural University https://orcid.org/0000-0001-7816-3860

Research Article

Keywords: BACTERIAL BLIGHT, RECOMBINANT INBRED LINES, DERIVED CLEAVED AMPLIFIED POLYMORPHIC SEQUENCES, SINGLE NUCLEOTIDE POLYMORPHISM, qRT-PCR, Oryza glaberrima

Posted Date: December 12th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3683558/v1

Version of Record: A version of this preprint was published at Molecular Biology Reports on May 8th, 2024. See the published version at https://doi.org/10.1007/s11033-024-09573-8.

Abstract

Bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most devastating diseases of rice leading to huge yield losses in Southeast Asia. The bacterial blight recessive resistance gene *xa-45(t)* from the *Oryza glaberrima* accession IRGC102600B was mapped to 80 Kb region with 9 candidate genes on Nipponbare reference genome IRGSP-1.0 on rice chromosome 8. The aim of this study was to precisely locate the target gene by utilizing a recombinant inbred line (RIL) population developed from a cross between Pusa 44 (susceptible parent) and an introgression line IL274 (resistant parent). The sequence comparisons between Pusa 44 and IL274 at 9 candidate genes, revealed 7 SNPs and an Indel that were preceded for the marker development. The dCAPS assay revealed 3 recombinant breakpoints for the locus LOC_Os08g42350, LOC_Os08g42370 and LOC_Os08g42400, 15 recombinants for LOC_Os08g423420 and 24 recombinants for LOC_Os08g42440 out of 190 individuals. The Indel marker at the locus LOC_Os08g42410 was found co-segregating with the phenotype thus indicating its candidacy towards *xa-45(t)*. Further, relative expression analysis of the candidate genes at 6-time intervals (0, 8, 24, 48, 72 and 96 hrs) of BB infection showed overexpression of LOC_Os08g42410 specific transcripts in IL274 as compared to Pusa44. At 72 hours after inoculation, a significant 4.46-fold increase in differential expression was observed, providing strong evidence for the involvement of LOC_Os08g42410 in the resistance conferred by the bacterial blight gene *xa-45(t).*

INTRODUCTION

Rice, scientifically known as *Oryza sativa* L. is a widely cultivated cereal crop that proliferates in diverse ecologies throughout the world. Worldwide statistics shows 510 million metric tons of milled rice production in year 2022 with the top rice-producing countries including China, India, Indonesia, Bangladesh, and Vietnam (https://www.statista.com/). However, the growth and development of rice face significant challenges due to various unfavourable factors, resulting in reduced yields and economic losses. Abiotic stresses encompass temperature fluctuations, drought, salinity, floods, nutrient deficiencies, and toxic conditions, while biotic stress factors involve a multitude of pathogens and insect pests. Out of various rice infecting diseases, Bacterial Blight (BB) is an ancient and acute disease attributable to bacteria, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Mew 1987; Vikal and Bhatia 2017). The first outbreak of this disease occurred in 1975 in Bihar, followed by the spread of disease in the Palakkad district of Kerala, Andhra Pradesh, Haryana, Kerala, Orissa, Punjab and, Uttar Pradesh (Rangaswami 1975; Venkatesan and Gnanamanickam 1999). This bacterium is responsible for causing 20–30% yield loss which may reach up to 75% annually specifically in irrigated and rainfed lowland rice-growing areas throughout Asia (Mew 1987; Bhasin et al. 2012; Chen et al. 2011).

The rapid proliferation of this disease highlights the urgency of adopting comprehensive approaches such as integrated pest management strategies, water management techniques, and soil fertility management practices. These measures are essential for reducing the adverse effects of both biotic and abiotic stresses on rice cultivation. However, the conventional measures include excessive use of pesticides and insecticides that poses a high risk to the environment and mankind. Thus, the best

possible eco-friendly and economical measure comprises breeding/ development of rice varieties with improved tolerance to various stresses. The utilization of host plant resistance in the form of available gene pool of wild and landraces germplasm represents effective approach to achieve the desired yield trait in the diseased state. Till date, 47 BB resistant genes, called *Xa /xa* genes, have been identified through various genetic mapping studies (Kim et al. 2015; Busungu et al. 2016; Kim 2018; Kim et al. 2019). Among these, 18 genes *viz. xa5* (Blair et al. 2003), *xa8* (Sidhu et al. 1978), *xa9* (Yoshimura et al. 1983; Sun et al. 2004; Xiang et al. 2006), *xa13* (Yoshimura et al. 1995; Zhang et al. 1996; Chu et al. 2006), *xa15* (Noda and Ohuchi 1989), *xa19* (Taura et al. 1991), *xa20* (Taura et al. 1992), *xa24* (Mir and Khush 1990; Khush and Angeles 1999), *xa25* (Gao et al. 2001,2005), *xa26* (Lee et al. 2003; Sun et al. 2004), *xa28* (Lee et al. 2003), *xa31* (Wang et al. 2009), *xa33* (Korinsak et al. 2009), *xa34* (Chen et al. 2011), *xa41* (Hutin et al. 2015), *xa42* (Busungu et al. 2016), *xa44* (Kim 2018) and *xa-45(t)* (Neelam et al. 2019) are found recessive and rest 29 genes are of dominant type.

Mapping of resistant genes is subsequently followed by the saturation of region so as devise specific gene sequence that allows efficient transfer of desirable gene to elite varieties. Various approaches are employed for fine mapping of genes including, map-based cloning of *Xa1* was employed by isolating 340 kb YAC clone spanning the locus, which was followed by construction of cDNA library of IRBB1. The position clones were used for high resolution linkage mapping followed by complement testing with different *Xoo* races and the composite cDNA clone revealed presence of exons (Yoshimura et al. 1998). Among the characterized gene, only *Xa21* and *Xa3/ Xa26* are involved in regulation of gene -for-gene type disease resistance (Xu et al. 2007). Furthermore, positional cloning including critical recombinant selection, SNP assay between recombinant markers followed by RT-PCR and in-silico sequence homology analysis led to narrowing of 100 kb *xa5* segment to 8.1 kb region encoding a transcription factor TFIIAγ (Iyer et al. 2004). In addition to this, *Xa10* locus was fine mapped to proximal side of marker E1981S with genetic distance at 0.93 cM belonging to 74 kb region on Nipponbare genome bearing, 6 candidate genes (Gu et al. 2008). Also, Chu et al. (2006) fine mapped *xa13* gene to a 14.8 kb region using BAC libraries. Another gene, *Xa23* was fine mapped using TAC and PAC libraries using EST marker's PCR product of 0.8 kb. The region was fine mapped to 0.4–0.6 kb region using 7 probes for RFLP survey (Wang et al. 2006). In summary, fine mapping efforts are crucial for identifying and characterizing resistance genes, enabling the development of resilient and productive crop varieties, ultimately enhancing agriculture and food security.

The O. *glaberrima* (2n = 24, AA) is a low-yielding African rice variety and its production is limited to West Africa. Various studies suggested that the African rice *O. glaberrima* (AA genome) pertains tremendous potential as a source for improving the cultivated rice produce. At PAU, a novel recessive BB resistant gene *xa-45(t)* was identified from the *O. glaberrima* accession IRGC 102600B limiting to 80 kb on Nipponbare reference genome IRGSP-1.0 harboring 9 candidate genes. The purpose of this study was to pinpoint a candidate gene and conduct expression analysis to reveal the probable gene responsible for the resistance.

MATERIALS AND METHODS

Plant material

A bacterial blight tolerant accession namely, *O. glaberrima* IRGC 102600B exhibits resistance against 7 *Xoo* pathotypes prevalent in Punjab (Neelam et al. 2019, Vikal et al. 2007). In order to develop RILs with desirable traits, a BC_3F_8 introgression line of *O. glaberrima* IRGC 102600B *viz.* IL274, a medium maturing semi-dwarf high yielding variety was selected along with the elite cultivar Pusa 44. The RILs were sown in the kharif season 2019 (F_6 generation) and 2020 (F_7 generation). The experimental design used for planting RILs was a Randomized Complete Block Design (RCBD) with standard row to row and plant to plant spacing of 20 x 15 cm for each RIL.

Bacterial Blight inoculation with PbXo-7

The *Xoo* bacterial inoculations were performed following Kauffman's leaf-clipping method to evaluate the disease reaction of population. The isolates of *Xoo* strain PbXo-7 were isolated from the ooze of cut leaves *viz.* reservoir of specific bacterial strains (Lore et al. 2011). The ooze so obtained was streaked on Walkimoto media (10g sucrose, 5g peptone and 20g agar/1000ml distilled water) plates/ slants and were incubated at 27–30°C for 72 h and these colonies were further stored as stock at 4°C. *Xoo* isolates were revived on the same media at 30°C and grown colonies were suspended in sterile distilled water to a concentration of about 10^8 cell/ml to prepare the inoculum. This *Xoo* inoculum was used to inoculate and assess the disease reaction of plant material at maximum tillering stage following the leaf-clipping method (Kauffman et al. 1973).

Phenotypic Assessment

The mean lesion length or SES qualitative score using 0–9 scale was used for phenotyping of the population. For this study, average lesion length of five leaves from each individual plant was inoculated with pathotype PbXo-7 in triplicates and its lesion length was recorded after 14 days of inoculation. The lesion length up to 5.0 cm (disease score 1–3) was classified as resistant, between 5 and 10 cm (disease score 3–5) as moderately resistant, 11–15 cm (disease score 5–7) as moderately susceptible and greater than 15 cm (disease score 7–9) as susceptible (Cottyn and Mew 2004) (Fig S1). The Chi-square test was used to test the goodness of fit for ascertaining the number of genes governing the BB resistance.

DNA extraction and Marker development

The large-scale DNA extraction protocol was followed to isolate DNA of Pusa 44 (susceptible parent), IL274 (resistant parent), along with the RIL population following standard CTAB (Cetyl Trimethyl Ammonium Bromide) method (Doyle & Doyle 1987). The high molecular weight genomic DNA was evaluated for its quality and integrity on 0.8% agarose gel electrophoresis. Further, DNA quantity and purity were determined using spectrophotometer at 260/280 nm absorbance ratio.

Marker development and Sequence analysis of candidate genes in Pusa 44 and IL274

For the 9 candidate genes locus-specific primers were designed using Perl primer offline tool, keeping the parameters to default. The genomic sequences of candidate genes were downloaded from the Nipponbare reference genome IRGSP 1.0 available at the Rice Genome Annotation Project database for designing overlapping primers for full-length gene amplification (http://rice.plantbiology.msu.edu/). A PCR reaction of 20–50 µl volume was carried out using hi-fidelity *Taq polymerase* Extaq (Takara) for the purpose of sequencing; that was preceded in triplicates in order to obtain stringency of data. The sequencing data involved AB1 files, that were first used to align each overlapping primer of a single gene so as to fetch a continuous stretch of sequence using DNA Baser Assembler. Following this, the individual forward and reverse sequences were aligned to generate full contig of genes so as to find the variations among Pusa 44 and IL274 with respect to all candidate genes using CLUSTAL X offline tool. The tools aided the identification of the putative SNPs and Indels for the described locus.

SNP and Indel-based analysis

For analysing and verifying the putative SNPs, dCAPS markers were designed using an online tool dCAPS finder 2.0. The genomic sequence of two haplotypes which was identical except for the putative SNP was used for designing these primers. The input included 25 nucleotide sequence with the SNP in between for wild and mutant alleles, where Pusa 44 was considered as wild while IL274 as mutant haplotype. Initially, the output from zero mismatches shows whether a CAPS marker is present or not. Further, the number of mismatches was increased in each run until a potential dCAPS marker was obtained.. The genotyping of population was conducted through dCAPS analysis, which included PCR amplification, amplicon confirmation through agarose gel electrophoresis followed by its restriction digestion using a suitable enzyme. The reaction involved 5 µl of PCR product, optimal units of restriction enzyme, buffer owing to 100% activity of enzyme and nuclease-free water, which was then incubated at optimal temperature and time. These enzymatic digestions were visualized by agarose gel electrophoresis for genetic profiling. Apart from SNPs, the sequential variations included Indels and thus, Indel-based markers were developed using Perl primer tool, in such a way that the insertion-deletion region was included within the amplicon.

RNA Extraction and cDNA preparation

To conduct quantitative expression analysis of candidate genes, leaf samples of susceptible parent (Pusa 44) and resistant parent (IL274) were collected in the time span on 8, 24, 48, 72 and 96 hours post *Xoo* inoculation and its total RNA was isolated along with the control leaf samples collected before *Xoo* inoculation. Prior to extraction, all the plasticware, glassware and pestle-mortars were treated with 1X DEPC (Diethyl pyrocarbonate) followed by its autoclaving to attain complete sterilization. Total RNA was isolated from leaf samples using TRIzol reagent (Takara), following manufacturer's instruction. The quality of isolated RNA was confirmed with denaturing agarose gel prepared in 1X MOPS [3-(N-Morpholino) propane sulfonic Acid] buffer. The total RNA, appropriately denatured was visualized as

ribosomal RNA with 28S, 18S and 5S subunits and quality was evaluated based on three distinct bands on gel. The concentration of RNA sample was measure using Thermo scientific NanoDrop™ 1000 spectrophotometer. The samples having 260/280 absorbance ratio between 1.9 to 2.1, were characterized as adequately good quality and pure RNA. From the normalised RNA samples, First-strand of cDNA was synthesised by reverse transcribing total RNA using PrimeScript 1st strand cDNA synthesis kit (Takara). Subsequently the intactness of cDNA was assessed by a PCR amplification of template cDNA with house-keeping *Actin* gene-specific primer, followed by agarose gel electrophoresis for visualising an amplicon of 67 bp(Fig. S2).

Real-Time PCR assay

To facilitate Quantitative Real-time PCR analysis (qRT-PCR) gene specific primers of the candidate genes were designed using Perl primer tool under default parameters. The primers designed for qRT-PCR comprises of an amplicon size within the range of 80–220 bp (Table S1). Relative expression of all the candidate gene at 0, 8, 24, 48, 72 and 96 hrs post inoculation with *Xoo* pathotype-7 was analysed keeping *Actin* as an internal control. This qRT-PCR assay was performed using 96-well StepOnePlus Applied Biosystem RT-PCR. The PCRs conditions for the all the primers were set up as: 94ºC for 3 minutes, followed by 45 cycles for 30 seconds at 94ºC, 42 seconds at T_a(ºC), 30 seconds at 72ºC. Expression dynamics were analysed subsequently by $2^{-(\Delta\Delta Ct)}$ method given by Livak and Schmittgen in 2001. The relative expression of target genes was normalized to reference gene expression for each sample where $2^{-(\Delta\Delta Ct)}$ value represents fold change in gene expression in stress conditions relative to the control conditions.

RESULTS

(A) FINE MAPPING

Inheritance pattern of BB resistance

To evaluate the inheritance pattern, we recorded the disease reaction of F_6 and F_7 population developed from the cross of Pusa 44 and IL274 an introgression line derived from *O. glaberrima* IRGC102600B (Fig. 1). The populations were consecutively screened for two years against PbXo-7. The phenotypic evaluation of the mean data of F_6 and F_7 disease reaction against PbXo-7, showed 1:1 segregation ratio for recessive gene *xa-45(t)* (Table S2). Among the 290 individuals, 152 were resistant while 141 were displaying susceptible disease reaction fitting to 1:1 segregation ratio. The Chi-square value was found non-significant at 5% level of significance (0.2, $2.33 \leq 3.8\ \chi^2_{0.05,1}$) (Table 1). Thus, the inheritance and segregation pattern from these results implies that a single gene is responsible for conferring resistance governed by *xa-45(t)*.

Table 1
Chi Square analysis of F_6 and F_7 RIL population indicating single recessive gene inheritance of xa-*45(t)*

F_6				
BB disease score	**0–3 (Resistant)**	**5–9 (Susceptible)**	**$\chi 2_{Calculated}$ (1:1)**	**$\chi 2_{Table}$ (0.05,1)**
Number of plants	161	132	2.8	3.84
F_7				
Number of plants	152	141	0.41	3.84

Sequential variations found among the candidate genes

The recessive *xa-45(t)* gene, confining to an 80 kb region harbours 9 candidate genes. The sequencing results obtained from overlapping primers for the candidate genes revealed 13 SNPs and 5 Indels among the two parents. To put in view, 4 SNPs were found for LOC_Os08g42370, a SNP and an Indel corresponding to LOC_Os08g42390, LOC_Os08g42400 having 2 SNPs and Indels, 3 SNPs and 10 bp deletion in LOC_Os08g42410, 1 SNP pertaining to LOC_Os08g42420 while LOC_Os08g42440 comprised of 2 SNPs and an Indel. The LOC_Os08g42360, LOC_Os08g42380 and LOC_Os08g42430 were not considered for the candidacy due to the absence of variation in the nucleotide sequence of resistant v/s susceptible parents. From the whole, 7 putative SNPs and Indels belonging to 6 candidate genes were selected in correspondence to IL274 (Table 2) for the marker development.

Table 2
SNPs and Indels identified in different candidate genes and its corresponding dCAPS assay

Gene_ID	Alleles	Position #	Confirmation assay
LOC_Os08g42360	-	-	-
LOC_Os08g42370	T/G**	723	
	A/T**	927	*NlaIII*
	T/C*	999	*NlaIII*
	T/C*	1087	
LOC_Os08g42380	-	-	-
LOC_Os08g42390	C/A**	1485	*AcuI*
	A/-	1838	
LOC_Os08g42400	T/G**	2157	*SwaI*
	A/G*	2374	
	A/-	2681	
	AT/--	2783	
LOC_Os08g42410	A/C**	1068	
	A/T**	1220	
	T/G**	1313	
	TCTCTCTCTC/----------	4458	Indel Marker
LOC_Os08g42420	C/G**	2526	*HgaI*
LOC_Os08g42430	-	-	-
LOC_Os08g42440	A/-	3311	
	T/C*	3455	*HpyCHIV*
	T/C*	3563	

Transitions* and transversions** observed as nucleotide substitution

The SNP position was calculated relative to the reference sequences of candidate gene from http://rice.plantbiology.msu.edu/

[+]Text highlighted in green depicts the SNPs used for dCAPS assay

Development of SNP and Indel-based marker for MAS

We conducted fine mapping of *xa-45*(t) using dCAPS (derived-Cleaved Amplified Polymorphic Sequences) markers designed for LOC_Os08g42370, LOC_Os08g42390, LOC_Os08g42400, LOC_Os08g42420, and LOC_Os08g42440. These polymorphic markers were employed for genotypic assay of F_7 populations (Fig. 2). Among the 190 individuals, three recombinant inbred lines (11, 124, and 125) exhibited breakpoints with respect to LOC_Os08g42350, LOC_Os08g42370, and LOC_Os08g42400. Intriguingly, these individuals displayed a resistant disease reaction to Pbxo-7, contrary to their genotypic data, which indicated susceptibility. For the markers LOC_Os08g42440 and LOC_Os08g423420, we identified 24 and 15 recombinants between genotype and phenotype, respectively, suggesting that these loci were not associated with *xa-45*(t) (Fig. 3). Apart from this, an Indel marker was designed for locus LOC_Os08g42410, which exhibited clear size differentiation between the parental lines and population (Forward primer: GTTGGCGCTGAAATATGGTC; Reverse primers: ACAAAGCAGCAGCCGTAAGT).

Expression dynamics of candidate genes

The expression study of putative candidate genes was conducted with three biological replicates of the parental lines at the specific time points including 0, 8, 24, 48, 72, and 96 hours after PbXo-7 inoculations. The transcript abundance assay for LOC_Os08g42370, LOC_Os08g42390 and LOC_Os08g42420 unveiled higher number of transcripts for Pusa 44 at 8, 24, 48, 72 and 96 h post inoculation experiments with maximum fold change of 3.47 at 24 h, 9.68 at 96 h and 5.45 at 24 h respectively. This enumeration indicated for Pusa 44, serve to eliminate LOC_Os08g42370, LOC_Os08g42390 and LOC_Os08g42420 as potential candidates. Besides these, the two potential candidate genes LOC_Os08g42380, LOC_Os08g42430 could not be validated due to difficulties encountered in obtaining amplification with their respective primers. In addition to these, differential expression among the resistant and susceptible reaction against PbXo-7 was not observed for LOC_Os08g42400, LOC_Os08g42440. Unparallelly significant expression was recorded for LOC_Os08g42410 in IL274 contrary to Pusa 44. The transcription of this gene exhibited a gradual upregulation ranging from 1.88- fold to 4.46-fold, during the time span of 8 to 72 h post *Xoo* inoculation (Fig. 4). The sequential variation confirms presumptive gene LOC_Os08g42410 as the putative gene for *xa-45(t)*, validated by expression studies. All these experiments offer compelling evidence that LOC_Os08g42410 is the putative gene among all the genes loacted within the 80 kb region.

Discussion

The present study advances our understanding regarding molecular and genetic mechanisms of disease resistance along with marker-assisted breeding of *xa-45(t)* into elite cultivars for providing broader resistance. The gene has been identified and transferred from *O. glaberrima* IRGC102600B. Among the cultivated rice worldwide, *O. glaberrima* (AA genome) has its spread restricted to West Africa only. The African rice has been brought to domestication from its putative progenitor *O. barthii*. The focus regarding development of new resistance sources can clearly indulge *O. glaberrima* species due to its important traits *viz.* weed competitiveness, drought tolerance, survival under low input conditions, tolerance to abiotic stresses and resistance to pests & diseases. Various studies suggests that *O.*

glaberrima is a novel source for drought related QTLs. Bimpong et al. 2011 derived the well-known fact that *O. glaberrima* accessions are efficiently involve in early stomatal closures and early maturity, that fundamentally provides tolerance to drought conditions. The species has the key advantage of its resistance gene pool along with its ability to grow in low input conditions (Sarla and Swamy 2005). Also, an alien introgression line derived from *O. glaberrima* and *O. sativa*, that bears higher yield than *O. sativa* during drought stress. In addition to these, 2000 accessions of this species tolerant to drought, have been evaluated by Shaibu et al. 2018. Thiemele et al. 2010, identified *RYMV2* gene for Rice Yellow Mottle Virus from *O. glaberrima* accession TOG5681. In few regions of Africa, phosphorus deficiency is generally observed; therefore, *OsPSTOL1* gene has been identified so as to tolerate phosphorus starvation conditions (Gamuyao et al. 2012). Pidon et al. (2017) reported a resistant gene RYMV3 from *O. glaberrima* against *Rice Yellow mottle virus*. Petitot et al. 2017, studied molecular responses for *Meloidogyne graminicola* in *O. glaberrima* accessions TOG5681. The responses were assessed using histological assay and root transcriptome profiling. Various QTLs were observed for chalcone synthase, isoflavone reductase, phenylalanine ammonia lyase, WRKY62 transcription factor, thionine, thaumatin, ATPase3 and stripe rust resistance that ultimately provides resistance against nematode virus. Many more genes for various important traits have been identified from *O. glaberrima*. Therefore, for increasing per capita production and fulfilling food requirements, more efforts should be directed towards *O. glaberrima* gene pool utilization.

The analysis of transcript abundance highlights LOC_Os08g42410 as the potential gene responsible for the region exhibiting peak expression at 72 hours after *Xoo* infestation. Other bacterial blight studies also involved differential expression as a tool to decipher the mechanism behind the disease resistance. The first BB resistant gene *Xa1* conferring resistance to Japanese race 1 of *Xoo*, been isolated by map-based cloning, belonging to NBS-LRR family of R proteins. The RT-PCR analysis conducted for *Xa1* gene revealed the induction of gene by wounding and pathogen inoculation in IRBB1, which was absent in T7 133 and IR24 (Yoshimura et al. 1998). Iyer et al. 2004 employed RT-PCR analysis to investigate the candidate genes Q94HL4 and TFIIAγ associated with *xa5*. They found these genes expressed at all time points in both wounded and control samples. However, the transcript abundance assay for Q94HL4 exhibited a similar pattern in susceptible and resistant isolines, leading to its exclusion as a potential gene. Consequently, the focus shifted to TFIIAγ, which harbored a mutation in its coding region and is now considered the likely contributor to *xa5* resistance. In year 2018, *xa44*(t) was identified as 120 kb segment harbouring 9 ORFs. Among these 9 ORFs, qRT-PCR analysis confirmed the significant differential expression levels of the candidate genes (Os11g0690066 and Os11g0690466) (Kim 2018). These studies collectively demonstrate the crucial role of expression analysis in unravelling the genetic basis of disease resistance in the context of bacterial blight.

The putative function of LOC_Os08g42410 belongs to transketolase activity. Its role has been verified under various biotic stress, abiotic stress, plant growth, development and diverse physiological processes. Many studies reveal the role of transketolase especially in photosynthetic activities of plants. Previously, Henkes et al. (2001) reported the downregulation of transketolase enzyme in tobacco transformants which further inhibits ribulose-1,5-bisphosphate, confirming its role in photosynthesis.

Similarly, in rice plants decreased amount of transketolase was observed under salt stress that ultimately hampers photosynthetic activity of seedlings (Kim et al. 2005). Transketolase enzymatic activity is found highly profound in signalling cascades and reactive oxygen species (ROS) production on the onset of disease reaction. Tunc-Ozdemir et al. (2009) verdicts the role of transketolase in production of cytosolic NADPH that further promotes ROS production during stress conditions. Fernandez et al. (2014) reported role of transketolase in providing resistance in rice against rice blast disease. Various reports suggest the protective role of transketolase for other crops like *Zea mays* (Rapala- Kozik et al. 2008) and *Solanaceum oleracea* (Kaiser 1976; Takabe et al. 1980). These findings collectively underscore the significance of transketolase in diverse physiological processes and its potential importance in enhancing plant resilience towards various diseases.

Conclusion

To sum it up, the locus LOC_Os08g42410 has been recognized as the primary candidate for the bacterial blight recessive gene *xa-45(t)*. This gene exhibits transketolase activity in plants, which plays a crucial role in various important processes such as biotic stress response, abiotic stress response, plant growth, development, and physiological processes.

Declarations

Funding

The authors gratefully acknowledge the financial support from Indo-U.S. Fellowship for Women in STEMM (WISTEMM_Fellowship_2020_0927).

Competing Interests

The authors declare no conflict of interest

Authors' Contributions

Kumari Neelam and Yogesh Vikal designed the experiment and developed the mapping population. Jagjeet Singh Lore provided *Xanthomonas* cultures and assisted in phenotyping of the mapping population. Ankita Babbar carried out the phenotyping and genotyping of F_6 and F_7 population along with qRT-PCR. Pavneet Kaur and Navdeep Singh assisted in data collection and analysis. Ankita Babbar and Kumari Neelan analysed the result and wrote the manuscript. Nidhi Rawat and Kumari Neelam proofread the manuscript and provided the critical feedback to shape the manuscript. All co-authors approved this manuscript before submission.

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors

Consent to Participate

Informed consent was obtained from all individual participating in the study

Consent to Publish

Additional informed consent was obtained from all individual participants included in this study

Acknowledgements

Authors considerately acknowledge Indo-U.S. Fellowship for Women in STEMM (WISTEMM_Fellowship_2020_0927) by Department of Science and Technology, Government of India, for providing financial support.

References

1. Bhasin H, Bhatia D, Raghuvanshi S, Lore JS, Sahi GK, Kaur B (2012) New PCR-based sequence-tagged site marker for bacterial blight resistance gene *Xa38* of rice. Mol Breed 30:607–611
2. Bimpong KI, Serraj R, Chin JH, Ramos J, Mendoza EMT, Hernandez JE, Mendioro MS, Brar DS (2011) Identification of QTLs for drought-related traits in alien introgression lines derived from crosses of rice (*Oryza sativa* cv. IR64) × *O. glaberrima* under lowland moisture stress. J Plant Biol 54:237–250. https://doi.org/10.1007/s12374-011-9161-z
3. Blair MN, Garris AJ, Iyer AS, Chapman B, Kresovich S, McCouch SR (2003) High resolution genetic mapping and candidate gene identification at the *xa5* locus for BB resistance in rice. Theor Appl Genet 107:62–73. https://doi.org/10.1007/s00122-003-1231-2
4. Busungu C, Taura S, Sakagami JI, Ichitani K (2016) Identification and linkage analysis of a new rice bacterial blight resistance gene from XM14, a mutant line from IR24. Breed Sci 66:636–645. https://doi.org/10.1270/jsbbs.16062
5. Chen S, Liu X, Zeng L, Ouyang D, Yang J, Zhu X (2011) Genetic analysis and molecular mapping of a novel recessive gene *xa34(t)* for resistance against *Xanthomonas oryzae* pv. *oryzae*. Theor Appl Genet 122:1331–1338. https://doi.org/10.1007/s00122-011-1534-7
6. Chu ZH, Fu B, Yang H, Xu C, Li Z, Sanchez AH, Park JJ, Bennetzen L, Zhang Q, Nang S (2006) Targeting *xa13*, a recessive gene for BB resistance in rice. Theor Appl Genet 112:455–461. https://doi.org/10.1007/s00122-005-0145-6
7. Cottyn B, Mew TW (2004) Bacterial blight of rice. In: Goodman RM (ed) Encyclopedia of plant and crop science. Marcel Dekker, New York, pp 79–83
8. Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15
9. Fernandez J, Marroquin-Guzman M, Wilson RA (2014) Evidence for a transketolase-mediated metabolic checkpoint governing biotrophic growth in rice cells by the blast fungus *Magnaporthe oryzae*. PLoS Pathog. https://doi.org/10.1371/journal.ppat.1004354

10. Gamuyao R, Chin JH, Pariasca-Tanaka J, Pesaresi P, Catausan S, Dalid C, Slamet-Loedin I, Tecson-Mendoza EM, Wissuwa M, Heuer S (2012) The protein kinase *Pstol1* from traditional rice confers tolerance of phosphorus deficiency. Nature 488:535–539. https://doi.org/10.1038/nature11346
11. Gao DY, Liu MA, Zhou AH, Cheng Y, Xiang YH, Sun LH, Zhai WX (2005) Molecular mapping of a bacterial blight resistance gene *Xa-25* in rice. J Genet Genom 32:183–188
12. Gao DY, Xu ZG, Chen ZY, Sun LH, Sun QM, Lu F, Hu BS, Liu YF, Tang LH (2001) Identification of a new gene for resistance to bacterial blight in a somaclonal mutant HX-3 (indica). Rice Genet Newsl 18:66–68
13. Gu K, Sangha JS, Li Y, Yin Z (2008) High-resolution genetic mapping of bacterial blight resistance gene *Xa10*. Theor Appl Genet 116:155–163. https://doi.org/10.1007/s00122-007-0655-5
14. Henkes S, Sonnewald U, Badur R, Flachmann R, Stitt M (2001) A small decrease of plastid transketolase activity in antisense tobacco transformants has dramatic effects on photosynthesis and phenylpropanoid metabolism. Plant Cell 13:535–551. https://doi.org/10.1105/tpc.13.3.535
15. Hutin M, Sabot F, Ghesqui ere A, Koebnik R, Szurek B (2015) A knowledge-based molecular screen uncovers a broad-spectrum *OsSWEET14* resistance allele to bacterial blight from wild rice. Plant J 84:694–703. https://doi.org/10.1111/tpj.13042
16. Iyer AS, McCouch SR (2004) The rice bacterial blight resistance gene *xa5* encodes a novel form of disease resistance. Mol Plant Microbe Interact 17:1348–1354. https://doi.org/10.1094/MPMI.2004.17.12.1348
17. Kaiser W (1976) The effect of hydrogen peroxide on CO_2 fixation of isolated intact chloroplasts. Biochim Biophys Acta 440:476–482. https://doi.org/10.1016/0005-2728(76)90035-9
18. Kauffman HE, Reddy APK, Hsieh SPY, Merca SD (1973) An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. Plant Dis Rep 57:537–541
19. Khush GS, Angeles ER (1999) A new gene for resistance to race 6 of bacterial blight in rice, *Oryza sativa* L. Rice Genet Newsl 16:92–93
20. Kim SM (2018) Identification of novel recessive gene *Xa44*(*t*) conferring resistance to bacterial blight races in rice by QTL linkage analysis using an SNP chip. Theor Appl Genet 131:2733–2743. https://doi.org/10.1007/s00122-018-3187-2
21. Kim SM, Reinke RF (2019) A novel resistance gene for bacterial blight in rice, *Xa43(t)* identified by GWAS, confirmed by QTL mapping using a bi-parental population. PLoS ONE 14(2): e0211775 https://doi.org/10.1371/journal.pone.0211775
22. Kim SM, Suh JP, Qin Y, Noh TH, Reinke RF, Jena KK (2015) Identification and fine-mapping of a new resistance gene, *Xa40*, conferring resistance to bacterial blight races in rice (*Oryza sativa* L). Theor Appl Genet 128:1933–1943. https://doi.org/10.1007/s00122-015-2557-2
23. Korinsak S, Sriprakhon S, Sirithanya P, Jairin J, Korinsak S, Vanavichit A (2009) Identification of microsatellite markers (SSR) linked to a new bacterial blight resistance gene *xa33(t)* in rice cultivar 'Ba7'. Maejo Int J Sci Technol 3:235–247

24. Lee KS, Rasabandith S, Angeles ER, Khush GS (2003) Inheritance of resistance to bacterial blight in 21 cultivars of rice. Phytopathol 93:147–152. https://doi.org/10.1094/PHYTO.2003.93.2.147
25. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using realtime quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262
26. Lore JS, Vikal Y, Hunjan MS, Goel RK, Bharaj TS, Raina GL (2011) Genotypic and pathotypic diversity of *Xanthomonas oryzae* pv. *oryzae*, the cause of bacterial blight of rice in Punjab state of India. J Phytopathol 159:479–487. https://doi.org/10.1111/j.1439-0434.2011.01789.x
27. Mew TW (1987) Current status of future prospects of research on bacterial blight of rice. Annu Rev Phytopathol 25:359–382. https://doi.org/10.1146/annurev.py.25.090187.002043
28. Mir GN, Khush GS (1990) Genetics of resistance to bacterial blight in rice cultivar DV86. Crop Res 3(2):194–198
29. Neelam K, Mahajan R, Gupta V, Bhatia D, Gill B, Komal R, Lore JS, Mangat G, Singh K (2019) High-resolution genetic mapping of a novel bacterial blight resistance gene *xa-45(t)* identified from *Oryza glaberrima* and transferred to *Oryza sativa*. Theor Appl Genet 135:689–705. https://doi.org/10.1007/s00122-019-03501-2
30. Noda T, Ohuchi A (1989) A new pathogenic race of *Xanthomonas campestris* pv. *oryzae* and inheritance of resistance of differential rice variety, Tetep to it. Jpn J Phytopathol 55:201–207. https://doi.org/10.3186/jjphytopath.55.201
31. Petitot AS, Kyndt T, Haidar R, Dereeper A, Collin M, Engler JDA, Gheysen G, Fernandez D (2017) Transcriptomic and histological responses of African rice (*Oryza glaberrima*) to *Meloidogyne graminicola* provide new insights into root-knot nematode resistance in monocots. Ann Bot 119:885–899. https://doi.org/10.1093/aob/mcw256
32. Pidon H, Ghesquière A, Chéron S, Issaka S, Hébrard E, Sabot F et al (2017) Fine mapping of *RYMV3*: a new resistance gene to Rice yellow mottle virus from *Oryza glaberrima*. Theor Appl Genet 130:807–818. https://doi.org/10.1007/s00122-017-2853-0
33. Rangaswami G (1975) Diseases of crop plants in India, p-520. Prentice Hall, New Delhi
34. Rapala-Kozik M, Kowalska E, Ostrowska K (2008) Modulation of thiamine metabolism in *Zea mays* seedlings under conditions of abiotic stress. J Exp Bot 59:4133–4143. https://doi.org/10.1093/jxb/ern253
35. Sarla N, Swamy BPM (2005) *Oryza glaberrima*: A source for the improvement of *Oryza sativa*. Curr Sci 89:955–963
36. Shaibu AA, Uguru MI, Sow M, Maji AT, Ndjiondjop MN, Venuprasad R (2018) Screening African rice (*Oryza glaberrima*) for tolerance to abiotic stresses: II. Lowland drought. Crop Sci 58(1):133–142. https://doi.org/10.2135/cropsci2017.04.0255
37. Sidhu GS, Khush GS, Mew TW (1978) Genetic analysis of bacterial blight resistance in seventy-four cultivars of rice, *Oryzae sativa L*. Theor Appl Genet 53:105–111. https://doi.org/10.1007/BF00272687

38. Sun X, Cao Y, Yang Z, Xu C, Li X, Wang S, Zhang Q (2004) *Xa26*, a gene conferring resistance to *Xanthomonas oryzae* pv. *oryzae* in rice, encodes an LRR receptor kinase-like protein. Plant J 37:517–527. https://doi.org/10.1046/j.1365-313X.2003.01976.x
39. Takabe T, Asami S, Akazawa T (1980) Glycolate formation catalyzed by spinach leaf transketolase utilizing the superoxide radical. Biochemistry 19:3985–3989. https://doi.org/10.1021/bi00558a015
40. Taura S, Ogawa T, Yoshimura A, Ikeda R, Iwata N (1992) Identification of a recessive resistance gene to rice bacterial blight of mutant line XM6, *Oryza sativa* L. Jpn J Breed 42:7–13. https://doi.org/10.1270/jsbbs1951.42.7
41. Taura S, Ogawa T, Yoshimura A, Ikeda R, Omura T (1991) Identification of a recessive resistance gene in induced mutant line XM5 of rice to bacterial blight. Jpn J Breed 41:427–432. https://doi.org/10.1270/jsbbs1951.41.427
42. Thiémélé D, Boisnard A, Ndjiondjop MN et al (2010) Identification of a second major resistance gene to Rice yellow mottle virus, *RYMV2*, in the African cultivated rice species, *O. glaberrima*. Theor Appl Genet 121:169–179. https://doi.org/10.1007/s00122-010-1300-2
43. Tunc-Ozdemir M, Miller G, Song L, Kim J, Sodek A, Koussevitzky S, Misra AN, Mittler R, Shintani D (2009) Thiamin confers enhanced tolerance to oxidative stress in Arabidopsis. Plant Physiol 151:421–432. https://doi.org/10.1104/pp.109.140046
44. Venkatesan BP, Gnanamanickam SS (1999) Occurrence of a sub-population of *Xanthomonas oryzae* pv. *oryzae* with virulence to rice cultivar IRBB21 (*Xa21*) in southern India. Plant Dis 83:781–781. https://doi.org/10.1094/PDIS.1999.83.8.781B
45. Vikal Y, Bhatia D (2017) Genetics and genomics of Bacterial Blight Resistance. Chapter 10 In: Li Jinquan (ed.) Advances in International Rice Research. 175–213
46. Vikal Y, Das A, Patra B, Goel RK, Sidhu JS, Singh K (2007) Identification of new sources of bacterial blight (*Xanthomonas oryzae pv. oryzae*) resistance in wild *Oryza* species and *O. glaberrima*. Plant Genet Resour 5:108–112. https://doi.org/10.1017/S147926210777661X
47. Wang CL, Chen LT, Zeng CG, Zhang QY, Liu PQ, Liu YG, Fan YL, Zhang Q (2006) Chromosome Walking for Fine Mapping of *Xa23* Gene Locus by Using Genomic Libraries. Chin J Rice Sci 20(4):355–360
48. Wang C, Wen G, Lin X, Liu X, Zhang D (2009) Identification and fine mapping of new bacterial blight resistance gene, *Xa31(t)*, in rice. Eur J Plant Pathol 123:235–240. https://doi.org/10.1007/s10658-008-9356-4
49. Xiang Y, Cao YL, Xu CQ, Li X, Wang S (2006) *Xa3*, conferring resistance for rice bacterial blight and encoding a receptor kinase-like protein, is the same as *Xa26*. Theor Appl Genet 113:1347–1355. https://doi.org/10.1007/s00122-006-0388-x
50. Xu S, Cao Y, Li X, Wang S (2007) Expressional and biochemical characterization of rice disease resistance gene *Xa3/Xa26* family. J Integr Plant Biol 49(6):852–862. https://doi.org/10.1111/j.1744-7909.2007.00494.x
51. Yoshimura A, Mew TW, Khush GS, Omura T (1983) Inheritance of resistance to bacterial blight in rice cultivar Cas 209. Phytopathology 73:1409–1412

52. Yoshimura S, Yamanouchi U, Katayose Y, Toki S, Wang ZX, Kono I, Kurata N, Yano M, Iwata N, Sasaki T (1998) Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. Proc Natl Acad Sci USA 95:1663–1668. https://doi.org/10.1073/pnas.95.4.1663
53. Yoshimura S, Yoshimura A, Iwata N, McCouch SR, Abenes ML, Baraoidan MR, Mew TW, Nelson RJ (1995) Tagging and combining bacterial-blight resistance genes in rice using RAPD and RFLP markers. Mol Breed 1:375–387. https://doi.org/10.1007/BF01248415
54. Zhang G, Angeles ER, Abenes MLP, Khush GS, Huang N (1996) RAPD and RFLP mapping of the bacterial blight resistance gene *xa13* in rice. Theor Appl Genet 93:65–70. https://doi.org/10.1007/BF00225728

Figures

Figure 1

Bacterial blight disease reaction of F_7 RILs against PbXo-7 according to standard evaluation system; P1: Pusa 44, P2: IL274, R: Resistant (Score 1-3), S: Susceptible (Score 7-9)

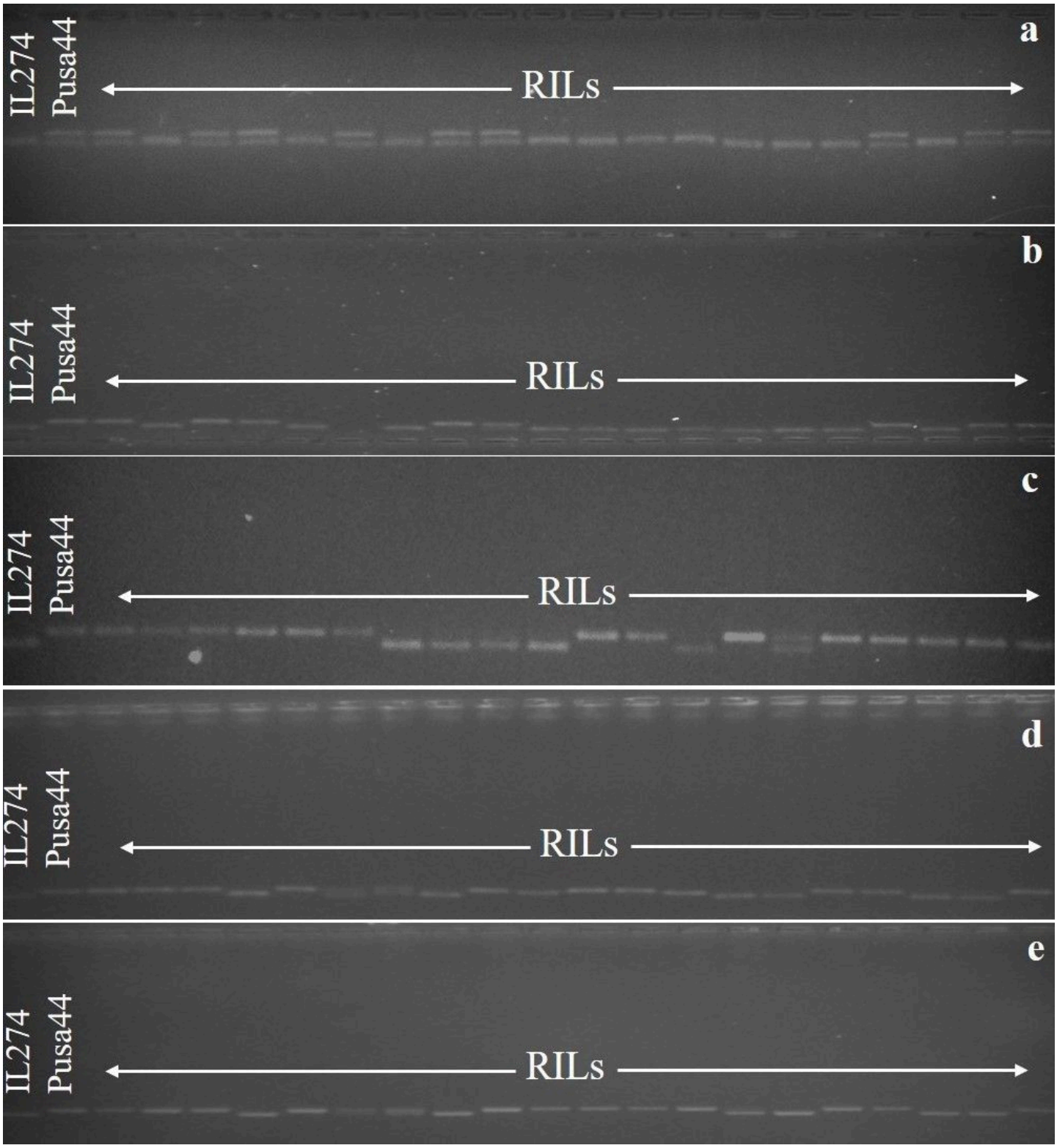

Figure 2

Genotyping of F_7 population a-d represents dCAPS assay a) LOC_Os08g42370 digestion using *NlaIII* b) LOC_Os08g42400 digested using *SwaI* c) LOC_Os08g42420 digested using *HgaI* d) LOC_Os08g42440 digested using *HpyCHIV* e) LOC_Os08g42410 Indel-specific marker

ID	Os0842350	Os0842370	Os0842400	Os0842410	PbXo-7 disease reaction	Os0842420	Os0842440
67	S	S	S	S	S	S	R
68	S	S	S	S	S	S	R
70	S	S	S	S	S	S	R
77	S	S	S	S	S	S	R
93	S	S	S	S	S	S	R
94	S	S	S	S	S	S	R
95	S	S	S	S	S	S	R
141	S	S	S	S	S	S	R
167	S	S	S	S	S	S	R
71	S	S	S	S	S	R	R
11	S	S	S	R	R	R	R
124	S	S	S	R	R	R	R
125	S	S	S	R	R	R	R
13	R	R	R	R	R	S	S
14	R	R	R	R	R	S	S
21	R	R	R	R	R	S	S
43	R	R	R	R	R	S	S
44	R	R	R	R	R	S	S
45	R	R	R	R	R	S	S
49	R	R	R	R	R	S	S
50	R	R	R	R	R	S	S
60	R	R	R	R	R	S	S
63	R	R	R	R	R	S	S
64	R	R	R	R	R	S	S
65	R	R	R	R	R	S	S
10	R	R	R	R	R	S	S
184	R	R	R	R	R	S	S

Figure 3

Recombination events in RIL population for LOC_Os08g42350, LOC_Os08g42370, LOC_Os08g42400, LOC_Os08g42410, LOC_Os08g42420, LOC_Os08g42440 indicating breakpoints for all the 5 loci except LOC_Os08g42410, where R depicts resistant and S depicts susceptible reaction

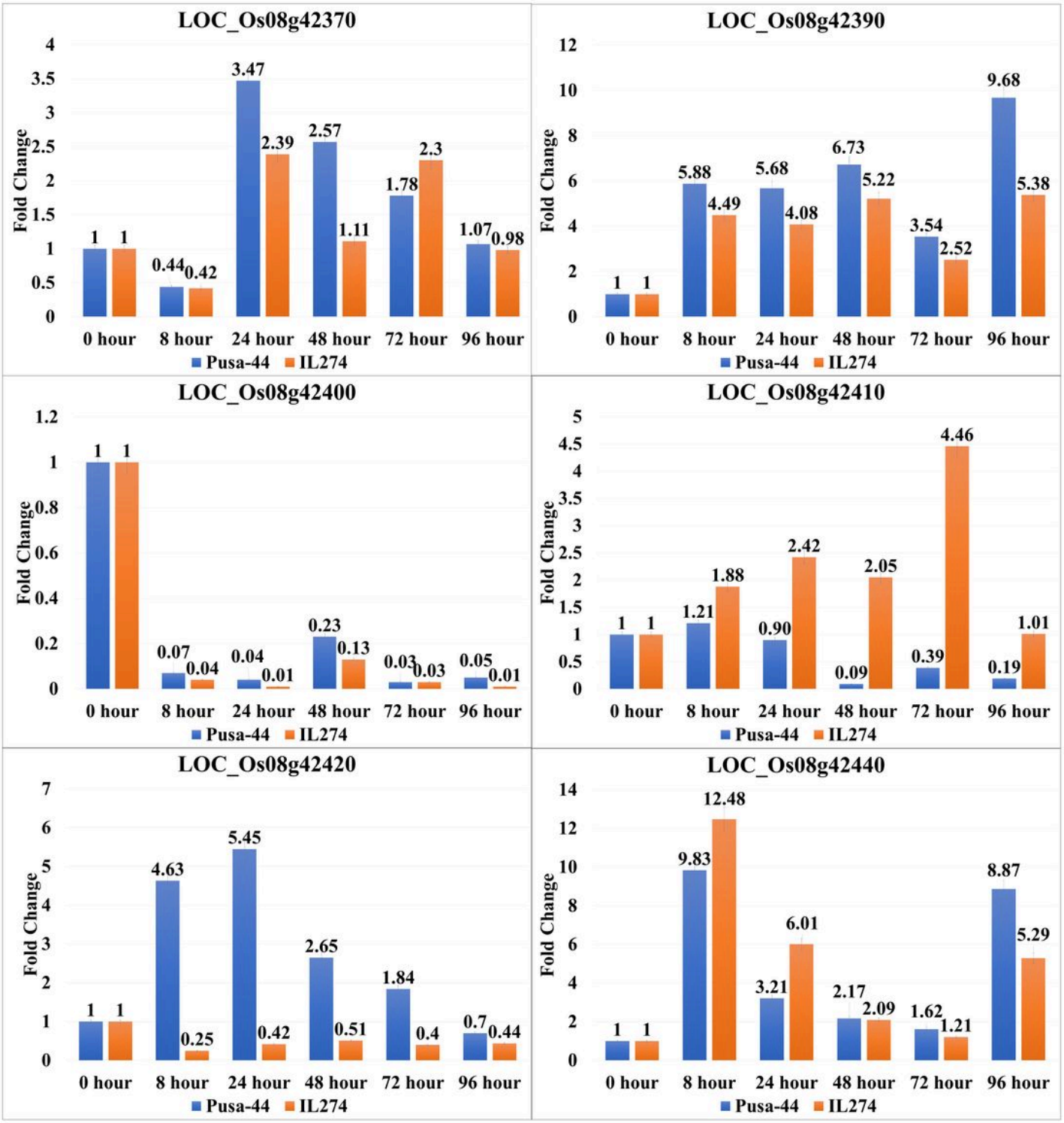

Figure 4

Transcript abundance assay conducted for candidate genes involving *Xoo* inoculation on parental lines Pusa 44 and IL274. The expression analysis of genes was considered at a time course 0 hr, 8 hr, 24 hr, 48 hr, 72 hr and 96 hr of *Xoo*infestation. For qRT-PCR, the average values were obtained for 3 technical replicates, and the error bar shows standard deviation

Supplementary Files

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

- FigS1.jpg
- TableS1.docx
- TableS2.docx