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ORIGINAL ARTICLE

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

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Abstract

Background 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 recessive resistance gene *xa-45(t)* from *Oryza glaberrima* IRGC102600B, mapped on rice chromosome 8, spans 80 Kb with 9 candidate genes on Nipponbare reference genome IRGSP-1.0. The *xa-45(t)* gene provides durable resistance against all the ten *Xanthomonas* pathotypes of Northern India, thus aiding in the expansion of recessive bacterial blight resistance gene pool. Punjab Rice PR127, carrying *xa-45(t)*, was released for wider use in breeding programs. This study aims to precisely locate the target gene among the 9 candidates conferring resistance to bacterial blight disease.

Methods and results Sanger sequencing of all nine candidate genes revealed seven SNPs and an Indel between the susceptible parent Pusa 44 and the resistant introgression line IL274. The genotyping with polymorphic markers identified three recombinant breakpoints for LOC_Os08g42370, and LOC_Os08g42400, 15 recombinants for LOC_Os08g423420 and 26 for LOC_Os08g42440 out of 190 individuals. Relative expression analysis across six time intervals (0, 8, 24, 48, 72, and 96 h) after bacterial blight infection showed over expression of LOC_Os08g42410-specific transcripts in IL274 compared to Pusa 44, with a significant 4.46-fold increase observed at 72 h post-inoculation.

Conclusions The Indel marker at the locus LOC_Os08g42410 was found co-segregating with the phenotype, suggesting its candidacy towards *xa-45(t)*. The transcript abundance assay provides strong evidence for the involvement of LOC_Os08g42410 in the resistance conferred by the bacterial blight gene *xa-45(t).*

Keywords Bacterial blight · Recombinant inbred lines · Derived cleaved amplified polymorphic sequences · Single nucleotide polymorphism · qRT-PCR · *Oryza glaberrima*

Introduction

Rice (*Oryza sativa* L.) is a crucial staple food, sustaining half of the world's population. While its production has steadily risen over the past decade, meeting the demands of a growing population necessitates further increases by 2050 (https://www.statista.com/). However, the emergence of various biotic and abiotic stresses under changing agro-climatic conditions poses a significant threat to rice production worldwide. Out of various rice-infecting diseases, Bacterial Blight (BB) stands out as an ancient and acute disease caused by the bacterium, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) [1, 2]. This bacterium is responsible for causing 20–30% yield loss which may reach up to 75% annually specifically in irrigated and rain-fed lowland rice-growing areas throughout Asia [1, 3, 4]. BB lesions cover the leaves, diminishing photosynthesis and consequently leading to a significant reduction in yield owing to incomplete grain filling. The initial incidence of this disease was recorded in 1975 in Bihar, with subsequent spread observed in the Palakkad district of Kerala, as well as in Andhra Pradesh, Haryana, Kerala, Orissa, Punjab, and Uttar Pradesh [5, 6]. In order to manage this disease, 47 BB-resistant genes,

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named *Xa/xa* have been identified from various sources [7], mostly documented with tightly linked molecular markers. Among these, 18 genes viz. *xa5* on the rice chromosome 5 (C5:437,010–443,270) [8], *xa8* on the rice chromosome 7 (C7:3,697,195–3,706,830) [9], *xa9* on the rice chromosome 11 (C11:27,778,967–27,605,189) [10–12], *xa13* on the rice chromosome 8 (C8:26,725,952–2,678,794, 26,816,798–26,813,624) [13–15], *xa15* [16], *xa19* on the rice chromosome 7 (C7:RM8262-RM6728) [17], *xa20* on the rice chromosome 3 (C3:KIC-33.88-KIC3-34.06) [18], *xa24* (C2:RM14222-RM14224) [19, 20], *xa25* on the rice chromosome 12 (C12:17,302,073–17305326) [21, 22], *xa26* on the rice chromosome 11 (C11:27,778,967–27,605,189) [10, 23], *xa28* on the rice chromosome 6 [23], *xa31* on the rice chromosome 4 (C4: G235-C600) [24], *xa33* on the rice chromosome 6 (C6: RMWR7.1-RMWR7.6) [25], *xa34* on the rice chromosome 1 (C1:11,237,861–11,475,805) [13], *xa41* on the rice chromosome 11 (C11:1,874,478–18,171,678) [26], *xa42* on the rice chromosome 3 (C3: KGC3_16.341& KGC3_16.399) [27], *xa44* (C11:11,964,077–11985463) [7] and *xa-45(t)* (C8:26,725,954–26,728,807) [28] have been found recessive and rest 29 genes are of dominant type.

Fine mapping of the identified resistance genes are required to pinpoint specific gene sequences facilitates the efficient transfer of desirable genes into elite varieties. Various methodologies are employed for fine mapping of genes. For instance, in the case of *Xa1*, map-based cloning involved isolating a 340 kb YAC clone spanning the locus, followed by constructing a cDNA library of IRBB1. The identified clones were then utilized for high-resolution linkage mapping, complemented with testing against various *Xoo* races [29]. Among the characterized genes, only *Xa21* and *Xa3/Xa26* are implicated in the regulation of gene-for-gene type disease resistance [30]. Furthermore, positional cloning, including critical recombinant selection, SNP assay between recombinant markers followed by RT-PCR, and in-silico sequence homology analysis, led to the narrowing of the 100 kb *xa5* segment to an 8.1 kb region encoding a transcription factor TFIIAγ [31]. The *Xa10* locus was fine-mapped to the proximal side of marker E1981S with a genetic distance of 0.93 cM, belonging to a 74 kb region on the Nipponbare genome bearing 6 candidate genes [32]. The recessive resistance gene *xa13* was precisely mapped to a 14.8 kb region with the aid of BAC libraries [13]. Similarly, *Xa23* underwent fine mapping to a 0.4–0.6 kb area utilizing TAC and PAC libraries, employing EST marker PCR products of 0.8 kb and 7 probes for restriction fragment length polymorphism (RFLP) survey [32]. Another resistant locus identified against Korean *Xoo* races, *Xa43(t)*, was fine-mapped to a 119 kb interval flanked by marker IBb27os11_14 and S_BB11.ssr_9 harboring 9 target ORFs [33]. It remains imperative to identify newer and stronger resistance genes against BB, especially in the diverse cultivars of rice, and transfer them to the elite BPH-susceptible cultivars to generate durable resistance against bacterial blight disease.

Wild rice species are rich sources of resistance to various disease and insect pests and has tremendous potential as a source for improving cultivated rice produce. Eleven BB resistance genes have been identified from eight wild species viz; *Xa21* from *O. longistaminata* [34], *Xa23* and *Xa30(t)* from *O. rufipogon* [35, 36], *Xa27* and *Xa35(t)* from *O. minuta* [37, 38], *Xa29(t)* from *O. officinalis* [39], *Xa32(t)* from *O. australiensis* [40], *Xa33(t)* and *Xa38(t)* from *O. nivara* [3, 41], *Xa34(t)* from *O. brachyantha* [42] and *xa41(t)* from *O. barthii* [26]. At PAU, a novel recessive BB-resistance locus designated as *xa-45(t)* was identified on rice chromosome 8 from *O. glaberrima* accession IRGC 102600B. The SNP markers C8.26737175 and C8.26818765 demonstrated a LOD score of 33.22. The peak marker, C8.26810477, explained 49.8% of the total phenotypic variance. The identified QTL cover an 80 kb segment on the Nipponbare reference genome IRGSP-1.0, encompassing nine candidate genes. Here, we aim to pinpoint a candidate gene and conduct expression analysis to reveal the probable gene responsible for the resistance.

Materials and methods

Plant materials

An introgression line IL274 was developed by backcrossing of the F_1's generated from a cross between bacterial blight susceptible parent Pusa 44 and the resistant parent *O. glaberrima* IRGC 102600B [28, 43]. The mapping population was developed by crossing this introgression line, IL274 with Pusa 44. The F_1's were selfed using single seed descent method till F_6 and F_7 generation. The recombinant inbred lines (RILs) were sown in the *kharif* season 2019 (F_6 generation) and 2020 (F_7 generation) for screening against *Xoo* pathotype 7 (PbXo-7). The experimental design used for planting RILs was a Randomized Complete Block Design with standard row to row and plant to plant spacing of 20 × 15 cm for each RIL.

Bacterial blight inoculation with Xoo pathotype PbXo-7

The *Xoo* bacterial inoculations were performed following Kauffman's leaf-clipping method to evaluate the disease reaction of population. The *Xoo* strain PbXo-7 was isolated from the ooze of cut leaves viz. reservoir of specific bacterial strains [44]. The ooze so obtained was streaked on Walkimoto media (10 g sucrose, 5 g peptone and 20 g agar/1000 ml distilled water) plates or 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 [45].

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 [46] (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 of RIL population

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 [47]. 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.

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, which was preceded in triplicates to attain stringent data. The sequencing data involved AB1 files, that were first used to align each overlapping primer of a single gene 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.

Identification of SNPs and indels for marker development

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 h 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 and 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.

Quantitative 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 h 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: 94 °C for 3 min, followed by 45 cycles for 30 s at 94 °C, 42 s at T_a(°C), 30 s at 72 °C. Expression dynamics were analysed subsequently by $2^{-(\Delta\Delta Ct)}$ method given by Livak and Schmittgen in 2001 [48]. 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

Inheritance pattern of BB resistance gene *xa-45 (t)*

To evaluate the inheritance pattern, we recorded the disease reaction of F_6 and F_7 population against *Xoo* pathotype 7 (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)*. 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)*.

Parental sequential variations

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.

Fig. 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)

Table 1 Chi square analysis of F_6 and F_7 Recombinant Inbred Lines (RILs) developed from the cross Pusa 44 × IL274 indicating single recessive gene inheritance of *xa-45(t)*

RIL population	No. of plants R	S	χ^2 Calculated (1:1)	χ^2 Table (0.05,1)
F_6	161	132	2.8	3.84
F_7	152	141	0.41	3.84

R: Resistant (**0–3**), S: Susceptible (**5–9**)

Genotyping of the mapping population using SNP and Indel-based marker

We conducted fine mapping of *xa-45*(t) gene using dCAPS markers designed for LOC_Os08g42370, LOC_Os08g42390, LOC_Os08g42400, LOC_Os08g42420, and LOC_Os08g42440 (Table 2). These polymorphic markers were employed for genotyping of F_7 population (Fig. 2,

Table 2 List of SNPs and Indels identified in the candidate genes and their corresponding Derived Cleaved Amplified Polymorphic Sequences (dCAPS) primer sequences

Gene_ID	Alleles	Position[c]	Primer sequence F (5′-3′) R (3′-5′)	Enzyme used for confirmation
LOC_Os08g42360[d]	–	–		–
LOC_Os08g42370	T/G[b]	723		
	A/T[b]	927	F-AGTTTAATGATCATGTTCAGTATCC R-GGCAGAACCAAATGTATCAC	*NlaIII*
	T/C[a]	999	F-GACTTTAGCTAAATTTAGAAGTTCA R-ATTTAGCATGAAGACCAGCG	*NlaIII*
	T/C[a]	1087		
LOC_Os08g42380[d]	–	–		–
LOC_Os08g42390	C/A[b]	1485	F-TCTTCTATGAAAGGAGTCAACTGGA R-ATGCTGAGGAAACATTTGAC	*AcuI*
	A/-	1838		
LOC_Os08g42400	T/G[b]	2157	F-TGCTCGTTTTGATTGAGAGAATATT R-TTCATGTAACAAGTTCAGATGGTTT	*SwaI*
	A/G[a]	2374		
	A/-	2681		
	AT/–	2783		
LOC_Os08g42410	A/C[b]	1068		
	A/T[b]	1220		
	T/G[b]	1313		
	TCTCTCTCTC/———	4458	F-GTTGGCGCTGAAATATGGTC R-ACAAAGCAGCAGCCGTAAGT	Indel Marker
LOC_Os08g42420	C/G[b]	2526	F-GCCCTAAATTTATGAACAGAACTGA R-CCCAGATTTGATATCTTCTGCA	*HgaI*
LOC_Os08g42430[d]	–	–		–
LOC_Os08g42440	A/-	3311		
	T/C[a]	3455	F-AAGCACCGGGAGCAACAAACGGAACG R-CTTCTTCTCCCTGTACCGCT	*HpyCHIV*
	T/C[a]	3563		

[a]Transitions

[b]Transversions observed

[c]The SNP position with respect to the reference sequences of candidate gene from http://rice.plantbiology.msu.edu/

[d]No sequence variations observed for LOC_Os08g42360, LOC_Os08g42380 and LOC_Os08g42430

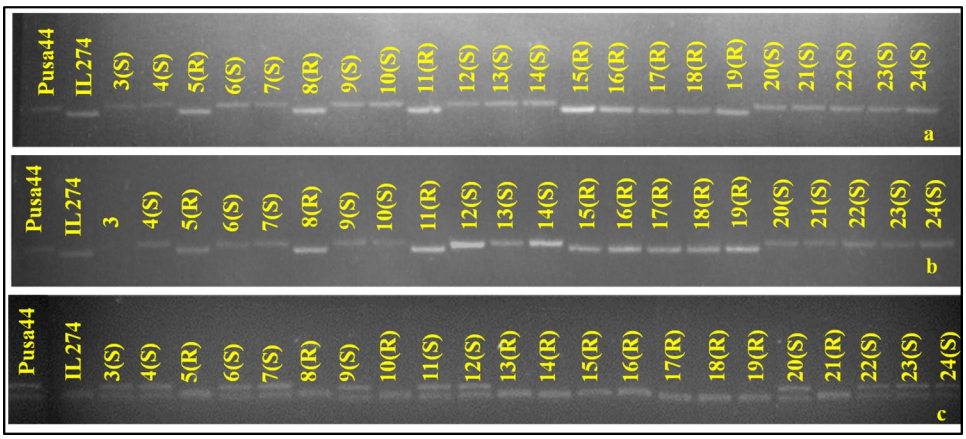

Fig. 2 Genotyping of F_7 population with dCAPS markers **a** LOC_Os08g42440 digested using *HpyCHIV*, **b** LOC_Os08g42420 digested using *HgaI*, **c** LOC_Os08g42370 digestion using *NlaIII*

Table S2). Among the 190 individuals, three recombinant inbred lines (11, 124, and 125) exhibited breakpoints with respect to 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_Os08g42420 and LOC_Os08g423440, we identified 15 and 26 recombinants between genotype and phenotype, respectively, suggesting that these loci were not associated with *xa-45*(t) (Fig. 3a). An Indel marker of the locus LOC_Os08g42410 exhibited clear size differentiation between the parental lines and were used for genotyping of F_7 population (Fig. S2). A refined genetic map of *xa-45(t)* is provided in Fig. 3b.

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 h after PbXo-7 inoculations. The transcript abundance assay for LOC_Os08g42370, LOC_Os08g42390 and LOC_Os08g42420 unveiled higher number of transcripts in 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. The two candidate genes LOC_Os08g42380, LOC_Os08g42430 could not be validated due to difficulties encountered in obtaining amplification with their respective primers. Differential expression among the resistant and susceptible reaction against PbXo-7 was not observed for LOC_Os08g42400, LOC_Os08g42440. A significant differential 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 located within the 80 kb region.

Discussion

The current study enhances our comprehension of the molecular mechanisms underlying disease resistance provided by the bacterial blight resistance gene *xa-45(t)* from *O. glaberrima* IRGC102600B. The *O. glaberrima* species is recognized as a valuable donor for essential traits including weed competitiveness, drought tolerance, resilience under low-input conditions, tolerance to other abiotic stresses, and resistance to pests and diseases in various studies. Bimpong et al. [49] evaluated alien introgression lines derived from *O. sativa* x *O. glaberrima* accessions RAM 54 and PAM 90, for tolerance to drought conditions. The study identified key QTLs for various traits and concluded that *O. glaberrima* accessions exhibit early stomatal closures and early maturity, enhancing drought tolerance [50]. In 2018, Shaibu et al. [51] screened 2106 accessions of *O. glaberrima* for over 3 years and identified 4 accessions namely, TOG 7400, TOG 6520, TOG 6519-A and TOG 7442-B exhibiting higher grain yield under drought stress conditions as compared to other accessions. Pariasca-Tanaka et al. [52] identified allelic variant for *PSTOL1* locus in upland NERICAs, inherited from *O. glaberrima* parent CG14. The overexpression of this gene was observed to have significantly higher grain yield in phosphorous-deficient soil [52]. The *RYMV2*¸ *RYMV3* gene for Rice Yellow Mottle Virus resistance have been identified from *O. glaberrima* accessions TOG 5681 and TOG 5672 respectively [53, 54]. Petitot et al. [55], 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 nematodes. In summary, the rich genetic diversity within *O. glaberrima* represents a promising resource for the development of robust rice cultivars with improved disease resistance and adaptability to harsh environmental conditions.

The analysis of transcript abundance highlights LOC_Os08g42410 as the potential gene responsible for the region exhibiting peak expression at 72 h 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. Mishra et al. [56] reported relative expression assay for *Xa21* gene for PB-1 and IPB-1. In considerate with the tillering stage of IPB-1, significantly higher fold-change (6.453) was observed at 72 h after inoculation. While higher expression was observed at 60 h of inoculation in adult plant, indicating the resistance mediated by *Xa21* is developmentally controlled in rice. Likewise, we observed similar pattern of transcript abundance for LOC_Os08g42410 exhibiting highest fold-change of 4.46 at 72 h post *Xoo* inoculation. In 2018, *xa44*(t) was recognized as a 120 kb segment harbouring 9 candidate genes. Quantitative expression analysis when compared in parental lines P6 and Ilpum, indicated up-regulation of Os11g0690066 and Os11g0690466 genes in P6, at 2 and 4 h respectively, following *Xoo* inoculations [7]. These studies collectively demonstrate the crucial role

A

ID	*Os08g42370*	*Os08g42400*	PbXo-7 disease reaction	*Os08g42410*	*Os08g42420*	*Os08g42440*
67	A	A	A	A	A	B
68	A	A	A	A	A	B
70	A	A	A	A	A	B
77	A	A	A	A	A	B
93	A	A	A	A	A	B
94	A	A	A	A	A	B
95	A	A	A	A	A	B
141	A	A	A	A	A	B
167	A	A	A	A	A	B
71	A	A	A	A	B	B
11	A	A	B	B	B	B
124	A	A	B	B	B	B
125	A	A	B	B	B	B
13	B	B	B	B	A	A
14	B	B	B	B	A	A
21	B	B	B	B	A	A
43	B	B	B	B	A	A
44	B	B	B	B	A	A
45	B	B	B	B	A	A
49	B	B	B	B	A	A
50	B	B	B	B	A	A
60	B	B	B	B	A	A
63	B	B	B	B	A	A
64	B	B	B	B	A	A
65	B	B	B	B	A	A
69	B	B	B	B	A	A
10	B	B	B	B	A	A
184	B	B	B	B	A	A
185	B	B	B	A	A	A

B

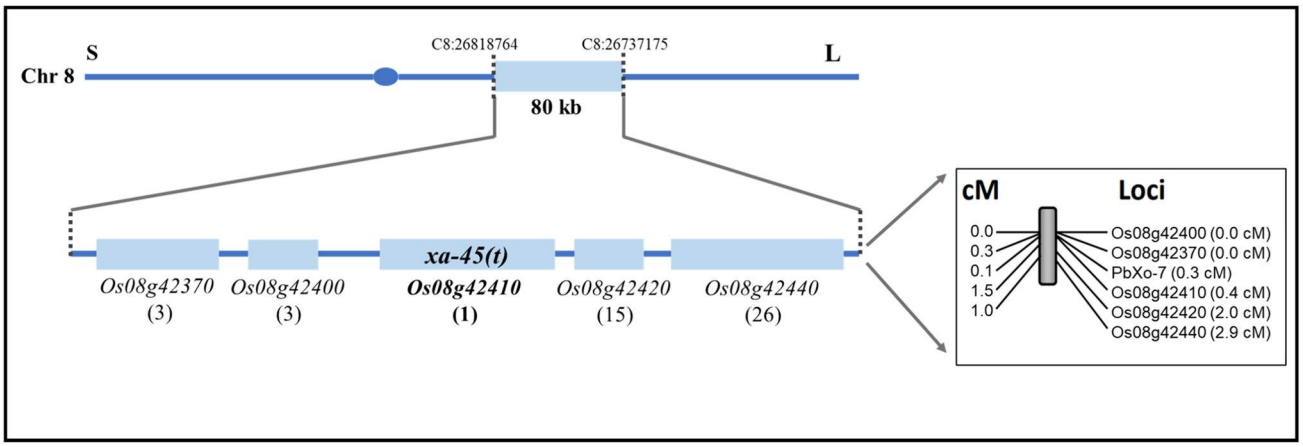

Fig. 3 **a** Recombination events in RIL population at five locus viz. LOC_Os08g42370, LOC_Os08g42400, LOC_Os08g42410, LOC_Os08g42420, LOC_Os08g42440 indicating breakpoints with the phenotype. A indicates homozygous allele for Pusa 44 (susceptible parent) whereas B indicates homozygous allele for IL 274 (resistant parent). **b** An integrated map of *xa-45(t)* within 80 kb interval on the long arm of rice chromosome 8. The numbers in bracket depict recombination events between genotype and phenotype

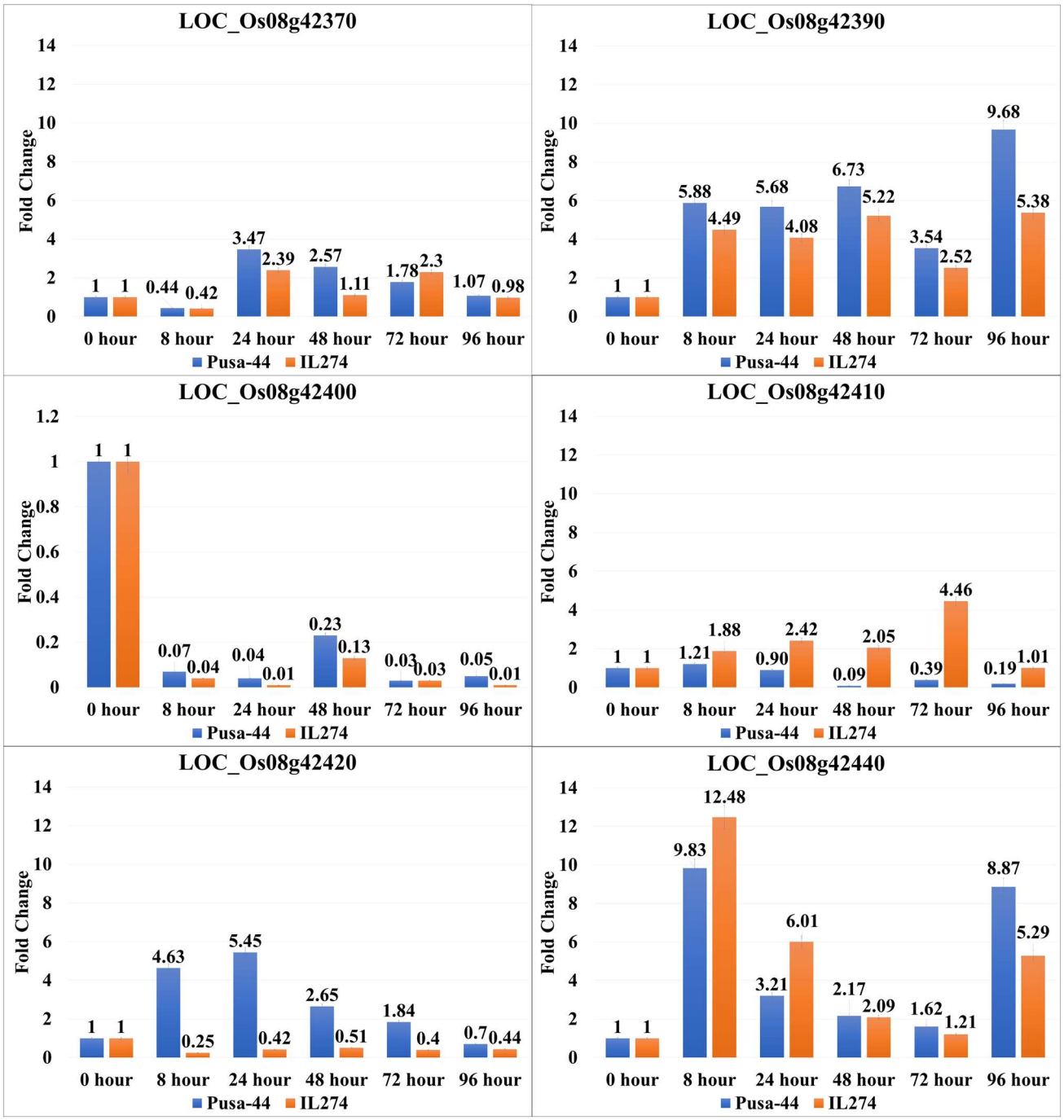

Fig. 4 Transcript abundance assay of candidate genes on parental lines Pusa 44 and IL274 at different time intervals after *Xoo* inoculation. The expression analysis of genes was considered at a time course 0, 8, 24, 48, 72 and 96 h of *Xoo* infestation. For qRT-PCR, the average values were obtained for 3 technical replicates, and the error bar shows standard deviation

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. [57] reported the down-regulation 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 [58]. Transketolase enzymatic activity was found highly profound in signalling cascades and reactive oxygen species (ROS) production on the onset of disease reaction. Tunc-Ozdemir et al. [59] verdicts the role of transketolase in production of cytosolic NADPH that further promotes ROS production during stress conditions. Fernandez et al. [60] 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* [61] and *Solanaceum oleracea* [62, 63]. These findings collectively underscore the significance of transketolase in diverse physiological processes and its potential importance in enhancing plant resilience towards various diseases.

Conclusion

In summary, 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.

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Author contributions All authors contributed to the study conception and design. 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 Neelam 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 read and approved the final manuscript.

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Data availability All the experimental data, generated and analyzed are available withing the paper and its Supplementary Information.

Declarations

Competing interests The authors declare no conflict of interest.

Ethical 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.

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