Physiological and Molecular Plant Pathology 113 (2021) 101583

Contents lists available at ScienceDirect

Physiological and Molecular Plant Pathology

journal homepage: www.elsevier.com/locate/pmpp

Virus-induced gene silencing of *Xa38* compromises the resistance towards bacterial blight disease of rice

R. Kant [a], K. Kumari [a], P. Mishra [b], R. Rai [b], K. Singh [c], I. Dasgupta [a,*]

[a] Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi, 110021, India
[b] ICAR-National Institute for Plant Biotechnology, Pusa Campus, New Delhi, 110012, India
[c] ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, 110012, India

ARTICLE INFO

Keywords:
Bacterial blight
VIGS
Xa38
Transcripts
Resistance

ABSTRACT

Bacterial blight (BB) is one of the most destructive diseases of rice, caused by the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Several BB resistant genes have been deployed in rice cultivars. One such gene is *Xa38*, which exhibits resistance against several Indian *Xoo* races. Since the detailed characterization of *Xa38* is still awaited, we have used Virus Induced Gene Silencing, using the rice-specific pRTBV-MVIGS system, to silence *Xa38* and study its effect on the symptom development of BB. We find that *Xa38* silencing compromises the resistance towards *Xoo*. Silencing results in increased BB symptoms and enhanced *Xoo* accumulation.

1. Introduction

Rice (*Oryza sativa* L.), the most important staple food crop, accounts for 35–60% of the calorie's consumption in Asian countries [1]. Bacterial blight (BB) disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most devastating diseases, mainly in irrigated and rain-fed lowland areas, prevalent in both tropical and temperate regions of the world, including majority of Asia, Northern Australia, United States of America and parts of West Africa. BB poses a significant threat to sustainable rice production in the regions mentioned above [2–4]. BB is managed primarily through the deployment of host resistance (R) genes. To date, more than 40 R-genes identified from various wild varieties, land races and mutant populations, have been reported to confer resistance against BB [5,6] (Table 1). Of the above, only twelve genes (*Xa1*, *Xa3/Xa26*, Xa4, *xa5*, *Xa10*, *xa13*, *Xa21*, *Xa23*, *xa25*, *Xa27, xa41*) have yet been characterized [75,76] at the molecular level and elucidate diverse mechanisms adapted by rice to confront *Xoo* (Table 2). Unlike other plant-pathosystems, including rice-fungi, where the major resistance genes encode polypeptides comprising nucleotide binding (NB) leucine-rich repeat (NB-LRR) domains [95], rice *Xoo* utilizes only one NB-LRR type gene, so far (*Xa1*, 7) and others encompass receptor-like kinases e.g.*Xa21* [46], *Xa26* [96]), MtN3/saliva gene family member (*xa13*, [97,98]), transcription factor (*xa5*, 19) and other biochemically different proteins e.g.*Xa10* [87] and *Xa27* [99]. The diversity of genes and molecular mechanisms utilized by rice uniquely against *Xoo*, underlines the need to characterize more *Xa* genes.

Xa38, a relatively recent R gene identified in *Oryza nivara* acc. IRGC 81825 and mapped on the chromosome 4 L in a 38.4 kb region [100], is reported to be highly effective against several Indian Xoo races [66]. Further refinement of its molecular map has suggested the locus LOC_Os04g53030 as the most probable candidate for *Xa38* [101]. Marker assisted introgression of Xa38 into popular Indian cultivated rice varieties, susceptible to BLB, underscores its importance in conferring BB resistance [102,103]. However, as with many other BB host resistance sources identified, understanding the molecular mechanism of resistance conferred by *Xa38* awaits its cloning and characterization.

23.	*xa25/ Xa25 (t)/ Xa25*	12	Chinese and Philippine races	Minghui 63, HX-3 (somaclonal mutant of Minghui 63)	[14, 54]
24.	*Xa27*	6	Chinese strains and Philippine race 2 to 6	*O. minuta* IRGC 101141, IRBB27	[54, 55]
25.	*xa28(t)*	–	Philippine race2	Lota sail	[14]
26.	*Xa29(t)*	1	Chinese races	*O. officinalis* (B5)	[56]
27.	*Xa30(t)*	11	Indonesian races	*O. rufipogon* (Y238)	[57]
28.	*xa31(t)*	4	Chinese races	Zhachanglong	[58]
29.	*Xa32(t)*	11	Philippine races	*Oryza australiensis* (introgression line C4064)	[59]
30.	*xa33(t)/ Xa33*	7	Thai races	Ba7, *O. nivara*	[60, 61]
31.	*xa34(t)*	1	Thai races	Pin Kaset, *O. brachyantha*	

(*continued on next page*)

* Corresponding author.
E-mail address: indasgup@south.du.ac.in (I. Dasgupta).

https://doi.org/10.1016/j.pmpp.2020.101583
Received 25 April 2020; Received in revised form 14 November 2020; Accepted 16 November 2020
Available online 30 November 2020

Table 1
List of BLB resistance genes identified.

Identified *Xa* resistance genes					
S. No.	*Xa* genes	Chromosome	Races of Xoo against which resistance exhibited	Donor variety	References
1.	*Xa1*	4	Japanese race-I	Kogyoku, IRBB1	[7–9]
2.	*Xa2*	4	Japanese race-II	IRBB2	[8,10]
3.	*Xa3/ Xa4b/ Xa6/ xa9/ xa26*	11	Chinese, Philippine races, and Japanese races	Wase Aikoku 3, Minghui 63, IRBB3, Zenith, Khao Lay Nhay and Sateng, Nep Bha Bong	[11–16]
4.	*Xa4 (Xa4a)*	11	Philippine race-I	TKM6, IRBB4	[16–18]
5.	*xa5*	5	Philippine races I, II, III	IRBB5	[17,19, 20]
6.	*Xa7*	6	Philippine races	DZ78	[21–24]
7.	*xa8*	7	Philippine races	PI231128	[25]
8.	*Xa10*	11	Philippine and Japanese races	Cas 209	[26–28]
9	*Xa11*	3	Japanese races IB, II, IIIA, V	IR8	[29,30]
10.	*Xa12*	4	Indonesian race V	Kogyoku, Java14	][31]
11.	*xa13*	8	Philippine race 6	BJ1, IRBB13	[32–36]
12.	*Xa14*	4	Philippine race 5	TN1	[37–39]
13.	*xa15*	–	Japanese races	M41 mutant	[40]
14.	*Xa16*	–	Japanese races	Tetep	[41]
15.	*Xa17*	–	Japanese races	Asominori	[42]
16.	*Xa18*	–	Burmese races	IR24, Miyang23, Toyonishiki	[43]
17.	*xa19*	–	Japanese races	XM5 (mutant of IR24)	[44]
18.	*xa20*	–	Japanese races	XM6 (mutant of IR24)	[45]
19.	*Xa21*	11	Philippine and Japanese races	*O. longistaminata*, IRBB21	[46–48]
20.	*Xa22*	11	Chinese races	Zhachanglong	[49,50]
21.	*Xa23*	11	Indonesian races	*O. rufipogon* (CBB23)	[51]
22.	*xa24 (t)*	2	Philippine and Chinese races	DV86	[52,53]

(*continued*)

					[60, 62]
32.	*Xa35(t)*	11	Philippine races	*Oryza minuta* (Acc. No.101133)	[63]
33.	*Xa36(t)*	11	Philippine races	C4059	[64]
34.	*xa37*	–	–	-	[65]
35.	*Xa38*	4	Indian Punjab races	*O. nivara* IRGC81825	[66, 67]
36.	*Xa39*	11		FF329	[68]

(*continued on next column*)

Table 2
Xa genes characterized at molecular level.

Characterized *Xa* genes					
S. No.	*Xa* genes	Encoding proteins			References
1	*Xa3/Xa26*	Leucine-rich repeat receptor like kinase [LRR-RLK]			[11,77,78]
2	*Xa21*	Leucine-rich repeat receptor like kinase [LRR-RLK]			[46,79]
3	*Xa4*	Wall-associated kinase/RLK			[80]
4	*xa13 (OsSWEET11)*	SWEET-type protein [MtN3/saliva]			[36,81]
5	*xa25 (OsSWEET13)*	SWEET-type protein [MtN3/saliva]			[82,83]
6	*xa41 (OsSWEET14)*	SWEET-type protein			[70, 84–86]
7	*Xa10*	Executor R protein, encodes 126 A A, with four potential transmembrane helices.			[87]
8	*Xa23*	Executor R protein, encodes 113 A A, with four potential transmembrane helices.			[88,89]
9	*Xa27*	Executor R protein [Apoplast, rice unique gene]			[90]
10	*Xa1*	NBS-NLR (Nucleotide binding domain or site and leucine rich repeat)			[7,91]
11	*xa5*	TFIIA transcription factor (TFIIA transcription factor IIA)			[92–94]

(*continued*)

			Chinese and Philippines races		
37.	*Xa40 (t)*	11	Korean BB races	IR65482-7-216-1-2	[69]
38.	*xa41(t)*	–	Various Xoo strains	Rice germplasm	[70]
39.	*xa42*	3	Japanese Xoo races	XM14, a mutant of IR24	[71]
40.	*Xa43*	11	Korean BB races	P8 (IRGC: 126,955)	[6]
41.	*xa44*	11	Korean BB races	IR73571-3B-11-3-K3	[72]
42.	*Xa45*	8 L	Indian Punjab races	*Oryza glaberrima* (IRGC:102600B)	[73]
43.	*Xa46(t)*	11	Chinese Xoo races	japonica line Lijiangxintuanheigu (LTH)	[74]

Virus Induced Gene Silencing (VIGS) technology, offers a rapid and convenient avenue for the functional analysis of plant genes [104–109]. VIGS exploits the plant defense pathway, based on RNA-interference (RNAi) for the sequence-specific degradation of transcripts through inoculation of recombinant viral vectors carrying fragments of the gene to be silenced. VIGS results in the transient loss of function of the target gene in the plant, which leads to a measurable phenotype, thereby revealing the gene function in a relatively short time [106,110,111]. Using VIGS, the functions of several plant genes have been revealed, both for dicots [112–115] as well as monocots [116,117]. Of the several VIGS vectors developed for gene silencing in monocots [109], one derived from the rice-infecting DNA virus, belonging to the species *Rice tungro bacilliform virus* (pRTBV-MVIGS, 117) has been demonstrated to be a suitable system for rice. In this study, we deployed pRTBV-MVIGS system to examine the candidature of locus LOC_Os04g53030in Xa38 mediated resistance.

2. Material and methods

2.1. Plant material and bacterial strains

The rice varieties PR114BB susceptible and PR114 (Xa38) BB resistant were grown in an insect-free growth chamber (Conviron S10H) at 30 °C, 60%–70% relative humidity and L14:D10 (light:dark) hours for two months. Bacterial Xoo strain PbXo7 was grown in peptone sucrose agar incubated at 28 °C for 2–3 days. Bacterial strain DH5α and

agrobacterium strain EHA105 was grown in Luria-Bertani agar incubated at 37 °C and 28 °C respectively.

2.2. Construction of gene silencing plasmids for VIGS

For VIGS, it is not necessary to clone the entire gene to be silenced in the vector and in most cases only part of the gene to be silenced is cloned [118]. To clone the putative *Xa38* gene fragments in pRTBV-MVIGS vector, 5988 bp of the candidate locus, LOC_Os04g53030_nivara [101] was targeted. The two longest ORFs, ORF-I (1703 bp) and ORF-III (1775 bp) out of four found using ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/), were selected for further analysis. Primers were designed (Table 3) to amplify 520 bp from each ORF separately, through PCR from cDNA prepared using total RNA extracted from PR114 (*Xa38*) plants. The amplified products were cloned in antisense orientation at *Mlu*I and *Pac*I restriction enzyme sites of pRTBV-MVIGS. The clones were named as pRTBV-MVIGS-Xa38(ORF-I) and pRTBV-MVIGS-Xa38 (ORF-III) respectively (Fig. 1). The above constructs including pRTBV-MVIGS (empty vector) were mobilized into the *Agrobacterium* strain EHA105 for gene silencing.

2.3. Agroinoculation

Agroinoculation was performed as described earlier [108]. Agrobacterium cells containing pRTBV-MVIGS, pRTBV-MVIGS-Xa38(ORF-I) and pRTBV-MVIGS-Xa38(ORF-III) were inoculated in 20-day old PR114 (*Xa38*) andPR114 rice plants. The inoculations were performed at the meristematic region, using 1 ml syringe. The inoculated plants were kept in controlled conditions at 28 ± 2 °C, 80% humidity in a growth chamber for 24 days to study the gene silencing effect on*Xoo* pathogenicity.

2.4. Real time PCR analysis of Xa38 transcripts

Total RNA was isolated from agroinoculated rice plants at 24 days post-inoculation (dpi) using RNeasy plant mini kit (Qiagen, Germany) as per manufacturer's instructions. About 2 μg isolated RNA was converted to cDNA using high capacity cDNA synthesis kit (Applied Biosystems™, USA) as per manufacturer's instructions. Real-time PCR was performed using KAPA SYBR® fast qPCR Master Mix (KAPA Biosystems, USA) according to manufacturer's instructions, to quantify the accumulation of transcripts specific to ORF-I and ORF-III of the candidate locus LOC_Os04g53030in PR114 (*Xa38*) plants silenced with pRTBV-MVIGS Xa38(ORF-I/III) and silenced with the empty vector (pRTBV-MVIGS), using Xa38-specific real-time PCR primers (Table 3, qRT Xa38-ORF I/III). The ubiquitin transcript (*UBQ5*, Genbank accession no. AK061988) was used as internal control and all real-time PCR primers were designed using Primer Express® software (Applied Biosystems™, USA). The relative transcript accumulations were calculated using comparative C_T method [119].

2.5. Challenging rice plants with Xoo and disease assessment

Rice plants of variety PR114, which is susceptible to *Xoo* strain PbXo7 from pathotype VII identified in Punjab [120] and the variety PR114 (*Xa38*), resistant against PbXo7 [66] were used for disease phenotyping. Bacterial suspension of *Xoo* strain PbXo7 (OD_{600} of 0.5) was used to artificially infect the plants using leaf clip inoculation method [121]. Challenge of plants with*Xoo* was carried out at 10 days post-agroinoculation for gene silencing. During all experiments plants were kept in the growth chamber conditions already mentioned in section 2.1. Lesion lengths were measured at 14 days post challenge, in both control (empty vector) and *Xa38*-silenced plants. The experiment was repeated thrice.

2.6. PCR-based detection of Xoo

Symptomatic and asymptomatic leaf samples were collected 14 days after *Xoo* challenge and genomic DNA was isolated from the collected leaf samples using DNAeasy plant mini kit (Qiagen, Germany) as per manufacurer's instructions. Isolated genomic DNA was further used for the PCR-based detection of *Xoo*. PCR was performed according to a previously published method [122] using the primers specific to *Xoo* insertion sequence element (*IS1113*, Table 3). The reaction mixture was incubated in thermal cycler for initial denaturation at 94 °C/2 min, denaturation at 94 °C/20 s, annealing at 56 °C/20 s, extension at 72 °C/50 s followed by final extension at 72 °C/7 min for 40 cycles.

2.7. Semi-quantitative PCR to quantify the levels of Xoo

After confirming the presence of *Xoo*, the leaf samples were further used for semi-quantitative PCR to quantify the levels of *Xoo* DNA in control plants, silenced using the empty vector pRTBV-MVIGS and plants silenced using pRTBV-MVIGS Xa38(ORF-I/III). The semi-quantitative PCR was performed by diluting the extracted DNA in autoclaved milliQ water in 1:10, 1:50, 1:100 and 1:200 dilutions. PCR was performed using the primers specific to *Xoo* insertion sequence element (*IS1113*, Table 3) and employed the conditions described in section 2.6.

3. Results

3.1. VIGS-based silencing of Xa38 ORF-I and ORF-III reduced target transcript accumulation

To test if LOC_04g53030 has any contribution towards resistance mediated by Xa38, we chose to individually silence two (ORF-I and ORF-III) of the four putative ORFs spanning the locus. The pRTBV based

Table 3
Primers used in the study.

Primer name	Primer sequence (5'→3')	Position on Loc_Os04g53030_nivara, UBQ5 nucleotide sequences
Xa38_(ORF I) FP	**TTAATTAA**TGTGGGAAGACAAGGAT	1173–1189
Xa38_(ORF I) RP	**ACGCGT**GACACAAAGCTTTGT	1677–1692
Xa38_(ORF III) FP	**TTAATTAA**ATTCACCTCCCTTGAGG	4198–4214
Xa38_(ORF III) RP	**ACGCGT**TGAAAGCGTCAAGT	4703–4717
qRT Xa38-(ORFI) FP	GCTTGCAACGCTGTTTCTCT	2234–2254
qRT Xa38-(ORFI) RP	CCAAATCCACCAATTTGTCC	2382–2402
qRT Xa38-(ORFIII) FP	GCTGTCCACTTCATGGGAAT	5038–5057
qRT Xa38-(ORFIII) RP	CGTCAAGTGTTGGAGGGAGT	5200–5183
qRT UBQ5-FP	ACCACTTCGACCGCCACTACT	508–528
qRT UBQ5-RP	ACGCCTAAGCCTGCTGGTT	558–576
TXT (XO)-FP[a]	GTCAAGCCAACTGTGTA	
TXT4R (XO)-RP[a]	CGTTCGCGCCACAGTTG	

Note: Bold letters in the primer nucleotide sequence represent restriction enzyme sites.
[a] Indicate primers used for *IS1113*.

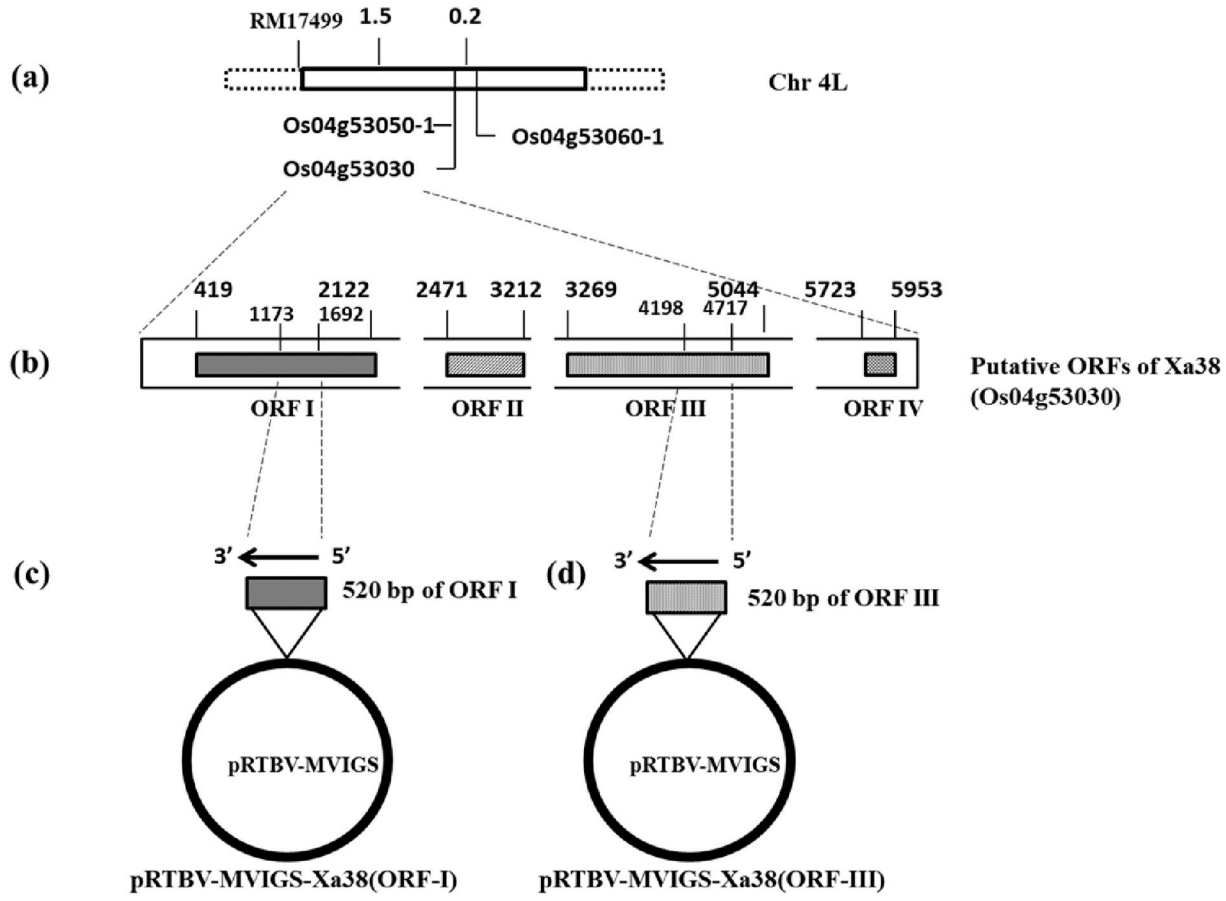

Fig. 1. Schematic representation of linkage map of *Xa38*, putative *Xa38* ORFs and recombinant pRTBV-MVIGS clones. **(a)** Linkage map of locus Os04g53030 (*Xa38*) along with markers on chromosome 4 L and numbers indicate the position of markers. **(b)** Indicates the identified four putative ORFs (I-IV) of *Xa38* and numbers show the nucleotide positions of all four ORFs and target gene fragments for cloning respectively. **(c–d)** Vector maps of recombinant pRTBV-MVIGS constructs for *Xa38*-ORFI and *Xa38*-ORFIII showing specific sizes of gene fragments.

silencing constructs were generated as schematically depicted in Fig. 1 and delivered into the PR114-Xa38 seedlings. Next, to assess the silencing efficiency of these constructs for their respective targets endogenously, we measured the transcript accumulation of both ORF-I and ORF-III in the treated seedlings. The transcript levels of ORF-I and ORF-III exhibited reduction by 50% and 55% respectively in PR114-Xa38 plants treated with silencing constructs, relative to their levels in plants treated with empty vector construct (Fig. 2). The results were reproducible in three independent experiments.

3.2. Silencing of Xa38 ORFs caused increase in BB symptoms

Having attenuated expression of the two ORFs, we next wanted to check if the attenuation affects the resistance mediated by Xa38. Xoo strain PbXo7, has been previously reported to be compatible with PR114 and incompatible with Xa38 introgressed in PR114. Both these rice lines were therefore included as controls for our disease assay experiment. The reported phenotype tested true in our experiments also, as PR114, in response to inoculation with PbXo7 exhibited strong disease symptoms in the form of lesions (about 11 cm, Fig. 3a). Whereas PR114-Xa38's response to PbXo7 was same as to that of water, evidencing resistant phenotype (Fig. 3b). To check if the vectors delivered through Agrobacterium amount to any symptomatic response from the plants being treated, we mock-inoculated the plants pre-infiltrated with empty vector. None of these plants resulted in disease symptoms (data not shown).

With the controls authenticated, working towards our hypothesis, we next challenged the PR114-Xa38 plants infiltrated with the silencing constructs, with *Xoo* strain PbXo7. Xoo inoculation in both ORF-I

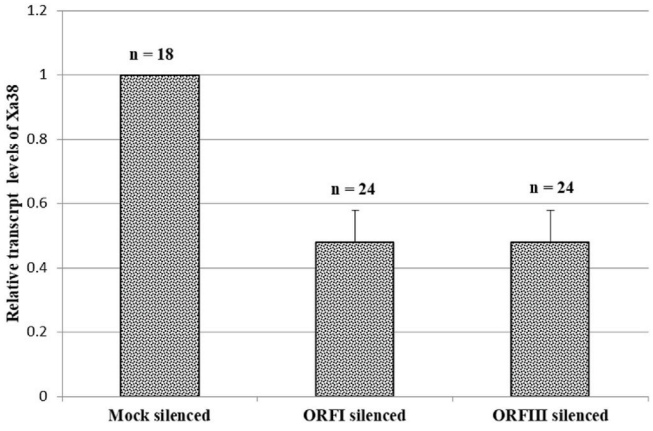

Fig. 2. Quantitative estimation of *Xa38* gene silencing. Relative transcript levels of *Xa38*-ORFI and *Xa38*-ORFIII in PR114-*Xa38* plants silenced with pRTBV-MVIGS-Xa38(ORFI) and pRTBV-MVIGS-Xa38(ORFIII) constructs as compared to control plants are shown by bars. Each set of bars represents average of three experiments. The transcript levels of *Xa38* ORF-I and *Xa38* ORF-III in the control plants inoculated with pRTBV-MVIGS (empty vector) have been set to 1. Error bars represent the standard deviation of mean. The data is an average of three individual experiments with three biological and technical replicates.

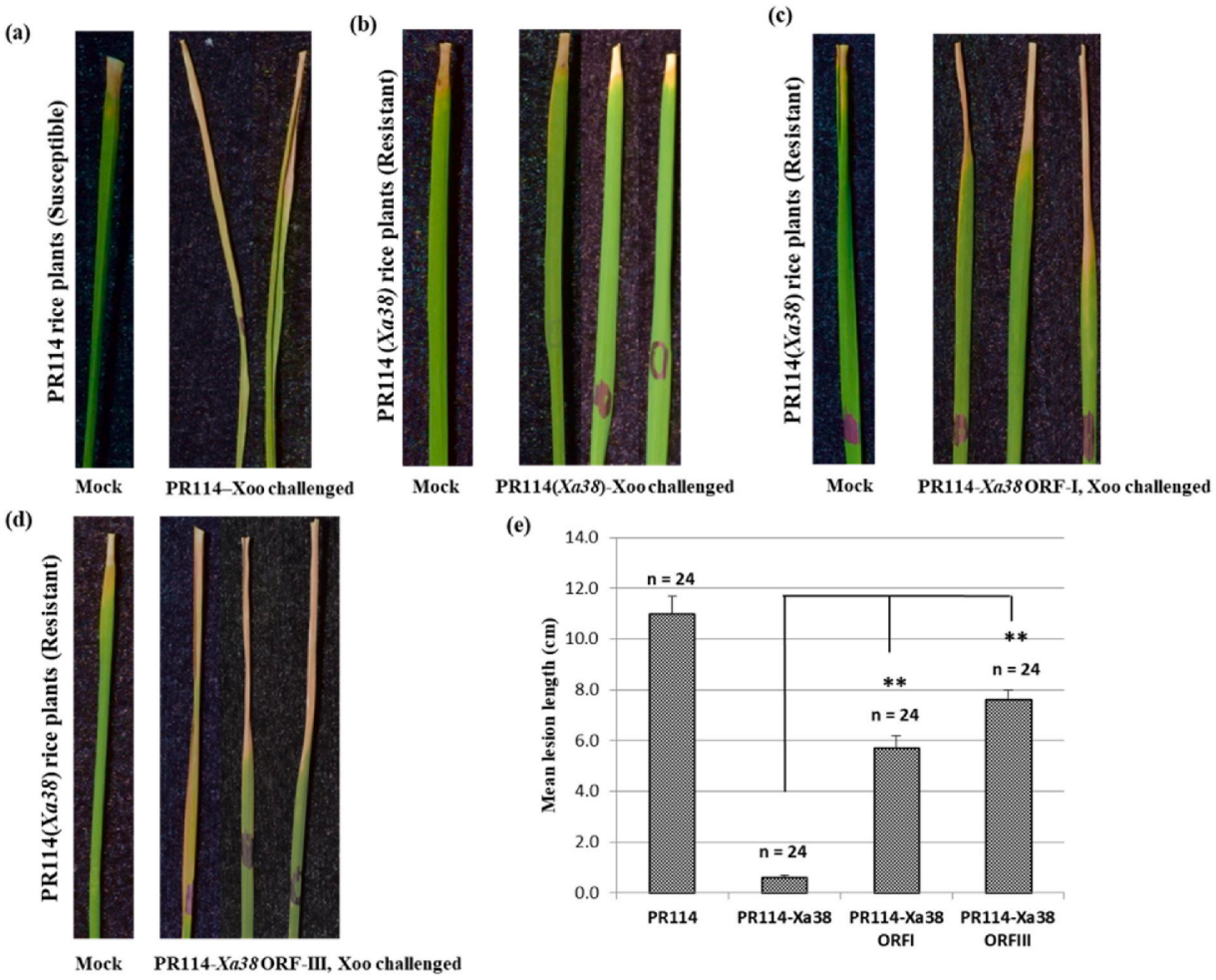

Fig. 3. Symptoms of BB 14 days after challenge of *Xoo*.**(a)** PR114 plants silenced with pRTBV-MVIGS (empty vector).**(b)** PR114-*Xa38* plants silenced with pRTBV-MVIGS (empty vector).**(c)** PR114-*Xa38* plants silenced with pRTBV-MVIGS-Xa38(ORF-I) and **(d)** PR114-*Xa38* plants silenced with pRTBV-MVIGS-Xa38(ORF-III). Mock-inoculated plants without *Xoo* infection (inoculated with water) are also shown in each case. **(e)** Representation of mean lesion lengths (cms) for *Xoo* inoculated leaves in PR114 susceptible plants, PR114-*Xa38* resistant plants silenced with empty vector, vector containing Xa38(ORF-I) and vector containing Xa38(ORF-III) respectively. Error bars represent the standard deviation of mean. Asterisks represent statistically significant differences in values between two treatments (**p > 0.01) using Student's t-test. The experiment was repeated three times and typical symptoms are shown.

silenced as well as ORF-III silenced plants elicited lesions significantly longer than the same treated with water (mock, Fig. 3c–e, about 6–8 cm).

3.3. Semi-quantitative PCR to quantify the levels of Xoo

To corroborate these visual symptoms, we also crudely quantified the Xoo population in the PbXo7 inoculated silenced plants. We deployed a semi-quantitative PCR based method to detect and quantify Xoo-IS1113, which is an insertion sequence used as a diagnostic marker for *Xoo* [44]. This was done by comparing the amplicon band intensity across several dilutions of genomic DNA samples from *Xoo* inoculated *Xa38* ORF I/III-silenced versusXoo inoculated control empty vector-silenced plants. We first analysed RT-PCR amplification of *Xoo-IS1113* in resistant and susceptible rice lines challenged with *Xoo*. As anticipated from a compatible reaction, *Xoo* population was high enough in PR114 leaves to show up in the most diluted template (1/100th, Fig. S1a) whereas, the incompatible environment in PR114-Xa38 did not facilitate the *Xoo* proliferation as witnessed from the fainter band intensities in the lesser diluted (1/10th) to none in the higher diluted templates (Fig. S1b). In contrast to these non-silenced plants, the plants silenced for *Xa38* ORF-I and those for *Xa38* ORF-III displayed higher presence of *Xoo* poulations (Fig. S1c & Fig. S1d). These results agreed well with the phenotyping data from each plant type tested.

4. Discussion

This study presents utilization of VIGS for assessment of a putative gene's candidature in resistance mediated by Xa38, an effective source of resistance against BB in rice. In post-genomics era, VIGS has been a useful technique in deciphering functions of plant genes, especially those responsive to biotic stresses. VIGS of *RCR1*, an NB-LRR gene in wheat, impaired its resistance against the necrotrophic fungal pathogen, *Rhizoctonia cerealis,* thereby proving the gene's requirement for immunity in wheat [123]. The technique has also been utilized to study susceptibility gene, *mlo*, where silencing resulted in powdery mildew resistant phenotype in wheat [124]. pRTBV-MVIGS,used in the present study, is a DNA-based vector [117], for gene silencing in rice. Its efficacy has been demonstrated with different genes in rice including a well characterized BB resistance gene, *Xa21* [108].

The candidate locus Os4g53030 studied here for its role in Xa38-mediated resistance is annotated to harbor four ORFs. Since this region is yet not cloned, we did not have adequate information about definite boundaries of the gene in this locus. So, we picked two ORFs from the region for knock-down to test the consequences of their loss of

function in terms of resistant phenotype. Gene silencing, using corresponding fragment cloned in antisense orientation, has been reported to be more pronounced than that with sense orientation [108,125,126]. Hence, we generated antisense constructs with short regions from ORF-I and ORF-III. The accumulation of corresponding transcripts in PR114-Xa38 plants inflitrated with pRTBV constructs, was reduced by half relative to that in plants infiltrated with empty vector. The effect of reduced transcript levels were further reflected in the disease assay with increae in lengths of lesions in silenced plants. This indicates that the regions targeted for silencing contribute to Xa38 mediated silencing. Being a gene silencing approach operational at the post-transcriptional level, the authors are acutely aware that VIGS cannot distinguish between the regions targeted and their possible orthologues distributed elsewhere in the rice genome, which could be simultaneously silenced, if they share sufficient nucleotide identities between them. Hence, the results obtained here do not accord the candidate locus LOC_Os04g53030 to constitute *Xa38* in entirety, but point towards its role in conferring resistance. Techniques such as CRISPR Cas-mediated disruption can lead towards a more conclusive proof of its function. Further research on cloning and characterization of specific gene(s) within *Xa38* mapped region is warranted to delineate the molecular mechanism underlying this resistance.

5. Conclusion

VIGS-mediated silencing of two ORFs in the locus LOC_Os04g53030 within the *Xa38* gene compromised the resistance towards *Xoo*, strongly indicating a functional significance of the locus in the above resistance.

Statement related to ethical practices used

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

Financial support

This work utilized funds available to ID from J. C. Bose Fellowship of the Science and Engineering Research Board (SERB), Government of India and DU-DST PURSE Grant. RK and KK acknowledge Research Fellowships from Indian Council of Medical Research, New Delhi and University Grants Commission, New Delhi, respectively. PM acknowledges support from ICAR Networking Project on Transgenic Crops.

Author statement

The following statement gives the roles played by each author in this work:

Conceptualization – RR, KS and ID.
Planning and research design – RR, KS and ID.
Methodology and experimentation – RK, KK and PM.
Data analysis – RK, KK, RR and ID.
Writing – RK, KK, RR, KS and ID.
Fund acquisition - ID.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The infrastructural facilities made available by the DST-FIST Grant to the Department of Plant Molecular Biology, University of Delhi South Campus are gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pmpp.2020.101583.

References

[1] G.S. Khush, What will it take to feed 5.0 billion rice consumers in 2030? Plant Mol. Biol. 59 (2005) 1–6.
[2] S.S. Gnanamanickam, V.B. Priyadarisini, N.N. Narayanan, P. Vasudevan, S. Kavitha, An overview of bacterial blight disease of rice and strategies for its management, Curr. Sci. 77 (1999) 1435–1443.
[3] H. Zhang, S. Wang, Rice versus *Xanthomonas oryzae* pv. *oryzae*: a unique pathosystem, Curr. Opin. Plant Biol. 16 (2013) 188–195.
[4] S. Kim, J.T. Song, J.Y. Jeong, H.S. Seo, Niclosamide inhibits leaf blight caused by *Xanthomonas oryzae* in rice, Sci. Rep. 6 (2016) 21209.
[5] C. Busungu, S. Taura, J.I. Sakagami, K. Ichitani, Identification and linkage analysis of a new rice bacterial blight resistance gene from XM14, a mutant line from IR24, Breed Sci. 66 (2016) 636–645.
[6] S.M. Kim, R.F. Reinke, 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 (2019), e0211775.
[7] S. Yoshimura, U. Yamanouchi, Y. Katayose, S. Toki, Z. Wang, I. Kono, N. Kurata, M. Yano, N. Iwata, T. Sasaki, Expression of Xa1, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation, Proc. Natl. Acad. Sci. U. S. A. A95 (1998) 1663–1668.
[8] S. Sakaguchi, Linkage studies on the resistance to bacterial leaf blight, Xanthomonas oryzae (Uyeda et Ishiyama) Dowson, in rice (in Japanese. English summary), D16, Bulletin of National Institute of Agricultural Science Series, 1967, pp. 1–18.
[9] S. Yoshimura, Y. Umehara, N. Kurata, Y. Nagamura, T. Sasaki, Y. Minobe, N. Iwata, Identification of a YAC Clone Carrying the Xa1 allele, a bacterial blight resistance gene in rice, Theor. Appl. Genet. 93 (1996) 117–122.
[10] Q. He, D. Li, Y. Zhu, M. Tan, D. Zhang, X. Lin, Fine mapping of Xa2, a bacterial blight resistance gene in rice, Mol. Breed. 17 (2006) 1–6.
[11] Y. Xiang, Y. Cao, C. Xu, X. Li, S. Wang, Xa3, conferring resistance for rice bacterial blight and encoding a receptor kinase-like protein, is the same as Xa26, Theor. Appl. Genet. 113 (2006) 1347–1355.
[12] A. Ezuka, O. Horino, K. Toriyama, H. Shinoda, T. Morinaka, Inheritance of resistance of rice variety Wase Aikoku 3 to Xanthomonas oryzae, Bull. Tokai Kinki Natl. Agric. Exp. Stn. 28 (1975) 124–130.
[13] S. Yoshimura, A. Yoshimura, N. Iwata, S. McCouch, M. Abenes, M. Baraoidan, T. W. Mew, R.J. Nelson, Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers, Mol. Breed. 1 (1995) 375–387.
[14] K.S. Lee, S. Rasabandith, E.R. Angeles, G.S. Khush, Inheritance of resistance to bacterial blight in 21 cultivars of rice, Phytopathology 93 (2003) 147–152.
[15] G.S. Sidhu, G.S. Khush, Dominance reversal of a bacterial blight resistance gene in some rice cultivars, Phytopathology 68 (1978) 461–463.
[16] R.J. Singh, G.S. Khush, T.W. Mew, A new gene for resistance to bacterial blight in rice, Crop Sci. 23 (1983) 558–560.
[17] V. Petpisit, G.S. Khush, H.E. Kauffman, Inheritance of resistance to bacterial blight in rice, Crop Sci. 17 (1977) 551–554.
[18] X. Sun, Z. Yang, S. Wang, Q. Zhang, Identification of a 47 kb DNA fragment containing Xa4, a locus for bacterial blight resistance in rice, Theor. Appl. Genet. 106 (2003) 683–687.
[19] A.S. Iyer, S.R. McCouch, The rice bacterial blight resistance gene xa5 encodes a novel form of disease resistance, Mol. Plant Microbe Interact. 17 (2004) 1348–1354.
[20] M.W. Blair, A.J. Garris, A.S. Iyer, B. Chapman, S. Kresovich, S.R. McCouch, High resolution genetic mapping and candidate gene identification at the xa5 locus for bacterial blight resistance in rice (Oryza sativa L.), Theor. Appl. Genet. 107 (2003) 62–73.
[21] G.S. Sidhu, G.S. Khush, T.W. Mew, Genetic analysis of bacterial blight resistance to seventy-four cultivars of rice (Oryza sativa L.), Theor. Appl. Genet. 53 (1978) 105–111.
[22] R. Kaji, T. Ogawa, Identification of the located chromosome of the resistance gene, Xa-7, to bacterial leaf blight in rice [in Japanese], Breed Sci. 45 (1995) 79.
[23] B.W. Porter, J.M. Chittoor, M. Yano, T. Sasaki, F.F. White, Development and Mapping of markers linked to the rice bacterial blight resistance gene Xa7, Crop Sci. 43 (2003) 1484–1492.
[24] S. Chen, Z.H. Huang, L.X. Zeng, J.Y. Yang, Q.G. Liu, X.Y. Zhu, High-resolution mapping and gene prediction of Xanthomonas oryzae pv. oryzae resistance gene Xa7, Mol. Breed. 22 (2008) 433–441.
[25] Y. Vikal, H. Chawla, R. Sharma, J.S. Lore, K. Singh, Mapping of bacterial blight resistance gene xa8 in rice (Oryza sativa L.), Indian J. Genet. Plant Breed. 74 (2014) 589–595.
[26] T.W. Mew, C.M. Vera Cruz, R.C. Reyes, Interaction of Xanthomonas campestris pv. oryzae and a resistant rice cultivar, Phytopathology 72 (1982) 786–789.
[27] A. Yoshimura, T.W. Mew, G.S. Khush, T. Moura, Inheritance of resistance to bacterial blight in rice cultivar Cas 209, Phytopathology 73 (1983) 1409–1412.
[28] K. Gu, J.S. Sangha, Y. Li, Z. Yin, High resolution genetic mapping of bacterial blight resistance gene Xa10, Theor. Appl. Genet. 116 (2008) 155–163.

[29] T. Ogawa, T. Yamamoto, Inheritance of resistance to bacterial blight in rice, in: Rice Genetics. Proceedings of International Rice Genetics Symposium, IRRI, Manila, Philippines, 1986, pp. 471–480.

[30] T. Goto, T. Matsumoto, N. Furuya, K. Tsuchiya, A. Yoshimura, Mapping of bacterial blight resistance gene Xa11 on rice chromosome 3, Jpn. Agric. Res. Q. 43 (2009) 221–225.

[31] T. Ogawa, T. Morinaka, K. Fujii, T. Kimura, Inheritance of resistance of rice varieties of Kogyoku and Java14 to bacterial group V of Xanthomonas oryzae, Ann. Phytopathol. Soc. Jpn. 44 (1978) 137–141.

[32] Z. Chu, B. Fu, H. Yang, C. Xu, Z. Li, A. Sanchez, Y.J. Park, J.L. Bennetzen, Q. Zhang, S. Wang, Targeting xa13, a recessive gene for bacterial blight resistance in rice, Theor. Appl. Genet. 112 (2006) 455–461.

[33] T. Ogawa, L. Lin, R.E. Tabien, G.S. Khush, A new recessive gene for resistance to bacterial blight of rice, Rice Genetics Newsletter 4 (1987) 98–100.

[34] G. Zhang, E.R. Angeles, M.L.P. Abenes, G.S. Khush, N. Huang, RAPD and RFLP mapping of the bacterial blight resistance gene xa13 in rice, Theor. Appl. Genet. 93 (1996) 65–70.

[35] A.C. Sanchez, L.L. Ilag, D. Yang, D.S. Brar, F. Ausubel, G.S. Khush, M. Yano, T. Sasaki, Z. Li, N. Huang, Genetic and physical mapping of xa13, a recessive gene bacterial blight resistance gene in rice, Theor. Appl. Genet. 98 (1999) 1022–1028.

[36] B. Yang, A. Sugio, F.F. White, Os8N3 is a host disease-susceptibility gene for bacterial blight of rice, Proc. Nat. Acad. Sci., USA 103 (2006) 10503–10508.

[37] S. Taura, T. Ogawa, R.E. Tabien, G.S. Khush, A. Yoshimura, T. Omura, The specific reaction of Taichung Native 1 to Philippine races of bacterial blight and inheritance of resistance to race 5 (Pxo112), Rice Genetics Newsletter 4 (1987) 101–102.

[38] Z. Tan, Q. Zhang, L. Zhu, C. Wang, RFLP mapping of a rice bacterial blight resistance gene Xa-14, Hereditas 20 (1998) 30–33.

[39] B. Si-Yuan, T. Ming-Pu, L. Xing-Hua, Genetic mapping of a bacterial blight resistance gene Xa14 in rice, Acta Agron. Sin. 36 (2010) 422–427.

[40] H. Nakai, K. Nakamura, S. Kuwahara, M. Saito, Genetic studies of an induced rice mutant resistant to multiple races of bacterial leaf blight, Rice Genetics Newsletter 5 (1998) 101–103.

[41] T. Noda, A. Ohuchi, A new pathogenic race of Xanthomonas campestris pv. oryzae and inheritance of resistance of differential rice variety, Tetep to it, Ann. Phytopathol. Soc. Jpn. 55 (1989) 201–207.

[42] T. Ogawa, H. Kaku, T. Yamamoto, Resistance gene of rice cultivar, Asaminori to bacterial blight of rice, Jpn. J. Breed. (1989) 196–197.

[43] T. Ogawa, T. Yamamoto, Inheritance of resistance to bacterial blight in rice, in: Rice Genetics, Proceedings of International Rice Genetics Symposium, IRRI, Manila, Philippines, 1986, pp. 471–480.

[44] S. Taura, T. Ogawa, A. Yoshimura, R. Ikeda, T. Omura, Identification of a recessive resistance gene in induced mutant line XM5 of rice to bacterial blight, Jpn. J. Breed. 4 (1991) 427–432.

[45] S. Taura, T. Ogawa, A. Yoshimura, R. Ikeda, N. Iwata, Identification of a recessive resistance gene to rice bacterial blight of mutant line XM6, Oryza sativa L. Jpn. J. Breed. 42 (1) (1992) 7–13.

[46] W.Y. Song, G.L. Wang, L. Chen, et al., A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*, Sci 270 (1995) 1804–1806.

[47] G.S. Khush, E. Bacalangco, T. Ogawa, A new gene for resistance to bacterial blight from Oryza longistaminata, Rice Genetics Newsletter 7 (1990) 121–122.

[48] P.C. Ronald, B. Albano, R. Tabien, L. Abenes, K.S. Wu, S. McCouch, S.D. Tanksley, Genetic and physical analysis of the rice bacterial blight disease resistance locus, Xa21, Mol. Gen. Genet. 236 (1992) 113–120.

[49] X.H. Lin, D.P. Zhang, Y.F. Xie, H.P. Gao, Q. Zhang, Identification and mapping of a new gene for bacterial blight resistance in rice based on RFLP markers, Phytopathology 86 (1996) 1156–1159.

[50] C. Wang, M. Tan, X. Xu, G. Wen, D. Zhang, X. Lin, Localizing the bacterial blight resistance gene, Xa22(t), to a 100-kilobase bacterial artificial chromosome, Phytopathology 93 (2003) 1258–1262.

[51] Q. Zhang, S.C. Lin, B.Y. Zhao, C.L. Wang, W.C. Yang, Y.I. Zhou, D.Y. Li, C.B. Chen, L.H. Zhu, Identification and tagging a new gene for resistance to bacterial blight (Xanthomonas oryzae pv. oryzae) from O. rufipogon, Rice Genetics Newsletter 15 (1998) 138.

[52] G.N. Mir, G.S. Khush, Genetics of resistance to bacterial blight in rice cultivar DV86, Crop Res. 3 (1990) 194–198.

[53] X. Wu, X. Li, C. Xu, S. Wang, Fine genetic mapping of xa24, a recessive gene for resistance against Xanthomonas oryzae pv. oryzae in rice, Theor. Appl. Genet. 106 (2008) 1467–1472.

[54] A. Amante-Bordeos, L.A. Sitch, R. Nelson, R.D. Dalmacio, N.P. Oliva, H. Aswidinnoor, H. Leung, Transfer of bacterial blight and blast resistance from the tetraploid wild rice Oryza minuta to cultivated rice, Oryza sativa, Theor, Appl. Genet. 84 (1992) 345–354.

[55] D.Y. Gao, Z.G. Xu, Z.Y. Chen, L.H. Sun, Q.M. Sun, F. Lu, B.S. Hu, Y.F. Liu, L. H. Tang, Identification of a new gene for resistance to bacterial blight in a somaclonal mutant HX-3 (indica), Rice Genetics Newsletter 18 (2001) 66–68.

[56] G.X. Tan, X. Ren, Q.M. Weng, Z.Y. Shi, L.L. Zhu, G.C. He, Mapping of a new resistance gene to bacterial blight in rice line introgressed from O. officinalis, J. Genet. Genomics. 31 (2004) 724–729.

[57] X.W. Jin, C.L. Wang, Q. Yang, Q.X. Jiang, Y.L. Fan, G.C. Liu, K.J. Zhao, Breeding of near-isogenic line CBB30 and molecular mapping of Xa30(t), a new resistance gene to bacterial blight in rice, Sci. Agric. Sin. 40 (2007) 1094–1100.

[58] C.T. Wang, G.S. Wen, X.H. Lin, X.Q. Liu, D.P. Zhang, Identification and fine mapping of the new bacterial blight resistance gene, Xa31(t), in rice, Eur. J. Plant Pathol. 23 (2009) 235–240.

[59] C.K. Zheng, C.L. Wang, Y.J. Yu, Y.T. Liang, K.J. Zhao, Identification and molecular mapping of Xa32(t), a novel resistance gene for bacterial blight (Xanthomonas oryzae pv. oryzae) in rice, Acta Agron. Sin. 35 (2009) 1173–1180.

[60] S. Korinsak, S. Sriprakhon, P. Sirithanya, J. Jairin, S. Korinsak, A. Vanavichit, T. Toojinda, Identification of microsatellite markers (SSR) linked to a new bacterial blight resistance gene xa33(t) in rice cultivar 'Ba7', Maejo Int. J. Sci. Tech. 3 (2009) 235–247.

[61] P. Nataraj kumar, K. Sujatha, G.S. Laha, B.C. Viraktamath, C.S. Reddy, B. Mishra, S.M. Balachandran, T. Ram, K. Srinivasarao, Y. Hari, R.M. Sundaram, Identification of a dominant bacterial blight resistance gene from Oryza nivara and its molecular mapping, Rice Genetics Newsletter 25 (2010) 54–56.

[62] T. Ram, G.S. Laha, S.K. Gautam, Ramdeen, M.S. Madhav, D.S. Brar, B. C. Viraktamath, Identification of a new gene introgressed from Oryza brachyantha with broad-spectrum resistance to bacterial blight of rice in India, Rice Genetics Newsletter 25 (2010) 57.

[63] S.B. Guo, D.P. Zhang, X.H. Lin, Identification and mapping of a novel bacterial blight resistance gene Xa35(t) originated from Oryza minuta, Sci. Agric. Sin. 43 (13) (2010).

[64] L.L. Miao, C.L. Wang, C.K. Zheng, J.Y. Che, Y. Gao, Y.C. Wen, G.Q. Li, K.J. Zhao, Molecular mapping of a new gene for resistance to rice bacterial blight, Sci. Agric. Sin. 43 (15) (2010) 3051–3058.

[65] G. Antony, Molecular Basis of AvrXa7 Mediated Virulence in Bacterial Blight of Rice, University of Kansas, USA, 2010. Ph.D. thesis.

[66] K.K. Cheema, K.K. Grewal, Y. Vikal, et al., A novel bacterial blight resistance gene from *Oryza nivara* mapped to 38 Kb region on chromosome 4L and transferred to *Oryza sativa* L, Genet. Res. 90 (2008) 397–407.

[67] R. Kaur, N. Grewal, A. Das, Y. Vikal, J. Singh, T.S. Bharaj, J.S. Sidhu, K. Singh, Inheritance of bacterial blight resistance in two accessions of wild rice, Oryza nivara, Rice Genetics Newsletter 22 (2006) 78–82.

[68] F. Zhang, D.L. Zhuoa, F. Zhang, L.Y. Huang, W.S. Wang, J.L. Xu, C. Vera Cruz, Z. K. Li, Y.L. Zhou, Xa39, a novel dominant gene conferring broad-spectrum resistance to Xanthomonas oryzae pv. oryzae in rice, Plant Pathol. 64 (2014) 568–575.

[69] S.M. Kim, J.P. Suh, Y. Qin, T.H. Noh, R.F. Reinke, K.K. Jena, 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 (2015) 1933–1943.

[70] M. Hutin, F. Sabot, A. Ghesquière, R. Koebnik, B. Szurek, A knowledge-based molecular screen uncovers a broad-spectrum OsSWEET14 resistance allele to bacterial blight from wild rice, Plant J. 84 (2015) 694–703.

[71] C. Busungu, S. Taura, J.I. Sakagami, K. Ichitani, Identification and linkage analysis of a new rice bacterial blight resistance gene from XM14, a mutant line from IR24, Breed Sci. 66 (2016) 636–645.

[72] S.M. Kim, 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 (12) (2018) 2733–2743.

[73] N.M. Kumari, R. Gupta, V. Bhatia, D. Gill, B. Komal, R. Lore, J. Mangat, G. Singh, K. Singh, 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. (2019) 133, https://doi.org/10.1007/s00122-019-03501-2.

[74] S. Chen, C. Wang, J. Yang, et al., Identification of the novel bacterial blight resistance gene *Xa46*(t) by mapping and expression analysis of the rice mutant H120, Sci. Rep. 10 (2020) 12642.

[75] Y. Vikal, D. Bhatia, Genetics and genomics of *Bacterial blight* resistance in rice, Adv. Int. Rice Res. (2017), https://doi.org/10.5772/67361 (Ed.), InTech.

[76] N. Jiang, J. Yan, Y. Liang, Y. Shi, Z. He, Y. Wu, Q. Zeng, X. Liu, J. Peng, Resistance genes and their interactions with bacterial blight/leaf streak pathogens (*Xanthomonas oryzae*) in rice (*Oryza sativa L.*)-an updated review, Rice 13 (2020) 3 (New York, N.Y.).

[77] X. Sun, Y. Cao, Z. Yang, C. Xu, X. Li, S. Wang, Q. Zhang, Xa26, a gene conferring resistance to Xanthomonas oryzae pv. oryzae in rice, encodes an LRR receptor kinase-like protein, Plant J. 37 (2004) 517–527.

[78] P. Li, J.Y. Long, Y.C. Huang, Y. Zhang, J.S. Wang, AvrXa3: a novel member of avrBs3 gene family from Xanthomonas oryzae pv. oryzae has a dual function, Prog. Nat. Sci. 14 (2004) 767–773.

[79] R.N. Pruitt, B. Schwessinger, A. Joe, N. Thomas, F. Liu, M. Albert, M.R. Robinson, L.J. Chan, D.D. Luu, H. Chen, O. Bahar, A. Daudi, D. De Vleesschauwer, D. Caddell, W. Zhang, X. Zhao, X. Li, J.L. Heazlewood, D. Ruan, M. Dajumder, M. Chern, H. Kalbacher, S. Midha, P.B. Patil, R.V. Sonti, C.J. Petzold, C.C. Liu, J. S. Brodbelt, G. Felix, P.C. Ronald, The rice immune receptor XA21 recognizes a tyrosinesulfated protein from a gram-negative bacterium, Sci. Adv. 1 (2015), e1500245.

[80] K. Hu, J. Cao, J. Zhang, F. Xia, Y. Ke, H. Zhang, W. Xie, H. Liu, Y. Cui, Y. Cao, X. Sun, J. Xiao, X. Li, Q. Zhang, S. Wang, Improvement of multiple agronomic traits by a disease resistance gene via cell wall reinforcement, Native Plants 3 (2017) 17009.

[81] Z. Chu, M. Yuan, J. Yao, X. Ge, B. Yuan, C. Xu, X. Li, B. Fu, Z. Li, J.L. Bennetzen, Q. Zhang, S. Wang, Promoter mutations of an essential gene for pollen development result in disease resistance in rice, Genes Dev. 20 (2006) 1250–1255.

[82] T. Li, B. Liu, M.H. Spalding, D.P. Weeks, B. Yang, High-efficiency TALEN-based gene editing produces disease-resistant rice, Nat. Biotechnol. 30 (2012) 390–392.

[83] J. Zhou, Z. Peng, J. Long, D. Sosso, B. Liu, J.S. Eom, S. Huang, S. Liu, C. Vera Cruz, W.B. Frommer, F.F. White, B. Yang, Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice, Plant J. 82 (2015) 632–643.

[84] G. Antony, J. Zhou, S. Huang, T. Li, B. Liu, F. White, B. Yang, Rice xa13 recessive resistance to bacterial blight is defeated by induction of the disease susceptibility gene Os-11N3, Plant Cell 22 (2010) 3864–3876.
[85] Y. Yu, J. Streubel, S. Balzergue, A. Champion, J. Boch, R. Koebnik, J. Feng, V. Verdier, B. Szurek, Colonization of rice leaf blades by an African strain of Xanthomonas oryzae pv. oryzae depends on a new TAL effector that induces the rice nodulin-3 Os11N3 gene, Mol. Plant Microbe Interact. 24 (2011) 1102–1113.
[86] J. Streubel, C. Pesce, M. Hutin, R. Koebnik, J. Boch, B. Szurek, Five phylogenetically close rice SWEET genes confer TAL effector-mediated susceptibility to Xanthomonas oryzae pv, Oryzae, New Phytol. 200 (2013) 808–819.
[87] D. Tian, J. Wang, X. Zeng, et al., The rice TAL effector-dependent resistance protein XA10 triggers cell death and calcium depletion in the endoplasmic reticulum, Plant Cell 26 (2014) 497–515.
[88] C.L. Wang, T.F. Qin, H.M. Yu, X.P. Zhang, J.Y. Che, Y. Gao, C.K. Zheng, B. Yang, K.J. Zhao, The broad bacterial blight resistance of rice line CBB23 is triggered by a novel transcription activator-like (TAL) effector of Xanthomonas oryzae pv. oryzae, Mol. Plant Pathol. 15 (2014) 333–341.
[89] C.L. Wang, X.P. Zhang, Y.L. Fan, Y. Gao, Q.L. Zhu, C.K. Zheng, T.F. Qin, Y.Q. Li, J. Y. Che, M.W. Zhang, B. Yang, Y.G. Liu, K.J. Zhao, XA23 is an executor R protein and confers broad-spectrum disease resistance in rice, Mol. Plant 8 (2015) 290–302.
[90] K. Gu, B. Yang, D. Tian, L. Wu, D. Wang, C. Sreekala, F. Yang, Z. Chu, G.L. Wang, F.F. White, Z. Yin, R gene expression induced by a type-III effector triggers disease resistance in rice, Nat 435 (2005) 1122–1125.
[91] Z. Ji, C. Ji, B. Liu, L. Zou, G. Chen, B. Yang, Interfering TAL effectors of Xanthomonas oryzae neutralize R-gene-mediated plant disease resistance, Nat. Commun. 7 (2016) 13435.
[92] G.H. Jiang, Z.H. Xia, Y.L. Zhou, J. Wan, D.Y. Li, R.S. Chen, W.X. Zhai, L.H. Zhu, Testifying the rice bacterial blight resistance gene xa5 by genetic complementation and further analyzing xa5 (Xa5) in comparison with its homolog TFIIAγ1, Mol. Genet. Genom. 275 (2006) 354–366.
[93] H. Zou, W. Zhao, X. Zhang, Y. Han, L. Zou, G. Chen, Identification of an avirulence gene, avrxa5, from the rice pathogen Xanthomonas oryzae pv, oryzae, Sci. China Life Sci. 53 (2010) 1440–1449.
[94] A. Sugio, B. Yang, T. Zhu, F.F. White, Two type III effector genes of Xanthomonas oryzae pv. oryzae control the induction of the host genes OsTFIIAγ1 and OsTFX1during bacterial blight of rice, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 10720–10725.
[95] DeYoung, R.W. Innes, Plant NBS-LRR proteins in pathogen sensing and host defense, Nat. Immunol. 7 (2006) 1243–1249.
[96] Y. Cao, X. Ding, M. Cai, J. Zhao, Y. Lin, X. Li, C. Xu, S. Wang, The expression pattern of a rice disease resistance gene *xa3/xa26* is differentially regulated by the genetic backgrounds and developmental stages that influence its function, Genetics 177 (2007) 523–533.
[97] Z. Chu, M. Yuan, J. Yao, et al., Promoter mutations of an essential gene for pollen development result in disease resistance in rice, Genes Dev. 20 (2006) 1250–1255.
[98] M. Yuan, Z. Chu, X. Li, C. Xu, S. Wang, The bacterial pathogen *Xanthomonas oryzae* overcomes rice defenses by regulating host copper redistribution, Plant Cell 22 (2010) 3164–3176.
[99] K. Gu, D. Tian, F. Yang, L. Wu, C. Sreekala, D. Wang, G.L. Wang, Z. Yin, High-resolution genetic mapping of Xa27(t), a new bacterial blight resistance gene in rice, *Oryza sativa L*, Theor, Appl. Genet. 108 (2004) 800–807.
[100] Y. Vikal, A. Das, B. Patra, R.K. Goel, J.S. Sidhu, K. Singh, Identification of new sources of bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) resistance in wild *Oryza* species and *O. glaberrima*, Plant Genet. Resource. 5 (2007) 108–112.
[101] H. Bhasin, D. Bhatia, S. Raghuvanshi, J.S. Lore, G.K. Sahi, B. Kaur, Y. Vikal, K. Singh, New PCR-based sequence-tagged site marker for bacterial blight resistance gene Xa38 of rice, Mol. Breed. 30 (2012) 607–611.
[102] R.K. Ellur, A. Khanna, S. Gopala Krishnan, et al., Marker-aided incorporation of *Xa38*, a novel bacterial blight resistance gene, in PB1121 and comparison of its resistance spectrum with *xa13* + *Xa21*, Sci. Rep. 6 (2016) 29188.
[103] A. Yugander, R.M. Sundaram, K. Singh, D. Ladhalakshmi, L.V. Subba Rao, M. S. Madhav, J. Badri, M.S. Prasad, G.S. Laha, Incorporation of the novel bacterial blight resistance gene *Xa38* into the genetic background of elite rice variety Improved Samba Mahsuri, PloS One 13 (2018), e0198260.
[104] D. Robertson, VIGS vector for gene silencing: many Targets, many Tools, Annu. Rev. Plant Biol. 55 (2004) 495–519.
[105] A. Purkayastha, I. Dasgupta, Virus-induced gene silencing: a versatile tool for discovery of gene functions in plants, Plant Physiol. Biochem. 47 (2009) 967–976.
[106] M. Senthil-Kumar, K.S. Mysore, New dimensions for VIGS in plant functional genomics, Trends Plant Sci. 16 (2011) 656–665.
[107] R. Kant, S. Sharma, I. Dasgupta, Virus-induced gene silencing (VIGS) for functional genomics in rice using *Rice tungro bacilliform virus* (RTBV) as a vector, in: Plant Gene Silencing: Methods Mol. Biol, vol. 1287, 2015, pp. 201–217.
[108] R. Kant, I. Dasgupta, Phenotyping of VIGS-mediated gene silencing in rice using a vector derived from a DNA virus, Plant Cell Rep. 36 (2017) 1159–1170.
[109] R. Kant, I. Dasgupta, Gene silencing approaches through virus-based vectors: speeding up functional genomics in monocots, Plant Mol. Biol. 100 (2019) 3–18.
[110] D. Baulcombe, RNA silencing in plants, Nature 43 (2004) 356–363.
[111] H. Vaucheret, Post-transcriptional small RNA pathways in plants: mechanisms and regulations, Genes Dev. 20 (2006) 759–771.
[112] Y. Liu, M. Schiff, S.P. Dinesh-Kumar, Virus-induced gene silencing in tomato, Plant J. 31 (2002) 777–786.
[113] A. Igarashi, K. Yamagata, T. Sugai, et al., *Apple latent spherical virus* vectors for reliable and effective virus-induced gene silencing among a broad range of plants including tobacco, tomato, *Arabidopsis thaliana*, cucurbits, and legumes, Virology 386 (2009) 407–416.
[114] P.K. Kandoth, R. Heinz, G. Yecke, A virus-induced gene silencing method to study soybean cyst nematode parasitism in *Glycine max*, BMC Res. Notes 6 (2013) 255.
[115] G.M.R. Eftekhariyan, F. Karimi, G.S.L. Mousavi, T.S.A. Hosseini, S.A. Salami, Assessing the *Tobacco rattle virus*-based vectors system as an efficient gene silencing technique in *Datura stramonium* (*Solanaceae*), Virus Gene. 49 (2014) 512–516.
[116] S. Holzberg, P. Brosio, C. Gross, G.P. Pogue, *Barley stripe mosaic virus*-induced gene silencing in a monocot plant, Plant J. 30 (2002) 315–327.
[117] A. Purkayastha, S. Mathur, V. Verma, S. Sharma, I. Dasgupta, Virus-induced gene silencing in rice using a vector derived from a DNA virus, Planta 232 (2010) 1531–1540.
[118] M.H. Hsieh, H.C. Lu, Z.J. Pan, et al., Optimizing virus-induced gene silencing efficiency with *Cymbidium* mosaic virus in *Phalaenopsis* flower, Plant Sci. 201 (2013) 25–41.
[119] T.D. Schmittgen, K.J. Livak, Analyzing real-time PCR data by the comparative CT method, Nat. Protoc. 3 (2008) 1101–1108.
[120] J.S. Lore, Y. Vikal, M.S. Hunjan, et al., Genotypic and pathotypic diversity of *Xanthomonas oryzae* pv. *oryzae*, the cause of bacterial blight of rice in Punjab State of India, J. Phytopathol. 159 (2011) 479–487.
[121] H.E. Kauffman, A.P.K. Reddy, S.P.Y. Hsieh, S.D. Merca, An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*, Plant Dis. Rep. 56 (1973) 537–541.
[122] N. Sakthivel, C.N. Mortensen, S.B. Mathur, Detection of *Xanthomonas oryzae pv. oryzae* in artificially inoculated and naturally infected rice seeds and plants by molecular techniques, Appl. Microbiol. Biotechnol. 56 (2001) 435–441.
[123] X. Zhu, C. Lu, L. Du, et al., The wheat NB-LRR gene TaRCR1 is required for host defence response to the necrotrophic fungal pathogen *Rhizoctonia cerealis*, Plant Biotechnol. J. 15 (2017) 674–687.
[124] J. Zhang, D. Yu, Y. Zhang, et al., Vacuum and co-cultivation agroinfiltration of (germinated) seeds results in *Tobacco rattle virus* (TRV) mediated whole-plant virus-induced gene silencing (VIGS) in wheat and maize, Front. Plant Sci. 8 (2017) 1–12.
[125] C. Zhang, C. Yang, S. Whitham, J.H. Hill, Development and use of an efficient DNA-based viral gene silencing vector for soybean, Mol. Plant Microbe Interact. 22 (2009) 123–131.
[126] C. Zhang, J.D. Bradshaw, S. Whitham, J.H. Hill, The development of an efficient multipurpose bean pod mottle virus viral vector set for foreign gene expression and RNA silencing, Plant Physiol. 153 (2010) 52–65.