A varied AvrXa23-like TALE enables the bacterial blight pathogen to avoid being trapped by Xa23 resistance gene in rice

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Introduction: Xa23 as an executor mediates broad-spectrum resistance to Xanthomonas oryzae pv. oryzae (Xoo), which contains a matching avirulence gene avrXa23, in rice for bacterial leaf blight (BLB). avrXa23 encodes a transcription activator-like effector (TALE) protein which binds to the EBE (effector-binding element) of the Xa23 promoter. It is unclear whether the considerable pressure of Xa23 leads to an emerging Xoo strain that overcomes Xa23 resistance.

Objectives: This study aimed to uncover new Xoo isolate(s) that overcome Xa23-mediated resistance and to investigate how the pathogen evades the resistance.

Methods: Totally 185 Xoo isolates were used to screen possibly compatible strain(s) with Xa23-containing rice CBB23 by pathogenicity test. Genome Sequencing, Southern blot, tal gene cloning, Western blot, qRT-PCR and electrophoretic mobility shift assays (EMSA) were conducted to determine the mechanism of one Xoo isolate being compatible with Xa23-containing rice.

Results: One isolate AH28 from Anhui province is compatible with CBB23. AH28 strain contains an ortholog of avrXa23, tal7b and has 17 tal genes. The 4th RVD (repeat-variable diresidue) in Tal7b is missed and the 5th and 8th RVDs changed from NG and NS to NS and S*, respectively. These alternations made Tal7b unable to bind to the EBE of Xa23 promoter to activate the expression of Xa23 in rice. The ectopic expression of tal7b in a tal7b-free mutant PH of PXO99A did not alter the virulence of the strain PH, whereas avrXa23 made AH28 from compatibility to incompatibility with Xa23 rice.
Introduction

As one of the most devastating rice diseases worldwide, bacterial leaf blight (BLB) is largely mediated by a gene-for-gene manner between the pathogen Xanthomonas oryzae pv. oryzae (Xoo) and the host plant rice [1,2]. Xoo is able to successfully colonize and infect rice in the absence of resistance (R) genes or when R genes are not activated [3]. The Xoo-rice pathosystem has followed a ‘zigzag’, co-evolving arms-race competition. The outcome of this interaction largely depends on the effector proteins translocated into host cells by the Xoo-encoded type III secretion system (T3SS) and their interaction with R gene products that are recognized in a cultivar/race-specific manner [3-5].

Approximately 46 genes conferring resistance to various Xoo races have been identified in cultivated and wild rice and artificial mutants [6,7]. At least 17 of these R genes have been cloned and characterized, including Xa1, Xa1, Xa2, Xa3/Xa26, Xa4, xa5, Xa7, Xa10, xa13, Xa14, Xa21, Xa23, xa25, Xa27, Xa31 (t), xa41 and Xa45 (t) [6,8-11]. The R gene Xa23 was originally derived from wild rice (Oryza rufipogon) and confers dominant, broad-spectrum resistance to BLB [12,13]. Xa23 is an executor R gene (E gene) that encodes a 113 amino acid protein (Xa23) [14]. The near-isogenic line CBB23 was obtained by transferring Xa23 from wild rice into the susceptible Oryza indica cultivar JG30 [13]. The improved cultivar CBB23 exhibits broad-spectrum resistance to Xoo, which largely depends on the transcriptional activation of Xa23 by effector AvrXa23 that is considered to be encoded by virtually all Xoo strains in the past [13,14], but skeptically in the future.

Xoo may evolve decoys or new effectors to evade R gene recognition and suppress the resistance triggered by effectors (ETI), resulting in effector-induced susceptibility (ETS) [3,15,16]. Among effectors, transcription activator-like effectors (TALEs) form a particular family of proteins in Xoo that bind effector-binding elements (EBE) in the promoter regions of plant genes encoding for resistance (R) or susceptibility (S) [17-19]. Therefore, TALEs are commonly referred to as avirulence or virulence proteins based on whether the targets are R or S genes [15,20,21]. The ability to recognize and bind EBE is due to the conserved architecture in TALEs which includes the typical components: (1) a highly conserved N-terminal region required for type III secretion; (2) a central repeat region containing a variable number of mostly 34-amino acid repeats that are polymorphic at positions 12 and 13, referred to as the repeat-variable di-residue (RVD) and determine EBE specificity; (3) two to three C-terminal nuclear localization signals (NLSs); and (4) an acidic transcription activation domain (AD) [17,22-25].

Among the 17 BLB resistance genes cloned, the functions of 14 R genes have been found to be related to TALEs except Xa3/Xa26, Xa4 and Xa21 [6,19]. Xa1 and its alleles (Xa1, Xa2, Xa14, Xa31(t), and Xa45(t)) encode NB-LRR type (NLR) proteins and confer resistance to Xoo by recognizing typical TALEs. However, the NLR resistance is suppressed independently on rice basal transcription factor TFIIA by iTALEs that are prevalent in Asian Xoo strains [8,9,16,26,27]. The recessive R gene xa5 encodes a gamma subunit 5 of the basal transcription factor IIA (TFIIAγ5) and is a substitution variant of a single amino acid V39E. TFIIAγ5 and TFIIAγ1 directly interact with TALEs and are required for the survival of Xoo in rice [27-29]. Other recessive R genes, xa13, xa25, and xa41, encode SWEET family transmembrane proteins, which are basically sugar transporters, and the dominant alleles of these genes are S genes specifically induced by TALEs for Xoo to establish infection [15,30-32]. The simultaneously disrupting TALE-EBEs of these S genes confers broad-spectrum resistance to rice against BLB [33,34]. Xa7, Xa10, Xa23 and Xa27 are known to be E genes triggered by TALEs AvrXa7/PthXo3, AvrXa10, AvrXa23 and AvrXa27, respectively [10,11,14,35,36]. The complexity of the interaction between TALEs and corresponding R genes is mystifying and has major implications for the continued deployment of stable resistance to Xoo in the field [6,37].

The Xa23 gene has been widely implemented in rice breeding programs, both singly and in combination with other R genes [13,14,38,39]. Thus, it is important to monitor whether new Xoo isolates threaten Xa23-mediated resistance. In the present study, we collected 185 indigenous Xoo strains from various regions of China and recovered one isolate designated AH28 that was highly virulent on Xa23-containing rice line CBB23. To gain insight into the mechanism(s) by which AH28 overcomes Xa23, we sequenced its whole genome and assembled the full repertoire of TALEs. Further, isolation and ectopic expression of tal7b from AH28 revealed that one ortholog of avrXa23 did not trigger resistance executed by Xa23 in rice.

Materials and Methods

Plant material, bacterial strains and growth conditions

The susceptible rice varieties Kitaake, IR24, Nipponbare and near-isogenic resistant lines IRB55 (harbouring xa5), IRB57 (harbouring Xa7), IRB810 (harbouring Xa10), IRB813 (harbouring xa13) and CBB23 (harbouring Xa23) were grown in experimental fields and greenhouse located at Shanghai Jiao Tong University (Shanghai, China). The bacterial strains used in this study are listed in Table S3. Escherichia coli strains were grown in Luria-Bertani medium (LB) supplemented with appropriate antibiotics at 37°C. Xoo strains collected [33] were grown in nutrient broth (NB) or NB supplemented with 1.5% agar (NA) at 28°C [40]. The rice lines and bacterial strains were stocked in G ongyou Chen’s laboratory at Shanghai Jiao Tong University (Shanghai, China). Antibiotics were used at the following concentrations (μg/mL) when required: ampicillin (Ap), 100; kanamycin (Km) 25; and spectinomycin (Sp), 50.

Southern blot

For tal gene detection, Xoo genomic DNA was extracted, digested with BamHI, separated in agarose gels, and transferred to membranes for blotting as previously reported [33,44]. The probe was made from a DNA fragment labeled with DIG containing the repetitive region of pthXo1 (GenBank accession number: AY495676). Bacterial Genomic DNA Miniprep Kit was purchased from Axygen (USA). Restriction endonucleases and DNA molecular weight markers were provided by TaKaRa Bio (Japan). DIG-labeled Southern Blot Kits were purchased from Roche (Switzerland) and Immobilon-Ny™ membranes were supplied by Millipore (USA).
AH28 genome sequencing, assembly, and annotation

AH28 Genomic DNA was extracted using the AxyPrep Bacterial Genomic DNA Miniprep Kit (Axygen, USA) and sequenced with the PromethION (Oxford Nanopore, UK) plus NovaSeq 6000 (Illumina, USA) by Shanghai OE Biotech Corporation (Shanghai, China) as described previously [41]. The sequencer was controlled with MinKNOW version 2.2.12 software. A de novo genome assembly was performed with the software Flye version 2.6 [42] via default parameters. The Circos software [43] was used to generate the circular genome map of AH28 to show annotation information.

Genomes of AH28 and PX099β starting from the gyrB gene were aligned using progressive MAUVE with default parameters (http://darlinglab.org/mauve). The TALE coding genes (tal genes) were scanned using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and software AnnoTALE v1.2 [44]. The whole-genome sequence of AH28 was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) and deposited in the NCBI BioProject Database (BioProject ID: PRJNA722377). The genome sequences of 20 Xoo strains were retrieved from the NCBI database and a phylogenetic tree was constructed by the Type Genome Server (TYGS) (https://tygs.dsmz.de).

Tal gene cloning and plasmid construction

tal7b of AH28 was isolated as described before [33,45]. Briefly, genomic DNA (2–3 µg) of AH28 was digested with BamHI (New England Biolabs, USA) and separated in 1.3% agarose gels. Since the BamHI fragment of tal7b is 4170 bp according to the genome sequence, DNA fragments larger than 4-kb were excised, ligated into BamHI-digested pBluescript II KS(−), and transferred into E. coli DH5α. The resulting plasmid library was screened for TALE-containing clones by in situ colony blot hybridization using the 3.2-kb SphI fragment of avrXa23 (GenBank accession no. GU732172) as a probe. Hybridizing colonies were further evaluated by PCR analysis with tal-specific primers TALN18-F and TALN18-R (Table S4). Putative tal-containing clones were confirmed by restriction enzyme digestion and Sanger sequencing. tal7b and avrXa23 were cloned into vector pZW in-frame with C-terminal Flag tag epitopes, resulting in pZW-tal7b and pZW-avrXa23 (Table S3). These plasmids were ligated into the broad-host-range vector pHM1 at the Hind III site, generating pHZW-tal7b and pHZW-avrXa23 (Table S3), which were then transferred into a tal-free strain PH (derived from PX099β) [16] and AH28, respectively, to obtain PH/tal7b, PH/avrXa23 and AH28/avrXa23 (Table S3).

Western blot

To detect the TALE proteins in Xoo strains, Western blotting was conducted as described previously [33]. The tested Xoo strains were cultured in NB to the logarithmic phase and harvested by centrifugation. Bacterial cells were washed twice, and adjusted to OD600 = 2.0 with sterile distilled water. Proteins added loading buffer were boiled, separated on an SDS-PAGE gel and transferred to a polyvinylidene difluoride membrane for immunoblotting with the 3.2-kb SphI fragment of avrXa23 (GenBank accession no. GU732172) as a probe. Hybridizing colonies were further evaluated by PCR analysis with tal-specific primers TALN18-F and TALN18-R (Table S4). Putative tal-containing clones were confirmed by restriction enzyme digestion and Sanger sequencing. tal7b and avrXa23 were cloned into vector pZW in-frame with C-terminal Flag tag epitopes, resulting in pZW-tal7b and pZW-avrXa23 (Table S3). These plasmids were ligated into the broad-host-range vector pHM1 at the Hind III site, generating pHZW-tal7b and pHZW-avrXa23 (Table S3), which were then transferred into a tal-free strain PH (derived from PX099β) [16] and AH28, respectively, to obtain PH/tal7b, PH/avrXa23 and AH28/avrXa23 (Table S3).

Disease assays

Xoo strains were cultured in NB supplemented with appropriate antibiotics at 28 °C for 20 h. Bacterial suspensions (OD600 = 0.8) were used to inoculate two-month-old rice plants by the tip-
Whole-genome sequence analysis of AH28

To gain insights into the reason why Xa23 resistance is overcome, we completed the whole genome sequence of AH28 using long-read Nanopore sequencing combined with Illumina genome sequencing. The genome was assembled into two contigs, corresponding to a circular chromosome of 4,923,022 bp (accession number CP074076) and a plasmid pAH28 of 42,144 bp (accession number CP074077), respectively (Fig. 1). The GC contents of the chromosome and plasmid were 63.71% and 61.95%, respectively (Table S1). The AH28 genome contains 4,816 predicted genes, of which 3,407 (70.7%) genes encode proteins categorized into clusters of orthologous groups (COG) category (Table S1). A comprehensive frame of the AH28 genome is presented in Fig. 1, including the predicted 17 tal genes (red color) and the COG function classification of genes.

To further investigate the population structure of Xoo strains, we performed the phylogenetic tree analysis using genome sequences of AH28 and other 19 fully sequenced Xoo strains, including 11 Chinese strains (Fig. 2, Table S2). The 11 Chinese strains could be divided into three clades (Fig. 2): Clade I contained two strains from Liaoning province of China (LN18 and LN4) and nine strains in Clade II were from Jilin, Jiangsu, Hunan, Sichuan, Taiwan and Anhui provinces, including the newly sequenced AH28. A strain from Yunnan (YN24) was in Clade III, which also contained an Indian strain (IX-280) and a Philippine strain PXO99A. These results implied that the geographical distribution of Xoo strains may be reflected in the genome features. Indeed, the

### Table 1

Inoculation results of Xoo strain AH28 and PXO99A with NILs.

| NILs* | Strains | Lesion length (cm) | Phenotypes | TALEs matched |
|-------|---------|--------------------|------------|---------------|
| IRBB5(xa5) | AH28 | 0.58 | R | PthXo7 absent |
|        | PXO99A | 6.32 | S | PthXo7 |
| IRBB7(Xa7) | AH28 | 0.33 | R | PthXo3 |
|        | PXO99A | 12.23 | S | AvrXa7 and PthXo3 absent |
| IRBB10(Xa10) | AH28 | 13.38 | S | AvrXa10 absent |
|        | PXO99A | 13.01 | S | |
| IRBB13(xa13) | AH28 | 13.02 | S | PthXo2 and PthXo3 |
|        | PXO99A | 1.87 | R | PthXo1 |
| CBB23(Xa23) | AH28 | 20.2 | S | AvrXa23-like TALE |
|        | PXO99A | 1.43 | R | AvrXa23 |
| Nipponbare | AH28 | 13.42 | S | |
|        | PXO99A | 13.45 | S | |
| IR24 | AH28 | 13.75 | S | |
|        | PXO99A | 12.17 | S | |

* NILs, near-isogenic lines rice.

* S, susceptibility with lesion length > 2.5 cm; R, resistance with lesion length ≤ 2.5 cm.

![Fig. 1. Circular genome map of AH28 strain. The outermost ring is genome size, with 0.1 Mb intervals. The second and the third circle are CDS on the sense and antisense strands. Different colors indicate CDS according to COG functional classification. The fourth circle represents noncoding RNA. The fifth circle shows the GC content. Central circle is the GC skew value.](image_url)
MAUVE alignments of AH28 and PXO99A genomes showed a high-level genomic rearrangements and inversions (Fig. 3A), adapting to different rice cultivars planted geographically.

**TALEs encoded by the AH28 genome**

Genome sequence analysis showed that there were seven tal gene loci in AH28 encoding 17 TALEs and eight loci encoding 19 TALEs in PXO99A (Fig. 3B). The TALE repertoire of AH28 consisted 15 typical TALEs and 2 iTALEs (Fig. 3B, 4, S1). The iTALEs are TALE variants with shortened and truncated N- and C-termini that function as the suppressors of the resistance mediated by Xa1/Xo1 and their alleles [8,9,16,26]. The predicted RVD numbers in each of AH28 TALome varies from 13 to 29 (Fig. 4). Seven TALEs in AH28 are identical to those in the genomes of the Xoo strains available in the NCBI database, and ten TALEs in AH28 displayed one to nine
RVDs different to those possessed by PXO99A (Fig. 4). These results suggest that the TALome of AH28 is acquired for the co-evolution of the pathogen virulence with its host rice in a particular rice-growing region.

The TALome overview of AH28 showed seven clustered tal genes without the cluster IV, which were present in PXO99A (Fig. 3B). The cluster I in AH28 contained one tal gene (tal1) encoding PthXo2, whereas PthXo7 correspondingly in PXO99A (Fig. 3B). These explained that the incompatibility of AH28 with IRBB5 (xa5) rice was due to the absence of PthXo7 in the AH28 genome (Table 1). The cluster II had three tal genes in AH28, including tal2c for PthXo3 (Fig. 3B, 4), which activated the expression of Xa7 for ETI and OsSWEET14 for ETS in rice [10,11,15]. This likely explains that the strain AH28 was incompatible with Xa7-containing rice IRBB13 (xa13), but are absent in PXO99A, which was incompatible with IRBB13 (Table 1). Interestingly, AvrXa10, matched by Xa10 [36], is absent both in AH28 and PXO99A, explaining the reason that these two strains are compatible with IRBB10 (Xa10) (Table 1, Fig. 4).

The compatibility of AH28 with rice CBB23 (Xa23) (Table 1) and no similar Xa23-hybridized band showed in AH28 (Fig. S1) compromises us to assume that the Xa23-matched avirulence gene avrXa23 may be absent or mutated in AH28. With the help of the Ah28 genome sequence, it is surprising to see an ortholog of avrXa23 gene, tal7b, is present in the strain AH28 (Fig. 3B, 4, S1). Then, tal7b was directly cloned by cutting the putative tal-hybridized band (Fig. S1) through the screening by in situ colony hybridization. The sequencing results, including the genome sequence, showed that tal7b is 4347 bp in size with 97.2% identity at the nucleotide levels to avrXa23 of PXO99A and encodes an AvrXa23-like TALE (designated Tal7b or AvrXa23C), totally 1448 amino acid residues with 26 RVD repeats (Fig. 4). Compared with AvrXa23, Tal7b has the 4th repeat missed, and the 5th and 8th RVDs changed from NG and NS to NS and S*, respectively (Table 2). The information above implies that Tal7b may be a mutation of AvrXa23.

Tal7b, an AvrXa23-like TALE, did not trigger resistance mediated by Xa23 in rice

To verify the hypothesis in the previous section, the isolated tal7b was subcloned as FLAG-tagged derivatives into pZW, resulting in pZW-tal7b (Table S3), and this construct was then inserted into the HindIII site of pHM1, resulting in the cosmid pHZW-tal7b (Table S3). The construct was then transferred into Xoo PH, a tal-free derivative of PXO99A [16], generating a strain PH/tal7b (Table S3). The cosmid pHZW-avrXa23 was constructed in the similar strategy mentioned above and then transferred into PH and AH28 strains, resulting in PH/avrXa23, and AH28/avrXa23, respectively (Table S3). Western blot analysis showed that both Tal7b and AvrXa23, about 150 kDa, were detectable in corresponding Xoo strains (Fig. 5A). Xoo strains containing pHZW-avrXa23, pHZW-tal7b or empty vector (EV) were infiltrated into young CBB23 seedling leaves using needleless syringes. As expected, Tal7b, the mutated version of AvrXa23, did not make the PH strain trigger HR on CBB23, while AvrXa23 enabled AH28 from compatibility (water-soaked symptoms) to incompatibility (HR phenotype) with CBB23 when AH28 and PH harbored the avrXa23 gene in trans

\[ \text{Fig. 4. RVD sequences of AH28 TALEs. RVDs in red color are different in PXO99A orthologs. An asterisk indicates that the second amino acid in the RVD is absent, resulting in a 33 aa repeat. A dagger indicates an iTALE.} \]
This, we investigated the distribution of Multiple alignment of the RVD sequences of AvrXa23-like TALEs. (Fig. 5B). The incompatibility was similar to that caused by Xoo strains contain AvrXa23-like TALEs RVDs displayed from the available genome sequences of (Table 2). These RVD differences showed diversities of AvrXa23-like TALEs in Xoo population, suggesting that the avrXa23 locus may undergo the resistance pressure by the cognate R gene. Moreover, AvrXa23D seems like the major version of AvrXa23-like TALEs displayed from the available genome sequences of Xoo strains so far (Table 2).

Limited by the plant quarantine policies among countries, four Xoo strains, PXO99A, PXO86, AH28 and LN18, which contain AvrXa23, AvrXa23A, AvrXa23C, and AvrXa23D, respectively, were chosen as the representatives of the Xoo collection to confirm the gene-for-gene manner between the AvrXa23-like TALEs and the Xa23 gene. Indeed, the AvrXa23C-harbored strain AH28 was compatible with Xa23-containing rice CBB23, while the other three strains were incompatible (Fig. S2), implying that AH28 is an emerging race to overcome Xa23 resistance in rice.

Discussion

Xaa23, a TALE-dependent E gene, confers the broadest resistance against Xoo strains without any exception before this report [13,14,47]. Here, we uncovered an Xa23-breaking Xoo isolate, AH28 from a rice-growing field in Anhui province of China (Table 1). The genome sequencing (Figs. 1, 3) and TALE analysis (Fig. 4, S1) of this isolate led to the finding that the Tal7b, with the 4th RVDs (NN) missed and the 5th (S*) changed in comparison with those of AvrXa23, is an AvrXa23-like TALE that makes the pathogen unable to trigger Xa23-mediated ETI (Fig. 5, S2). This is consistent with the binding affinity of AvrXa23, but not Tal7b, to the EBE of Xa23 promoter (Fig. 5F).

The concept accepted is that the induction of S or E genes via the EBEs bound by the presence of major virulence factors (PthXo) or matching avirulence factors (TALEs) confers susceptibility or resistance to rice. The completed genomes show that the strain AH28 has PthXo2 and PthXo3, but not PthXo1 and PthXo7, which are present in PXO99A (Fig. 3B), explaining the incompatibility of AH28 with Xa7-containing rice IRBB7 and xa5-containing rice IRBB5 vs the compatibility of PXO99A with IRBB7 and IRBB5, respectively (Table 1), since OsSWEET1 is targeted by PthXo1 [20], OsSWEET13 by PthXo2 [31], Xa7 by PthXo3 or AvrXa7 [10,11], and OsTFL1A1 by PthXo7 [48]. The absence of AvrXa10 both in AH28 and PXO99A

Table 2

| TALEs     | Strainsa   |
|-----------|------------|
| AvrXa23   | PXO99a, PXO79, ICMP3125, Ixo1088 |
| AvrXa23A  | MAFF 311018(T7174), PXO68, PXO211, PXO236, PXO563, K3, K3a, JLI25, JLI28, YC11, JP01, Scyc-b, XM9, PXO145 |
| AvrXa23B  | NX0260 |
| AvrXa23C  | AH28 |
| AvrXa23D  | LN18, LN4, PXO61, PXO71, PXO524, PXO602, PXO704, IX-280, SK2-3, BXO1, PXO404, PXO421, PXO513, JW10189, Xf89b, K1, K2, KX065, AUST2013 |
| AvrXa23E  | PXO282 |
| AvrXa23F  | PXO142 |

a RVDs that are different from those of the reference (AvrXa23) at the same positions are colored in red font. S* are rare RVDs in TALomes of Xanthomonas species. b Strains in blue means incompatible with Xa23 rice tested by the report (Wang et al., 2014).
(Fig. 3B) makes the strains compatible with Xa10-containing rice IRBB10 (Table 1). It also could be predicted that AH28 is compatible with Xa27-containing rice (though we did not test this in the report), since it does not have the TALE AvrXa27 (Fig. 3B). Several Xa27-breaking Xoo strains have been reported before [13]. Thus, there is no exception that the resistance mediated by these four E genes could not be overcome by the pathogen presently, suggesting that durable broad-spectrum resistance for BLB mediated by an E gene is relative to an emerging race, whether dominant or minority.

The arms-race between the pathogen and the host normally occurs in a gene-for-gene manner. PthXo2 secreted by Xoo as a major virulence TALE targets the S gene OsSWEET13 to induce ETS [31]. Five PthXo2 variants and ten haplotypes of OsSWEET13 have been identified in Xoo strains and rice varieties, respectively, which is the result of arms-race between Xoo and rice [32]. For ETI triggered by an avr gene and the corresponding R gene, pathogen may mutate or just discard the avr gene under high selection pressure generated by the R gene [15]. In this study, we found that AH28 loses avrXa10 but contain a defective copy of avrXa23, tal7b (Table 1, Fig. 3B). Seven versions of AvrXa23-like TALEs were also identified in different Xoo strains (Table 2). These results demonstrate that diversified strategies are used by Xoo population to evade recognition by E genes in rice.

Combining the results of this study and previous reports [10,11,14,20,31], we propose that avrXa23-containing Xoo strains utilize major TALEs to target the corresponding S genes and induce ETS, which can be suppressed by Xa23-mediated ETI triggered by AvrXa23, leading to host resistance to these strains; The Xa23-breaking isolate AH28 modifies the RVDs of AvrXa23 to avoid binding to the EBE and activating the expression of Xa23 in rice, which would impede the inhibitory effect of ETI on ETS induced by major
TALEs (PthXo2 and PthXo3), resulting in host susceptibility to the strain (Fig. 6). However, the mechanism of Xa23-mediated ETI is still unclear and further research is needed.

None of the seven AvrXa23-like TALEs was present in the genomes of six Asian Xoo strains including three Chinese strains (Table S2). One of the strains, HuN37 originally isolated from Hunan province of China, was inoculated to the Xa23-containing rice line CBB23 (Table S2, Fig. S2). Surprisingly, this strain is incompatible in CBB23 (Fig. S2), which may be explained by the inaccurate assembly of this genome or other effector(s) in this strain activating host resistance. This requires further experiments to determine.

The broad-spectrum resistance mediated by Xa23 to Xoo has led to its inclusion in rice breeding programs, and several Xa23-containing elite inbred and hybrid rice varieties have been released to growers [13,14,38,39]. However, our discovery shows that there is a potential, emerging Xoo population that can overcome Xa23-mediated BLB resistance. Thus it is important to continually monitor the Xoo population in the world, particularly in China by genome sequencing and TALome analysis. Meanwhile, it is necessary to avoid planting rice varieties with a single R gene (like Xa23) in a large area. The diversified implementation of multiple resistance strategies, including those that exploit the EBE site of E genes and S genes, are promising approaches to stay abreast of the ongoing ‘arms race’ between Xoo and cultivated rice [33,34,37,47,49-51].

Conclusion

In conclusion, Xoo may survive by changing few RVDs of an avirulence TALE to avoid being trapped by the EBE of a matched executor R gene in rice. This may impede the inhibitory effect of ETI on ETS to keep host susceptibility (Fig. 6).

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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jare.2022.01.007.

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