The Xa7 resistance gene guards the rice susceptibility gene SWEET14 against exploitation by the bacterial blight pathogen

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https://doi.org/10.1016/j.xplc.2021.100164

ABSTRACT
Many plant disease resistance (R) genes function specifically in reaction to the presence of cognate effectors from a pathogen. Xanthomonas oryzae pathovar oryzae (Xoo) uses transcription activator-like effectors (TALes) to target specific rice genes for expression, thereby promoting host susceptibility to bacterial blight. Here, we report the molecular characterization of Xa7, the cognate gene to the TALes AvrXa7 and PthXo3, which target the rice major susceptibility gene SWEET14. Xa7 was mapped to a unique 74-kb region. Gene expression analysis of the region revealed a candidate gene that contained a putative AvrXa7 effector binding element (EBE) in its promoter and encoded a 113-amino-acid peptide of unknown function. Genome editing at the Xa7 locus rendered the plants susceptible to avrXa7-carrying Xoo strains. Both AvrXa7 and PthXo3 activated a GUS reporter gene fused with the EBE-containing Xa7 promoter in Nicotiana benthamiana. The EBE of Xa7 is a close mimic of the EBE of SWEET14 for TALe-induced disease susceptibility. Ectopic expression of Xa7 triggers cell death in N. benthamiana. Xa7 is prevalent in indica rice accessions from 3000 rice genomes. Xa7 appears to be an adaptation that protects against pathogen exploitation of SWEET14 and disease susceptibility.

Keywords: Xa7, TAL effector, Xanthomonas, bacterial blight, disease resistance, SWEET14

INTRODUCTION
Crop plants suffer detrimental effects from plant diseases and pests, which cause global yield losses of about 20% each year (Oerke 2006; Savary et al., 2019). To counteract disease, host plants have evolved innate immunity mechanisms that work against pathogens mainly through a diverse array of plant genes and gene products that recognize molecular signals from the pathogens (Spoel and Dong 2012). Conceptually, resistance is mediated by two general pathways. In the first pathway, membrane-bound receptors recognize conserved small molecules, often of pathogen origin, the so-called pathogen-associated molecular patterns, and trigger basal and broad immunity against the invading pathogens (Jones and Dangl 2006). Many host- and cultivar-specific pathogenic fungi and proteobacteria have evolved processes that suppress basal immunity (Cook et al., 2015; Monteiro and Nishimura 2018). Plants, in turn, have evolved a second layer of defense, the so-called effector triggered immunity (ETI), which involves the specific recognition of immunity-suppressive effectors (Jones and Dangl 2006). Major resistance (R) genes are adaptive components of the plant defense system that arise from selective pressure exerted by virulent pathogen populations. In some cases, pathogens can overcome ETI through mutation or loss of R gene-specific effector genes or the acquisition of new effectors that, in turn, suppress ETI (Jackson et al., 1999; Feng and Zhou 2012).
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Bacterial blight (BB) of rice, caused by the γ-proteobacterium Xanthomonas oryzae pathovar oryzae (Xoo), is among the most damaging diseases in a wide range of South Asian rice-producing areas and also poses a threat in some African countries (Niño-Liu et al., 2006). R gene deployment is the most economically sound and environmentally friendly means to control BB, and many BB-specific R genes have been identified and characterized at the molecular level (Song et al., 1995; Yoshimura et al., 1998; Yang et al., 2000; Iyer and McCouch 2004; Sun et al., 2004; Gu et al., 2005; Chu et al., 2006; Xiang et al., 2006; Liu et al., 2011; Tian et al., 2014; Wang et al., 2015; Hu et al., 2017; Ji et al., 2020; Zhang et al., 2020). The cognate effectors that recognize R genes, as in other proteobacterial disease complexes, are commonly effectors of the type III secretion pathway. Historically, type III effectors with a cognate R gene are named Avr effectors (Leach and White 1996). All the known cognate type III Avr effector/R gene pairs in BB involve a subset of type III effectors known as transcription activator-like effectors (TALes). TALes of Xanthomonas represent the largest subgroup of type III effector proteins in plant pathogenic bacteria. For the most part, they function as transcription factors that promote the expression of host genes by binding to sequence-specific promoter segments, referred to here as effector binding elements (EBEs). Consequently, expression of the host susceptibility (S) gene enhances the disease process. TALes of Xoo that have a dramatic effect on virulence and host susceptibility are referred to as major TALes and are known to target three members of the sucrose transporter, or SWEET, gene family. In the absence of SWEET gene expression, Xoo strains are virtually nonpathogenic, and every Xoo strain examined to date has at least one gene for a major TALe (Oliva et al., 2019). Rice cultivars have adapted to TALe-mediated virulence by the acquisition of a genetically dominant R gene class defined by TALe-specific expression that triggers a state of resistance. Rice R genes with TALe-specific expression include Xa27, Xa10, and Xa23 (Gu et al., 2005; Tian et al., 2014; Wang et al., 2015). TALe-mediated R gene expression has also been demonstrated for B33 and B54C-R in pepper (Capsicum sp.) (Römer et al., 2009; Strauss et al., 2012).

Xa7 is a dominant R gene of rice that confers resistance to Xoo strains that harbor the cognate major TALe AvrXa7 (Hopkins et al., 1992). The AvrXa7 effector has a dual function: as a virulence factor, it induces the rice S gene SWEET14, which encodes a sucrose efflux transporter, and as an avirulence factor, it also triggers Xa7-mediated resistance. AvrXa7 targets an overlapping EBE of the S gene SWEET14 with a second major TALe, PthXo3. Although the identity and mechanism of Xa7 are unknown, it has been shown to confer resistance to all six Japanese Xoo races or sub-races and 4 of 10 Philippine Xoo races (Ogawa et al., 1991). Xoo races are defined by the set of R genes in a given group of rice cultivars with which the strains are incompatible. The broad spectrum of Xa7 makes it an important R gene in rice breeding programs (Ogawa et al., 1991; Hsu et al., 2020). The pathogen gene avrXa7 was found in 11 of 33 fully sequenced Asian Xoo strains, whereas pthXo3 was found in 12 of the 33 strains. No strain contained both avrXa7 and pthXo3 (Oliva et al., 2019). Xa7 has been found to retain effectiveness under field conditions (Bai et al., 2000) and to perform better at high temperatures (Webb et al., 2010; Dossa et al., 2020), which are reported to compromise the function of some R genes. Efforts to map Xa7 have placed the gene on chromosome 6 (Kaji and Ogawa 1995; Porter et al., 2003; Chen et al., 2008; Zhang et al., 2009). In this study, we present evidence for the identity of Xa7 based on fine mapping, gene expression assays, and CRISPR-mediated gene editing.

RESULTS
Fine mapping of Xa7 from IRBB7

To fine map Xa7 in IRBB7 (an indica rice variety carrying Xa7), the first mapping population was created by crossing the near-isogenic line IRBB7 with the recurrent parental cultivar IR24 (Ogawa et al., 1991). For mapping, 220 F2 plants were phenotyped by inoculation with the avrXa7-carrying Xoo strain PXO86, and genotyped using the previously reported Xa7-linked marker M5 (Porter et al., 2003). A set of 10 newly developed molecular markers linked to M5 were also identified and used to further genotype the plants (see Supplemental Table 1 for markers and Supplemental Table 2 for oligonucleotides). Xa7 was mapped to an interval defined by the markers RM7243 (three recombinants) and RM20593 (two recombinants) and was shown to co-segregate with the markers M5, M5-5k, and M5-48k (Figure 1A). These results indicate that Xa7 is located within a region of 512 kilobases (kb) between RM7243 and RM20593 relative to the reference genome of the cultivar Nipponbare. An additional 17,000 members from an F2 population of IRBB7 and Nipponbare were screened for recombinants between RM7243 and RM20593, and the recombinants were phenotyped for genetic association analysis. Based on a number of polymorphic markers (Supplemental Table 1), Xa7 was shown to reside within a region corresponding to the 41.3-kb region between M5 and M5-48k on the Nipponbare reference genome (Figure 1B).

Short and long sequencing reads obtained from IRBB7 DNA by Illumina and Nanopore sequencing were used for de novo assembly of the Xa7 region across markers M5 and M5-48k, resulting in a genomic sequence of 74 kb. PCR amplification and sequencing of the amplicons were also performed to validate the accuracy of the sequencing data, and the sequence was aligned with the related region from Nipponbare (Figure 1B). The regions are syntenic and include homologous genes that encode the IRGSP GenBank protein accessions XP_025882165.1 (only the 402 amino acid [aa] C-terminal exon is conserved in IRBB7), XP_015642179.1 (not present in IRBB7), XP_015644250.1 (perfectly conserved), and XP_015641982.1 (perfectly conserved). All proteins are annotated as “uncharacterized.” XP_015641982.1 contains a common protein–protein interaction motif of about 100 aa, known as the BTB/POZ domain. The IRBB7 contig has a GC content of approximately 45%, and about 62% of the sequence matches transposable elements as determined by RepeatMasker (version open-4.0, http://www.repeatmasker.org) using the RITE database (Copetti et al., 2015).

Deletion of the Xa7 region

To further confirm the location of Xa7, a line named NB7, which was derived from an IRBB7 and Nipponbare cross and is resistant to PXO66, was used to delete 53 kb of the IRBB7 Xa7 region using CRISPR-Cas9 with two guide RNAs (Figure 1B, gRNA1 and
gRNA2). NB7 is transformable due to its Nipponbare genetic background. PCR with deletion-specific and internal primers showed that one transgenic line, nb7-1, contained a large 53-kb deletion delimited by the two guide RNAs in one of its chromosomal copies (Supplemental Figure 1; e eSupplemental Table 2 for oligonucleotide information). The nb7-1 line was resistant to disease after inoculation with Xoo strain PXO86, indicating that it retained a copy of Xa7 and was heterozygous for the deletion. Susceptibility was shown to co-segregate with the homozygous deletion in the T1 population (n = 5/24) using primers that could detect the wild-type and deleted regions (Figure 2).

Gene expression from the Xa7 region

We hypothesized that Xa7 could be distinguished from the other annotated genes in the 53-kb region based on its TALe-mediated expression. RNA samples from IRBB7 infected with PXO86 and the avrXa7 mutant MX53 were subjected to RAMPAGE analysis, which combines RNA annotation and mapping of the respective promoters (Batut and Gingeras 2013; Raborn and Brendel 2019). The transcription start site (TSS)-adjacent sequences of transcribed genes in the unique 53-kb region were captured, and the RAMPAGE reads were used to project the transcript abundance of individual genes in the two treatments (with and without avrXa7). We used the Bioconductor TSRchitect package (Raborn et al., 2017) to identify transcription start regions (TSRs) in the contig, and the edgeR package (Robinson et al., 2010; McCarthy et al., 2012) to assess differential expression. Only one strongly induced TSR was found in the 53-kb region, in positions 63 494–63 513 (predominant TSS at 63 503; Figure 1), and it showed a 147-fold increase in expression after treatment with PXO86 relative to MX53 (Supplemental Figure 2). We refer to this transcript as R-Xa7 (Figure 1B, red arrow).

Sixty-eight base pairs downstream of the predominant R-Xa7 TSS site is an open reading frame (ORF) of 342 bp (including the stop codon) designated ORF113. A 726-bp cDNA, encompassing ORF113, a 68-bp 5’ UTR, and a 316-bp 3’ UTR, was isolated by screening a cDNA library derived from IRBB7 infected with PXO86 (Figure 1C, Supplemental Information 1). ORF113 was predicted to encode a small protein of 113 aa that showed no
significant similarity to known R proteins in the database (Figure 1D). The gene from IRBB7 is hereafter tentatively referred to as Xa7.

To corroborate its involvement in Xa7-mediated resistance, two sites in Xa7 were targeted by CRISPR in NB7. Two CRISPR target sites in Xa7 were chosen to construct two guide RNAs for the transformation of NB7 (Figure 3A, gRNA3 and gRNA4). Sequence analysis of 15 T0 transgenic plants revealed three independent T0 plants in which both alleles were knocked out: x7cr-1, x7cr-2, and x7cr-3. The mutations (1-bp deletion) at the first guide RNA target in x7cr-1 led to a premature stop codon; the mutations (79-bp deletion) in x7cr-2 also led to a premature stop codon in Xa7; and the two alleles in x7cr-3 also led to two null mutations in Xa7 (Figure 3A and 3B). All three altered lines were susceptible to PXO86, indicating that Xa7-mediated resistance to PXO86 requires functional Xa7 (Figure 3C).

To identify the putative EBE for AvrXa7 in Xa7 (designated EBE_{AvrXa7}), the sequence upstream of the Xa7 cDNA was analyzed by the EBE prediction programs TALVEZ and TALE-NT (Doyle et al., 2012; Booher and Bogdanove 2014). Both programs predicted consensus sequences of 26 nucleotides for AvrXa7 and 29 nucleotides for PthXo3 located 134–109 and 136–107 bp upstream of the Xa7 ATG that exhibited the DNA binding specificity predicted for the repeat regions of AvrXa7 and PthXo3, respectively (Figure 4A). The EBEs of Xa7 for AvrXa7 and PthXo3 are similar to the corresponding EBEs of SWEET14 (Figure 4A), and their predicted binding scores are comparable to those of the previously characterized overlapping SWEET14 EBEs for AvrXa7 and PthXo3 (Figure 4B) (Antony et al., 2010).

**Ectopic expression of Xa7 induces cell death in N. benthamiana**

Although all the TALe-dependent executor R proteins of rice share some amino acid identities, it is unclear whether the proteins are phylogenetically related, with the exception of XA10 and XA23 (Figure 6A). Ectopic expression of Xa10 and Xa23 triggers cell death in N. benthamiana (Tian et al., 2014; Wang et al., 2015), but the effect of Xa27 and Xa7 is unknown. To investigate whether Xa7 can function in N. benthamiana, the 3SS promoter (3SS) was placed immediately upstream of the translation start codon of the Xa7, Xa10, Xa23, and Xa27 ORFs, and each construct was delivered into N. benthamiana leaves by agroinfection. A weak HR (hypersensitive response) for Xa7, Xa10, and Xa23 was visible at 16 h after infiltration, and HR was
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pronounced at 48 h after infiltration, although the degree of cell death induced by Xa7 appeared lower than that induced by Xa10 and Xa23 (Figure 6B). No visible HR occurred after the transient expression of Xa27 (Figure 6B).

Spectrum of Xa7 resistance against various Xoo strains

We next examined the spectrum of Xa7 resistance against all known major TALe genes and representative Xoo strains. Six major TALe genes (avrXa7, pthXo1, pthXo2, pthXo3, TalC, and TalF) that target three cognate SWEET-related S genes in rice were transferred into ME2. ME2 is not pathogenic in either IR24 or IRBB7 due to loss of the endogenous copy of pthXo1 and the resulting inability to induce any SWEET gene. Individual ME2 complementation strains carrying avrXa7 and pthXo3 caused susceptibility in IR24 and resistance in IRBB7, whereas the other four TALe genes in ME2 caused susceptibility in both IR24 and IRBB7, indicating that Xa7 is specific to avrXa7 and pthXo3 (Figure 7). Seven representative field isolates of Xoo are known to carry avrXa7 or pthXo3 or lack either gene (Oliva et al., 2019). Only isolates carrying avrXa7 or pthXo3 triggered resistance (Figure 7). ME2(pthXo3) resulted in an incompatible interaction, and PXO61, which contains pthXo3, was scored as moderately susceptible in comparison to the PXO99 and PXO86 reactions. IRBB7 has been scored as moderately susceptible to resistant PXO61 in previous tests (Institute 2006).

Prevalence of Xa7 locus in other species and rice cultivars

Xa7 homologs have been identified in other species. Homologs exist in wild rice species (O. punctata and O. longistaminata), sorghum, Setaria, and panicgrass (P. hallii and D. oligosanthes). The C termini of XA7 homologs are more conserved than the N termini (Supplemental Figure 3A and 3B). To determine whether representative genes could cause cell death in N. benthamiana, the coding sequences of six homologous genes were each constructed under the control of the 35S promoter and expressed ectopically by agroinfiltration of N. benthamiana (Supplemental Information 1). Among these constructs, only Ol_Xa7 resulted in an HR at 48 h. Op_Xa7 appeared to cause a weak cell death response compared with that of Xa7 (Supplemental Figure 3C). No obvious cell death phenotype was observed with the remaining homologs (Supplemental Figure 3C).

A total of 294 accessions from a scan of 3000 rice genome sequences contained Xa7 coding sequences (Supplemental Data 1). A 3171-bp region of the IRBB7 Xa7 locus, including 842 bp upstream and 2014 bp downstream of the Xa7 coding sequence, was used as the reference to assemble reads from these 294 accessions, generating 294 contigs that contained Xa7 and its flanking sequences. Due to the lower sequencing coverage of some accessions and the limitations of next generation sequencing technology to reveal 13 consecutive Cs, only nine contigs contained the Xa7 EBEs for AvrXa7 and PthXo3. The EBEs could not be unambiguously identified in the majority of contigs (Supplemental Data 1).

Among the 294 accessions, the majority (n = 185, 63%) are indica, 72 are japonica (24%), 22 are Aus/boro, 14 are Basmati/sadri, and seven are intermediate types (Supplemental Data 1). Geographically, India has the most accessions (n = 58), followed by China (n = 41), Bangladesh (n = 34), Indonesia (n = 26), the Philippines (n = 22), and Cambodia (n = 19) (Supplemental Data 1).

DISCUSSION

Xa7 has mysteriously evaded cloning endeavors for the past 20 years. Here, multiple approaches provide evidence that our postulated candidate gene is indeed the cognate R gene for the TALe AvrXa7. Three critical observations are that (1) the gene lies within the region of markers previously associated with the Xa7 locus; (2) the deletion of the region and specific mutations in ORF113 eliminate Xa7-mediated resistance; and (3) the gene...
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**Figure 4.** AvrXa7 and PthXo3 are predicted to target two overlapping EBEs in Xa7.

(A) Individual RVDs of the AvrXa7 and PthXo3 central repeats match single nucleotides of the predicted EBE regions in the Xa7 promoter. Single letters are used for amino acids at the 12th and 13th positions of individual repeats. * The missing amino acid at the 13th position of a particular repeat. The EBE regions for AvrXa7 and PthXo3 in the S gene SWEET14 are shown for comparison.

(B) The scores of matches between the DNA sequences of putative Xa7 and SWEET14 (as controls) and the repeats of AvrXa7 and PthXo3 as predicted by two programs (TALE-NT and TALVEZ). A lower score indicates a higher binding affinity between the RVDs and the target sequence.

is expressed in an AvrXa7-dependent manner upon infection. That the AvrXa7-mediated expression of Xa7 is directed by a sequence-specific element in its promoter was corroborated by transient expression assays in *N. benthamiana* leaves. The GUS fusion was not expressed in the absence of AvrXa7 or another TALe (PthXo1) that has an alternate binding specificity. Disruption of the EBE by a 20-bp deletion also interfered with AvrXa7-dependent transient expression. The experiments also revealed that the TALe PthXo3 functions as an allele of AvrXa7 and directs Xa7-mediated resistance. The PthXo3-dependent activity of Xa7 explains its relatively broad activity against extant Asian strains of *Xoo*. The gene for PthXo3 was originally cloned from PXO61, a strain isolated in the Philippines. IRBB7 has been scored variously as moderately susceptible (or resistant) to resistant to PXO61 (Ogawa et al., 1991; Lee et al., 2003; Zhang et al., 2009; Xu et al., 2012). Here, we demonstrate that the ability of four executor genes cloned from rice to induce cell death in *N. benthamiana* is unique in that its EBE mimics that of the S gene, guarding against pathogen exploitation of SWEET14 in healthy cells by triggering the death of infected cells that are injected with either of the two major TALEs.

Several examples show that the transient overexpression of executor *R* genes in *N. benthamiana* can induce cell death, which may mimic the HR phenotype in the host plant/microbe interaction (Romer et al., 2009; Strauss et al., 2012; Tian et al., 2014; Wang et al., 2015; Wang et al., 2017; Wang et al., 2018). In this study, we compared the ability of four executor genes cloned from rice to induce cell death in *N. benthamiana*. The appearance of the HR showed that Xa7 has a moderate ability to induce cell death relative to Xa10 and Xa23, both of which trigger cell death much more rapidly and strongly. No visible cell death was observed in *N. benthamiana* when Xa27 was overexpressed (Figure 6B) (Tian et al., 2014). Xa27 was reported as an *R* gene that triggered a strong HR in response to AvrXa27 in rice (Gu et al., 2005), and Xa27-like genes activated by designer TALEs that targeted the promoters of Xa27-like genes also mediated strong HR in rice (Li et al., 2013). Together with the observation of the diverse abilities of six Xa7 homologs to induce cell death in *N. benthamiana*, our results therefore show that the mechanism of cell death induction in *N. benthamiana* may differ from the HR triggered in rice by the corresponding avirulence TALE gene.

All four executor *R* genes in rice encode small proteins: XA27, XA23, and XA7 consist of 113 aa, and XA10 consists of 126 aa.

| TALEs | Score Xa7 | Score OsSWEET14 | Software |
|-------|-----------|-----------------|----------|
| AvrXa7 | 14.908 | 10.069 | TALVEZ |
| PthXo3 | 12.590 | 8.285 | TALVEZ |

| TALEs | Score Xa7 | Score OsSWEET14 | Software |
|-------|-----------|-----------------|----------|
| AvrXa7 | 19.85 | 29.95 | TALE-NT |
| PthXo3 | 26.72 | 31.87 | TALE-NT |
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(Figure 6A). XA27, the first executor R gene identified, is predicted to contain two transmembrane domains (Gu et al., 2005). The N terminus of XA27 is also predicted to contain a signal anchor-like sequence, leading to the cellular localization of XA27-green fluorescent protein to the apoplast and wall of xylem cells. Alterations to the hydrophobic nature of the signal anchor-like sequence change the location and resistance activity of XA27 to an avirulent strain of Xoo (Wu et al., 2008). On the other hand, XA10 is predicted to contain four transmembrane domains and localizes in the endoplasmic reticulum (ER) (Tian et al., 2014). XA10 was found to be associated with ER Ca\(^{2+}\) depletion in plant and HeLa cells. Mutations that render the protein unable to deplete ER Ca\(^{2+}\) and to cause cell death in N. benthamiana concomitantly abolish Xa10-mediated resistance in rice (Tian et al., 2014). Similarly, Bs4C-R, induced by AvrBs4 for the resistance of pepper to Xanthomonas campestris pathovar vesicatoria, encodes a 164-aa protein of unknown function (Strauss et al., 2012). Bs4C-R is predicted to contain four transmembrane motifs, and a fluorescent fusion protein of BS4C-R was shown to localize in the ER membrane in N. benthamiana. Bs4C-R causes cell death in N. benthamiana when ectopically expressed (Wang et al., 2018). Significantly, Xa10 promoter-Bs4C genes confer rice resistance to Xoo strains carrying avrXa10 (Wang et al., 2018). One exception to the small TALe-induced R proteins is Bs3 of pepper, which encodes 342 amino acids. Its gene product is homologous to the flavin-dependent monoxygenases (Römer et al., 2007), a group of enzymes that catalyze a wide range of chemo-, regio-, and enantioselective oxygenation reactions (Huijbers et al., 2014). In this study, XA7 is predicted to possess two transmembrane domains (Figure 6A), but its cellular localization remains to be characterized. Genome editing, especially base editing that induces DNA substitutions (Anzalone et al., 2020), will provide a robust tool with which to dissect the structure-function requirements of XA7 in an endogenous context.

METHODS

Plant materials, bacterial strains, medium, and growth conditions

The indica rice variety IR24, the recurrent near-isogenic line IRB77 with R gene Xa7, and the japonica variety Nipponbare were kindly provided by the International Rice Research Institute and the U.S. National Small Grains Collection. The rice line NB7 was a segregant from a cross of IRB77 and Nipponbare that exhibited Xa7-mediated resistance activity and the tissue culture trait of Nipponbare. N. benthamiana seeds were kindly provided by Dr. Gregory Martin. Xoo strains PXO86, PXO99A, and PXO99A\(^{\text{m}}\) mutant ME2 and transformants ME2(avrXa7), ME2(pthXo3), and ME2(pthXo1) were from the collection of the Yang laboratory.

All rice plants were grown in the greenhouse and growth chambers with a 12-h 30°C light period and a 12-h 28°C dark period at 60%–75% relative humidity. Escherichia coli cells strains were grown in Luria-Bertani medium supplemented with appropriate antibiotics at 37°C. Agrobacterium tumefaciens strains were grown at 30°C. All Xoo strains were grown at 28°C on TSA (10 g/l tryptone, 10 g/l sucrose, 1 g/l glutamic acid, 1.5% Difco agar). Antibiotics were used at the following concentrations if required: 100 µg/ml ampicillin, 10 µg/ml cephalaxin, 25 µg/ml rifampicin, 25 µg/ml kanamycin, and 100 µg/ml spectinomycin.

Disease assays

The leaf tip-clipping method was used to measure the lesion lengths of blight disease as described previously (Yang and Bogdanove 2013). In brief, an aliquot of the appropriate Xoo glycerol stock, stored at –80°C, was streaked onto TSA containing appropriate antibiotics and grown at 28°C for about 3 days. The bacterial cells were harvested from plates, suspended in sterile water, washed twice with water, and resuspended in water; the solution was adjusted to an optical density of 0.5 at 600 nm. Scissors blades were immersed in the Xoo suspension and used to clip the tip of a fully expanded leaf. The lesion lengths were measured at 14 days or at the specified days after inoculation. Three replicates with multiple leaves per replicate were examined for each Xoo strain. Data were plotted using BoxPlotR (http://shiny.chemgrid.org/boxplots/). One-way analysis of variance was performed on all
CRISPR-Cas9-based gene editing in rice

The CRISPR-Cas9 system used to generate a large chromosomal deletion in the Xa7 locus and mutations within the Xa7 coding region was described previously (Zhou et al., 2014). Two guide RNA genes (gRNA1 and gRNA2) targeting two sites spanning the 53-kb Xa7 coding region were constructed in the intermediate guide RNA vector pgRNA-1. The guide RNA cassette was mobilized into the Cas9 destination vector pBY02-Cas9-GW through the Gateway reaction using LR clonase (Thermo Fisher Scientific), resulting in pCas9-gRNA1+2. Similarly, two guide RNA genes targeting two sites (gRNA-3 and gRNA-4) in the Xa7 coding region were combined into pBY02-Cas9-GW, resulting in pCas9-gRNA3+4. The Xa7 coding region was depleted, reverse complementary DNA synthesis with custom RAMPAGE-specific oligos, cap-trapping of the 5’-complete cDNA and RNA double-stranded DNA/RNA, streptavidin-based pull-down of the biotinylated DNA/RNA, PCR amplification and size selection of double-stranded DNA, and Illumina-based paired-end sequencing. Library quality was assessed using the Agilent 2200 TapeStation instrument (Agilent Technologies, Santa Clara, CA, USA) at the Indiana University Center for Genomics and Bioinformatics. All computational analyses are documented for reproducibility at https://github.com/BrendelGroup/AllRice following the guidelines proposed in Brendel (2018).

Gene expression assays

For RAMPAGE experiments, young leaves of IRBB7 were inoculated with PXO86 and the avrXa7 knockout mutant MX33 (Hopkins et al., 1992). Total RNA was extracted using the TRizol reagent (Thermo Fisher Scientific) 24 h after inoculation. Three replicates for each Xoo strain were used to construct RAMPAGE libraries for paired-end sequencing as described previously (Batut and Gingeras 2013; Raborn and Brendel 2019). In brief, total RNA was subjected to DNase I treatment, ribosomal RNA depletion, reverse complementary DNA synthesis with custom RAMPAGE-specific oligos, cap-trapping of the 5’-complete cDNA and RNA double-stranded DNA/RNA, streptavidin-based pull-down of the biotinylated DNA/RNA, PCR amplification and size selection of double-stranded DNA, and Illumina-based paired-end sequencing. Library quality was assessed using the Agilent 2200 TapeStation instrument (Agilent Technologies, Santa Clara, CA, USA) at the Indiana University Center for Genomics and Bioinformatics. All computational analyses are documented for reproducibility at https://github.com/BrendelGroup/AllRice following the guidelines proposed in Brendel (2018).

Transgenic TALE-specific Xa7 promoter activity in N. benthamiana

Xa7 promoter fusions to GUS reporter constructs were made using the 2.7-kb promoter region upstream of the Xa7 ATG after amplification with the oligos Pr02.7kHind-F and Xa7ATG-R from IRBB7 genomic DNA. The ampiclon was cloned into pCAMBIA1305 at HindIII and NcoI through Gibson cloning (Gibson et al., 2009). To construct the Xa7 promoter-GUS reporter with a mutant AvrXa7 binding element, two fragments of the promoter were amplified with Pr02.7kHind-F and DeLEBE-F3 and Xa7ATG-R from IRBB7 genomic DNA and inserted into pCAMBIA1305 at HindIII and NcoI. The constructs were transferred into the Agrobacterium strain EHA105. The TALE expression constructs were made by cloning the coding regions of avrXa7, pthXo3, and pthXo1 under the 35S promoter in pCAMBIA1300 at BamHI and SpeI sites. N. benthamiana plants were grown under 12 h of light and 12 h of darkness at 25°C and approximately 40%–60% relative humidity. Leaves of 4-week-old N. benthamiana plants were used for infiltration with a 1-mL needleless syringe. Agrobacterium strain EHA105 that harbored the construct of interest was cultured in Luria-Bertani medium containing 25 mg/l rifampin, 25 mg/l kanamycin, and 100 μM acetosyringone. The bacterial
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Figure 7. Xa7 confers resistance to Xoo strains containing avrXa7 or pthXo3.
Rice plants of IR24 (clear boxes) and IRBB7 (filled boxes) were inoculated with strain ME2 carrying different major TALE genes. Individual strains are identified on the basis of the major TALE gene. Field isolates are specified below individual graphs to the left of the ME2-derived strains. Center lines indicate the median lesion length; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend to the minimum and maximum values; box width is proportional to the square root of the sample size; and data points (n = 26–42) are plotted as open circles. Treatments with the same lowercase letter are not significantly different at p < 0.05.

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cells were collected through centrifugation and resuspended in Murashige and Skoog medium containing 100 μM acetosyringone, pH 5.8. The cell suspension was adjusted to an OD_{600} of 0.2 for infiltration. For co-inoculation, cells of two Agrobacterium strains were mixed in equal volume before infiltration into N. benthamiana leaves.

Sequencing and annotation of the Xa7 region
Genomic DNA of IRBB7 was extracted using the CTAB method (Porebski et al., 1997). Sequencing was conducted using long-read Oxford Nanopore and Illumina technologies. The assembler Flye (Kolmogorov et al., 1997) was used to create a de novo assembly of the IRBB7 genome. The resulting contigs were first corrected by re-mapping Nanopore reads and correcting with the medaka tool. A second correction was performed by mapping highly accurate Illumina reads using Bowtie 2 (Langmead and Salzberg 2012) and the Pilon correction tool (Walker et al., 2014). Gene structure annotation was based on spliced alignment of homologous proteins and transcripts using Genomethredder (Gremme et al., 2006).

Survey of the Xa7 locus from 3000 rice genomes
The complete 3000 rice genome project (3K RGP) database was downloaded from http://gigadb.org/dataset/2000001. The database contains ~11 Tb of raw paired-end Illumina reads from 3010 diverse cultivated rice (Oryza sativa L.) accessions. Reads were mapped against the 4-kb region spanning the Xa7 locus using Bowtie 2 (Langmead and Salzberg 2012) and the Pilon correction tool (Walker et al., 2014). Gene structure annotation was based on spliced alignment of homologous proteins and transcripts using Genomethredder (Gremme et al., 2006).

FUNDING
This work was partially supported by the United States Department of Agriculture National Institute of Agriculture and Food (2017-67013-26521 to B.Y.), the National Science Foundation (1238189 to F.F.W., V.P.B., and B.Y.; 1741090 to F.F.W.), and subawards to University of Missouri and University of Florida from the Heinrich Heine University Dusseldorf funded by the Bill & Melinda Gates Foundation [OPP1155704] (B.Y. and F.F.W.).

ACKNOWLEDGMENTS
We are grateful to the US National Small Grains Collection (NSGC) and the International Rice Research Institute for providing rice accessions, and to Drs. Xingu Mao and Lifeng Zhao for help with Xa7 mapping experiments. No conflict of interest declared.

Received: December 28, 2020
Revised: January 14, 2021
Accepted: January 15, 2021
Published: January 19, 2021

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Supplemental Information is available at Plant Communications online.
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