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Xa7, a new executor *R* gene that confers durable and broad-spectrum resistance to bacterial blight disease in rice

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ABSTRACT

Bacterial blight (BB) is a globally devastating rice disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). The use of disease resistance (*R*) genes in rice breeding is an effective and economical strategy for the control of this disease. Nevertheless, a majority of *R* genes lack durable resistance for long-term use under global warming conditions. Here, we report the isolation of a novel executor *R* gene, *Xa7*, that confers extremely durable, broad-spectrum, and heat-tolerant resistance to *Xoo*. The expression of *Xa7* was induced by incompatible *Xoo* strains that secreted the transcription activator-like effector (TALE) AvrXa7 or PthXo3, which recognized effector binding elements (EBEs) in the *Xa7* promoter. Furthermore, *Xa7* induction was faster and stronger under high temperatures. Overexpression of *Xa7* or co-transformation of *Xa7* with *avrXa7* triggered a hypersensitive response in plants. Constitutive expression of *Xa7* activated a defense response in the absence of *Xoo* but inhibited the growth of transgenic rice plants. In addition, analysis of over 3000 rice varieties showed that the *Xa7* locus was found primarily in the *indica* and *aus* subgroups. A variation consisting of an 11-bp insertion and a base substitution (G to T) was found in EBE_{AvrXa7} in the tested varieties, resulting in a loss of *Xa7* BB resistance. Through a decade of effort, we have identified an important BB resistance gene and characterized its distinctive interaction with *Xoo* strains; these findings will greatly facilitate research on the molecular mechanism of *Xa7*-mediated resistance and promote the use of this valuable gene in breeding.

Keywords: *Xa7*, executor *R* gene, durable resistance, TALE, bacterial blight, rice

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INTRODUCTION

Plants are frequently attacked by pathogenic microbes in the ecosystem (Chisholm et al., 2006; Boller and He, 2009). In response to pathogen invasion, plants have evolved mechanisms that rapidly recognize pathogen-associated molecular patterns (PAMPs) via receptors on the cell surface and activate PAMP-triggered immunity (PTI) to defend against pathogen attack (Jones and Dangl, 2006; Tang et al., 2017). In turn, pathogens have successfully deployed a mechanism to secrete so-called effectors, which can interfere with PTI in host cells, resulting in effector-triggered plant susceptibility (Jones and Dangl, 2006; Antony et al., 2010). Evidence from crop research has shown that pathogen effectors can target the so-called susceptibility (*S*) genes in host cells, and genetic variability in *S* genes may cause a recessive resistance to pathogens (Liu et al., 2009; White and Yang, 2009; Antony et al., 2010). Interestingly, plants have also co-evolved dominant resistance (*R*) genes that can specifically recognize pathogen effectors to activate effector-triggered immunity (Jones and Dangl, 2006; Zhang et al., 2020a, 2020b). Therefore, plants undergo endless attacks by pathogens and in

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turn evolve novel *R* genes for survival. More than 300 *R* genes have been cloned from various plants, but the molecular mechanisms underlying their functions are understood for only a small fraction of *R genes* (Kourelis and van der Hoorn, 2018).

Rice is an economically important staple food crop for an enormous number of people worldwide (Ainsworth, 2008). Rice production is always affected by various diseases in the field, among the most devastating of which is bacterial blight (BB), which is caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*; Mew, 1987). BB can affect rice growth, development, or reproduction, and it causes severe yield losses of up to 50% (Liu et al., 2014). Breeding rice varieties with genetic resistance is an effective, economical, and environmentally friendly strategy for the control of BB disease in rice production ([1]; Jiang et al., 2020). To date, at least 46 genes that confer dominant or recessive host resistance to *Xoo* have been identified (Chen et al., 2020). Among them, 15 genes (or alleles) have been cloned, including *Xa1* (Yoshimura et al., 1998) and its alleles *Xa2*, *Xa14*, *Xa31(t)*, and *Xa45(t)* (Ji et al., 2020; Zhang et al., 2020a, 2020b); *Xa3/Xa26* (Sun et al., 2004; Xiang et al., 2006); *Xa4* (Hu et al., 2017); *xa5* (Iyer and McCouch, 2004; Jiang et al., 2006); *Xa10* (Tian et al., 2014); *xa13* (Yang et al., 2006); *Xa21*(Song et al., 1995); *Xa23* (Wang et al., 2015); *xa25* (Liu et al., 2011); *Xa27* (Gu et al., 2005); and *xa41(t)* (Hutin et al., 2016). Based on the functions of their encoded proteins, these *R* genes or *S* genes (dominant alleles of the recessive resistance genes) can be classified into four categories: nucleotide-binding leucine-rich repeat receptor genes (*Xa21*, *Xa3/Xa26*, and *Xa4*), sugars will eventually be exported transporter (SWEET) genes (*Xa13/OsSWEET11*, *Xa25/OsSWEET13*, and *Xa41/OsSWEET14*), executor *R* genes (*Xa10*, *Xa23*, and *Xa27*), and others (*Xa1* and *Xa5*). Interestingly, eight of the above genes (*Xa1*, *Xa5*, *Xa10*, *Xa13, Xa23, Xa25, Xa27*, and *Xa41*) have been shown to interact with a type of pathogen effector called transcription activator-like effectors (TALEs) during the defense response of host cells (Jiang et al., 2020).

TALEs, the virulent or avirulent proteins secreted by *Xoo*, are translocated into host cells by the bacterial Type III secretion system (Bogdanove et al., 2010). TALE protein sequences are highly conserved, with the exception of the repetitive central region that consists of 33- to 35-amino-acid repeats. In the host cell, TALEs are located in the nucleus where they bind to specific DNA sequences called effector binding elements (EBEs) in order to regulate the expression of host genes. The recognition between TALE and EBE is determined by the repeat variable di-residues (RVDs) at the 12th and 13th positions of each repeat in the central TALE region (Boch et al., 2009; ([1])Moscou and Bogdanove, 2009). Binding of pathogen TALEs to EBEs transcriptionally activates target genes in the host cell, resulting in susceptibility or resistance of the host plant (Bogdanove et al., 2010; Bogdanove and Voytas 2011). For instance, *Xoo* TALEs can directly target corresponding EBEs in the promoters of the *OsSWEET* genes *Xa13/OsSWEET11* (Antony et al., 2010), *Xa25/OsSWEET13* (Zhou et al., 2015), and *Xa41/OsSWEET14* (Hutin et al., 2016), upregulating their expression in rice and causing BB disease. The SWEET proteins are cellular sugar transporters, and induction of *OsSWEET* genes may provide sufficient sugars for the nutrition of the pathogen (Chen, 2014; Bezrutczyk et al., 2017). Therefore, natural variations or CRISPR-Cas9-mediated genome editing in EBE regions of the *OsSWEET* genes abolished cognate TALE binding and produced recessive resistance to *Xoo* (Yang et al., 2006; Liu et al., 2011; Hutin et al., 2016; Oliva et al., 2019; Xu et al., 2019). Alternatively, the expression of the rice executor *R* genes *Xa10*, *Xa23*, and *Xa27* can be transcriptionally activated by the TALEs AvrXa10, AvrXa23, and AvrXa27, respectively, triggering a hypersensitive response (HR) to restrict *Xoo* growth in rice, resulting in dominant resistance to BB disease (Gu et al., 2005; Tian et al., 2014; Wang et al., 2015). However, all the known executor *R* genes encode small proteins that lack conserved domains or display little homology with one another (Wang et al., 2014). The biological functions and molecular mechanisms of the executor *R* genes are still unclear.

Xa7 is a dominant *R* gene that provides broad-spectrum and extremely durable resistance to *Xoo* (Vera Cruz et al., 2000; ([1]) White et al., 2009; Zhang et al., 2015). Over the past decades, *Xa7* has been the subject of ongoing research since its original identification from the Bangladeshi rice cultivar DV85 in the 1970s (Sidhu et al., 1978). Field tests over continuous years confirmed that the BB resistance of *Xa7* was more durable than that of *Xa4* and *Xa10* (Vera Cruz et al., 2000). Furthermore, evidence from different studies has shown that *Xa7* is more effective against BB at high temperatures, a characteristic that differentiates it from most other *R* genes (Webb et al., 2010; Cohen et al., 2017; Dossa et al., 2020). Likewise, the TALE AvrXa7, encoded by the avirulent gene *avrXa7*, triggers the resistance of *Xa7* in rice and is found in various *Xoo* strains (Hopkins et al., 1992; Yang et al., 2000). AvrXa7 has also been shown to be an important virulence factor for *Xoo* because isolated strains that lost the ability to induce *Xa7*-associated resistance were weakly virulent, presumably due to mutations in AvrXa7 (Vera-Cruzetal., 2000). In addition, AvrXa7 can induce the expression of *Xa41/OsSWEET14* by binding to a specific EBE in its promoter (Antony et al., 2010; ([1])). Because the BB resistance conferred by *Xa7* is particularly persistent and tolerant of high temperature, and because its cognate TALE, AvrXa7, plays double roles as both an avirulent and virulent factor in *Xoo* strains, the cloning and functional identification of *Xa7* may provide insight into a novel molecular mechanism of plant–pathogen interaction.

In 1995, *Xa7* was initially located at 107.5 cM on the current Rice Genome Research Project map (Kaji and Ogawa, 1995). It was later finely mapped to a 2.7-cM region by Porter et al. (2003) using the BB-resistant rice variety IRBB7, and it was ultimately mapped to a 118.5-kb region on chromosome 6 by Chen et al. (2008) based on the Nipponbare reference genome. However, the molecular nature of *Xa7* remains to be revealed. We began work on *Xa7* in 2005 and finely mapped it to a 200-kb physical region in 2009 using the Chinese BB-resistant rice variety Zhen-hui 084, which was bred from DV85 (Zhang et al., 2009). After another 11 years of effort, we now report the molecular cloning and characterization of the mysterious *Xa7* gene. Our results show that *Xa7* is a new executor *R* gene that encodes a novel protein significantly different from the known executor R proteins XA10, XA27, and XA23, and it has no significant homolog in any other plants. We identified a putative EBE targeted by AvrXa7 in the *Xa7* promoter and documented *Xa7* expression patterns in response to various *Xoo* strains and

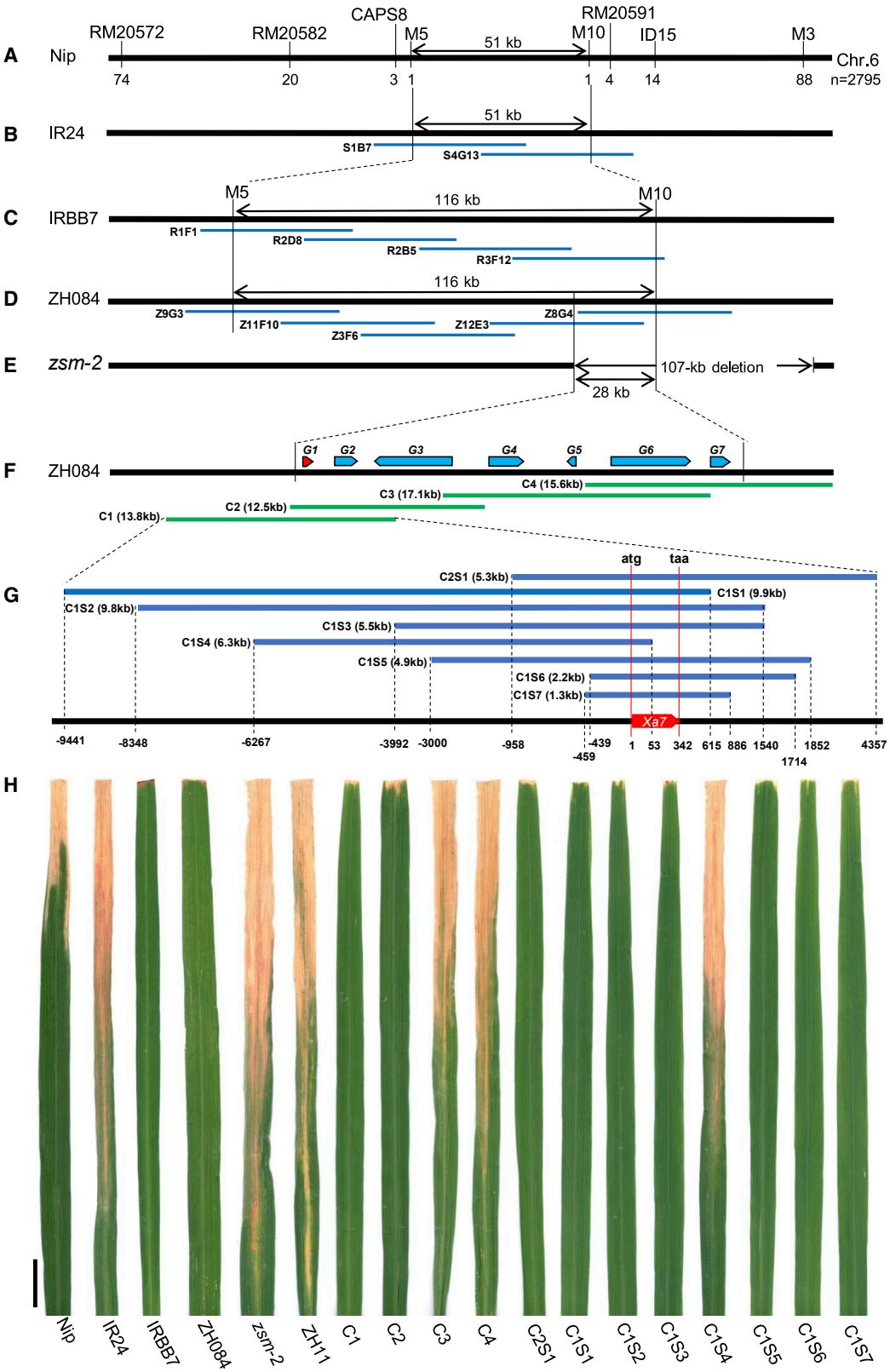

Figure 1. Map-based cloning of *Xa7*.

(A) Physical map of *Xa7* on rice chromosome 6 based on the reference genome of Nipponbare (Nip); n refers to the total number of BB-susceptible F_2 plants used for fine mapping; the number under each marker represents the number of recombinants detected by the corresponding marker.

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at different temperatures. These results help to explain why *Xa7* confers a broad-spectrum and durable BB resistance in rice and provide a solid foundation for future investigations of its molecular mechanisms during host defense.

RESULTS

Fine mapping of *Xa7*

To narrow down the *Xa7* mapping region, an F_2 population with 11 285 individual plants was derived from a cross between Zhen-hui 084 (which contains *Xa7*) and the BB-susceptible variety Cheng-hui 448. The BB-resistant or -susceptible phenotypes of the F_2 plants were observed after inoculation with the *Xoo* strain PXO86 (which contains *avrXa7*) (Supplemental Figure 1). Statistical results showed that 8490 plants were highly resistant, and 2795 plants were highly susceptible, with a significant segregation ratio of 3:1 (χ^2 = 0.326, $P < 0.05$; Supplemental Figure 1). All the susceptible F_2 plants were used for genetic analysis by DNA markers, and *Xa7* was placed in a 51-kb interval flanked by the markers M5 and M10 on the Nipponbare rice genome (https://rapdb.dna.affrc.go.jp; Figure 1A). To develop new polymorphism markers for fine mapping, PCR primers inside the 51-kb region were designed based on Nipponbare, and DNA fragments were successfully amplified from Cheng-hui 448 and other varieties, but not from *Xa7*-containing varieties such as Zhen-hui 084, IRBB7, and DV85 (data not shown). This result indicated that the sequences in the *Xa7*-mapping region might be specific for *Xa7*-containing varieties.

To identify the *Xa7* sequence, a Zhen-hui 084 genomic library was constructed using a fosmid strategy and screened by PCR using markers M5 and M10. The positive clones were subjected to paired-end sequencing using the fosmid vector's primers to obtain the insertion sequences, which were in turn used to develop PCR primers to screen new overlapping clones. Finally, five clones (Z9G3, Z11F10, Z3F6, Z12E3, and Z8G4) were screened out, constituting a contig that covered the whole *Xa7* mapping region (Figure 1D), and the assembled sequences were obtained using the Illumina HiSeq 2500 platform. Surprisingly, the physical distance from M5 to M10 was 116 kb in Zhen-hui 084 (Supplemental Data 2), very different from the 51 kb distance in Nipponbare (Supplemental Figure 2). To confirm this result, genomic libraries of IRBB7 and its near-isogenic line IR24, a BB-susceptible variety, were constructed and screened. Among the positive clones sequenced, two (S1B7 and S4G13) from IR24 covered the mapped 51-kb region in IR24 (Figure 1B), and four (R1F1, R2D8, R2B5, and R3F12) from IRBB7 covered the 116-kb region in IRBB7 (Figure 1C). Remarkably, the 116-kb sequence of IRBB7 was identical to that of Zhen-hui 084 (Supplemental Figure 2), which was completely different from that of IR24 (Supplemental Figure 2). By contrast, the sequence between markers M5 and M10 in IR24 was almost identical to that in Nipponbare (Supplemental Figure 2).

Bioinformatic analysis of the 116-kb sequence was performed using online databases, but it was not helpful in identifying the *Xa7* candidate gene. Therefore, we began to screen BB-susceptible mutants in our Zhen-hui 084 radiation-mutagenesis library in 2015. More than 20 000 M_1 lines were phenotyped by inoculation with the *Xoo* strain PXO86, and nine highly susceptible mutant lines were identified. All mutant lines were assayed by PCR using primers located in the 116-kb region to find any possible variation. Fortunately, we found one mutant line, *zsm-2* (Zhen-hui 084 susceptible mutant 2), in which the expected DNA fragments could not be amplified by several primers consecutively distributed in the 116-kb region (data not shown). A 107-kb deletion on chromosome 6 was then detected in the *zsm-2* mutant by high-throughput sequencing (Supplemental Figure 3) and further verified by PCR amplification and sequencing. We compared the 107-kb fragment with the 116-kb region and found a 28-kb sequence overlap (Figure 1E) in which *Xa7* may reside.

Functional complementation of *Xa7* by genetic transformation

Seven putative genes were predicted in the 28-kb sequence (Figure 1F). However, none were homologous or related to known *R* genes. Therefore, four overlapping fragments (designated C1 to C4) that contained different putative genes were used for a transgenic complementation test (Figure 1F; Supplemental Data 2). The fragments were amplified from fosmid clone Z12E3 or Z8G4 by high-fidelity proofreading PCR and then cloned separately into the pCAMBIA1300 vector. The correct constructs were transformed into Zhong-hua 11, a *japonica* rice variety that is highly susceptible to the *Xoo* strain PXO86. Among 261 hygromycin phosphotransferase-resistant plants in the T_0 generation, 15 T_0 plants were highly resistant to PXO86 (Supplemental Table 1). All the resistant plants were derived from transformation with the C1 or C2 constructs, whereas all the T_0 plants that contained C3 or C4 constructs were highly susceptible to PXO86 (Supplemental Table 1; Figure 1F and 1H). The T_1 resistant plants from line C1 were then inoculated with 10 *Xoo* strains from the Philippines that represented different types of *Xoo* physiological races identified

(B–D) Physical maps of *Xa7* based on the rice varieties **(B)** IR24, **(C)** IRBB7, and **(D)** Zhen-hui 084 (ZH084). Maps were built using contigs of clones isolated from the genomic libraries of the corresponding varieties.
(E) The 28-kb overlapping region between the 116-kb *Xa7* mapping region and the 107-kb deletion in a BB-susceptible mutant, *zsm-2*, mutagenized from ZH084.
(F) Predicted genes (*G1* to *G7*) in the 28-kb overlapping region and relative positions and sizes of the four fragments (C1 to C4) used for complementation transformation in Zhonghua 11 (ZH11).
(G) Contigs and sizes of subclones (C2S1, C1S1 to C1S7) used for the transgenic complementation test in ZH11. Numbers under the line represent positions relative to the start codon (atg) of the *Xa7* gene. Negative numbers refer to positions before atg, and positive numbers refer to positions starting at atg. taa refers to the stop codon of *Xa7*, and the sequence from atg to taa is 342 bp in length.
(H) Leaves inoculated with *Xoo* strain PXO86. Photographs were taken 2 weeks after inoculation. Nip, IR24, and ZH11 are BB-susceptible rice varieties. IRBB7 and ZH084 are *Xa7*-containing rice varieties. *zsm-2* refers to a BB-susceptible mutant mutagenized from ZH084. C1 to C4, C1S1 to C1S7, and C2S1 are the transgenic complementation lines of ZH11. Bar, 2 cm.

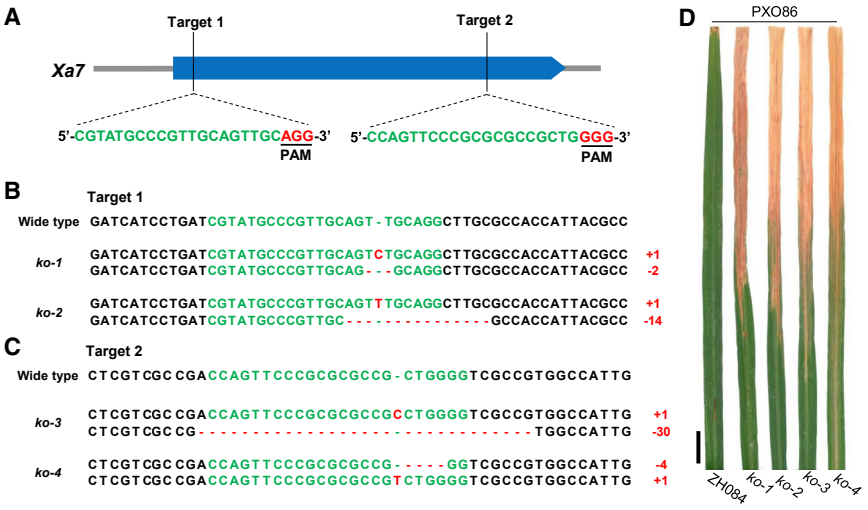

Figure 2. CRISPR-Cas9-mediated knockout of *Xa7*.

(A) The CRISPR-Cas9 editing sites of targets 1 and 2 in *Xa7*. The blue box represents the exon of *Xa7*. The sgRNA sequences of targets 1 and 2 are shown in green, and the protospacer adjacent motif (PAM) is shown in red.

(B and C) Sequences of targets 1 **(B)** and 2 **(C)** in the CRISPR-Cas9 transgenic lines of rice variety Zhen-hui 084 (ZH084). Wild type refers to the sequence of ZH084. *ko-1* to *ko-4* refer to the different types of knockout mutants. The sgRNA sequence regions are shown in green, and mutations are shown in red. Numbers in red are the base-mutation changes in the single chromosome, and base deletions and insertions are indicated with minus and plus, respectively.

(D) Leaves of the *ko1* to *ko4* mutants of *Xa7* and the wild-type control ZH084 inoculated with *Xoo* strain PXO86. Photographs were taken 2 weeks after inoculation. Bar, 2 cm.

by the International Rice Research Institute and have been widely used to evaluate BB-resistance genes. The results showed that the resistance spectrum of transgenic line C1 was identical to that of Zhen-hui 084 (Supplemental Figure 4).

To isolate the *Xa7* sequence, eight constructs containing different-sized fragments amplified from the C1 clone (Figure 1H; Supplemental Data 2) were constructed and used to transform Zhong-hua 11. In total, 68 of 465 T_0 transgenic plants showed resistance to PXO86 (Supplemental Table 2). All the BB-resistant plants were derived from transformation with constructs that contained the predicted gene *G1*, with the exception of construct C1S4, which had partially lost the *G1* sequence (Figure 1G and 1H). In addition, CRISPR-Cas9-mediated gene editing was used to knock out *G1* in Zhen-hui 084. To increase the efficiency of gene editing, two sgRNAs targeting different sites in the *G1* coding sequence (CDS) were designed and cloned into CRISPR-Cas9 vectors for the transformation of Zhen-hui 084 (Figure 2A). In total, 49 T_0 transgenic plants were characterized by PCR-based sequencing of *G1* and inoculation with PXO86. Twelve *G1*-knockout plants with different editing types were highly susceptible to PXO86 (Figure 2B–2D; Supplemental Tables 3 and 4). By contrast, the other 41 transgenic plants in which *G1* was not edited or only one allele was edited maintained the BB resistance of wild-type Zhen-hui 084 (Supplemental Table 3 and 4). All these results indicated that *G1* was the BB-resistant gene *Xa7*.

Xa7 is an executor *R* gene whose TALE-induced transcription increases at high temperature

PCR amplification and high-throughput sequencing showed that the open reading frame (ORF) of *Xa7* and its promoter (>3000 bp) were completely identical in IRBB7 and Zhen-hui 084 but did not exist in PXO86-susceptible varieties Nipponbare, IR24, and Zhong-hua 11. The *Xa7* gene has only one exon, with a 342-bp ORF that encodes a 113-aa unknown protein (Supplemental Figure 5). Coincidentally, the amino acid number of the deduced XA7 protein is equal to that of executor R proteins XA23 (Wang et al., 2015) and XA27 (Gu et al., 2005), and XA7 contains putative transmembrane domains similar to those of XA10, XA23, and XA27 (Zhang et al., 2015). Nevertheless, XA7 shares few sequence similarities with known executor R proteins (Figure 3A) and has no homologs in current plant databases. These results suggest that *Xa7* may encode a novel executor R protein.

Executor *R* genes are reported to be transcriptionally induced by TALEs that recognize and bind to EBEs in *R gene* promoters (Zhang et al., 2015). To search for a possible EBE in the *Xa7* promoter, the 2000-bp sequence before the start codon was scanned using TALE-NT with a 3.0 cutoff (Doyle et al., 2012). A 26-bp putative EBE (Figure 3B) for AvrXa7 was detected from −135 to −110 bp with a high TALE-NT score (Table 1) (Figure 3D). Smaller scores indicate greater binding capacities of EBEs to TALEs, and the EBE in the *Xa7* promoter (score: 19.65) was therefore predicted to have a greater binding capacity than that in the *OsSWEET14* promoter (score: 26.72) identified by Antony et al. (2010; Table 1). In addition, based on the principle of recognition between TALEs and EBEs (Boch et al., 2009), the 25.5 RVDs of AvrXa7 could be perfectly manually aligned with the 26-bp EBE in the *Xa7* promoter, and this alignment was better than that of the *OsSWEET14* promoter (Figure 3B). To distinguish between these two EBEs, we designated that in the *Xa7* promoter *Xa7-EBE*$_{AvrXA7}$ and that in the *OsSWEET14* promoter *OsSWEET14-EBE*$_{AvrXA7}$. We also found an EBE in the *Xa7* promoter that may be recognized by the TALE PthXo3 (designated *Xa7-EBE*$_{PthXo3}$; Table 1; Figure 3C). Interestingly, the *Xa7-EBE*$_{AvrXA7\ and\ Xa7\text{-}EBEPthXo3}$ sequences overlap (Figure 3D), just like the EBEs in the *OsSWEET14* promoter (Antony et al., 2010).

To demonstrate that *Xa7* could be transcriptionally induced by AvrXa7 and PthXo3, the *Xoo* strains PXO86 (AvrXa7), PXO61 (PthXo3), and PXO99 (neither AvrXa7 nor PthXo3; Oliva et al., 2019) were used to infect the leaves of IRBB7, and *Xa7* expression was analyzed by qRT–PCR 24 h after inoculation. Compared with the control, *Xa7* was highly induced by PXO86 and PXO61 but not by PXO99 (Figure 3E). Because high temperature can increase the BB resistance conferred by *Xa7*

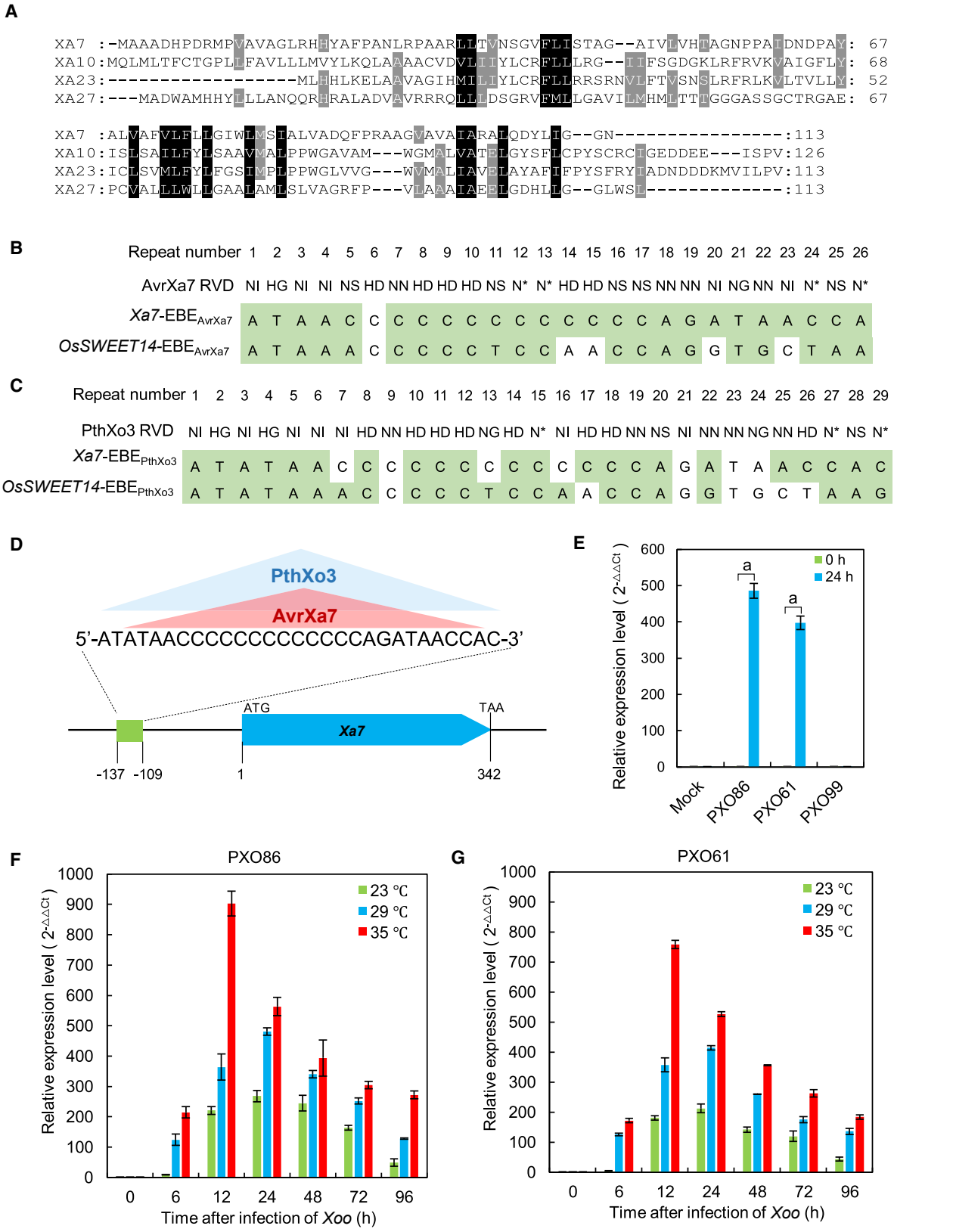

Figure 3. Sequence characteristics and induced expression of *Xa*.

(A) Deduced amino acid sequence alignment of XA7 with other executor R proteins, XA10, XA23, and XA27. Conserved residues are boxed in black or highlighted in gray based on the degree of conservation. Dashes indicate missing sequences or gaps generated by the alignment.

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Gene	TALE	[a] EBE	[b] Score
Xa7	AvrXa7	*Xa7-EBE*$_{AvrXa7}$	19.65
	PthXo3	*Xa7-EBE*$_{PthXo3}$	29.95
OsSWEET14	AvrXa7	*OsSWEET14-EBE*$_{AvrXa7}$	26.72
	PthXo3	*OsSWEET14-EBE*$_{PthXo3}$	31.87

Table 1. EBE prediction scores for *Xa7* and *OsSWEET14* genes by TALE-NT 2.0 (cutoff: 3.0).

[a]Effector binding element in the promoter of Xa7 or SWEET14.

[b]EBE prediction scores for Xa7 and OsSWEET14.

(Webb et al., 2010; Supplemental Figure 6), the *Xoo* induction of *Xa7* was compared under different temperatures (23°C, 29°C, and 35°C) by qRT–PCR. After inoculations with PXO86 and PXO61, *Xa7* was highly induced at 6 h under 29°C and 35°C, but its induction was delayed at 12 h under 23°C (Figure 3F and 3G). *Xoo-induced* expression of *Xa7* was therefore proportional to temperature and gradually increased from 23°C to 35°C (Figure 3F and 3G).

Xa7 activates plant defense response

The executor *R* genes *Xa10* and *Xa23* are reported to activate defense responses such as HR in plants other than rice (Tian et al., 2014; Wang et al., 2015). We transferred an *avrXa7 overexpression* construct into a *Xa7*-compatible strain, PXO99, which was designated PXO99^{avrXa7}. This strain and the control PXO99 were infiltrated into the leaves of IRBB7. Three days after injection, the leaves with PXO99^{avrXa7} displayed a strong HR and resistance phenotype, whereas those infiltrated with PXO99 did not (Figure 4A). Moreover, a vector overexpressing *Xa7* driven by the CaMV35S promoter was constructed and infiltrated into tobacco leaves by the *Agrobacterium*-mediated method. Vectors that overexpressed *Xa10* or *Xa23* were used as positive controls. The overexpression of *Xa7* triggered a strong HR, just like *Xa10* and *Xa23* (Figure 4B and 4C). A construct of *Xa7* driven by its native promoter (C1S3) also induced HR in tobacco leaves (Figure 4B and 4C), and co-infiltration of C1S3 with the *avrXa7*-expressing vector triggered a stronger HR (Figure 4B and 4C; Supplemental Figure 7).

The plants of some *Xa7* transgenic lines displayed abnormal phenotypes such as dwarfism, short leaves, and small panicles. We speculated that *Xa7* might be constitutively expressed and activate a defense reaction in these lines, resulting in the inhibition of plant growth or development. Three types of *Xa7-transgenic plants*, normal (C1S3-N), semi-dwarf (C1S3-SD), and dwarf (C1S3-D), were selected from the transgenic line C1S3 (Figure 4D) for *Xa7* expression assay by qRT–PCR. In the absence of *Xoo*, the expression levels of *Xa7* were 5-, 48-, and 142-fold higher in the C1S3-N, C1S3-SD, and C1S3-D plants than in the Zhen-hui 084 control (Figure 4E). The higher the expression level, the lower the plant height. Expression of the defense response marker genes *PR1a* and *PBZ1* ([1]Peng et al., 2008) was measured by qRT–PCR. Both were induced in the three plant types, and expression was particularly high in the C1S3-SD and C1S3-D plants (Figure 4F and 4G). These results indicated that the induction of *Xa7* could activate a defense response. Furthermore, the BB resistance of the three plant types was tested using the *Xa7*-incompatible strain PXO86 and the compatible strain PXO99. The IRBB7 and Zhen-hui 084 controls were resistant to PXO86 and susceptible to PXO99 (Figure 4H and 4I). As expected, all three types of *Xa7* transgenic plants were resistant to PXO86 (Figure 4H). However, they exhibited different responses to PXO99: the C1S3-N plants were highly susceptible, the C1S3-SD plants were moderately susceptible, and the C1S3-D plants were resistant (Figure 4I).

Genetic diversity of *Xa7* in rice germplasm

Xa7 was initially identified from DV85, an *indica* rice variety from Bangladesh (Sidhu et al., 1978). The *Xa7* sequence was used for the analysis of genetic diversity in a database of 3010 cultivated rice varieties (Wang et al., 2018), and 493 varieties were found to contain the *Xa7* CDS (Supplemental Data 2). These varieties were mainly from the *indica* and *aus* subgroups (Figure 5A), and their origins were geographically distributed in South Asia (India and Bangladesh), Southeast Asia (Laos, Vietnam, Thailand, Philippines, and Indonesia), and the south of China. These are the exact cultivation areas of the *indica* and *aus* subgroups (Figure 5B). Compared with the subgroup distribution of the 3010 varieties, the *Xa7* locus showed an obvious expansion in the *aus* subgroup (Figure 5A).

Sequence alignments showed that most of the 493 varieties had an *Xa7* CDS identical to that of Zhen-hui 084 (Supplemental Data 2). However, we were unable to determine whether the EBE sequences of the 493 varieties were consistent with that of *Xa7-EBE*$_{AvrXa7}$ in Zhen-hui 084, particularly at the site of polycytosine (C) bases (Supplemental Figure 5). There are 13 C bases in the *Xa7-EBE*$_{AvrXa7}$ sequence (Figure 3D), and they are difficult to sequence completely by high throughput methods and then assemble correctly. To confirm the genetic diversity of the *Xa7* gene in rice germplasm, 27 of the 493 varieties from

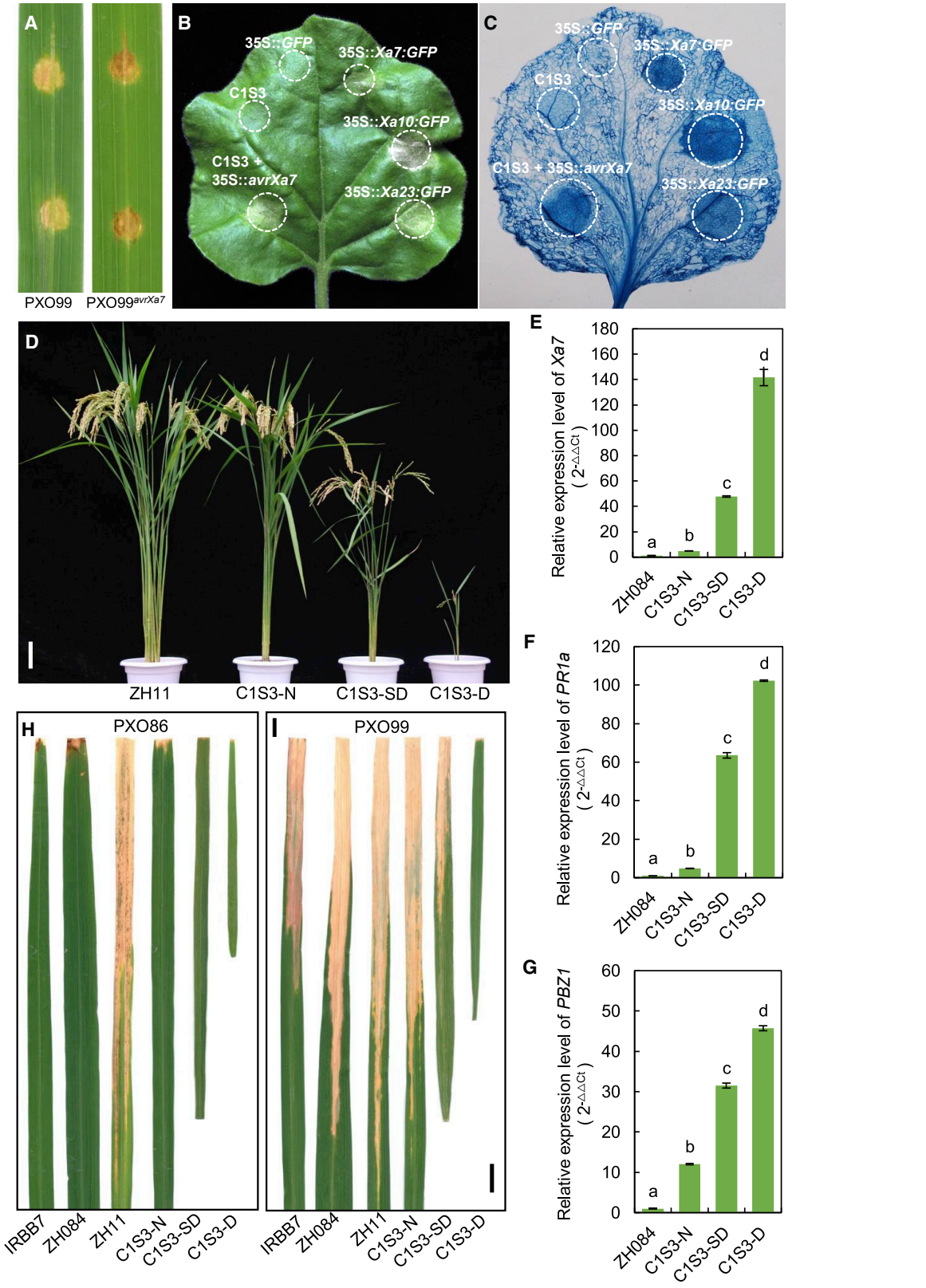
(B) Pairing recognition relationship between AvrXa7 and the putative *Xa7-EBE*$_{AvrXa7}$ compared with *OsSWEET14-EBE*$_{AvrXa7}$.

(C) Pairing recognition relationship between PthXo3 and the putative *Xa7-EBE*$_{PthXo3}$ compared with *OsSWEET14-EBE*$_{PthXo3}$. Repeat number refers to the specific repeat in the central region of the AvrXa7 or PthXo3 protein. RVD represents the repeat variable di-residues at the 12th and 13th positions of each repeat in the central region of AvrXa7 or PthXo3. Perfect recognition between the bases of EBE and the RVD of the TALE is highlighted in green.

(D) Overlapping EBEs corresponding to AvrXa7 and PthXo3 in the promoter of *Xa7*. The blue box represents the *Xa7* gene from ATG to TAA. The green box represents the EBEs. Numbers indicate the sites of EBEs. Red and blue triangles show the EBEs recognized by AvrXa7 and PthXo3, respectively.

(E) Expression of *Xa7* in rice variety IRBB7 in response to different *Xoo* strains: PXO86 (AvrXa7), PXO61 (PthXo3), PXO99 (neither AvrXa7 nor PthXo3), and mock inoculation (H_2O). Leaves were sampled at 0 and 24 h after inoculation. Data are presented as the mean of three independent replicates ± SD. Statistical analysis was performed by *t*-test, and significant differences ($P < 0.01$) between 0 and 24 h inoculation with each strain or the mock control are indicated by "a" on the bars.

(F and G) Expression of *Xa7* in IRBB7 in response to strains PXO86 **(F)** and PXO61 **(G)** under different temperatures (23°C, 29°C, and 35°C). Leaves were sampled at 0, 6, 12, 24, 48, 72, and 96 h after inoculation. Gene expression levels were measured by qRT–PCR, and data are presented as the means of three independent replicates ± SD.



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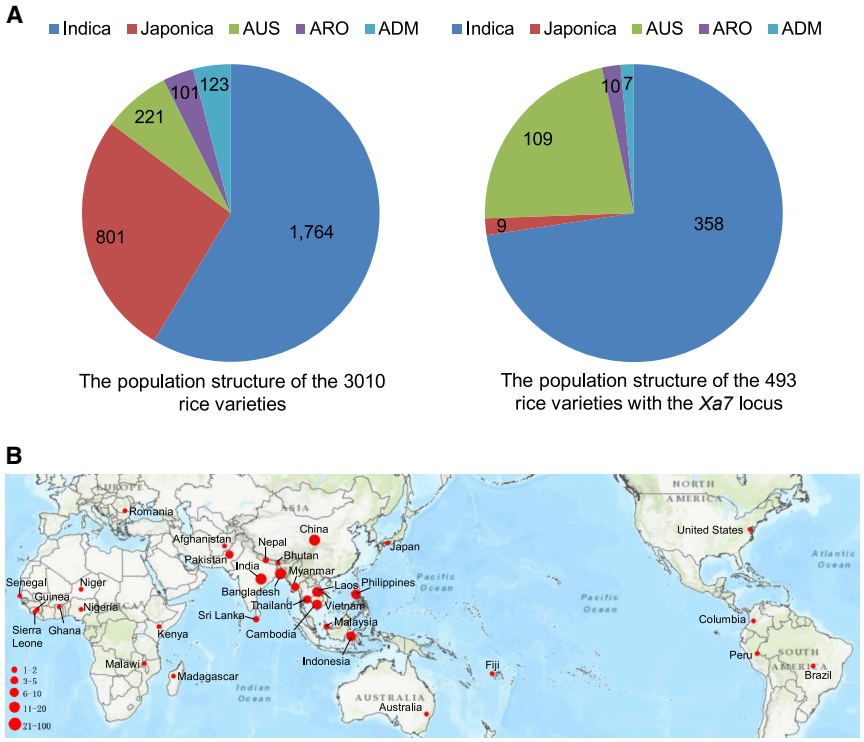

Figure 5. Geographic distribution and genetic variation of the *Xa7* locus.

(A) Comparison of the population structure of 3010 rice accessions and 493 rice varieties that contain the *Xa7* locus. Indica, Japonica, AUS, ARO, and ADM are different subgroups of rice. Numbers refer to the number of varieties in each subgroup.

(B) Geographic distribution of the *Xa7* locus among the 3010 cultivated rice accessions. The size of the red dots represents the number of varieties distributed in the indicated countries.

(C) Comparison of EBEs in the *Xa7* locus based on the sequencing results of the 12 varieties (shown in Supplemental Figure 8). Type I (*Xa7*) belongs to the PXO86-resistant varieties, and Type II (*xa7*) belongs to the PXO86-susceptible varieties.

our lab were used for identification (Table 2). PCR primers were designed based on the sequences of Zhen-hui 084, and amplification was performed using genomic DNA from the 27 varieties as templates. *Xa7 CDS* sequences were amplified from all 27 varieties (Table 2), and all the amplified sequences were identical to that of Zhen-hui 084 (data not shown). However, the promoter sequences of *Xa7* could be amplified from only 12 varieties, and two types of sequence diversity were found in the EBE region (Supplemental Figure 8). The Type I sequences from five varieties (IRBB7, DV85, AUS 242, AUS 299, and AUS 308) were identical to that of Zhen-hui 084, and the Type II sequences from the other seven varieties had an 11-bp (5′-CCCCCCC GCCC-3′) insertion and a G-to-T base substitution (Figure 5C; Supplemental Figure 8). This insertion directly broke the EBE sequence and may result in nonrecognition of AvrXa7. The promoter sequences of the remaining 15 varieties were unknown, as they could not be amplified; there may be other types of EBE in their promoters. The 27 varieties were then inoculated with PXO86 to test their BB resistance. Consistent with the PCR results, the Type I varieties were highly resistant, whereas the Type II varieties and the remaining 15 varieties were highly susceptible (Table 2). These results indicated that the EBE_{AvrXa7} in the *Xa7* promoter was essential for the BB resistance in rice.

DISCUSSION

Map-based cloning remains a classical and effective method for the mapping and isolation of important genes from crops (Song et al., 1995; Tian et al., 2019; Wang et al., 2020). Nevertheless,

Figure 4. Phenotypes of hypersensitive response (HR) and defense response triggered by *Xa7*.

(A) IRBB7 in response to inoculation with different *Xoo* strains. HR resistant (dark brown inoculation spots) or susceptible (pale inoculation spots and spreading) symptoms were scored 3 days after inoculation. PXO99 is an *Xa7*-compatible strain used as a control, and $PXO99^{avrXa7}$ is the modified strain transformed with the *avrXa7* gene in PXO99.

(B) HR assays in tobacco leaves. *Agrobacterium tumefaciens* strains harboring different constructs were injected individually or co-infiltrated with *avrXa7*-overexpressing *A. tumefaciens* into leaves using a needle-less syringe. 35S::*Xa7:GFP*, 35S::*Xa10:GFP*, and 35S::*Xa23:GFP* refer to constructs that overexpressed *Xa7*, *Xa10*, and *Xa23* fused with *eGFP*, respectively, and 35S::*GFP* refers to the negative control. C1S3 refers to the C1S3 construct that contained *Xa7* with its native promoter. The infiltrated areas are shown by dashed circles. The picture was taken at 4 days after infiltration.

(C) Cell death histochemical staining assay. The corresponding leaf from **(B)** was stained with trypan blue and cleared in ethanol to visualize cell death. The infiltrated areas are shown in dashed circles.

(D) Phenotypes of transgenic plants transformed with the construct C1S3. Zhong-hua 11 (ZH11) is the wild-type rice variety selected as the receptor for genetic transformation. C1S3-N, C1S3-SD, and C1S3-D refer to normal, semi-dwarf, or dwarf plants, respectively, from the C1S3 transgenic lines. Bar, 10 cm.

(E–G) Expression of *Xa7* **(E)**, *PR1a* **(F),** and *PBZ1* **(G)** analyzed by qRT–PCR in the transgenic plants in the absence of *Xoo*. Zheng-hui 084 (ZH084) was used as the wild-type control. Data are presented as the means of three independent replicates ± SD and were statistically analyzed by one-way ANOVA. Bars with different letters are significantly different from each other ($P < 0.01$).

(H and I) Leaves of the transgenic plants and the wild-type control inoculated with *Xoo* strain PXO86 **(H)** and PXO99 **(I)**. Photographs were taken 2 weeks after inoculation. Bar, 2 cm.

[a] Accession ID	Variety name	Origin	[b] Group	[c] EBE	[d] Lesion length	[e] Phenotype
IRGC 117725	DV85	Bangladesh	AUS	Type I	0.4 ± 0.154	R
IRIS_313-11049	AUS 219	Bangladesh	AUS	–	30.4 ± 2.08	S
IRIS_313-11051	AUS 242	Bangladesh	AUS	Type I	0.3 ± 0.06	R
IRIS_313-11055	AUS 299	Bangladesh	AUS	Type I	0.4 ± 0.08	R
IRIS_313-11057	AUS 308	Bangladesh	AUS	Type I	0.4 ± 0.07	R
B120	HONG WAN 1	China	IND	–	27.8 ± 3.15	S
B207	AI HE CHI	China	IND	–	24.8 ± 2.33	S
B246	LAO ZAO GU	China	IND	Type II	29.2 ± 2.50	S
IRIS_313-9555	CHUA DAU	China	IND	–	32.2 ± 1.96	S
IRIS_313-10875	ARC 12021	India	AUS	–	30.3 ± 1.85	S
IRIS_313-11175	SONA AUS	India	AUS	–	31.1 ± 1.97	S
IRIS_313-11374	W 398	India	AUS	–	31.8 ± 2.65	S
IRIS_313-11454	BARI SUTAR	India	AUS	Type II	33.2 ± 5.78	S
IRIS_313-11456	KOLAMBA	India	AUS	–	27.2 ± 3.00	S
IRIS_313-9137	ARC 10100	India	AUS	Type II	37.1 ± 2.37	S
IRIS_313-9610	DANGAR	India	AUS	Type II	26.6 ± 1.99	S
IRIS_313-11809	MHARAKA	Kenya	AUS	Type II	34.4 ± 2.22	S
IRIS_313-11071	CHAO HAI	Laos	IND	–	30.4 ± 2.47	S
IRIS_313-12139	MANSARA DHAN	Nepal	AUS	Type II	31.3 ± 0.92	S
IRIS_313-11020	BAMLA SUFFAID 320	Pakistan	AUS	–	37.2 ± 1.25	S
IRIS_313-11028	MOTIA	Pakistan	AUS	Type II	28.0 ± 3.67	S
IRGC 135948	IRBB7	Philippines	IND	Type I	0.6 ± 0.287	R
IRIS_313-7809	WAS 174-B-3-5	Senegal	IND	–	28.6 ± 2.50	S
IRIS_313-11510	A 69-1	Sri Lanka	IND	–	29.7 ± 2.46	S
IRIS_313-10928	KHAO SIM	Thailand	IND	–	28.0 ± 1.54	S
IRIS_313-8073	DAWN CI 9534	United States	AUS	–	29.1 ± 1.05	S
IRIS_313-8341	BAT DO	Vietnam	IND	–	21.2 ± 4.70	S

Table 2. Information on 27 rice varieties that contain the *Xa7* CDS.

[a]Accession ID of variety in the 3K Rice database or IRGC.

[b]SNP-based subgroups of varieties.

[c]EBE types in the *Xa7* promoter. Type I is identical to Zhen-hui 084, Type II is a variation shown in Figure 6, and '–' indicates that the type is unknown.

[d]Lesion length (mean ± SD) of leaves measured at 2 weeks after inoculation with *Xoo* strain PXO86. More than 15 individual leaves were measured for each variety.

[e]Resistant (R) or susceptible (S) phenotype of the variety against PXO86.

we encountered great difficulties in map-based cloning of the BB resistance gene *Xa7* in rice. It took us many years to overcome these challenges and finally isolate the gene. We had successfully fine mapped *Xa7* to a 51-kb interval using available DNA markers (Figure 1A). However, no products could be amplified using primers located in the 51-kb region based on the rice reference genome, indicating that there must be a large structural variation in this region of *Xa7*-containing varieties such as IRBB7 and Zhen-hui84. In that situation, map-based cloning of a target gene could not continue until the sequence had been determined. By constructing and sequencing BAC libraries, we identified a 116-kb sequence from Zhen-hui84 and IRBB7 that corresponded to the 51-kb region in IR24 (Figure 1D). Moreover, there was almost no homology between the 116-kb and 51-kb sequences (Supplemental Figure 2). Therefore, it was likely that chromosome recombination of the *Xa7* mapping region was inhibited in our F_2 population, thereby blocking the fine mapping of *Xa7*. Because no candidate genes were predicted in the interval, we subsequently turned to screening for *Xa7* mutants in a Zhen-hui84 radiation-mutagenesis library. After 5 years of effort, we screened out one BB-susceptible mutant from 20 000 lines; it contained a 107-kb deletion overlapping the *Xa7* mapping region (Supplemental Figure 3). Combining the regions identified by fine mapping and the 107-kb deletion, we finally placed the target gene in a 28-kb candidate region and identified *Xa7* through the analysis of a large number of transgenic plants. Our experiences in this study showed that if difficulties are encountered in the map-based cloning of a gene of interest, other approaches such as mutant screening can be carried out simultaneously.

Just like *Xa23* (Wang et al., 2015) and *Xa27* (Gu et al., 2005), *Xa7* encodes an executor R protein that confers broad-spectrum

resistance to *Xoo* strains. However, *Xa7* has some unique characteristics, including extremely durable and heat-tolerant resistance (Vera Cruz et al., 2000; Webb et al., 2010). First, the durable resistance of *Xa7* may be partly explained by the distinctive functions of its cognate avirulence gene *avrXa7* in *Xoo*. The cognate avirulence genes of the *R* genes *Xa23* and *Xa27* are *avrXa23* and *avrXa27*, respectively. The AvrXa23 and AvrXa27 proteins are not major virulent TALEs in *Xoo* and function only as avirulence factors whose mutation or loss of function does not affect *Xoo* survival (Oliva et al., 2019). Under the pressure of long-term, continuous use of a single *R* gene in crop breeding and production, pathogens accumulating gene mutations may overcome this *R* gene in host plants (Quibod et al., 2019). However, *avrXa7* plays dual roles in *Xoo*, not only encoding an avirulent TALE that induces the *R* gene expression of host plants, but also acting as a virulence gene that maintains the toxicity of *Xoo* for survival. Thus, an *Xoo* strain that contains *avrXa7* is subject to defense by the cognate *R* gene in plants, but the loss of *avrXa7* causes a risk of being eliminated in nature (Vera Cruz et al., 2000). This places the *Xoo* strain in a dilemma but enables *Xa7* to become a durable *R* gene in plants. Our results suggested that the expression of *Xa7* could be induced by a *Xoo* strain that contained *avrxa7* (Figure 3E and 3F), thereby activating a defense response (like HR) to inhibit *Xoo* (Figure 4). *Xa7 induction appeared to result from the binding of AvrXa7 to EBE$_{AvrXa7}$ in the Xa7 promoter* (Figure 3B). We provided a preliminary demonstration of an interaction between *Xa7* and AvrXa7 and shed light on the durable resistance of *Xa7*.

Second, *Xa7* provides broad-spectrum resistance to BB, suggesting that *avrXa7* is generally widespread in *Xoo* strains. We searched for *avrXa7* in the genomes of hundreds of *Xoo* strains using Blast but found that it was not widely present in *Xoo* (data not shown). It has been reported that some *Xoo* strains such as PXO61 that lack a credible *avrXa7* gene are still incompatible with *Xa7* (Oliva et al., 2019). Naturally, a question arises: how does *Xa7* provide broad-spectrum resistance to *Xoo*? In fact, PXO61 contains the virulent gene *pthXo3*, which encodes a major TALE, PthXo3 (Yang and White, 2004), and is highly homologous to *avrXa7* in genomic DNA (data not shown). We speculated that *avrXa7* and *pthXo3* may have originated from the same ancestor. Interestingly, a sequence containing two overlapping EBEs in the *OsSWEET14* promoter could be recognized by both AvrXa7 and PthXo3 (Antony et al., 2010). We also found that two putative EBEs for AvrXa7 and PthXo3 overlapped in the *Xa7* promoter (Figure 3D), and *Xa7* expression was significantly induced by both PXO86 (*avrXa7*) and PXO61 (*pthXo3*) with similar expression patterns (Figure 3F and 3G). This means that *pthXo3* may be another cognate avirulent gene for *Xa7* in host plants. Therefore, two major TALEs of *Xoo*, AvrXa7 and PthXo3, target *Xa7* in host plants, enabling this *R* gene to confer broad-spectrum disease resistance. More evidence should certainly be collected to confirm this speculation.

Third, global warming is affecting the survival of human beings and plants. Studies have confirmed that heat stress reduces the effectiveness of plant resistance against pathogens (De Jong et al., 2002; Zhu et al., 2010). At present, the underlying mechanisms by which *R* genes lose effectiveness in disease resistance at high temperatures are poorly understood. Fascinatingly, *Xa7* acts in the completely opposite manner, enhancing the BB resistance of rice to a greater extent at higher temperatures (Cohen et al., 2017; Dossa et al., 2020; Webb et al., 2010; Supplemental Figure 6). Here, we showed that *Xa7* induction by *Xoo* was faster and stronger at higher temperatures (Figure 3F and 3G), which may promptly activate and enhance the defense response to inhibit *Xoo* in rice. This trait is very valuable for rice breeding in the face of global warming.

The isolation of *Xa7* provides a unique model with which to investigate the co-evolution between host plants and pathogens. Although the molecular mechanism of *Xa7*-mediated defense response remains unclear, our results provide valuable clues for uncovering it in the future and will greatly facilitate the use of this gene in breeding. This study also sheds light on breeding varieties with broad-spectrum and durable disease resistance, for example by mimicking the recognition of specific TALEs (like AvrXa7) by *R* genes or by pyramiding different EBEs of the major TALEs into the promoter of a single *R* gene. These strategies may provide an effective means of breeding durable disease-resistant varieties to control BB disease.

METHODS

Plant materials and growth conditions

The rice (*Oryza sativa* L.) varieties Zhen-hui 084 and IRBB7 both contain *Xa7* with broad-spectrum resistance to *Xoo*, and the varieties Nipponbare, IR24, Cheng-hui 448, and Zhong-hua 11 are highly susceptible to *Xoo*. For fine mapping of the *Xa7* gene, Cheng-hui 448 was selected as the pollen accepter and crossed with Zhen-hui 084; the heterozygous F_1 plants were self-crossed to develop the F_2 population. Rice plants were grown in the field. For temperature treatment, rice seedlings at the tillering stage were transferred into a growth chamber (14 h light/10 h dark) with different temperatures. Tobacco (*Nicotiana benthamiana*) plants were cultured in a growth chamber at 25°C (16 h light/8 h dark).

Xoo strains and inoculation

The *Xoo* strains used in this study included ten races from the Philippines. Strains were cultured on agar medium that contained 20 g sucrose, 5 g peptone, 0.5 g $Ca(NO_3)_2$, 0.43 g Na_2HPO_4, and 0.05 g $FeSO_4$ per liter and were allowed to grow at 28°C for 2–3 days. The bacterial colony was suspended in sterile distilled water at an optical density of $OD_{600} = 1.0$ and immediately used for plant inoculation. The leaf-tip clipping method (Kauffman et al., 1973) was used for *Xoo* inoculation. From the inoculated leaves, 1 cm of leaf tissue below the cut edge was collected for gene expression analysis. Lesions on the inoculated leaves were measured for the evaluation of BB resistance 2 weeks after inoculation (Yin et al., 2000). Based on the genomic sequence of PXO86 (NCBI: CP031463.1), the coding sequence of AvrXa7 was cloned by PCR amplification and inserted into the pHM1 vector by homologous recombination. The constructed vector was transformed into strain PXO99 by electroporation. The resulting PXO99^{AvrXa7} strain was injected into tobacco or rice leaves through a needle-less syringe, and photos were taken 3 days after injection.

Genomic library construction and sequencing

Seeds of rice were germinated and grown in the dark, and the etiolated seedlings were sent to the Takara Biomedical Technology (Beijing) Company in China for the construction of genomic fosmid libraries. The library from each variety provided greater than 10-fold coverage of the rice genome, and the inserted chromosome fragments of each clone were 35 kb on average. Positive clones were screened by normal PCR from

the constructed libraries and sequenced on the Illumina HiSeq 2500 platform. The clean reads were assembled using SPAdes 3.5.0.

Complementary vector construction and rice transformation

Complementary fragments C1 to C4, C1S1 to C1S7, and C2S1 (Supplemental Data 2) were amplified from the plasmid of fosmid clones by proofreading PCR using a PrimeSTAR HS DNA Polymerase Kit (TaKaRa) and cloned into the pCAMBIA1300 vector using an In-Fusion HD Cloning Kit (TaKaRa) according to the manufacturer's instructions. CRISPR-Cas9 technology was used to knock out the *Xa7* gene. Two gRNAs targeting the CDS of *Xa7* were designed by the CRISPR Design program (http://crispr.mit.edu). The sequences of Target-1 and Target-2 were 5′-CGTATGCCCGTTGCAGTTGCAGG-3′ and 5′-CCAGTTCCCGCGCGCCGCTGGGGG-3′, respectively. The underlined bases represent the protospacer adjacent motif sequences. The pCAMBIA1300-pYAO-cas9 vector was used to make the two CRISPR/Cas9 constructs. All of the vectors were transformed into rice by the *Agrobacterium*-mediated method described previously (Nishimura et al., 2006).

RNA extraction and qRT–PCR analysis

Total RNA was extracted from leaves using the TRIzol reagent (Life Technologies) and purified using an RNeasy mini kit (QIAGEN) and RNase-Free DNase Set (QIAGEN) following the manufacturer's instructions. Synthesis of first-strand cDNAs from RNA was performed using the M-MLV Reverse Transcriptase kit (Promega) according to the manufacturer's instructions. Fast SYBR Green Master Mix reagent (Applied Biosystems) was used for the real-time PCR experiment. The thermal cycle was performed on a StepOne Real-Time PCR system (Applied Biosystems) with the following program: 95°C for 20 s, followed by 40 cycles of 95°C for 3 s and 60°C for 30 s. A rice housekeeping gene, *Actin*, was used as the standardization control, and the relative expression level of each gene was analyzed by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Standard errors were calculated based on a minimum of three biological replicates. Primers are listed in Supplemental Table 5.

HR analysis in tobacco leaves

CDSs of *Xa7*, *Xa10*, and *Xa23* were amplified and inserted into the pCAMBIA1300-GFP vector by homologous recombination. The CDS of *avrXa7* was amplified from *Xoo* strain PXO86 and inserted into the pCAMBIA1300 vector driven by the CaM35S promoter. All constructs were transformed into *Agrobacterium tumefaciens* strain EHA105. The bacteria were cultured in 5 ml of LB liquid medium with kanamycin (50 mg/ml) and grown at 28°C for 2 days. A single clone was picked to inoculate in 5 ml LB liquid medium with kanamycin (50 mg/ml) until the density reached OD_{600} = 0.8 and was then subcultured in 20 ml of LB liquid medium until the density reached OD_{600} = 0.6. Qualified bacterial cells were collected by centrifugation at 4°C for 10 min at 3000 rpm, resuspended in 5 ml buffer (10 mM MES, 10 nM $MgCl_2$, 200 μM AS, [pH 5.7]), and activated at 28°C for 4 h. Leaves of 4-week-old tobacco plants were used for infiltration as described by Kay et al. (2007). The infected leaves were photographed and then stained with trypan blue 2–3 days after infiltration (Wilson and Coffey, 1980).

Bioinformatic analysis

Gene prediction in the *Xa7* mapping region was performed with Fgenesh (http://www.softberry.com/). Sequence alignment was performed with ClustalX 2.0, and the output was colored using GENEDOC 2.1. TALE-NT 2.0 (https://tale-nt.cac.cornell.edu/) was used with a stringency cutoff of 3.0 to search for putative EBEs recognized by AvrXa7 and PthXo3 (Doyle et al., 2012). The RFGB database (http://www.rmbreeding.cn/Blast; Wang et al., 2018) was used to investigate the genetic evolution of *Xa7* in different rice varieties. The geographic distribution of rice varieties was visualized with ArcGIS (version 10.5) based on longitude and latitude of the varieties obtained from the MBKbase database (http://www.mbkbase.org/rice; Peng et al., 2020).

SUPPLEMENTAL INFORMATION

Supplemental information is available at *Plant Communications Online*.

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AUTHOR CONTRIBUTIONS

B.M. and Q.Q. designed and supervised the research. X.C., P.L., L.M., and X.H. performed the pivotal experiments. L.C., H.L., S.S., Z.J., X.Z., and Y.Z. contributed the preparatory work. Z.G. and D.Z. provided the rice varieties. X.C., P.L., and L.M. wrote the manuscript with input from all other co-authors.

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