Interfering TAL effectors of *Xanthomonas oryzae* neutralize *R*-gene-mediated plant disease resistance

Zhiyuan Ji\(^{1,2,*}\), Chonghui Ji\(^{2,*}\), Bo Liu\(^{2}\), Lifang Zou\(^{1}\), Gongyou Chen\(^{1}\) & Bing Yang\(^{2}\)

Plant pathogenic bacteria of the genus *Xanthomonas* possess transcription activator-like effectors (TALEs) that activate transcription of disease susceptibility genes in the host, inducing a state of disease. Here we report that some isolates of the rice pathogen *Xanthomonas oryzae* use truncated versions of TALEs (which we term interfering TALEs, or iTALEs) to overcome disease resistance. In comparison with typical TALEs, iTALEs lack a transcription activation domain but retain nuclear localization motifs and are expressed from genes that were previously considered pseudogenes. We show that the rice gene *Xa1*, encoding a nucleotide-binding leucine-rich repeat protein, confers resistance against *X. oryzae* isolates by recognizing multiple TALEs. However, the iTALEs present in many isolates interfere with the otherwise broad-spectrum resistance conferred by *Xa1*. Our findings illustrate how bacterial effectors that trigger disease resistance in the host can evolve to interfere with the resistance process and, thus, promote disease.
Plant diseases are largely a consequence of molecular interactions between pathogens and their host plants and, when battles are won by the pathogens, they can inflict significant yield loss in crop production. Pathogenic microbes and their host plants have followed a ‘zigzag’ course that has co-evolved new virulence strategies in pathogens and counteracting resistance mechanisms in hosts. Pathogenesis of many bacterial pathogens depends in part on the effector proteins translocated into host cells by a type-III secretion system. Plants use diverse resistance (R) genes to recognize the cognate bacterial type-III effectors in a gene-for-gene manner, resulting in cultivar/race-specific disease resistance that prevents a state of disease susceptibility in plants. Bacteria, in turn, diversify or inactivate the effector genes to evade the R gene recognition or evolve new effectors to suppress the resistance triggered by other distinct type-III effectors.

Transcription activator-like effectors (TALEs) represent the largest type-III effector family that are highly conserved at the nucleotide and amino acid level, and are distinguishable by the varying number of central repeats of 34 amino acids and composition of the variable 12th and 13th amino acids of each repeat (so-called repeat variable di-residue). TALEs also contain characteristic nuclear localization motifs and transcription activation domain at their carboxy termini (Fig. 1a, exemplified by PthXo1). The repeat number and composition determine the specificity of each TAL effector for its DNA recognition in the promoter of host target gene, a feature that has spawned the development of TALE-based biotechnologies including TALE nucleases for genome editing.

TALEs play an important role in the pathogenesis of some Xanthomonas bacteria, including X. oryzae pv. oryzae (Xoo) and X. oryzae pv. oryzicola (Xoc), two pathogens that cause leaf blight by colonizing the vascular tissue and causing leaf streak by infecting the mesophyll tissue, respectively, in rice. Bacterial TALEs target host genes of susceptibility (S gene) in a sequence-specific manner, resulting in enhanced bacterial growth and development of disease symptom. To counteract such virulence strategy, host plants diversify the TALE-binding elements in the promoters of S genes, resulting in recessive R genes. In addition, plants have also evolved so-called executor R genes to lure TALEs into triggering resistance similar to TALEs inducing host susceptibility. Finally, in one case, tomato uses the nucleotide-binding leucine-rich repeat (NLR)-type R gene Bs4 to activate resistance in response to AvrBs4 independent of gene activation.

Here we demonstrate that Xa1, an NLR-type R gene in rice, initiates resistance including the hypersensitive response cell death (HR) in response to all tested full-length TALEs, whereas such resistance is suppressed by two groups of TALE variants expressed from previously annotated pseudogenes in Xoo and Xoc.

Results

Deletion of the Tal3 cluster triggers disease resistance. PXO99, a representative strain of Xoo, is virulent to a large number of rice varieties and contains 9 gene clusters totalling 19 individual TALE genes (Fig. 1b), some of which are important pathogenesis factors in bacterial blight of rice. We generated a series of PXO99 mutant strains that are depleted of different and complete complements of TALE genes by sequentially deleting individual TALE gene clusters (Supplementary Fig. 1). Disease assays with those mutants on 36 rice varieties of different genetic backgrounds were performed to assess the pathogenesis role of each gene cluster. PXO99 is virulent to or compatible with 25 rice varieties, but avirulent to or incompatible with others due to some recessive (xa13) and dominant (Xa21, Xa27 and Xa23) R genes (Supplementary Table 1, column 1 and 2). In agreement with prior study, mutant PB with deletion of pthXo1-containing cluster lost the ability to cause disease in susceptible rice varieties. To our surprise, mutant PB with deletion of the cluster 3 (Tal3a/Tal3b) showed resistance in rice varieties IRBB1 and Kogyoku but not in other rice lines all susceptible to PXO99 (Fig. 1b and Supplementary Table 1).

Figure 1 | TALE and variants in PXO99. (a) Unique structure of TALEs typified by PthXo1. PthXo1 contains 23.5 nearly identical repeats of 34 amino acids in the central repeat region (CRR), with polymorphic repeat variable di-residue (RVDs; two residues, in red). Single letter is used for each amino acid and the asterisk (*) denotes the missing residue. The dots represent repeats not shown. The C terminus of PthXo1 contains three NLSs and the transcription activation domain (AD). (b) Nine clusters (enumerated boxes) of TALE genes (pentagons, not to scale) are located in the genome of PXO99. Previously annotated pseudogenes (truncated genes or genes with premature stop codons, in red) are 3a, 3b and 6b. pbXo1 corresponds to 2b of cluster 2. PXO99 mutants PB, PH and ΔTal3 are cluster 3 + 7 + 8, all clusters and cluster 3 deleted (denoted by the carets, ‘†’), respectively. (c) Three TALE variants deduced from the possible open reading frames (ORF) of three pseudogenes in PXO99. The asterisk (*) denotes either the insertion (for a frame-shift) or the change of C to T for a stop codon, and the caret (‘†’) indicates the base pair deletion, relative to pbXo1.

TALE variants in PXO99.

| TALE Variants | Number of Amino Acids | CRR Length | NLS AD | Notes |
|---------------|-----------------------|------------|--------|-------|
| Tal6b         | 33+48                 |            |        | G-insertion |
| Tal3a         | 102                   | CRR (17.5 repeats) | NLS AD |        |
| Tal3b         | 102                   | CRR (17.5 repeats) | NLS AD | -688 bp |

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PXO99 genome contains three TALE pseudogenes that have been annotated and previously reported. Tel6b has a...
1 bp insertion at the 97 bp position in the 5’-coding sequence. The nucleotide change may enable the gene to encode a new open-reading frame of 82 codons (Fig. 1c). Tal3a carries a premature stop codon due to a C to T change at the 3,013 bp position of the gene, probably encoding a protein with a C-terminal truncation of 103 amino acids, whereas Tal3b undergoes a large deletion (688 bp) at the 2,560 bp position relative to Tal3a and presumably encodes a product with 229 amino acids deleted and additional 10 amino acids due to a frame shift. Both genes contain two deletions (129 and 45 bp) within the 5’-regions (Fig. 1c and Supplementary Figs 1 and 2). Tal3a and Tal3b, if expressed, are predicted to contain identical amino-termini, distinct central repetitive and C-terminal domains; both effectors contain the nuclear localization motifs but lack the transcriptional activation domains (Fig. 1c and Supplementary Fig. 2). Indeed, reverse transcription–PCR (RT–PCR) on bacterial RNA revealed the expression of both pseudogenes (Supplementary Fig. 3).

iTALe Tal3a and Tal3b are virulence factors. A mutant of PXO99A was constructed with only the cluster 3, containing the two TALE pseudogenes, deleted (ΔTal3; Fig. 1b) to assess the role of the pseudogenes in pathogenesis with other 17 TALE genes intact. ΔTal3 triggered HR in IRBB1 but not in IR24 when injected directly into the leaf blade (Fig. 2a). Similarly, ΔTal3 was able to cause disease in IR24 but not in IRBB1, on the basis of lesion length when the bacteria were introduced at the leaf tip (Fig. 2b). Tal3a and Tal3b were cloned and introduced, with an added FLAG epitope, individually to ΔTal3. Each clone enabled ΔTal3 to cause disease in IRBB1 comparable to the parent strain PXO99A (Fig. 2a,b). Western blotting probed with the anti-FLAG antibody showed the presence of Tal3a and Tal3b in the complementing strains of ΔTal3 (Supplementary Fig. 4). The results indicate that the iTALEs Tal3a and Tal3b are not pseudogenes as previously annotated but instead are expressed and function as TALE variants in PXO99A for virulence by interfering with the host resistance in IRBB1. Both effector variants and their relatives are referred to hereinafter as interfering TALEs (iTALeS).

iTALeS interfere with Xa1-mediated resistance in rice. IRBB1 and IR24 are near-isogenic rice lines for the R gene Xa1 (ref. 19), which was identified as an NLR-type R gene from Kogyoku and IRBB1 with no cognate elicitor (or avirulence) gene identified yet. To test whether the resistance to ΔTal3 and the suppressive effect of Tal3a and Tal3b were, in fact, specific to Xa1 and not due to another gene in the IRBB1 background, the Xa1 locus was PCR amplified from IRBB1 and transferred into the rice cultivar Kitaake, which is susceptible to PXO99A and ΔTal3. As expected, Xa1 transgenic lines (n = 7), still susceptible to PXO99A, were resistant to ΔTal3 in terms of HR and lesion length, but became susceptible to ΔTal3 in the presence of either Tal3a or Tal3b (Fig. 2c,d). The results demonstrate that PXO99A gains virulence by deploying its iTALE genes Tal3a and Tal3b, to mask the otherwise resistance in Xa1-containing plants.

iTALeS need their unique structures to function. Tal3a was characterized in more detail, to determine the requirement of each domain for activity of the iTALEs in Xa1 context. Internal deletions of the central repeats resulted in three Tal3a variants that were expressed at a similar level in bacterial cells (Supplementary Fig. 5); all except one with 2.5 repeats retained the ability to suppress the resistance responses to ΔTal3 in PXO99A and Xa1 transgenic Kitaake (Fig. 3a,b). Similarly, Tal3a variants swapped with repeat domains from AvrXa7, AvrXa10 and PthXo1 were also able to suppress the resistance responses to ΔTal3 in IRBB1 (Supplementary Fig. 6). The results suggest the indispensability but not the repeat number and repeat variable.

Figure 2 | iTALE genes Tal3a and Tal3b interfere with Xa1 resistance. (a) Disease reactions of IRBB1 and IR24. Strains are indicated above the leaves. Brown colouration indicates HR and clearing spots indicate disease reaction. (b) Lesion lengths in IR24 and IRBB1 caused by Xoo strains as measured at 12 days post inoculation (DPI). (c) Lesion lengths in Xa1 transgenic lines (n = 4) at 12 DPI. (d) Xa1 transgenic line #17 showed HR (brown colouration) to ΔTal3 but disease reactions to ΔTal3 complementing strains and PXO99A. Photos were taken at 3 DPI. Error bars (s.d., n = 10, 3 replicates) with the same letter do not differ from each other at P < 0.05 (Tukey’s test).
di-residue composition of the repeat domain for suppressive activity of Tal3a. The N terminus unique in two internal deletions in Tal3a was also tested for its contribution to the suppression. The N terminus of PthXo1 was swapped with the Tal3a corresponding region, the resultant Tal3a variant containing the N-terminal region of PthXo1 and Tal3a repetitive and C-terminal regions lost the ability to suppress the resistance triggered by ΔTal3 in IRBB1 and Xa1 transgenic plants (Fig. 3c and Supplementary Fig. 7a). Similarly, swapping AvrXa7 N terminus into Tal3a also resulted in the loss of suppressive activity of Tal3a (Supplementary Fig. 7b). Likewise, Tal3a with the full-length C-terminal region of PthXo1 due to domain swapping lost its ability to suppress the resistance to ATa3 in IRBB1 and Xa1 transgenic plants (Fig. 3c and Supplementary Fig. 7a). In their truncated C-termini, Tal3a still retains two nuclear localization signals (NLSs) and Tal3b acquires an NLS because of frame shift at its 3′-end (Supplementary Fig. 2); the NLS motifs were functional in directing the green fluorescent protein (GFP)-tagged Tal3a and Tal3b, to the nuclei of rice protoplasts. NLS mutations in Tal3a and Tal3b resulted in fluorescent signals present in cytosols, whereas the addition of the SV40 T-antigen NLS to the Tal3a and Tal3b variants with mutated NLS lost their abilities to suppress the resistance triggered by ΔTal3 in IRBB1; the addition of the SV40 T-antigen NLS restored their activities (Fig. 4c). The results indicate that the unique N- and C-terminal structures of Tal3a and Tal3b are essential and their NLSs (although not necessarily unique) are also needed for the iTALEs to interfere with the disease resistance controlled by Xa1.

Xa1 activates resistance in response to full-length TALEs. In the initial disease assay with the TALE cluster deletion mutants, the resistance in IRBB1 appeared when the clustered Tal3a and Tal3b were deleted and retained till remaining TALE clusters were deleted. We surmised that Xa1 might recognize TALEs and confer resistance against the pathogen only in the absence of iTALE genes. To test the hypothesis, we introduced TALE genes (pthXo1, Tal4 and Tal9d) from PXO99A individually into PH, the TALE-free mutant of PXO99A (Fig. 1b). The resulting TALE-containing strains induced strong HR in Xa1 transgenic Kitaake (Supplementary Fig. 8). Similarly, Tal3a and Tal3b variants that contain the full-length C termini due to domain swapping with PthXo1 also triggered HR in Xa1 transgenic plants (Supplementary Figs 7a and 8) and IRBB1 (Supplementary Fig. 9). However, PthXo1 and AvrXa7 variants with the NLS mutated lost their abilities to trigger HR in IRBB1 and Xa1 transgenic plants, whereas addition of SV40 T-antigen NLS restored their activities, suggesting a nuclear site of action of XA1/TALEs (Supplementary Fig. 10).

Tal3a does not interfere with Xa1 expression. To determine whether Xa1, similar to Xa27 and other executor R genes, recognizes TALEs through its promoter-specific transcription activation and iTALE overcomes resistance by suppressing Xa1 induction, we made a construct expressing Xa1 coding sequence under the promoter of a rice ubiquitin gene (Os02g06640). The UbiXa1 transgenic Kitaake lines (n = 4) were completely resistant to ΔTal3 and the resistance was suppressed in the presence of Tal3a (Supplementary Fig. 11). The results indicate that the mode of action by iTALE is not through interference with Xa1 transcription activation. To characterize the molecular role of iTALE in suppression of Xa1 resistance in rice, three typical defense genes (peroxidase, PBZ and PR1) that are highly activated particularly during resistance response were assessed using the quantitative RT–PCR (qRT–PCR) approach. Xa1 was induced slightly by wounding and bacterial infection, in agreement with the previous study14. In a contrast, in the incompatible interaction (Xa1/ΔTal3), all three defense genes were highly activated relative to non-infection and compatible
The prevalence of iTALE genes in the majority of Xoo and Xoc populations is high. For example, Tal3a (referred to as type A) and Tal3b (type B) types of iTALE genes exist in three of four Xoo and all nine Xoc strains sequenced and well annotated to date (Supplementary Fig. 13)\(^{18,23–25}\). The known iTALE genes (\(n=18\)) are highly conserved at the nucleotide level (>99% identity) and, if expressed, encode effectors that have nearly identical N termini in both types and nearly identical C termini in each type. The predicted iTALEs contain distinct central repeat domains (Supplementary Fig. 14).

We further assessed the prevalence of the two types of iTALE genes among 36 Xoo strains using a PCR approach with type-specific primers. Seven Xa1-incompatible strains contain either no detectable iTALE gene (three strains including AXO1947) or only type-B iTALE genes (four strains). AXO1947 has been sequenced and contains no iTALE gene\(^{25}\), with which our PCR result from AXO1947 is in agreement. The remaining 29 Xa1-compatible strains indeed contain iTALE genes either of only type A (3 strains) or B (6 strains), or of both type A and B (19 strains; Supplementary Table 2). The four Xa1-incompatible strains, which include strain T7174 and contain only type-B iTALE genes, may either not express iTALE genes or express iTALE genes at a level not adequate to suppress Xa1-mediated resistance. To investigate this possibility, the iTALE gene Tal3a or Tal3b from PXO99\(^{A}\) or the T7174 iTALE gene Tal6 (type B) constructed under the lacZ gene promoter were introduced into T7174. Introduction of each plasmid-borne iTALE gene enabled T7174 to overcome the resistance in IRBB1 (Supplementary Fig. 15). The results appear to be in an agreement with our hypothesis. However, when transcripts of the type-B iTALE genes in bacterial strains that contain only type-B genes and are either Xa1 incompatible (four strains) or compatible (six strains) were quantified using a qRT–PCR approach, no obvious correlation between expression levels of type-B iTALE genes in bacterial cells grown in medium and the disease phenotypes was observed (Supplementary Fig. 16). Therefore, the inability of the endogenous type-B iTALE genes to suppress the Xa1 resistance by some Xoo strains needs further investigation in future.

We also cloned Tal3 (type A) and Tal6 (type B) from PX086 of Xoo, Tal11h (type B) and Tal12 (type A) from BX01, and Tal5e (type B) from RS105, two Xoc strains. All five iTALE genes, when transferred into A Tal3, were functional in suppression of Xa1-mediated resistance (that is, in IRBB1 and Xa1-transgenic Kitaake) (Fig. 5a and Supplementary Fig. 17). Furthermore, for Xoc pathogen, when Tal5e, the only iTALE gene in RS105, was inactivated, the mutant was incompatible with IRBB1, and transfer of Tal5e or any of the four iTALE genes from Xoo enabled Xa1 compatibility with the RS105 mutant (Fig. 5b,c and Supplementary Fig. 18). The results indicate that the type-A and type-B iTALE genes are evolutionarily conserved and functionally equivalent to contribute strain virulence by interfering with the Xa1 gene in Xoo strains.
localization and transcription activation domains of AvrBs4, suggesting cytoplasmic perception of AvrBs4 by Bs4 in tomato. Most recently, a locus, Xo1, mapped in a 1.09 Mbp region in chromosome 4 of the heirloom rice variety Carolina Gold Selection, was found to activate resistance in response to various X. oryzae TALEs and TALE PthXo1 mutant with truncation of its C-terminal transcription activation domain. Similar to Xa1, Xo1-mediated resistance is independent of the number of repeats (if > 3.5 repeats) and the composition of the 12th and 13th amino acid residues of each repeat. It would be interesting to see whether Xa1 and Xo1 are the same gene or form a Xa1 R gene family. On the other hand, AvrBs4 can also be recognized by an executor R gene, Bs4C and trigger resistance in pepper. The recognition requires a match between the promoter element of Bs4C and the central repeats of AvrBs4 for tight expression of Bs4C, entailing a functionality of the full-length AvrBs4 (ref. 30). In contrast, Xa1, an NLR-type R gene unrelated to Bs4, recognizes all tested TALEs and initiates resistance in rice; the resistance elicitation requires the functional nuclear localization motif of TALEs. Furthermore, truncated TALEs (that is, iTALEs) as loss-of-function mutants avoid triggering Xa1 resistance and are also as gain-of-function mutants able to suppress Xa1 resistance triggered by full-length TALEs, in a way analogous to the dominant, negative regulators in host innate immunity.

Rice, evolutionarily speaking, has appeared to ‘hit the jackpot’ in the acquisition of an R gene that recognizes all or most TAL effectors. From the pathogen’s stand point, exposure of multiple TALE targets to a cognate host R gene would be conundrum in that at least one TALE is critical for virulence in all strains. We show that X. oryzae pathogens have evolved a potent adaptation to counteract the Xa1-controlled disease resistance in rice triggered by the large number of TALE genes of the pathogens using the very same genetic components. Understanding how one or two iTALEs efficiently mask the host immunity derived from recognition of multiple targets may enable engineering of more effective R genes that, for example, are less sensitive to the suppressive iTALE genes. Xa1 and derived R genes may be an efficient genetic source to combat several other important crop diseases (for example, citrus canker and wheat blight) wherein the causative Xanthomonas agents possess TALE genes but not iTALE genes. In a broader light, our results suggest that the seemingly pseudogenes in a variety of bacterial genomes may warrant further examination.

Methods

Plant and bacterial materials. Seeds of all rice varieties were kindly provided by the International Rice Research Institute, the U.S. National Small Grains Collection and collaborators. All plants were grown in growth chambers with photoperiod of 12 h, temperature of 28 °C daytime and 26 °C at night. Escherichia coli strains were grown in Luria–Bertani medium supplemented with appropriate antibiotics at 37 °C. All Xoo and Xoc strains were grown at 28 °C in nutrient broth with agar (NA) (1% peptone, 0.5% yeast extract, 1% sucrose, and 1.5% agar), nutrient broth without agar (NB), NA without sucrose, NA with 10% sucrose or TSA (10 g l⁻¹ tryptone, 10 g l⁻¹ sucrose and 1 g l⁻¹ glutamic acid). Antibiotics were used at the following concentrations, if required: cephalaxin 10 µg ml⁻¹, kanamycin 25 µg ml⁻¹, ampicillin 100 µg ml⁻¹ and spectinomycin 100 µg ml⁻¹. Strains and plasmids used in this study are listed in Supplementary Table 3.

TALE gene cluster deletion. Suicide vector pKMS1 was used to generate PXO99ΔA gene cluster deletion mutants using a method as described. Nine clusters of TALE genes were sequentially deleted from PXO99ΔA as indicated in Supplementary Fig. 1.
As DNA sequences of TALE genes are nearly identical, unique sequences flanking individual TALE clusters were chosen for knockouts. Based on the PXO99b genomic sequence (NCBI accession CP00099b), two pairs of primers, TalF1/D1 and TalF2/D2 (Φ represents a TALE gene cluster), were used to amplify the upstream and downstream regions flanking the target TALE loci by using the PXO99b genomic DNA as the template (primer information is provided in Supplementary Table 4). The two PCR products for each cluster deletion were digested and cloned into the multiple cloning sites of pKM105 and confirmed by sequencing for accuracy. The first round of mutagenesis was carried out on PXO99b targeting the duplicated clusters Tal7 and Tal8. Plasmid was sequenced, to confirm the accuracy of deletions.

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To construct the NLS mutant of Tal3a, primers Tal3aMF1–Tal3aMR along with pZWtal3aF as template were used for the first round of PCR; the amplicon was used for the second round of PCR with primers Tal3aMF2–Tal3aMR. One mutation was incorporated into Tal3a in each round of PCR. The final PCR product was cloned back into pZWtal3aF by EcoBb and HindIII digestion, followed by ligation, resulting in pZWTal3aM. Primers Tal3aMF2–Tal3aMVR along pZWtal3aM as template were used to add the SV40 NLS coding sequence into Tal3aM through a PCR approach and subsequently cloning through EcoKll/HindIII digestion and ligation. Similarly, Tal3bM (NLS mutant) was constructed. pZWtal3bF was digested with HindIII and HincII and then ligated into pZWtal3bF, resulting in pZWtal3bSV. The resulting plasmids were sequenced for the accuracy of PCR-amplified regions. All pZW versions of plasmids were ligated into pH1M through HindIII digestion and ligation.

GFP-tagged Tal3a and Tal3b were constructed using PCR and standard cloning approaches. Primers GFFP1 and GFFBamR along with an enhanced GFP (eGFP) template were used to PCR amplify the GFP coding region. The PCR product cloned into pGEM-T vector through A/T cloning and sequenced for accuracy. The eGFP coding region was cut out with KpnI and BamHI. The digested eGFP fragment along with the HindIII-digested pH1M fragment was ligated with the CaMV 35S promoter and Nos terminator in pUC19 (digested with KpnI and HindIII), respectively.

Gene encoding the PfXo1 NLS mutation was constructed by swapping the whole 3′ region (813 bp) downstream of AatII recognition site in pZWphta1x01 with a gBlock synthesized from the Integrated DNA Technologies. The gBlock encoding the PfXo1 NLS mutations was used to replace the corresponding regions of pZWtal3aF at AatII and HindIII cleavage sites using Gibson cloning method. Similarly, gBlock encoding the NLS mutations and additional SV40 NLS was swapped into the corresponding region of pZWtal3aF in pZWphta1x01. The NLS mutation and addition of the SV40 NLS for avrXa7 in pZWavRXa7M123 (referred to as avrXa7M) and pZWavRXa7M40 (referred to as avrXa7SV), respectively, were described. The pZW versions of pfxtal3aF each were subcloned into pH1M at the HindIII restriction sites.

To clone iTALE genes Tal11h and Tal12f from BXOR1, primers BXOR1F and BXOR1R that are complementary to the flanking regions of both genes were used to amplify the respective fragments from the genomic DNA. The amplicons were cloned into pH1M (BamH I digested) directly through Gibson cloning.

DNA manipulation and plasmid construction. DNA manipulation and PCR were conducted according to standard protocols. Plasmids were introduced by electroporation into E. coli bacterial cells as described previously. Primers for PCR were synthesized by Invitrogen Biotechnology Co., Ltd (Shanghai, China) and Integrated DNA Technologies (Corvallis, IA, USA); PCR was performed with Ex-Taq (TakaRa Biotechnology, Dalian, China) and Phusion High-Fidelity DNA Polymerase (New England BioLabs, Ipswich, MA, USA).

Construction of genomic libraries for Tal3a, Tal3b and other iTALE genes was completed as following. Genomic DNA of PXO99b was digested with Clal and separated in 1% agarose gel. DNA fragments of ~4–6.5 kb were purified from the agarose gel and ligated into the Clal-digested pBlueScript SK− (Stratagene, La Jolla, CA, USA). The ligation reaction was transferred into E. coli DH5 αX cells. The library was screened for Tal3a and Tal3b using probe derived from the Spl fragment (repetitive region) of avrXa7. Candidate clones were sequenced for confirmation of Tal3a and Tal3b. To isolate the iTALE genes from PXO86 and RS105, genomic DNA was digested with BamHI and AatII, respectively, and the BamHI and AatII restriction sites were purified from the agarose gel. The DNA fragments were subcloned into BamHI-digested pBlueScript KS− and then transferred into DH5 αX cells for screening of positive clones of iTALE genes Tal3a and Tal3b of PXO86 and RS105 of Tal3a and Tal3b, respectively. To construct the FLAG epitope-tagged Tal3a and Tal3b, primers Tal3aMF–Tal3aHR and Tal3bMF–Tal3bHR were used to amplify the respective fragments of Tal3a and Tal3b, respectively. The purified PCR products were first digested with HincII and HindIII, and then along with BamHI–HindIII fragments of Tal3a and Tal3b, ligated into the backbone of pZWtal3aF (BamHI–HindIII digested), resulting in pZWtal3aF and pZWtal3bF, respectively. Both pZWtal3aF and pZWtal3bF were digested with HindIII and ligated into pH1M (HindIII digested) to generate pHZWtal3aF and pHZWtal3bF. PH1M is a plasmid replicable in Xanthomonas, whereas the pZW version derived from pBlueScript SK− is not replicable in Xanthomonas.

For construction of the internal control reporter delets, pZWtal3aF was first completely digested with AatII, then partially with MscI; fragments in a range of 200 to 1,800 bp were recovered and ligated back pZWtal3aF (digested with MscI–AatII). Clones with various sizes of repeat regions were selected and sequenced, to confirm the accuracy of deletions.

For domain swapping of Tal3a and Tal3b, primers Tal3aMF–Tal3aHR and Tal3bMF–Tal3bHR were used to amplify the respective fragments of Tal3a and Tal3b, respectively. The purified PCR products were first digested with HincII and HindIII, and then along with BamHI–HindIII fragments of Tal3a and Tal3b, ligated into the backbone of pZWtal3aF (BamHI–HindIII digested), resulting in pZWtal3aF and pZWtal3bF, respectively. Both pZWtal3aF and pZWtal3bF were digested with HindIII and ligated into pH1M (HindIII digested) to generate pHZWtal3aF and pHZWtal3bF. PH1M is a plasmid replicable in Xanthomonas, whereas the pZW version derived from pBlueScript SK− is not replicable in Xanthomonas.

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For domain swapping of avrXa7, avrXa10 and pfxtal3aF into Tal3a, the respective Spl coding sequence of each gene was cloned into the corresponding region of Tal3a, resulting in pZWavRXa7a, pZWavRXa10a and pZWpfxtal3aXo1a (see Supplementary Fig. 6a). The resulting plasmids were ligated into pH1M at the HindIII cleavage site.

Genotyping of Xoo strains for the presence of iTALE genes. Primers Tal3aMF1–Tal3aMR and Tal3bMF1–Tal3bMR were used along with the genomic DNA of individual strains for detection of the Tal3a- and Tal3b-type iTALE genes, respectively.
iScript cDNA synthesis kit (Bio-Rad). cDNA derived from 25 ng of total RNA was used for each real-time PCR, which was performed on Stratagene’s Mx4000 multiplex qPCR system using iQ SYBR Green Supermix (Bio-Rad). The gene-specific primer sequences are provided in Supplementary Table 4. The average threshold cycle (Ct) was used to determine the fold change of gene expression. As an internal control, rice actin gene was used. The 2^ΔΔCt method was used for relative quantification.

Rice transformation. For construction of Xa1, primers Xa1F1–Xa1R1 and Xa1F2–Xa1R2 were used to amplify two fragments from Xa1 locus in IRBB1. The two overlapping amplicons were joined and inserted into SacI site in pCAMBIA1300 using Gibson Assembly Master Mix (New England Biolabs). Xa1 with ubiquitin promoter was constructed with a synthetic DNA fragment (751 bp) of the 5′-and a PCR amplicon (4,664 bp) of the 3′-end of Xa1 under the rice ubiquitin 2 gene promoter in pCAMBIA1300. Both cDNA clone pUbiXa1 and genomic clone pC3000Xa1 were electroporated into Agrobacterium tumefaciens strain EHA105. Calli from immature embryos of Kitaake were initiated and transformed by using A. tumefaciens as described. Transgenic plants were genotype with primers (Xa1F3 and U) located at the 5′ of Xa1 and in the backbone of pCAMBIA1300, respectively.

Disease assays. Hypersensitive cell death response (HR) and virulence assays were conducted as described previously. Briefly, Xoo strains were grown in NB with antibiotics at 28°C. Bacterial cells were collected from culture by low-speed (4,000 r.p.m.) centrifugation, washed twice and suspended in sterile water. The suspensions were adjusted to an optical density of 0.5 at 600 nm and were used to infiltrate into leaves of seedlings (about 3 weeks old) with the needleless syringe to assess the strain ability to trigger HR in plants. The cells of the same concentration were also used to inoculate two fully expanded leaves of five to ten adult plants (about 2 months old) using the leaf-tip clipping method, to evaluate the strain ability to cause disease or trigger resistance in plants by measuring the lesion lengths. Similarly, inoculum of Xoc was infiltrated into rice leaves using the needleless syringe to measure the rice reactions (susceptible or resistant). The disease assays were performed at least twice. One-way analysis of variance statistical analyses were performed on all measurements. The Tukey’s honest significant difference test was used for post analysis of variance pair-wise tests for significance, set at 5% (P<0.05).

Data availability. The data that support the findings of this study are available within the paper and its Supplementary Information files.

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**Author contributions**
Z.J., C.J., B.L. and L.Z. performed the experiments. Z.J., G.C. and B.Y. conceived the experiments. Z.J., C.J. and B.Y. prepared the manuscript with input from all other co-authors.

**Additional information**

**Supplementary Information** accompanies this paper at http://www.nature.com/naturecommunications

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