LETTERS

R gene expression induced by a type-III effector triggers disease resistance in rice

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Disease resistance (*R*) genes in plants encode products that specifically recognise incompatible pathogens and trigger a cascade of events leading to disease resistance in the host plant[1]. *R*-gene specificity is dictated by both host *R* genes and cognate avirulence (*avr*) genes in pathogens[2,3]. However, the basis of gene-for-gene specificity is not well understood. Here, we report the cloning of the *R* gene *Xa27* from rice and the cognate *avr* gene *avrXa27* from *Xanthomonas oryzae* pv. *oryzae*. Resistant and susceptible alleles of *Xa27* encode identical proteins. However, expression of only the resistant allele occurs when a rice plant is challenged by bacteria harbouring a*vrXa27*, whose product is a nuclear localized type-III effector. Induction of *Xa27* occurs only in the immediate vicinity of infected tissue, whereas ectopic expression of *Xa27* resulted in resistance to otherwise compatible strains of the pathogen. Thus *Xa27* specificity towards incompatible pathogens involves the differential expression of the *R* gene in the presence of the AvrXa27 effector.

Many *R* genes corresponding to diverse pathogens have been isolated, and fall primarily into five classes[3]. The majority of *avr* genes identified from bacterial pathogens encode effectors, also known as Avr proteins, which are secreted by type-III secretion pathways and, in many cases, internalized in the host cells[4]. Bacterial blight, a disease affecting rice and caused by *X. oryzae* pv. *oryzae*, is a significant agronomic problem in many rice-growing regions and is an ideal model system for the study of the interaction between plants and their bacterial pathogens. Nearly thirty rice loci have been recognized for resistance to bacterial blight, and four have been cloned[5–8]. However, no bacterial *avr* gene corresponding to the cloned rice *R* gene has been identified.

The *Xa27* locus of rice confers resistance to diverse strains of *X. oryzae* pv. *oryzae*, including PXO99^{A} (ref. 9). We cloned *avrXa27*, an *avr* gene corresponding to *Xa27*, from PXO99^{A} by transferring the gene to compatible recipients. The gene with *Xa27*-dependent elicitor activity resided in a region of the PXO99^{A} genome containing at least five similar genes, which differed according to the number of direct repeats in the central repetitive region (Fig. 1a). The genes are bounded by sequences related to phage and transposon genes, indicating that the genes were subjected to horizontal transfer and gene amplification. *avrXa27* encodes a protein of 1,136 amino-acid residues and 16.5 thirty-four amino-acid direct repeats (Fig. 1b and Supplementary Fig. 1a). AvrXa27 is similar to members of the AvrBs3/PthA family of type-III effectors (not shown), and has a conserved carboxy-terminal region containing three nuclear localization signal (NLS) motifs and a transcription activation domain, which are found in all members of the family (Fig. 1b). *avrXa27* inhibited bacterial growth on rice plants containing *Xa27* when introduced into the compatible field strain AXO1947 (Fig. 1c).

AvrXa27 is distinct from other AvrBs3/PthA family members based on the repeat organization (Supplementary Fig. 1b). AvrBs3, AvrXa7 and AvrXa10 differ primarily in the number and arrangement of the repeats, which contribute to specificity, and require nuclear localization and the activation domain for function[10–13]. We replaced the repetitive region of AvrXa10 with that of AvrXa27, resulting in a fully active effector with *Xa27*-dependent activity and no AvrXa10 activity (not shown). Thus, the repetitive region of AvrXa27, at least in part, contributes to the specificity of the

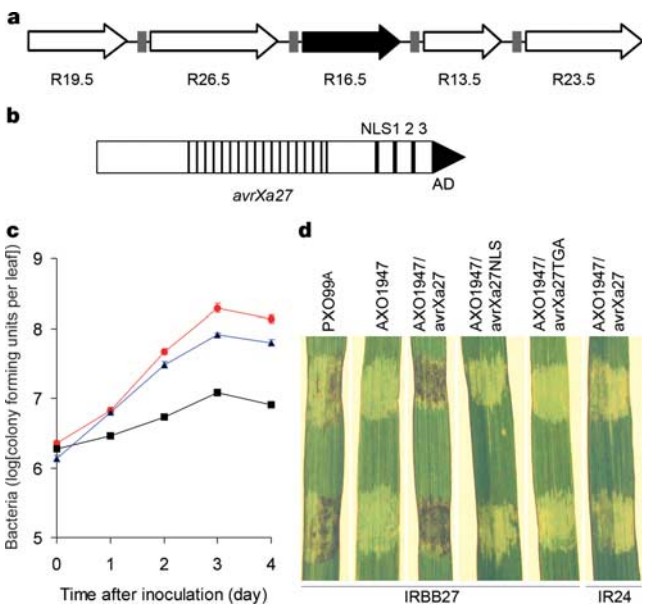

Figure 1 | AvrXa27 is an AvrBs3/PthA type-III effector. a, *avrXa27* (black arrow) resides among four additional homologues (R indicates the number of repeats). Grey blocks indicate sequences related to a phage gene (NP_536678). **b**, *avrXa27* contains 16.5 repeats (thin boxes), three NLS motifs and an activation domain. **c**, Growth of AXO1947 with (black squares) and without *avrXa27* (red circles) in IRBB27, and with *avrXa27* in IR24 (blue triangles). **d**, *avrXa27* requires the NLS and activation-domain features for *Xa27*-dependent resistance. Resistance is indicated by tissue browning at the inoculation site within 48–72 h.

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Xa27-dependent resistance. To determine the NLS and activation-domain requirements, the respective coding sequences of *avrXa27* were replaced with non-consensus or truncated sequences (Supplementary Fig. 1c). In the absence of the NLS motifs, *avrXa27* did not confer the ability to elicit *Xa27*-dependent resistance to bacterial blight (Fig. 1d). Similarly, removal of the activation domain of *avrXa27* also resulted in the loss of resistance-elicitor activity (Fig. 1d and Supplementary Fig. 1c). Thus, AvrXa27 is typical of this family of effectors that require NLS and activation-domain features and for which no *R* gene has been cloned[14].

Xa27 was isolated from rice variety IRBB27 by map-based cloning. Two of sixteen subclones from the *Xa27* region on chromosome 6, AA17.6 and NN9.9, resulted in plants with *Xa27*-dependent resistance (Supplementary Table 1 and Supplementary Fig. 2c). Further subcloning associated resistance with the 5.2-kilobase (kb) *Nsi*I/*Avr*II (NA5.2), 7.0-kb *Pst*I (PP7) and 3.2-kb *Nsi*I/*Apa*I (NA3.2) fragments, indicating that *Xa27* resided within a 2.4-kb *Pst*I–*Apa*I region (Fig. 2a and Supplementary Table 1). Challenge with bacteria resulted in resistance only if strains contained *avrXa27* (Fig. 2b). A complementary DNA was isolated from an IRBB27 cDNA library, and the 5′ sequence was obtained by a 5′RACE polymerase chain reaction (PCR). *Xa27* is an intronless gene and encodes a protein of 113 amino acids (Fig. 2d). Xa27 has no discernible sequence similarity to proteins from organisms other than rice (Supplementary Fig. 3). At least four paralogues were identified in cultivar Nipponbare, including one (BAD32948) that is allelic to *Xa27*. However, the allele in Nipponbare has diverged considerably (64% identity) from the gene of IRBB27. Structural analysis of Xa27 provided little or no clues as to the mode-of-action of the protein. A TMpred (http://www.ch.embnet.org/software/TMPRED_form.html) analysis predicted two α-helix domains in Xa27 (Fig. 2d). However, the significance of the predictions has not been tested.

The allele from IR24 was also characterized and shares a near-identical DNA sequence with *Xa27* from IRBB27 (Fig. 2c). Two striking differences occur in the presumed promoter. One difference is the presence of a 10-base-pair (bp) sequence at the −1.4-kb position in the susceptible allele (Fig. 2c). The second is the presence of a 25-bp duplication 18 bp upstream of the putative TATA box, also in the susceptible allele (Fig. 2c). The differences raised the possibility that the two alleles might differ in their expression. Indeed, hybridization analysis revealed that only the resistance allele was expressed,

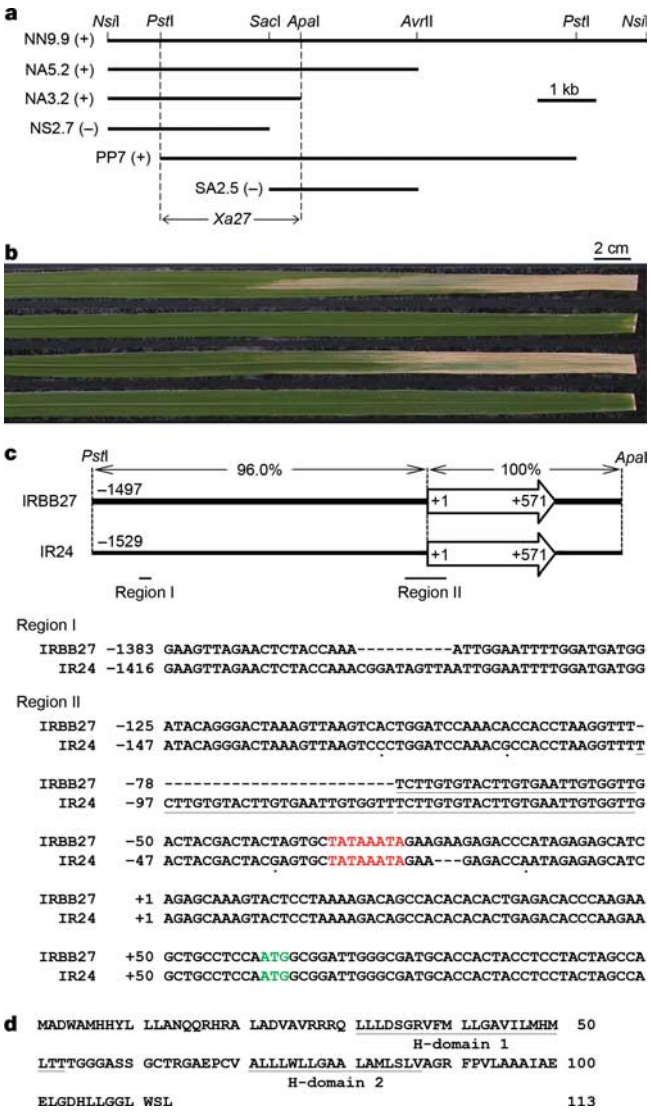

Figure 2 | **Identification of *Xa27*.** **a**, Clones from IRBB7 tested for *Xa27* activity. **b**, Transgenic plant TN8 conferred resistance to PXO99^{A} (second leaf from top) and PXO99^{A}ME1 (*avrXa27*) (bottom leaf), but not to PXO99^{A}ME1 (third leaf from top). Nipponbare parent was susceptible to PXO99^{A}ME1 with *avrXa27* (top leaf). **c**, Genomic organization and sequence alignment of the polymorphic promoter regions (regions I and II) of *Xa27* alleles. Open arrow, predicted mRNA. The 25-bp elements are underlined. TATA boxes (red) and start codons (green) are indicated. **d**, Predicted amino-acid sequence of Xa27 and α-helix domains (underlined).

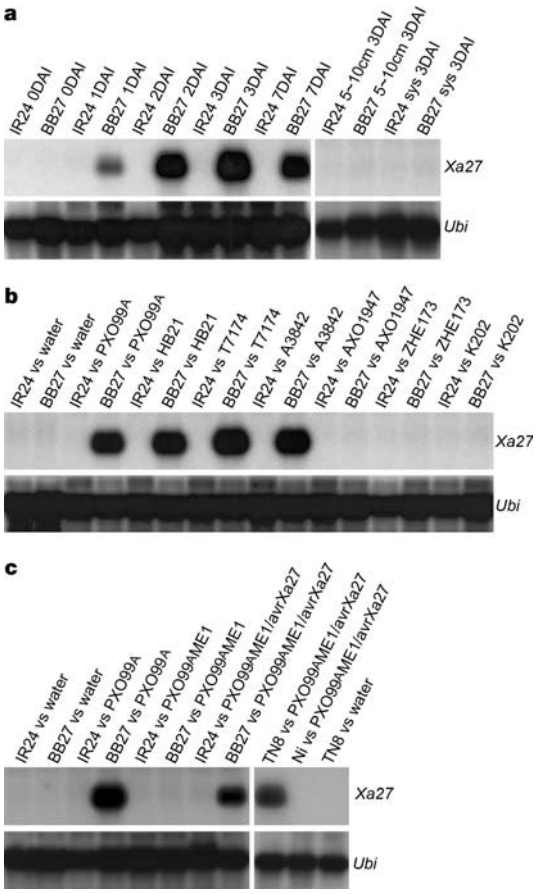

Figure 3 | ***Xa27* is induced specifically during challenge by bacteria containing *avrXa27*.** **a**, *Xa27* in IRBB27, but not in IR24, was induced after challenge with PXO99^{A}. Induction occurs in inoculated leaf tips, but not in tissue 5–10 cm below the infection sites or un-inoculated systemic leaves (sys). DAI, days after inoculation. **b**, *Xa27* was induced by incompatible strains (PXO99^{A}, HB21, T7174 and A3842), but not by compatible strains (AXO1947, ZHE173 and K202). **c**, *Xa27* was induced in IRBB27 or TN8 by the strains harbouring *avrXa27*, but not by the *avrXa27* mutant PXO99^{A}ME1.

and expression of *Xa27* was only detected upon inoculation with the incompatible *X. oryzae* pv. *oryzae* strains, reaching the highest level three days after inoculation (Fig. 3a and b). *Xa27* was not induced by challenge with the mutant PXO99^{A}ME1 strain, but the ability to induce *Xa27* was restored by the re-introduction of *avrXa27* (Fig. 3c). No expression of *Xa27* was detected in leaf tissue immediately below the inoculation site or from untreated leaves, indicating that induction is not systemic and is limited to the vicinity of infected tissues (Fig. 3a). No expression was detected in the susceptible plants or in *Xa27* plants challenged with compatible strains of the pathogen (Fig. 3a and b).

Expression of *Xa27* in IRBB27 rice appears to be tightly controlled as might be expected for a potentially lethal effect upon expression. However, several *Xa27* transgenic lines, including TT41, showed resistance to compatible as well as incompatible strains, indicating that these lines might have increased expression levels of *Xa27*, possibly due to the location of the T-DNA insert (Supplementary Table 2). TT41 plants, although normal in outward appearance, showed thickened vascular elements in the absence of bacterial challenge, which are typical of plants such as IRBB27 after challenge with incompatible bacteria (Fig. 4a). Unlike IRBB27, *Xa27* expression was detected in unchallenged TT41 plants (Fig. 4b). The phenotype of TT41 indicated that the expression of *Xa27* is a critical feature for the resistance reaction. To further examine the consequence of ectopic expression, the *Xa27* coding sequence was placed under the control of the rice *PR1* promoter, which is not activated specifically by AvrXa27. However, *PR1* is induced in a variety of conditions including infection by compatible and incompatible bacteria (unpublished data). Six independent P_{PR1}*Xa27* transgenic lines were examined for *Xa27* expression and resistance with similar results. The plants expressed *Xa27* in a pattern similar to the native *PR1* (Fig. 4c) and were resistant to both incompatible and compatible strains of the pathogen (Fig. 4d, Supplementary Table 2).

R genes are often expressed constitutively in uninfected plants. In a few cases, *R* gene expression is elevated upon infection[8,15,16]. *Xa1*, for example, is a race-specific resistance gene in rice to *X. oryzae* pv. *oryzae* and induced by bacterial inoculation. However, expression of *Xa1* is increased by wounding and challenge with compatible and incompatible bacteria[8]. In none of the above cases is specificity proposed to be the result of differential expression of the *R* gene. With a few exceptions, *Xa27* is not expressed at detectable levels in rice leaves unless it has been challenged with the incompatible bacteria and, more specifically, in the presence of AvrXa27. The localization of *Xa27* expression is consistent with the hypothesis that induction only occurs within those cells that receive AvrXa27 via the type-III secretion system. Ectopic expression of *Xa27* circumvented the requirement for AvrXa27 and conferred resistance on compatible strains, indicating that resistance is a post-transcriptional consequence of *Xa27* expression and that specificity is attributable to the *Xa27* promoter. The presence of the 10-bp sequence and the 25-bp duplication in the null allele implies that these regions may be necessary for expression that is influenced by AvrXa27.

Xa27 and *avrXa27*, in addition to being the first bacterial avirulence and *R*-gene combination from rice, provide the first insight into the molecular basis of pathogen recognition directed by effectors requiring nuclear localization for function[17–19]. AvrXa27 and the related type III effectors have features similar to transcription activator and may function as transcription factors—both as elicitors and virulence factors[20]. The induction of *Xa27* in the presence of AvrXa27 is consistent with the above model. However, whether AvrXa27 acts directly at the *Xa27* promoter or indirectly through the activation of an endogenous transcription factor is unknown.

The prevailing models for specificity propose that *R*-gene products recognize elicitors or a catalytic product of an effector enzyme, and initiate resistance through a signalling cascade[21]. In models involving the AvrRpt2 protease, for example, the plant appears to have evolved an alternate or mimic substrate to the virulence targets, which are at present unknown[22,23]. Effectors related to AvrXa27 are critical virulence factors, whose activities are dependent on nuclear localization and transcription activation[11,18,24]. Indeed, we have recently identified changes in rice gene expression during compatible (disease) interactions that are due to specific AvrXa27-related effectors (unpublished data), and host expression changes specific to AvrBs3 have also been observed[25]. Specificity in the case of *Xa27* is the result of host gene induction. Here, again, the plant appears to deploy a mimic, in this case a mimic promoter, that triggers a defence response and

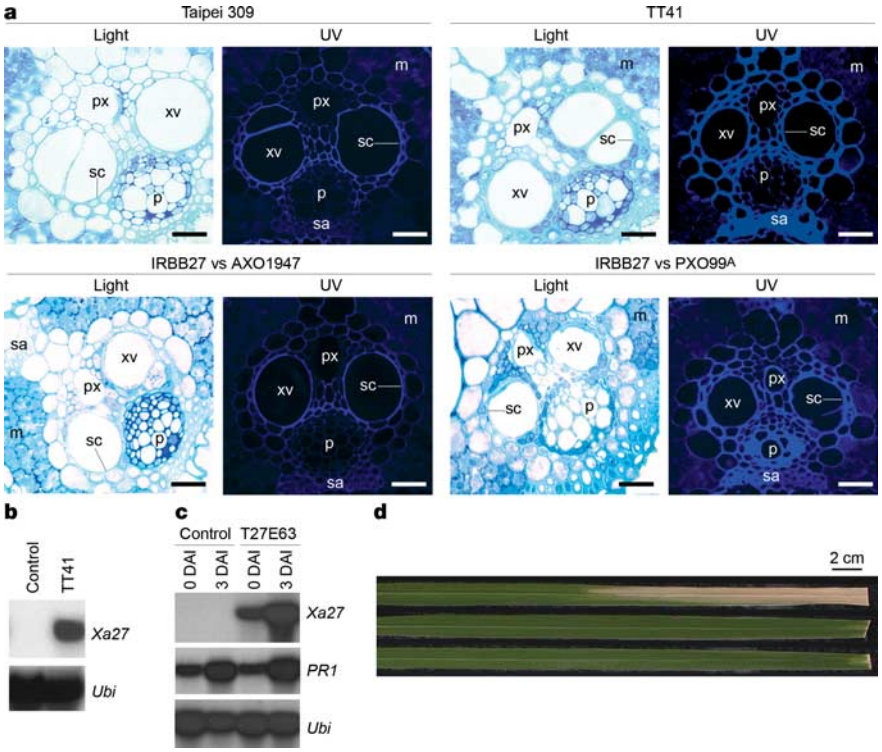

Figure 4 | Ectopic expression of *Xa27* confers resistance to compatible strains of *X. oryzae* pv. *oryzae*. **a**, Secondary cell-wall thickening in vascular bundle elements of transgenic line TT41 and from IRBB27 at 3 DAI with incompatible strain PXO99^{A}. Normal elements are shown for parental (Taipei 309) plants and IRBB27 plants at 3 DAI with compatible pathogen AXO1947. Secondary cell walls appear light blue under light microscopy and fluorescent blue under confocal microscopy. Abbreviations: m, mesophyll cells; p, phloem; px, protoxylem lacuna; sa, sclerenchyma cells; sc, secondary cell wall; xv, xylem vessel; UV, ultraviolet. Scale bars, 20 μm. **b**, Elevated expression of *Xa27* in TT41 in the absence of bacterial challenge. **c**, Expression of P_{PR1}*Xa27* in transgenic line T27E63 was elevated after inoculation with compatible strain AXO1947 (lanes 3 and 4). **d**, P_{PR1}*Xa27* line T27E63 conferred resistance to both incompatible strain PXO99^{A} (middle leaf) and compatible strain AXO1947 (bottom leaf). Control line was susceptible to compatible strain AXO1947 (top leaf). For control experiments in **b**, **c** and **d**, transgenic Taipei 309 (**b**) or Nipponbare (**c** and **d**) lines with the pC1300 vector were used.

confounds the desired effects of the pathogen. Molecular mimicry is a term sometimes used to describe pathogens that mimic host molecules to confound host recognition and defence[26]. The evidence from plant recognition of pathogenic bacteria suggests a general model of molecular mimicry in the adaptation of plants in response to pathogens and their associated virulence strategies.

METHODS

Isolation of *avrXa27*. PXO99^{A}ME1 was identified from a collection of mutants of PXO99^{A} (ref. 24). A subgenomic library of *avrXa7*-related sequences was introduced into PXO99^{A}ME1, and strains were tested for *Xa27*-dependent reactions on six-week-old IRBB27 (*Xa27/Xa27*) plants. *avrXa27* was subcloned from 99-avrXa27-20. The NLS mutation was generated by replacing the *Pml*I-*Hind*III fragment of pZWavrXa27 with the corresponding fragment from pZWavrXa7M123 (ref. 11). *avrXa27TGA* was generated by exchanging the *Sph*I fragment of pZWavrXa27 with that of pZWavrXa10TGA, which had a stop codon at the 1046 position[10].

Isolation of *Xa27*. BAC libraries of IRBB27 and IR24 were made in pIndigoBAC-5 as described[27]. Clones 43L18 and 109L05 were identified from IRBB27 using markers M964 and M499 (ref. 9), respectively, and sequenced (Supplementary Fig. 2). A clone containing allele of the *Xa27* gene was isolated from IR24 and sequenced. Subclones from 43L18 and 109L05 were inserted into the pC1300 vector and transferred to rice cultivars Nipponbare or Taipei 309 with *Agrobacterium tumefaciens* strain AGL1[28]. Six-week-old T_0 transgenic plants were evaluated for resistance using the leaf-clipping method[29]. Homozygous T_2 plants were tested for resistance specificity with four IRBB27 incompatible strains and three IRBB27 compatible strains. For ectopic expression, the *Xa27* coding region was fused to the promoter of rice *PR1* gene from cultivar Co39 (accession number U89895).

***Xa27* cDNA and northern blot analyses.** A cDNA library was constructed from PXO99^{A}-inoculated leaf tissue of IRBB27 in Uni-ZAP XR (Stratagene). RNA was isolated by using TRIZOL reagent (Invitrogen), and mRNA was purified with a messenger RNA kit (Qiagen). The 5′ end of the *Xa27* cDNA was determined with SMART RACE cDNA Amplification Kit (Clontech). For northern hybridization, 5 μg mRNA from induced IRBB27 plants or 30 μg total RNA from *Xa27* ectopic lines were used and probed with ^{32}P-labelled DNA. The mRNA loading was assessed by hybridization to rice *Ubi1*.

Histological analysis. Leaf tips 3 mm long were fixed in 50 mM $NaPO_4$ buffer (pH 7.4), containing 2.5% glutaraldehyde. After dehydration with ethanol, samples were embedded using a Leica Historesin Embedding Kit. The 3-μm sections were stained with 0.05% toluidine blue O and viewed with a Leica DMRB microscope. Unstained sections were examined for autofluorescence from phenolic compounds by confocal microscopy (Zeiss LSM 510) at 405 nm with a band pass 420–480 nm emission filter.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions K.G., B.Y., D.T. and L.W. are all co-first authors. G.-L.W. initiated the preliminary studies of *Xa27*. Z.Y. designed and carried out data analysis. K.G., D.T. and L.W. conceived the experiment, and together with D.W., C.S., F.Y., Z.C. and Z.Y. carried it out. For *avrXa27*, B.Y., F.F.W. and Z.Y. designed and carried out data analysis, B.Y., together with D.T. and K.G., conducted the experiment. F.F.W. and Z.Y. co-wrote the paper.

Author Information Sequences of *O. sativa Xa27* IR24 allele (*xa27*), *Xa27* IRBB27 allele (*Xa27*), *Xa27* full-length cDNA and *X. oryzae* pv. *oryzae* PXO99^{A} *avrXa27* locus are deposited in GenBank under accession numbers AY986491, AY986492, AY986493 and AY986494. Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The authors declare competing financial interests: details accompany the paper on www.nature.com/nature. Correspondence and requests for materials should be addressed to Z.Y. (yinzc@tll.org.sg).