Os8N3 is a host disease-susceptibility gene for bacterial blight of rice

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Many bacterial diseases of plants depend on the interaction of type III effector genes of the pathogen and disease-susceptibility genes of the host. The host susceptibility genes are largely unknown. Here, we show that expression of the rice gene *Os8N3*, a member of the *MtN3* gene family from plants and animals, is elevated upon infection by *Xanthomonas oryzae* pv. *oryzae* strain PXO99^A and depends on the type III effector gene *pthXo1*. *Os8N3* resides near *xa13*, and PXO99^A failed to induce *Os8N3* in rice lines with *xa13*. Silencing of *Os8N3* by inhibitory RNA produced plants that were resistant to infection by strain PXO99^A yet remained susceptible to other strains of the pathogen. The effector gene *avrXa7* from strain PXO86 enabled PXO99^A compatibility on either *xa13*- or *Os8N3*-silenced plants. The findings indicate that *Os8N3* is a host susceptibility gene for bacterial blight targeted by the type III effector PthXo1. The results support the hypothesis that *X. oryzae* pv. *oryzae* commandeers the regulation of otherwise developmentally regulated host genes to induce a state of disease susceptibility. Furthermore, the results support a model in which the pathogen induces disease susceptibility in a gene-for-gene manner.

avrXa7 | *Oryza sativa* | *pthXo1* | *xa13* | *Xanthomonas*

A virulence genes and their cognate host R genes, contribute to the basis of the so-called gene-for-gene model of host–pathogen incompatibility, and many avirulence and R gene pairs have been characterized, along with attempts to explain the associated signaling pathways for resistance (1). The outcome of host–pathogen interactions is also controlled by compatibility factors, which consist of virulence factors in the pathogen and susceptibility factors of the host. In plant pathogenic bacteria, important contributions to compatibility are provided by the type III secretion system (TTSS), which functions in the secretion and injection of substrate proteins (type III effectors) into eukaryotic host cells (2, 3). Type III effectors of plant pathogens presumably function similarly to effectors of animal pathogens and target various host-susceptibility factors (4, 5). In contrast to the effectors of the animal pathogens and R genes of plants, however, few host-susceptibility targets of type III effectors from plant bacterial pathogens have been identified.

Bacterial blight of rice is a vascular disease caused by *Xanthomonas oryzae* pv. *oryzae*, which spreads systemically through the xylem tissue, and is an important disease throughout many regions of rice production. A functioning TTSS is essential for the pathogenicity of *X. oryzae* pv. *oryzae*, and members of the *avrBs3/pthA* gene family of TTSS substrate effectors are essential for the ability of *X. oryzae* pv. *oryzae* to cause disease (6, 7). *avrBs3/pthA* products have characteristics of nuclear transcription activation factors and are referred to here as transcription activator-like (TAL) effectors (8). TAL effectors constitute a large family of closely related proteins, are individually distinguishable by the nature and number of direct repeats within a central repetitive region, and contribute to virulence in a variety of other important disease complexes of citrus, cotton, and cassava (9).

TAL effectors are secreted through the TTSS and targeted to host nuclei, a process that is mediated by the conserved N-terminal and C-terminal regions, respectively (10, 11). Each effector also has a C-terminal transcription activation-like domain, which is required in all cases for virulence activity and for avirulence activity (the ability to elicit a gene-specific resistance reaction in the host) in many cases (11–14). The TAL effector AvrBs3 is associated with the induction of host genes in pepper when introduced ectopically (15). The role of the pepper genes in host susceptibility is unknown. AvrXa27, a TAL effector from *X. oryzae* pv. *oryzae* PXO99^A, is associated with the specific induction of the R gene *Xa27* (16). AvrXa27 is an elicitor of resistance and is not known to be involved in strain virulence.

We hypothesized that *Xa27* is a plant adaptation to mimic the activity of the TAL effectors as virulence factors and that *X. oryzae* pv. *oryzae* virulence depended on the induction of one or more key host genes (16). To examine the hypothesis, we used whole-genome-based microarray of rice genes to identify genes that are possibly up-regulated after challenge by bacteria. Here, we present the identification of a host gene that is differentially expressed during disease and a potential target of a type III TAL effector of *X. oryzae* pv. *oryzae*.

Results

***Os8N3* Is Induced in a TAL Effector-Dependent Manner.** Microarray hybridization analysis with RNA from infected rice leaves was conducted by using the Affymetrix full genome arrays of rice (www.affymetrix.com/products/arrays/specific/rice.affx). RNA was isolated from two sets of rice leaves 24 h after inoculation with two strains of *X. oryzae* pv. *oryza*. One strain was the fully virulent strain PXO99^A. The second strain was the mutant PXO99^{A}ME2 (hereafter ME2), which has a mutation in the TAL effector gene *pthXo1* and is severely reduced in virulence (7). The gene *Os8N3* was induced 103-fold higher after inoculation with strain PXO99^A relative to the value for ME2 and represented the target sequence from the array with the highest fold increase (see Data Sets 1 and 2 and Table 1, which are published as supporting information on the PNAS web site). The increase in *Os8N3* expression was corroborated by Northern hybridization analysis using a 598-bp gene-specific probe for *Os8N3* derived from a unique nucleotide sequence in the 3′ region of the *Os8N3* cDNA. The probe sequences are provided in Table 2, which is published as supporting information on the PNAS web site. *Os8N3* was not induced by inoculation of rice plants with water, or the TTSS-deficient strain PXO99^{A}ME7 (6), or the *pthXo1*mutant ME2, and reintroduction of *pthXo1* to ME2 restored *Os8N3* induction (Fig. 1*A*). *Os8N3* is induced within 6 h after inoculation (Fig. 1*B*), and expression increased only upon challenge with PXO99^A and not with several additional strains of the pathogen (Fig. 1*C*). Although expressed at low levels in leaves and roots in unchallenged plants, *Os8N3* is expressed at higher

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Abbreviations: HR, hypersensitive resistance; iRNA, interfering RNA; TAL, transcription activator-like; TTSS, type III secretion system.

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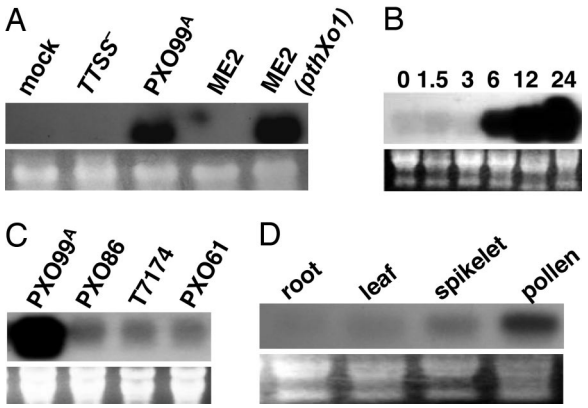

Fig. 1. Northern hybridization analysis of *Os8N3* expression. (*A*) *Os8N3* is induced in a TAL effector-dependent manner upon infection. Rice leaves were inoculated with water (mock), type III secretion mutant PXO99^{A}ME7 (TTSS^{-}), wild-type parent (PXO99^{A}), *pthXo1*$^{-}$ (ME2), and ME2 with *pthXo1* (ME2/*pthXo1*) reintroduced on pZWpthXo1 in pHM1, as indicated above each lane. Total RNA was extracted 24 h after inoculation and probed with a ^{32}P-labeled gene-specific 3′ probe of *Os8N3*. (*B*) *Os8N3* is induced within 6 h after bacterial infection. Rice leaves were inoculated with PXO99^{A}, and RNA was extracted at the indicated time points (in hours). Filter was probed as in *A*. (*C*) Expression of *Os8N3* is not elevated after infection of rice leaves with other strains of the pathogen. Rice leaves were inoculated with strains of *X. oryzae* pv. *oryzae* as indicated above each lane, and RNA was extracted 24 h after inoculation. Filter was probed as in *A*. (*D*) *Os8N3* is expressed in developing inflorescence. Total RNA was extracted from the uninoculated plant tissues, as indicated above each lane. Filter was probed as in *A*. Total RNA loading is shown below the lanes (tRNA) for each blot.

levels in developing spikelets and pollen, indicating that *Os8N3* is developmentally regulated (Fig. 1*D*).

***Os8N3* Is a Member of a Gene Family.** The *Os8N3* coding region starts at position 26,721,365 on chromosome 8 of the rice cultivar Nipponbare [National Center for Biotechnology Information (NCBI) accession no. AP008214] and is represented by a 1,516-bp full-length cDNA clone (NCBI accession no. AK070510) (Fig. 2*A*). The predicated protein consists of 307 amino acid residues and eight membrane-spanning domains (Fig. 2*B*). *Os8N3* is named after *MtN3*, a differentially expressed gene in the root nodules of *Medicago truncatula* (17). Additional related genes are found in nematodes, insects, and animals, although the biochemical functions of the proteins are unknown (Fig. 2*C*). The rice genome contains ≈17 genes encoding related proteins scattered throughout the genome, although none cross hybridize with the 5′ and 3′ gene-specific probes used in this study. Alignments of portions of the four closest relatives of *Os8N3* are provided in Figs. 8 and 9, which are published as supporting information on the PNAS web site.

***Os8N3* Is Not Inducible in Plants Homozygous for the R Gene *xa13*.** *Os8N3* is located in the region of bacterial blight-resistance gene *xa13*, which was recently mapped to within a 14-kb region on chromosome 8, and PXO99^{A} is one of the few strains that is incompatible with *xa13* (18, 19) (Fig. 3*A*). The ability of PXO99^{A} to induce *Os8N3* expression in plants containing *xa13* was therefore tested. Rice cultivars BJ1 and Juma, which are traditional cultivar sources for *xa13*, and IRBB13, which is a *xa13*-containing near-isogenic line derived from the recurrent susceptible parent lines IR24 and BJ1, all failed to show increased levels of *Os8N3* expression after inoculation with PXO99^{A} (Fig. 3*B*). Sequence analysis of the promoter regions from IR24 and IRBB13 revealed sequence polymorphisms that might account for the differences in responsiveness to PXO99^{A}. The most conspicuous difference is the presence of a 243-bp insertion of repetitive sequences near the

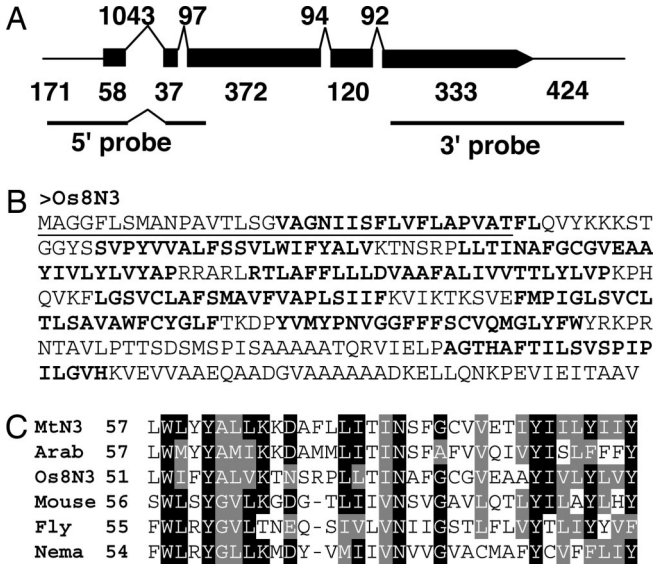

Fig. 2. *Os8N3* is a member of the MtN3 family. (*A*) *Os8N3* is expressed as a 1.6-kb messenger RNA. *Os8N3* is shown as represented by the full-length cDNA AK070510. Exons are represented by the solid black bars, and introns are indicated above the bars. Numbers indicate the number of base pairs of nucleotides. The first and last numbers for exons indicate the predicted 5′ and 3′ untranslated regions, respectively. 5′ and 3′ probes used throughout the study for hybridization (see *Results*) are indicated below the diagram. (*B*) *Os8N3* product is predicted to encode a 307-aa integral membrane protein. The underlined region is a predicted uncleaved leader peptide. Eight predicted transmembrane regions are in bold. (*C*) Alignment of representative related proteins of the MtN3 family. Sequences were aligned by using CLUSTALW (38) and displayed by using BOXSHADE (www.ch.embnet.org/software/BOX_form.html). Protein sequence files: *Medicago truncatula* nodulin protein CAA69976 (MtN3), *Arabidopsis* MtN3-like protein AAL47380 (Arab); recombination activating gene 1 gene activation protein AAH14292 (Mouse), *Drosophila melanogaster* saliva protein AAD03390 (Fly), and *Caenorhabditis elegans* protein AAC02609 (Nema).

TATA box of *Os8N3* in IRBB13 (Fig. 3*C*). The sequences of the *Os8N3* promoter regions from IRBB13 and IR24 are provided in Fig. 10, which is published as supporting information on the PNAS web site.

***Os8N3*-Silenced Plants Are Incompatible with PXO99^{A}.** If expression of *Os8N3* is required for susceptibility, loss or reduction of *Os8N3* expression in susceptible plants because of interfering RNA (iRNA) as mediated by double-stranded RNA was predicted to result in plants that are resistant to PXO99^{A} infection. Fifty-four plants from 14 independent transgenic rice lines were generated by using a short segment of the 3′ region of *Os8N3* expressed in a manner to create a hairpin RNA (Fig. 4*A*). The plants were assayed for *Os8N3* hairpin RNA expression by using the 598-bp gene-specific 3′ region (see Fig. 2*A*, 3′ probe). Hybridization with the 3′ probe revealed those plants expressing the silencing construct at high levels but could not distinguish the transgene RNA from the *Os8N3* endogenous RNA. The 292-bp gene-specific 5′ probe, whose sequences are not present in the iRNA construct, was used to determine the expression level of the full *Os8N3* before and after challenge by PXO99^{A} (see Fig. 2*A*, 5′ probe). Control plants, containing only vector sequences, showed high levels of *Os8N3* induction (Fig. 4*B*, vector), whereas four plants that expressed high levels of the iRNA construct (Fig. 4*B*, plants 1–4, 3′ probe) had little or no *Os8N3* induction after bacterial challenge (Fig. 4*B*, plants 1–4, 5′ probe). We hereafter refer to the four plants as *Os8N3*-silenced plants. The susceptibility of the transgenic plants was measured on the basis of bacterial populations and lesion length in comparison with control plants. Control plants supported typical disease symp-

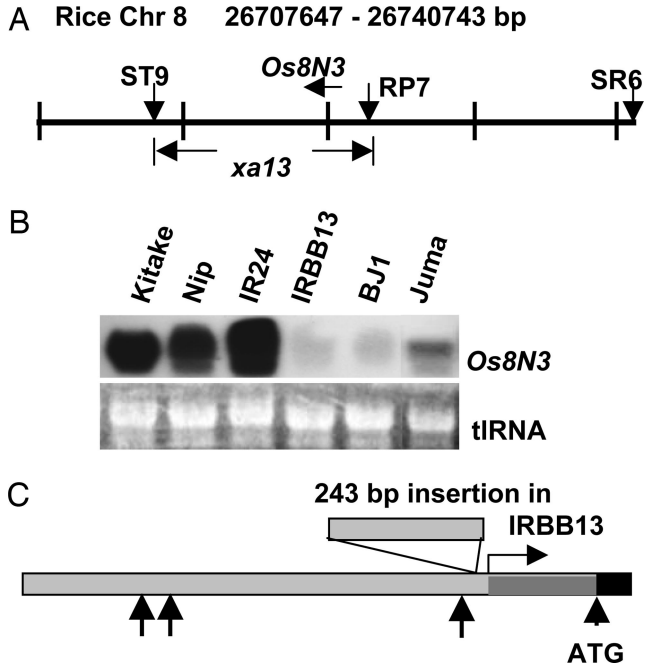

Fig. 3. *Os8N3* is not induced in *xa13* plants. (*A*) *Os8N3* is located on rice chromosome 8 in the region of the recessive resistance gene *xa13*. *xa13* has been mapped to within a 14-kb region between two sequence-tagged markers, ST9 and RP7 (19). The position of *Os8N3* within the interval is indicated by the horizontal arrow above the map. (*B*) *Os8N3* is not induced in rice cultivars homozygous for *xa13*. Leaf RNA was isolated from the indicated rice cultivars 24 h after challenge with PXO99^{A} and probed with the 3′ probe for *Os8N3*. (*C*) Sequence polymophisms are present between IR24 and IRBB13 in the *Os8N3* promoter. The *Os8N3* promoter is shown, including 60 bp of coding sequence. The start of transcription is shown by the horizontal arrow, and the start of the coding sequence is indicated by ''ATG.'' A 243-bp insertion of DNA found in the IRBB13 sequence is indicated by the box on the promoter region. Other arrows indicate one to three nucleotide insertions present in either IRBB13 or IR24 in comparison with each sequence.

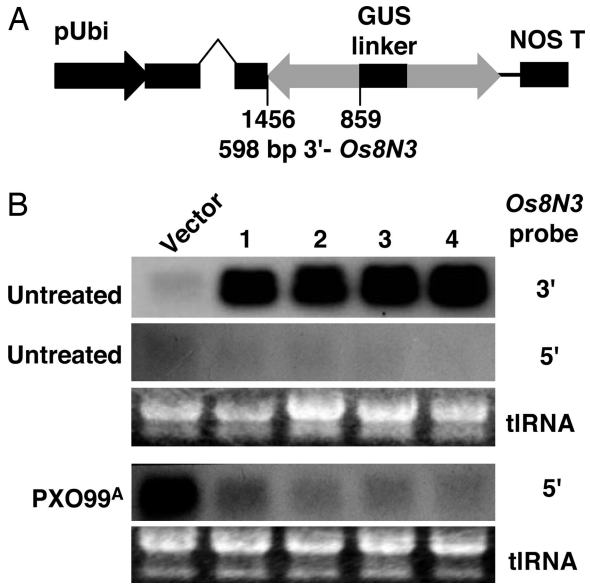

Fig. 4. Silencing of *Os8N3* expression by using iRNA. (*A*) Schematic of silencing vector for inhibition of *Os8N3* mRNA. A 598-bp fragment from the 3′ region of *Os8N3* (see Fig. 2*A*, 3′ probe) was cloned in two orientations in pANDA with a fragment of the *uidA* gene as an internal linker (GUS linker) behind the maize Ubi1 promoter. (*B*) Northern analysis of *Os8N3*-silenced rice plants. RNA was isolated from leaves of control plants containing only transformation vector sequences (Vector) and four independent transformed plants expressing the dsRNA sequence as indicated by the 3′ probe (*Top*, untreated, lanes 1–4). Expression was then analyzed with a 5′ probe to determine the level of expression for the endogenous *Os8N3* before (*Middle*, untreated) and after challenge by strain PXO99^{A} (*Bottom*, PXO99^{A}).

toms, whereas silenced plants had lower bacterial leaf populations (Fig. 5*A*) and shorter leaf lesions (Fig. 5*B*) in comparison with control plants. The normal expression pattern of *Os8N3* was also disrupted in silenced plants, and low expression was observed in spikelets and pollen (data not shown). The silenced plants had low fertility, and most pollen grains were defective, as indicated by their starch content, or lack thereof, in comparison with normal pollen grains (Fig. 5*C*, *a* and *b*, respectively). However, the leaves were normal in appearance and visibly affected only in susceptibility.

***avrXa7* Enables PXO99^{A} Compatibility on IRBB13 and *Os8N3*-Silenced Plants.** The reduced ability of PXO99^{A} to incite disease on IRBB13 and *Os8N3*-silenced plants indicated that the strain depends on *Os8N3* induction for compatibility. Strain PXO86 did not induce *Os8N3* in normal plants, yet the strain is compatible with most rice cultivars, including IRBB13 (18). The TAL effector gene *avrXa7* is a major contributor to the virulence of strain PXO86 (14, 20). We therefore transferred *avrXa7* to strains ME2 and PXO99^{A} and compared their abilities to cause disease on IRBB13 and the near-isogenic susceptible parent line IR24. ME2 was weakly virulent on both IRBB13 and IR24 (Fig. 6*A*, ME2). Virulence on IR24 was restored to ME2 by the reintroduction of *pthXo1* but not IRBB13 (Fig. 6*A*, *ME2/pthXo1*). Virulence was restored to ME2 on both IRBB13 and IR24 by the introduction of *avrXa7* (Fig. 6*A*, *ME2/avrXa7*). PXO99^{A} behaves similarly to ME2 on IRBB13 and is virulent on IR24 (Fig. 6*A*, PXO99^{A}). The addition of *avrXa7* to PXO99^{A} also restored virulence on IRBB13 (Fig. 6*A*, PXO99^{A}/*avrXa7*). ME2 (data not shown) and PXO99^{A} are also not virulent on *Os8N3*-silenced plants (Fig. 6*B*), whereas PXO86 caused disease on *Os8N3*-silenced plants (Fig. 6*B*, PXO99^{A} and PXO86, respectively). The introduction of *avrXa7* to ME2 or PXO99^{A} restored

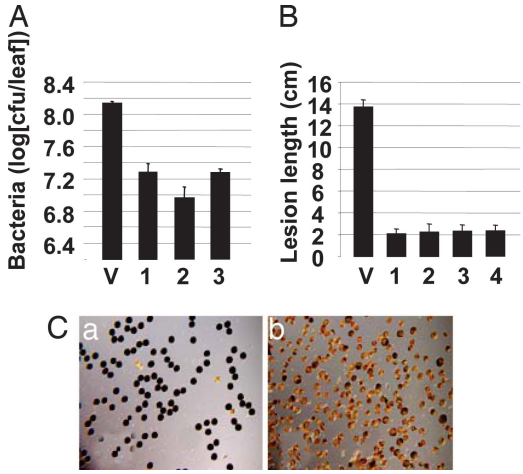

Fig. 5. Virulence assay on *Os8N3*-silenced plants. (*A*) Silenced plants have reduced bacterial populations. Bacterial populations at 8 days were measured from 10 plants of the control plant line with vector-only sequences (V) and three silenced plant lines (columns 1–3). Bacterial populations were measured as cell-forming units (cfu) per leaf. (*B*) Silenced plants display reduced lesion lengths. Lesion measurements in cm were taken at 8 days after inoculation from 10 leaves of the control line (V) and 10 leaves of each of four silenced lines (columns 1–4). (*C*) *Os8N3*-silenced plants have low pollen viability. Low pollen viability was assessed by the presence of starch (which stains dark blue) accumulation in pollen. Mature pollen grains from control plant (*Ca*) and silenced plant (*Cb*) were stained with 1% I-KI solution.

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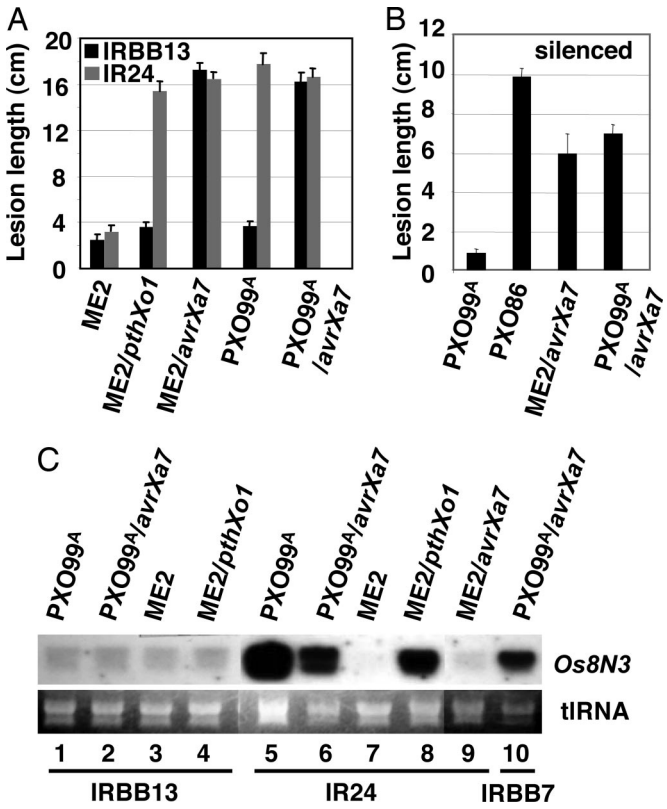

Fig. 6. Strain-specific susceptibility mediated by *Os8N3* and PthXo1. (*A*) *avrXa7* enables PXO99^{A} compatibility on IRBB13. Leaves of 60-day-old IRBB13 plants containing *xa13* and the near-isogenic parent IR24 were inoculated with the bacterial strain as indicated below each column. The average lesion length for 10 plants after 16 days was determined. (*B*) *avrXa7* enables PXO99^{A} compatibility on *Os8N3*-silenced plants. Leaves of 40-day-old *Os8N3*-silenced plants were inoculated with the bacterial strain as indicated below each column. The average lesion length for six plants was determined after 8 days. (*C*) *avrXa7* does not direct elevated expression of *Os8N3* in IRBB13 or IR24. IRBB13 and IR24 plants were inoculated with the strain indicated above each lane. Total RNA was extracted at 24 h after inoculation and analyzed with the 3′ probe of *Os8N3* (as in Fig. 1*A*). Total RNA loading is shown below the blots (tIRNA). Lanes are numbered for reference in the text. Plant variety is indicated below the lane numbers.

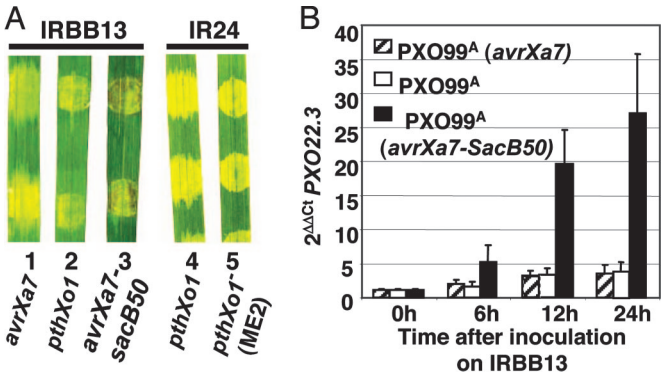

Fig. 7. PXO99^{A} incites an atypical resistance reaction on IRBB13. (*A*) PX099^{A} does not elicit a typical hypersensitive reaction on leaves of IRBB13. Each leaf was inoculated with PXO99^{A} containing the gene indicated below the leaf. The number below each leaf photograph is included for reference in the text. The HR (brown tissue in the region of inoculation of leaf 3) is due to the presence of a dominant R gene present in IRBB13 and the avirulence gene *avrXa7-sacB50*. The avirulence gene was derived from a deletion analysis of *avrXa7* and directs the elicitation of an HR in IR24 and related near-isogenic lines such as IRBB13 (22). The leaves were photographed 4 days after inoculation. (*B*) Rice peroxidase gene *POX22.3* is not induced during challenge of IRBB13 with PXO99^{A}. Three sets of IRBB13 plants were inoculated as in *A* with PXO99^{A} (*avrXa7*), PXO99^{A}, and PXO99^{A} (*avrXa7-SacB50*), and total RNA was isolated at 0, 6, 12, and 24 h after inoculation. cDNA prepared from the samples was subjected to quantitative RT-PCR using *POX22.3*-specific primers. Gene-specific primers for the rice gene *OsTFIIAγ5* were used for control.

virulence to both strains on the silenced plants (Fig. 6*B*, ME2/*avrXa7* and PXO99^{A}/*avrXa7*, respectively).

Northern analysis of infected IRBB13 plants indicated that PXO99^{A} (*avrXa7*), despite the ability to incite disease, failed to induce *Os8N3* (Fig. 6*C*, lane 2). PXO99^{A} (*avrXa7*) maintained the ability to induce *Os8N3* in the susceptible variety IR24 (Fig. 6*C*, lane 6). Induction of *Os8N3* in IR24 was affected only by the presence or absence of *pthXo1*, because ME2 and ME2 (*avrXa7*) both failed to induce *Os8N3* (Fig. 6*C*, lanes 7 and 9). Reintroduction of *pthXo1* into ME2 restored *Os8N3* induction (Fig. 6*C*, lane 8). PXO99^{A} (*avrXa7*), as shown in previous work, triggers a *Xa7*-mediated resistance reaction in IRBB7 (21). The induction of *Os8N3* also occurs in plants undergoing a *Xa7*-mediated resistance reaction, indicating that expression of *Os8N3* does not suppress a dominant R gene reaction due to *Xa7* (Fig. 6*C*, lane 10). The resistance profile of both *xa13*-containing and *Os8N3*-silenced plants, therefore, is narrow, and the alternative virulence factor *avrXa7* can bestow virulence to the incompatible strain regardless of the presence or absence of *pthXo1*.

***xa13* Confers an Atypical Resistance Response to PXO99^{A}.** The resistance of IRBB13 to PXO99^{A} also does not have the typical phenotype of a hypersensitive resistance (HR) reaction in rice. The compatible reaction on IRBB13, as evidenced by PXO99^{A} (*avrXa7*), presents itself as clearing of the infected area in which the lesion begins to spread out from the immediately infected region (Fig. 7*A*, leaf 1) and is similar to the compatible reaction of PXO99^{A} on the susceptible line IR24 (Fig. 7*A*, leaf 4). In contrast, PXO99^{A} without *avrXa7* caused reduced clearing on IRBB13 with no browning (Fig. 7*A*, leaf 2). The reaction of PXO99^{A} is most similar in appearance to the phenotype of ME2 on IR24 (Fig. 7*A*, leaf 5). In contrast, a typical HR reaction, here due to the avirulence gene *avrXa7-sacB50* (22) that occurs on IRBB13 (and IR24, data not shown), is characterized by the collapse and browning of the infected tissue (Fig. 7*A*, leaf 3). The defense gene *POX22.3* encodes a peroxidase and is strongly induced specifically during resistance reactions in rice (23, 24). PXO22.3 expression in IRBB13 was measured by quantitative RT-PCR and POX22.3-specific primers in a time course experiment after inoculation with PXO99^{A} and compared with PXO22.3 expression in IRBB13 after inoculation with the compatible strain PXO99^{A} (*avrXa7*) and the incompatible strain PXO99^{A} (*avrXa7-sacB50*). Levels of PXO22.3 began to rise within 6 h of inoculation and continued to rise through the 24-h period after inoculation with PXO99^{A} (*avrXa7-sacB50*) (Fig. 7*B*). PXO22.3 expression remained relatively low in IRBB13 during the same time period in both the susceptible reaction to PXO99^{A} (*avrXa7*) and the incompatible reaction with PXO99^{A} (Fig. 7*B*).

Discussion

We used a comparison of host gene expression after inoculation of rice plants with PXO99^{A} and the *pthXo1* mutant to identify genes that are differentially expressed in the presence of *pthXo1*. *Os8N3* emerged from the analysis as the gene with the greatest relative change in expression after the inoculations. *Os8N3* expression was nearly 5-fold greater than the next-nearest candidate gene, which encoded a dehydrin-related protein and may be related to desiccation of the infected tissue. The location of *Os8N3* within the region of a previously mapped recessive resistance gene, *xa13*, added to the evidence that *Os8N3* may have a critical role in compatibility. Although not demonstrated here, we believe it is likely that *Os8N3* and *xa13* are allelic. Plants deficient in bacterial-

mediated *Os8N3* induction, by either engineered disruption of *Os8N3* transcription or natural genetic variation (*xa13*), were not susceptible to infection by strain PXO99^{A}, which depends on the major TAL effector gene *pthXo1* for full virulence. At the same time, both silenced and *xa13*-containing plants were not generally resistant to bacterial infection. Infectivity by strain PXO86, for example, was unaffected by either silencing or *xa13*, and PXO86 did not cause *Os8N3* induction in normal or *xa13*-containing plants. PXO86, in turn, depends for virulence on the TAL effector *avrXa7*, which was originally identified as the cognate avirulence gene to the R gene *Xa7*. The effect of *avrXa7* is dominant over *pthXo1* in the sense that PXO99^{A} with *pthXo1* and *avrXa7* is also compatible on IRBB13 and the silenced plants.

We conclude that these observations are consistent with the hypothesis that *Os8N3* is a host gene for strain-specific susceptibility to bacterial blight disease of rice. We feel compelled to consider the gene as a susceptibility factor, as opposed to a resistance reaction, based on the following features: (*i*) The lack of compatibility of PXO99^{A} can be overcome by the addition of a virulence factor, in this case *avrXa7*, and not, as typically occurs with resistance reactions, by the loss of an avirulence gene. (*ii*) Loss of compatibility is due to the loss of a virulence gene in the pathogen. (*iii*) The loss of compatibility resulted from the loss of a host function, as inferred from the loss of expression of the *Os8N3* product regardless of whether the loss was due to silencing or the *xa13* configuration. (*iv*) The phenotypic response of IRBB13 has the appearance of a weak compatibility reaction and not the typical HR associated with R gene-specific avirulence gene products. Here, we used a previously discovered variant of *avrXa7* (*avrXa7-sacB50*) that elicits resistance in IR24 and derived near-isogenic lines to compare the PXO99^{A} reaction to a typical HR on rice (22). Gene-profiling experiments with *xa13* have also noted atypical defense gene expression profiles in IRBB13 plants after challenge with incompatible bacteria (25). Here, we demonstrated that the defense-related peroxidase gene *PXO22.3* was not elevated in IRBB13 after challenge with PXO99^{A}.

Elevated expression of *Os8N3* was strictly correlated with the presence of *pthXo1* in the pathogen. Whether *Os8N3* is a direct target of the TAL effector PthXo1 is unknown, because the mode of action of TAL effectors in general is unknown. PthXo1 may act directly at the *Os8N3* promoter, binding to an as-yet-unidentified sequence motif. TAL effectors are predicted to form dimers, a common feature of promoter-specific transcription factors and, in at least one case, have been shown to have double-stranded-DNA-binding activity (14, 26). Alternatively, TAL effectors may interact with specific host transcription factors, which, in turn, affect the expression of host genes. Regardless of the exact mode of action, the different effectors appear to be associated with the induction of specific host genes. PthXo1 is the second TAL effector to be associated with the elevated expression of a host gene that has known consequences for the outcome of the host–pathogen interaction. *Xa27* is a dominant resistance gene whose expression is specifically elevated in the presence of the TAL effector AvrXa27 (16).

We postulated that the loss is related to sequence polymorphism in the *Os8N3* promoter and, in fact, simulated the effect of *xa13* by perturbation of *Os8N3* expression after bacterial challenge by iRNA. We predicted that, upon induction of *Os8N3* in plants expressing the hairpin construct, the gene would be subject to gene-specific silencing (27). Indeed, the results from probing the leaf RNA content with a 3′ probe, which assessed the level of hairpin RNA expression, and a 5′ probe, which assessed the progression of the silencing to the complete mRNA, indicated that *Os8N3* mRNA could not accumulate to appreciable levels after challenge by bacteria. Nonetheless, the possibility of the progressive silencing reaching more conserved regions of some of the other related genes raises the possibility that the lack of susceptibility is due to the silencing of another family member. This possibility is not consistent with the lack of *Os8N3* expression in IRBB13, nor is it consistent with the results of silencing in the *OsRac* gene family (28). In the study of *OsRac*, silencing of individual members of the family by using a unique 3′ region did not lead to transitive silencing of the other gene members unless a gene-specific region was included in the iRNA construct (28).

The discovery of *Os8N3*, which encodes a predicted integral membrane protein, provides a window through which to address a variety of issues in host susceptibility to disease. The biochemical functions of the MtN3 family members are unknown. The mammalian member *Rga* (recombination-activating gene 1 gene activation) is expressed in neurons and immune system cells and is associated with cell-surface presentation of an ion-channel protein called TRPV2 (29, 30). *Os8N3* product may function similarly and involve ion-channel recruitment, which, in turn, may alter signal transduction or regulate molecular trafficking across the cell membrane. In any event, *Os8N3* appears also to have a developmental role in inflorescence development. Loss of expression affects pollen development and may have deleterious effects in other floral tissues. Otherwise, the outward appearances of the leaves and roots are normal. Changes in expression of *NEC1*, which encodes another member of the MtN3 family in petunia, have been associated with changes in phloem development and anther maturation (31, 32). Bacterial-stimulated *Os8N3* expression could possibly alter the source–sink relationships of the infected tissue, resulting in enhanced nutritional conditions.

Strains of *X. oryzae* pv. *oryzae* have multiple copies of genes for TAL effectors, and alternate genes function in avirulence and virulence (7, 21). Addition of an alternate virulence factor, in this case, the TAL effector AvrXa7 from *X. oryzae* pv. *oryzae* strain PXO86, resulted in compatibility between PXO99^{A} and both *Os8N3*-silenced and IRBB13 plants without concomitant induction of *Os8N3*. AvrXa7 presumably targets an alternate host factor that facilitates bacterial growth and disease, possibly another gene encoding a related MtN3 family member. The number of alternate TAL effectors is unknown. In an analysis of six strains, four alternate genes, which we refer to as major TAL effector genes, were identified based on the sequence relatedness. Each of these genes could replace *pthXo1* in PXO99^{A} with full restoration of virulence (7). Thus, in addition to *avrXa7*, *pthXo2* or *pthXo3* may also induce host susceptibility by alternate strategies, and more major TAL genes may yet be discovered. The discovery that AvrXa7 and PthXo1 exploit different features of the host serves to emphasize that the outcomes of host–pathogen interactions are the result of gene-for-gene interactions of pathogen virulence factors and host susceptibility genes (33).

Materials and Methods

Plant Material, Plasmids, and Bacterial Strains. The seeds of rice varieties Nipponbare (accession no. PI 514663), BJ1 (PI221109), and Juma (PI 574797) were provided by the U.S. Department of Agriculture–Agricultural Research Service National Small Grains Collection. IR24 and IRBB13 seeds were obtained from the International Rice Research Institute (courtesy of Nollie Vera Cruz). All rice plants were grown in growth chambers with temperature of 28°C, relative humidity of 85%, and photoperiod of 12 h. The plasmids and bacterial strains used in this study are listed in Table 3, which is published as supporting information on the PNAS web site.

Microarray Analysis. Leaves of 14-day-old rice (cv. Nipponbare) were inoculated with bacterial suspensions with optical density of 0.5 at 600 nm ($\approx 5 \times 10^7$ cell-forming units per ml) by using a needleless syringe. Total RNA was extracted 24 h after inoculation by using TRI Reagent (Ambion) following the manufacturer's instruction. Five micrograms of total RNA of each sample was used for synthesis of cDNA and biotin-labeled cRNA according to the manufacturer's manual by using the One-Cycle Eukaryotic Target Labeling kit (Affymetrix). The processed cRNA was used for

hybridization to the GeneChip Rice Genome Array (Affymetrix) on the Affymetrix GeneChip instrument system at Kansas State University.

Northern Blot and Quantitative RT-PCR Analyses. The rice leaves were inoculated, and total RNA was extracted as described above at the time points indicated in the text. Fifteen micrograms of total RNA for each sample were subjected to electrophoresis in 1% agarose gels. The RNA was transferred to Hybond N+ (Amersham Pharmacia) and hybridized with specific probe at 65°C in hybridization buffer [5 × SSC, 0.5% SDS, 20 mM Na_2HPO_4/NaH_2PO_4 (pH 6.5), 5 mM EDTA, and 10 mM Tris·HCl]. The ^{32}P-labeled probes were prepared by using the Rediprime II Random Prime Labeling kit (Amersham Pharmacia) according to the manufacturer's instruction. The 5′ and 3′ probes were prepared from cDNA by using primers listed in Table 4, which is published as supporting information on the PNAS web site. Quantitative real-time PCR was performed on RNA extracted from leaves inoculated with bacteria as indicated in the text. One microgram of total RNA was subjected to DNase I (Invitrogen) treatment and first-strand cDNA synthesis by using the iScript cDNA Synthesis kit (Bio-Rad). cDNA derived from 0.025 μg of total RNA was used for each real-time PCR, which was performed on iCycler iQ (Bio-Rad) using iQ SYBR green Supermix (Bio-Rad). The gene-specific primer sequences are provided in Table 4. The average threshold cycle (Ct) was used to determine the fold change of gene expression. As an internal control, *OsTFIIAγ5* was used. The $2^{-\Delta\Delta Ct}$ method was used for relative quantification (34).

Construction of *Os8N3* Gene-Silencing Vector. A 598-bp fragment, corresponding to 859 to 1,457 nucleotides of full-length cDNA sequence of *Os8N3* (accession no. AK070510), was PCR amplified from a cDNA pool synthesized from mRNA extracted from leaves of rice (cv. Nipponbare) by using 3′ probe primers provided above. The product was cloned into pCR 2.1-TOPO (Invitrogen) and sequenced. The fragment was excised with BamHI and XhoI and cloned into Gateway vector pENTR™4 for LR recombinase reaction with pANDA (35) according to the manufacturer's instruction (Invitrogen). The RNA interference vector was electroporated into *Agrobacterium tumefaciens* strain EHA105.

PCR, Cloning, and Sequencing of Promoter Regions of *Os8N3*. Four pairs of specific primers (listed in Table 4) were used to amplify the promoter regions of *Os8N3*. PCRs were performed by using the Accuprime kit (Invitrogen). The products were cloned into pCR 2.1-TOPO (Invitrogen) and sequenced.

Stable Rice Transformation. Cali initiated from immature embryos of rice (cv. Kitake) were transformed by using *Agrobacterium* as described (36). Multiple plantlets were regenerated from individual transgenic lines, and the primary transgenic plants were used for analyses.

Virulence Assay. The fully expanded leaves were inoculated by leaf-tip clipping with scissors contaminated with bacterial suspensions of optical density of 0.25 at 600 nm ($\approx 2.5 \times 10^7$ cell-forming units per ml) as described (37). Symptoms were scored by measuring lesion length. Significance between treatments was assessed on the basis of a P value of <0.05 by using the Tukey test for analysis after ANOVA. Bacterial growth in rice leaves was measured by harvesting 10 leaves for each treatment after leaf-clip inoculation. The leaves were ground with a mortar and pestle in sterile water. The solution was diluted serially and spread on trypticase soy agar plates with appropriate antibiotics. The mean number of colonies in three plates of the proper dilution (10–100 colonies) was calculated.

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