the plant journal

SEB Society for Experimental Biology

The Plant Journal (2016) **86,** 186–194 doi: 10.1111/tpj.13164

The broadly effective recessive resistance gene *xa5* of rice is a virulence effector-dependent quantitative trait for bacterial blight

Sheng Huang[1,†], Ginny Antony[2,‡], Ting Li[1], Bo Liu[1], Ken Obasa[3], Bing Yang[1,*] and Frank F. White[3,*]

[1]*Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011, USA,*

[2]*Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA,*

[3]*Department of Plant Pathology, University of Florida, Gainesville, FL 32611, USA, and*

Received 13 January 2016; revised 2 March 2016; accepted 8 March 2016; published online 15 March 2016.

*For correspondence (e-mails byang@iastate.edu or ffwhite@ufl.edu).

[†]Present address: State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, College of Life Science and Technology, Guangxi University, Nanning, Guangxi, 530005, China.

[‡]Present address: Department of Plant Science, Central University of Kerala, Riverside Transit Campus, Kerala, 671314, India.

ABSTRACT

Mutations in disease susceptibility (S) genes, here referred to as recessive resistance genes, have promise for providing broad durable resistance in crop species. However, few recessive disease resistance genes have been characterized. Here, we show that the broadly effective resistance gene *xa5*,for resistance to bacterial blight of rice (*Oryza sativa*), is dependent on the effector genes present in the pathogen. Specifically, the effectiveness of *xa5* in preventing disease by strains of *Xanthomonas oryzae* pv. *oryzae* is dependent on major transcription activation-like (TAL) effector genes, and correlates with reduced expression of the cognate S genes. *xa5* is ineffective in preventing disease by strains containing the TAL effector gene *pthXo1*, which directs robust expression of the S gene *OsSWEET11*, a member of sucrose transporter gene family. Incompatibility is associated with major TAL effectors that target the known alternative S genes *OsSWEET14* and *OsSWEET13*. Incompatibility is defeated by transfer of *pthXo1* to otherwise *xa5*-incompatible strains or by engineering a synthetic designer TAL effector to boost *SWEET* gene expression. In either case, compatible or incompatible, target gene expression and lesion formation are reduced in the presence of *xa5*. The results indicate that *xa5* functions as a quantitative trait locus, dampening effector function, and, regardless of compatibility, target gene expression. Resistance is hypothesized to occur when S gene expression, and, by inference, sucrose leakage, falls below a threshold level.

Keywords: recessive resistance, *xa5*, TFIIAγ, *Xanthomonas oryzae*, *Oryza sativa*, TAL effector.

INTRODUCTION

Broadly functional plant resistance (R) genes are genes that provide disease resistance against a broad range of pathogen strains in a given host. Characterization of widely functioning resistance may provide insight into genetic strategies for durable resistance in plants. One class of broadly functional R genes comprises recessive resistance genes that reduce host susceptibility (van Schie and Takken, 2014; Sanfaçon, 2015). A good example is the *mlo* locus of barley (*Hordeum vulgare*). Alleles of *mlo* were first identified in varietal germplasm, and new alleles were selected from radiation-treated cultivars, giving protection against all races of *Blumeria graminis* f. sp. *hordei*, the causal agent of powdery mildew in barley (Acevedo-Garcia *et al.*, 2014). MLO is a seven transmembrane domain protein whose function is required by the fungus for susceptibility. Null mutations protect the plant from infection, and resistance has not been defeated by adaptions in the fungal population. Recessive resistance to bacterial blight disease of rice takes in several forms. The first is typified by the genes *xa13* and *xa25*. Expression of genes *xa13* and *xa25* is dependent on specific type III transcription activation-like (TAL) effector genes of the invading organism, *Xanthomonas oryzae* pv. *oryzae* (Chu *et al.*, 2006; Yang *et al.*, 2006; Liu *et al.*, 2011; Zhou *et al.*, 2015). TAL effectors are synthesized in the bacterium, and injected into host cells, where they function as sequence-specific DNA transcription factors. The specificity is determined by central repeats of 34 amino acids that match nucleotides in

the target promoters of host genes (Boch and Bonas, 2010). TAL effectors are hypothesized to target specific host genes for expression, and the consequence of their expression is enhanced plant susceptibility (Doyle *et al.*, 2013). All pathogenic *X. oryzae* pv. *oryzae* strains harbor at least one major TAL effector that targets one of three SWEET genes in clade III of the plant SWEET gene family (Yang and White, 2004; Antony *et al.*, 2010; Chen *et al.*, 2010; Streubel *et al.*, 2013; Zhou *et al.*, 2015). Strains expressing the major TAL effector gene *pthXo1* fail to induce *OsSWEET11* alleles that have promoter variants, known collectively as *xa13*, in the TAL effector binding site (Antony *et al.*, 2010; Römer *et al.*, 2010). The *xa13* gene is only effective against strains that are dependent on PthXo1 for SWEET gene induction, while *xa25* is only effective for strains that rely entirely on the major TAL effector PthXo2 (Liu *et al.*, 2011; Zhou *et al.*, 2015). SWEET proteins have been shown to mediate sugar transport, and clade III members have a preference for sucrose (Chen *et al.*, 2010, 2012). However, the mechanism by which SWEET proteins facilitate susceptibility is unknown (Yuan and Wang, 2013).

The recessive R gene *xa5*, on the other hand, provides broad resistance against many strains of *X. oryzae* pv. *oryzae* (Huang *et al.*, 1997; Garris *et al.*, 2003; Iyer and McCouch, 2004; Jiang *et al.*, 2006; Mishra *et al.*, 2013). How *xa5* confers broad resistance, while at the same time being ineffective against some strains of the pathogen, is unknown. The gene is located on rice chromosome 5, and encodes a protein with a valine to glutamic acid change (V39E) in TFIIAγ5 (Xa5), one of two small subunits of the highly conserved general transcription factor TFIIA, which forms part of the widely conserved transcription pre-initiation complex (Mediator) in eukaryotic organisms (Iyer and McCouch, 2004). TAL effectors have a potent transcription activation domain, which stabilizes the complex during the transcription initiation and re-initiation events that occur during high levels of activated gene transcription (Zhu *et al.*, 1998, 1999; Yudkovsky *et al.*, 2000; Szurek *et al.*, 2001). Mutations in TFIIAγ, specifically, are known to result in defective interaction with the activation domain of transcription factors (Ozer *et al.*, 1996). The rice resistance gene *Xa27* is dependent on the TAL effector AvrX27 (Gu *et al.*, 2005), and *Xa27* expression is lower and resistance is diminished in the *xa5* background (Gu *et al.*, 2009). A similar suppression of the TAL effector-dependent R gene *Xa10* was also observed in the *xa5* background (Tian *et al.*, 2014). However, a few strains of *X. oryzae* pv. *oryzae* are compatible on rice plants with *xa5*, including *X. oryzae* pv. *oryzae* strains PXO99^A and PXO71 from The Philippines. Both strains were shown previously to harbor the major TAL effector gene *pthXo1* (Yang and White, 2004). We therefore hypothesized that the modified TFIIAγ subunit encoded by *xa5* interferes with TAL effector-dependent susceptibility in a TAL effector-specific manner. Here, we examined the effectiveness of *xa5* in relation to the major TAL effector gene complement of the pathogen.

RESULTS

Strains expressing *pthXo1* are compatible with plants expressing *xa5*

The virulence of the strains PXO99^A and PXO71 was measured on recurrent rice lines IR24 and IRBB5, which are near-isogenic for *xa5*, with IRBB5 containing the active recessive allele. Plants with lesion lengths less than 5 cm were scored as resistant. Both strains were pathogenic on both lines when the bacteria were inoculated at the leaf tip (Figure 1a). The gene *pthXo1* is the only major TAL effector gene in strain PXO99^A, and loss of *pthXo1* as observed in PXO99^{A}ME2 (hereafter ME2) results in low virulence or resistance on both IR24 and IRBB5 rice lines (Figure 1b, column 1). ME2 therefore provides an opportunity to test the effect of major TAL effector gene content on strain compatibility to *xa5* resistance. Re-introduction of *pthXo1* to ME2 resulted in compatibility on IRBB5 and IR24,

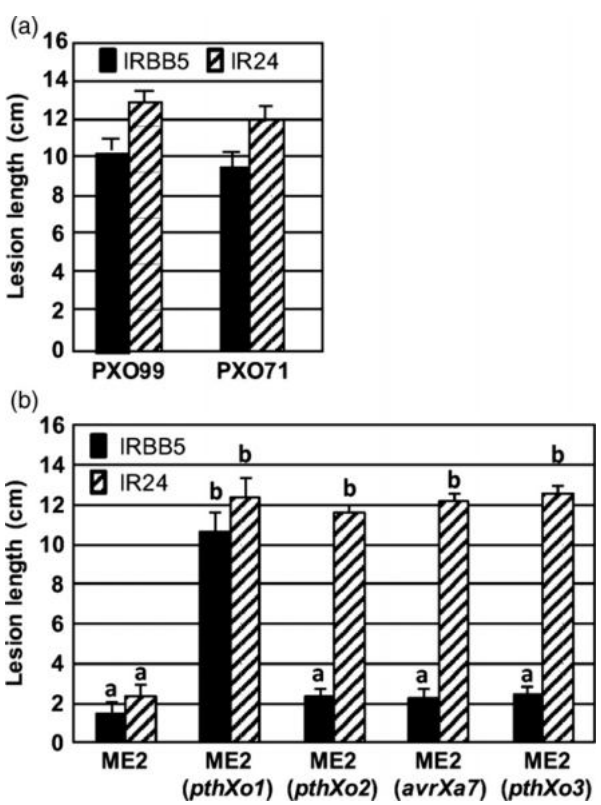

Figure 1. PthXo1 is associated with compatibility on IRBB5 (*xa5/xa5*) plants.
(a) Compatibility on *xa5* is associated with *pthXo1*. Lesion lengths for IRBB5 and IR24 were measured 13 days after inoculation of 5-week-old plants with PXO99^A or PXO71.
(b) IRBB5 is resistant to strains containing *pthXo2*, *avrXa7* or *pthXo3*. ME2 is a *pthXo1* mutant of PXO99. The assay was performed as in (a) using different strains. Bars with the same letter do not differ from each other at $P < 0.05$ (Tukey test).

although the lesions are shorter on IRBB5 (Figure 1b, column 2). Replacement of *pthXo1* by one of three alternate major TAL effector genes (*pthXo2*, *avrXa7* or *pthXo3*) restored compatibility on IR24 but not IRBB5 (Figure 1b, columns 3–5). Thus, of the four major TAL effector genes tested here, only *pthXo1* conferred compatibility to ME2 on IRBB5.

A converse test of the hypothesis is to introduce *pthXo1* into incompatible strains to determine whether the gene has a dominant effect over their resident major TAL effector genes with respect to compatibility on IRBB5. The gene *pthXo1* was introduced into four strains that are incompatible on IRBB5. PXO86 contains the major TAL effector gene *avrXa7* (Hopkins *et al.*, 1992). Strain T7174 (MAFF 311018) harbors both *pthXo2* and *avrXa7*, while PXO61 contains *pthXo3* (Yang and White, 2004; Ochiai *et al.*, 2005). AXO1947, a strain from Africa, has a gene that is identical to *talC* from the African isolate BAI3 (Yu *et al.*, 2011). When inoculated on IR24 and IRBB5, each wild-type strain without *pthXo1* was incompatible on IRBB5, with the exception of PXO99^{A} (Figure 2a, leaves 2, 4, 6 and 8), and, when *pthXo1* was introduced, all wild-type strains were compatible on IR24 and IRBB5 (Figure 2a, leaves 1, 3, 5, 7 and 9). Quantitatively, the presence of *pthXo1* confers compatibility on IRBB5 on the basis of lesion length for each strain (Figure 2b). Addition of *pthXo1* had no effect on lesion lengths on IR24 in comparison with the parent strain (Figure 2b). However, lesions on IRBB5 for all strains with *pthXo1* are shorter than lesions for the same strains on IR24 (Figure 2b).

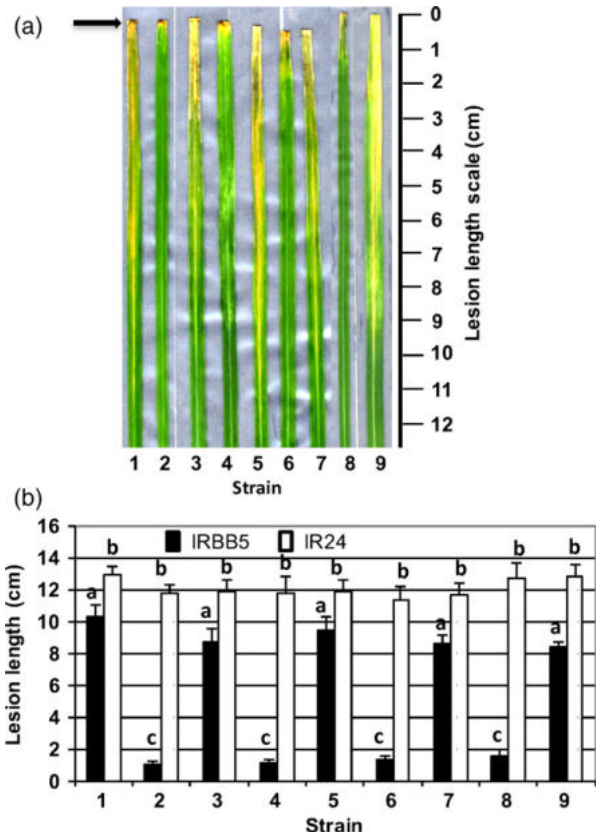

Figure 2. PthXo1 converts incompatible strains into compatible strains on IRBB5.
(a) Single leaves from rice cultivar IRBB5 (*xa5/xa5*) inoculated with strains of *X. oryzae* pv. *oryzae*. The arrow indicates the site of inoculation, and yellowish areas indicates lesions. The leaves were infected with the following strains: (1) PXO99^{A}; (2) PXO86; (3) PXO86 (*pthXo1*); (4) T7174 (MAFF 311018); (5) T7174 (*pthXo1*); (6) PXO61; (7) PXO61 (*pthXo1*); (8) AXO1947; (9) AXO1947 (*pthXo1*).
(b) Lesion lengths after inoculation on IR24 or IRBB5. Column numbers correspond to the strains detailed in (a). Plants were 5 weeks old, and lesion length was determined 13 days after inoculation. Bars with the same letter do not differ from each other at $P < 0.05$ (Tukey test).

Expression levels of the cognate S gene are altered in IRBB5

The effect of *xa5* on S gene expression was measured by quantitative real-time RT-PCR 24 h after inoculation of IR24 or IRBB5, using gene-specific primers for the respective SWEET genes. Expression of *OsSWEET14*, which is the S gene target of the AvrXa7 and PthXo3 effectors, was reduced by approximately 50% in the incompatible combination of IRBB5 and ME2 with *avrXa7* or *pthXo3*, compared to expression in IR24 (Figure 3a). Expression of *OsSWEET11*, which is the target of PthXo1, decreased by approximately 20% in IRBB5 compared to IR24 (Figure 3b), and was 68% and 60% higher than *OsSWEET14* expression in the incompatible combination of IRBB5 and ME2 with *avrXa7* or *pthXo3*, respectively. *OsSWEET11* expression due to *pthXo1* was approximately 36% and 32% higher than *OsSWEET14* expression due to *avrXa7* or *pthXo3*, respectively, in IR24 and the absence of *xa5* (Figure 3).

Transfer of *TFIIAγ5/Xa5* alters effector-dependent incompatibility to compatibility

IR24 and IRBB5 may differ at loci other than *xa5* due to the inexact nature of backcrossing. Therefore, the strict dependence of TAL effector-mediated virulence on *TFIIAγ* alleles (*Xa5* or *xa5*) was tested by introduction of the constitutive dominant allele of *xa5* (*Xa5*, also called *TFIIAγ5*) into to the recessive resistant rice line NipB5 (*xa5/xa5*), which was derived from a cross of Nipponbare (*Xa5*/*Xa5*) and IRBB5 (*xa5*/*xa5*). Transgenic NipB5 plants expressing *Xa5* under the control of the ubiquitin 1 promoter (OXγ5 lines 4 and 6) were generated, and tested for susceptibility to strains of ME2 expressing various major TAL effector genes. Similar to IR24 and IRBB5, the NipB5 and OXγ5 lines are susceptible to ME2 (*pthXo1*) as indicated by leaf reactions and the lesion length measurements (Figure 4a,b). However, the NipB5 rice line was resistant to strains ME2 (*avrXa7*) and ME2 (*pthXo3*), and the transgenic lines expressing *Xa5* under the control of the ubiquitin 1 promoter (OXγ5 lines 4

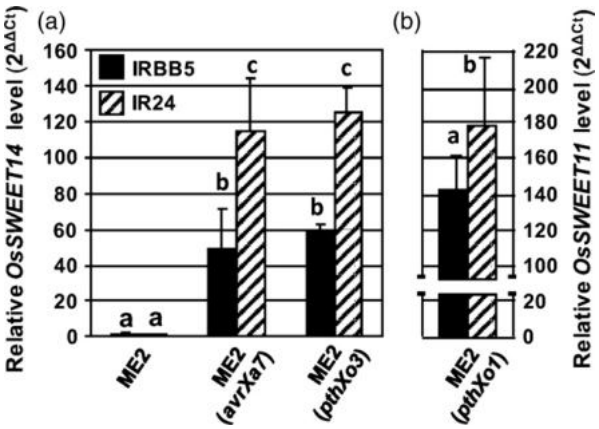

Figure 3. SWEET gene expression levels are correlated with strain compatibility and incompatibility on IRBB5.
The transcript levels of *OsSWEET14* (a) and *OsSWEET11* (b) activated by AvrXa7, PthXo3 or PthXo1 were measured by quantitative real-time RT-PCR in 5-week-old IR24 (*Xa5/Xa5*) and IRBB5 (xa5/*xa5*) plants 24 h after inoculation. Note the difference in scale between (a) and (b). Bars with the same letter do not differ from each other at $P < 0.05$ (Tukey test).

and 6) were susceptible (Figure 4a,b), indicating that susceptibility to strains other than those containing *pthXo1* is specifically dependent on expression of *TFIIAγ5* (*Xa5*). No differences in lesion lengths were observed between NipB5 and OXγ5 lines after inoculation with ME2 (*pthXo1*), which is predicted to be compatible on both lines (Figure 4b). *OsSWEET14* expression in NipB5 plantsplants after inoculation with ME2 (*avrXa7*) was reduced by approximately 60% in comparison with OXγ5 lines 4 and 6, which were susceptible to infection (Figure 4c).

Enhanced expression of *OsSWEET14* restores strain compatibility to IRBB5

To test whether qualitative or quantitative differences between S genes are responsible for the differences in compatibility of strains with various major TAL effector genes, designer TAL effector (dTALe) was constructed corresponding to an alternate DNA sequence from both AvrXa7 and PthXo3 (Figure 5a, dTALe-SWT14). The dTALe was designed with so-called optimized repeat variable diamino acid (RVD) repeats with the intention of enhancing *OsSWEET14* expression in comparison with the expression mediated by AvrXa7 or PthXo3. The dTALe binds 28 bp upstream of the effector binding site for AvrXa7 (Figure 5b). In two independent trials, *OsSWEET14* expression in IRBB5 upon inoculation by ME2 (dTALe-SWT14) as measured by quantitative real-time RT-PCR was approximately double that observed for ME2 (*avrXa7*) (Figure 5c). Concomitant with the higher expression levels of *OsSWEET14*, ME2 (dTALe-SWT14) was compatible on IRBB5, with lesion lengths in excess of 5 cm, and lesion lengths were comparable to those for ME2 (*avrXa7*) on IR24 and ME2 (*pthXo1*) on IRBB5 (Figure 5d). Conversely, two TAL effectors that target *OsSWEET11* at predicted alternative binding sites do not confer compatibility on IRBB5. PthXo4 and PthXo5 are RVD variants of AvrXa7 that were previously identified as having lost the ability to trigger resistance in rice lines expressing the R gene *Xa7*. Nonetheless, the variants retained the ability to confer virulence on ME2 (Yang *et al.*, 2005). Promoter binding predictions based on the RVD repeats of each variant indicate that the effectors probably target *OsSWEET11*, and microarray assays of SWEET gene

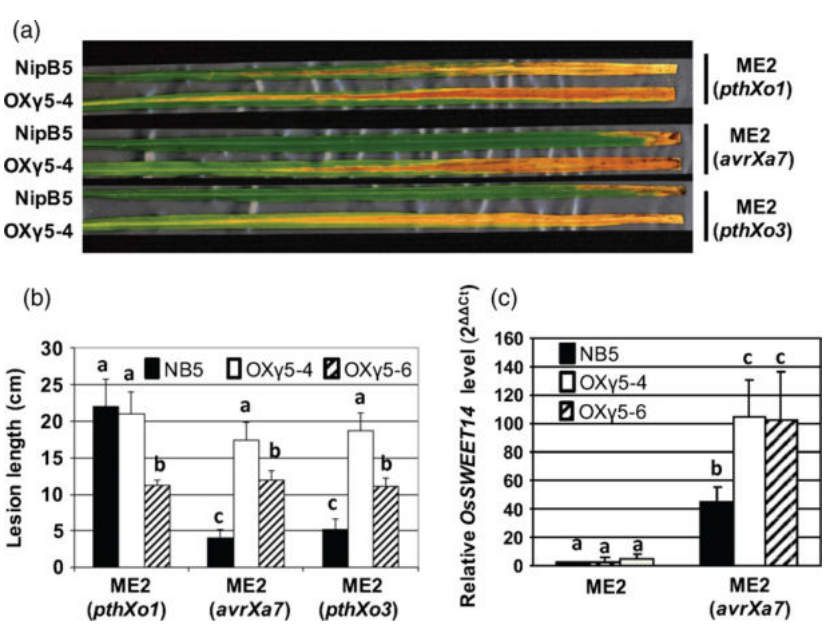

Figure 4. Compatibility on rice is specifically dependent on *Xa5* (*OsTFIIAγ5*).
(a) Symptomatic lesions on Nipponbare (*xa5/xa5*) (NipB5) and a derived *OsTFIIAγ5* transgenic plant (OXγ*5*). The strains and relevant gene are indicated on the right. The rice cultivar is indicated on the left.
(b) Lesion lengths caused by the indicated strains on rice leaves of *xa5* (NB5) and two independent *OsTFIIAγ5* (*Xa5*) transgenic lines (OXγ5 lines 4 and 6). Five-week old plants were inoculated, and lesion lengths were measured 13 days after inoculation.
(c) The expression level of *OsSWEET14* is correlated with the presence of *OsTFIIAγ5* allele *Xa5* versus *xa5* 24 h after inoculation of 5-week-old plants.
Bars with the same letter do not differ from each other at $P < 0.05$ (Tukey test).

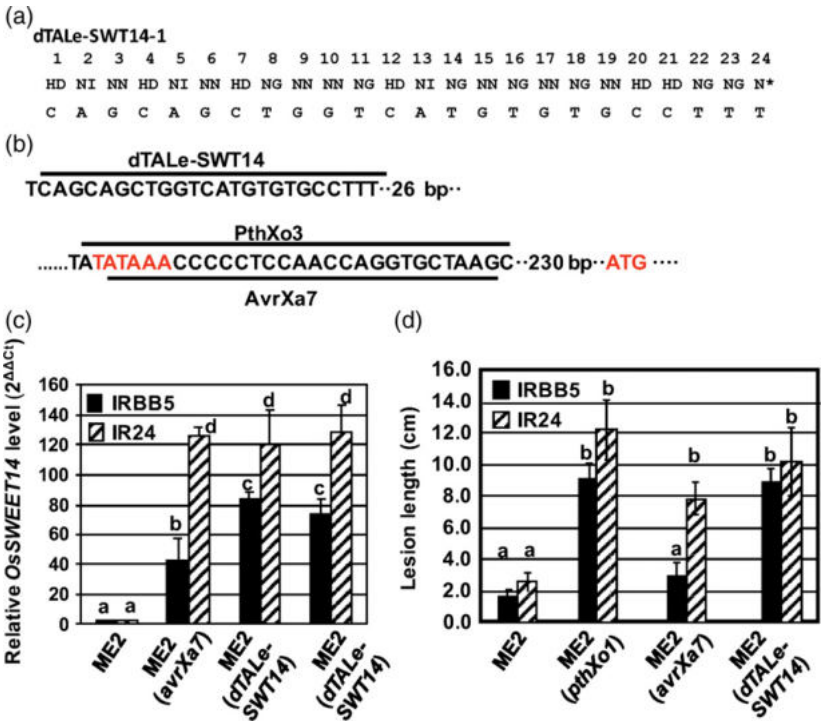

Figure 5. The designer TAL effector dTALe-SWT14 enhances *OsSWEET14* expression in IRBB5 and overcomes *xa5* resistance.
(a) dTALe-SWT14 was designed to target a 24 bp effector binding element (EBE) in the *OsSWEET14* promoter.
(b) Schematic of the *OsSWEET14* promoter, showing EBEs for dTALe-SWT14 and the natural TAL effectors PthXo3 and AvrXa7. The predicted TATAA binding protein motif (TATAA box) and start codon (ATG) are highlighted in red.
(c) Relative expression of *OsSWEET14* by quantitative real-time RT-PCR 24 h after inoculation of 5-week-old IR24 and IRBB5 plants.
(d) Lesion lengths of 5-week-old IR24 and IRBB5 plantsinoculated with the strains indicated below each column. The lesion lengths were measured 13 days after inoculation.
Bars with the same letter do not differ from each other at $P < 0.05$ (Tukey test).

expression confirmed this, although the expression levels were lower than expression levels for PthXo1 (Figure S1). In contrast to ME2 (*pthXo1*), ME2 expressing *pthXo4* or *pthXo5* is incompatible on IRBB5 and compatible on IR24 (Figure 6).

A combination of *xa5* and *xa13* confers broad and robust resistance to blight disease

Thirty-five strains of *X. oryzae* pv. *oryzae* from various geographic regions were tested for induction of *OsSWEET11*, *OsSWEET14* and *OsSWEET13* in IR24. All combinations of S gene induction were found, with the exception of strains capable of inducing both *OsSWEET11* and *OsSWEET13* (Table 1). No strains were identified that did not induce one of the three known S genes. The bacterial strains were tested in IR24 and IRBB53, the latter being the near-isogenic line of IR24 pyramided with *xa13* and *xa5* (Jeung *et al.*, 2006). The assay revealed broad resistance of IRBB53 against all the tested strains that were virulent on IR24 (Table 1).

DISCUSSION

Despite its broad effectiveness, the results indicate that the major recessive resistance gene *xa5* is dependent on the TAL effector gene content of *X. oryzae* pv. *oryzae*. All strains tested in this study that contained the major TAL effector gene *pthXo1* were compatible on IRBB5. The effect of *pthXo1* was tested by replacing it with other major TAL effector genes in PXO99^{A}, the strain from which the gene originated, and by introducing *pthXo1* into field strains that express other major TAL effector genes. Replacement of *pthXo1* by *pthXo2*, *pthXo3* or *avrXa7* in PXO99^{A} results in loss of compatibility on IRBB5. Conversely, compatibility on IRBB5 was observed if *pthXo1* was introduced into strains that are otherwise incompatible on IRBB5. In the latter cases, the effect of *pthXo1* is dominant over other major effectors present in the strain. IR24 and IRBB5 are so-called near-isogenic lines, meaning that IRBB5 is the progeny of extended backcrossing of the *xa5* donor and the recurrent parent IR24. However, near-isogenic lines do not generally differ by a single gene, and may show other co-incidental chromosomal introgression from the donor line; thus we were concerned that compatibility may be affected by other unknown genes in the IRBB5 background. We demonstrated that the effect of major TAL effector gene content is indeed dependent on alleles of *TFIIAγ5*, by addition of the dominant allele *Xa5* to an introgression line of the more easily transformable line Nipponbare expressing *xa5* (NipB5). NipB5 differs from lines OXγ5 lines 4 and 6 only in that the latter have the wild-type copy of *TFIIAγ5* or dominant *Xa5*. Nonetheless, strain ME2 containing either *pthXo1*, *avrXa7* or *pthXo3* is compatible on lines ectopically expressing *Xa5*, while ME2 containing *avrXa7* or *pthXo3* is incompatible on NipB5.

The results also indicate that *xa5* effectiveness is not strictly dependent on the specific SWEET gene, but that levels of the sugar transporter product must reach some threshold level for effectiveness. The expression levels of *OsSWEET11* mRNA as mediated by PthXo1 are exceptionally high, significantly higher than the relative levels of

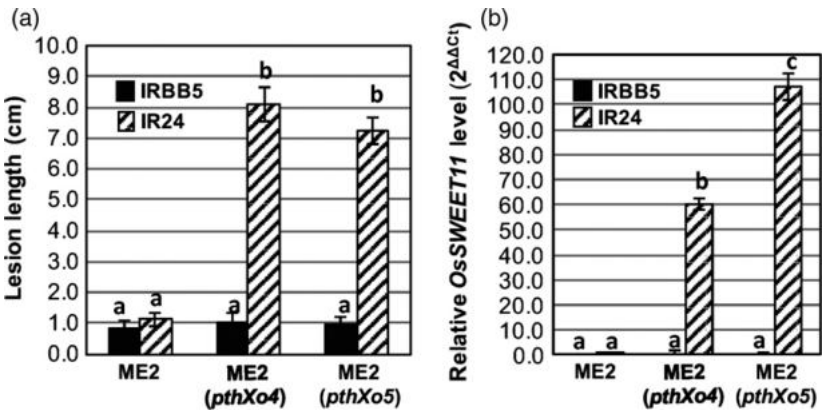

Figure 6. Strains with PthXo4 and PthXo5 target *OsSWEET11* and are incompatible on IRBB5.
(a) Lesion lengths for IRBB5 and IR24 leaves were measured 7 days after inoculation of 3-week-old plants with the strains indicated below each column.
(b) Relative expression level of *OsSWEET11* by quantitative real-time RT-PCR 24 h after inoculation of 3-week-old plants.
Bars with the same letter do not differ from each other at $P < 0.05$ (Tukey test).

OsSWEET14 mRNA as mediated by PthXo3 or AvrXa7, as indicated by quantitative real-time RT-PCR. Higher expression levels of *OsSWEET11* in comparison to *OsSWEET14* are also observed in both IR24 and IRBB5. Nonetheless, SWEET gene expression is elevated in both compatible and incompatible strain/host combinations. Further evidence that compatibility to *xa5* is mediated by levels of SWEET gene expression, regardless of the specific SWEET gene, was obtained by use of a dTALe to boost expression of *OsSWEET14*. Native major TAL effectors do not necessarily display the most optimal structure for binding to the S gene promoters. Increasing *OsSWEET14* expression above the levels that occur naturally with PthXo3 or AvrXa7 in the presence of *xa5* mimicked the phenotypic effect of PthXo1, and resulted in strain compatibility on IRBB5. As for the native major TAL effectors, S gene expression levels in the presence of dTALe-SWT14 are lower in IRBB5 than in IR24, showing a general dampening of gene expression in the *xa5* background. High levels of expression of either *OsSWEET11*, as induced by PthXo1, or *OsSWEET14*, as mediated by dTALe-SWT14, correlated with compatibility on plants homozygous for *xa5*. Conversely, variants of AvrXa7 that target *OsSWEET11* but do not induce the gene to the levels observed with PthXo1 did not confer compatibility on IRBB5, supporting a model whereby SWEET transporter levels are required to reach a threshold amount for disease to occur.

An alternative means to defeat *xa5* may be to induce two SWEET genes simultaneously, with the additive effect allowing the levels to surpass the hypothetical threshold. However, strain T7174 has two major TAL effectors, PthXo2 and AvrXa7, targeting *OsSWEET13* and *OsSWEET14*, respectively, but the strain is incompatible on *xa5*-expressing plants. In addition, 14 of the 34 strains that were tested for S gene induction induced both *OsSWEET12* and *OsSWEET13*. However, all of the 14 strains were incompatible on IRBB53, which is homozygous for *xa5*. The lack of effectiveness of dual S gene expression may indicate that mRNA expression levels in themselves do not correspond directly to the levels of SWEET protein. Further work on actual SWEET product levels in relation to *xa5* compatibility is required before firm conclusions may be drawn regarding levels of SWEET gene expression and susceptibility. PthXo1 is the only known native major TAL effector that mediates compatibility on IRBB5. Evidence from strain surveys also indicated that PthXo1 is an important effector in the field, as strains that are reported to be compatible on *xa5*-expressing plants also tend to be incompatible on plants that are homozygous for *xa13*, which is an allele that affects the PthXo1 binding site (Mishra *et al.*, 2013). Deployment of both *xa13* and *xa5* targets both *xa5*-compatible and *xa5*-incompatible classes of TAL effectors and provides even broader resistance than *xa5* alone.

The requirement for high levels of SWEET gene induction has implications for the role played by SWEET genes in disease susceptibility. All of the three known S genes for bacterial blight in the field are members of clade III of sugar transporters (Yang *et al.*, 2006; Antony *et al.*, 2010; Yu *et al.*, 2011; Streubel *et al.*, 2013; Zhou *et al.*, 2015). Two additional members of clade III are also capable of functioning as S genes for rice bacterial blight but have not been found to be associated with a native major TAL effector (Streubel *et al.*, 2013). Clade III members are predicted to transport sucrose preferentially in comparison to glucose. If the results with *OsSWEET11* and *OsSWEET14* may be extrapolated to all clade III members, then, regardless of the member, expression levels must reach a threshold for susceptibility. The exact level of the threshold remains unknown, nor is the precise mechanism by which the SWEET transporters enhance susceptibility known. In addition to the level of expression, individual SWEET transporters may have varying intrinsic effects on susceptibility. One hypothesis for the function of sugar transporters in disease is in release of sugars into extracellular spaces for utilization by the pathogen for growth. In this scenario, high levels of sugar transporters (e.g. SWEET proteins) may ensure release of internal cellular stores of sucrose in the face of competition with mechanisms of the host plant that clear sugars from extracellular compartments. Alternatively, high levels of the transporters may

Table 1 S gene induction and *xa13/xa5* compatibility for strains of *Xanthomonas oryzae* pv. *oryzae*

Country of origin	Strain	*OsSWEET11*	*OsSWEET13*	*OsSWEET14*	IR24[a]	IRBB53[b]
The Philippines	PXO61			+	S	R
	PXO71	+		+	S	R
	PXO86			+	S	R
	PXO99^{A}	+			S	R
	PXO112	+	+	+	S	R
	PXO125		+		S	R
Republic of Korea	KXO85			+	S	R
	JW89011			+	S	R
	K202		+	+	S	R
Japan	T7174		+	+	S	R
	H75373			+	S	R
Thailand	Xoo2	+	+	+	S	R
India	A3842	+			S	R
	A3857	+			S	R
	PbXo7	+			S	R
Indonesia	IXO56		+	+	S	R
Nepal	NXO260	+			S	R
Colombia	CIAT1185	+			S	R
China	ZHE 173		+	+	S	R
	C1		+	+	S	R
	C3			+	S	R
	C4			+	S	R
	C5			+	S	R
	C6			+	S	R
	C7		+	+	S	R
	GD1358		+	+	S	R
	HB17		+	+	S	R
	HB21			+	S	R
	HLJ72		+	+	S	R
	JS49-6		+	+	S	R
	LN57			+	S	R
	NX42		+	+	S	R
Australia	Aust-R3			+	S	R
Cameroon	AXO1947			+	S	R

Plus symbols indicate induction of the gene by the presence of the strains.
[a]Disease reaction is characterized as 'S', indicating susceptible to bacterial infection, when lesion lengths were >5 cm 12 days after inoculation.
[b]Disease reaction is characterized as 'R', indicating resistant to bacterial infection, when lesion lengths were <2 cm at 12 days after inoculation.

also interfere with normal membrane function or sucrose signaling pathways.

Proposed models of *xa5* action must accommodate previous findings. A TAL effector called AvrXa5 from a strain that is incompatible on *xa5* converted an *xa5*-compatible strain to incompatibility on IRBB5 plants (Zou *et al.*, 2010). We suggest that these results may be due to the presence of an additional R gene in IRBB5 that is co-incidental to the action of *xa5*. In addition, PXO99^{A} also has a TAL effector, PthXo6, that induces expression of *TFIIAγ1*, which is a second *TFIIAγ* gene in rice (Sugio *et al.*, 2007). However, PthXo6 only has a small effect on virulence in *xa5*-incompatible strains, in agreement with our results showing that PthXo1 plays the dominant role in PXO99^{A} compatibility on *xa5*-expressing plants. Strain PXO71, which harbors PthXo1, does not induce expression of *TFIIAγ1* (Sugio *et al.*, 2007). In fact, PXO99^{A} is the only strain known to harbor PthXo6. The function of PthXo6, or the possible need for two *TFIIAγ* genes in rice, for that matter, remain unknown.

The results do not provide information on the exact mechanism of the effect of TFIIAγ5 on TAL effector function. The *xa5* product shows an amino acid substitution, which may alter interaction of the transcription pre-initiation complex with the potent TAL effector activation domain and affect TAL effector efficiency for gene expression or, alternatively, reduce interaction with other components of the pre-initiation complex and have a general effect on activated host transcription that is not specific to TAL effectors. The latter possibility raises the concern that there may be a cost to the plant in some other respect, for example loss of yield, susceptibility to abiotic stress or

other pathogens. Fitness trade-offs have been identified with other recessive genes in terms of enhanced susceptibility to other pathogens, lesion-mimic phenotypes, and yield losses (McGrann *et al.*, 2015). No yield costs have been reported for expression of *xa5*. Deployment of *xa5* does have the potential to reduce the effectiveness of certain forms of dominant TAL effector-dependent resistance. *Xa27* and *Xa10* are dominant R genes for bacterial blight of rice, and their expression is dependent on TAL effectors AvrXa27 and AvrXa10, respectively, upon infection by bacteria containing the genes for the effectors (Gu *et al.*, 2005; Tian *et al.*, 2014). The presence of *xa5* dampens expression of the R genes but does not eliminate the resistance response (Gu *et al.*, 2009; Tian *et al.*, 2014). At present, R genes that require TAL effector binding and promotion are not known to be widely deployed in rice, and the effect of *xa5* expression on this type of resistance in the field is untested.

EXPERIMENTAL PROCEDURES

Plant material, plasmids and bacterial strains

Seeds of rice variety Nipponbare (*Oryza sativa* ssp. *japonica*, accession PI 514663) were provided by the US Department of Agriculture/Agricultural Research Service National Small Grains Collection. Seeds for IR24 and IRBB5 (both *O. sativa* ssp. *indica*) were obtained from the International Rice Research Institute (courtesy of Casiana vera Cruz). All rice plants were grown in growth chambers with a temperature of 28°C, relative humidity 85%, and a photoperiod of 12 h. *Escherichia coli* was grown in Luria–Bertani medium using a standard culture technique (Ausubel *et al.*, 1993). *X. oryzae* pv. *oryzae* cells were grown in either nutrient broth (Becton Dickinson, www.bd.com) or tryptone sucrose medium (10 g L^{-1} tryptone, 10 g L^{-1} sucrose, 1 g L^{-1} glutamic acid) at 28°C. The antibiotics used were carbenicillin (100 mg L^{-1}), kanamycin (50 mg L^{-1}), and spectinomycin (100 mg L^{-1}). The plasmids and *X. oryzae* pv. *oryzae*/*E. coli* strains used are listed in Table S1.

Gene expression analyses

The second youngest rice leaf of 3- or 5-week old plants was inoculated with the indicated bacterial strains, and inoculated portions were sampled for total RNA extraction at the indicated time points. Bacterial were re-suspended in sterile distilled water at an OD_{600} of 0.5, and infiltrated into rice leaves using a needle-less syringe. The RNA extraction buffer used was TRIzol® reagent (Thermo Fisher Scientific, ww.thermofisher.com). The RNA concentration and quality were measured using an ND-1000 Nanodrop spectrophotometer (Nanodrop Technologies, www.nanodropcom). Quantitative real-time RT-PCR was performed using 1 μg RNA from each sample after treatment with amplification-grade DNase I (Thermo Fisher Scientific), and cDNA synthesis was performed using an iScript Select cDNA synthesis kit (Bio-Rad, www.bio-rad.com). cDNA derived from 25 ng total RNA was used for each quantitative real-time RT-PCR reaction with gene-specific primers. The actin gene was amplified as a control, using primers OsActin-F and OsActin-R. Quantitative real-time RT-PCR was performed on an Mx4000 multiplex quantitative PCR system (Thermo Fisher Scientific) using an iQ™ SYBR Green Supermix kit (Bio-Rad). The mean threshold cycle (C_T) from three samples was used to determine the fold change of gene expression. The $2^{\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001) was used for quantification. The primer sequences are provided in Table S2.

For microarray hybridization, 5 μg total RNA for each sample was used for synthesis of cDNA and biotin-labeled cRNA using a One-Cycle eukaryotic target labeling kit (Affymetrix, www.affymetrix.com) according to the manufacturer's instructions. The processed cRNA was used for hybridization to a GeneChip rice genome array (Affymetrix), which was processed at the Iowa State University DNA Core Facility.

Rice transformation and gene construction

For construction of OsTFIIc5 with the maize ubiquitin promoter, primers TF2OX-F and TF2OX-R were used to amplify the coding region of *TFIIγ5* from the Nipponbare cDNA, and the PCR amplicon was first cloned into pENTR/D-TOPO (Thermo Fisher Scientific) and sequenced for accuracy before cloning into pUbi at the *Eco*RI and *Bam*HI sites. pUbi is pCAMBIA1300 modified by insertion of an over-expression cassette comprising the maize ubiquitin 1 promoter and NOS terminator (Christensen *et al.*, 1992; www.cambia.org). The construct, named OXc5, was electroporated into *Agrobacterium tumefaciens* strain EHA105. Calli from immature embryos of rice line NipB5 (*xa5/xa5*) were initiated and transformed using *Agrobacterium tumefaciens* as described previously (Hiei *et al.*, 1994).

Disease assays

Three- or five-week-old plants with fully expanded leaves were inoculated using the leaf clipping method for lesion measurement (Kauffman *et al.*, 1973). Bacterial inoculum with an OD_{600} of 0.5 (approximately 5.0×10^7 cfu per ml) was used for inoculation. Symptoms were scored by measuring lesion length. One-way analysis of variance (ANOVA) statistical analyses were performed on all measurements. The Tukey honest significant difference test was used for post-ANOVA pairwise tests for significance, set at 5% ($P < 0.05$).

Construction of the designer TAL effector

A library of four basic repeats derived from *avrXa7* encoding RVDs NI, NG, NN and HD corresponding to nucleotides A, T, G and C, respectively, was used to assemble the TAL effector central repeats based on the DNA sequence of the target site as described previously (Li *et al.*, 2013). The repetitive fragment was cloned into a repeat-deleted version of pZWavrXa10, resulting in a synthetic gene for dTALe-SWT14 consisting of the 5′ and 3′ coding sequences of *avrXa10* and the custom-made repeat domain (Li *et al.*, 2013). The dTALe-SWT14 fragment was linearized using *Hin*dIII and ligated into pHM1 (Yang and White, 2004).

ACKNOWLEDGEMENTS

This research was supported by US National Science Foundation research grants 2012-1238189 (to F.F.W. and B.Y.), IOS-1258103 (to B.Y.) and IOS-1258028 (to F.W.). The authors thank Pete Lelonek for maintaining greenhouse and growth chamber spaces.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1. PthXo4 and PthXo5 target *OsSWEET11*.

Table S1. Strains and plasmids used in the study.

Table S2. Primers and sequences used in the study.

REFERENCES

Acevedo-Garcia, J., Kusch, S. and Panstruga, R. (2014) Magical mystery tour: MLO proteins in plant immunity and beyond. *New Phytol.* **204**, 273–281.

Antony, G., Zhou, J., Huang, S., Li, T., Liu, B., White, F. and Yang, B. (2010) Rice *xa13* recessive resistance to bacterial blight is defeated by the induction of disease susceptibility gene *Os-11N3*. *Plant Cell*, **22**, 3864–3876.

Ausubel, F., Brent, R., Kingston, R., Moore, D., Seidman, J. and Struhl, K. (1993) *Current Protocols in Molecular Biology*. New York: John Wiley and Sons.

Boch, J. and Bonas, U. (2010) *Xanthomonas* AvrBs3 family-type III effectors: discovery and function. *Annu. Rev. Phytopathol.* **48**, 419–436.

Chen, L.Q., Hou, B.H., Lalonde, S. *et al.* (2010) Sugar transporters for intercellular exchange and nutrition of pathogens. *Nature*, **468**, 527–532.

Chen, L.Q., Qu, X.Q., Hou, B.H., Sosso, D., Osorio, S., Fernie, A.R. and Frommer, W.B. (2012) Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. *Science*, **335**, 207–211.

Christensen, A.H., Sharrock, R.A. and Quail, P.H. (1992) Maize polyubiquitin genes: structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplasts by electroporation. *Plant Mol Biol.*, **18**, 675–689.

Chu, Z., Yuan, M., Yao, J. *et al.* (2006) Promoter mutations of an essential gene for pollen development result in disease resistance in rice. *Genes Dev.* **20**, 1250–1255.

Doyle, E.L., Stoddard, B.L., Voytas, D.F. and Bogdanove, A.J. (2013) TAL effectors: highly adaptable phytobacterial virulence factors and readily engineered DNA-targeting proteins. *Trends Cell Biol.* **23**, 390–398.

Garris, A.J., McCouch, S.R. and Kresovich, S. (2003) Population structure and its effect on haplotype diversity and linkage disequilibrium surrounding the *xa5* locus of rice (*Oryza sativa* L). *Genetics*, **165**, 759–776.

Gu, K., Yang, B., Tian, D. *et al.* (2005) R gene expression induced by a type-III effector triggers disease resistance in rice. *Nature*, **435**, 1122–1125.

Gu, K., Tian, D., Qiu, C. and Yin, Z. (2009) Transcription activator-like type III effector AvrXa27 depends on OsTFIIAγ5 for the activation of *Xa27* transcription in rice that triggers disease resistance to *Xanthomonas oryzae* pv. *oryzae*. *Mol. Plant Pathol.* **10**, 829–835.

Hiei, Y., Ohta, S., Komari, T. and Kumashiro, T. (1994) Efficient transformation of rice (Oryza sativa L.) mediated by Agrobacterium and sequence analysis of the boundaries of the T-DNA. *Plant J.* **6**, 271–282.

Hopkins, C.M., White, F.F., Choi, S.H., Guo, A. and Leach, J.E. (1992) Identification of a family of avirulence genes from *Xanthomonas oryzae* pv. *oryzae*. *Mol. Plant-Microbe Interact.* **5**, 451–459.

Huang, N., Angeles, E.R., Domingo, J., Magpantay, G., Singh, S., Zhang, G., Kumaravadivel, N., Bennett, J. and Khush, G.S. (1997) Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. *Theor. Appl. Genet.* **95**, 313–320.

Iyer, A.S. and McCouch, S.R. (2004) The rice bacterial blight resistance gene *xa5* encodes a novel form of disease resistance. *Mol. Plant Microbe Interact.* **17**, 1348–1354.

Jeung, J.U., Heu, S.G., Shin, M.S., Vera Cruz, C.M. and Jena, K.K. (2006) Dynamics of xanthomonas oryzae pv. oryzae populations in Korea and their relationship to known bacterial blight resistance genes. *Phytopathology.*, **96**, 867–875.

Jiang, G.H., Xia, Z.H., Zhou, Y.L., Wan, J., Li, D.Y., Chen, R.S., Zhai, W.X. and Zhu, L.H. (2006) Testifying the rice bacterial blight resistance gene *xa5* by genetic complementation and further analyzing *xa5* (*Xa5*) in comparison with its homolog TFIIAγ1. *Mol. Genet. Genomics*, **275**, 354–366.

Kauffman, H.E., Redd, A.P.K., Hsiek, S.P. and Marca, S.D. (1973) An improved technique for evaluating resistance of race varieties to *Xanthomonas oryzae*. *Plant. Dis. Rep.* **57**, 537–541.

Li, T., Huang, S., Zhou, J. and Yang, B. (2013) Designer TAL effectors induce disease susceptibility and resistance to *Xanthomonas oryzae* pv. *oryzae* in rice. *Mol. Plant.* **6**, 781–789.

Liu, Q., Yuan, M., Zhou, Y., Li, X., Xiao, J. and Wang, S. (2011) A paralog of the MtN3/saliva family recessively confers race-specific resistance to *Xanthomonas oryzae* in rice. *Plant, Cell Environ.* **34**, 1958–1969.

Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods*, **25**, 402–408.

McGrann, G.R., Steed, A., Burt, C., Nicolson, P. and Brown, J.K. (2015) Differential effects of lesion mimic mutants in barley on disease development by facultative pathogens. *J. Exp. Bot.*, **66**, 3417–3428.

Mishra, D., Vishnupriya, M.R., Anil, M.G., Konda, K., Raj, Y. and Sonti, R.V. (2013) Pathotype and genetic diversity amongst Indian isolates of *Xanthomonas oryzae* pv. *oryzae*. *PLoS ONE*, **8**, e81996.

Ochiai, H., Inoue, Y., Takeya, M., Sasaki, A. and Kaku, H. (2005) Genome sequence of *Xanthomonas oryzae* pv. *oryzae* suggests contribution of large numbers of effector genes and insertion sequences to its race diversity. *Jpn. Agric. Res. Q.* **39**, 275–287.

Ozer, J., Bolden, A.H. and Lieberman, P.M. (1996) Transcription factor IIA mutations show activator-specific defects and reveal a IIA function distinct from stimulation of TBP–DNA binding. *J. Biol. Chem.* **271**, 11182–11190.

Römer, P., Recht, S., Strauss, T., Elsaesser, J., Schornack, S., Boch, J., Wang, S. and Lahaye, T. (2010) Promoter elements of rice susceptibility genes are bound and activated by specific TAL effectors from the bacterial blight pathogen, Xanthomonas oryzae pv. oryzae. *New Phytol.* **187**, 1048–1057.

Sanfaçon, H. (2015) Plant translation factors and virus resistance. *Viruses*, **7**, 3392–4319.

van Schie, C.C. and Takken, F.L. (2014) Susceptibility genes 101: how to be a good host. *Annu. Rev. Phytopathol.* **52**, 551–581.

Streubel, J., Pesce, C., Hutin, M., Koebnik, R., Boch, J. and Szurek, B. (2013) Five phylogenetically close rice *SWEET* genes confer TAL effector-mediated susceptibility to *Xanthomonas oryzae* pv. *oryzae*. *New Phytol.* **200**, 808–819.

Sugio, A., Yang, B., Zhu, T. and White, F.F. (2007) Two type III effector genes of *Xanthomonas oryzae* pv. *oryzae* control the induction of the host genes *OsTFIIAγ1* and *OsTFX1* during bacterial blight of rice. *Proc. Natl Acad. Sci. USA*, **104**, 10720–10725.

Szurek, B., Marois, E., Bonas, U. and Van den Ackerveken, G. (2001) Eukaryotic features of the *Xanthomonas* type III effector AvrBs3: protein domains involved in transcriptional activation and the interaction with nuclear import receptors from pepper. *Plant J.* **26**, 523–534.

Tian, D., Wang, J., Zeng, X. *et al.* (2014) The rice TAL effector-dependent resistance protein XA10 triggers cell death and calcium depletion in the endoplasmic reticulum. *Plant Cell*, **26**, 497–515.

Yang, B. and White, F.F. (2004) Diverse members of the AvrBs3/PthA family of type III effectors are major virulence determinants in bacterial blight disease of rice. *Mol. Plant Microbe Interact.* **17**, 1192–1200.

Yang, B., Sugio, A. and White, F.F. (2005) Avoidance of host recognition by alterations in the repetitive and C-terminal regions of AvrXa7, a type III effector of *Xanthomonas oryzae* pv. *oryzae*. *Mol. Plant Microbe Interact.* **18**, 142–149.

Yang, B., Sugio, A. and White, F.F. (2006) *Os8N3* is a host disease susceptibility gene for bacterial blight of rice. *Proc. Natl Acad. Sci. USA*, **103**, 10503–10508.

Yu, Y., Streubel, J., Balzergue, S., Champion, A., Boch, J., Koebnik, R., Feng, J., Verdier, V. and Szurek, B. (2011) Colonization of rice leaf blades by an African strain of *Xanthomonas oryzae* pv. *oryzae* depends on a new TAL effector that induces the rice nodulin-3 *Os11N3* gene. *Mol. Plant Microbe Interact.*, **24**, 1102–1113.

Yuan, M. and Wang, S. (2013) Rice MtN3/saliva/SWEET family genes and their homologs in cellular organisms. *Mol. Plant*, **6**, 665–674.

Yudkovsky, N., Ranish, J.A. and Hahn, S. (2000) A transcription re-initiation intermediate that is stabilized by activator. *Nature*, **408**, 225–229.

Zhou, J., Peng, A., Long, J. *et al.* (2015) Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice. *Plant J.* **82**, 632–643.

Zhu, W., Yang, B., Chittoor, J.M., Johnson, L.B. and White, F.F. (1998) AvrXa10 contains an acidic transcriptional activation domain in the functionally conserved C-terminus. *Mol. Plant Microbe Interact.* **11**, 824–832.

Zhu, W., Yang, B., Wills, N., Johnson, L.B. and White, F.F. (1999) The C-terminus of AvrXa10 can be replaced by the transcriptional activation domain of VP16 from the herpes simplex virus. *Plant Cell*, **11**, 1665–1674.

Zou, H., Zhao, W., Zhang, X., Han, Y., Zou, L. and Chen, G. (2010) Identification of an avirulence gene, *avrxa5*, from the rice pathogen *Xanthomonas oryzae* pv. *oryzae*. *Sci. China Life Sci.* **53**, 1440–1449.