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A resistance locus in the American heirloom rice variety Carolina Gold Select is triggered by TAL effectors with diverse predicted targets and is effective against African strains of *Xanthomonas oryzae* pv. *oryzicola*

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Summary

The rice pathogens *Xanthomonas oryzae* pathovar (pv.) *oryzae* and pv. *oryzicola* produce numerous transcription activator-like (TAL) effectors that increase bacterial virulence by activating expression of host susceptibility genes. Rice resistance mechanisms against TAL effectors include polymorphisms that prevent effector binding to susceptibility gene promoters, or that allow effector activation of resistance genes. This study identifies, in the heirloom variety Carolina Gold Select, a third mechanism of rice resistance involving TAL effectors. This resistance manifests through strong suppression of disease development in response to diverse TAL effectors from both *X. oryzae* pathovars. The resistance can be triggered by an effector with only 3.5 central repeats, is independent of the composition of the repeat variable diresidues that determine TAL effector

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binding specificity, and is independent of the transcriptional activation domain. We determined that the resistance is conferred by a single dominant locus, designated *Xo1*, that maps to a 1.09 Mbp fragment on chromosome 4. The *Xo1* interval also confers complete resistance to the strains in the African clade of *X. oryzae* pv. *oryzicola*, representing the first dominant resistance locus against bacterial leaf streak in rice. The strong phenotypic similarity between the TAL effector triggered resistance conferred by *Xo1* and that conferred by the tomato resistance gene *Bs4* suggests that monocots and dicots share an ancient or convergently evolved mechanism to recognize analogous TAL effector epitopes.

Keywords

TAL effector; Carolina Gold Select; *Xanthomonas oryzae*; Bs4; *Oryza sativa*; bacterial leaf blight; bacterial leaf streak

Introduction

The bacterial species *Xanthomonas oryzae* comprises pathovar (pv.) *oryzae*, which causes bacterial blight (BB) of rice (*Oryza sativa* L.), and pv. *oryzicola*, which causes bacterial leaf streak of rice (BLS). Both diseases are globally distributed and cause significant yield losses. Numerous resistance (*R*) genes have been identified that confer complete resistance to BB; however, only one recessive, race-specific resistance locus to BLS, tentatively named *bls1*, has been mapped (He *et al.* 2012). BLS is an important crop threat in Asia and Africa (Verdier *et al.* 2012b), and sources of resistance are highly sought.

The genomes of African and Asian strains of *X. oryzae* contain between eight and 26 genes encoding transcription activator-like (TAL) effectors (Bogdanove *et al.* 2011, White *et al.* 2009). TAL effectors bind host DNA to activate gene transcription, and DNA binding specificity is mediated by a central repeat region (CRR) made up of a variable number of repeats, each 34 amino acids in length. Some repeats from the *X. gardneri* effector AvrHah1 have 35 amino acids. The sequence of each repeat is nearly identical to the others except in the repeat variable residue (RVD), the pair of hypervariable residues at positions 12 and 13. Each RVD specifies a corresponding nucleotide in the host promoter, such that the RVD composition determines which nucleotide sequences are targeted in the host (Boch *et al.* 2009, Moscou and Bogdanove 2009). A C-terminal activation domain (AD) activates host gene transcription.

Several TAL effectors play a role in *X. oryzae* pv. *oryzae* virulence (Boch and Bonas 2010). Among these, TAL effectors that activate transcription of a group of sugar transporter genes called SWEET genes are particularly effective as virulence factors, hypothetically by increasing the extracellular concentration of carbohydrates to favor bacterial growth (Chen *et al.* 2012). A TAL effector can also act as an avirulence factor in rice varieties where a corresponding binding site lies upstream of a dominant resistance (*R*) gene. The rice resistance genes *Xa10*, *Xa23*, and *Xa27* are expressed after activation by corresponding specific TAL effectors, resulting in hypersensitive response (HR) and cell death (Gu *et al.* 2005, Hopkins *et al.* 1992, Wang *et al.* 2015). A second mechanism of rice resistance to TAL effectors is genetically recessive and results from escape of effector-induced susceptibility,

occurring if both copies of a host promoter have polymorphisms that prevent TAL effector binding. For example, a mutation in the TAL effector binding site upstream of the SWEET gene *OsSWEET11* prevents the increase in susceptibility mediated by the corresponding TAL effector, PthXo1 (Yang *et al.* 2006).

In addition to the two mechanisms of TAL effector-targeted resistance characterized in rice, a third mechanism, conferred by the nucleotide-binding leucine-rich repeat (NLR) resistance protein Bs4 in tomato, is independent of direct gene activation by the effector. Unlike Xa10 and Xa27, Bs4 recognizes even a severely truncated version of its corresponding effector, AvrBs4, containing the N-terminal signal sequence and only three repeats of the CRR, without the AD and nuclear localization signals (Schornack *et al.* 2004). Bs4 also exhibits broad specificity, triggering defense in response to the effectors Hax3 and Hax4 (Kay *et al.* 2005), in addition to the effector AvrBs3 expressed to high levels *in planta* (Schornack *et al.* 2005). Thus, it was hypothesized that Bs4 recognizes a general feature of the TAL effector CRR in the cytoplasm. The mechanism of TAL effector targeted resistance exemplfied by Bs4 is so far rare: to date, no other *R* protein has been found that recognizes inactive TAL effectors.

Although TAL effectors are central to the virulence of *X. oryzae* pathovars, strains of *X. oryzae* isolated from rice in the United States have none. The US *X. oryzae* strains form a clade phylogenetically distinct from *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*, and cause weak BB-like symptoms (Jones *et al.* 1989, Triplett *et al.* 2011). Heterologous expression of selected TAL effectors in US *X. oryzae* strain X11-5A caused a substantial increase in virulence on 14 of 21 diverse rice varieties, supporting the hypothesis that the lack of TAL effectors underlies the weak pathogenicity of the US strains (Verdier *et al.* 2012a). Given the strong fitness advantage conferred by TAL effectors and the widespread distribution of *X. oryzae* in the rice-producing world, it is intriguing that TAL effector-producing *X. oryzae* strains have never been identified in the US. It is possible that populations of US *X. oryzae* have been geographically isolated from populations of TAL effector-harboring bacteria, preventing horizontal transfer. Alternately, TAL effectors might have conferred a virulence disadvantage on rice genotypes originally introduced to the US, restricting their acquisition or causing their loss.

In this paper, we describe the identification and mapping of a resistance gene in Carolina Gold Select, a genetically purified line of the heirloom rice variety Carolina Gold, that might explain the absence of TAL effectors in US strains of *X. oryzae*. Carolina Gold is thought to be one of the earliest varieties of rice cultivated in colonial America, grown from the early 18th century (Schulze 2012). Valued for disease resistance and grain cooking quality traits, it was the dominant US variety for well over a century, playing a significant role in the early American economy and cuisine (Schulze 2012). Carolina Gold is the progenitor of several modern rice cultivars, including Dawn, Lemont and Gulfmont, as well as the aromatic hybrid Charleston Gold (Durham and Avant 2011). We found that diverse *X. oryzae* TAL effectors, including variants lacking an activation domain or lacking all but 3.5 repeats, triggered resistance on Carolina Gold Select rice when expressed and delivered heterologously by *X. oryzae* X11-5A. The Bs4-like resistance is conferred by a single dominant locus that maps to a 1.09 Mb interval on chromosome 4 and co-segregates with

complete resistance to isolates of *X. oryzae* pv. *oryzicola* from Africa, indicating its potential for deployment there to control bacterial leaf streak.

Results

Diverse TAL effectors from *X. oryzae pv. oryzae* and *X. oryzae pv. oryzicola*, but not from *X. gardneri* or *X. axonopodis*, trigger resistance on Carolina Gold Select rice

Our previous work demonstrated that diverse TAL effectors are expressed and translocated from the US strain *X. oryzae* X11-5A, and that several TAL effectors augment virulence in this strain in many rice varieties (Verdier *et al.* 2012a). To determine whether TAL effectors confer a selective disadvantage to strains inoculated to US heirloom rice, Carolina Gold Select was inoculated with *X. oryzae* X11-5A and X11-5A transformants expressing constructs based on nine different *X. oryzae* TAL effectors, cloned into the high-copy vectors pKEB31 or pSKX1. Three of these constructs, *avrXa7CRR*, *avrXa10CRR*, and *pthXo1CRR*, consist of the CRR-containing SphI fragment of the effector gene of interest cloned in the context of the *tal1C* effector gene of *X. oryzae* pv. *oryzicola* strain BLS256. The other six constructs express either the full native TAL effector gene or the BamHI fragment, encoding all but 17 C-terminal amino acids of the native TAL effector, cloned into *tal1C*. These constructs will be heretofore referred to by the names of the TAL effector genes (Table 1). The TAL effectors tested in X11-5A have CRRs with diverse origins and target specificities and include those of *pthXo1*, *avrXa7*, and *avrXa10* from Asian *X. oryzae pv. oryzae*, *talC* and *tal5* from African *X. oryzae pv. oryzae*, and *tal1c*, *tal2a*, *tal8*, and *tal2g* from *X. oryzae pv. oryzicola* (Table 1).

Following clip inoculation, wild-type *X. oryzae* X11-5A produces short lesions on Carolina Gold Select in conditions of high humidity (>85%, Figure 1A); a test at 70% humidity did not yield lesions. Eight of the nine *X. oryzae* effector constructs caused a drastic decrease in the length of lesions caused by X11-5A at high humidity; *tal2g* caused an intermediate decrease (Figure 1A and 1B). However, effector genes *avrHah1* from *X. gardneri* and *tale1Xam* from *X. axonopodis* pv. *manihotis* did not cause a significant change. *tal1c* missing the central repeat region likewise did not cause a change in lesion length, but a truncation of *pthXo1CRR* missing the region encoding the C-terminal activation domain still did (Figure 1A and 1B). Thus, the AD is not required for resistance. No dark coloration indicative of a HR was observed in clip inoculation experiments (Figure 1B). In a leaf infiltration assay, *pthXo1CRR* and *pthXo1CRR*(ΔAD) inhibited the watersoaking normally caused by X11-5A by 48 h (Figure 1C), although constructs lacking the CRR did not. Two rice varieties related to Carolina Gold, Dawn and Lemont, were also inoculated, but no TAL effectors triggered resistance in these lines (Figure S1). Together, these results indicate that Carolina Gold Select has a mechanism of resistance triggered by *X. oryzae* TAL effectors independently of their transcription activation activity.

Carolina Gold Select resistance triggered by *pthXo1CRR(ΔAD)* inhibits bacterial growth

Decreased *X. oryzae* lesion length is often, but not always, associated with decreased bacterial population growth in the rice leaves (Verdier *et al.* 2012a). To determine whether the TAL effector-triggered resistance restricts bacterial multiplication, we measured the

populations of wild-type X11-5A and X11-5A expressing TAL effector variants on Carolina Gold Select rice, in the topmost 5 cm of clip-inoculated leaves. X11-5A(*pthXo1CRR*) and X11-5A(*pthXo1CRRΔAD*) multiplied in the leaf, but reached population sizes significantly smaller than X11-5A or X11-5A(*tal1C*ΔCRR) (Figure 2). The population size of X11-5A(*tal1C*ΔCRR) was slightly smaller than that of X11-5A after 7 days, but not after 14 days. Whether that is due to a plant response to the CRR-less effector, which does not affect lesion development, or to a slight negative effect of expression of the effector on the doubling rate of the bacterium, is not clear. Nevertheless, the data indicate that the TAL effector-triggered resistance in Carolina Gold Select restricts population growth, and that this is AD-independent.

Resistance does not depend on the composition of RVDs

As shown in Figure 1, in contrast to the *X. oryzae* effectors, *avrHah1* from *X. gardneri* does not trigger resistance in Carolina Gold Select. Because AvrHah1 is more distantly related to *Xo* TAL effectors than they are to one another, we hypothesized that sequence differences between *X. oryzae* TAL effectors and AvrHah1 might be critical for determining whether resistance is triggered. The domain swap construct *avrHah1S*, consisting of the CRR of *avrHah1* cloned into the N- and C-terminal context of *tal1C,* did not trigger resistance when expressed from X11-5A (Figure 3A). X11-5A(*avrHah1S*) triggered resistance mediated by the AvrHah1 target gene Bs3 in pepper, demonstrating that AvrHah1S is expressed and secreted from X11-5A and is a functional TAL effector *in planta* (Figure S2). The lack of *avrHah1S* activity in triggering resistance in Carolina Gold Select suggests that the composition of the TAL effector CRR is critical for this phenotype. To determine whether the RVD or non-RVD portions of the CRR are key, we introduced an engineered *avrHah1* "analog" (*avrHah1A*)(Cermak *et al.* 2011) into X11-5A. The *avrHah1A* construct expresses an effector of which the repeat sequences match the consensus for PthXo1, except for the RVD composition, which is that of AvrHah1. X11-5A (*avrHah1A*) triggered full resistance in Carolina Gold Select (Figure 3A), and was also able to trigger Bs3 in pepper (Figure S2). This confirms that the RVD composition is not relevant to the ability of *X. oryzae* TAL effectors to trigger resistance in Caroina Gold Select.

A TAL effector with only 3.5 repeats is sufficient to trigger resistance in Carolina Gold Select

The above experiments demonstrate that TAL effector activity is not required for triggering resistance in Carolina Gold Select, but suggest that at least part of the CRR is required. To ascertain whether a long CRR is required to trigger resistance, we used a modular gene construction approach (Supporting Methods S1) to design *pthXo1CRR*-based TAL effectors with small numbers of central repeats. We constructed effector genes with full N- and C-terminal coding regions, but containing only the first 0.5, 1.5, 2.5, 3.5, and 4.5 repeats of the *pthXo1* CRR. In X11-5A, the TAL effector constructs encoding 0, 0.5, 1.5 or 2.5 repeats did not cause a reduction in lesion length on Carolina Gold Select, but the effector constructs encoding 3.5 or 4.5 repeats triggered the resistance response (Figure 3B). This demonstrates that a TAL effector with 3.5 repeats is sufficient to trigger resistance on Carolina Gold Select.

Carolina Gold Select is resistant to strains of *X. oryzae* from Africa, but not from Asia

Because diverse *X. oryzae* strains express TAL effectors, we hypothesized that Carolina Gold Select might have broad-spectrum resistance to strains of both *X. oryzae* pathovars. To test this, Carolina Gold Select plants were inoculated with African and Asian strains of *X. oryzae* pv. *oryzicola* and *X. oryzae* pv. *oryzae*. African *X. oryzae* pv. *oryzicola* strains BAI3, MAI5, and MAI10 triggered a strong HR, while the Asian strain BLS256 produced lesions (Figure 4A). The Asian *X. oryzae* pv. *oryzae* strains PXO86 and PXO99A caused long lesions indicative of full susceptibility; however, the African strains CFBP1947 and CFBP1951 produced lesions shorter than 5 cm, a limit conventionally used to classify varieties as resistant to a strain (Gonzalez *et al.* 2007). The two African strains produced long lesions indicative of full susceptibility on another US tropical japonica variety, Lemont (Figure 4B). To determine the breadth of resistance to African *X. oryzae* strains, Carolina Gold Select was inoculated with 12 strains each of *X. oryzae* pv. *oryzicola* and *X. oryzae* pv. *oryzae,* respectively, collected from rice fields throughout Mali (Table S1). All *X. oryzae* pv. *oryzicola* strains tested triggered the HR. Carolina Gold Select was also resistant to all African *X. oryzae* pv. *oryzae* strains, although like CFBP1947 and CFBP1951 these formed short lesions and did not trigger HR (Table S1). Thus, the resistance of Carolina Gold Select triggered by TAL effectors heterologously expressed from X11-5A is not effective against many Asian *X. oryzae* strains, but may account for the resistance of Carolina Gold Select to a broad range of African *X. oryzae* isolates.

Because Carolina Gold Select was not resistant to Asian strains, in which the TAL effectors are encoded in the chromosome, we next tested the possibility that the resistance we observed in response to TAL effectors expressed from pKEB31 or pSKX1 in strain X11-5A is due to relative overexpression of the TAL effectors from these plasmids. To do this, we tested effector genes *avrXa7* and *avrXa10* cloned in the low-copy plasmid pHM1. Expressed from pHM1 in X11-5A, both triggered full resistance on Carolina Gold Select (Figure S3A). Conversely, introduction of *pthXo1CRR* cloned in the high-copy vector pKEB31 into Asian *X. oryzae* pv. *oryzae* strains PXO86 and PXO99A did not result in strong resistance to these strains (Figure S3B). Introduction of *pthXo1CRR(ΔAD)* on this vector into PXO99A did cause a small reduction in lesion length (Figure S3B), but since this was not observed for PXO86, we conclude that this is likely due to *pthXo1CRR(ΔAD)* interfering with activation of *OsSWEET11* by the native PthXo1 protein of PXO99A by competing at the binding site. In sum, the results demonstrate that the resistance is independent of TAL effector gene copy number and suggest that Asian *X. oryzae* strains in some way suppress or overcome the TAL effector-triggered resistance of Carolina Gold Select.

Carolina Gold Select resistance to TAL effectors is controlled by a dominant locus that co-segregates with resistance to African isolates of *X. oryzae* pv. *oryzicola*

Toward identifying the genetic determinant(s) of the resistance triggered by *X. oryzae* TAL effectors and of the resistance to African *X. oryzae* pv. *oryzicola*, we crossed Carolina Gold Select with Kitaake, a Japonica variety in which susceptibility to X11-5A is enhanced by PthXo1 (Verdier *et al.* 2012a). A total of 157 F_2 individuals from 4 independent hybrid lines were tested for susceptibility to X11-5A, X11-5A(*pthXo1CRR*), and the African *X. oryzae* pv. *oryzicola* strain MAI10 on separate leaves. Due to the limited number of testable leaves

per plant, resistance to African *X. oryzae* pv. *oryzae* was not assayed in this study. In both the Kitaake and Carolina Gold Select parent lines, X11-5A caused short lesions; X11-5A(*pthXo1CRR*) caused long lesions in Kitaake and very short lesions in Carolina Gold Select (Figure 5A). The phenotypes of F_2 hybrids were similar either to the Kitaake or Carolina Gold Select parent, with the TAL effector-expressing strain eliciting lesions either longer or much shorter than the X11-5A control. About 80% of the F_2 individuals were resistant to X11-5A(*pthXo1CRR*) (Figure 5A). Hybrid plant responses to *X. oryzae* pv. *oryzicola* strain MAI10 also segregated into complete resistance (the Carolina Gold Select parent phenotype) and susceptibility (the Kitaake parent phenotype), and these showed 100% co-segregation in 157 lines with the resistant and susceptible responses to X11-5A (*pthXo1CRR*), respectively. Unlike Carolina Gold Select, F_2 hybrids did not develop a clear HR to MAI10. Instead, qualitative resistance in the F_2 lines manifested in a complete lack of lesion formation. To determine whether a weak HR was occurring, 14 additional F2 hybrids and four additional Carolina Gold Select individuals were inoculated with MAI10 in a growth chamber under high-intensity light conditions that favor HR development. After 72 hours, dark color development was visible to a variable degree at the inoculation sites of resistant lines, but this phenotype was weak compared to the response of Carolina Gold Select (Supplemental Figure S4). This suggests that additional loci in the Carolina Gold Select genome contribute to development of a strong HR. Together, these results demonstrate that the TAL effector-triggered resistance phenotype in Carolina Gold Select is governed by a single dominant locus, and that this locus is also the source of resistance to the African strain MAI10. Because the TAL-effector triggered resistance activity is not a typical bacterial leaf blight resistance gene, and is triggered by and effective against genes from both *X. oryzae* pathovars, we name this locus *Xo1*.

Xo1 maps to a 1.09 Mbp segment of the long arm of Chromosome 4

We used a genotyping-by-sequencing approach to map *Xo1*. DNA from 110 phenotyped F_2 hybrids and three individuals of each parental line was sequenced to low coverage from *Rsa*I digestion sites. From an initial set of 207,303 non-reference calls, 18,696 high-quality SNPs were recovered after filtering and data imputation. SNPs in a single region of chromosome 4 correlated strongly with the resistance to X11-5A(*pthXo1*) and MAI10, placing the locus in the interval from 31,358,156 bp to 32,448,509 bp (Figure 5B). This interval contains approximately 275 annotated genes in Nipponbare (Kawahara *et al.* 2013), including 10 full or partial NLR genes; one of these is bacterial leaf blight resistance gene *Xa1* (Loc_Os04g53120). Five other dominant resistance genes for bacterial leaf blight, *Xa12*, *Xa14*, *Xa2*, *Xa30*(t), and *Xa31*(t), have been mapped to intervals near the Xo1 locus of chromosome 4, demonstrating that this is an important genomic region for blight resistance (reviewed in (Xia *et al.* 2012)). Further research is underway to determine whether *Xo1* is conferred by an allele of *Xa1* or by another NLR gene.

Discussion

Rice production has increased substantially in West Africa in recent years, but with limited yields and increases in consumption, many countries still rely heavily on foreign imports (http://www.fao.org/3/a-i4337e.pdf). Intensified production and the lack of resistant

germplasm has facilitated a rapid spread of BLS to multiple new rice-growing areas, affecting up to 80% of the plants in fields surveyed (Afolabi *et al.* 2015, Afolabi *et al.* 2014, Wonni *et al.* 2011). With yield losses from BLS reaching up to 30% (Mew 1993), identification of effective genetic resistance will be a critical step toward increasing rice production in the region. Here, we report that Carolina Gold Select rice has a resistance locus, *Xo1*, conferring resistance triggered by *X. oryzae* TAL effectors with different DNA binding specificities and from both pathovars, independent of the ability of the TAL effector to directly activate gene expression. The *Xo1* locus confers complete resistance to African strains of *X. oryzae* pv. *oryzicola*. TAL effector-related resistance in rice was previously only known to hinge on the ability of a particular TAL effector to activate a gene, deriving either from an activation trap that turns on an *R* gene or a binding site polymorphism that prevents activation of a susceptibility gene. The broad efficacy of *Xo1* against African strains of *X. oryzae* pv. *oryzicola* makes it a promising resource for breeding efforts against BLS. Interestingly, strong resistance specific to African strains of *X. oryzae* pv. *oryzicola* was recently identified in two African varieties of the cultivar indica (Wonni *et al.* 2015); further gene characterization will determine whether these varieties share *Xo1*.

Our findings suggest that *Xo1* could encode a receptor that triggers resistance upon direct recognition of the TAL effector, similar to the way Bs4 has been hypothesized to recognize AvrBs4 in tomato (Schornack *et al.* 2006). Both *Xo1* and *Bs4* are characterized by activity against closely related TAL effectors with diverse RVD composition, activity against effectors containing only a few central repeats, and independence from the transcriptional activation domain. Together, these similarities suggest that *Bs4* and *Xo1* could both be mechanisms for plants to recognize epitopes of the highly conserved TAL effector structure.

Bs4 encodes a member of the TIR-NLR family of resistance proteins, which has only been observed in dicots (Tarr and Alexander 2009). Bs4 signals through the EDS1 pathway (Schornack *et al.* 2004), distinct from the mechanism used by monocot CC-NLR-type resistance proteins (Heidrich *et al.* 2012). The phenotypic similarities between *Bs4* and *Xo1* raise the intriguing possibility that rice and tomato independently evolved NLRs that recognize activity-less TAL effectors. Convergent evolution of R proteins has been observed within dicots: *Arabidopsis* and bean have non-orthologous R proteins that each recognize the *Pseudomonas* effector AvrB through its activity on RIN4 (Ashfield *et al.* 2004). In the case of *Xo1* and Bs4, it is striking that plant phyla diverging 150 million years ago recognize the same class of pathogen effector, independent of any activity of those effectors on a conserved plant target. Identifying the gene(s) responsible for *Xo1* activity will help determine whether *Xo1* and Bs4 are mechanistically similar to one another.

Our discovery of Xo1 in rice and its functional similarity to Bs4 in tomato raises the question of whether resistance triggered by RVD-independent recognition of TAL effectors is prevalent in plants, or other organisms. As TAL effectors are rapidly being adapted for diverse biotechnological and therapeutic applications, this question may be increasingly important. Of 24 diverse rice varieties we have tested for TAL effector responses ((Verdier *et al.* 2012a) and this work), Carolina Gold Select is the only variety demonstrating resistance triggered by multiple TAL effectors and gene activation-deficient TAL effectors. *Bs4* activity was detected in 15 of 17 tomato accessions tested, however (Ballvora *et al.* 2001). Our

results with the Asian *X. oryzae* strains also suggest that bacteria can suppress *Xo1* activity, possibly through other TAL or non-TAL effectors, just as Asian *X. oryzae* pv. *oryzicola* strains BS303 and BLS256 suppress *Xa10* and *Xa7*-mediated resistance in a Type III-dependent manner (Makino *et al.* 2006). Widespread suppressive ability would obscure identification of other TAL effector-detection resistance loci through traditional race typing studies. Additionally, effectors could avoid (*e.g.*, AvrHah1) or partially escape (*e.g.*, Tal2g) detection through polymorphisms in the recognized epitope. Understanding the basis of *Xo1* suppression by Asian strains of *X. oryzae* will be important to determine how widespread *Xo1*-like resistance mechanisms may be, and how broadly they affect TAL effector evolution. Understanding these recognition mechanisms in both tomato and rice could lead to strategies for engineering receptors that recognize a broad range of pathogen TAL effectors.

For *Xo1*, additional research is first needed to determine whether the response to TAL effectors and the resistance to African *X. oryzae* pv. *oryzicola* strains are controlled by one or by different genes at the locus. While Carolina Gold Select exhibits a strong hypersensitive response upon infiltration with African *X. oryzae* pv. *oryzicola,* little to no HR phenotype was observed following infiltration with X11-5A transformed with single TAL effectors. However, the broad efficacy of the resistance of Carolina Gold Select against African *X. oryzae* pv. *oryzicola* isolates from different sites might be consistent with its resulting from the non-specific TAL effector recognition conferred by *Xo1*. Determining the genetic basis of the resistance to African *X. oryzae* pv. *oryzicola* strains and testing a wider collection strains will help predict whether *Xo1* can provide durable resistance that will be useful for rice breeders fighting BLS in Africa.

Experimental Procedures

DNA cloning and bacterial culture techniques

Bacterial strains and plasmids are listed in Table 1. Strains of *E. coli* were cultured on LB media containing the appropriate antibiotics, and *X. oryzae* was cultured on PSA (Karganilla *et al.* 1973). pCS503, pCS510, pCS541, and pCS553 were constructed as part of a comprehensive series of vectors with inserts of 1, 2, or 3 TAL effector repeats in every possible RVD combination (described in Methods S1, Table S2, and Table S3). TAL effector CRR truncation clones pSPC03, pSPC04, and pSPC07 were generated by subcloning the *Psp*XI-*Xho*I fragments from pCS503, pCS510, and pCS541 into the *Xho*I site of pCS495. pSPC09 was generated during attempts to clone the *Psp*XI-*Xho*I fragment from pCS553 into pSPC07 to total 6.5 repeats, but this instead resulted in a construct with 4.5 in-frame repeats with RVDs of NN-HD-NG-NG-NG, possibly due to an unexpected restriction site in pCS553. To generate *Xanthomonas* expression clones pSPC01, 05, 06, 08, and 10, inserts from pCS495-derived vectors were recombined into the Gateway destination vector pKEB31 (Cermak *et al.* 2011) using LR Clonase Enzyme Mix II (Life Technologies, lifetechnologies.com) according to the manufacturer's instructions. pKEB31-*avrHah1S* was constructed by cloning the *SphI* fragment from pKEB31-*avrHah1* into the *Sph*I site of pCS466 (Verdier *et al.* 2012a). pAH338 was constructed by PCR site-directed mutagenesis of pCS466 to change the codon encoding residue W1334, immediately upstream of the

activation domain, to a stop codon. This was followed by recombination into pKEB31 and insertion of the *Sph*I fragment from *pthXo1*. TAL effector gene inserts were sequenced at the Colorado State University Proteomics and Metabolomics facility. TAL effector constructs were transformed into *X. oryzae* X11-5A by electroporation, and confirmed by colony PCR.

Plant inoculations

Experiments were performed on Carolina Gold Select (GRIN accession CSOR301024), a Carolina Gold line purified using genetic markers (Duitama *et al.* 2015), obtained from Dr. Anna McClung. Rice accessions Lemont (CSOR301093) and Kitaake (GSOR 300108) from the Leach lab seed collection, and Dawn (CIor9534), obtained through the USDA-ARS National Plant Germplasm System, were also used. Lines of pepper (*Capsicum annuum)* ECW-10R and ECW-30R were obtained through the USDA-ARS U.S. National Plant Germplasm System as part of the Pepper Bacterial Spot Differential Host Set (accessions CPPSIH_1_02 and 1_04).

For inoculations with *X. oryzae* pv. *oryzae* or the US *X. oryzae* strain X11-5A, fully-expanded leaves of 6 week-old plants were inoculated with bacterial suspensions of 10^8 cfu/mL using a standard scissor-clip inoculation technique (Kauffman *et al.* 1973). A leaf infiltration technique was used for inoculations with *X. oryzae* pv. *oryzicola* (Reimers and Leach 1991). Lesion lengths were measured at 14 d post-inoculation for *X. oryzae* pv. *oryzae* and US *X. oryzae* and at 7 d for *X. oryzae* pv. *oryzicola*. Figure 1A represents combined data from two experiments performed in different months; two prior experiments yielded similar results but did not include all of the *X. oryzae* TAL effector genes. Inoculations depicted in Figures 2, 3, and 5 show results from one representative experiment out of three experiments yielding similar results.

Inoculation of pepper differential lines ECW-10R and ECW-30R was performed by infiltration of leaves on six-week-old plants with fresh cultures of 10^8 cfu/mL. Leaves were imaged five days after inoculation. Decoloring was performed in a 19:1 ethanol/glycerol solution.

For population count experiments, a 5 cm segment of each leaf terminus was macerated in 2 ml sterile water, followed by serial dilution plating at 7 and 14 days as previously described (Verdier *et al.* 2012a). All inoculations for lesion length analysis were performed in a greenhouse kept at >85% humidity. Inoculations for bacterial population growth analyses were performed in a growth chamber (28° days, 24° nights, 16 h days, 85% humidity).

Carolina Gold Select (maternal line) was crossed with Kitaake (paternal line) using a standard procedure (Herrera and Coffman 1975). F_1 plants were selected as likely crosses based on short (<65 d) flowering time. 38 to 42 F_2 individuals were grown from each of 4 independent lines, for a total of 157 plants. For phenotyping of individual F_2 segregants, five fully expanded leaves of each 6-week-old plant were inoculated: two were clip-inoculated with *X. oryzae* X11-5A, two were clip-inoculated with X11-5A (*pthXo1CRR*), and one leaf was inoculated by infiltration in four spots with *X. oryzae* pv. *oryzicola* MAI10. X11-5A

lesions were measured at 14 d, and MAI10 presence or absence of lesions was observed at 7 d post-inoculation.

Data analyses and image generation

For the inoculations in Figure 1A, differences among means were determined using one-way ANOVA and Tukey's HSD test. For all other figures, pairwise differences with the X11-5A wild type treatment were determined using Welch's T-Test. Statistical analyses were conducted in R version 3.2.1 (http://www.r-project.org/), using the package agricolae for Tukey's HSD (http://tarwi.lamolina.edu.pe/~fmendiburu/). For all lesion and infiltration images, leaf scans or photographs were taken separately of separate leaves and combined into one figure. Any adjustments in size, brightness, and contrast were then made equally on the combined images.

Genotyping-by-Sequencing

Leaf tissue was collected from both parents and 110 Carolina Gold Select X Kitaake F_2 plants that originated from a single F_1 plant, and all plants were phenotyped as described above. Genomic DNA was extracted via standard protocols (Dellaporta 1994). Genomic DNA was used to generate GBS libraries as previously described (Heffelfinger *et al.* 2014) using *Rsa*I as the restriction enzyme. Libraries were pooled, then sequenced on 40% of one lane of an Illumina HiSeq 2500 at the Yale Center for Genome Analysis, yielding a total of 60,437,829 reads across all samples.

Data processing

Raw reads were aligned to the MSU 7.0 rice reference genome (Kawahara *et al.* 2013) using NovoAlign (www.novocraft.com) under default parameters. Variants were then called from aligned reads using GATK (McKenna *et al.* 2010). Sites that failed to meet the following criteria were removed: a quality depth of at least 2, at least two non-reference and reference allele calls, a Fisher strand-bias score of at least 60, a fraction of heterozygous calls between 0.1 and 0.9, a haplotype score less than or equal to 10, mapping quality of at least 40, mapping quality rank-sum score of at least −12.5, read position rank sum of at least −8, Phred score of at least 40, and overall heterozygosity (the ratio of reference to non-reference alleles) between 0.1 and 0.9. Following these hard filters, non-independent sites were collapsed into single markers. A site was declared non-independent if it was identified from the same sequencing read as another polymorphic site. Finally, retained variants were required to have a mean r^2 correlation of at least 0.05 with the five variants immediately upstream and downstream. These filters were performed using custom scripts.

Following initial filtering, data imputation was performed using an impute-filter-impute process. First, missing markers within the parents were imputed. Next, variants were filtered such that only variants that were homozygous within and polymorphic between the parents were retained. Finally, offspring markers were imputed. Imputation was performed using LB-Impute (Heffelfinger *et al.*, submitted) under default settings.

Trait mapping

The *Xo1* locus was mapped from the filtered and imputed data via a generalized linear model using TASSEL 5.2.11 (Bradbury *et al.* 2007) under default settings. Data were visualized via the qqman package in R (Turner 2014).

Gene name registration

Xo1 was registered and approved as a rice gene name according to the guidelines of the Committee on Gene Symbolization, Nomenclature, and Linkage (CGSNL) of the Rice Genetics Cooperative (McCouch 2008).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Afolabi O, Milan B, Amoussa R, Koebnik R, Poulin L, Szurek B, Habarugira G, Bigirimana J, Silue D. First report of *Xanthomonas oryzae* pv. *oryzicola* causing bacterial leaf streak of rice in Burundi. Appl Environ Microbiol. 2015; 81:688–698. [PubMed: 25398857]

Afolabi O, Milan B, Poulin L, Ongom J, Szurek B, Koebnik R, Silue D. First report of *Xanthomonas oryzae* pv. *oryzicola* causing bacterial leaf streak of rice in Uganda. Plant Dis. 2014; 98:1579–1579.

Ashfield T, Ong LE, Nobuta K, Schneider CM, Innes RW. Convergent evolution of disease resistance gene specificity in two flowering plant families. The Plant cell. 2004; 16:309–318. [PubMed: 14742871]

Bai J, Choi SH, Ponciano G, Leung H, Leach JE. *Xanthomonas oryzae* pv. *oryzae* avirulence genes contribute differently and specifically to pathogen aggressiveness. Mol Plant Microbe Interact. 2000; 13:1322–1329. [PubMed: 11106024]

Ballvora A, Pierre M, Van Den AG, Schornack S, Rossier O, Ganal M, Lahaye T, Bonas U. Genetic mapping and functional analysis of the tomato *Bs4* locus governing recognition of the *Xanthomonas campestris* pv. *vesicatoria* AvrBs4 protein. Mol Plant Microbe Interact. 2001; 14:629–638. [PubMed: 11332727]

Boch J, Bonas U. *Xanthomonas* AvrBs3 family-type III effectors: discovery and function. Annu Rev Phytopathol. 2010; 48:419–436. [PubMed: 19400638]

Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, Lahaye T, Nickstadt A, Bonas U. Breaking the code of DNA binding specificity of TAL-type III effectors. Science. 2009; 326:1509–1512. [PubMed: 19933107]

Bogdanove AJ, Koebnik R, Lu H, Furutani A, Angiuoli SV, Patil PB, Van Sluys MA, Ryan RP, Meyer DF, Han SW, Aparna G, Rajaram M, Delcher AL, Phillippy AM, Puiu D, Schatz MC, Shumway M,

Sommer DD, Trapnell C, Benahmed F, Dimitrov G, Madupu R, Radune D, Sullivan S, Jha G, Ishihara H, Lee SW, Pandey A, Sharma V, Sriariyanun M, Szurek B, Vera-Cruz CM, Dorman KS, Ronald PC, Verdier V, Dow JM, Sonti RV, Tsuge S, Brendel VP, Rabinowicz PD, Leach JE, White FF, Salzberg SL. Two new complete genome sequences offer insight into host and tissue specificity of plant pathogenic *Xanthomonas* spp. J Bacteriol. 2011; 193:5450–5464. [PubMed: 21784931]

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES. TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics. 2007; 23:2633–2635. [PubMed: 17586829]

Cermak T, Doyle EL, Christian M, Wang L, Zhang Y, Schmidt C, Baller JA, Somia NV, Bogdanove AJ, Voytas DF. Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. Nucleic Acids Research. 2011; 39:e82. [PubMed: 21493687]

Chen LQ, Hou BH, Lalonde S, Takanaga H, Hartung M, Qu XQ, Guo WJ, Kim JG, Underwood W, Chaudhuri B. Sugar transporters for intercellular exchange and nutrition of pathogens. Nature. 2012; 468:527–532. [PubMed: 21107422]

Dellaporta, S. The Maize Handbook. Springer; 1994. Plant DNA miniprep and microprep: versions 2.1–2.3; p. 522-525.

Durham S, Avant S. ARS and CGIAR: Working To Provide International Food Security. Agricultural Research. 2011; 59:4.

Gonzalez C, Szurek B, Manceau C, Mathieu T, Sere Y, Verdier V. Molecular and pathotypic characterization of new *Xanthomonas oryzae* strains from West Africa. Mol Plant Microbe Interact. 2007; 20:534–546. [PubMed: 17506331]

Gu K, Yang B, Tian D, Wu L, Wang D, Sreekala C, Yang F, Chu Z, Wang GL, White FF, Yin Z. R gene expression induced by a type-III effector triggers disease resistance in rice. Nature. 2005; 435:1122–1125. [PubMed: 15973413]

Heffelfinger C, Fragoso CA, Moreno MA, Overton JD, Mottinger JP, Zhao H, Tohme J, Dellaporta SL. Flexible and scalable genotyping-by-sequencing strategies for population studies. BMC Genomics. 2014; 15:979. [PubMed: 25406744]

Heidrich K, Blanvillain-Baufumé S, Parker JE. Molecular and spatial constraints on NB-LRR receptor signaling. Curr Opin Plant Biol. 2012; 15:385–391. [PubMed: 22503757]

Herrera, R.; Coffman, R. Emasculation of rice by vacuum extraction. 5. Scientific Meeting of the Crop Science Society of the Philippines; Naga City (Philippines). 16 May 1974; CSSP; 1975.

Hopkins CM, White FF, Choi SH, Guo A, Leach JE. Identification of a family of avirulence genes from *Xanthomonas oryzae* pv. *oryzae*. Mol Plant Microbe Interact. 1992; 5:451–459. [PubMed: 1335800]

Jones RK, Barnes LW, Gonzalez CF, Leach JE, Alvarez AM, Benedict AA. Identification of low virulence strains of *Xanthomonas campestris* pv. *oryzae* from rice in the United States. Phytopathology. 1989; 79:984–990.

Karganilla A, Paris-Natural M, Ou SH. A comparative study of culture media for *Xanthomonas oryzae*. Philipp Agric. 1973; 57:141–152.

Kauffman H, Reddy A, Hsiek S, Merca S. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. Plant Dis Rep. 1973; 57:537–541.

Kawahara Y, de la Bastide M, Hamilton JP, Kanamori H, McCombie WR, Ouyang S, Schwartz DC, Tanaka T, Wu J, Zhou S. Improvement of the *Oryza sativa* Nipponbare reference genome using next generation sequence and optical map data. Rice. 2013; 6:4. [PubMed: 24280374]

Kay S, Boch J, Bonas U. Characterization of AvrBs3-like effectors from a Brassicaceae pathogen reveals virulence and avirulence activities and a protein with a novel repeat architecture. Mol Plant Microbe Interact. 2005; 18:838–848. [PubMed: 16134896]

Makino S, Sugio A, White F, Bogdanove AJ. Inhibition of resistance gene-mediated defense in rice by *Xanthomonas oryzae* pv. *oryzicola*. Mol Plant Microbe Interact. 2006; 19:240–249. [PubMed: 16570654]

McCouch SR. Gene Nomenclature System for Rice. Rice. 2008; 1:72–84.

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010; 20:1297–1303. [PubMed: 20644199]

Mew T, Cruz V, Reyes R. Interaction of Xanthomonas campestris pv. oryzae and a Resistant Rice Cultivar. Phytopathology. 1982; 72:786–789.

Mew, TW. *Xanthomonas oryzae* pathovars on rice: Cause of bacterial blight and bacterial leaf streak. In: Swings, JG.; Civerolo, EL., editors. Xanthomonas. London: Chapman & Hall; 1993. p. 30-40.

Mew TW, Vera C, CM, Medalla ES. Changes in race frequency of *Xanthomonas oryzae* pv. *oryzae* in response to rice cultivars planted in the Philippines. Plant Dis. 1992; 76:1029.

Moscou MJ, Bogdanove AJ. A simple cipher governs DNA recognition by TAL effectors. Science. 2009; 326:1501. [PubMed: 19933106]

Reimers PJ, Leach JE. Race-specific resistance to *Xanthomonas oryzae* pv. *oryzae* conferred by bacterial blight resistance gene *Xa-10* in rice *Oryza sativa* involves accumulation of a lignin-like substance in host tissues. Physiol Mol Plant Pathol. 1991; 38:39–55.

Schornack S, Ballvora A, Gürlebeck D, Peart J, Ganal M, Baker B, Bonas U, Lahaye T. The tomato resistance protein Bs4 is a predicted non-nuclear TIR-NB-LRR protein that mediates defense responses to severely truncated derivatives of AvrBs4 and overexpressed AvrBs3. Plant J. 2004; 37:46–60. [PubMed: 14675431]

Schornack S, Meyer A, Römer P, Jordan T, Lahaye T. Gene-for-gene-mediated recognition of nuclear-targeted AvrBs3-like bacterial effector proteins. Journal of Plant Physiology. 2006; 163:256–272. [PubMed: 16403589]

Schornack S, Peter K, Bonas U, Lahaye T. Expression Levels of *avrBs3*-like genes affect recognition specificity in tomato Bs4- but not in pepper Bs3-mediated perception. Mol Plant Microbe Interact. 2005; 18:1215–1225. [PubMed: 16353556]

Schulze, R. Carolina Gold Rice: The Ebb and Flow History of a Lowcountry Cash Crop. Charleston, SC: The History Press; 2012.

Streubel J, Pesce C, Hutin M, Koebnik R, Boch J, Szurek B. Five phylogenetically close rice SWEET genes confer TAL effector-mediated susceptibility to Xanthomonas oryzae pv. oryzae. New Phytol. 2013; 200:808–819. [PubMed: 23879865]

Tarr DEK, Alexander HM. TIR-NBS-LRR genes are rare in monocots: evidence from diverse monocot orders. BMC research notes. 2009; 2:197. [PubMed: 19785756]

Triplett LR, Hamilton JP, Buell CR, Tisserat NA, Verdier V, Zink F, Leach JE. Genomic analysis of *Xanthomonas oryzae* isolates from rice grown in the United States reveals substantial divergence from known *X. oryzae* pathovars. Appl Environ Microbiol. 2011; 77:3930–3937. [PubMed: 21515727]

Turner SD. qqman: an R package for visualizing GWAS results using QQ and manhattan plots. 2014 bioRxiv.

Verdier V, Triplett LR, Hummel AW, Corral R, Cernadas RA, Schmidt CL, Bogdanove AJ, Leach JE. Transcription activator-like (TAL) effectors targeting *OsSWEET* genes enhance virulence on diverse rice (*Oryza sativa*) varieties when expressed individually in a TAL effector-deficient strain of *Xanthomonas oryzae*. New Phytol. 2012a; 196:1197–1207. [PubMed: 23078195]

Verdier V, Vera Cruz C, Leach JE. Controlling rice bacterial blight in Africa: needs and prospects. J Biotechnol. 2012b; 159:320–328. [PubMed: 21963588]

Wang C, Zhang X, Fan Y, Gao Y, Zhu Q, Zheng C, Qin T, Li Y, Che J, Zhang M. XA23 is an executor R protein and confers broad-spectrum disease resistance in rice. Mol Plant. 2015; 8:290–302. [PubMed: 25616388]

White FF, Potnis N, Jones JB, Koebnik R. The type III effectors of *Xanthomonas*. Mol Plant Pathol. 2009; 10:749–766. [PubMed: 19849782]

Wonni I, Djedatin G, Ouédraogo L, Verdier V. Evaluation of Rice Germplasm against Bacterial Leaf Streak Disease Reveals Sources of Resistance in African Varieties. Journal of Plant Pathology & Microbiology. 2015

Wonni I, Ouedraogo L, Verdier V. First Report of Bacterial Leaf Streak Caused by Xanthomonas oryzae pv. oryzicola on Rice in Burkina Faso. Plant Dis. 2011; 95:72–73.

Xia C, Chen H, Zhu X. Identification, mapping, isolation of the genes resisting to bacterial blight and application in rice. Molecular Plant Breeding. 2012:3.

Yang B, Sugio A, White FF. *Os8N3* is a host disease-susceptibility gene for bacterial blight of rice. Proc Natl Acad Sci USA. 2006; 103:10503–10508. [PubMed: 16798873]

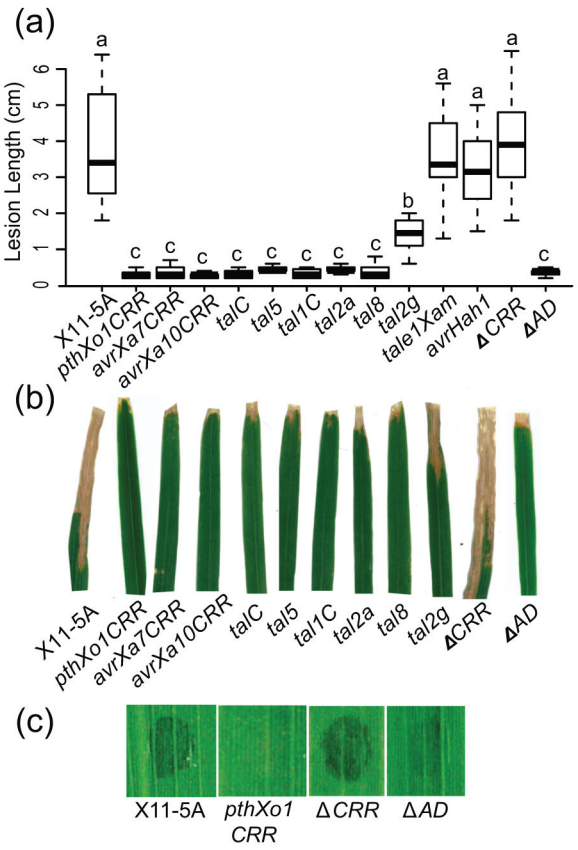

Figure 1.

Response of rice variety Carolina Gold Select to US *X. oryzae* strain X11-5A and X11-5A transformed with diverse TAL effectors. (a) Lesion lengths on Carolina Gold Select rice 14 d after inoculation. Boxes represent first through third quartiles of at least 12 independent leaves, and bars show the range of observed values. Letters denote significance groups (α = 0.05). Data were combined from two independent experiments that included the same group of treatments. (b) Representative inoculated leaves, taken 21 d after inoculation, show light-colored inoculation sites in resistant lines. Leaf scans were taken separately and combined into one image. (c) Watersoaking responses at the inoculation site 48 h after infiltration with selected strains.

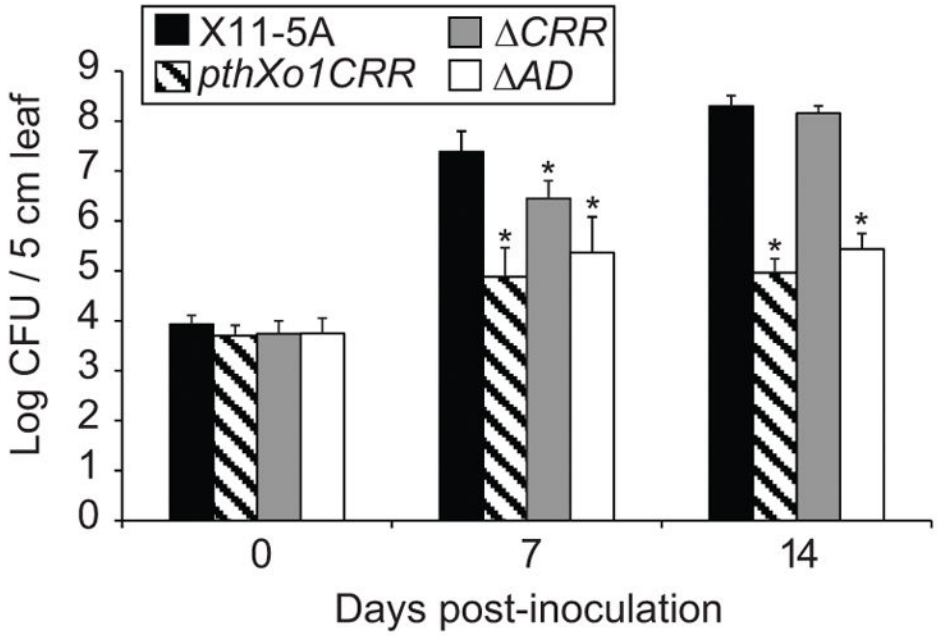

Figure 2.

Bacterial population growth in leaves of Carolina Gold Select rice inoculated with US *X. oryzae* strain X11-5A, X11-5A(*pthXo1CRR*), X11-5A(*tal1CΔCRR*), and X11-5A(*pthXo1CRRΔAD*). Population sizes were measured in the leaf segment up to 5 cm from the inoculation site. Error bars represent standard deviation of four independent leaves, and asterisks denote treatments significantly different from WT X11-5A ($p<0.01$).

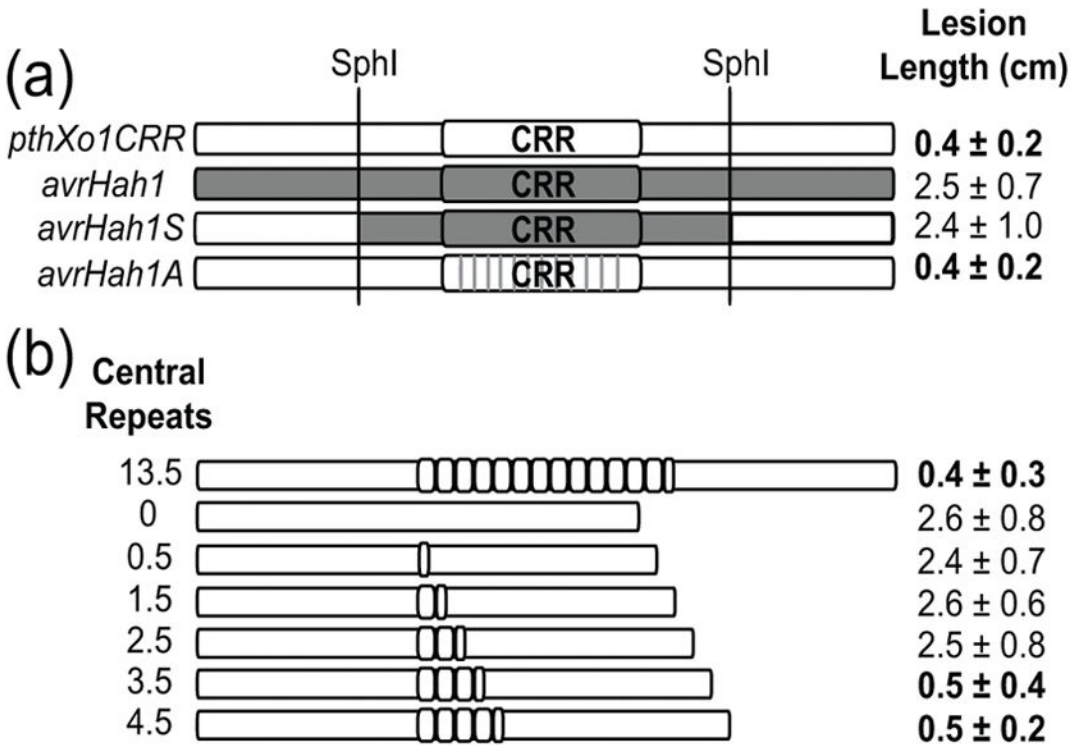

Figure 3.

Lesion lengths elicited on Carolina Gold Select by US *X. oryzae* strain X11-5A carrying domain swap or internal deletion variants of TAL effector genes. (a) Lesion lengths elicited by X11-5A transformed with the *X. oryzae* effector construct *pthXo1CRR*, the *X. gardneri* effector gene *avrHah1,* the *Sph*I fragment of *avrHah1* cloned into *pthXo1CRR* (*avrHah1S*), or the RVD codons of *avrHah1* in the *pthXo1CRR* context (*avrHah1A*). (b) Lesion lengths of X11-5A transformed with *pthXo1* (encoding 13.5 central repeats) and internal deletions engineered to encode 0.5-4.5 central repeats. Values are means and standard deviations of values from nine inoculated leaves. Resistant responses are shown in bold.

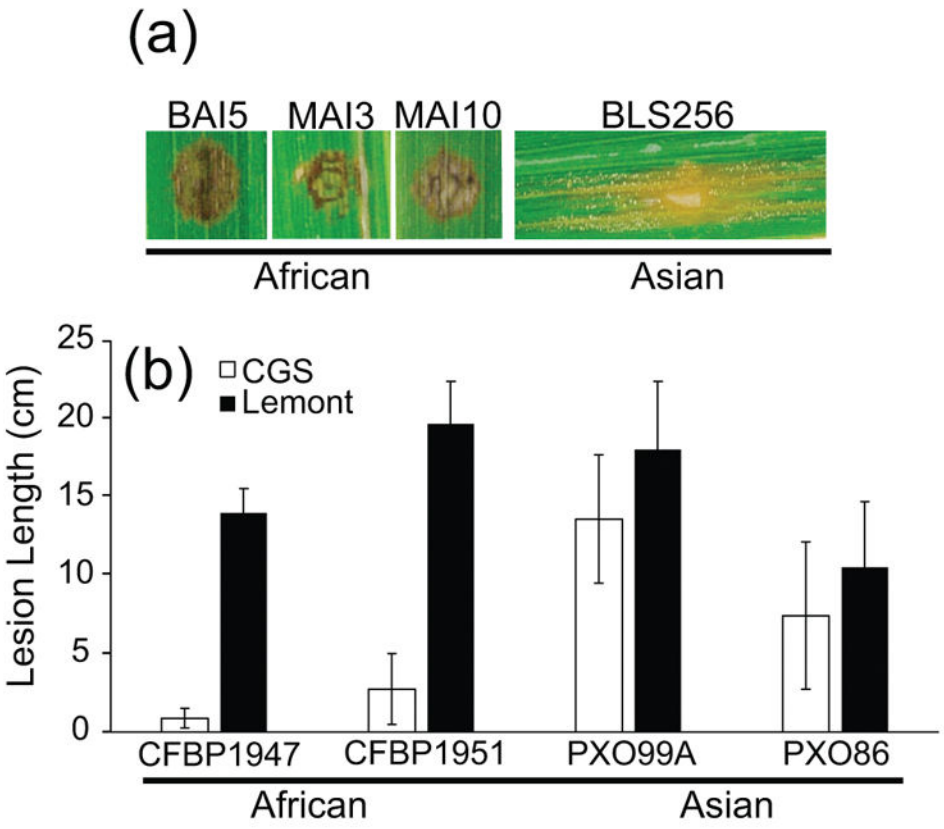

Figure 4.

Carolina Gold Select is resistant to African strains of *X. oryzae*. (a) Carolina Gold Select leaves infiltrated with African *X. oryzae* pv. *oryzicola* strains BAI5, MAI3, and MAI10 caused a strong hypersensitive response, while Asian *X. oryzae* pv. *oryzicola* strain BLS256 produced disease symptoms. Images were collected at 7 dpi. (b) Carolina Gold Select (CGS) is resistant (i.e., <5 cm lesions) to African *X. oryzae* pv. *oryzae* strains CFBP1947 and CFBP1951, but susceptible to Asian strains PXO99A and PXO86. Inoculation on a related rice variety, Lemont, was performed for comparison. Values and error bars represent the means and standard deviations of at least nine biological replicates. Experiments were repeated two additional times with similar results. Refer to Table S1 for lesion lengths of additional African strains.

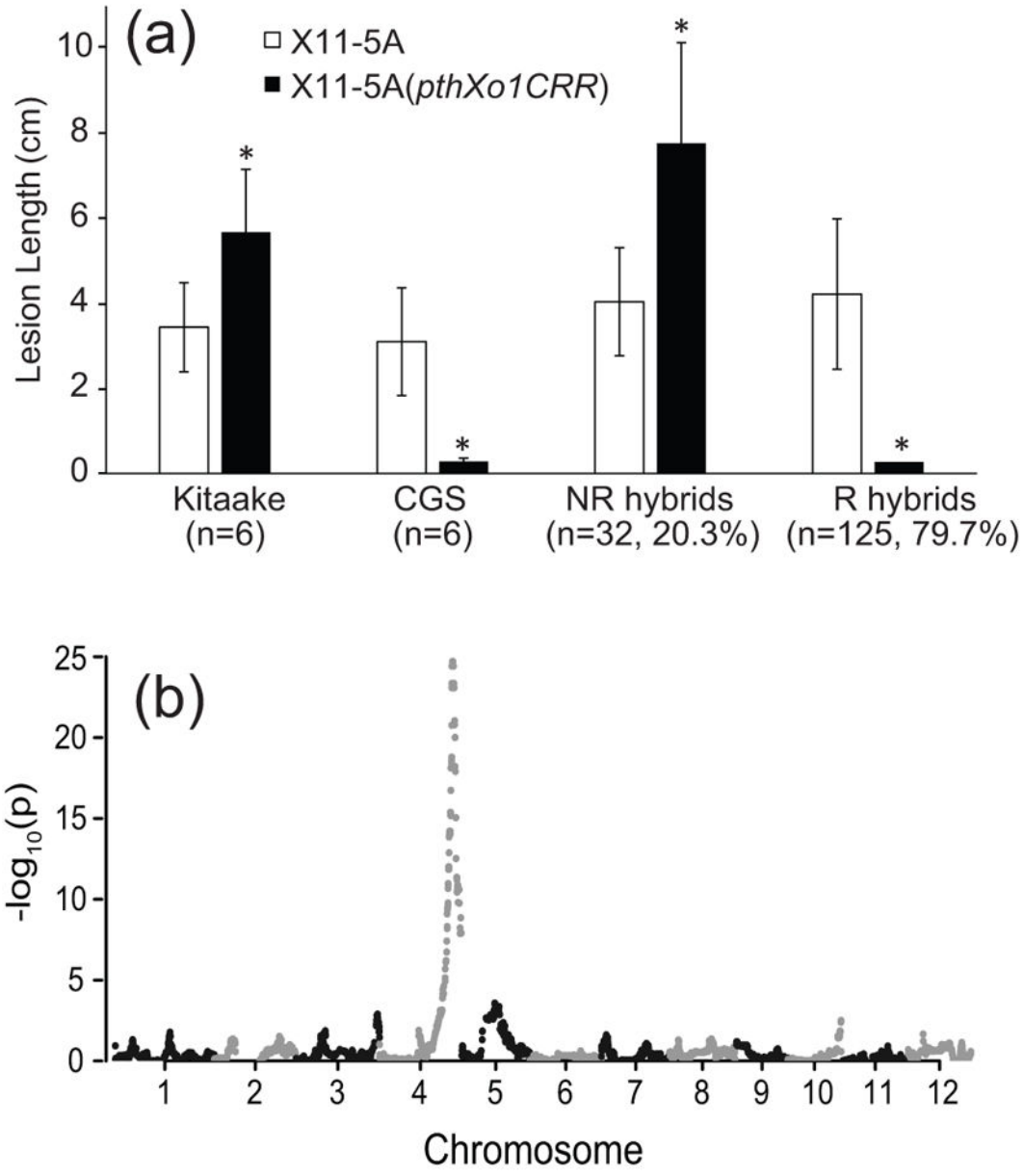

Figure 5.

Resistance triggered by TAL effectors and African *X. oryzae* pv. *oryzicola* is conferred by a single locus on Chromosome 4. (a) Lesion lengths of X11-5A with and without the TAL effector gene *pthXo1CRR* inoculated onto hybrid parent lines Kitaake and Carolina Gold Select, 32 F_2 hybrid lines lacking resistance to African *X. oryzae* strain MAI10 (NR hybrids), and 125 lines resistant to MAI10 (R hybrids). All lines resistant to MAI10 were also resistant to X11-5A (*pthXo1CRR*). Values and error bars represent the means and standard deviations of 2 inoculated leaves per plant, or 2n, measured at 14 d after inoculation. Asterisks represent values significantly different from X11-5A for each group ($p<0.001$). Resistance to MAI10 was judged by the absence of lesions in all of four inoculation sites on a single leaf at 7 dai. (b) TASSEL plot of SNPs significantly associated

with resistance in 110 F_2 hybrid individuals, indicating a single causative locus on Chromosome 4.

Table 1

Bacterial strains and plasmids used in this study.

Strains	Relevant characteristics[a]	Source
X. oryzae		
BLS256	pv. oryzicola from Asia (Philippines)	CFBP7109[b]
PXO99A	pv. *oryzae* from Asia (Philippines)	(Mew *et al.* 1992)
PXO86	pv. *oryzae* from Asia (Philippines)	(Mew *et al.* 1982)
CFBP1947	pv. *oryzae* from Africa (Cameroon)	CFBP
CFBP1951	pv. *oryzae* from Africa (Mali)	CFBP
MAI10	pv. *oryzicola* from Africa (Mali)	CFBP7331
MAI3	pv. *oryzicola* from Africa (Mali)	CFBP7326
BAI5	pv. *oryzicola* from Africa (Burkina Faso)	LMG 25979[c]
X11-5A	no pv. designation; wild type; Ap^r	(Triplett *et al.* 2011)
X11-5A(*avrXa7CRR*)	X11-5A derivative containing pKEB31-*avrXa7CRR*	(Verdier *et al.* 2012a)
X11-5A(*avrXa10CRR*)	X11-5A derivative containing pKEB31-*avrXa10CRR*	This study
X11-5A(*pthXo1CRR*)	X11-5A derivative containing pKEB31-*pthXo1CRR*	(Verdier *et al.* 2012a)
X11-5A(*talΔCRR*)	X11-5A derivative containing pKEB31-*talΔCRR*	This study
X11-5A(*pthXo1CRR*ΔAD)	X11-5A derivative containing pKEB31-*pthXo1CRR*ΔAD	This study
X11-5A(*tal1c*)	X11-5A derivative containing pKEB31-*tal1c*	(Verdier *et al.* 2012a)
X11-5A(*tal2a*)	X11-5A derivative containing pKEB31-*tal2a*	(Verdier *et al.* 2012a)
X11-5A(*tal2g*)	X11-5A derivative containing pKEB31-*tal2g*	(Verdier *et al.* 2012a)
X11-5A(*tal8*)	X11-5A derivative containing pKEB31-*tal8*	(Verdier *et al.* 2012a)
X11-5A(*talC*)	X11-5A derivative containing pSKX1-2-*talC*	(Verdier *et al.* 2012a)
X11-5A(*tal5*)	X11-5A derivative containing pSKX1-*tal5*	This study
X11-5A(*avrHah1*)	X11-5A derivative containing pKEB31-native *avrHah1*	This study
X11-5A(*avrHah1S*)	X11-5A derivative containing pKEB31-*avrHah1*S	This study
X11-5A(*avrHah1A*)	X11-5A derivative containing pKEB31-*avrHah1A*	This study
X11-5A (pK107)	X11-5A derivative containing pK107 (*avrXa7*)	This study
X11-5A(pK110)	X11-5A derivative containing pK110 (*avrXa10*)	This study
X11-5A (pHM1-AD)	X11-5A derivative containing pHM1-*pthXo1*ΔAD	This study
X11-5A(pHM1)	X11-5A derivative containing pHM1	This study
X. campestris		
Xcv 81-23	Race 2 strain of pv. *vesicatoria*	Stall, R.E.
Xcv 82-8	Race 1 strain of pv. *vesicatoria*	Stall, R.E.
Plasmids		
pKEB31	pDD62 derivative containing Gateway destination vector cassette (Invitrogen) between *Xba*I and *Bam*HI sites; Tc^r	(Cermak *et al.* 2011)
pKEB31-*talΔCRR*	pKEB31 containing *tal1c* gene of *X. oryzae* pv. *oryzicola* BLS256 without repeat-containing *Sph*I fragment; Tc^r	(Verdier *et al.* 2012a)
pKEB31-*tal1c* (pCS472)	pKEB31 containing *tal1c* gene of *X. oryzae* pv. *oryzicola* BLS256; Tc^r	(Verdier *et al.* 2012a)
pKEB31-*avrXa10CRR* (pCS481)	pKEB-*tal1c* with repeat-containing *Sph*I fragment replaced by that of *avrXa10* gene of *X. oryzae* pv. *oryzae* PXO86; Tc^r	This study

Strains	Relevant characteristics[a]	Source
pKEB31-*avrXa7CRR* (pCS718)	pKEB-*tal1c* with repeat-containing *Sph*I fragment replaced by that of *avrXa7* gene of *X. oryzae* pv. *oryzae* PXO86; Tc^r	(Verdier *et al.* 2012a)
pKEB31-*pthXo1CRR* (pMP45)	pKEB-*tal1c* with repeat-containing *Sph*I fragment replaced by that of *pthXo1* gene of *X. oryzae* pv. *oryzae* $PXO99^A$; Tc^r	(Verdier *et al.* 2012a)
pKEB31-*pthXo1CRR*ΔAD (pAH338)	pMP45 derivative with a stop codon inserted upstream of the activation domain; Tc^r	This study
pKEB31-*tal2a* (pCS695)	pKEB-*tal1c* with repeat-containing *Bam*HI fragment replaced by that of *tal2a* gene of *X. oryzae* pv. *oryzicola* BLS256; Tc^r	(Verdier *et al.* 2012a)
pKEB31-*tal2g* (pCS587)	pKEB-*tal1c* with repeat-containing *Bam*HI fragment replaced by that of *tal2g* gene of *X. oryzae* pv. *oryzicola* BLS256; Tc^r	(Verdier *et al.* 2012a)
pKEB31-*tal8* (pCS696)	pKEB-*tal1c* with repeat-containing *Bam*HI fragment replaced by that of *tal8* gene of *X. oryzae* pv. *oryzicola* BLS256; Tc^r	(Verdier *et al.* 2012a)
pSKX1-2-*talC*	pSKX1-2 derivative containing the *talC* gene of *X. oryzae* pv. *oryzae* BAI3; Gm^r	(Verdier *et al.* 2012a)
pSKX1-*tal5*	pSKX1 derivative containing the *tal5* gene of *X. oryzae* pv. *oryzae* MAI1; Gm^r	(Streubel *et al.* 2013)
pKEB31-*avrHah1*	pKEB-*tal1c* with repeat-containing *Bam*HI fragment replaced by that of *avrHah1* from *X. gardneri,* Tc^r	(Cermak *et al.* 2011)
pKEB31-*avrHah1A*	pKEB31 containing a *pthXo1*-based gene with *avrHah1* RVDs, Tc^r	(Cermak *et al.* 2011)
pKEB31-*avrHah1S*	pKEB-*tal1c* with repeat-containing *Sph*I fragment replaced by that of *avrHah1*, Tc^r	This study
pCS495	Gateway starter plasmid containing tal1C N- and C-termini for cloning designer TAL effectors, Km^r (see Table S3)	This study
pCS503	Single repeat module plasmid, RVD=HD, Ap^r (see Table S3)	This study
pCS510	Double repeat module plasmid, RVD=HD-NI, Ap^r (see Table S3)	This study
pCS541	Triple repeat module plasmid, RVD=HD-NI-NG, Ap^r (see Table S3)	This study
pCS553	Triple repeat module plasmid, RVD=HD-NG-NG, Ap^r (see Table S3)	This study
pSPC03	pCS495 containing the PspXI-XhoI fragment of pCS503 (1.5 repeats)	This study
pSPC04	pCS495 containing the PspXI-XhoI fragment of pCS510 (2.5 repeats)	This study
pSPC07	pCS495 containing the PspXI-XhoI fragment of pCS541(3.5 repeats)	This study
pSPC09	pSPC07 with an extra NG repeat added (4.5 repeats)	This study
pSPC01	0.5 repeat TAL effector gene from pCS495 in pKEB31	This study
pSPC05	1.5 repeat TAL effector gene from pSPC03 in pKEB31	This study
pSPC06	2.5 repeat TAL effector gene from pSPC04 in pKEB31	This study
pSPC08	3.5 repeat TAL effector gene from pSPC07 in pKEB31	This study
pSPC10	4.5 repeat TAL effector gene from pSPC09 in pKEB31	This study
pHM1	Broad host-range cosmid derivative of pRI40, Sp^r, Sm^r	(Hopkins *et al.* 1992)
pK107	*avrXa7* gene in pHM1, Sp^r, Sm^r	(Hopkins *et al.* 1992)
pK110	*avrXa10* gene in pHM1, Sp^r, Sm^r	(Bai *et al.* 2000)
pHM1- *pthXo1*ΔAD	pHM1 containing the HindIII fragment from pAH338	This study

[a] Tc^r, tetracycline resistance; Gm^r, gentamycin resistance; Ap^r, ampicillin resistance; Km^r, kanamycin resistance; Sp^r, spectinomycin resistance; Sm^r, streptomycin resistance.

[b] CFBP: in the collection of plant-associated bacteria at the Institut National de la Recherche Agronomique (INRA), Angers, France

[c] LMG: in the the Belgium Co-ordinated Collections of Microorganisms, Ghent, Belgium