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Designer TAL Effectors Induce Disease Susceptibility and Resistance to *Xanthomonas oryzae* pv. Oryzae in Rice

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ABSTRACT TAL (transcription activator-like) effectors from *Xanthomonas* bacteria activate the cognate host genes, leading to disease susceptibility or resistance dependent on the genetic context of host target genes. The modular nature and DNA recognition code of TAL effectors enable custom-engineering of designer TAL effectors (dTALE) for gene activation. However, the feasibility of dTALEs as transcription activators for gene functional analysis has not been demonstrated. Here, we report the use of dTALEs, as expressed and delivered by the pathogenic *Xanthomonas oryzae* pv. oryzae (Xoo), in revealing the new function of two previously identified disease-related genes and the potential of one developmental gene for disease susceptibility in rice/Xoo interactions. The dTALE gene *dTALE-xa27*, designed to target the susceptible allele of the resistance gene *Xa27*, elicited a resistant reaction in the otherwise susceptible rice cultivar IR24. Four dTALE genes were made to induce the four annotated *Xa27* homologous genes in rice cultivar Nipponbare, but none of the four induced *Xa27*-like genes conferred resistance to the dTALE-containing Xoo strains. A dTALE gene was also generated to activate the recessive resistance gene *xa13*, an allele of the disease-susceptibility gene *Os8N3* (also named *Xa13* or *OsSWEET11*, a member of sucrose efflux transporter SWEET gene family). The induction of *xa13* by the dTALE rendered the resistant rice IRBB13 (*xa13*/*xa13*) susceptible to Xoo. Finally, *OsSWEET12*, an as-yet uncharacterized SWEET gene with no corresponding naturally occurring TAL effector identified, conferred susceptibility to the Xoo strains expressing the corresponding dTALE genes. Our results demonstrate that dTALEs can be delivered through the bacterial secretion system to activate genes of interest for functional analysis in plants.

Key words: TAL effector; rice; *Xanthomonas*; *Xa27*; disease susceptibility; disease resistance; designer TAL effector.

INTRODUCTION

Plant pathogens of *Xanthomonas* bacteria essentially depend on a type III secretion system for pathogenesis in their host plants (Bonas et al., 1991; Zhu et al., 2000; Cho et al., 2008). The type III secretion system in *Xanthomonas* secretes a suite of effector proteins, including TALEs (transcription activator-like effectors), into host cells (White et al., 2009). Once internalized, TALEs function as transcription activators to mediate host gene expression by binding to the promoters of host resistance (R) genes or susceptibility (S) genes, triggering resistance responses or inducing disease susceptibility, respectively (Gu et al., 2005; Yang et al., 2006; Kay et al., 2007; Antony et al., 2010). In addition to the nuclear localization motif and *trans*-activating domain, each TALE contains a central region of multiple 34- to 35-amino acid direct repeats that are nearly identical except the 12th and 13th amino acid residues (so-called repeat variable di-amino-acids, or RVD) (Boch and Bonas, 2010). The combination of repeat number and composition of RVDs of individual TALEs determine the specificity of the targeted genes (Boch and Bonas, 2010; Bogdanove et al., 2010). Each repeat of TALE recognizes contiguously one nucleotide of target DNA, hereinafter referred to as EBE for effector binding element, in a simple cipher (Boch et al., 2009; Moscou and Bogdanove,

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2009). Among many native TALE repeats, four predominant types of repeats each recognize preferentially one of four nucleotides of target DNA. Therefore, the TALE recognition 'code' can be used to guide custom-engineering of DNA-binding domains with novel specificity to the user-chosen DNA sequences (Morbitzer et al., 2010; Li et al., 2011; Zhang et al., 2011). A variety of methods have been developed and used to synthesize TALE repeats that can be subsequently used for different fusions, in most cases for TAL effector nucleases (TALENs) (see review by Bogdanove and Voytas (2011)).

Bacterial blight of rice, caused by *Xanthomonas oryzae* pv. oryzae (Xoo), is a major rice disease in Asia and western Africa, often resulting in yield losses of up to 50% and sometimes loss of the entire crop (Mew, 1987). The TALEs are important pathogenicity determinants in Xoo/rice interaction. Several TALEs have been found to be essential virulence factors of Xoo in susceptible rice, inducing host S genes and subsequently promoting pathogen infection and disease development (Yang and White, 2004; Yang et al., 2006; Antony et al., 2010; Yu et al., 2011). For example, some Xoo strains use TALE PthXo1 to induce the S gene *Os8N3* (*Xa13* or *OsSWEET11*) for disease in susceptible rice. Intriguingly, some genetic variations in the promoter of *Os8N3* are non-responsive to PthXo1 and confer disease resistance to PthXo1-dependent Xoo strains, and those S gene alleles are collectively named as the recessive resistance gene *xa13* (Chu et al., 2006; Yang et al., 2006; Chen et al., 2010). The 'resistance' is due not to the active defense, but to a lack of S gene induction and loss of disease susceptibility (Yang et al., 2006). To encounter TALEs, plants have evolved R genes, such as *Xa27*, that recognize TALEs through the promoter sequence and activate defense process once induced. *Xa27*, recognizing the cognate TALE AvrXa27, is the representative of an unusual class of dominant R genes in plants (Gu et al., 2004, 2005). Both resistant (*Xa27*) and susceptible (*xa27*) alleles contain identical coding sequences but only resistant cultivars express *Xa27* upon infection by Xoo strains expressing AvrXa27 (Gu et al., 2005). Expression of *Xa27* depends on the direct interaction between its promoter and AvrXa27 (Romer et al., 2009). When fused to the non-specific pathogen-inducible *OsPR1* promoter, the induced Xa27 conferred resistance to Xoo strains regardless of the presence of AvrXa27 (Gu et al., 2005). Two additional, as-yet uncharacterized rice R genes (*Xa10* for AvrXa10 and *Xa7* for AvrXa7) have similar requirements for the transcription activation domain and nuclear localization motifs of their cognate TALEs as required for AvrXa27-mediated induction of *Xa27* (Zhu et al., 1998; Yang et al., 2000). However, it remains unclear how many, if any, other TALE-recognizing R genes exist in rice genome.

In this work, we describe the feasibility of using custom-engineered TALEs to investigate the functionality of host target genes involved in Xoo/rice interaction. Specifically, by using artificial TALEs, we show that *xa13* can be induced by *Xanthomonas oryzae* pv. oryzae and confer disease susceptibility, lending further evidence for *Os8N3* (*OsSWEET11* or *Xa13*) as an S gene. This approach allowed us to identify another SWEET gene (*OsSWEET12*) that can act as an S gene, provided that the Xoo pathogen contains a corresponding TALE. We also show that the *xa27* allele can be activated and triggers resistance to the bacterium expressing a corresponding dTALE. However, four other *Xa27* family members do not appear to have the potential to act as R genes, since their activation by dTALEs did not trigger resistance.

RESULTS

Design of TAL Effectors for Targeted Gene Activation

We used an improved modular assembly method to synthesize TAL effector DNA-binding domains. Four types of modular repeats with RVDs of NI, NG, NN, and HD were used to recognize the respective target nucleotides of A, T, G, and C. Instead of ligating eight pre-digested PCR-derived single-repeat units in one reaction as developed in our previous study (Li et al., 2011), the improvement involved digesting and ligating eight or fewer plasmid-borne single-repeat units into one receptor plasmid in one tube by adapting the 'Golden Gate' cloning strategy described by Engler et al. (2008). Three arrays of 8-mers were ligated into scaffold of repeat-less *avrXa10*, resulting in a full-length dTALE presumably retaining specificity for the user-chosen DNA sequence (Figure 1). The target DNA sequences in the promoter of genes of interest were selected and included based on the criteria: (1) potential or obvious TATA-box upstream of the transcription initiation site or predicted translation start site (if no cDNA sequence existed); (2) 'T' preceding each EBE sequence (or at zero position of target site); and (3) EBE about 23 bp long.

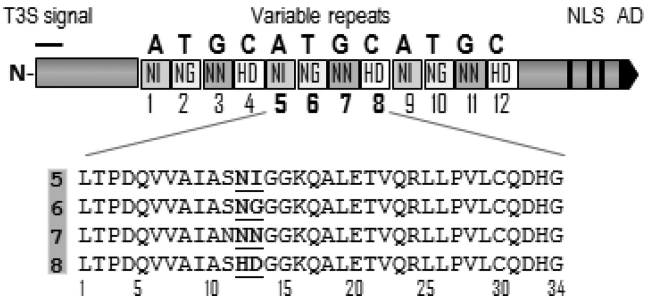

Figure 1. Structure of a Typical Design TAL Effector (dTALE).

The dTALEs consist of a scaffold of the repeat-less AvrXa10 and the custom-engineered TAL effector repeat domains. Each dTALE contains an N-terminal type III secretion signal (T3S signal), three functional nuclear localization motifs (NLS), and an acidic *trans*-activating domain (AD), and a varying number of repeats as each represented by the 12th and 13th amino acids (Variable repeats). Four types of 34-amino-acid repeats, each recognizing one of four nucleotides, are used to assemble the repeat domain of each dTALE.