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Host mechanisms for resistance to TAL effectors: Thinking outside the UPT box☆

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ABSTRACT

Transcriptional activator like (TAL) effectors are important virulence factors of several plant pathogenic species of *Xanthomonas*. Members of this secreted protein family may activate transcription of; susceptibility factors, favoring disease, or resistance genes, triggering immunity. Accordingly, most research in this area has focused on the responses of a few model varieties of plants to the transcriptional activities of TAL effectors. However, a few studies suggest that plants may have evolved a diversity of additional responses to TAL effectors that are not well understood. These could include resistance to the effects of the TAL-activated susceptibility factors such as carbohydrate transporters, genomic multiplication of off-target effector binding sites, direct receptor recognition of the conserved TAL effector structure, or mutation of the host machinery required for TAL effector function. A better understanding of the diversity of plant responses to TAL effectors will be important for harnessing the potential of these proteins for agricultural applications.

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Transcriptional Activator Like (TAL) effectors are type III secreted proteins produced by at least nine species of bacterial plant pathogens in the genus *Xanthomonas*. True to their name, they function as eukaryotic transcription factors in the host nucleus. TAL effectors bind to specific sequences in host gene promoters, sometimes termed UPT (UP-regulated by TAL) boxes, and activate genes that may increase the disease susceptibility of the host [1]. While many TAL effectors have predicted targets of unknown function, susceptibility-enhancing transcriptional targets may include genes that induce hyperplasia, act as transcriptional or small RNA regulators, or manipulate the nutrient supply in the plant (Grau et al., 2013). Multiple TAL effectors in several bacterial species activate members of the SWEET family of sucrose/glucose transporters, which are thought to increase the availability of carbohydrates to bacteria in the extracellular space [2]. TAL effectors that target SWEET genes are particularly effective at enhancing bacterial virulence [2].

TAL effectors are highly conserved, with homologs across *Xanthomonas* species sharing greater than 90% amino acid identity and several conserved structural features, reviewed in Ref. [3]. These features include two targeting signals: an N-terminal type III secretion signal mediates translocation from the bacterial cell into the plant, and a C-terminal nuclear localization signal mediates entry into the plant nucleus. A large middle section of the protein consists of a Central Repeat Region (CRR), comprised of a series of 13–26 repeats that are 34 or 35 amino acids in length. The repeats are nearly identical in their amino acid composition, except for residues 12 and 13 of each repeat, which are variable. The CRR mediates DNA binding by wrapping around the host DNA helix. The composition of residues making up position 12 and 13 of each repeat, termed the Repeat Variable Diresidue (RVD), determines which nucleotide will be bound by that repeat. In this way, the composition of RVDs in each TAL effector determines which host DNA sequence(s) the effector binds. The final structural feature of each functional TAL effector is an activation domain (AD) at the extreme C-terminus, which allows the TAL effector to employ the host transcriptional machinery for gene activation. In addition to *Xanthomonas* sp., TAL effector structural homologs have also been identified in *Ralstonia solanacearum* as well as in the metagenomes of uncultured marine bacteria not known to be pathogens [4,5]. The

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presence of TAL effector-like proteins in diverse organisms suggests that they represent an ancient family of proteins with roles beyond plant pathogenesis.

The greatest number and diversity of TAL effectors are found in the *Xanthomonas* pathogens of rice, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *X. oryzae* pv. *oryzicola (Xoc)*. *Xoo* causes bacterial leaf blight, a vascular disease characterized by spreading marginal lesions and typical yield losses of 20–50% [6]. *Xoc* is a mesophyll-inhabiting pathogen that causes bacterial leaf streak of rice, which typically reduces yields by 8 to 20%. While *Xoc* is limited to tropical rice-growing areas of Asia and Africa, *Xoo* is found in most major rice-growing areas other than the United States and the Mediterranean region. Although *Xoc* has a more limited range and lower potential yield impact of the two pathogens, there are 40 genes identified that confer resistance to *Xoo*, and yield loss is reduced to negligable levels where resistance has been deployed [7]. Several *Xoo* resistance genes are triggered by or escape the effects of TAL effector activity. Some rice varieties have evolved mutations in TAL effector binding sites, inhibiting the activity of the effector; the recessive *xa13* resistance activity is conferred by an insertion in the PthXo1 binding site of *OsSWEET11* [8]. In other cases, TAL effector binding sites are found upstream of dominant resistance genes, termed executor R genes, and TAL effector activity results in a hypersensitive cell collapse and complete resistance to the pathogen. Specific *Xoo* TAL effectors activate the executor genes Xa10, Xa23, and Xa27 [9].

Despite the successes in genetic control of *Xoo*, efforts to identify single-gene resistance to *Xoc* have been largely unsuccessful. One recessive, race-specific resistance gene, *bls1*, was mapped in *Oryza rufipogon* [10], and the dominant maize gene *Rxo1* confers resistance to *Xoc* strains from Asia when transgenically expressed in rice [11], but no effective resistance is available in cultivated rice. This lack of genetic resistance severely limits control options for bacterial leaf streak. As a result, the disease has become a significant problem for rice production in Africa, where *Xoc* has been reported in several new areas in recent years [12,13].

Genome sequencing of global *Xoo* and *Xoc* isolates revealed clear genetic distinctions between the African and Asian isolates in each pathovar, although geographic *Xoc* clades are more tightly clustered than those of *Xoo*. Genome sequences also confirmed the diversity of TAL effectors, which number up to 18 for *Xoo* strains and up to 27 for *Xoc* [14]. Some TAL effectors have long been known to act as effective virulence and avirulence factors, but because multiple TAL effectors may target the same gene families or could possibly even inhibit one another's function, it is difficult to discern a functional role for many individual *Xo* TAL effectors using gene inactivation studies. However, one clade of *X. oryzae,* isolated from rice in the United States, has been found to lack TAL effectors entirely [15,16]. The "*USXo*" strains are highly genetically distinct from *Xoo* and *Xoc* strains from Asia and Africa, likely descending from a common ancestor of both pathovars (Fig. 1). Expression of the SWEET-targeting TAL effector PthXo1 more than doubled the lesion length of the *USXo* strain X11-5A on rice variety Azucena, demonstrating that this strain could perform as a suitable platform for testing the roles of different TAL effectors in different host backgrounds [17].

In a study comparing the effects of three SWEET-targeting TAL effectors in *USXo* virulence on diverse rice varieties, TAL effectors were capable of increasing *USXo* lesion length on 14 of 21 varieties [17]. However, only eight varieties were rendered more susceptible by all three TAL effectors, and the degree of change in lesion length varied greatly among effectors and varieties (Fig. 2). Six varieties had increased susceptibility in response to only one or two TAL effectors. While insensitivity to TAL effectors can sometimes be explained by a mutation in both copies of the TAL effector binding

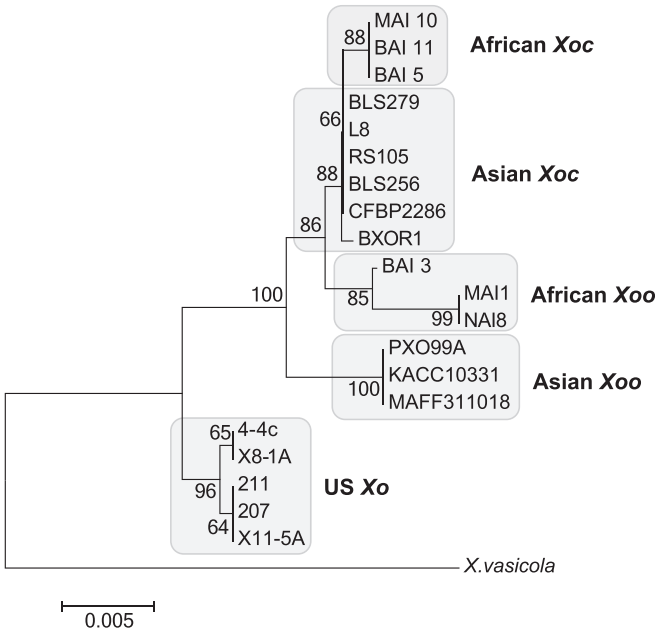

Fig. 1. Phylogenetic groups of *X. oryzae* are separated by pathovar and geographic origin. Analysis was performed as in Triplett et al. 2011, with the addition of corresponding sequence from six Asian *Xoc* genomes published in Wilkins et al. 2015.

site, sequencing of promoter segments of several varieties found no polymorphisms among TAL effector binding sites, even when the TAL effector caused little to no increase in susceptibility [17]. RT-PCR further demonstrated that TAL effectors causing little to no increase in lesion length were fully active in the host cell, inducing a large (>50 fold) increase in transcription of the SWEET gene targets. Therefore, while SWEET-targeting TAL effectors greatly increase susceptibility in some plants, some varieties appear to resist or overcome the effects of SWEET gene activation by TAL effectors. Varieties with similar patterns of response to TAL effectors tended to cluster phylogenetically, indicating that genetic factors may underlie an insensitivity to SWEET gene activation [17].

If the proposed role of SWEET genes is simply to enhance bacterial growth through increased extracellular sugar levels, why is this strategy ineffective in some varieties? One possibility is that increased levels of basal resistance, in the form of enhanced preformed or induced defenses, may negate the effects of SWEET gene activation. However, this would not explain the observed contrasts in phenotypes between two effectors that both target SWEETs (Fig. 2). For example, why does *pthXo1* enhance virulence in Pokkali, while *avrXa7* or *talC* do not? A thorough comparison of TAL effector target sequences and target gene expression levels in more varieties would be needed to address this question. Interestingly, even effectors targeting the same gene for activation may have inconsistent phenotypes. *avrXa7* and *talC* target the same susceptibility gene, OsSWEET14, from distinct binding sites in the promoter. Of the 13 varieties in which Verdier et al. found that *avrXa7* and *talC* increased lesion length, *talC* caused the greater increase in lesion length in eight (61.5%) of the varieties (Tainung 67, LTH, Dom-sufid, Azucena, Kitaake, Zhenshan 97B, N22, and FR13A, Fig. 2). It could be possible that the *avrXa7* binding site is disrupted in some of these varieties, or that *talC* is simply a more efficient activator.

However, another explanation might be suggested by a feature of AvrXa7 noted by multiple authors [18,19]. In several TAL effector target prediction programs, the validated target OsSWEET14 is predicted to be among the top two most favorable TalC binding