Xanthomonas axonopodis Virulence Is Promoted by a Transcription Activator-Like Effector–Mediated Induction of a SWEET Sugar Transporter in Cassava

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The gene-for-gene concept has historically been applied to describe a specific resistance interaction wherein single genes from the host and the pathogen dictate the outcome. These interactions have been observed across the plant kingdom and all known plant microbial pathogens. In recent years, this concept has been extended to susceptibility phenotypes in the context of transcription activator-like (TAL) effectors that target SWEET sugar transporters. However, because this interaction has only been observed in rice, it was not clear whether the gene-for-gene susceptibility was unique to that system. Here, we show, through a combined systematic analysis of the TAL effector complement of *Xanthomonas axonopodis* pv. *manihotis* and RNA sequencing to identify targets in cassava, that TAL20_{Xam668} specifically induces the sugar transporter *MeSWEET10a* to promote virulence. Designer TAL effectors (dTALs) complement TAL20_{Xam668} mutant phenotypes, demonstrating that *MeSWEET10a* is a susceptibility gene in cassava. Sucrose uptake-deficient *X. axonopodis* pv. *manihotis* bacteria do not lose virulence, indicating that sucrose may be cleaved extracellularly and taken up as hexoses into *X. axonopodis* pv. *manihotis*. Together, our data suggest that pathogen hijacking of plant nutrients is not unique to rice blight but also plays a role in bacterial blight of the dicot cassava.

Xanthomonas axonopodis pv. *manihotis*, the causal agent of bacterial blight of cassava, delivers type III effector proteins into the plant cell to suppress or modulate host innate immunity and promote pathogenesis. Most *X. axonopodis* pv. *manihotis* strains studied to date contain between one and five transcription activator-like (TAL) effectors (Bart et al. 2012; Castiblanco et al. 2013) which, in other xanthomonads, have been shown to elicit disease symptoms by inducing expression of host genes that allow the pathogen to achieve full

virulence (Antony et al. 2010; Cernadas et al. 2014; Hu et al. 2014; Li et al. 2014; Verdier et al. 2012; Yang et al. 2006). TAL effectors are delivered into host cells via the *Xanthomonas* type III secretion system and are translocated to the plant nucleus, where they physically interact with DNA in a sequence-specific manner to activate host gene expression (Boch and Bonas 2010; Bogdanove et al. 2010; Deng et al. 2012; Kay et al. 2007; Mak et al. 2012; Römer et al. 2007). Consistent with their function, the modular structure of TAL effectors consists of an N-terminal type III secretion signal, C-terminal nuclear-localization sequences, eukaryotic acidic activation domain, and central repeat domain that are responsible for sequence-specific protein–DNA interactions. This central repeat domain consists of a variable number of 33 to 35 amino acid repeats which are nearly identical, with most polymorphism residing at amino acids 12 and 13, known as the repeat variable diresidues (RVD). The amino acid identity of these RVD dictates the TAL effector–DNA-binding code, with each RVD specifying the binding of a single consecutive nucleotide through direct interaction (Boch et al. 2009; Moscou and Bogdanove 2009). Structural studies have shown that residue 13 in each repeat makes specific contact with the DNA, while residue 12 plays a stabilizing role (Deng et al. 2012; Mak et al. 2012). The nucleotide sequence bound by TAL effectors is called the effector-binding element (EBE).

TAL effectors can function either in effector-triggered immunity (ETI) if recognized by resistance (*R*) genes or as virulence determinants activating plant genes whose increased or ectopic expression facilitates bacterial growth, disease symptom formation, or both (Schornack et al. 2013). Several TAL effector-targeted genes have been identified as susceptibility genes, providing insight into strategies used by *Xanthomonas* during infection. *UPA20*, activated by the TAL effector AvrBs3 from *X. euvesicatoria*, is a transcription factor that regulates cell size in pepper (*Capsicum annuum*), leading to the cell hypertrophy that is a symptom of infection by *X. euvesicatoria* (Kay et al. 2007). *CsLOB1*, a transcription factor in sweet orange (*Citrus sinensis*) associated with expression of genes involved in cell expansion, is activated by multiple TAL effectors of *X. citri* subsp. *citri*. Expression of *CsLOB1* promotes citrus bacterial canker pustule formation and in planta bacterial growth (Hu et al. 2014; Li et al. 2014). TAL2g of the rice (*Oryza sativa*) mesophyll colonizer *X. oryzae* pv. *oryzicola* activates the predicted sulfate transporter *OsSULTR3;6* to promote lesion expansion and bacterial exudation from the leaf

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surface (Cernadas et al. 2014). *OsSWEET11* and *OsSWEET14* from rice, each activated by at least one *X. oryzae* pv. *oryzae* TAL effector, are members of the SWEET family of sugar transporters and were shown to transport both glucose and sucrose (Antony et al. 2010; Chen et al. 2010, 2012; Yang et al. 2006). Expression of host SWEET transporters at the site of bacterial infection is thought to cause an excess of sucrose to be transported into the apoplast, providing bacteria with a source of carbon (Chen 2014).

The recessive rice resistance gene *xa13* was found to be an allele of *OsSWEET11* that contains a modified promoter sequence that can neither be bound nor activated by the TAL effector PthXo1, rendering strains that utilize PthXo1 as their primary determinant of virulence unable to cause disease (Chu et al. 2006; Yang et al. 2006). A second recessive rice resistance gene, *xa25*, encodes *OsSWEET13* and is thought to avoid targeting by an as of yet unidentified TAL effector in a similar manner (Liu et al. 2011; Yuan and Wang 2013). Inspired by naturally occurring plant alleles, researchers have shown that plant disease resistance can be engineered by employing genome editing technologies to manipulate EBE in the promoters of susceptibility genes, such that they can no longer be activated in a TAL effector-dependent manner (Li et al. 2012). Engineering recessive resistance by modifying host susceptibility gene promoters is a strategy that could be combined (stacked) with dominant *R*-gene-mediated strategies employed by breeders and researchers (Schornack et al. 2013). However, in order to be a widely applicable strategy, susceptibility targets must first be identified in the pathosystem of interest.

Cassava (*Manihot esculenta*) is a root crop grown in Africa, Asia, and South America and is the third most important source of calories for people living in the tropics behind rice and corn (FAO 2008). Cassava production is increasing in part because of the low input costs and drought tolerance of this staple food source. However, cassava remains a relatively understudied crop (Howeler et al. 2013). Outbreaks of cassava bacterial blight (CBB), incited by *X. axonopodis* pv. *manihotis*, can cause extensive crop damage and constrain production (López and Bernal 2012; Lozano 1986). CBB symptoms include water-soaked angular lesions on leaves, shoot dieback, and necrosis. The development of resistant cassava varieties could protect farmers from the crop losses associated with CBB (Wydra et al. 2004). A robust cassava transformation protocol has been developed which will allow genetic engineering approaches to address this problem (Bull et al. 2009). However, few reports of phenotypic resistance to CBB exist and the genes underlying resistance have not been extensively studied, resulting in a lack of resources for genetic engineering of resistant cultivars (López and Bernal 2012). In addition, efforts at attaining CBB resistance through breeding have been largely unsuccessful, most likely due to genetic diversity among *X. axonopodis* pv. *manihotis* strains (Bart et al. 2012; Restrepo et al. 2000, 2004). All sequenced *X. axonopodis* pv. *manihotis* strains contain TAL effector-like sequences, indicating that TAL effectors are a conserved component of *X. axonopodis* pv. *manihotis*'s virulence arsenal (Arrieta-Ortiz et al. 2013; Bart et al. 2012). In addition, Castiblanco and associates (2013) demonstrated a virulence role for TALE1_{Xam} from *X. axonopodis* pv. *manihotis* CFBP1851. Therefore, the development of resistance based on modification of TAL effector susceptibility targets in the plant is a promising approach to developing cassava that is resistant to CBB.

In this study, we characterize the TAL effectors of highly virulent *X. axonopodis* pv. *manihotis* Xam668 and identify TAL14_{Xam668} and TAL20_{Xam668} as important contributors to *X. axonopodis* pv. *manihotis* virulence. We use RNA sequencing

(RNA-Seq) to identify host gene targets for all TAL effectors of Xam668, and identify a SWEET sugar transporter, *MeSWEET10a*, that is directly activated by TAL20_{Xam668}. We use designer TAL effectors (dTALE) to show that *MeSWEET10a* is the primary virulence target of TAL20_{Xam668}.

RESULTS

Xam668 TAL effectors contribute unequally to virulence and symptom development.

To assess the role of TAL effectors in *X. axonopodis* pv. *manihotis* virulence, we generated mutant strains for each of the five TAL effectors present in virulent *X. axonopodis* pv. *manihotis* Xam668. The Xam668 TAL effector RVD sequences were published previously (Bart et al. 2012). We subsequently identified two additional repeat units present in TAL18_{Xam668}, henceforth referred to as TAL20_{Xam668} (Supplementary Table S1) (Bart et al. 2013). TAL effector knockout strains were generated through a saturating screen of suicide vector integration events in the TAL effector coding region. Thirty-four independent insertions were screened by Western blot analysis and, in each case, at least one TAL effector was disrupted. We note that this method does not preclude the possibility of multiple TAL effectors of the same size being simultaneously knocked out. However, the five Xam668 TAL effectors were originally identified and sequenced from a cosmid library and the number of TAL effectors was estimated through restriction digest profiling. The data presented here combined with our previous work suggest that each differently sized TAL effector is unique.

Single knockouts for each of the five Xam668 TAL effectors were selected and virulence levels were assessed through observation of watersoaking symptom development and measurement of bacterial populations in planta over time. Disruption of TAL13_{Xam668}, TAL15_{Xam668}, and TAL22_{Xam668} did not cause a visible alteration in the development of watersoaked lesions or measurable differences in bacterial growth (Fig. 1A and B). Disruption of either TAL14_{Xam668} or TAL20_{Xam668} resulted in a reduction in bacterial growth in both the leaf midvein (Fig. 1B and C), and the leaf apoplast (Supplementary Fig. S1A). In addition to its growth defect and in contrast to TAL14_{Xam668}, disruption of TAL20_{Xam668} resulted in a reduction in watersoaking symptom development (Fig. 1A).

The Xam668 genome contains three differently sized plasmids in addition to chromosomal DNA, as visualized by agarose gel electrophoresis. Plasmid-cured strains assayed for TAL effector expression allowed us to determine that TAL20_{Xam668} is the only TAL effector encoded on the smallest plasmid. The TAL20_{Xam668} plasmid-cured strain (Xam668 ΔTAL20p) mimicked the symptom defect observed for Xam668 ΔTAL20, and reintroducing TAL20_{Xam668} into the plasmid-cured strain [Xam668ΔTAL20p(TAL20_{Xam668})] fully complemented the symptom defect phenotype (Fig. 1A). Because the plasmid-cured strain was marker free, we were able to generate a Xam668ΔTAL14/ΔTAL20p double knockout. The double mutant showed a reduction in watersoaking when compared with wild-type Xam668 and less growth than Xam668 and either single mutant (Fig. 1A and B). These results suggest that TAL14_{Xam668} and TAL20_{Xam668} make distinct contributions to *X. axonopodis* pv. *manihotis* virulence.

TAL20_{Xam668} increases watersoaking conferred by TAL20-deficient *X. axonopodis* pv. *manihotis* CIO151.

X. axonopodis pv. *manihotis* CIO151 lacks a TAL20_{Xam668} homolog and causes less watersoaking than Xam668 (Bart et al. 2012). To test whether TAL20_{Xam668} would increase watersoaking of *X. axonopodis* pv. *manihotis* CIO151, native pro-

motor-driven *TAL20_{Xam668}* was transferred to strain CIO151 by conjugation. Watersoaking caused by CIO151(*TAL20_{Xam668}*) was greater than that caused by CIO151 (Fig. 1D). However, CIO151(*TAL20_{Xam668}*) did not grow to significantly higher levels in planta than CIO151.

RNA-Seq identifies cassava genes upregulated in response to *X. axonopodis* pv. *manihotis* infection.

To identify Xam668 TAL effector targets in cassava, we first used the program TALE-NT 2.0 to computationally predict possible targets based on the TAL effector DNA-binding code

Fig. 1. *Xanthomonas axonopodis* pv. *manihotis* strain Xam668 contains five distinct transcription activator-like (TAL) effectors that contribute unequally to bacterial growth and symptom development. **A**, Knockouts (red arrows) were generated for each TAL effector and confirmed via Western blot analysis. TAL effector knockouts, *TAL20* plasmid-loss, and double mutant lines were inoculated into cassava leaves (optical density at 600 nm [OD_{600}] = 0.01) and symptom development was recorded after 5 days. **B**, Bacterial populations in leaf midveins were measured at day 0 and day 6. Data are represented as mean CFU per 6 cm of leaf midvein ($\pm$ standard deviation). Asterisks: * denotes significantly lower growth than Xam668, two-tailed *t* test, *P* value < 0.005 and ** denotes lower growth than Xam668 Δ TAL14 and Xam668 Δ TAL20, two-tailed *t* test, *P* value = 0.006 and 0.06, respectively. Growth assays were repeated at least three times with similar results. **C**, Schematic of midvein growth assay protocol showing site of wounding and placement of bacterial suspension and midvein section from which CFUs were counted. **D**, *TAL20_{Xam668}* increases watersoaking symptoms of *X. axonopodis* pv. *manihotis* strain CIO151. Native promoter-driven *TAL20_{Xam668}* was introduced into strain CIO151 by bacterial conjugation. Strains were inoculated into cassava leaves (OD_{600} = 0.01) and symptom development was recorded after 6 days. T = TAL, p = plasmid.

(Doyle et al. 2012). Even when the search area was narrowed to the 300-bp promoterome, there were still far too many targets to validate using molecular characterization alone (Fig. 2A). In addition, our experimental cassava cultivar of choice, 60444, may contain promoter polymorphisms with respect to the reference genome AM560-2, hindering accurate TAL effector target prediction. Therefore, we undertook a transcriptomic approach to identify Xam668 TAL effector targets in cassava.

We designed an RNA-Seq experiment to identify the full repertoire of genes induced by Xam668 infection in cassava, as well as genes induced by TAL20_{Xam668}, chosen because of the virulence defects seen in the mutant strain. A cosmid clone from our previously published cosmid library (Bart et al. 2012) encoding TAL20_{Xam668} was conjugated into *X. euvesicatoria* 85-10. Notably, *X. euvesicatoria* 85-10 does not have any endogenous TAL effectors, does not elicit a hypersensitive reaction or disease symptoms when infiltrated into cassava leaves even at high inoculum, and is able to express TAL effectors encoded on cosmid clones or the pVSP61 plasmid (Supplementary Fig. S2). Our RNA-Seq experimental design encompassed three time points (8, 24, and 50 h postinoculation [hpi]) and four experimental conditions (mock, Xam668, *X. euvesicatoria*, and *X. euvesicatoria*[TAL20_{Xam668}]). Genes upregulated in Xam668 compared with mock infiltration were considered potential targets of the five Xam668 TAL effectors, whereas genes upregulated in *X. euvesicatoria*(TAL20_{Xam668}) compared with *X. euvesicatoria* were considered to be TAL20_{Xam668} specific (Fig. 2B). Twelve libraries per replicate were indexed, pooled, and sequenced in a single lane on an Illumina HiSeq2000. In total, three biological replicates for each condition were compared. Illumina reads were aligned to the cassava reference genome AM560-2 using the TUXEDO suite of tools and default settings (Trapnell et al. 2012). Because our experiments were performed in cassava 60444, we allowed for two mismatches per read to accommodate possible single-nucleotide polymorphisms.

TAL20_{Xam668} directly targets *MeSWEET10a*.

We initially focused on identifying genes induced similarly in timing and intensity between mock versus Xam668 and *X. euvesicatoria* versus *X. euvesicatoria*(TAL20_{Xam668}). These analyses yielded a single putative target for TAL20_{Xam668}, *cas-sava4.1_013474*, henceforth referred to as *MeSWEET10a*, a member of the SWEET sugar transporter family of TAL effector-targeted susceptibility genes (Chen et al. 2012, 2010) (Fig. 3A; Supplementary Fig. S3). TAL effector dependence for activation was tested using *X. euvesicatoria* delivering individual Xam668 TAL effectors. Only TAL20_{Xam668} was able to activate *MeSWEET10a* (Fig. 3B). In addition, *MeSWEET10a* was not activated by Xam668ΔTAL20, suggesting that TAL20_{Xam668} is solely responsible for induction of this gene (Fig. 4). Semi-quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) verified *MeSWEET10a* activation by *X. axonopodis* pv. *manihotis* CIO151(TAL20_{Xam668}) and Xam668ΔTAL14/TAL20p(TAL14,TAL20), indicating that *MeSWEET10a* plays a role in the virulence and watersoaking phenotypes seen in these strains (Supplementary Fig. S4A and B).

To test whether *MeSWEET10a* activation is directly induced by TAL20_{Xam668}, we pursued a transient approach in which the *MeSWEET10a* promoter was used to drive expression of the reporter gene luciferase in an *Agrobacterium* binary vector. The resulting construct was coexpressed with 35S-driven TAL20_{Xam668} delivered via *Agrobacterium tumefaciens* in *Nicotiana benthamiana* leaf cells. Luciferase activity was measured 24 h after inoculation. *Agrobacterium tumefaciens* delivering TAL20_{Xam668} specifically induced expression of the *MeSWEET10a* promoter (Fig. 3C). Previous research has demonstrated that an *X. oryzae* pv. *oryzae* TAL effector-targeted EBE placed in the *Bs3* promoter from pepper (normally targeted by *X. euvesicatoria* TAL effector AvrBs3) retains its function (Römer et al. 2009); therefore, we tested whether this was true for the predicted TAL20_{Xam668}-targeted EBE from the *MeSWEET10a* promoter (Supplementary Table S2). Although

Fig. 2. Transcription activator-like (TAL) effector target prediction and strategy for experimental identification. **A**, Pie charts show the number of computationally predicted targets for each Xam668 TAL effector within the full cassava genome (left), 1,000 bp from an annotated start codon (middle), and 300 bp from an annotated start codon (right). Predictions were made using TALE-NT 2.0 (Doyle et al. 2012) scanning the genome of cassava AM560-2. **B**, Cartoon of experimental strategy for identifying TAL effector targets; hpi = hours postinoculation.

TAL20_{Xam668} did not induce expression of the wild-type *Bs3* promoter, when the predicted TAL20_{Xam668} EBE was inserted into the *Bs3* promoter, activation was observed (Fig. 3C). We note that the tested promoters do not display identical background levels and speculate that this is a result of their differential interaction with endogenous transcription activators or repressors.

Electromobility shift assays confirmed direct interaction between TAL20_{Xam668} and an oligonucleotide encompassing the predicted TAL20_{Xam668} EBE from the *MeSWEET10a* promoter (Supplementary Fig. S5A). Additionally, *MeSWEET10a* gene activation was seen in the presence of 50 μ M cycloheximide (CHX), an inhibitor of eukaryotic protein synthesis. Taken together, our data show that the *MeSWEET10a* promoter is directly bound and activated at the predicted EBE by TAL20_{Xam668}.

Fig. 3. TAL20_{Xam668} directly activates expression of *MeSWEET10a*. **A**, RNA sequencing (RNA-Seq) data showing expression of *MeSWEET10a* in mock- (wounding), Xam668- (five distinct transcription activator-like [TAL] effectors), *Xanthomonas euvesicatoria* 85-10- (no TAL effectors), and *X. euvesicatoria* 85-10 (TAL20_{Xam668})-inoculated cassava leaves 8, 24, and 50 h postinoculation (hpi). Heat map of log₁₀FPKM values represents the mean of three biological replicates. **B**, Semiquantitative reverse-transcriptase polymerase chain reaction of *MeSWEET10a* expression 24 hpi in leaf tissue inoculated at an optical density at 600 nm of 0.5 with *X. euvesicatoria* 85-10 delivering each of the five Xam668 TAL effectors. Experiment was repeated three times with similar results. **C**, *Agrobacterium tumefaciens* was used to deliver promoter-luciferase fusion constructs and 35S-driven TAL20_{Xam668} into *Nicotiana benthamiana* cells. Luciferase activity was quantified 24 hpi. Data are displayed as mean technical replicates ($\pm$ standard deviation). Experiments were repeated three times with similar results; EBE = effector-binding element. T = TAL.

dTALE restore Xam668ΔTAL20 virulence defects.

To test the role of *MeSWEET10a* as a host susceptibility gene, dTALE targeting distinct places within the *MeSWEET10a* promoter (dT_{MeSWEET10a-3} and dT_{MeSWEET10a-4}) were assembled with code-optimized RVD sequences (Supplementary Fig. S6) (Boch et al. 2009; Moscou and Bogdanove 2009). dT_{MeSWEET10a-3} and dT_{MeSWEET10a-4} were conjugated into Xam668ΔTAL20 and the resulting strains were assessed for restoration of virulence phenotypes. Both dT_{MeSWEET10a-3} and dT_{MeSWEET10a-4} were able to activate expression of *MeSWEET10a*, and both complemented symptom development (Fig. 4) and bacterial growth (Fig. 1B) in Xam668ΔTAL20. Complementation of the Xam668ΔTAL20 mutant phenotype by dTALE that activate *MeSWEET10a* shows that TAL20_{Xam668} contributes to *X. axonopodis* pv. *manihotis* virulence through the activation of *MeSWEET10a*, and that *MeSWEET10a* carries out a function in the plant that, when coopted by *X. axonopodis* pv. *manihotis*, promotes disease susceptibility.

MeSWEET10a is a sugar transporter.

Phylogenetic analysis indicates that *MeSWEET10a* belongs to clade III of the SWEET sugar transporter family and is most closely related to the *Arabidopsis* sucrose transporter *AtSWEET10* (Chen et al. 2012) (Fig. 5A). Several recent studies indicate that pathogens induce sucrose export from cells into the apoplast to be used as a carbon source (Chen 2014; Chen et al. 2010, 2012; Sonnewald 2011). If, indeed, the *X. axonopodis* pv. *manihotis* virulence strategy follows this model, we hypothesized that disrupting the ability of *X. axonopodis* pv. *manihotis* to import sucrose should result in a phenotype similar to that observed from blocking induction of *MeSWEET10a* (Xam668ΔTAL20). *SuxC* was identified as part of an operon from *X. campestris* pv. *campestris* that is specific for sucrose import and essential to *X. campestris* pv. *campestris* fitness (Blanvillain et al. 2007). To test the above hypothesis, we generated a deletion mutant in the orthologous *SuxC* gene of *X. axonopodis* pv. *manihotis* and confirmed that Xam668Δ*suxC* was unable to grow when sucrose was provided as the only carbon source (Fig. 5B). Notably, in minimal media, Xam668Δ*suxC* could still utilize glucose. We inoculated this mutant into cassava leaves and observed no significant reduction in growth (Fig. 5C). Although the watersoaking caused by Xam668ΔTAL20 was reduced, watersoaking by Xam668Δ*suxC* was similar to wild-type Xam668 (Fig. 5D). These results indicate that the induction of *MeSWEET10a*

Fig. 4. *MeSWEET10a*-targeted designer transcription activator-like effectors (dTALE) restore virulence defects of Xam668ΔTAL20. dTALE designed to target locations within the *MeSWEET10a* promoter were conjugated into Xam668ΔTAL20. Semiquantitative reverse-transcriptase polymerase chain reaction was used to confirm *MeSWEET10a* transcript induction 24 h postinoculation. Watersoaking symptom development was assessed at 5 days postinoculation for Xam668ΔTAL20 and dTALE-complemented strains inoculated at an optical density at 600 nm of 0.1. Experiments were repeated three times with similar results. T = TAL.

Fig. 5. MeSWEET10a is a member of the clade III family of sugar transporters. **A**, SWEET proteins from *Arabidopsis thaliana* (At), *Oryza sativa* (Os), and *Homo sapiens* (Hs) were chosen to represent clades of the SWEET superfamily (Chen et al. 2010). The phylogenetic tree was made using the neighbor-joining method in MEGA version 5.2.2 (Tamura et al. 2011). Bootstrap values (percentage of 1,000) are displayed. **B**, A full gene knockout was constructed for the *SuxC* gene in *Xam668*. Bacterial growth of *Xam668*ΔsuxC was compared with wild-type *Xam668* and *Xam668*ΔsuxC(SuxC) in minimal media supplemented with either sucrose or glucose. **C**, In planta growth of *Xam668*ΔsuxC was compared with *Xam668* and *Xam668*ΔsuxC(SuxC). Bacterial populations in leaf midveins were measured at day 0 and day 6. Data are represented as mean CFU per 6-cm leaf midvein ($\pm$ standard deviation). Growth assays were repeated at least three times with similar results. **D**, Symptom development was compared for *Xam668*, *Xam668*ΔsuxC, *Xam668*ΔTAL20, and complemented mutant lines. Pictures representative of seven independent replicates were taken 5 days after inoculation (optical density at 600 nm = 0.1). **E**, MeSWEET10a mediates sucrose transport activity as detected by coexpression with the cytosolic Förster resonance energy transfer (FRET) sucrose sensor FLIPsuc90Δ1a in HEK293T cells (Chen et al. 2012). Individual cells were analyzed by quantitative ratio imaging of eCFP and Aphrodite emission (acquisition interval of 10 s). HEK293T cells were perfused with culture medium, followed by square pulses of increasing sucrose concentrations. Blue line indicates cells expressing sensor alone; red line indicates cells coexpressing sensor and MeSWEET10a; accumulation of sucrose is indicated by a negative FRET ratio change (mean + standard error [s.e.]; $n > 19$). Experiments were repeated with comparable results at least four times. **F**, MeSWEET10a mediates glucose transport activity as detected by coexpression with the cytosolic FRET glucose sensor FLII¹²Pglu700Δ6 in HEK293T cells (Chen et al. 2010). Individual cells were analyzed by quantitative ratio imaging of eCFP and Citrine emission (acquisition interval of 5 s). HEK293T cells were perfused with culture medium, followed by square pulses of increasing glucose concentrations. Blue line indicates cells expressing sensor alone; red line indicates cells coexpressing sensor and MeSWEET10a; accumulation of glucose is indicated by a positive FRET ratio change (mean + s.e.; $n > 15$). Experiments were repeated with comparable results at least four times.

contributes to bacterial growth and watersoaking independently of the bacteria's ability to directly utilize sucrose in the apoplast. Although clade III transporters typically function as sucrose transporters, some members of this clade have been found to transport both glucose and sucrose. To test the sugar transport capabilities of MeSWEET10a, the protein was expressed in mammalian cells together with sucrose and glucose Förster resonance energy transfer (FRET) sensors (Chen et al. 2010). Sucrose and glucose-induced FRET responses indicate that MeSWEET10a can transport both sucrose and glucose (Fig. 5E and F).

Correlating induced genes with specific TAL effectors.

To identify targets of TAL13_{Xam668}, TAL14_{Xam668}, TAL15_{Xam668}, and TAL22_{Xam668}, we examined our RNA-Seq data for genes that were highly induced by Xam668 as compared with mock in at least two of the three time points (8 hpi and 24 hpi, 24 hpi and 50 hpi, or in all three time points). We cross-referenced the set of induced genes with the list of putative EBE predicted by TALE-NT 2.0 and found that many induced genes contained EBE with what are considered good scores (within a threefold cutoff of the best possible score for the given TAL effector) within their promoters (Doyle et al. 2012). A heat map for a manually selected subset of the most highly induced candidate TAL effector targets is presented (Fig. 6, left).

We assigned TAL effector specificity to the subset of putative target genes by carrying out semiquantitative RT-PCR on cassava leaves inoculated with *X. euvesicatoria* strains delivering individual Xam668 TAL effectors. Of the 17 genes tested,

we found that 1 was upregulated by TAL13_{Xam668}, nine by TAL14_{Xam668}, two by TAL15_{Xam668}, and four by TAL22_{Xam668}. No additional genes were found to be upregulated by TAL20_{Xam668} (Fig. 6, right). To test whether the TAL effectors were directly activating these target genes, we carried out inoculations in the presence of 50 μ M CHX. All genes tested were activated in a TAL effector-dependent manner in both the presence and absence of CHX, indicating that activation is direct (Supplementary Fig. S7). A slight decrease in expression was observed for 024404 and 026646, suggesting that these two genes may be indirect targets. However, the expression was not reduced to background levels; therefore, the decrease may be due to biological variation between samples. Most of the validated targets have one or more EBE specific to the TAL effectors by which they are activated in their promoters. The 020499 promoter contains multiple TAL14_{Xam668} EBE when the stringency for EBE score is relaxed to be within 3.5-fold of the best possible score. Similarly, the 029452 promoter contains two TAL13_{Xam668} EBE when the score stringency is reduced to be within fourfold of the best possible score. EBE for TAL22_{Xam668} were identified in the promoters of 018388 and 020556 by the program TALEVZ but not by TALE-NT, presumably because of differences in the programs' predictive RVD-nucleotide association matrices (Pérez-Quintero et al. 2013).

Finally, because our Western blot did not convincingly show TAL14_{Xam668} complementation of our TAL14_{Xam668}/TAL20_{Xam668} double mutant (Xam668 Δ TAL14/TAL20p(TAL14,TAL20) (Fig. 1A), we used semiquantitative RT-PCR to detect activation of the TAL14 target 007568, confirming TAL14 complementation.

Fig. 6. Specific gene upregulation during *Xanthomonas axonopodis* pv. *manihotis* infection can be attributed to individual transcription activator-like (TAL) effectors. Subset of RNA sequencing (RNA-Seq) data showing genes induced by Xam668 (five distinct TAL effectors) but not in mock (wounding)-inoculated cassava leaves shows potential TAL effector targets. Heat map of log₁₀FPKM values represents the mean of three biological replicates. Semiquantitative reverse-transcriptase polymerase chain reaction (PCR) of leaf tissue inoculated (optical density at 600 nm = 0.5) with *Xanthomonas euvesicatoria* 85-10 delivering each of the five Xam668 TAL effectors shows TAL effector-dependent gene activation. Experiment was repeated three times with consistent results. In all, 25 cycles of PCR were carried out, unless otherwise indicated (* = 28 cycles, ** = 23 cycles, and *** = 30 cycles).

Presence of TAL20 in diverse *X. axonopodis* pv. *manihotis* strains correlates with *MeSWEET10a* induction and watersoaking.

To determine the level of conservation among *X. axonopodis* pv. *manihotis* isolates in the ability to induce *MeSWEET10a* during infection, we selected a variety of naturally occurring field isolates of *X. axonopodis* pv. *manihotis* (Bart et al. 2012) and tested *MeSWEET10a* induction by semiquantitative RT-PCR. In addition, we assessed the number and approximate sizes of TAL effectors in these strains by Western blot (Fig. 7A). We found that *MeSWEET10a* induction is conserved in nine of 11 strains and correlates with relatively higher levels of watersoaking (Fig. 7B). All strains capable of inducing *MeSWEET10a* expressed a TAL effector similar in size to TAL20_{Xam668}.

DISCUSSION

TAL effectors constitute a major class of virulence factors used by xanthomonads during infection of their host plants. In this work, an RNA-Seq approach was used to study transcriptional changes induced during TAL effector introduction into plant cells. We chose as our pathosystem the important food crop cassava and its major bacterial pathogen *X. axonopodis* pv. *manihotis*. A differential contribution to virulence was found for the five TAL effectors of strain Xam668, with TAL20_{Xam668} influencing bacterial growth and symptom formation; TAL14_{Xam668} contributing to growth; and TAL13_{Xam668}, TAL15_{Xam668}, and TAL22_{Xam668} showing no detectable effect on

virulence. This variation is consistent with what is reported in the literature for other TAL effector family members and makes sense, given that TAL effectors are defined as transcriptional activators but their specific function in the host is dependent on their RVD sequence and the interaction of their RVD specificity with the host genotype. Like TAL20_{Xam668}, TAL effectors avrXa7 and PthXo1 of *X. oryzae* pv. *oryzae*, PthA of *X. campestris* pv. *campestris*, and TALE1_{Xam} of *X. axonopodis* pv. *manihotis* CFBP1851 have been reported to contribute to both growth and symptom formation (Bai et al. 2000; Castiblanco et al. 2013; Swarup et al. 1991; Yang and White 2004). The phenotype of Xam668ΔTAL14 shows that an influence on bacterial growth can be uncoupled from symptom development. Similarly, Tal2g of *X. oryzae* pv. *oryzicola* contributes to lesion length and watersoaking in rice but not to bacterial growth (Cernadas et al. 2014), and AvrB6 from *X. campestris* pv. *malvacearum* contributes to watersoaking and necrosis but not to growth in cotton (*Gossypium hirsutum*) (Yang et al. 1994). TAL effectors that have no effect when mutated have also been reported numerous times in the literature (Bai et al. 2000; Cernadas et al. 2014; Yang et al. 1996).

Our RNA-Seq experimental design allowed us to identify transcriptional changes induced by all five TAL effectors encoded by the Xam668 genome (within the mock versus Xam668 data set) and also to distinguish the transcriptional changes caused specifically by the TAL effector TAL20_{Xam668} (*X. euvesicatoria* versus *X. euvesicatoria* delivering TAL20_{Xam668}). Despite the fact that 457 annotated cassava promoters contain potential

Fig. 7. Multiple *Xanthomonas axonopodis* pv. *manihotis* strains from Asia, Africa, and South America (highlighted in orange, gray, and light blue, respectively) activate *MeSWEET10a*. **A**, Transcription activator-like (TAL) effector sizes for each strain were estimated based on Western blot. **B**, Semiquantitative reverse-transcriptase polymerase chain reaction shows activation of *MeSWEET10a* by various strains. Results of the Western blot are displayed figuratively, with blue and white squares indicating the presence or absence of TAL effectors with the specified number of repeat variable diresidues, respectively. Symptom development for each strain is shown. Leaves were imaged 7 days postinoculation (optical density at 600 nm = 0.01).

TAL20_{Xam668} EBE, only a single transcript, *MeSWEET10a*, was induced to similar levels by both Xam668 and *X. euvesicatoria*(TAL20_{Xam668}). Notably, there are predicted EBE for TAL13_{Xam668} and TAL14_{Xam668} in the *MeSWEET10a* promoter in addition to the TAL20_{Xam668} EBE, yet activation is dependent only on TAL20_{Xam668}. Mechanisms of TAL effector target specificity not mediated by RVD–base pair interaction are still being elucidated. It has been shown that TAL effector-dependent activation is limited by epigenetic modifications; therefore, chromatin state is a likely factor contributing to specificity (Bultmann et al. 2012). Recently, it was shown that adding constraints such as a lower relative EBE score (ratio of actual score to the score of a hypothetical perfect match) requirement and increased stringency for distance from the transcriptional start site can reduce the incidence of falsely predicted targets (Cernadas et al. 2014). Other factors contributing to specificity may include precision gained by members of the transcriptional complex or currently undiscovered specificity constraints built into TAL effectors themselves.

To identify targets of TAL13_{Xam668}, TAL14_{Xam668}, TAL15_{Xam668}, and TAL22_{Xam668}, we turned to our mock versus Xam668 RNA-Seq data set. Seventeen likely targets were selected based on expression profile and were subsequently shown to be activated by TAL effectors. Some targets lend themselves to hypotheses of function: TAL14_{Xam668}-targeted 007568, a pectate lyase, may aid bacterial colonization of the leaf and vascular tissues. Pectate lyases have been reported to promote disease in plants: bacteria in the genus *Erwinia* secrete pectate lyase enzymes via the type II secretion pathway to break down plant cell walls and cause symptoms of soft rot (Brencic and Winans 2005); *PMR6*, an *Arabidopsis* pectate lyase-like gene, is required for susceptibility to powdery mildew infection (Vogel et al. 2002); and cell wall pectic structures were previously shown to be highly altered during *X. axonopodis* pv. *manihotis* infection of cassava (Boher et al. 1995). Interestingly, *X. euvesicatoria* AvrBs3 was found to induce pepper pectate lyase *upa8*, and the *X. campestris* pv. *campestris* PthA-targeted *CsLOB1* transcription factor was associated with expression of pectate lyase *Cit.39387* (Hu et al. 2014; Marois et al. 2002). A metallothionein, 020499, may be induced in order to sequester toxic metals away from *X. axonopodis* pv. *manihotis* (Leszczyszyn et al. 2013). Genes 030094, 029949, and 027342 are annotated as transcription factors, which is consistent with reports of TAL effectors affecting multiple changes in the host transcriptome by directly activating a host transcription factor (Hu et al. 2014; Kay et al. 2007; Li et al. 2014). Although TAL20_{Xam668} has a single host target, TAL14_{Xam668} has many which may contribute collectively to virulence. The absence of a mutant phenotype for TAL13_{Xam668}, TAL15_{Xam668}, and TAL22_{Xam668} despite having bona fide host targets may be attributed to effector functional redundancy or to the challenge of measuring bacterial fitness in a laboratory setting. Laboratory experiments are hindered by an inability to include all potential pathogen life stages or environmental stresses, measure infection over long periods of time, or include large numbers of replicates. Additionally, TAL13_{Xam668}, TAL15_{Xam668}, and TAL22_{Xam668} may have susceptibility targets in host cultivars other than our experimental 60444.

MeSWEET10a is a sugar transporter related to the susceptibility genes *OsSWEET11* and *OsSWEET14* in rice. *OsSWEET11* and *OsSWEET14* are activated by multiple TAL effectors from *X. oryzae* pv. *oryzae*, and their activation promotes disease progression (Antony et al. 2010; Streubel et al. 2013; Yang et al. 2006; Yu et al. 2011). *MeSWEET10a* resides in clade III of the SWEET transporters along with *OsSWEET11* and *OsSWEET14*. Interestingly, another clade III SWEET, *cassava4.1_014124*, was upregulated in our mock versus Xam668

data set but its TAL effector dependence was not tested in this study. Clade III SWEET transporters appear to be particularly relevant for disease promotion, because a clade I SWEET from citrus, *CsSWEET1*, was identified as an *X. campestris* pv. *campestris* TAL effector target but no role in susceptibility was found (Hu et al. 2014). Clade III SWEET transporters in rice not yet shown to be TAL effector targets increased virulence of *X. oryzae* pv. *oryzae* when specifically activated by dTALE, while SWEET transporters from other clades did not (Streubel et al. 2013). The cassava genome encodes 23 putative SWEET members, 13 in clade III, based on annotations available on Phytozome (Goodstein et al. 2012; Prochnik et al. 2012). The reason for the specific role of *MeSWEET10a* in susceptibility is unknown.

SWEET transporters facilitate glucose and sucrose efflux from mesophyll cells into the apoplast, and are likely involved in phloem loading (Chen 2014). Because clade III SWEET transporters were found to preferentially transport sucrose, it was hypothesized that pathogens induce expression of *SWEET* genes to increase sucrose export from cells into the apoplast to be used as a pathogen carbon source (Chen et al. 2010, 2012). We explored the proposed link between SWEET transporter induction and pathogen sucrose utilization by comparing disease phenotypes of Xam668ΔTAL20, which does not activate *MeSWEET10a*, and Xam668ΔsuxC, which cannot import sucrose. Xam668ΔsuxC did not show a significant defect in growth in planta, which is likely explained by its ability to utilize carbon sources in the apoplast other than sucrose. Glucose exported by *MeSWEET10a* may be used directly, while exported sucrose may be broken down into hexose sugars by a host cell wall invertase enzyme (Kocal et al. 2008; Sturm 1999). This hypothesis is supported by our RNA-Seq data that show that one cassava gene annotated as an invertase is expressed and another is specifically induced during Xam668 infection (1.9-fold induction at 50 hpi). Both contain a signal peptide predicted by SignalP 4.1 (Petersen et al. 2011). In contrast to the phenotype of Xam668ΔTAL20, watersoaking was largely unaffected by the mutation of *suxC*. Taken together, our results show that *MeSWEET10a*'s contribution to virulence can be independent of *X. axonopodis* pv. *manihotis*'s ability to directly utilize apoplastic sucrose.

Although both the absence of TAL14_{Xam668} and TAL20_{Xam668} lead to a reduction in bacterial growth, only when *MeSWEET10a* is not activated (Xam668ΔTAL20) do we observe a significant reduction in watersoaking. In addition, TAL20_{Xam668} increases watersoaking by TAL20-deficient strain *X. axonopodis* pv. *manihotis* CIO151 but does not cause a significant increase in growth. The fact that growth can be uncoupled from watersoaking indicates that the role of symptom formation is not solely to aid in, nor is it necessarily a result of, bacterial growth. Watersoaking is caused by most xanthomonads and it is thought to contribute to bacterial dissemination (Brunings and Gabriel 2003). Mutation of *Tal2g* in *X. oryzae* pv. *oryzicola* and *AvrB6* in *X. campestris* pv. *malvacearum* resulted in decreased bacterial exudation from the leaf surface, a phenotype that was attributed to the decreased watersoaking conferred by the mutant strains. Notably, neither *Tal2g* nor *AvrB6* directly promote bacterial growth (Cernadas et al. 2014; Yang et al. 1994). AtSWEET9, a clade III SWEET sucrose uniporter from *Arabidopsis thaliana*, was recently shown to be involved in nectar secretion. AtSWEET9 exports sucrose into the apoplast, where it is likely broken down into hexose sugars by a plant invertase, thereby maintaining a sufficient osmotic gradient for water secretion (Lin et al. 2014). An intriguing possibility is that *MeSWEET10a* aids bacterial growth by providing an apoplastic carbon source and independently promotes water accumulation by altering the osmotic gradient of

the mesophyll, leading to *X. axonopodis* pv. *manihotis*'s characteristic watersoaking symptoms.

The identification of *X. axonopodis* pv. *manihotis*-induced susceptibility genes will contribute to developing cassava that is resistant to CBB. Li and colleagues (2012) reported an experiment in rice in which TAL effector nucleases were used to generate promoter mutations that abolished TAL effector induction of downstream susceptibility genes, resulting in *X. oryzae* pv. *oryzae*-resistant rice. This type of resistance strategy will prove effective in cassava if targeting of a given susceptibility gene promoter is a conserved strategy among *X. axonopodis* pv. *manihotis* strains. Although we were previously able to shed light on effector conservation across a collection of *X. axonopodis* pv. *manihotis* isolates using next-generation sequencing, this type of work applied to TAL effectors remains complicated because of their highly repetitive sequences. We considered a panel of diverse *X. axonopodis* pv. *manihotis* strains for induction of *MeSWEET10a*, and found activation to be conserved in nine of 11 strains and correlated with the presence with a TAL20_{Xam668}-sized TAL effector. Our results suggest that an effective resistance strategy against CBB would be one that abolishes the ability of *X. axonopodis* pv. *manihotis* to induce *MeSWEET10a*; however, additional susceptibility genes must be identified to ensure resistance. Multiple *X. oryzae* pv. *oryzae* TAL effector-targeted SWEET susceptibility genes have been identified; therefore, it is possible that the *X. axonopodis* pv. *manihotis* strains that do not activate *MeSWEET10a* target a different yet functionally equivalent member of the cassava SWEET family (Antony et al. 2010; Liu et al. 2011). We propose that a combination of identifying plant resistance genes that recognize members of a core set of effectors coupled with the genome editing of cassava susceptibility gene promoters will provide a multipronged strategy that will hinder pathogen growth and reduce the possibility of selecting for mutants that can overcome disease resistance. This approach should provide a durable resistance in the host, saving farmers from the hardships associated with crop loss (Dangl et al. 2013).

MATERIALS AND METHODS

Additional methods are provided in Supplementary Text.

Plant inoculations and virulence assays.

Xanthomonas strains were resuspended in 10 mM MgCl₂. For watersoaking assays and inoculations for semiquantitative RT-PCR, abaxial nicks were made on leaves of cassava 60444 with a razor blade and culture was injected into the leaf via a 1-ml needleless syringe. For midvein growth assays, 2-mm holes were made with a glass Pasteur pipette in the midvein one-quarter of the way in from the leaf tip. A 5- μ l drop of bacterial suspension at an optical density at 600 nm (OD₆₀₀) = 0.2 was immediately placed in the hole and allowed to dry undisturbed for 15 min. At each time point, 3-cm midvein sections starting 0.5 cm in from the inoculation point were cut out and ground in 10 mM MgCl₂ with a 3-mm glass bead in a bead-beater. Two midvein sections were combined per replicate, for a total of three replicates. Serial dilutions were plated on the appropriate selection plus CHX to inhibit fungal growth.

RNA-Seq.

Strain construction for RNA-Seq. *X. euvesicatoria* (TAL20_{Xam668}) was made by conjugating a cosmid library clone (pLAFR3) containing TAL20_{Xam668} into *X. euvesicatoria* 85-10 by triparental mating, using *Escherichia coli* helper strain pRK600. Expression of TAL20_{Xam668} was verified by Western blot.

Plant inoculations for RNA-Seq. *X. axonopodis* pv. *manihotis* and *X. euvesicatoria* grown on nutrient yeast glycerol agar plates supplemented with rifampicin (100 μ g/ml) or rifampicin and tetracycline (10 μ g/ml) for *X. euvesicatoria*(TAL20_{Xam668}) were resuspended in 10 mM MgCl₂ at OD₆₀₀ = 0.5. Abaxial nicks were made on leaves of cassava 60444 with a razor blade and culture was injected into the leaf via a 1-ml needleless syringe. Mock infiltrations were done with 10 mM MgCl₂.

NGS library preparation. Total RNA from inoculated leaf tissue (30 to 50 mg) was extracted with the Spectrum Plant total RNA kit (Sigma-Aldrich), with an on-column DNaseI digestion step included. RNA quality was checked by Bioanalyzer (RNA pico chip; Functional Genomics Laboratory [FGL], University of California [UC] Berkeley). RNA-Seq libraries were made using the TruSeq RNA sample preparation kit (v2; Illumina), starting with 3 μ g of total RNA. Library quality was assessed by Bioanalyzer (DNA 1000 chip; FGL, UC Berkeley). Quantification by quantitative PCR and pooling of samples was carried out at UC Berkeley's Genomics Sequencing Laboratory (GSL). Libraries were sequenced at the GSL on an Illumina HiSeq 2000, generating 100-bp paired-end reads. Raw reads were submitted to Short Read Archive (BioProject: PRJNA-257332).

Data analysis. Raw read files were trimmed for quality and adaptor removal using Trimmomatic (LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36) (Lohse et al. 2012). After trimming, gene expression changes were identified using the Tuxedo suite of tools with the default setting (Trapnell et al. 2012). In brief, bowtie-build was used to make a bowtie index from the only publicly available and annotated cassava genome, AM560-2 v 4.1, available from Phytozome (Prochnik et al. 2012). TOPHAT (-I 1000-i20) was used to align reads to identify gene models, map reads to gene models, and estimate expression levels. Analyses were first conducted individually on three biological replicates and global gene expression patterns were examined using the R-implementation CummeRbund (Trapnell et al. 2012) to identify outlying biological replicates. Because all three replicates had similar expression profiles, final analysis was conducted on the union of all three biological replicates for increased statistical power. Cufflinks was run with default settings on the "accepted hits" bam files from the TOPHAT analysis. Cuffmerge was used to join all gene models from all Cufflinks analyses and then Cuffdiff was used to estimate differential gene expression values. By default, Cuffdiff analyzes all possible combinations of input conditions. In-house python scripts were used to pare data down to comparisons of mock versus Xam668 and *X. euvesicatoria* 85-10 versus *X. euvesicatoria*(TAL20) for each time point. Comparisons that showed at least threefold induction at one time point were chosen for further consideration. In addition, comparisons that yielded nonstatistically significant data were excluded. A heat map of the resulting list was generated to compare expression patterns across all samples. Rows that showed highly variable or inconsistent trends in expression were excluded.

Prediction of Xam668 TALE-targeted EBE. TALE-NT was used to identify potential binding sites in the cassava promoterome (1 kb upstream of annotated start codons) for Xam668 TAL effectors (Doyle et al. 2012). Each TAL effector was assigned a best binding score given its RVD sequence, and sites within a threefold cut-off of this score were considered potential EBE. Functional annotations for each gene model were obtained from the Mesculenta_147_annotation_info text file (last modified 12.26.12) available from Phytozome.

Semiquantitative RT-PCR analysis.

X. axonopodis pv. *manihotis* strains were inoculated as described at OD₆₀₀ = 0.5. Total RNA was extracted with the

Spectrum plant total RNA kit (Sigma-Aldrich), with an on-column DNaseI digestion step included. Starting with 1 µg of total RNA, cDNA was made using the SuperScript III first-strand synthesis system for RT-PCR (Invitrogen) and diluted 1:10. The 25-µl RT-PCR reactions contained 10 µl of the diluted cDNA sample, 0.2 µM forward primer, 0.2 µM reverse primer, and 1× OneTaq Master Mix with Standard Buffer (New England Biolabs). Reactions cycled 25 times (unless otherwise stated in figure legends) and were run on high percent Tris-acetate-EDTA agarose gels. For CHX treatments, bacterial infiltrations were done with suspensions containing 50 µM CHX. Actin (gene ID *cassava4.1_009807*) was used as a control for all RT-PCR. Primer sequences can be found in Supplementary Table 3.

N. benthamiana transient expression analyses.

Leaves of *N. benthamiana* were injected with *Agrobacterium tumefaciens* GV3101 suspensions ($OD_{600} = 0.4$) using a 1-ml needleless syringe. At 24 hpi, sites of *Agrobacterium* inoculation were infiltrated with 1 mM luciferin. For each site, four 0.28-cm² leaf punches were taken and placed in separate wells of a black microtiter plate, suspended on 100 µl of water. Luciferase activity was read using a Wallace Envision machine.

Construction of dTALE and complemented strains.

The DNA binding domain of dTALE were assembled as described by Morbitzer and associates (2010) and used to replace the RVD of AvrBs3. We designed two distinct dTALE with *MeSWEET10* promoter-specific, code-optimized RVD sequences (dTALE_{MeSWEET10a-3} – EBE score 5.02 and dTALE_{MeSWEET10a-4} – EBE score 4.88) (Boch et al. 2009; Moscou and Bogdanove 2009). No dTALE target sites overlap with validated TAL20_{Xam668} EBE. For both dTALE, the ratio of the EBE score to the hypothetical best possible score is 1. dTALE constructs driven by the *TAL20_{Xam668}* promoter in pVSP61 were conjugated into Xam668ΔTAL20 using *E. coli* helper strain pRK600.

Characterization of SuxC mutant.

X. axonopodis pv. *manihotis* strains were grown in a 28°C shaker in 20 ml of minimal MME medium (K₂HPO₄, 10.5 g/liter; KH₂PO₄, 4.5 g/liter; (NH₄)₂SO₄, 1 g/liter; MgSO₄, 1 mM; casamino acids, 0.15 g/liter) (Arlat et al. 1991) containing 20 mM sucrose or glucose and rifampicin (100 µg/ml). Following inoculation at $OD_{600} = 0.1$ from a washed starter culture, optical densities at 600 nm were measured every 10 to 20 h over a period of 100 h.

FRET analysis.

Mammalian cell culture, transfection, image acquisition, and FRET analysis were performed essentially as described (Hou et al. 2011; Takanaga and Frommer 2010). In short, HEK293T cells were grown in DMEM (Invitrogen) with 10% fetal bovine serum, penicillin at 50 U/ml, and streptomycin at 50 µg/ml in a 37°C incubator at 5% CO₂. For FRET imaging, cells were cultured on collagen-coated glass cover slips. Cells were transfected when the confluence of cells reached 35 to 40%. FLIP-suc90µΔ1a (a FRET sucrose sensor modified from FLIPsuc90µ [Lager et al. 2006] carrying full eCFP at the N terminus and Aphrodite [Deuschle et al. 2006] at its C terminus) in pcDNA3.1(–) and FLII¹²Pglu700µΔ6 in pcDNA3.1(–) (Takanaga et al. 2008) were used to monitor sucrose and glucose uptake activities, respectively.

Western analysis.

Minimal media expression and protein extraction. Overnight liquid cultures of *X. axonopodis* pv. *manihotis* were resus-

suspended in 125 µl of minimal media A (pH 5.14) to $OD_{600} = 0.2$ along with appropriate antibiotics—*X. axonopodis* pv. *manihotis* strains: rifampicin (100 µg/ml), ΔTALE rifampicin (100 µg/ml), and tetracycline (10 µg/ml) and Xam668ΔTALE(pVSP61 TALE): rifampicin (100 µg/ml), tetracycline (10 µg/ml), and kanamycin (25 µg/ml)—and shaken at 28°C for 4 h. An equal volume of 3× Laemmli buffer was added and samples were boiled 6 min, vortexed, spun down, and stored as aliquots at –80°C.

Western blot. Protein lysates were separated via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using the NuPAGE Novex 4 to 12% Bis-Tris precast gels in NuPAGE morpholinepropanesulfonic acid SDS running buffer (Life Technologies). After 4.5 h at 120 V, TAL effector content was analyzed using standard immunoblotting techniques with a polyclonal antibody capable of recognizing diverse TAL effectors. Full-length PthB, renamed TALE1_{Xam} (Castiblanco et al. 2013), was expressed in *E. coli* from the vector pETDuet, which added a C-terminal 6xHis tag. TALE1_{Xam} protein was purified on a nickel column and sent to Covance for antibody production in rabbit.

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