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Research article

Phytohormone priming elevates the accumulation of defense-related gene transcripts and enhances bacterial blight disease resistance in cassava

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ABSTRACT

Cassava bacterial blight (CBB) disease caused by *Xanthomonas axonopodis* pv. *manihotis* (*Xam*) is a severe disease in cassava worldwide. In addition to causing significant cassava yield loss, CBB disease has not been extensively studied, especially in terms of CBB resistance genes. The present research demonstrated the molecular mechanisms underlining the defense response during *Xam* infection in two cassava cultivars exhibiting different degrees of disease resistance, Huay Bong60 (HB60) and Hanatee (HN). Based on gene expression analysis, ten of twelve putative defense-related genes including, leucine-rich repeat receptor-like kinases (*LRR-RLKs*), resistance (*R*), *WRKY* and pathogenesis-related (*PR*) genes, were differentially expressed between these two cassava cultivars during *Xam* infection. The up-regulation of defense-related genes observed in HB60 may be the mechanism required for the reduction of disease severity in the resistant cultivar. Interestingly, priming with salicylic acid (SA) or methyl jasmonate (MeJA) for 24 h before *Xam* inoculation could enhance the defense response in both cassava cultivars. The disease severity was decreased 10% in the resistant cultivar (HB60) and was remarkably reduced 21% in the susceptible cultivar (HN) by SA/MeJA priming. Priming with *Xam* inoculation modulated *cassava4.1_013417, cassava4.1_030866* and *cassava4.1_020555* (highest similarity to *MeWRKY59, MePR1* and *AtPDF2.2*, respectively) expression and led to enhanced resistance of the susceptible cultivar in the second infection. The putative *cis*-regulatory elements were predicted in an upstream region of these three defense-related genes. The different gene expression levels in these genes between the two cultivars were due to the differences in *cis*-regulatory elements in their promoter regions. Taken together, our study strongly suggested that the induction of defense-related genes correlated with defense resistance against *Xam* infection, and exogenous application of SA or MeJA could elevate the defense response in both cultivars of cassava. This finding should pave the way for management to reduce yield loss from disease and genetic improvement in cassava.

1. Introduction

Cassava (*Manihot esculenta* Crantz.) is a dicotyledonous crop belonging to the family Euphorbiaceae. It is the third most important source of carbohydrates for over 700 million people in tropical and subtropical areas (FAO, 2008). Many countries are large worldwide exporters of cassava products, which are used as raw material for various industries including human food, animal feed, biofuel production and starch-based industries. However, cassava yields are continuously decreased by several viral, bacterial and fungal diseases. One of the most severe diseases is cassava bacterial blight (CBB) disease caused by the pathogenic bacteria *Xanthomonas axonopodis* pv. *manihotis* (*Xam*). *Xanthomonas* spp. are hemibiotrophic pathogens that initially feed on living host tissue, but in later infection stages, cause the death of plant cells (Verdier et al., 1994; Boher et al., 1995; Buttner and Bonas, 2010). Critically, this disease has been reported to cause yield losses of 50–75% in different areas, depending on the severity of the disease in Latin America and Africa (Wydra and Verdier, 2002), and 22% in the eastern part of Thailand, depending on the cassava cultivars, developmental stages, severity of the disease and environment (Tokhun, 2014).

During evolution, plant mechanisms developed for the detection of pathogens have relied on innate immunity in each cell and on systemic signals emanating from the sites of infection. Plants commonly use at least two mechanisms, including (1) the innate immunity, which is

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initially triggered by transmembrane plant pattern-recognition receptors (PRRs) to recognize microbial- or pathogen-associated molecular patterns (MAMP-/PAMP-triggered immunity, PTI); and (2) largely inside the cell, a plethora of resistance (R) proteins that can recognize given pathogen secreted-effector proteins, resulting in highly specific effector-triggered immunity (ETI) (Jones and Dangl, 2006). The hallmark of PTI is the ability of the plant to detect pathogens using PRRs. Receptor-like kinases (RLKs) are among the well-characterized PRRs. RLKs with leucine-rich repeat-containing extracellular domains (LRR-RLKs) comprise the largest subfamily of transmembrane RLKs in plants. LRR-RLKs have been reported to regulate a wide variety of developmental and defense-related processes, including cell proliferation, stem cell maintenance, hormone perception, host-specific as well as non-host-specific defense responses, wound responses, and symbiosis (Torii, 2004). LRR-RLKs have been identified in many plants such as *Arabidopsis*, tomato, maize and citrus (Kemmerling, 2011); however, little is known about the LRR-RLK gene family in cassava. Some plant pathogenic bacteria deliver effectors into host cells using type III secretion systems, and the effectors contribute to pathogen virulence, often by mimicking or inhibiting cellular functions. The ETI response is initiated by the secretion of effector molecules, called Avr (avirulence) proteins, which are detected by the plant through recognition by host specific intracellular disease resistance (R) proteins containing polymorphic nucleotide binding (NB) and leucine-rich repeat (LRR) domains. NB-LRR activation results in a network of response pathways. Although PTI and ETI employ distinct immune receptors, they seem to modulate a complex of signaling pathways and subsequently induce a number of overlapping sets of defense mechanisms. Signaling receptors can trigger rapid and convergent downstream signaling networks controlled by calcium-activated protein kinases and mitogen-activated protein kinase (MAPK) cascades that phosphorylate downstream transcription regulators to control early defense gene expression. These protein kinase signaling networks serve specific and overlapping roles in controlling a large spectrum of protein targets, such as transcription factors, metabolic enzymes, plasma membrane proteins, and cytoskeletal proteins, contributing to resistance against the pathogen (Bigeard et al., 2015).

Xanthomonas spp. are classified as hemibiotrophic pathogen that live and obtain nutrients from living host tissue as a biotroph at the initial infection stage, and then transit to the necrotroph and feeds on nutrients obtained from killing the plant cell at the later infection stage (Boher et al., 1995; Buttner and Bonas, 2010). *Xam* enters the plant through natural openings such as stomata, hydathodes or wounds. The disease symptoms are characterized as angular leaf spots, followed by blight, defoliation, wilting of the immature shoot and finally dieback (Verdier et al., 2004). To cause the disease, *Xam* produces and secretes virulence factors such as lipopolysaccharides and extracellular polysaccharides and effectors to increase the bacterial population and disease progression in host cassava plants. One of the best characterized is the family of effectors called Transcriptional Activator-Like proteins (TALEs), which play an important role in the modulation of host plant genes by mimicking eukaryotic transcription factors (Bogdanove et al., 2010). The intriguing characteristics of the repeat domain of TALE proteins has allowed the prediction of putative target sequences in the promoter regions of specific genes in the host plant. Recently, the possible binding sites for $TALE1_{Xam}$ were predicted in the promoter regions of genes in the available draft cassava genome using the prediction program TALVEZ. The top 100 target genes in cassava with a promoter region containing possible binding sites for TALE1 encoded transcription factors, enzymes, membrane transporter, lipid metabolism, hormone responsive and unknown function proteins, suggesting that $TALE1_{Xam}$ interferes with as yet unknown host functions (Castiblanco et al., 2013).

Previously, expressed sequence tags (ESTs) have been developed to study global gene expression profiles in cassava, and 199 genes showed differential expression patterns between *Xam*-infected and non-infected plants, especially at 7 days post-inoculation. Six genes, including degradation enzymes and disease resistance (R) proteins, showed higher expression in the resistant cassava cultivar compared with the susceptible cultivar. In addition, oxidative burst enzymes and potential pathogen recognition (putative leucine rich repeat), cell death and WRKY transcription factors were also up-regulated in infected plants (Lopez et al., 2005). The RNA-seq analysis in cassava inoculated with pathogenic *Xam* was compared with non-pathogenic *Xam* inoculation. Both strains triggered similar responses, such as the up-regulation of genes related to photosynthesis, phenylpropanoid biosynthesis, pathogenesis and repression of genes related to jasmonic acid signaling (Muñoz-Bodnar et al., 2014). In addition, approximately 30,000 genes in the cassava oligonucleotide-DNA microarray were used to demonstrate the gene expression of *Colletotrichum gloeosporioides* f. sp. Manihotis-infected HB60 and HN. The expression of various plant defense-related genes, such as PR genes, cell wall-related genes, detoxification enzymes, genes related to the response to bacterium, MAPK, and genes related to SA, JA and ethylene pathways were higher in HB60 compared with HN (Utsumi et al., 2016).

In Lozano et al., 2015 identified 228 NBS-LRR type genes and 99 partial NBS genes in the cassava genome (Lozano et al., 2015). In the same year, a genetic map of cassava with integrated physical mapping of immunity-related genes was constructed using the genotyping-by-sequencing approach. Based on the cassava genes coding for immune-related proteins (IRPs) in the cassava genome, a total of 569 IRPs were mapped, 198 of which belonged to the LRR, 160 to the LRR-kinase, 88 to the NB-ARC-LRR, 80 to the WRKY, 8 to the NB-ARC, 13 to the TIR-NB-ARC-LRR, 8 to the LysM, 6 to the TIR and 9 to the LysM-kinase class (Soto et al., 2015). In 2017, an F1 mapping population of 117 full cassava sibs was tested for resistance to two strains of CBB at two locations in Colombia, and genotyping by environmental analysis was performed. The three QTL (Quantitative Trait Loci) conferring resistance to CBB were detected and localized on the genetic map of cassava. DNA sequence analysis of the QTL intervals revealed 29 candidate defense-related genes, two of which contained domains related to plant immunity proteins such as NB-ARC-LRR and WRKY (Soto et al., 2017).

In defense signaling pathways, the phytohormones salicylic acid (SA) and jasmonic acid (JA) function as an important signaling molecule that play a central role in response to different types of pathogens. SA is generally involved in the activation of the defense response against biotrophic and hemibiotrophic pathogens, as well as the establishment of systemic acquired resistance (SAR), while JA is required for induced systemic resistance (ISR) in response to necrotrophic pathogens (Grant and Lamb, 2006; Vos et al., 2015). Moreover, exogenous application of SA and methyl-jasmonate (MeJA) as defense elicitors promotes the plant primed state of an enhanced defense response through the induction of SAR and ISR, respectively. In the primed state, defense responses can be activated more rapidly and more effectively when plants are infected by pathogens (Conrath et al., 2015). During plant defense, the importance of hormone signaling networks is reflected by the observation that many microbes interfere with hormone signaling pathways or produce hormones that increase microbial fitness. However, very little has been reported about the defense response at the molecular level or the application of phytohormones for activating immunity in *Xam*-infected cassava.

Taken together, previous information indicates that defense-related genes should be key genes in response to *Xam* infection in cassava. Therefore, the aims of this study were to study the molecular mechanisms underlying the complex signaling pathway and the utilization of phytohormones for activating the defense response by focusing on the expression of defense-related genes, including R proteins, WRKY transcription factors and PR proteins, as well as LRR-RLKs. Although *RLKs* are not defense genes, they are required for detecting pathogens and signal transduction. Two cultivars of cassava with different degrees of CBB disease resistance were used to demonstrate the correlation of the response to *Xam* and the differential expression of defense-related