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Colloquium Abstract

Type III transcription activator like effector (TAL) proteins drive the expression of susceptible genes in citrus plants

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Abstract

The economy of the world's agricultural market is experiencing a major setback due to introduction of large number of invasive species into the crops. *Xanthomonas* is one of the pathogenic species introduced into the agricultural crops. This group of bacteria comprise 27 species and are Gram-negative, aerobic, rod-shaped bacterium measuring 1.5-2.0 x 0.5-0.75 µm having a single polar flagellum for motility *[Ryan, R. P. et.al., 2011]*. Xanthomonas is associated with ~400 plant hosts causing serious disease in them. Among the diseases caused by Xanthomonas, citrus canker disease caused by Xanthomonas citri subsp.citri pathotype A (Xcc) is the most widespread [Pitino, M et.al., 2015]. It is the main cause of Asiatic citrus canker *[Ferreira, C. B. Et.al., 2017]*. It is the most virulent pathogenic bacteria with wide range of host plants *[Graham, J. H. et.al., 2004]*. Citrus canker disease is associated with necrotic lesions on leaves, fruits and stems. With the disease progression, premature drop of fruits, twig die back occurs resulting in severe economic loss *[Pitino, M et.al., 2015]*.

The bacteria marks its entry into the host through wounds and stomatal openings. Once attached to the host plant through flagellum and adhesin proteins, the extracellular receptors present on the plasma membrane of the host recognises the pathogen as pathogen associated molecular pattern (PAMPs). This recognition triggers the host basal defence system to prevent the bacterial colonisation *[McCann, H. C. et.al., 2008]*. However, the pathogens have evolved mechanisms to evade the host defense system. Type III secretary system (T3SS) is one of the best characterised mechansim whose primary role is to translocate effector proteins to suppress the defense system. Transcription activator like effector proteins (TALEs) are one of the most virulent effectors *[Ma, L. et.al., 2018]*.

TALEs are substrates of T3SS and are translocated into the host nucleus via type 3 secretory system (T3SS) *[Zhang, M.,et 2014]*. This translocation mechanism is said to be the key feature of *Xanthomonas* pathogenesis. TALES have a central DNA binding domain that is comprised of tandem repeats of 33-34 amino acids. Numerous TALES with varying tandem repeats are identified in different Xanthomonas species *[Duchateau, P. et.al., 2016]*. Every TALE is unique in their 12th and 13th position. The pair of monomers at these positions direct the binding to specific nucleotide base in the target gene. These pair of monomers are called as repeat variable di residue (RVDs). The most common pair of monomers at 12 and 13th position of every TALE are HD, NG, HG, NN. *[Mak, A. N. S. et.al., 2012]*. HD is recognizes cytosine, NG and HG recognizes thymine and NN recognizes guanine of the Effector Binding Elements (EBEs) of the targeted gene. Such binding allows the activation of target gene by mimicking the host transcriptional machinery.

Regulation of gene expression can lead to disease symptoms in the hosts and such genes are called as susceptible genes. However, in some cases the TALEs proteins are recognized as avirulence proteins by the host and activate the resistance mechanisms, such genes are called as resistance gene *[Denancé, N. et.al., 2018]*. PthA transcription factor is one of the most virulent factor that is recognized in X.citri pv citri (Xcc). PthA drives the expression of susceptible gene CsLOB1 in citrus plants *[Hu et al., 2014; Yan and Wang, 2012]*.

Here, I describe if the PthA transcription factor activated susceptible gene CsLOB1 is capable of inducing one of the important citrus canker symptoms, the pustule formation and molecular mechanism of causing the disease symptoms.

Citrus sinensis lateral organ boundary (CsLOB1) is one of the member of Lateral organ boundary domain (LBD) gene family. Studies by Zhang. J, Duan. S, Hu and their colleagues have shown that CsLOB1 gene is a susceptible gene responsible in assisting Xcc pathogen to induce pustule formation in citrus plants.

Duan. S, (2018) and his colleagues tested if CsLOB1 S gene was indeed responsible for inducing pustule formation in citrus plants. For which, they constructed a plasmid having CsLOB1 gene, fused with glucocorticoid receptor at C-terminus, green fluorescent protein (GFP) and neomycin phosphotransferase II as the markers. This construct was used to develop transgenic Duncan grapefruit by *Agrobacterium* mediated transformation. *Agrobacterium* transformation is the widely used transformation protocol in producing transgenic citrus plants as it is easy and do not require embryonic calli. Dexamethasone (DEX), a synthetic inducer of nuclear targeting via glucocorticoid receptor was exploited to induce the expression of CsLOB1-GR. The results demonstrate that DEX successfully directed the nuclear localization of CsLOB1-GR and its expression that lead to the induction of pustule formation along with induction of downstream genes in the transgenic plants. The downstream genes included cell wall degrading genes such as pectate lyase genes. This gene is responsible for inducing water soaking in plants. Previous studies had demonstrated that PthA gene also induces the expression of pectate lyase gene *[Hu.et.al.,2014]*. Therefore, the expression of downstream genes in DEX mediated transgenic plants confirmed that PthA exerts its effects through CsLOB1.

Studies suggest that plant might develop resistance to canker via recessive resistance strategies by modifying the effector binding elements in the promoter region of CsLOB1. To confirm there can be no resistance developed, Zhang. J (2017) and his colleagues demonstrated that homologous of CsLOB1 gene also contributes to the disease development in citrus plants. Custom designed TALEs identified two sets of homologous genes namely CsLOB2 and CsLOB3. These homologous genes showed 67.9% and 71.0% identity respectively. The study demonstrated restoration of canker symptoms on inducing the expression of CsLOB2 and CsLOB3. RNA sequencing analysis showed that CsLOB1, CsLOB2, CsLOB3 all functional similarly and regulate the expression of cell wall metabolic genes which are required in the canker symptom development. Therefore, this redundancy in the three target genes proves that mutation in the CsLOB1 alone will not help in developing resistance against pathogen colonization and disease development *[Zhang, J. Et.al., 2017]*.

Conclusion: Successful translocation of transcription Activator like effector protein, PthA is sufficient to induce the expression of susceptible genes CsLOB1 resulting in develompment of canker symptom.

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