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Phytoplasma Effectors and their Role in Plant-Insect Interaction

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A B S T R A C T

Phytoplasmas are plant pathogenic prokaryotes lacking cell wall, inhabiting phloem sieve elements in infected plants and have a unique life cycle among pathogens as they invade organisms of two distinct kingdoms, namely Plantae (plants) and Animalia (insects) and replicate intracellularly in both of them. Effectors are proteins secreted by a microbial pathogen into a host cell to enhance colonization and facilitate multiplication of the pathogens. In plants these effectors unload from the phloem to access distant tissues and alter basic developmental processes. The effectors provide phytoplasmas with a fitness advantage by modulating their plant and insect hosts. Phytoplasmas have a functional Sec-dependent translocation pathway for the secretion of effectors. Fifty six (56) candidate effectors were identified and named as secreted AY-WB proteins (SAPs), 45 in OY, 41 in AUSGY, 13 in AP, 25 in MBSP. Among these effectors, only some have been functionally characterized like SAP 11 which enhance insect vector reproduction by manipulating plant development and defence hormone biosynthesis, SAP 54 which induces indeterminate leaf like flower development and discovery of SAP54 is novel as there is no pathogen effector identified tills date that interferes with floral development. Further research on phytoplasma effectors and dissection of plant defense responses that these effectors may target is expected to generate knowledge for the design of novel benign control strategies for phytoplasmas and hemipteran insects.

Introduction

Phytoplasmas are obligate plant pathogenic prokaryotes without cell wall. They belong to class Mollicutes of phylum Tenaricutes (Hogenhout *et al.*, 2008) and are thought to have been evolved from fermicutes through the loss of cell wall and reduction in genome size (Weisburg *et al.*, 1989; Woese, 1987). So the genome of phytoplasmas is the smallest among known cellular plant parasites and pathogens of size530 to 1350 kb (Marcone *et al.*, 1999), present as one large circular double stranded DNA chromosome. The development of molecular tools has enabled identification and preliminary classification of phytoplasmas, several of which have achieved *Candidatus* (non-culturable bacterial species) status (Firrao *et al.*, 2004). Within a plant, phytoplasmas inhibit the phloem (Doi *et al.*, 1967; Whitcomb and Tully, 1989) and are injected directly into the cytoplasm of phloem

sieve cells via the feeding activity of insect vector (Nault, 1997). Phytoplasma have a unique biology among bacteria, because they need plants and insects for survival in nature and they can multiply intra cellularly in both of them. Insects capable of transmitting phytoplasma belong to order Hemiptera, including sap sucking leaf hoppers, plant hoppers and psyllids (Markham, 1983; Weintraub and Beanland, 2006). Phytoplasmas have a plant host range that is in part depend upon the feeding range of their insect vectors (Hogenout *et al.,* 2008). It causes more than 400 plant disease worldwide. In India it is associated with 45 plant species i.e., fruits, vegetables, ornamentals, trees and other agriculturally important crop species belonging to 12 groups. Mostly it affects 4 economically important crops in India viz. sugarcane (Sugarcane grassy shoot disease; 16Sr XI group), coconut (Coconut root wilt disease, 16Sr XI group), sesame (Sesame phyllody disease: 16SrI, II and VI group) and toria (toriaphyllody: 16SrIXgroup). Common symptom cause by phytoplasma infection is phyllody, the production of leaf-like structures in place of flowers.

Other symptoms, such as the yellowing of leaves, are thought to be caused by the phytoplasma's presence in the phloem, affecting its function and changing the transport of carbohydrates. Phytoplasma-infected plants may also suffer from virescence, the development of green flowers due to the loss of pigment in the petal cells. Many plants infected by phytoplasma gain a bushy or "witches' broom" appearance due to changes in their normal growth patterns (Bertaccini, 2007). Most plants show apical dominance, but phytoplasma infection can cause the proliferazation of auxiliary (side) shoots and an increase in size of the internodes. Other symptoms include grassy shoot, little leaf wilt etc.

Effectors

Plant pathogens typically employ a range of specific proteins, called effectors that enable these organisms to manipulate developmental processes within the host to the benefit of the pathogen (Hogenhout *et al.*, 2009) so that they can successfully multiply in their plant hosts. The word effector typically denotes a protein that is secreted by a microbial pathogen or insect into a host cell to enhance colonization and facilitate multiplication of the pathogens/insects (Bos *et al.*,, 2010; Wu *et al.*,, 2010), but in a broader definition, effectors can also include elicitors, toxins, phytohormone analogs, cell wall degradation enzymes, and other molecules that alter host plants (Hogenhout *et al.*, 2009). Phytoplasma effectors include SAP11, SAP54, SAP09, SAP11, TENGU etc. Fifty six candidate effectors were identified in Aster Yellow-Witches Broom and named as Secreted AY-WB proteins (SAPs) (Bai *et al.*, 2009), 45 in Onion Yellow, 41 in Australian Grape Yellow, 13 in Apple Proliferation, 25in Maize Bushy Stunt phytoplasmas (Zhang *et al.*,, 2004; Lee *et al.*,, 2000). Phytoplasma produce effectors to gain fitness advantages by inducing morphological and physiological changes (Hogenhout *et al.*, 2009) which are speculated as phytoplasmas incur a competitive advantage through the increased generation of young vegetative tissues (witches' broom symptom and virescence), which attract phytoplasma vectors. As phytoplasmas require insect vectors for transmission to other plants; hence, an increase in insect vector fitness would also result in an increase in phytoplasma fitness. Another possibility is that phytoplasmas have a competitive advantage by extending the lifespan of the plant host. Many herbaceous plants die upon completion of the reproductive phase and therefore reverting flower development (phyllody) may prolong the vegetative growth phase of the plant and

delay plant death. Also, phytoplasmas may alter the production of phytoharmones like jasmonic acid that is having a fundamental role in plant defense signaling (Sugio *et al.,* 2011).

Identification and Localization of Effectors

Effector secretion

Phytoplasmas being limited to the phloem sieve cells of their plant hosts, effectors are also released into the cytoplasm of sieve cells upon secretion. Phytoplasma genomes lack genes present in the Type-III or Type-IV secretory systems (Hogenhout *et al.,* 2009). Phytoplasmas appear torely predominantly on Sec-dependent secretion system (via SecA, SecEandSecY) for translocation of proteins (including the majority of candidate effectors) across the phytoplasma membrane into host cytoplasm (Kakizawa *et al.,* 2004). Phytoplasma genomes also encode YidC (Bai *et al.,* 2006) that function in integration of proteins into bacterial membranes (Hennon *et al.,* 2015). Secreted proteins often possess a N'-terminal signal peptide sequence of about 20 amino acids long that is recognized by the Sec-dependent secretion system and cleaved off during translocation of proteins across the membrane. Signal peptide sequences are conserved among diverse organisms and often consist of specific sequence of hydrophobic, polar and acidic/basic amino acids that can be searched for with prediction software, such as SignalP (Nielsen *et al.,* 1997; Bendtsen *et al.,* 2004). SignalP identified in about 75 proteins with signal peptides in all predicted proteins of the AY-WB genome. 19 of these had one or more predicted transmembrane regions, whereas 56 did not. These56 proteins are likely to be secreted to the extracellular environment of the phytoplasmas and were named secreted AY-WB proteins (SAPs) (Bai *et al.,* 2009). SecA-secreted proteins are candidate effectors, as they are likely to interact with host cell components upon secretion from the phytoplasma cell.

Sec dependent pathway

Proteins (effectors) secreted in cytoplasm of phytoplasma cells are immediately captured by SecB protein which keeps it in unfolded state. Afterwards SecB effector complex binds with secA protein which takes the complex to translocase complex present in cell membrane formed of SecEYG proteins etc. SecA has also ATPase activity, by this activity the effector protein is pushed outside the membrane but N terminal of protein is still attached with secA protein. Certain peptidases like LepB, snip off the aminoacid terminal signal sequence of protein as a result effector protein gets transported to outside phytoplasma cell into host plant cell (Akimaru *et al.,* 1991; Michael *et al.,* 2012; Kakizawa *et al.,* 2004).

Systemic movement

Phytoplasma localisation has been demonstrated to be limited to the phloem tissues of infected plants via various microscopical methods such as fluorescence *in situ* hybridisation (Bulgari *et al.,* 2011) or immune labelling of anti-AMP (phytoplasma membrane protein) (Hoshi *et al.,* 2009; Arashida *et al.,* 2008). Work by Arashida *et al.,* (2008) has shown phytoplasma localisation in the phloem of infected flowers from hydrangea plants. Similarly, when *Arabisdopsis* vegetative leaves are infected with phytoplasma using infected leafhoppers, phytoplasma can be visualised in the phloem of flowers (Hoshi *et al.,* 2009), suggesting systemic movement of phytoplasma via phloem. A common method of insect-free phytoplasma propagation in greenhouse is grafting an infected scion onto a healthy rootstock. The lateral branches developing from the original rootstock later show the

characteristic symptoms of phytoplasma infection and are PCR-positive for phytoplasma of the infected scion (Hodgetts *et al.,* 2013). Moreover, phytoplasmas can be transferred from plant to plant via parasitic plant *Cuscuta spp*. (dodder), which forms vascular connections between the infected donor and healthy recipient plant hosts. Similar to grafting experiments, the parasitized recipient plant develops the characteristic disease symptoms and is PCR-positive for the phytoplasma strain from the infected donor plant (Pribylova and Spak, 2013). Together, these experiments suggest systemic movement of phytoplasmas via plant phloem. While phytoplasma is limited to the phloem, secreted phytoplasma effector proteins have been also visualised in other tissue types than phloem alone. For example, OY secreted protein TENGU was labelled with TENGU-specific antibody in parenchyma cells, shoot apical meristems and axillary buds of OY infected plants (Hoshi *et al.,* 2009). AY-WB effector SAP11 has a nuclear localisation signal (NLS) required for targeting cell nuclei (Bai *et al.,* 2009). Since phloem sieve elements are anucleate, the presence of NLS suggested potential transport and function of SAP11 beyond plant phloem. In support of this hypothesis, SAP11 was visualised in nuclei of mesophyl cells and trichomes of AY-WB-infected plants (Bai *et al.,* 2009). Although lacking a characteristic NLS, TENGU also targets cell nuclei (Hoshi *et al.,* 2009).

Proteins translocate out of a sieve cell and move between plant cells. It is most likely that the proteins are transported across the plasmodesmata that connect the plant cells. The size exclusion limits (SELs) of plasmodesmata that connect sieve cells and companion cells in the loading phloem are reported to be larger than 67 kDa (Stadler *et al.,* 2005) whereas the SELs of other cells vary from 10 kDa to 50 kDa (Imlau *et al.,* 1999). Plasmodesmata SELs differ between source and sink tissues of the plant. Source tissues, which produce most of the carbohydrates that are transported in the phloem have SELs of approximately 10 kDa, whereas sink tissues (which require carbohydrates for growth) have SELs of about 50 kDa (Imlau *et al.,* 1999). It was noted that most AY-WB effector candidates are less than 40 kDa (Bai *et al.,* 2009). Hence, the majority of effector proteins may unload from the phloem through plasmodesmata, particularly in sink tissues (Hogenhout and Loria, 2008). This is in agreement with the symptomatology of phytoplasma infected plants, as the symptoms are frequently observed in sink tissues, such as the shoot apical meristem and flowers.

Alternatively, phytoplasmas may degrade plant cell walls or generate holes in plant cell membranes to facilitate effector translocation between plant cells. However, genes encoding proteins that may modify plant cell walls and membranes have yet to be identified in the phytoplasma genomes sequenced thus far, and it is also unclear how such proteins would travel alongside the effectors upon secretion into the sieve cell cytoplasm (Sugio *et al.,* 2011).

Location of effector genes

Phytoplasmas have reduced genome sizes and restricted metabolic capabilities. Nonetheless, these genomes encode large AT repeat rich regions organized into units of up to approximately 20 kb that include genes with similarities to the IS*3* insertion sequences found in many bacterial genomes (Lee *et al.,* 2005). These units have been termed potential mobile units (PMUs) in AY-WB (Bai *et al.,* 2006) or sequence variable motifs (SVGs) in clover phyllody (CPh) (Jomantiene and Davis, 2006; Jomantiene *et al.,* 2007). Potential mobile units (PMUs) resemble large

conjugative transposons (Toruno *et al.,* 2010). Similar units have been identified in OY-M, AUSGY and other unpublished phytoplasma sequences (Jomantiene *et al.,* 2007; Hogenhout *et al.,* 2008). The PMUs comprise several genes encoding DNA recombination functions along with others organized in a conserved order, and they tend to congregate as tandem or multiple repeats (Bai *et al.,* 2006; Hogenhout and Seruga Music, 2010). Several PMUs truncate and lack the sequence requirements for transposition, suggesting that these are degenerate remnants of insertion sequences.

However, the largest of these PMUs in the AY-WB genome, PMU1, apparently meets all of the requirements for mobilization, including 327 bp inverted repeats flanking the unit, a *tra5* gene encoding a putative transposase, and a number of genes encoding DNA replication and recombination functions. This unit also contains one gene encoding a protein with an N-terminal secretion signal peptide and seven genes encoding proteins predicted to localize to the phytoplasma membrane (Bai *et al.,* 2006). These secreted and membrane-associated proteins may be key virulence determinants in phytoplasma pathogenicity.

Interestingly, the majority of the 49 chromosomally encoded AY-WB effectors lie within PMU-like regions (Toruno *et al.,* 2010), thus PMUs may contribute towards phytoplasma virulence and enhance phytoplasma fitness. This is consistent with the general observation that genes involved in bacterial pathogenicity and symbiosis frequently lie on pathogenicity/ symbiosis islands derived from transposon and phage integrations into the bacterial chromosome and plasmids (Gal-Mor and Finlay, 2006). The largest repeats in the AY-WB genome are PMU1 through PMU4, and the SAP11 pathogenicity island, which encodes AY-WB effector SAP11 (Bai *et al.,* 2009; Bai *et al.,* 2006; Hogenhout *et al.,* 2008).

Functional characterization of phytoplasmas

Induce stem and leaf proliferation

Phytoplasma infection can induce stunting and stimulate the production of large numbers of axillary shoots, resulting in a witches' broom in infected plants. Yeast two-hybrid screening and immunoprecipitation studies revealed that phytoplasma effectors interacts with plant TCP (*TEOSINTE BRANCHED1*, *CYCLOIDEA*, *PROLIFERATING CELL FACTORS 1* and *2*) transcription factors (Maramorosch, 1958). One functionally characterized effector is SAP11, which was shown to interfere with various aspects of plant development as well as modulate plant insect interactions (Sugio *et al.,* 2011). SAP11 has a nuclear localisation signal (NLS), allowing to enter plant cell nucleus (Bai *et al.,* 2009), where the effector interacts with and destabilises TCP transcription factors (Sugio *et al.,* 2011, 2014). TCPs play important roles in regulating plant circadian clock, hormone pathways, mitochondrial biogenesis as well as cell differentiation and proliferation; these processes are key in gametophyte development, seed germination and patterning of vegetative and reproductive organs. Based on structural domains, TCPs are divided into two classes (Martin-Trillo and Cubas, 2010). SAP11 appears to destabilise all class II TCP transcription factors, including CINCINNATA (CIN) and CYC/TB1 clades of TCPs, which results in overproduction of immature cells that lead to production of large and curly leaves, increased stem numbers. OY effector proteins that induce such morphological changes the transient expression of OY phytoplasma effector candidates in *Nicotiana benthamiana* identified a gene that induces witches' broom

and dwarfism. The encoded protein is estimated to be 4.5 kDa in size, and a mature protein (lacking a signal peptide) is only 38 amino acids in length. The corresponding gene was named *tengu* (a class of supernatural creatures found in Japanese folklore), as witches' broom–like symptoms are called tengu-su (nest of Tengu) in Japanese. Transgenic *Arabidopsis* lines that express TENGU show a variety of morphological alterations, including witches' broom, dwarfism (i.e., short internodes), defects in phyllotaxis, and production of sterile flowers. Microarray analysis of *tengu*-expressing *Arabidopsis* lines revealed a down regulation of several auxin responsive genes and auxin efflux carrier genes. Although TENGU may directly interfere with auxin biosynthetic and signaling pathways, it is also possible that TENGU alters plant morphology by manipulating other molecular pathways, and auxin physiology is altered as an indirect consequence of this activity (Hoshi.*et al.*, 2009).

Alteration of plant-insect interactions

SAP11 down-regulates jasmonate (JA) production in plants, a phytohormone that is involved in the defense response against the AY-WB leafhopper vector *Macrosteles quadrilineatus*, resulting in enhanced fecundity of aster leafhopper, the principal vector of AY-WB phytoplasma (Sugio *et al.*, 2010). When eggs hatch, early instar leafhopper nymphs remain and feed on the plant and will acquire phytoplasmas and transmit these bacteria to other plants when they become adults. Thus, SAP11-mediated modulation of plant processes leads to an increase in the number of phytoplasma-carrier vectors thereby promoting phytoplasma spread (Beanland *et al.*, 2000). Destabilisation of CYC-TCPs like BRC1 results in organ proliferation which may benefit phytoplasmas by generating more phloem sink tissue for phytoplasma replication and, additionally attracting insect vectors. Healthy *M. quadrilineatus* produces about 60% more progeny on AY-WB-infected plants compared with non-infected plants, using the model plant *Arabidopsis* (Sugio *et al.*, 2010). For example SAP11 destabilizes *Arabidopsis* CIN-TCPs and one of these, TCP4, positively regulates expression of *LIPOXYGENASE 2*(*LOX2*), which produces oxylipins that are precursors of JA synthesis (Schommer *et al.*, 2008). Effectors are expected to provide the pathogen with a competitive advantage compared with pathogens that do not have these effectors. For example SAP11 enhances the fitness of the phytoplasma AY-WB. Indeed, nymphs that hatch from the eggs will immediately commence feeding from the AY-WB-infected plants and will thereby acquire phytoplasmas (Purcell, 1988). The AY-WB-carrying leafhoppers will subsequently migrate to other plants as they age and will introduce the phytoplasma into native plant hosts (Kingdom *et al.*, 2007). Thus, increasing leafhopper fecundity (mediated by the effector SAP11) is an effective strategy of increasing the dispersal of phytoplasmas in nature (Sugio *et al.*, 2011).

Iinterference with flower development

The most dramatic symptoms in phytoplasma infected plants include alterations in flower morphology, such as virescence, phyllody, sepal hypertrophy, big bud symptoms, and the production of influorescence shoots from flowers (indeterminate growth of flower organs) (Bertaccini, 2007). Virescence is a condition in which nongreen floral organs (such as petals) remain green due to the abnormal presence of chlorophylls. In phyllody, the floral organs are converted into green leaf–like organs (Irish, 2010). Plants with big bud symptoms typically show enlarged calyces with aborted whorls (no petals, stamens, and carpels) (Sablowski,

2007). Flower development in *A. thaliana* involves a sequence of steps: (i) transition from the vegetative phase to the reproductive phase (formation of an inflorescence meristem), (ii) establishment and maintenance of floral meristem identity (stages 1 and 2), and (iii) development of floral organs (after stage 3) (Alvarez-Buylla *et al.,* 2010). In step (iii), the identity of each floral organ is determined bya specific combination of floral homeotic genes constituting the ABCE model (Pelaz *et al.,* 2000; Honma and Goto, 2001; Theissen and Saedler, 2001). Most of these homeotic genes encode members of the MADS domain family of transcription factors, of which the class E SEPALLATA3 (SEP3) and class A APETALA1 (AP1) genes have critical roles. In step (iii), they repress transcription of flowering time genes including SHORT VEGETATIVE PHASE (SVP), SUPPRESSOR OF OVER EXPRESSION OF CO 1 (SOC1) and AGAMOUS-LIKE 24 (AGL24), which are involved in steps (i) and (ii) (Gregis *et al.,* 2008; Immink *et al.,* 2012). The repression of the flowering-time genes contributes to prevention of the outgrowth of ectopic secondary flowers (Liu *et al.,* 2007). Subsequently, SEP3 and AP1 up-regulate the class B genes APETALA3 (AP3) and PISTILLATA (PI) (Ng and Yanofsky, 2001; Wu *et al.,* 2012), which are required for specifying petals and stamens, thereby inducing floral organ development. AGAMOUS is a C-class protein that is involved in stamen and carpel development and in the termination of flower development. Various genes involved in floral organ formation are misregulated in phytoplasma-infected plants (Pracros *et al.,* 2006; Cettul and Firrao, 2010). Homolog of SAP54 from OY–W phytoplasma (‘Ca. P. asteris’, OY strain; wild-type line) called PHYL1 has been identified that also induces phyllody-like floral abnormalities. PHYL1 interacts with and induces ubiquitin–proteasome-dependent degradation of the MADS domain proteins SEP3, AP1 and CAULIFLOWER (CAL), leading to inhibition of their functions. PHYL1 homologs from other phytoplasma species retain the ability to interact with and induce degradation of MADS domain proteins, suggesting a role for degradation of MADS domain proteins in the changes in floral morphology generally caused by phytoplasma infection (Maejima *et al.,* 2014).

Semiquantitative reverse-transcription polymerase chain reaction experiments revealed down regulation of tomato homologs of *WUSCHEL* (*WUS*), *CLAVATA 1* (*CLV1*), *APETALA 3* (*AP3*), and *AGAMOUS* (*AG*), and upregulation of *LEAFY* (*LFY*) in flowers of infected tomatoes. In agreement with Pracros *et al.,* Himeno *et al.,* reported that homologs of *WUS* and some class B genes that regulate floral organ identity are down regulated in OY phytoplasma infected petunia (genus *Petunia*) flowers, showing virescence of petals and leaf-like carpels (Himeno *et al.,* 2010). Furthermore, Cettul and Firrao reported that *SEPALLATA3* (*SEP3*) is down regulated in Italian clover phyllodyphytoplasma-infected *Arabidopsis,* exhibiting altered flowers.The expression of various flower developmental gene homologs are up- or down regulated or expressed at the wrong time (misregulated).

Defence suppression

Different groups of plant pathogenic microorganisms possess virulence factors – proteins or small non-protein molecules, known as effectors. The function of these effector molecules is primarily modulation or suppression of innate plant immune responses to evade recognition by plants and ensure successful colonisation and reproduction in the host (Boller and He, 2009; Dodds and Rathjen, 2010). Interestingly, bacteria, fungi or root nematodes are also associated with

morphological changes in plant tissues, and potential effectors from these organisms are implicated in the modulation of plant development (Evangelisti *et al.,* 2014; Le Fevre *et al.,* 2014). Nevertheless, in many cases the fitness benefits of microbe-induced morphological changes in plants have not yet been empirically tested. Suppression of plant defences allows successful invasion and utilisation of host resources. When microbial pathogens invade plant tissue, plants encounter conserved microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs), such as fungal chitin or bacterial flagellin, on the surface of an invading pathogen. These molecules can be recognised by specific pattern recognition receptors (PRRs) at plant cell membrane. Detection of these extracellular molecular patterns by plant receptors elicits plant immune response, known as PAMP-triggered immunity or PTI (Couto and Zipfel, 2016). PTI leads to a set of immediate plant responses, including Ca^{2+} influxes into cells, production of reactive oxygen species, callose deposition and activation of genes involved in plant defences. Plant pathogens deliver effector proteins in the host cell to suppress PAMP-triggered signalling events and PTI responses, leading to effector-triggered susceptibility (ETS). However, an effector molecule that suppresses PTI can be recognised by a specific resistance gene (R-gene) in plant, eliciting effector-triggered immunity (ETI) (Cui *et al.,* 2015). ETI gives rise to hypersensitive response (HR) – rapid localised cell death that may restrict replication and spread of (biotrophic) pathogens. Interestingly, pathogens have evolved effectors that suppress ETI and, thus, avoid HR. The PTI, ETS and ETI together are referred as the ‘’Zig-Zag Model’ of plant immunity and often leads to an ‘’arms-race’’ between plant ability to recognise the pathogen and pathogen evading the immunity (Jones and Dangl, 2006). According to this model, evasion of host defence is possible when pathogens possess effectors that suppress PTI as well as ETI and when plants lack R-genes that would recognise such effectors. Pathogens possess a collection of effectors, many of which may promote virulence via other mechanisms than suppression of PTI or ETI. For example, *Pseudomonas syringae (Pto*DC3000) effectors induce extracellular accumulation of cytoplasmic proteins from host cells with a potential role to aid assimilation of host nutrients (Kaffarnik *et al.,* 2009). Another *P. syringae* effector HopW1 targets plant actin cytoskeleton to modulate the actin-dependent processes that were shown to be required to restrict pathogen growth (Kang *et al.,* 2014). Phytoplasmas have no outer cell wall and no flagella, and hence lack the peptidoglycans and flg22 PAMPs. However, phytoplasmas have genes encoding CSPs and the EF-Tu, and these gene products may induce PTI. All the plant PRRs identified till date seem to receive the ligands in the extracellular space, whereas R-mediated ETI can be triggered by extracellular and intracellular effectors (Bent and Mackey, 2007), but it is unclear whether sieve cells induce PTI/ETI. Because the intracellular phytoplasmas reside within the sieve cell cytoplasm, the bacteria may be hidden from the plant detection apparatus, resulting in the absence of PTI/ETI. Phytoplasmas may avoid detection by a plant host via an absence of both PAMPs and recognizable (avirulent) effectors or by secreting effectors that suppress PTI/ETI and/or by virtue of residence within nonresponsive phloem sieve cells.

Future issues

Functional characterization of these effectors may contribute to develop strategies to suppress phytoplasma multiplication in crop plants. Phytoplasma effector studies may lead to a greater understanding of how sieve cells

unload macromolecules for transport to other plant tissues, and how these plant cells detect pathogens and sap-sucking insect vectors. The research on phytoplasma effectors and dissection of plant defense responses that these effectors may target is expected to generate knowledge for the design of novel benign control strategies for phytoplasmas and hemipteran insects. Phytoplasmas have a unique life cycle among pathogens, as they invade organisms of two distinct kingdoms, *viz* Plantae and Animalia replicate intra cellularly in both of them. Effectors that increase the production of stems (SAP11) or produce leafy flowers (SAP54) may stimulate leafhopper feeding, increasing the frequency of phytoplasma acquisition by its vector. Phytoplasmas have a functional Sec-dependent translocation pathway that enables these pathogens to secrete effectors, such as SAP11TENGU, SAP54 into the host cells of plants and insects. Jasmonic acid induces defence in plants against the insects like leafhoppers. Effectors may provide phytoplasmas as well as insect with fitness advantages

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