Genome biology of plant pathogen effectors: An overview of plant's response

By

Sivadharshanapriya R.

(2018-11-146)

M.Sc Plant Pathology

Seminar report

Submitted in partial fulfilment of requirement of the course

Pl. Path. 591 Seminar (0+1)

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF HORTICULTURE

KERALA AGRICULTURAL UNIVERSITY

VELLANIKKARA- 680656

THRISSUR, KERALA, INDIA

DECLARATION

I, Sivadharshanapriya, R. (2018-11-146), hereby declare that the seminar report entitled '**Genome biology of plant pathogen effectors: An overview of plant's response'**, has been completed by me independently after going through the reference cited herein and I have not copied from any of the fellow students or previous seminar reports.

Vellanikkara — Sivadharshanapriya, R.

Date: 25-01-2020 — 2018-11-146

CERTIFICATE

Certified that the seminar report entitled '**Genome biology of plant pathogen effectors: An overview of plant's response'**, for the course Pl. Path. 591 has been prepared by Sivadharshanapriya, R. (2018-11-146), after going through various references cited herein under my guidance, and he has not copied or borrowed from any of his fellow students.

Dr. ReshmyVijayaraghavan
Assistant Professor
Department of Plant Pathology

Vellanikkara
25-01-2020

CERTIFICATE

Certified that the seminar report entitled **"Genome biology of plant pathogen effectors: An overview of plant's response"** is a record of seminar presented by Sivadharshanapriya, R. (2018-11-146) on 12th December, 2019 and is submitted for partial requirement of the course Pl. Path. 591.

Dr.Anil Kuruvila
Professor
Department of Agricultural Economics
College of Horticulture, Vellanikkara

Dr. Reshmy Vijayaraghavan
Assistant Professor
Department of Plant Pathology
College of Horticulture, Vellanikkara

Dr. Sangeetha Kutty M.
Assistant Professor
Department of Vegetable science
College of Horticulture, Vellanikkara

CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. Introduction

Plant pathogens like bacteria, fungi, oomycetes and nematodes pose a major threat to sustainable crop production. Damage by pathogens is ubiquitous and affects all major food crops. When a pathogen attack host plants, they secrete proteins into the host plant to modulate plant defense mechanisms and enable colonization. Understanding the molecular function of these secreted molecules, collectively known as effectors, is widely accepted as critical for a mechanistic understanding of the processes underlying host colonization and pathogenicity (Davis *et al*., 2008). This understanding significantly increased our knowledge of effectors from a diversity of plant pathogens, their host targets, how and where these molecules interact and affect the outcome of the plant-pathogen interaction.

Despite extensive differences in the biology of plant infections, pathogens share common themes in their infection processes. A major determinant of a pathogen's ability to infect a host plant is determined by a set of proteins called effectors. Here, we outline how effector genes emerge in pathogen genomes, how genome compartmentalization is crucially linked to the mechanisms generating variability in effector loci and different mechanisms favouring pathogenicity and defense response in plants. Finally, we suggest directions for future research to build upon our emerging understanding of effector genome biology in plant pathogens.

2. Effectors-definition:

Effectors are defined as pathogen proteins and small molecules that alter host-cell structure and function. These alterations either facilitate infection (virulence factors and toxins) or trigger defense responses (avirulence factors and hypersensitive responses) or both (Hogenhout *et al.*, 2009).

2.1 Types of effectors:

Pathogens can secrete effector proteins either in the extracellular space or inside the host cytoplasm and are subsequently classified as apoplastic and cytoplasmic effectors respectively (Sonah *et al.*, 2016) (Fig.1).

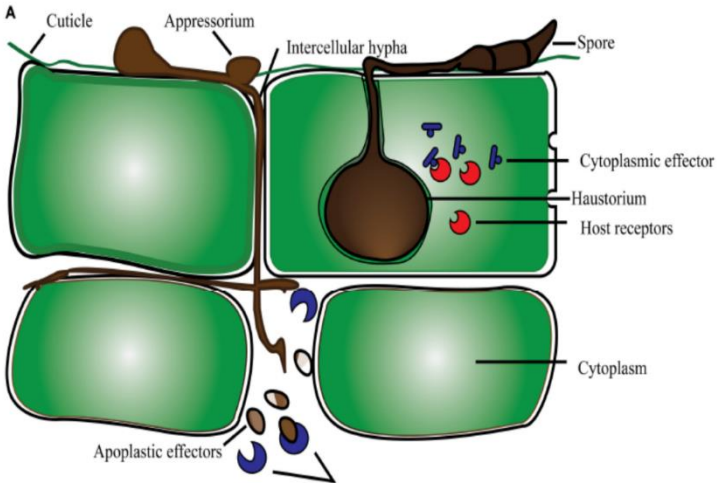

Fig. 1. Types of effectors

2.2 Protein organisation of effectors:

The standard protein organization of effectors contains a signal peptide within the initial 60 amino acids at the N terminus followed by multiple domains towards C terminus. These types of effectors are comparatively small and rich in cysteine residues like most of the serine or cysteine protease inhibitor proteins (Sonah *et al.,* 2016) (Fig.2).

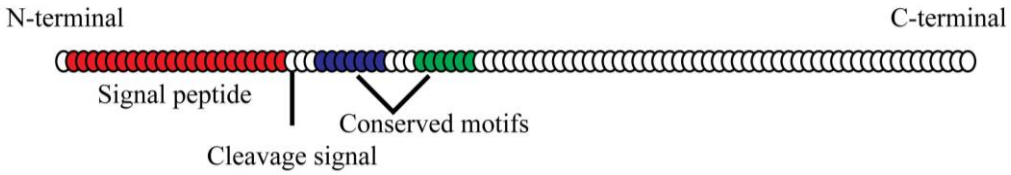

Fig. 2. Standard protein organization of effectors

2.3 Role of effectors in susceptible genotypes:

Infectious pathogens like bacteria, fungi, oomycetes, virus and even nematodes deliver effectors either at the interface of the host plant or inside the cell. Host translocated cytoplasmic effectors are delivered into the host cytoplasm through Type Three Secretion System(T3SS), in case of bacteria or through specialized structures called haustoria, in case of fungi that form within the cells. Pathogen effectors traffic to various components bind and manipulate different host proteins called targets. Depending on their localization in the cells these targets are classified into Apoplastic Effector Target (AET) and Cytoplasmic Effector Target (CET) Effector target interaction imparts the outcome of interaction between pathogen and its host

In susceptible genotypes, the molecular interactions alters the host cell process and suppress the plant immune response leads to Effector Triggered Susceptibility (ETS) thereby promote host colonization (Win *et al.*, 2012)

2.4 Role of effectors in resistant genotypes:

In resistant genotypes, effector pathogen interactions are perceived by key sensing receptors of plant immune system that in turn stop pathogen growth. The receptors may be present in plant's cell surface called Pattern Recognition Receptors (PRR) detect Pathogen Associated Molecular Pattern (PAMPs) or Apoplastic Effectors (AE) or Apoplastic Effector Target (AET) interaction to initiate PAMP-Triggered Immunity (PTI). If PTI fails, pathogen secrete effectors that enter into the host cytoplasm. At that time, receptors also present in the host cytoplasm called Intracellular NB-LRR receptors, also called as R gene induce NB-LRR -Triggeed Immunity or Effector Triggered Immunity(ETI) on recognition of Cytoplasmic Effectors (CE) or Cytoplasmic Effector Target (CET) interaction

3. Effector gene evolution:

As plant-pathogen interactions evolve, plants are selected for an incompatible (resistant) interaction and pathogens are selected for a compatible (susceptible) interaction. The underlying principle for this antagonistic coevolution is based on the gene-for-gene model. In this model, *R* gene from the host plant detect Avr effectors from the pathogen, leading to an incompatible interaction. By contrast, a failure of detection, resulting from either allelic variation or the absence of at least one of the components, results in a compatible interaction (Lo presti *et al.*, 2015). This relationship can result in boom-and-bust cycle.

3.1 Boom and bust cycle:

A resistant cultivar with single major resistant gene is introduced into an agroecosystem to control a plant disease. If the resistant cultivar has good agronomic characters and is widely accepted by farmer because it is a disease resistant, the cultivar spread and planted over a large area. This is the boom part of the cycle, characterized by an increase in the area planted to the resistant gene. In the pathogen population exposed to their gene, a mutation from avirulence to virulence occurs. This increase in frequency of pathogen strains with virulence mutation. This shadows the increase in frequency of resistant gene. The virulent pathotypes spread and infect all the field with resistant cultivar causing an epidemic and leading to loss of effectiveness of

resistant gene. Because of the resistant broken, farmers stops planting the resistant cultivar. So, the corresponding resistant gene decrease in frequency. This is the bust part of the cycle (Fig.3). The cycle begins again the introduction of new resistant cultivar (Brown *et al.*, 2011).

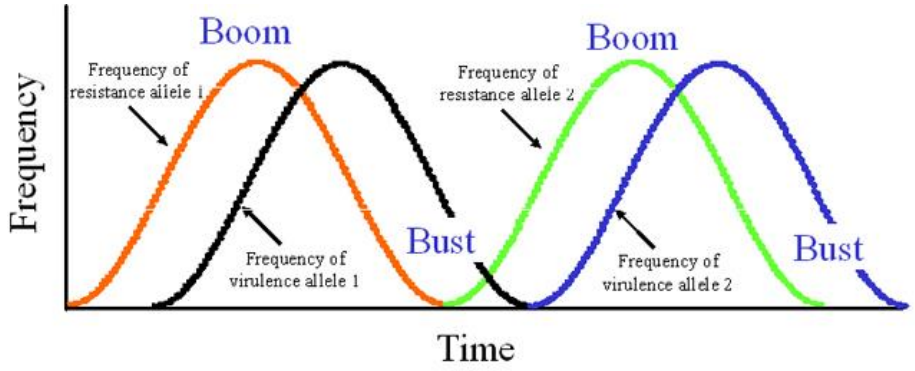

Fig. 3. Boom and Bust cycle

This boom and bust cycle leads to the evolution of two basic models that is Arms race model and Trench warfare model

3.1.1 Arms race model:

Coevolutionary struggle between host and pathogen. In arm race, the pathogen evolves an effector allele that enhances its fitness through manipulation of host physiology or suppression of host defense. So, frequency of such allele is rapidly increasing in pathogen population eventually replaces the older allele. In turn, a new allele of a gene for the host effect target that help the host to evade the effect of the pathogen effector evole and its allele frequency rapidly increases to finally fix in population. This cycle is repeated indefinitely (Fig.4). It is common in agricultural system, because of the constant needs of human on plant cultivars (Dawkins and krebs,1979).

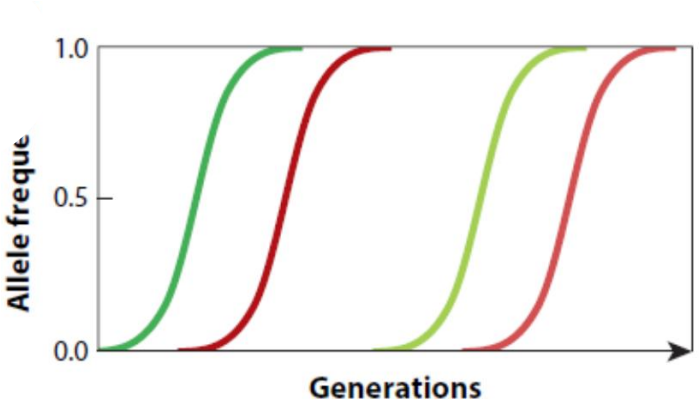

Fig. 4. Arms race model

3.2.2 Trench warfare model:

This model also called as red queen model. Two or more alleles of effector genes exist in the pathogen population, and the allele with the largest contribution to pathogen fitness increases in frequency. This model represents negative frequency dependent selection and the allele frequency of matching genes in the pathogen and host oscillate over time (Fig.5). Host and pathogen are engaged in never ending struggle. Effector and plant target alleles, due maintained in the population, but their frequencies oscillate over time (Stahl *et al*., 1999)

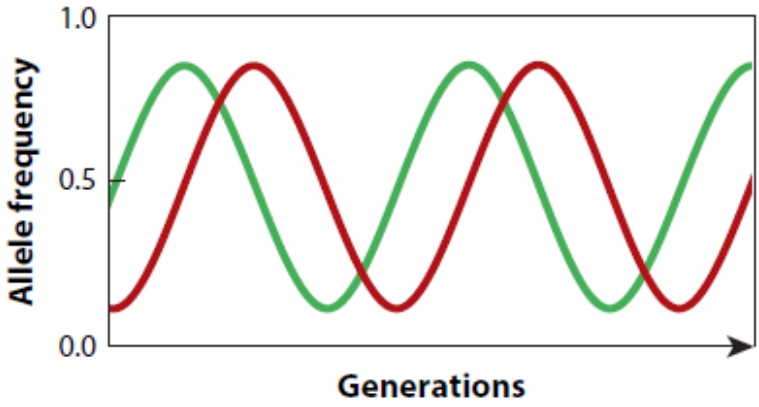

Fig. 5. Trench warfare model

4. Evolutionary birth of effectors:

The effector repertoire of a pathogen is a major determinant of host specialization. A successful pathogen must continuously maintain the ability to escape host recognition and maintain virulence (i.e., the ability to reproduce on the host). Evolution toward evasion of recognition and functional optimization is achieved by gene duplication, neofunctionalization, alteration of expression of existing effector genes, the gain of new effectors and horizontal gene transfer. Through the same process, pathogens evolved existing effectors or gained new effectors to specialize on a new host.

4.1 Gene duplication:

It is the major mechanism through which new genetic material is generated during molecular evolution, and arrive as the product of several types of errors in DNA replications of repair.

4.2 Neofunctionalization:

Due to duplications, two copies of gene are produced, if one copy of a gene experience a mutation that affect its original functions, the second copy can serve as spare part and continue to function correctly. Thus, duplicate gene accumulate mutation much faster than a functional single copy gene. It is possible for one of the two copies to develop a new and different strains.

4.3 Horizontal gene transfer:

Horizontal gene transfer, also known as **lateral gene transfer**, refers to nonsexual transmission of genetic material between unrelated genomes; hence, horizontal gene transfer involves gene transfer across species boundaries, alternative way to gain new virulence traits. If the transfer between two organisms belonging to same species then it is called as Intra-species horizontal gene transfer Ex: Transfer of mobile genetic chromosomes in *Fusarium* spp (Ma *et al*., 2010). If the transfer between two organisms belonging to different species then it is called as Inter-species horizontal gene transfer Ex: Transfer of host specific toxin ToxA from cereal pathogen *Stagnospora nodorum* to *Pyrenophora tritici-repentis*, led to emergence of highly virulent pathogen causes tan leaf spot in wheat fields worldwide (Friesen *et al*., 2006).

5. Evolutionary death of effectors

The major mechanism leading to the loss of effector gene is the presence of activity of nearby transportable elements. The major effects of TE can include Repeat-Induced Point (RIP mutations), epigenetic silencing or disruption of the gene sequences. Escape from recognition can also be mediated by chromosomal rearrangements or fixation of beneficial mutations. Rearrangements and selection for beneficial mutations are also major routes for loss of effectors to optimize their functions.

6. Genome compartmentalization:

Usually, pathogen use different strategies of genomic compartmentalization to outsource effector gene evolution. It is a balancing act of effector gene evolution.

Plant pathogens are faced with an evolutionary conflict, in which effector genes require fast and flexible evolution but the majority of the genome requires evolution at moderate rates. This balancing act is carried out by the compartmentalization of the genome. Many pathogenic fungi and oomycetes (e.g., *Phytophthora* spp) have gene-sparse genomic regions that are highly

enriched in repetitive elements and putative effector genes (Raffaele and Kamoun, 2012). The compartmentalization culminates in accessory chromosomes that are devoid of essential genes and harbor solely pathogenicity-relevant genes. Effectors are located on mobile, conditionally dispensible chromosomes Eg: *Fusarium* spp (Ma *et al*., 2010). In *verticillium dahlia*, various isolates display chromosomal reshuffling of the chromosomal break points are enriched for virulence related gene of effectors. It leads to genetic variations (De Jonge *et al*., 2013). Smut fungi have small genomes with a low content of repetitive DNA. In these genomes, many genes encoding secreted proteins reside in the clusters of three or more genes. These clusters may originate from gene duplications without subsequent dispersal. This compartmentalization determines both the life styles and host range of the pathogen (Kamper *et al*., 2006). These examples illustrate that fungal pathogens use different strategies of genomic compartmentalization to outsource effector gene evolution (Fig.6).

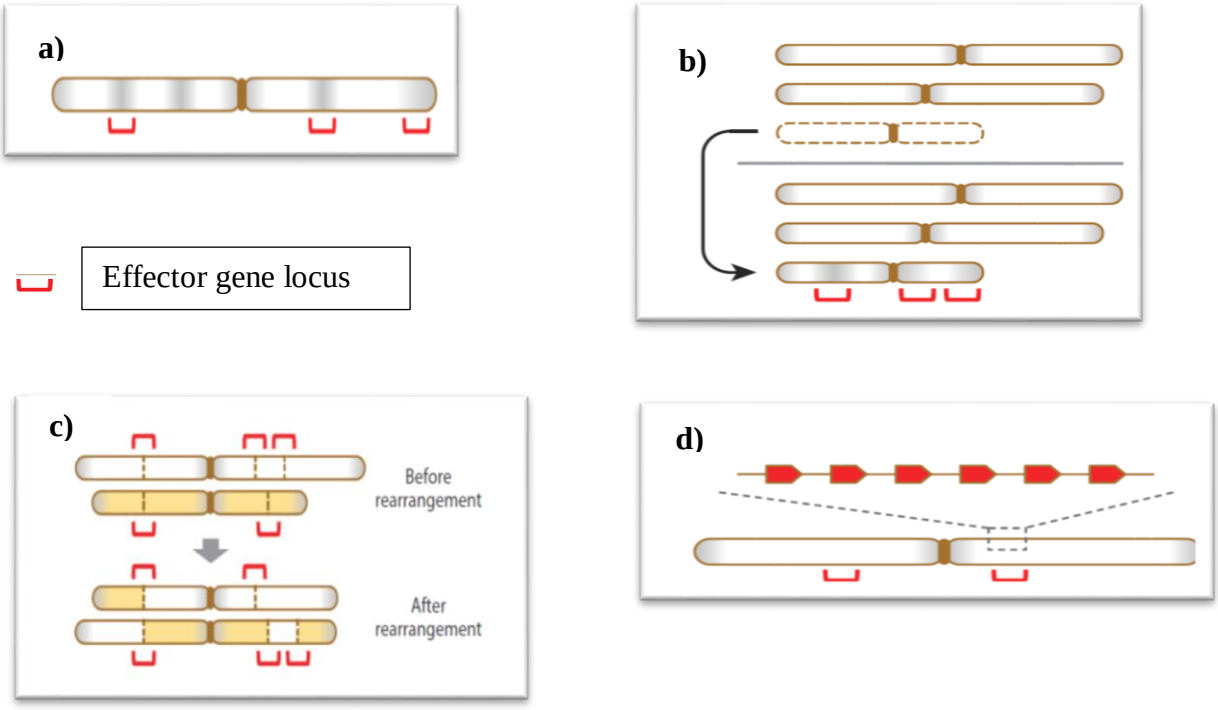

Fig. 6. Genome compartmentalization of effector genes a) Effector genes are located in gene-sparse genomic regions b) Effectors located on mobile chromosomes c) Effectors located in chromosomal break points d) Effector proteins reside in clusters of gene

7. Delivery mechanisms of effector proteins by pathogens:

Plant pathogenic bacteria, fungi, oomycetes, and nematodes have evolved the capacity to deliver effector proteins inside host cells through a diversity of mechanisms. They are:

7.1 Bacteria:

Bacterial effectors which are defined as bacterial produced proteins or component that contribute to an induced plant response either disease or defense (resistance). Effectors molecules are produced to obtain nutrients from host plant and cultivate the right environment in which to develop infections.

Phytopathogenic bacteria uses a number of secretion pathway to deliver the effectors molecules.

There are four basic types of secretion pathways:

Type I and II – secrete proteins to the supernatant or host intercellular spaces.

Type III and IV – secrete protein and deliver them directly to the host cells.

Type I pathway - Structure is simple, it allows direct secretion of effectors from the bacteria cytosol to the external environment. Eg. plant pathogen effectors secreted via., type I pathway are proteases and lipases from the soft rot pathogens, *Erwinia chrysanthemi.*

Type II pathway – More complex secretion system and two steps are involved :

1. Transport to the periplasm
2. Secretion across the outer membrane

Involve in the cell wall degradation, such as pectate lysine, polygalacturonase and cellulase from *Erwinia* and *Xanthomonas.*

Type III and IV pathway are complex structures.

Flagella, conjucation structures to interact with eukaryotic host cells and deliver their effectors. The gene encoding the TTSS are called the hrp (hyper-sensitive response and pathogenicity), genes in phytopathogenic bacteria. Characteristic of TTSS is a needle like protruding structure with a channel along with protein travel is resemblance of bacteria flagella (Ponciano *et al.*, 2003) (Buttner, 2016) (Fig.7).

Type IV pathway – best known form studies on *A. tumefaciens,* vir-De/t-DNA nucleo-protein complex delivers through TFSS directly to the plant.

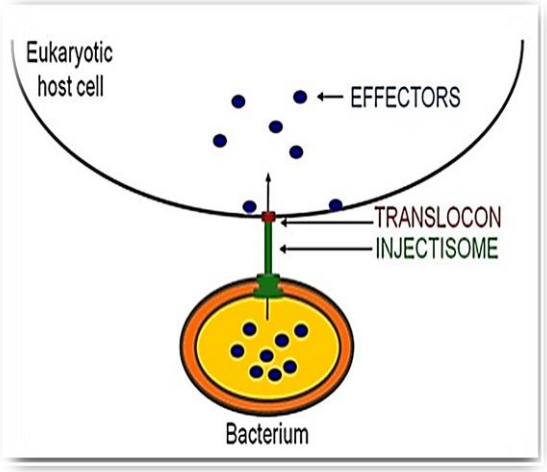

Fig. 7. Type III secretion system of delivering effectors

S.No	Effectors	Microbes	Target plants	References
1.	AvrB	*Pseudomonas syringae*	Tomato, Arabidopsis	Liu *et al.* (2011) Cui *et al*. (2010)
2.	AvrBsTXcv	*Xanthomonas* spp	Pepper	Kim *et al*. (2014)
3.	GALA proteins	*Ralstonia solanacearum*	Arabidopsis	Remigi *et al.* (2011)
4.	PopP2	*Ralstonia solanacearum*	Arabidopsis	Tasset *et al*. (2010)

Table 1: Examples of bacterial effectors and its host targets

7.2 Fungi:

The initial phase of infection involving fungal adhesion to the cuticle followed by growth of germ tubes on the plant surface and differentiation of infection structure viz., haustoria in plant pathogenic fungi and hyphopodium in beneficial fungi can perform in response to plant topographical cues such as stomatal pores, plant chemical cues such as epicuticular waxes. Pathogenic fungi with different lifestyles produces or develop different types of haustoria.

Sl. No.	Effector protein	Fungi/Oomycetes	References

1	Avr2	*Cladosporium fulvum*	De wit *et al.* (2009)
2	Avr3a	*Phytophthora infestans*	Bos *et al.* (2010)
3	Avr-Pita2	*Magnaporthe oryzae*	De wit *et al.* (2009)
4	Avr4	*Cladosporium fulvum*	De wit *et al.* (2009)
5	BEC4	*Blumeria graminis*	Schmidt *et al.* (2014)

Table 2: Examples of fungal/Oomycetes effectors

7.3 Virus

Viruses deliver effectors through vectors. Vectors employ effectors from obligate pathogen to interfere host phytohormone pathways.

7.4 Nematodes:

Nematode secrete effectors and inject into plant cells through stylet

8. Effectors in disease development:

Hogenhout *et al.* (2009) reported that ,for developing or promoting disease in plants, effectors possess certain mechanisms, they are,

- ✓ Inhibition of proteases in plants
- ✓ Inhibition of ubiquitin system
- ✓ Alteration in plant hormone signaling
- ✓ Molecular mimicry by effectors
- ✓ Alteration of plant behavior and development

8.1 Inhibition of proteases in plants

Some effectors act in the extracellular space at the plant-microbe interface, where they interfere with apoplastic plant defences like inhibition of proteases in plants to promote

pathogenicity in plants, because the of protease in plants degrade misfold, damaged and harmful proteins thereby supply aminoacids the plant cells (Table 3).

Effector	Pathogen	Target	Reference
Pit2	*Ustilago maydis*	Maize cysteine proteases inhibitor	Mueller *et al*. (2013)
EPIC1 & EPIC2B	*Phytophthora infestans*	Inhibition of tomato apoplastic proteases	Tian *et al.* (2007)

Table 3: Examples of effectors against plant proteases

8.2 Inhibition of ubiquitin system

Some fungal and oomycete effectors interact with ubiquitine ligase which is present in plant thereby promoting compatibility in plants, as ubiquitine ligase is essential for growth, hormone signalling etc., (Table 4).

Effector	Pathogen	Target	References
GALA	*Ralstonia solanacaerum*	Interact with ubiquitin proteasome	Angot *et al.* (2006)
Avr3a	*Phytophthora infestans*	Interact with ubiquitin proteasome	Gonzalez-Lamothe *et al.* (2009)

Table 4: Examples of effectors against plant ubiquitin system

8.3 Alteration in plant hormone signalling.

Systemic Acquired Resistance (SAR) in plants is mediated by hormone salicylic acid (SA). Bacterial and fungal effectors interfere with the SA pathway to promote virulence function in plants.

Eg: XopJ effector produced by *Xanthomonas campestris*. XopJ appear to benefit the pathogen by interfere with proteasome subunit, suppressing the accumulation of this defence related hormone during infection (Ustun and Bornke, 2015) (Fig.8).

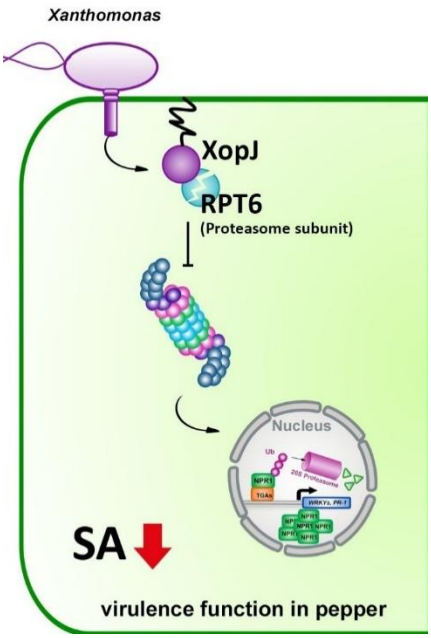

Fig. 8. Role of XopJ effector protein in salicylic acid production in pepper

8.4 Molecular mimicry by effectors

Many effectors produce analogs and mimics of plant hormones. Eg: Coronatine, a toxin secreted by several pathovars of *Pseudomonas syringae* that is structural and functional mimics of plant hormone jasmonyl-isoleucine (Fig.9). Coronatine has many effects that enhance the bacterial colonization of plants. These include impacting phytohormone pathways such as jamming the induction of salicylic acid mediated resistance response and increasing the opening of plant stomata (Bender *et al.*, 1999)

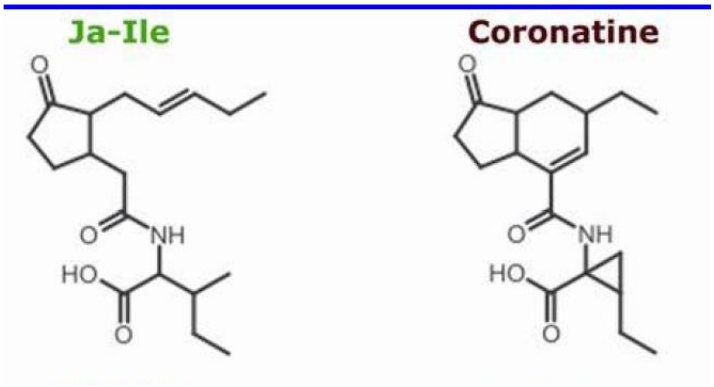

Fig. 9. Coronatine mimicking plant hormone jasmonyl-isoleucine

8.5 Alteration in plant behaviour and development:

Because of the action of effectors there should be alteration in plant behaviour and development. Ex: Coronatine. Usually plant stomata close upon the detection of PAMP from bacteria *Pseudomonas syringae*. However, Phytotoxin Coronatine (COR) inhibit stomatal closure, resulting in bacterial entry into plant leaves through reopening of stomata (Fig.10)

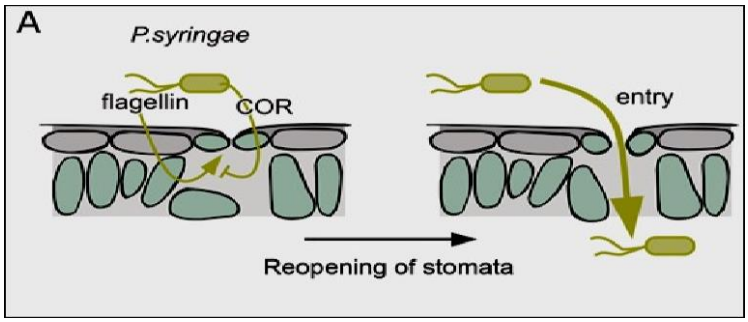

Fig. 10. Effect of **c**oronatine on alteration in plant behavior

Another example is the fungus *Gibberella fujikori* produce yellow spots infects a single rice seedling. The fungus produce the growth hormone Gibberellin, which induces plant elongation resulting in elongated (foolish)seedling several inches taller than non-infected seedlings (Fig.11). The height of the plant facilitates the spread of air borne fungi spores by the wind.

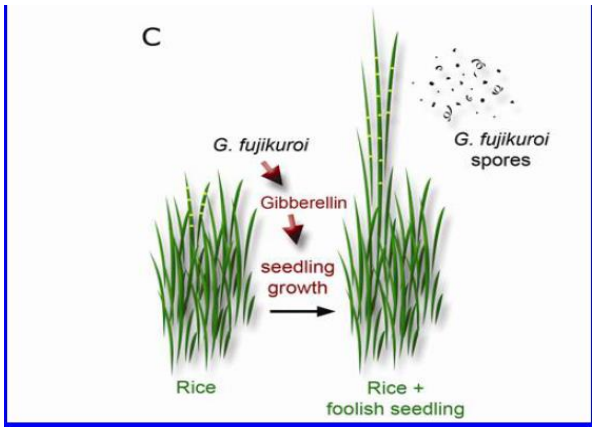

Fig. 11. *Gibberella fujikori* enhances pathogen infection through gibberellin production

9. Role of effectors in disease resistance.

There are 2 main strategies that plants use to restrict the invasion and growth of pathogens.

1. Basal defence
2. Secondary defence

9.1 Basal defense:

Also called as Pattern Recognition Receptors Triggered Immunity (PRR-T-I) or Pathogen Associated Molecular Pattern Triggered Immunity (PAMP T-I). When pathogen effector proteins interact with extracellular pathogen-receptors (PRR)in the plasma membrane of the host cell then PTI is activated. It leads to multiple defence responses like generation of ROS, defence gene expression, biosynthesis of defence hormones, phytoalexin, biosynthesis and cell wall strengthening is happened in the host cell. PTI offers protection against the majority of organisms that plant face (Nicaise *et al*. 2009)

9.2 Secondary defence

If the basal defence fails, the pathogen enters in to the host and deliver effectors that enters into the host cytoplasm. Plant contain resistant (R) proteins that specifically recognize pathogen effectors to activate Effector Triggered Immunity (ETI). It is associated with hypersensitive response or programmed cell death (Hatsugai *et al*., 2017).

R gene present in the host detect the effectors by two different ways

1. Direct detection of effector by receptor-ligand model
2. Indirect detection of effector by guard hypothesis

9.2.1 Receptor-ligand model:

Resistance proteins detect pathogen infection by directly interacting with avirulence proteins, triggering defence signaling in plants.

9.2.2 Guard hypothesis:

Here, pathogen secretes effectors into plant cell, these effectors modify the morphology of a protein called guardee, this change in the morphology of guardee protein will be detected by R gene/guard gene and this will initiate the defense responses in plant.

10. Case studies

10.1. Li *et al.* (2019) reported that A *Phytophthora capsici* virulence effector associates with NPR1 and suppresses the plant immune responses

Objective : Role of RxLR48 effector that target NPR1, facilitates *P. capsici* infection and is required for pathogen virulence

Plant pathogen delivers molecules termed effectors to manipulate host immunity during infection. Usually by targeting vital immune components, *Phytophthora* pathogens secrete hundereds of effectors called RxLR effectors. It contains conserved (Arginine -any AA-Leucine-Arginine) in N terminal for translocation in the plant cell.

Salicylic acid is the key plant hormone that is required for both local and systemic resistance. Plants are defective in Salicylic acid synthesis or accumulation always exhibit enhanced susceptibility to pathogens and the SA receptor NPR-1 (non expressor of pathogenesis related -1) is the central signalling regulator during SAR so the pathogen effector could target NPR-1 and PTI signalling pathway for virulence activity.

To investigate virulence function of RXLR48 in *P.capsici* infection, GFP-RXLR48 was expressed in *Nicotiana benthamiana* and GFP alone was used as a negative control. The leaves are inoculated with *P.capsici*. The photograph of disease symptoms and statistical analysis of lesion diameter together showed the ectopic expression of RXLR48 led to the development of bigger lesions compared to GFP (Fig.12).

RXLR48 contributes to pathogen virulence

To explore whether RXLR48 contributes to pathogen virulence. For that, we silenced RXLR 48 in *P.capsici*. Two such silenced transformants are named T12 and T129,where transcription level was significantly decreased to 0.5% and 3.8% of the wild type strain LT263 respectively.

Here T108 was selected as control in which RXLR48 expressed remained unaffected. Next we evaluated the virulence of RXLR48 silenced transformants on *N.benthamiana* leaves. The leaves were drop inoculated with zoospore suspension of T12 or T129 on the right side which T108 zoospores on left side.T12 and T129 develop smaller lesions compared to T108 at 36h post inoculation. Meanwhile statistical analysis showed that the lesion diagram caused by

T12 and T129 was reduced to 31% and 77% compared to that caused by T108 respectively. Thus, these results indicate that RXLR48 require for pathogen virulence (Fig.13).

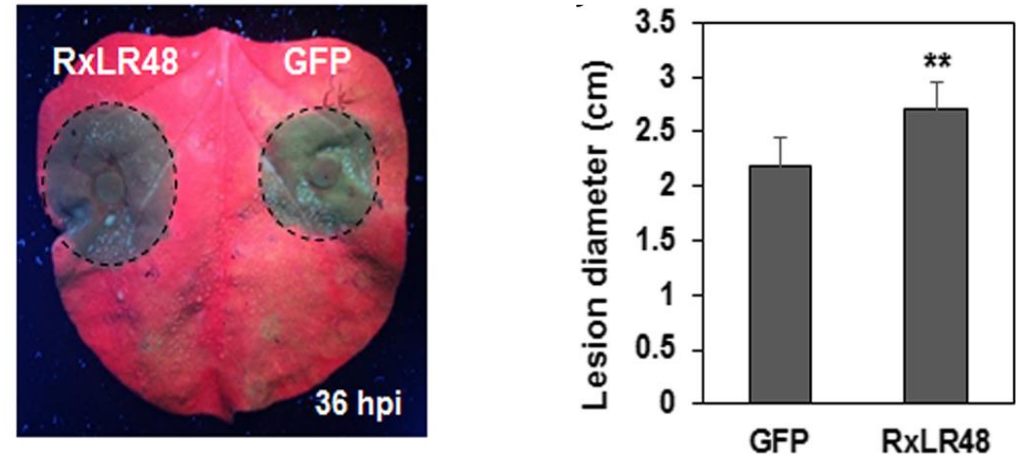

Fig. 12-. RxLR48 facilitates *Phytophthora capsici* infection in *Nicotiana benthamiana*. a, b Enhanced infection of P. capsici by RxLR48 at 36 hpi.

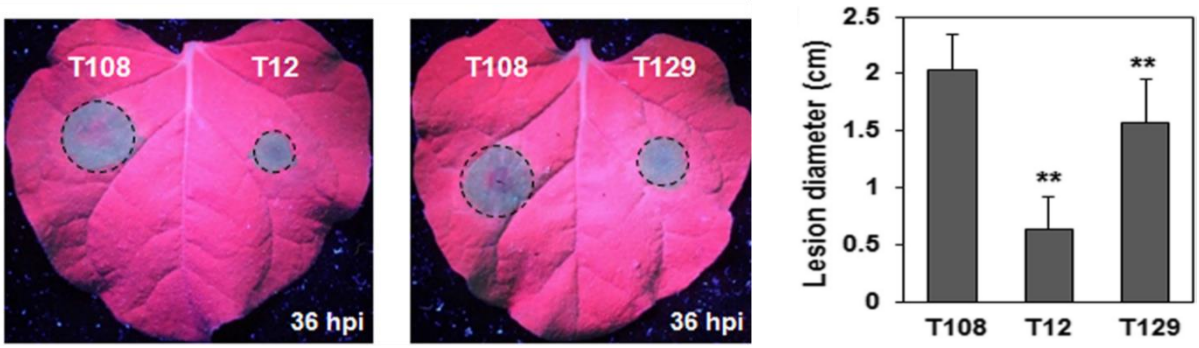

Fig. 13. Relative expression levels of RxLR48 in *P. capsici* transformants

RXLR 48 contributes to suppression of PTI related immune response:

They conducted inoculation assay using the RxLR48-silenced transformant T12. Here T108 was selected as control in which RXLR48 expressed remained unaffected. Detached leaves of *N. benthamiana* were inoculated with RxLR48-silenced transformant T12 on the right side and T108 on the left side. The ROS production was visualized by DAB staining. The black dashed circle indicates inoculation sites (Fig.14). And also Callose deposition after *P. capsici* infection. The results showed that T12 showed higher amount of ROS and callose deposition whereas T108 showed reduced amount. Because ROS and callose deposition are the indicators of plant immune system, therefore it is higher in RxLR48 silenced transformant compared to control.

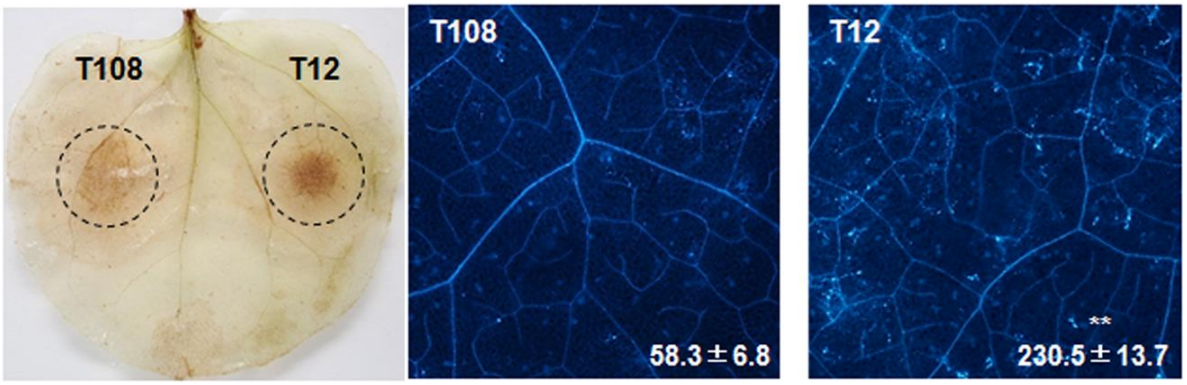

Fig. 14. RXLR 48 contributes to suppression of PTI immune response. T12 is RxLR48 silenced transformant and T108 is control A. ROS production is higher in RxLR48 silenced transformant. B. callose deposition is higher in RxLR48 silenced transformant.

Inference of the case study:

Virulence effector RxLR48 from the pathogen *P. capsici* interferes with plant immunity and suppresses PTI responses

10.2. Case study

Kim et al. (2002) reported that "Two distinct Pseudomonas effector proteins interact with the Pto kinase and activate plant immunity".

Objective : Role of *Pseudomonas* effectors in the activation of plant immunity

The Pto serine/threonine kinase of tomato confers resistance to speck disease by recognizing strains of *Pseudomonas syringae* that express the protein AvrPto and AvrPtoB. Pto and AvrPto physically interact, and this interaction is required for activation of host resistance. AvrPtoB is delivered into the plant cell by the bacterial type III secretion system and it elicits Pto-specific defenses, led to the production of hypersensitive response (HR) or Programmed Cell Death (PCD).

Interaction of *Pseudomonas* effector proteins with Pto Kinase

First they identified the effector proteins present in Pseudomonas that can interact with pto kinases, because in plants pto kinases are the mediators of effector triggered immunity. They found that avrpto and avrptob interact with pto kinases present in the plant (Fig.15). So they understood that avrpto and avrptob are involved in the defence response that is HR

Expression of effector proteins in tomato leaves:

To confirm this, Pto kinases and AVrPto effectors are infiltrated separately into plants. Effectors or pto kinases alone will not produce defence responses and found that coexpression of pto and effetors are needed for the production of HR (Fig.16). This indicates effectors has to interact with host proteins to triggers immune response in plants (HR).

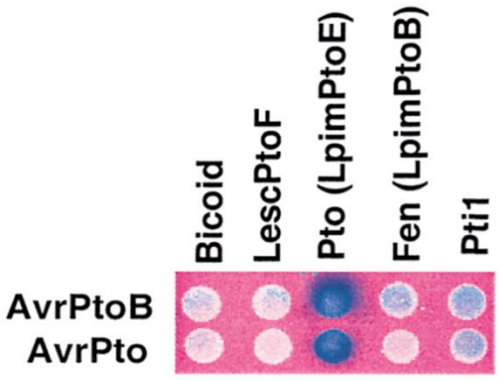

Fig. 15. Interaction of *Pseudomonas* effector proteins with Pto Kinase. Dark blue colour indicates interaction

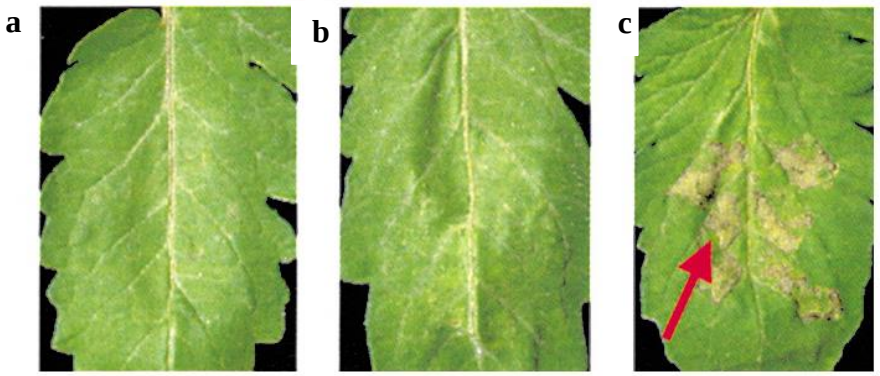

Fig. 16. Expression of effector proteins in tomato leaves. a,b. pto kinases and effectors are infiltrated separately into plants. C. coexpression of pto and effetors are needed for the production of HR

Conclusion

Devising durable pathogen control strategies is a primary challenge to improve agricultural yields and sustainability. An improved understanding of effector functions will enable a wide range of approaches that can be used to improve breeding for disease resistance With these advances, we have gained many insights into both how plants detect and respond

to pathogens, and how those pathogens cause disease on plants. Thus effectors play an integral role in host-pathogen interactions and can impact the outcome of an infection both positively and negatively depending on the host genotype

Discussion:

1. Difference between PTI and ETI?

   PTI is PAMP Triggered Immunity. It is also called as basal defense. It isactivated in cell membrane of the plant cell. ETI is Effector Triggered Immunity. It is also called as secondary defense. It is activated in the host cytoplasm.
2. Give examples of some of receptors present in the plant?

   They are serine, kinases, proteases, salicylic acid.
3. Which one of the immunity is best?

   Both are required for defense response by the plant to prevent pathogen attack.
4. Define horizontal gene transfer?

   Transfer of genetic material between two organisms beongs to same or different species.
5. Why in inoculation studies vector is called as prey vector?

   Because the vector acts as a base for the study and also the particular strain acts with the help of the vector.

References:

Angot, A., Peeters, N., Lechner, E., Vailleau, F., Baud, C., Gentzbittel, L., Sartorel, E., Genschik, P., Boucher, C., and Genin, S. 2006. *Ralstonia solanacearum* requires F-box-like domain-containing type III effectors to promote disease on several host plants. *Proc. Natl. Acad. Sci. 103*(39): 14620-14625.

Bender, C. L., Alarcon-Chaidez, F., and Gross, D. C. 1999. *Pseudomonas syringae* phytotoxins: mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. *Microbiol. Mol. Biol. Rev. 63*(2): 266-292.

Bos, J. I., Prince, D., Pitino, M., Maffei, M. E., Win, J., and Hogenhout, S. A. 2010. A functional genomics approach identifies candidate effectors from the aphid species *Myzus persicae* (green peach aphid). *PLoS genet. 6*(11).

Brown, J. K. and Tellier, A. 2011. Plant-parasite coevolution: bridging the gap between genetics and ecology. *Annu. Rev. phytopathol.* 49: 345-367.

Buttner, D. 2016. Behind the lines–actions of bacterial type III effector proteins in plant cells. *FEMS Microbiol. Rev. 40*(6): 894-937.

Cui, J., Yao, Q., Li, S., Ding, X., Lu, Q., Mao, H., Liu, L., Zheng, N., Chen, S., and Shao, F. 2010. Glutamine deamidation and dysfunction of ubiquitin/NEDD8 induced by a bacterial effector family. *Science 329*(5996): 1215-1218.

Davis, E. L., Hussey, R. S., Mitchum, M. G., and Baum, T. J. 2008. Parasitism proteins in nematode-plant interactions. *Curr. Opin. Plant Biol.11*(4): 360-366.

Dawkins, R. and Krebs, J.R. 1979. Arms races between and within species. *Proc. R. Soc. Lond. Ser. B. Biol. Sci. 205*(1161): 489-511.

De Jonge, R., Bolton, M. D., Kombrink, A., van den Berg, G. C., Yadeta, K. A., and Thomma, B. P. 2013. Extensive chromosomal reshuffling drives evolution of virulence in an asexual pathogen. *Genome Res.* 23(8): 1271-1282.

De Wit, P. J., Mehrabi, R., Van den Burg, H. A. and Stergiopoulos, I., 2009. Fungal effector proteins: past, present and future. *Mol. plant pathol. 10*(6): 735-747.

Friesen, T. L., Stukenbrock, E. H., Liu, Z., Meinhardt, S., Ling, H., Faris, J. D., Rasmussen, J. B., Solomon, P. S., McDonald, B. A., and Oliver, R.P. 2006. Emergence of a new disease as a result of interspecific virulence gene transfer. *Nat. Genet. 38*(8): 953.

Gonzalez-Lamothe, R., Mitchell, G., Gattuso, M., Diarra, M. S., Malouin, F., and Bouarab, K. 2009. Plant antimicrobial agents and their effects on plant and human pathogens. *Int. J. Mol. Sci. 10*(8): 3400-3419.

Hatsugai, N., Igarashi, D., Mase, K., Lu, Y., Tsuda, Y., Chakravarthy, S., Wei, H.L., Foley, J.W., Collmer, A., Glazebrook, J., and Katagiri, F. 2017. A plant effector-triggered immunity signaling sector is inhibited by pattern-triggered immunity. *EMBO J. 36*(18): 2758-2769.

Hogenhout, S. A., Van der Hoorn, R. A., Terauchi, R., and Kamoun, S. 2009. Emerging concepts in effector biology of plant-associated organisms. *Mol. Plant Microbe Interact.* 22(2): 115-122.

Kamper, J., Kahmann, R., Bolker, M., Ma, L. J., Brefort, T., Saville, B. J., Banuett, F., Kronstad, J. W., Gold, S. E., Muller, O., and Perlin, M. H. 2006. Insights from the genome of the biotrophic fungal plant pathogen Ustilago maydis. *Nature* 444(7115): 97.

Kim, S. H., Son, G. H., Bhattacharjee, S., Kim, H. J., Nam, J. C., Nguyen, P. D. T., Hong, J. C., and Gassmann, W. 2014. The Arabidopsis immune adaptor SRFR 1 interacts with TCP transcription factors that redundantly contribute to effector-triggered immunity. *Plant J. 78*(6): 978-989.

Kim, Y. J., Lin, N. C., and Martin, G. B. 2002. Two distinct Pseudomonas effector proteins interact with the Pto kinase and activate plant immunity. *Cell* 109(5): 589-598.

Li, Q., Chen, Y., Wang, J., Zou, F., Jia, Y., Shen, D., Zhang, Q., Jing, M., Dou, D., and Zhang, M. 2019. A Phytophthora capsici virulence effector associates with NPR1 and suppresses plant immune responses. *Phytopathol. Res.* 1(1): 6.

Liu, T., Ye, W., Ru, Y., Yang, X., Gu, B., Tao, K., Lu, S., Dong, S., Zheng, X., Shan, W., and Wang, Y. 2011. Two host cytoplasmic effectors are required for pathogenesis of *Phytophthora sojae* by suppression of host defenses. *Plant physiol. 155*(1): 490-501.

Lo Presti, L., Lanver, D., Schweizer, G., Tanaka, S., Liang, L., Tollot, M., Zuccaro, A., Reissmann, S., and Kahmann, R. 2015. Fungal effectors and plant susceptibility. *Annu. Rev. plant Biol*. 66: 513-545.

Ma, L. J., Van Der Does, H. C., Borkovich, K. A., Coleman, J. J., Daboussi, M. J., Di Pietro, A., Dufresne, M., Freitag, M., Grabherr, M., Henrissat, B., and Houterman, P. M. 2010. Comparative genomics reveals mobile pathogenicity chromosomes in Fusarium. *Nature* 464(7287): 367.

Mueller, A. N., Ziemann, S., Treitschke, S., Aßmann, D., and Doehlemann, G. 2013. Compatibility in the *Ustilago maydis*-maize interaction requires inhibition of host cysteine proteases by the fungal effector Pit2. *PLoS pathog. 9*(2).

Nicaise, V., Roux, M., and Zipfel, C. 2009. Recent advances in PAMP-triggered immunity against bacteria: pattern recognition receptors watch over and raise the alarm. *Plant physiol. 150*(4): 1638-1647.

Ponciano, G., Ishihara, H., Tsuyumu, S., and Leach, J. E. 2003. Bacterial effectors in plant disease and defense: keys to durable resistance?. *Plant Dis. 87*(11): 1272-1282.

Raffaele, S. and Kamoun, S. 2012. Genome evolution in filamentous plant pathogens: why bigger can be better. *Nat. Rev. Microbiol.* 10(6): 417.

Remigi, P., Anisimova, M., Guidot, A., Genin, S., and Peeters, N. 2011. Functional diversification of the GALA type III effector family contributes to *Ralstonia solanacearum* adaptation on different plant hosts. *New Phytol. 192*(4): 976-987.

Schmidt, S. M., Kuhn, H., Micali, C., Liller, C., Kwaaitaal, M., and Panstruga, R. 2014. Interaction of a *Blumeria graminis f. sp. hordei* effector candidate with a barley ARF-GAP suggests that host vesicle trafficking is a fungal pathogenicity target. *Mol. plant pathol. 15*(6): 535-549.

Sonah, H., Deshmukh, R. K., and Belanger, R. R. 2016. Computational prediction of effector proteins in fungi: opportunities and challenges. *Front. plant sci.* 7: 126.

Stahl, E. A., Dwyer, G., Mauricio, R., Kreitman, M., and Bergelson, J. 1999. Dynamics of disease resistance polymorphism at the Rpm1 locus of Arabidopsis. *Nature* 400(6745): 667

Tasset, C., Bernoux, M., Jauneau, A., Pouzet, C., Brière, C., Kieffer-Jacquinod, S., Rivas, S., Marco, Y., and Deslandes, L. 2010. Autoacetylation of the *Ralstonia solanacearum* effector PopP2 targets a lysine residue essential for RRS1-R-mediated immunity in Arabidopsis. *PLoS pathog. 6*(11).

Tian, M., Win, J., Song, J., van der Hoorn, R., van der Knaap, E., and Kamoun, S. 2007. A *Phytophthora infestans* cystatin-like protein targets a novel tomato papain-like apoplastic protease. *Plant physiol. 143*(1) :364-377.

Ustun, S. and Bornke, F. 2015. The *Xanthomonas campestris* type III effector XopJ proteolytically degrades proteasome subunit RPT6. *Plant physiol. 168*(1): 107-119.

Win, J., Chaparro-Garcia, A., Belhaj, K., Saunders, D. G. O., Yoshida, K., Dong, S., Schornack, S., Zipfel, C., Robatzek, S., Hogenhout, S.A., and Kamoun, S. 2012, Effector biology of plant-associated organisms: concepts and perspectives. (In:) *Cold Spring Harbor symposia on quantitative biology*. 77: 235-247.

KERALA AGRICULTURAL UNIVERSITY
COLLEGE OF HORTICULTURE, VELLANIKKARA
Department of Plant Pathology

Pl. Path. 591: Masters Seminar

Name	: Sivadharshanapriya R.	Venue	: Seminar Hall
Admission No.	: 2018-11-146	Date	: 12-12-19
Major Advisor	: Dr. Reshmy Vijayaraghavan	Time	: 10:00 am

Genome biology of plant pathogen effectors: An overview of plant's response

Abstract

Plant pathogens pose major threat to sustainable crop production. Damage caused by pathogens is ubiquitous and affects all major food crops. The mechanisms underlying the threat of pathogens to crops requires both the mechanistic understanding of the infection process and appreciation of the evolutionary trajectory of host-pathogen interactions (Sanchez-Vallet *et al.*, 2018).

Phytopathogens usually have diverse life styles and infection strategies, but in common they attempt to colonize and live at the expense of their host. A major determinant of a pathogen's ability to infect a host plant is determined by set of proteins called effectors (Lo Presti *et al*., 2015). Secreted effectors can act either in the apoplast (apoplastic effectors) or inside the cytosol (cytoplasmic effectors) of the host (Sonah *et al*., 2016). The main aim of the effector is to shield the pathogen, suppress the host immune response and modulate plant physiology to support growth and colonization.

As plant-pathogen interactions evolve, plants are selected for an incompatible (resistant) interaction and pathogens are selected for a compatible (susceptible) interaction. The underlying principle for this antagonistic coevolution is based on the gene-for-gene model which in turn result in boom and bust cycle, leading to the evolution of arms race model and trench warfare model (Lo Presti *et al*., 2015).

Effector evolution is a trade-off between escaping from host recognition and maintaining virulence. Evolution towards evasion of recognition and functional optimization is achieved by duplication, gene deletion, alteration of existing effector genes, gain of secretion

function and horizontal gene transfer. Through these processes, pathogens gain new effectors to specialize on the host (Sanchez-Vallet *et al.*, 2018).

The functions of well-characterized effectors are highly diverse and include the protection of fungal cell walls from hydrolytic enzymes, protease inhibitors, interaction with ubiquitin-proteasome system, disruptors of the hormone signalling pathway and alteration in plant behaviour which favours disease development (Pelgrom and Van den Ackerveken, 2016).

Plants have an innate immunity system to defend themselves against pathogens. In primary immune system, plants recognize pathogen associated molecular patterns (PAMPs) of potential pathogens through pattern recognition receptors (PRRs) that mediate a basal defense response termed as PAMP Triggered Immunity (PTI). Plant pathogens suppress this basal defense response by means of secreting effectors that enable them to cause disease. Although secreted effectors are key players in supressing PTI, with the help of secondary immune system, plants have gained the ability to recognize effector-induced perturbations of host targets through resistance proteins that mediate a strong local defense response or hypersensitive response that prevent the pathogen development in plants (de Wit, 2007).

Thus, effectors play an integral role in host-pathogen interactions and can impact the outcome of an infection, both positively and negatively depending on the host genotype.

References

de Wit, P. J. 2007. How plants recognize pathogens and defend themselves. *Cell Mol. Life Sci.* 64(21): 2726-2732.

Lo Presti, L., Lanver, D., Schweizer, G., Tanaka, S., Liang, L., Tollot, M., Zuccaro, A., Reissmann, S., and Kahmann, R. 2015. Fungal effectors and plant susceptibility. *Annu. Rev. Plant Biol.* 66: 513-545.

Pelgrom, A. J. and Van den Ackerveken, G. 2016. Microbial pathogen effectors in plant disease. In: *Encyclopaedia of Life Sciences*, Wiley-Blackwell, pp. 1-10.

Sanchez-Vallet, A., Fouche, S., Fudal, I., Hartmann, F. E., Soyer, J. L., Tellier, A., and Croll, D. 2018. The genome biology of effector gene evolution in filamentous plant pathogens. *Annu. Rev. Phytopathol.* 56: 21-40.

Sonah, H., Deshmukh, R. K., and Belanger, R. R. 2016. Computational prediction of effector proteins in fungi: opportunities and challenges. *Front. Plant Sci.* 7: 126.