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REVIEW

Molecular mechanism of disease establishment and host tolerance in *Ralstonia solanacearum* and tomato pathosystem

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Bacterial wilt in tomato caused by *Ralstonia solanacearum* is directly responsible for 25%-70% yield loss in India and it is a major threat. For a successful disease management, it is necessary to fully understand the underlying dynamics between *R. solanacearum* and tomato at molecular level. In the cycle of entering into a healthy host and finally leading to its succumb, *Ralstonia* has been observed to regulate its pathogenicity in a controlled manner by different genes and virulence factors among which PhcA, PhcR and Type III secretion system are most important ones. These core components are engaged to perceive bacterial density and gets regulated accordingly to produce cell wall degrading enzymes like cellulase, pectinase etc. Also these virulence factors upregulate bacterial motility genes and exopolysaccharides for facilitating successful disease establishment. At the same time, host plants depending upon their tolerance level, engaged to minimize damages caused by the bacteria by deploying different PR proteins, phytohormones like salicylic acid, ethylene etc. Due to their collective functions, plant cells undergo changes like lignification of cell wall to prevent bacterial entry, synthesis of different proteases to counter bacterial cells and to develop a PAMP-triggered immunity (PTI). This review discussed and analyzed the research progress so far made on this pathosystem focusing on the molecular mechanism of disease establishment as well plant defense. This comprehensive analysis will give a clear idea about the lacunae still existing on this area and will help to determine future research directions.

Key words: Bacterial wilt, disease tolerance, host defense, pathogen triggered immunity, *Ralstonia solanacearum*, siderophores, tomato, virulence factors

INTRODUCTION

Ralstonia solanacearum is a rod shaped, motile, aerobic, soil born, gram negative bacteria causing devastating wilt disease of solanaceous vegetable tomato (Guo *et al.* 2021). Bacterial wilt is very common in warm, tropical and subtropical parts of the world (Popoola *et al.* 2014) where this disease is chronicin nature due to high temperature and high humidity condition which is favoring factors for the pathogen.

Other factors like susceptible host, virulence of the particular strain of bacteria also affects the disease severity (Singh *et al.* 2014).

The pathogen primarily invades root of tomato plant through elongated zones or through the wounds caused by insects, nematodes etc. Bacteria infested tomato plants totally collapse within 2-5 days (Rivard *et al.* 2012). The infected vascular tissues showed brown discoloration and whitish to yellowish bacterial ooze. These are stream of bacteria cells which gets visible after cross section of infected stem (Seleim *et al.* 2014). Upon entry, bacteria migrate through vascular tissues fromroot towards the shoot. To facilitate the invasion, different pathogenic factors are produced by bacteria such as exopolysaccharide, type III effectors, cellulose, pecticlyase to promote cell wall degradation for further movement of the pathogen. In xylem, bacteria multiply their numbers and the amount of viral exopolysaccharides also increased thus inhibiting water flow in the shoot, causing shoot wilting and ultimately plant death (Xue *et al.* 2020).

Plant pathogen interaction is a dynamic process where plant's constitutive physical and chemical

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defense factors and pathogen's virulence factors are engaged with each other. Pathogen produces different type elicitors like glycoprotein, protein, peptide, carbohydrates and toxins which are recognized by cell membrane receptors of plant and helps to build up induced or acquired resistance. Other key factors like salicylic acid (SA) and pathogenesis related (PR) protein are produced in plant after primary infection and developed systemic acquired resistance (SAR) (Agrios 2005).

Hypersensitive resistance (HR) or systemic acquired resistance (SAR) developed in host plant after pathogen infection, by the production of defense proteins that are translated from plant genes. These defense proteins may be either structural proteins that are situated in extracellular matrix of host cells and interact with pathogen binding or proteinaceous enzymes that helps in secondary metabolites production and plant antibiotics biosynthesis. Third significant factor is pathogenesis related (PR) protein that helps to resist acidic pH and proteolytic cleavage both of which are detrimental for plant survival (Dahal *et al.* 2012).

Plant pathogen interaction at molecular level in every pathosystem is an intriguing field and this present review aims to conglomerate different studies on this particular pathosystem of *R.solanacearum* and tomato mainly focusing on their molecular strategies towards disease establishment and plant defense responses as well.

Disease establishment strategies of Ralstonia solanacearum in tomato

Infection source of this bacteria can be through irrigation, contaminated tools, infected seed materials and insect vectors (Deberdt*et al.* 2014). Primary entry site of *R. solanacearum* is through root of susceptible tomato plants by means of several stages. At first, bacteria colonize the root surface either in elongated zone of root or at the point of outgoing lateral root. Secondly, 2-3 days later, bacteria infect root epidermis and cortex followed by bacterial entry into vascular bundle including vessel and xylem parenchyma. At this stage, cell walls started to degrade and at final stages of infection, bacteria increase their number by multiplication and move towards the shoot and secrete exoplysaccharides which blocks the xylem. This causes water movement in root and shoot to halt and thus leading the plant to wilt (Kim *et al.* 2016).Secondary infection is common in case of *R. solanacearum* infection because of increase in phenolic acid release from tomato roots leading to attraction of pathogenic fungi *Fusarium solani*. Other favorable microbial community disappear due to the abundant of these two (Su *et al.* 2020).

Bacterial interaction with plant root surface occurs through irreversible and reversible attachment via adhesion protein, polysaccharide and bacterial outer appendages like pili. Type IV pili helps to attach on root surface that occur in polar fashion (Lowe-Power *et al.* 2018).Attachment process is also influenced by high temperature, low pH and physicochemical factors like host roots surface, xylem vessel wall etc. (Hoffman *et al.* 2015). Once in vicinity, *R. solanacearum* cells invade into roots of tomato plant through natural opening or wounds and moves into vascular tissue within next 24 hours (Caldwell *et al*. 2017). These solitary cells grow inside the vessel and aggregates in a biofilm matrix and produces EPS, which gradually block the vessel to stop the water flow thereby causing wilt (Caldwell *et al.* 2017; Minh Tran *et al.* 2016). *R. solanacearum* infected tomato plant is rich of many metabolites which in fact act as a positive feedback loop (Lowe-Power *et al.* 2018) because recent studies suggested that tomato sap consists of amino acid, sugar and organic acid helps bacteria to multiply and colonize vigorously within the host tissue (Zuluaga *et al.* 2013; Fatima and Senthil-Kumar, 2015).

Molecular regulation of virulence in Ralstonia solanacearum

The pathogenicity of *Ralstonia* sp. is exerted through a cascade of virulence gene expression which span altogether starting from initiation of infection to the severe establishment of wilt. The functions of these genes are in wide range like stress tolerance (bcp,dps, acrA and acrB), motility regulator (pilA, fliC), exopolysaccharide synthesizing factors, cell wall degrading enzyme synthesis (like pehA, pehB ,pehC, cbhA and egl)etc. (Flores-Cruz and Allen 2009).

PhcA, a virulence factor, plays a central role in regulation of expression of EPS and cellulase encoding genes as well as to regulate bacterial motility. PhcA also negatively regulates T3SS which is a family of siderophore genes (Genin and Denny 2012). During infection, *R.solanacearum* at first colonized on

root surface and comes in contact with plant cell followed by activation of T3SS system but PhcA not get activated due to low cell density. In next step, bacteria enter into root and increase their number in xylem which triggers PhcA to become activated and to induce EPS and cellulose production causing T3SS and siderophore repression (Mole *et al.* 2007). Homeostasis of this PhcA is maintained by cell density dependent mechanism where 3-hydroxy palmitic acid methyl ester is involved which originally is produced from inner membrane protein PhcB (Gennin and Denny 2012). Another regulatory component to regulate PhcA is PhcR which is activated upon bacterial cell density above 10^7 cell / ml or high level of 3-OH PAME. In presence of either of those two factors, PhcS/PhcR complex gets activated (Peeters *et al.* 2013) and phosphorylated. Phosphorylated PhcR then mediates PhcA activation. PhcA, upon activation, regulates several downstream genes like XpsR which induces the transcription of EPS operon that leads to production of EPS (Geninand Denny 2012). Notably, EPS production signaling is not only dependent on PhcA but also on two components system of VsrA/VsrD (Peeters *et al.* 2013). Separate other functions of VsrA/VsrDis repress the transcription of flagellin gene (fgl22) that reduces the swimming motility. EPS synthesis also controlled by another two component system namely VsrB/VsrC which can also repress the transcription of pectinase pglA (Peeters *et al.* 2013).

As discussed earlier, T3SS encoding genes which are under regulation of PhcA, are highly conserved across species (Tang *et al.* 2006). Its regulation is monitored by several factors which are to be discussed now. In R. solanacearum, HrpB regulator binds to hrpII box of promoter region of T3SS and induces the transcription of T3SS genes and HrpG regulator controls its expression (Peeters*et al*. 2013). HrpG regulator can also control T3SS unrelated genes and these genes are involved in plant pathogen interaction such as adhesion factors (lectins), catalase enzyme, ethylene producing enzyme etc. (Valls *et al.* 2006). Virulence determinants like pectinolytic and cellulase activity are also affected by HrpG regulator (Plener*et al*. 2012). Outer membrane receptor PrhA receive the signal of T3SS and transfer it to prhL and prhR protein that are present within the membrane. Then this signal trigger the expression of hrp/hrc genes. Chemotaxis of the bacteria within the plant vascular system and biosynthesis of Hrp dependent factors (HDF) monitored by a HrpB regulator. HDF, a low molecular weight chemical compound, triggers cell density dependent LuxR system (Occhialini *et al.* 2005). Thus, at earlier timepoints, multiple number of transcription factors and genes interplay between themselves to successfully establish the disease within host (Fig.1).

Host defense response in tomato against Ralstonia solanacearum

Tomato plant has multilayered and complex immune system to protect themselves from environmental microbes. At early stages, *R. solanacearum* fight for the infection with border cells, which produces extracellular matrix consists of polysaccharides, protein and DNA (Tran *et al* 2016). Then plant try to inhibit invasion of bacteria by recognition of various pathogen molecules that activates plant immune system. Plant immune system consist of defense systems that receive numerous general pathogen elicitors. Conserved microbes or pathogen associated molecular patterns (MAMP or PAMP) are detected by plant cell receptors of innate immune systems pattern recognition receptors (PRR) (Newman *et al.* 2013). PRR trigger immunity (PTI) of plant helps to resist from several microbes (Nguyen *et al.* 2021).

Bacterial flagellin (most abundant protein in bacterial flagellum) protein called flg22, composed of 22 amino acid peptide is the best known PAMP of tomato plant. Elongation factors EF-Tu, peptidoglycan (PGN), lipopolysaccarides (LPS) are common PAMP (Newman *et al* 2013). The flg22 can be recognized by the pattern recognition receptor (PRR) Flagellin Sensing 2 (FLS2) and elongation factors Ef-Tu, which recognized by EF-Tu receptor (EFR) and initiate the signaling cascade that ultimately activate the PTI of tomato plant (Gomez-Gomez and Boller, 2000;Kunze *et al.* 2004; Zipfel *et al.* 2006).

The flg22 of *R. solanacearum* shows sequence polymorphism and hence these are undetectable for all plants. Another PAMP is flgII-28 which is not a polymorphic and also detected by the PRR of certain plant of solanaceae family. These evidences give a hint that flagellin of *R.solanacearum* has been potentially evolved to avoid perception by plant PRRs (Clarke *et al.* 2013;Wei *et al.*2018). Heat shock protein csp22 of *R. solanacearum* can be detected by tomato plant and induces the immune system during bacterial penetration into root

(Wei *et al.* 2018). After entering into root, initially *R. solanacearum* does not activate PRR trigger immunity (PTI). Subsequent damage and cell death along bacterial colonization activate the PTI in neighboring cells (Zhou *et al.* 2020).

AvrE, DspE and WtsE family of effector molecules repress the plant defense signaling and leading to cell death (Kvitko *et al.* 2009; Ham *et al.* 2009). PopS is an AvrE family effector of *R. solanacearum* that suppress salicylic acid mediated defense of tomato. PopS mutant shows delayed in colonization and virulence but ultimately bacterial wilt disease occur (Jacobs *et al.* 2013). Effector protein molecules of *R. solanacearum* can enter into plant cells via type III secretion system (T3SS) (Cunnac *et al.* 2008).These effectors proteins known as type III effectors (T3S) are major virulence factors of most pathogenic gram negative bacteria and are able to alter plant cell's signal transduction and function. As a result, T3S permit proliferation of bacteria and development of disease symptoms (Deslandes and Rivas 2012; Toruno *et al;* 2016). Nucleotide binding and leucine rich repeat domain containing T3Ss are also detected by intracellular receptors of plant and activate effector triggered immunity (ETI) and prevent bacterial proliferation (Khan *et al.* 2016).

Regulation of tomato plant defense genes against Ralstonia solanaceraum

Transcriptomic studies of tomato plants during pathogenesis of *R.solanacearum* revealed a differentialgenetic expression profile between susceptible and resistant plants. Whether susceptible plant alters its transcriptome significantly or not, 146 upregulated genes and 10 down regulated genes are identified in resistant plant. Upregulated genes in resistant cultivators were related with different hormonal synthesis like jasmonic acid, ethylene and auxin. Pathogenicity related (PR) genes, hydroxyl cinnamic acid and lignin biosynthesis genes are also enhanced resulted in structural change in cell wall of xylem vessel of resistant tomato plant (Ishihara *et al.* 2012). *R.solanacearum* infection immediately activate auxin related gene expression in the roots of susceptible tomato plant compare to the resistant one. Mutation of dgt1-1of tomato increases resistance to *R.solanacearum* by altering auxin transport and defective secondary roots formation (French *et al.* 2018). Methionine cycle play crucial role in conferring disease resistance in tomato. Several genes such as SAHH, SAM, GAD positively regulate disease resistance. Transcriptome and proteomic study helps to know different pathogenesis related molecules, as 60 expressed protein are identified in tomato plant after infection with two strains of *R. solanacearum*, RsH and RsM. GABA and MTC biosynthesis pathway also involved in *R. solanacearum* infection. GABA catabolism genes SSAGH and GABAT were upregulated where biosynthesis gene GAD was downregulated. Transcriptome analysis shows MTC related gene such as MS and SAMS gene were down regulated and SAHH gene was upregulated. So in susceptible tomato plant participation of GAD is important (Wang *et al.* 2019). Hawaii 7996 is a resistant tomato plant that activate papain like cysteine proteases (PLCPs) and serine hydrolases (SHs) on R.solanacearum infection (Planas-Marquès *et al.* 2018).

Ethylene has the positive roles to play in tomato resistance against bacterial wilt. Ethylene biosynthesis is regulated by ACO gene, which inactivates CTR1 gene (Gao *et al.* 2003). The inactivation of CTR1 gene regulate de-repression of EIN2 gene and followed by activation of ERF1 gene induces defense responses (Ouaked *et al.* 2003). Jasmonic acid also has the positive responses in tomato plant defense against bacterial wilt. MAPK cascades also involved in tomato defense responses against *R.solanacearum* (Chen *et al.* 2009). Activation of MAPKs in tomato plant induces by MAPK kinase (MAPKK) such as MKK2, MKK3,MKK4 etc. MAPK kinase MKK2 and MKK4 in tomato plant can phosphorylate MAPK2 and MAPK3 (Pedley and Martin 2004). Thus tomato plant defense responses are regulated by ethylene, salicylic acid, jasmonic acid and MAPK cascades (Chen *et al* 2009).

CONCLUSION

Host pathogen interaction is a dynamic process where both projects their survival strategies to overcome its counterpart. A tolerant host has a higher chance of survival to reduce detrimental effects of pathogen or even to successfully block its entry into its internal tissues,whereas, a more virulent strain and a less tolerant host interaction leads to succumbing of the host plant. The above discussion of host pathogen interaction between *R.solanacearum* and tomato gives a detailed account on the interaction between these host and pathogen at molecular level. A successful invasion

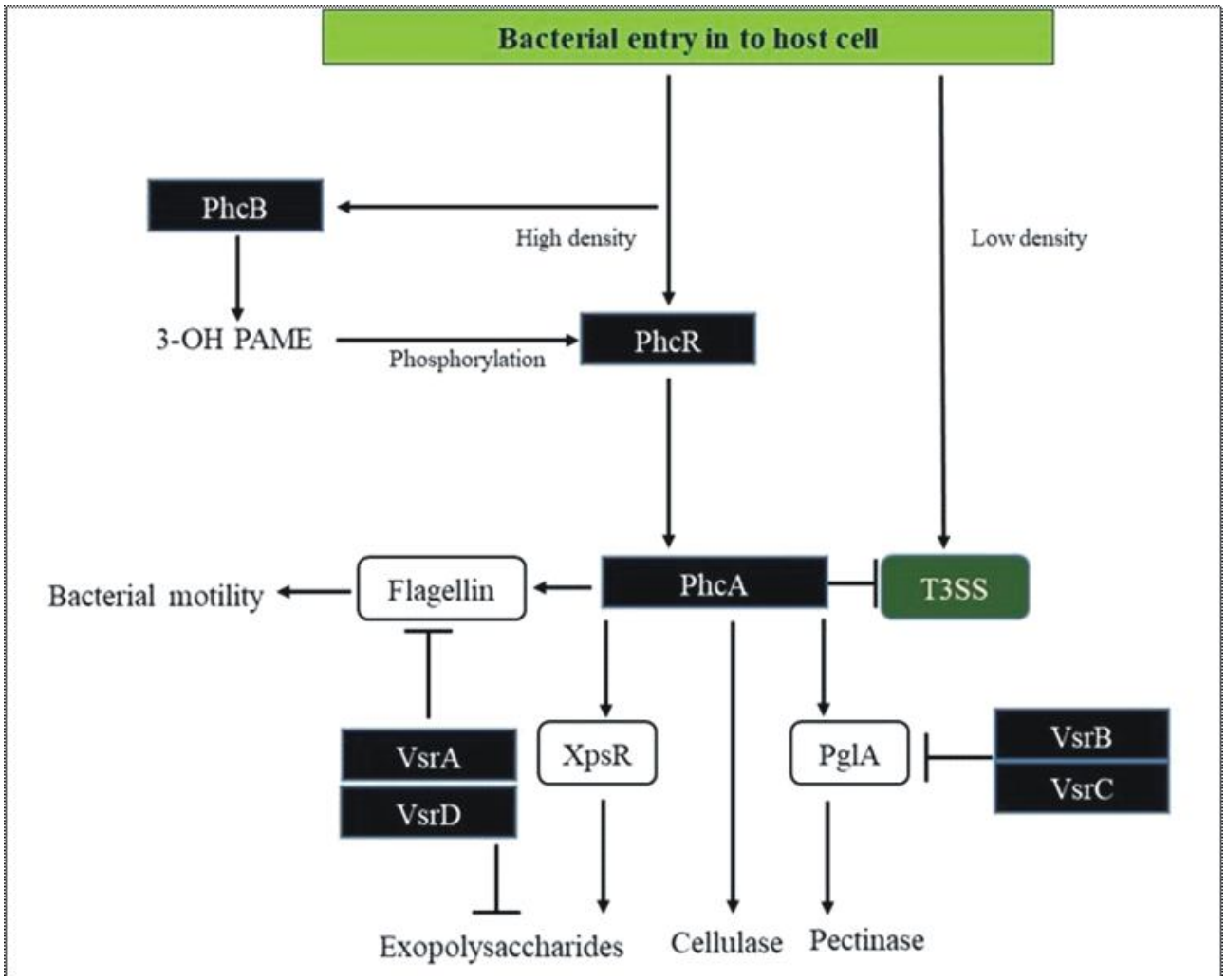

Fig. 1 : Interplay between bacterial transcription factors and other virulence genes to synthesize components essential for disease establishments

and establishment of disease is marked by wilting of the host plant due to complete blockage of water flow within the vessel. All these are regulated by several virulence factors and other related gene expressions. Whereas, a more tolerant tomato host will enforce its complex defense system of phytohormones, PR proteins, antimicrobials etc. to inhibit internal colonization of bacteria.

From this detailed study of the literatures, evidently there is a lack of information of more genes of *Ralstonia* associated with disease establishment. More studies need to be done in future on plant genes which are specifically involved in defense against *Ralstonia* and how they have evolved to match with that specific pathogen. Further, by gathering all those information about the molecular mechanism between *Ralstonia* and tomato, a sustainable biocontrol mechanism can be applied and there with a possibility of developing efficient tomato breeds in respect to bacterial wilt disease tolerance.

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