REVIEW

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Moleculer defence response in tomato against *Alternaria* blight : an over view

ANUPRIYA GHOSH #, SUVANWITA LAHIRI#,ARPAN DAS AND SUREKHA KUNDU*
Molecular and Applied Mycology and Plant Pathology Laboratory, Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Kolkata 700019, India

Early blight disease is one of the most important and dreadful diseases of tomato plant which is caused by different species of *Alternaria.* At the time of Early blight disease development, constant battle occurs between tomato plant and *Alternaria solani* pathogen. Plants try to resist pathogen invasion by creating physical barriers like callose deposition, secondary cell wall thickening or produce anti-microbial secondary metabolites to limit the growth of the pathogen. The first line of defense after recognition of pathogen is mediated by molecular signaling cascades which mainly involve generation of reactive oxygen species (ROS), defense gene like pathogenesis related (PR) proteins, plant defensins, regulation by transcription factors like ERF, MYB, WRKY, interaction with components of the JA/ET and SA signaling to control defense responses, to name a few. Due to *Alternaria* stress, several mRNAs are expressed differentially. This review highlights some of these most commonly used yet immensely important defense responses exhibited by tomato plants at the molecular level when they come in contact with the Early blight causing pathogen *Alternaria* sp.

Key words: *Alternaria solani,* Early blight disease, plant defense, plant pathogen interaction, ROS, secondary metabolites, tomato, transcription factors.

INTRODUCTION

Plants possess several defense mechanisms to defend themselves from pathogen attack. Defense responses are mainly generated by complex signaling pathways which affect cellular and molecular process leading to resistance. These responses comprise of ROS generation, lignification of cell wall, defense related gene activation, accumulation antimicrobial compounds, to name a few (Seo and Choi, 2015). Production of phytohormones such as SA (Salicylic Acid), JA (Jasmonic Acid), and Ethylene (ET) also play an important role in systemic signaling that facilitates resistance against pathogen (Bakshi and Oelmullar, 2014).So it is necessary to identify regulatory factors which form a part of the defense response in plants. In *Arabidopsis*, a number of transcription factors are identified which directs signal crosstalk. Among which, WRKYs play significant roles in combating biotic and abiotic stress (Tsuda and Somssich, 2015).

A number of genes (zinc finger and ribosomal protein transcription factors) play an important role in defense against *Alternaria solani* by operating the PR protein synthesis. (Upadhyay *et al.* 2016). Tomato is one of the most important food crops cultivated all around the world.Global production of tomatoes is 161.7 million metric tons, with China and India as major producers followed by USA (Adhikari *et al.* 2017). Apart from culinary uses, tomato possess medicinal properties. Lycopene found in tomato helps in protecting the body against cardiovascular diseases, cancer etc. It also contains Vitamin A, Vitamin C and antioxidants. Often production of tomato has been hampered by viral, bacterial, fungal diseases (Adhikari *et al.* 2017). Early blight is one such fungal disease which causes huge agricultural losses. Several types of diseases are found in tomato among which Early blight of tomato is a common disease (Nikam *et al.* 2015).

Alternaria solani and several important pathogens including *Alternaria alternata, Alternaria brassicicola* causes early blight and many

*Corresponding author: surekha_kundu@yahoo.com
# Both the authors contributed equally to the review and are joint first authors

necrotrophic diseases (Moghaddam *et al.* 2019; Sarker *et al,* 2017). It belongs to Phylum Ascomycota and Sub phylum-Pezizomycotina. It falls under the class- Dothideomycetes, subclass-Pleosporomycetidae, order-Pleosporales and family-Pleosporaceae.For successful infection, warm and humid conditions with a temperature range between 24-29°C are advantageous. Temperatures between 28-30°C along with moisture are needed for germination of conidia (Roy *et al.* 2019).

When the weather is helpful, it mainly affects stem, leaves and fruits. Symptoms mainly occur in leaf (Early blight), stem (stem lesions) and fruit of tomato plant. The main symptoms are found on leaves as circular lesions formation (Gulzar *et al.* 2018). In leaf, it is characterized by lesions which develop on lower leaves as small brownish black spots that expand as concentric rings in the darkened area. As the disease progresses, the area around the lesions become yellow and at last the whole leaf turns yellow. Later, lesions gradually occur in upper leaves, defoliation occur in the lower part of the plant. The infected leaves dry up and are detached from the plants. In stem, at first dark and sunken lesions are found that become bigger to form lens shaped lesions as found in leaves. In young seedlings, lesions girdle the stem destroying the vascular cambium and "collars" are formed (Gulzar *et al.* 2018). In fruits, sunken, leathery, dark and purple lesion appears at the end of the stem. These lesions become bigger and cover the fruits. So, those defected fruits fall down prematurely (Adhikari *et al.* 2017).

Mechanism of infection

The fungus mainly survives in infected debris and contaminated seeds. For the production of inoculum, these are the primary source. The conidia of *Alternaria* are club shaped and beaked. Due to melanin, hyphal cells are pigmented darkly. The melanin gives protection to spores and hyphae from environmental stress and helps the spores to persist in soil (Adhikari *et al.* 2017).After the conidia successfully germinates, germ tubes are formed which penetrate the host by appressoria formation or hyphae or by entering through stomata or wounds thus infection occurs. The host cell wall is degraded by some enzymes released by the pathogen and some toxins are also generated which kills the host cells so that

pathogen draw nutrients from the necrosed host cells. Pectin methyl galacturonase is also present which helps in host colonization (Sreenivasulu *et al*. 2019).Many enzymes and secondary metabolites are produced by fungal pathogen to infect the host plant. Before invasion, those secondary metabolites can penetrate within the cells or may destroy or operate host cells. *Alternaria* species also generates various types of low molecular weight secondary metabolites and they are involved in pathogenicity at the time of host pathogen interaction (Adhikari *et al.* 2017).

Defense mechanism of tomato plant against Early blight disease

When plants are challenged with pathogen attack, they protect themselves through an array of biochemical and structural responses (Ray *et al.* 2015). Formation of structural barriers and secretion of antimicrobial compounds, lignin, phytoanticipins and cutin waxes serve as the first line of defense (Ali *et al.* 2017; Esfahani *et al.* 2017). Other active defense mechanisms such as hypersensitivity response, lignification and PR protein and phytoalexin production are also present in plants (Yang *et al.* 2015; Abdelrahman *et al.* 2019).Plants produce several types of PR proteins as their defense response against fungal attack (Adhikari *et al.* 2017).The genes which are responsible for the generation of PR proteins have improved resistance in different crops from various pathogens (McNeece *et al.* 2019).

The enigmatic role of oxidative burst, production and regulation of ROS accumulation in defense response

An important event for pathogen recognition is redox status changes of the infected cells due to the reactive oxygen species (ROS) accumulation. Through oxidative bursts, ROS mediated defenses are mainly exhibited. The initial phase of oxidative bursts may occur after few minutes of infection in both susceptible and resistant host plant cells and after some hours second phase of oxidative burst occurs only in case of resistant interactions. Oxidative burst or the production of reactive oxygen species (ROS) by utilising oxygen molecules during host-pathogen interaction involves NADPH respiratory burst oxidase homolog (RBOH) proteins and play a role in resistance to a number of necrotrophic pathogens like *Alternaria*

brassicicola, *Phytophthora parasitica*, *Rhizoctonia solani*, *Botrytis cinerea*, and hemibiotroph like *Magnaporthe oryzae*. ROS exists in various forms and the most important are singlet oxygen ($^1O_2$), hydroxyperoxyl radical ($HO_2\cdot$), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) (Foley *et al.* 2016). ROS accumulation is generally noticed around the penetration site of cell wall, intracellular inoculation sites like chloroplast and mitochondria, plasma membrane, and it also participates in programmed cell death to restrict infection spread to neighbouring tissues at later stages of infection (Ali *et al.* 2017). ROS scavenging and producing mechanisms are observed in organelles such as mitochondria, chloroplasts and peroxisomes. ROS accumulation has been shown to be an initial mode of defense against necrotrophic pathogen *Alternaria* sp., followed by activation of MAP kinase cascades to trigger further activation of plant innate immunity. Plants possess antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), which confer increased resistance when produced in higher amounts (Choudhary *et al.* 2020; Dey *et al.* 2020; Thinakaran *et al.* 2020).

High levels of ROS accumulation at the beginning of infection in *A. solani*-infected tomato has been shown to enhance early defense response and a higher level of such compounds may act as a second messenger in a signaling pathway which leads to activation of downstream defense genes (Thinakaran *et al.* 2020). Expression of a NADPH oxidase or Rboh (Respiratory burst oxidase homolog) *Sl Rboh B* gene of tomato was increased during interaction with another necrotroph *B. cinera*(Li *et al.* 2015).A recent research has pointed out greater accumulation of H_2O_2 and superoxide anion in less tolerant tomato cultivars infected with *A. solani* and *A. alternata.* Membrane stability, as indicated by the rate of membrane-lipid peroxidation, followed a similar trend in inoculated plants than their respective controls(Sallam *et al.* 2021). In another study, a resistant tomato variety PR showed 1.24 fold higher ROS accumulation in the leaves than the susceptible PED variety at 72 hours post inoculation (hpi) during interaction with *A. solani*. This difference in disease tolerance is the result of the differential ROS accumulation in the PR and PED varieties. However, a higher activity of ROS scavenging enzyme SOD was found in PR at 48 hpi and 72 hpi. A possible explanation may

be provided by the fact that necrotrophs try to take advantage of ROS-mediated cell death in host and continue to grow rapidly inside the necrosed host tissue before spreading to other healthy parts. So, plants can counter the pathogen attack by successful ROS scavenging and limit ROS-mediated cell deaths. This claim was further validated by their findings on DNA damage which revealed lesser damage in resistant PR due to better ROS scavenging (Ray *et al.* 2015). Similar observations have been made by other research groups in case of pathosystems involving *R. solani*-wheat and *Macrophomina phaseolina*-sesame (Foley *et al* 2016; Chowdhury *et al.* 2017). Significant upregulation of anti-oxidant enzymes SOD, CAT, POX (peroxidase) and PPO (polyphenol oxidase) by 30%-50% in *A.solani* infected tomato plants was shown by Awan *et al.* (2019). Increased CAT activity causes the breakdown of H_2O_2 and regulate its cellular concentration to prevent oxidative damage. This regulation generally occurs during the early phase of infection, whereas enzymes like POX and PPO may be induced at a later stage than CAT. POX converts H_2O_2 to water using polyphenols as hydrogen donor and PPO catalyzes oxidation of phenolic substrates and produce quinones that are fungitoxic in nature (El-Nagar *et al.* 2020). Greater PPO activity correlates highly with greater disease tolerance in resistant tomato cultivar during its interaction with *A. solani* and lesser PPO activity was noticed in susceptible variety (Ray *et al.* 2015).

Physical barrier between plant and pathogen in the form of cell wall appositions

The α-1, 3-glucan polymer callose is a ubiquitous and abundant component of cell wall appositions which exists in higher plants. Callose deposition is a defense strategy which acts as a primary barrier and prevents fungal penetration within host cells. It mainly obstructs the virulence effectors secretion and decreases nutrient availability to the pathogen and helps in disease resistance (Ray *et al.* 2015). In case of pathogen attack, callose is deposited between preexisting cell wall and plasma membrane. To strengthen plant cell wall, this pathogen induced callose deposition acts as physical and chemical defense mechanism. Callose deposition also plays an important role in defense response to invade pathogens.At the place of pathogen attack (in vascular tissues, plasmodesmata) pathogen elicitor activates plant

innate immune response signaling which increases callose deposition at that site. Callose rich papilla forms at the point of infection which helps to restrict the penetration as well as colonization by pathogens. When the rate of callose deposition increase at plasmodesmata, it closes and helps to restrict pathogen spread (Wang *et al.* 2021).

Plant cell walls are also related to structural inductive mechanisms. At the infection site, cell wall thickening occurs due to lignification and other modifications (chemical) of plant cells. Plants mainly detect pathogen associated molecular patterns (PAMP's), damage associated molecular patterns (DAMP's) or microbe associated molecular patterns (MAMP's) by their pattern recognition receptors (PRR's) which is a trigger for PAMP triggered plant immunity (PTI) initiation with the factors released by pathogenic attacks (Malinovsky *et al.*, 2014). Immune responses (HR and SAR) are triggered by effector which targets pathogenic virulent effector proteins to disrupt components of PTI. It leads to specific gene expression which activates signaling pathway. For lignification or callose deposition, release of phytoalexins, PR and hormones like SA, JA these signals are responsible (Ali *et al.* 2017).

Cell wall appositions which mainly prevent penetration of pathogen have more than 30% higher amounts of cross-linked proteins in case of resistant tomato varieties than susceptible tomato varieties at 24 hpi and 48 hpi. The susceptible variety showed an overall delay in the deposition of these proteins, leading to lesser tolerance to *A. solani* (Ray *et al.* 2015). Callose deposition as a pathogen induced defense response was noted in case of *B. cinerea* infection in tomato leaves. Similar responses may be expected in case of *Alternaria* sp. induced pathogenesis too (Scalschi *et al.* 2015). In fact callose deposition peaked at 72 hpi in tomato leaves under *A. solani* stress in a recent study, which is in line with the observations made by others in this field of research (Sarkar *et al.* 2017).

Role of Secondary metabolites in early blight resistance

In the cells, presence of secondary metabolites restricts pathogen growth. Secondary metabolites like phenolic compounds, isoflavonoids, saponins, alkaloids form indispensable parts of plant defense.

They act as signaling molecules in defense response (induced) or a part of obstacles that are formed against infection.Peroxidases which are included in the enzymatic H_2O_2 scavenging, are linked in phenolic compound accumulation at the time of defense response in host cell walls. Phenolic compound deposition directly increases resistance in case of disease. Deposition of phenolic compounds and polyphenol oxidases, which catalyze phenol oxidation to quinones, are involved in defense against pathogen in plants. Phenolic compounds are deposited at much larger amounts, approximately 5-fold higher, in resistant tomato cultivar challenged with *A. solani* infection than its susceptible counterparts (Sallam *et al.* 2021). Similar trend was observed in another resistant tomato variety showing about 1.24 fold greater phenolic compound accumulation at 72 hpi (Ray *et al.* 2015). Application of exogenous gallic acid provided better resistance to *A. solani* infection and positively enhanced the content of total chlorophyll, total soluble phenolics, flavonoids, as well as increased the activities of enzymes catalase, peroxidase and polyphenol oxidase. This throws light upon the relation between secondary metabolic substances produced by plants to cope with pathogen invasion (El-Nagar *et al.* 2020). Total phenol was more even in resistant wild derivatives of tomato and contributed to resistance against early blight (Singh *et al.* 2017). Similarly, total phenolics, total tannins and total flavonoids were consistently higher in *A. solani* infected tomato fruits in all the tested cultivars. Infected fruits contained 45.18-76.85 mg/100g for infected fruits and 22.02-50.33 mg/100g fruit weight (FW) for healthy fruits total phenolics, 3.54 to 15.64 mg/100g total tannins for healthy and 10.13-26.06 mg/100g FW for infected fruits, and total flavonoids ranged from 1.77 to 3.70 mg/100 g FW for healthy fruits, while in the infected fruits the content of TF was from 4.05 to 6.60 mg/100g FW (Sladana *et al.* 2015).Greater accumulation of transcripts of important genes of the phenylpropanoid pathway and other genes leading to increased lignin biosynthesis viz., phenylalanine ammonia lyase (PAL), cinnamic acid 4-hydroxylase, 4-coumarate CoA, p-coumarate-3-hydroxylase, cinnamoyl-CoA reductase was observed in resistant accession of a wild relative of tomato when infected with *A.solani*. This study also showed upregulation of chalcone synthase (CHS) responsible for flavonoid synthesis in the resistant plants during early blight (Shinde *et al.* 2018)

Production of PR proteins for defense

PR proteins are plant species-specific proteins which are mainly produced in response to attacks by fungi, bacteria and virus and thus have an important role in plant defense. It functions either directly on the pathogen or indirectly by generating physical barriers for fungal infection process or also by intrinsic upstream PR signaling. In resistant and hypersensitive reactions, they are produced in large amounts Most of the PR proteins directly disrupts the fungal cell wall (basic endochitinase, glucan-endo-1,3-beta glucosidase and endo-1,4-beta glucanase) or by ribonuclease activity obstruct fungal germination. Those proteins also take part in the increased tolerance to *A. solani* induced early blight by decreasing damages in the host tissues (Upadhay *et al*. 2016). A number of defense -related PR proteins viz., PR-1, β-1,1, 3-glucanase (PR-2), chitinases (PR-3,PR-4, PR-8, PR-11), thaumatin (PR-5), proteinase inhibitors (PR-9), ribonuclease like defensin (PR-10), lipid transfer protein (PR-14) etc. have been identified in various plant species (Checker *et al.* 2018). According to a recent study, NPR1 gene was significantly upregulated in resistant tomato cultivar in response to *A. alternata* induced early blight. NPR1 encodes Non-expressor of Pathogenesis-Related gene1, acts as a co-TF that regulates transcription in response to early blight, thus functioning as a component of the basal defense system of tomato. It is suggested that NPR1 positively regulates the expression of other PR genes and helps to establish systemic acquired resistance (SAR) (Moghaddam *et al*. 2019).

After fungal infection colonize, plants generate chitinase (PR-3), for dismantling the main component of fungal cell wall, i.e. chitin (Adhikari *et al*. 2017).Transgenic expression of a rice chitinase gene in tomato provides protection against early blight (Khan *et al.* 2017).Glucanase and chitinase accumulation as hydrolytic products of induced and constitutive genes acts on fungal viability and upgrade plant immunity responses. High enzyme activity of PR-2 and PR-3 has been linked to resistance against early blight in resistant tomato genotypes like H.a.s 2274, Turkish Cherry, Esfahan local and Rio Grande where both α-1, 3-glucanase (PR-2) and chitinase (PR-3) were highly upregulated. Other PR genes like PR-7, PR3-ACHI and PR3-BCHI also showed higher expression levels in the resistant cultivars (Moghaddam *et al*., 2019).

Regulation of a multitude of defense signaling pathways requires an active participation of transcription factors

A transcription factor (TF) is a molecule regulating the expression of a gene positively or negatively. The Ethylene Responsive Transcription factor belongs to AP2/ERF family, the largest TF family. AP2/ERF TFs have been identified in Arabidopsis, rice, tomato that enable the plants to protect themselves from pathogen attack. Some ERFs, called jasmonic acid-responsive transcription factors (JREs), mediate transcriptional regulation of metabolic genes involved in biosynthesis of cytotoxic steroidal glycoalkaloids (SGA). The latter is said to function in defense responses against biotic stress in some solanaceous plants (Thagun *et al.*, 2016). A recent research suggests ERFs are known to bind to promoters of many jasmonic acids and PR proteins that act as the deciding factors for resistance and susceptibility of plants.They have observed the upregulation of six ERFs that confers resistance to *A. solani* induced early blight in a resistant accession of tomato EC-520,061. ERF-3 was significantly upregulated in EC-520,061 than in the susceptible accession CO-3 (Upadhyay *et al*. 2016).

WRKY gene has an important role in plant defense. Most of the WRKY genes act as positive regulators of plant response in tomato (Aljaafria *et al*. 2017). In case of most plant defense genes, they contain W Boxes situated in promoter region. The WRKY proteins interact with those W Boxes that are mainly found in cluster within promoters and indicates potential interactions of different WRKYs (Shinde *et al.* 2018). Pathogenic elicitors can stimulate the production of WRKY3 and WRKY4, both of which are known to enhance defense against necrotrophic pathogens. Reports on silencing of tomato WRKY33 genes (SIWRKY33A and SIWRKY33B) suggest their similarity to *Arabidopsis* AtWRKY33 which positively regulate defense against necrotrophs *Alternaria brassicicola* and *Botrytis cinerea* (Aamir *et al*. 2017). So, SIWRKY33A and SIWRKY33B may play a role in defense against *A. solani* induced early blight disease too. Recently in a wild relative of tomato, *Solanum arcanum*, it was shown that WRKY1 provides resistance against early blight, making it a possible source of resistance genes in *S. lycopersicum.* It interacts with cytosolic NPR1 in the SA signaling pathway to regulate plant defense

response. Exogenous SA application has proven to enhance resistance against early blight (Shinde *et al.* 2018). In fact, SlWRKY1 was shown to be considerably upregulated in *S. lycopersicum, A. alternata* interaction in resistant cultivars like H.a.s 2274, Esfahan local and Shiraz local variety. Other WRKY TFs like WRKY39, WRKY53 and WRKY70 were also observed to have high levels of expression in resistant cultivars as compared to susceptible cultivars (Moghaddam *et al.*2019).

MYELO BLAST (MYB) transcription factors are also known to participate in plant defense responses against fungal pathogens. One such study reveals the significant upregulation of six MYB TFs in resistant tomato cultivar during its interaction with *A. solani,* four MYB TFs were insignificantly expressed in the resistant variety and none were significantly expressed in the susceptible tomato cultivar (Upadhyay *et al.* 2016).

NAC TFs constitute a large family of transcriptional regulators and have been suggested to play regulatory roles in the transcriptional reprogramming of defense genes in plants. In the model plant *Arabidopsis*, NAC TFs called ATAF1 and ATAF2 are induced by JA signaling and confer resistance to plants during abiotic and biotic stresses (Ruan *et al.*, 2019). Similarly, in *A. solani* infected tomato at 72 hpi, several NAC TFs were markedly upregulated viz., SlNACMTF2 (2.8-fold), SlNACMTF8 (7.8 fold) and SlNACMTF11 (2.3 fold) suggesting their role as potential mediators of pathogen stress response in tomato (Bhattacharjee *et al.* 2017).

Zinc-finger proteins (ZFPs) are a class of DNA-binding transcription factors that possess a zinc-finger domain (Nordberg *et al.* 2015). Two such ZFPs, ZFP 12 and ZF-2 showed greater levels of expression in *A. solani* infected tomato plants at 24 hours post inoculation. The same group of researchers also suggested that MADS box TFs do not play any role in defense against pathogens as they were down regulated in both resistant and susceptible tomato genotypes (Upadhyay *et al.* 2016).

Role of stress hormones and corresponding responsive genes in defense arsenal of tomato

Plants have several mechanisms against biotic stresses to recognize external signals which permit them to develop an optimal response.

Phytohormones like salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) respond through signaling crosstalk to stress (Checker *et al.* 2018). SA and JA mediated signaling are connected in resistance of plants, encouraging plant responses to pathogen infection by making resistance gene expression. SA plays a pivotal role in defense against biotrophic pathogens and JA/ET signal transductions are activated in defense against necrotrophic pathogens (Ruan *et al.* 2019).

In JA/SA crosstalk, MYC2 mainly binds with NAC promoter and their transcription are activated which inhibit Isochorismatesynthase1 (ICS1) expression and triggers Benzoic Acid/ SA Carboxyl Methyltransferase 1(BSMT1). When exogenous SA is present, activation of Non-expressor Of PR Genes 1 (NPR1) induces WRKY70 transcriptional activity that promotes PR1 expression in *Arabidopsis* and defense response are induced. GPX480, a monomer are regulated positively by MPK4 in signaling of SA and in JA signaling MYC2 are regulated negatively that is important for JA responsive gene plant defensin 1.2 (PDF1.2) and THI1.2 (Yang *et al.*2015). It has been observed in a study that biosynthetic genes of JA (AOS and OPR3) and SA (PAL and ICS1) represented contrasting forms which fits with the early blight disease development. In SA biosynthesis and lignification, PAL isoforms plays an important role and high amount of lignin assemblage mainly observed in resistant than susceptible plants on *A. solani* inoculation and high SA levels are also displayed by pathogen treated resistant accessions (Shinde *et al.* 2018).

It has been already mentioned in this review that the SA receptor NPR1 has an important role in SA defense gene activation. It also has a key role in antagonistic talks of JA and SA. SA helps in down regulation of JA. NPR1 mainly bind to SA specifically and dimer form of NPR1 are formed which releases C terminal domain from N terminal. About 30 SA binding proteins (SABs) are identified which show affinity towards SA. The transcription factor WRKY 70 family also has a role in SA and JA antagonistic interactions. Overexpressing line of recombinant WRKY70 represses the JA responsive PDF 1.2. JA responsive gene like lipoxygenase 2 (LOX2), PDF1.2. (Checker *et al.* 2018).

The JA and ET pathways are synergistically activated in plants to defend themselves against

nectrophic pathogen infection. In agreement to this fact, noticeable upregulation of defense genes associated with JA/ET pathways was seen during *A. solani* stress response in tomato. The study identified 106 genes associated with the ET pathway, out of which 76 were upregulated and 34 genes among them were found to participate in ET biosynthesis (1- Aminocyclopropane-1-Carboxylate Synthase or ACS, 1-Aminocyclopropane-1-Carboxylate Oxidase or ACO) and 43 out of 62 genes were associated with ET signal transduction (Ethylene-Responsive transcription Factors, ERFs). As many as 16 genes responsible for JA biosynthesis (Allene Oxide Cyclase or AOC) and lipoxygenase or (LOX) and for JA signal transduction (Jasmonate ZIM domain, JAZ) were also significantly upregulated (Sarkar *et al.* 2017).

In JA/ET crosstalk, JAZs-MYC2, ET insensitive3 (EIN3) and EIN3-like 1 (EIL1) homologue are involved. The JAZ domain family proteins negatively regulate the JA pathway and play an important role in defense against herbivory as well as necrotrophic pathogen attack. JAZ degradation are mainly triggered by JA, so MYC2 gets released and control ORA59/ERF1 and VSP2 (wound responsive gene) thus resisting herbivorous insects. In the ET signaling the transcriptional activity of EIL2/EIN3 are inhibited by JAZ and downstream ORA59/ERF1 are activated which targets PDF1.2 promoter and its expression are induced to resist necrotrophic and hemibiotrophic pathogen infection (Yang *et al.*2015). Recent reports suggest that downregulation of SlJAZ1 in *A. solani* infected tomato leads to activation of JA regulated defense genes through MYC2 TF. The downregulation of SlJAZ1 might cause an increase in the biosynthesis of antifungal glycoalkaloids, hindering the growth of *A. solani* (Panda *et al.* 2019). Upregulation of other JA marker genes PR3, LOXD and JERF3 was noted in another study involving the same pathosystem (Gulzar *et al.* 2021).

Most miRNA mediated defense involves antagonistic expression of cognate mRNAs

Plants always try to protect themselves from phytopathogens. So, in the affected cells, some defense related transcriptional reprogramming is coordinated by plants. For this process, many transcription factors and signaling are the main elements. MicroRNAs or miRNAs regulate some of these gene expressions. miRNAs are single stranded, short, 21-24 nt, non-coding RNAs (Wang *et al.* 2015).miRNAs target mRNAs through complementary base pairing in order to regulate gene expression and during pathogen attack these miRNAs have specific upregulation and downregulation patterns (Luan *et al.* 2015). miRNAs act as important molecules in interaction between plant pathogens. So, specific roles are played by them in case of tomato diseases. The expression of genes which encodes serine/threonine protein kinases, disease resistance proteins and transcription factors are controlled primarily by some identified miRNAs (Upadhyay *et al.* 2016). A plant's reaction towards pathogen can be determined by regulation of set of genes and transcriptional reprogramming.It can be said that a set of mRNAs and miRNAs expression are altered during *Alternaria* tomato interaction. For the observation and study of modification of the expression levels of mRNAs or miRNAs in different species of plants under stress condition, next generation sequencing (NGS) technology has been utilized to acquire complete sequencing data (Jinand Wu, 2015; Tan *et al.*2015; Manzo *et al.*2016).Previous studies provide important information on the role of tomato miRNAs during pathogen stress caused by *B. cinera* and *Phytophthora infestans*. These works show that most miRNAs are downregulated during infection leading to the upregulation of genes associated with basal defense mechanisms (Tsushima *et al.*, 2015; Wang *et al.* 2015). Recent research has also revealed that as many as 102 tomato miRNA- mRNA pairs show opposite regulation in response to *A.solani* stress, i.e., pathogen infection has caused a significant downregulation of miRNAs and a consequential upregulation of cognate mRNAs leading to transcription of defense genes including signaling molecules or sensors like calmodulin binding proteins, transcription factors like MYB, proteins participating in redox reactions etc., which ultimately helps the plant to alleviate the effect of pathogen attack (Sarkar *et al.* 2017).

Participation of a number of protein kinases in tomato provide better resistance by coordinating signal transduction post pathogen attack

It is a well-known fact that protein kinases play a crucial role in signal transduction. In tomato, a

receptor-like cytoplasmic kinase (RLCK) is localized to the plasma membrane which is encoded by Tomato Protein Kinase 1 (TPK1b) (Li *et al*. 2021). TPK1b has been earlier shown to be required for protection against *B. cinerea*. Pathogen infection, oxidative stress etc. promote TPK1b expression and decreasing TPK1b gene expression by RNA silencing enlarges susceptibility to necrotropic fungus. TPK1b expression was higher as early as 4hpi in resistant tomato cultivar, PR, than in the susceptible variety,PED, which took 24 hours to show a distinct response (Ray *et al*. 2015).

Calcium and calmodulin dependent protein kinase (CCaMK) is found in Calcium/ CaM dependent protein kinase superfamily. Tomato CCaMK (SlCCaMK) gene was differentialy and widely responsive to different stimuli of pathogen. By ROS accumulation regulation, SlCCaMK is able to give resistance to tomato plants to different pathogen (Wang *et al*. 2015). Recent study points out the upregulation of 16 calmodulin-like proteins (CAM), calcium-dependent protein kinase (CDPKs), 2 CBL-interacting protein kinase (CIPK) and the downregulation of calcium/proton pumps during *A. solani* stress, indicating the necessity of intracellular calcium accumulation which may act as second messenger in downstream defense gene regulation (Sarkar *et al*. 2017).

MAPK (Mitogen activated protein kinase) cascade, a signaling transduction module have been identified in tomato and other horticultural plants. MAPK cascade has 3 parts- MAPKK, MAPKKK and MAPK. In tomato, 5 MAPKKs, 89 MAPKKKs and 16 MAPKs have been identified. MAPKKs are also divided into 4 groups on the basis of amino acid sequence analysis, among which SlMAPKK1 and SlMAPKK4 are mainly involved in many defense responses against necrotrophic pathogens via ROS generation, defense hormone signaling, HR cell death and activation of defense gene (He *et al*. 2020; Guo *et al*. 2021). SlMKK2 protein regulates immunity associated PCD in plants (Ding *et al.,* 2018). Other than these, a large number of genes encoding transmembrane receptor-like kinases (RLKs), receptor-like proteins (RLPs) and intracellular nucleotide-binding site leucine-rich repeat (NB-LRR) have been reported to be upregulated during early blight caused by *A. solani* (Sarkar *et al.,* 2017).

CONCLUSION AND FUTURE PROSPECT

Early blight disease of tomato plant caused by *Alternaria* sp. is a major concern for agriculturists all over the world. Researches till date have mainly focused on controlling the disease by applying fungicides and biocontrol agents. But the usage of these materials cannot provide a permanent solution to curb the disease outbreak. So, proper understanding of the underlying defense mechanisms of tomato at the molecular level provides useful insights into the host-pathogen interaction during early blight. It could be said that in tomato-*Alternaria* sp. interaction, there are several defense mechanisms which can protect themselves from the pathogen, starting from perception of pathogen signal to activation and regulation of downstream defense genes. In future, this knowledge can be of immense importance in improving tomato lines and help plant breeders to come up with new early blight-resistant cultivars by resistant breeding using QTL analysis and/or genetic engineering.

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