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The role of microRNAs and phytohormones in plant immune system

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Abstract
The plant-pathogen interaction is a multifactor process that may lead to resistance or susceptible responses of plant to pathogens. During the arms race between plant and pathogens, various biochemical, molecular and physiological events are triggered in plant cells such as ROS signaling, hormone activation and gene expression reprogramming. In plants, microRNAs (miRNAs) are key post-transcriptional regulators of gene expression and are involved in several cellular processes including response to environmental stress. In recent years, plant pathologists have presented a logical approach of plant immune system as zigzag based model that includes two phases of immunity, PTI and ETI in which miRNA molecules are determinant regulators. Here, we present an overview of miRNA biology, a brief explanation of plant immune systems in zigzag model, the role of phytohormones and miRNAs in plant immunity with a main focus on *Arabidopsis-Pseudomonas* interactions and finally we discuss our results on miRNA expression in lemon-*Xanthomonas* interactions.
Keywords: Effector-Triggered immunity, miRNAs, PAMP-Triggered immunity, Plant immune systems.

Introduction

Crop plants are often exposed to various environmental stress factors which severely affect crop production (Board and Kahlon, 2011). Plant responses to different stresses are highly complex and involve changes at the transcriptome, cellular, and physiological levels. Through an evolutionary process, plants have evolved specific mechanisms that allow them to detect precise environmental changes and respond to the stress condition, minimizing damage while conserving valuable resources for growth and reproduction. (Atkinson and Urwin, 2012).

Under conditions generated by pathogen attack, host plants must be able to orchestrate adaptive responses according to these circumstances in order to survive (Dodds and Rathjen, 2010). Plant immunity is controlled by a complex signaling network depending on cell-autonomous events. Indeed, plants rely on the innate immunity of each cell and on systemic signals emanating from infection sites (Ausubel, 2005; Dangl and Jones, 2001). Some parts of plant immunity systems may be

established on natural or modified mineral (Hassabi *et al*., 2014a), organic (Hassabi *et al*., 2014b) and biochemical (Hassabi *et al*., 2014c) compositions of plant tissues. The sensing of biotic stress conditions induces signaling cascades that activate ion channels, kinase cascades, production of reactive oxygen species (ROS) and accumulation of hormones such as salicylic acid (SA), ethylene (ET), jasmonic acid (JA) and abscisic acid ABA (Bari and Jones, 2009; Jones and Dangl, 2006). These signals ultimately induce expression of specific subsets of defense genes that lead to the assembly of the overall defense reaction (Jones and Dangl, 2006). In an attempt to reduce the damage of stress and adapt to their environment, plants have evolved multiple gene regulatory mechanisms involving transcriptional, post-transcriptional and post-translational regulation (Hirayama and Shinozaki, 2010).

Small non-coding RNAs (ncRNAs), which consist of 20–24 nucleotides (nt), have been increasingly investigated as important regulators of protein-coding gene expression; these small RNAs function by causing either transcriptional (TGS) or post-transcriptional gene silencing (PTGS) (Baulcombe, 2004). Our understanding of the complexity of plant's responses to stress has been enhanced by the discovery of ncRNA species which play crucial regulatory roles (Ruiz-Ferrer and Voinnet, 2009). MicroRNAs (miRNAs) are a class of ncRNAs that exist in most eukaryotic genomes. Over the past decade, miRNA molecules have emerged as critical post-transcriptional regulators of animal and plant genomes (Bartel, 2004; Carrington and Ambros, 2003). miRNAs are involved in development, signal transduction, protein degradation, response to environmental stress and pathogen invasion, and regulate their own biogenesis (Unver *et al*., 2010; Dugas and Bartel, 2004). Plants miRNAs were initially described in *Arabidopsis thaliana* (Ehrenreich and Purugganan, 2008) and since then, an increasing number of miRNAs has been identified in plants (Jones-Rhoades and Bartel, 2004). The levels of conserved and species-specific miRNAs change in response to different pathogens in plants, providing new avenues for the investigation of plant signalling in biotic stresses (Ruiz-Ferrer and Voinnet, 2009). Recently, authors successfully detected and analyzed three conserved miRNAs (mir159, mir167 and mir398) in *Citrus*×*Limon* (lemon) infected by *Xanthomonas* using stem-loop qRT-PCR (Alizade *et al*., 2014).

This review explains in detail the miRNA biogenesis and function in plants. Subsequently, plant immune system and the role of phytohormones and plant miRNAs in this system will be discussed with a focus on bacteria-responsive miRNAs.

Plant immune systems

Over the last 25 years, researches have led to an increasingly clear conceptual understanding of the molecular components of the plant immune system (Dangl *et al*., 2013). Currently, the evolutionary development of the plant immune system is represented as a zigzag model (Figure 1) (Jones and

Dangl, 2006). In accordance with this model, plant pathologists discriminate two phases of plant immunity: PTI (PAMP-Triggered Immunity) and ETI (Effector-Triggered Immunity).

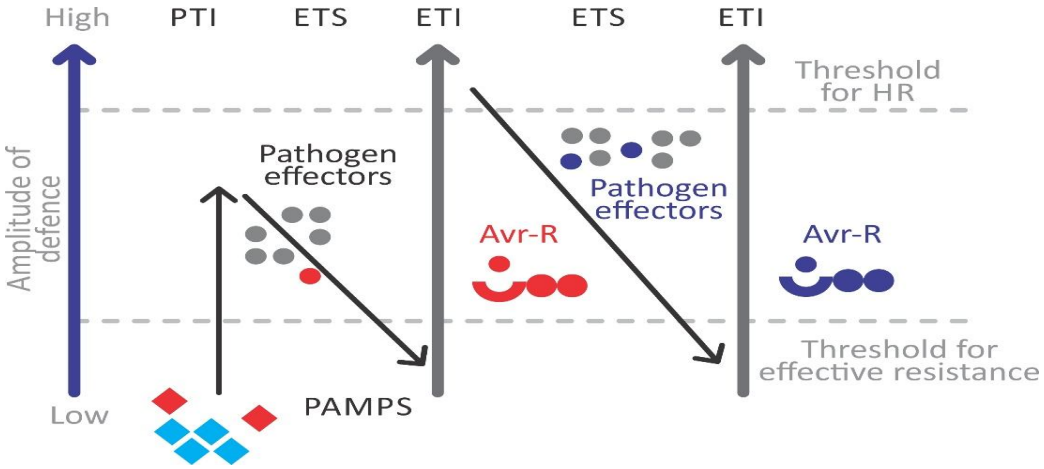

Figure 1. The recommended zigzag model in plant immunity (Jones and Dangl, 2006). In phase 1, plants detect PAMPs (red diamonds) via PRRs to trigger PTI. In phase 2, successful pathogens deliver effectors that interfere with PTI, or otherwise enable pathogen nutrition and dispersal, resulting in effector-triggered susceptibility (ETS). In phase 3, one effector (indicated in red) is recognized by an NB-LRR protein, activating ETI, an amplified version of PTI that often passes a threshold for induction of HR. In phase 4, pathogen isolates are selected that have lost the red effector, and perhaps gained new effectors through horizontal gene flow (in blue)—these can help pathogens to suppress ETI.

PTI is induced where the first level of microbe recognition is performed by membrane proteins termed pattern recognition receptors (PRRs), which perceive molecular signatures characteristic of a whole class of microbes, termed pathogen-associated (or microbe-associated) molecular patterns (MAMPs/PAMPs) (Medzhitov and Janeway., 1997). ETI as a second phase of plant immunity is mediated by intracellular nucleotide-binding leucine-rich repeat receptors (NLR) that recognize the presence or the activity of specific microbial effectors (García and Hirt, 2014). Although PTI and ETI employ distinct immune receptors, they seem to use a similar signaling network (Tsuda *et al.*, 2009) and activate a largely overlapping set of genes (Zipfel *et al.*, 2006; Navarro *et al.*, 2004), with the paradigm that activated immune responses in ETI occur quicker and are more prolonged and more robust than those in PTI (Jones and Dangl, 2006; Tao *et al.*, 2003). ETI amplifies PTI responses and is normally associated with the appearance of localized cell death lesions known as hypersensitive response (HR) (Figure 1) (Heidrich *et al.*, 2012). In plants, HR is defined as a rapid cell death that causes necrosis to restrict the growth of a pathogen (Morel and Dangl, 1997). Following PAMPs perception, a series of downstream defense responses are triggered including ion fluxes, MAPK (mitogen-activated protein kinase) cascade

activation, ROS (ROS) production, defense gene expression, callose (β-1->3 glucose polymer) deposition, stomatal closure, hormone activation and gene silencing (Nicaise *et al*., 2009).

Recent progresses have been made in understanding the complex hormone network that governs plant immunity. Downstream of PTI or ETI activation, diverse plant hormones act as central players in triggering of the plant immune signaling network (Pieterse *et al*., 2009; Bari and Jones, 2009).

The role of phytohormones in plant immunity

The plant hormones ethylene, jasmonic acid and salicylic acid play a central role in the regulation of plant immune responses (Robert-Seilaniantz *et al*., 2011; Vlot *et al*., 2009). In addition, other plant hormones, such as auxins, ABA, cytokinins, gibberellins and brassinosteroids that have been thoroughly described to regulate plant development and growth, have recently emerged as key regulators of plant immunity (Kazan and Manners, 2009; Ton *et al*., 2009).

SA plays a crucial role in plant defense and is generally involved in the activation of defense responses against biotrophic and hemi-biotrophic pathogens as well as the establishment of systemic acquired resistance (SAR) (Grant and Lamb, 2006). HR development is usually accompanied by an increase in SA and an accumulation of defense related proteins such as the pathogenesis related (PR) proteins (Vlot *et al*., 2008). By contrast with SA, JA and ET are involved in resistance to necrotrophic pathogens and herbivorous insects (Beckers and Spoel, 2006). Although SA and JA/ET defense pathways are mutually antagonistic, evidences of synergistic interactions have also been reported (Mur *et al*., 2006; Kunkel and Brooks, 2002).

The phytohormone ABA plays regulatory functions in many aspects of plant growth and development including seed germination, embryo maturation, leaf senescence, stomatal aperture and adaptation to environmental stresses (Wasilewska *et al*., 2008). In general, ABA is shown to be involved in the negative regulation of plant defense against various biotrophic and necrotrophic pathogens (Thaler and Bostock, 2004; Audenaert *et al*., 2002). ABA was shown to attenuate SA-mediated resistance at later infection stages and can also suppress callose deposition in response to PAMPs (De Torres-Zabala *et al*., 2007).

Many biotrophic pathogens could synthesize auxin or auxin-like molecules to promote disease symptoms in many plants (Navarro *et al*., 2006). Treatments with the auxin analogs 2,4-dichlorophenoxyacetic acid (2,4-D) or 1-naphthalacetic acid (NAA) enhance disease symptoms in *Arabidopsis* infected by *Pseudomonas syringae* pv. *tomato* (*Pst*) *DC3000* (Chen *et al*., 2007). Gene expression analysis of sweet orange leaves treated with auxin analogs suggested that auxin affects GA synthesis in citrus as it occurs in numerous plant species (Cernadas and Benedetti, 2009). Auxin transport inhibitor, naphthylphthalamic acid can attenuate canker development of sweet

orange infected by *Xanthomonas citri* pv. *citri*, but NAA can provoke more serious disease symptoms (Cernadas and Benedetti, 2009). As auxin and gibberellin hormones are core signals in cell division and growth, they are suggested to play key roles in contributing to citrus canker symptoms (Cernadas and Benedetti, 2009).

A comprehensive overview of miRNA

History of discovery

miRNAs were first found and characterized in a worm, *Caenorhabditis elegans*; *Lin-4*, a mutant worm which lost many adult structures and developmental plasticity (Lee and Ambors, 2001; Lau *et al*., 2001). It was observed that no protein sequences were encoded by this gene but were transcribed into RNA in wild-type worms (Lee *et al*., 1993). Another worm mutant, *let-7*, followed a similar pattern in gene expression (Moss, 2000). In both cases, the primary transcripts were sliced into smaller RNA fragments and finally into a sRNA with about 21nt in length, which is now known to be a miRNA. Lin-4 or let-7 miRNAs act as negative regulators of gene expression by annealing with their target mRNAs (Moss, 2000), resulting in time-dependent regulation of developmental phase change.

The importance of miRNA in plants was first demonstrated by Palatnik *et al* (2003). They showed that the gene locus responsible for the mutation in *Arabidopsis* mutants (*JAW* mutants), did not encode any protein. The transcript generated from this locus, had the potential to produce a miRNA. They showed that the miRNA was produced in the wild-type plants but not in *jaw* mutants (Palatnik *et al*., 2003). The miRNA partially complemented to mRNA sequence encoding the so-called TCP proteins, which are a class of transcription factors (TCP) (Palatnik *et al*., 2003).

Genomic location of miRNA-encoding genes (MIR genes)

In the recent years, the vast majority of conserved and novel microRNAs have been discovered by small RNA deep sequencing. These technologies are making it quickly possible to identify novel microRNAs as well as they are published and submitted to a database. Among the databases exist for miRNA information, miRBase (www .mirbase .com) is the most valid source for the biological studies. miRBase is the source for miRNA information includes databases of sequences and predicted targets, as well as an official name registry for new miRNA genes. In the *Arabidopsis* plant whose genome has been fully sequenced, over 100 miRNA encoding loci have been identified (Ehrenreich and Purugganan, 2008; Bonnet *et al*., 2004). miRNA-encoding (*MIR*) genes are frequently expressed individually, but many exist in clusters of 2–7 genes with small intervening sequences. Experimental results suggest that they are expressed co-transcriptionally, which indicates that they are under the control of common regulatory sequences (Lee *et al*., 2002; Lau *et al*., 2001). Other miRNA genes are usually located in intergenic regions, some in the introns of known genes, and

even within the expressed sequence tags (ESTs) (Lim *et al*., 2003). In addition, *MIR* genes are excised from the introns and exons of non-coding genes (Rodriguez *et al*., 2004), or even from the 3′-UTR of protein-coding genes (Cai *et al*., 2004). In mammalian genomes, it is also possible to find miRNAs in repetitive regions, and some studies suggest that transposable elements may be involved in the creation of new miRNAs (Smalheiser and Torvik, 2005).

miRNAs Biogenesis

Most characterized eukaryotic *MIR* genes are RNA polymerase II (Pol II) transcription units that generate a primary miRNA transcript called a pri-miRNA, therefore pri-miRNAs can be subjected to elaborate transcriptional control (Lee *et al*., 2004). miRNA biogenesis in animals is a two-step process (Figure 2) (Lee *et al*., 2002). In the first step, pri-miRNAs, which are several hundred nucleotides long, are processed by a nuclear multiprotein complex (Microprocessor) containing an enzyme called Drosha (nuclear RNase III type) into a 70~90nt hairpin long precursor miRNA (pre-miRNA) which is then exported to the cytoplasm (Lee *et al*., 2003). This cleavage event is important because it predetermines mature miRNA sequence and generates optimal substrate for the subsequent events (Lund *et al*., 2004; Lee *et al*., 2003). The nuclear export is elicited by a complex of Exportin 5 (Exp5) and Ran-GTP which selectively bind pre-miRNAs and protect them from exonucleolytic digestion (Lund *et al*., 2004). In the cytoplasm, the second step takes place where the pre-miRNA is cleaved by cytoplasmic RNase III Dicer into ~22nt miRNA duplex (miRNA: miRNA∗ duplex), with each strand originating from opposite arms of the stem–loop (Hutvágner and Zamore, 2002). The duplex strand with the weakest 5′ end base pairing is then selected as the mature miRNA and the remaining strand, called miRNA*, is degraded (Tomari *et al*., 2004). In general, the miRNA strand is then integrated in a ribonucleoprotein complex known as the (mi)RNA-induced silencing complex (miRISC or RISC) or miRNA-containing ribonucleoprotein particles (miRNPs) (Lau *et al*., 2001).

miRNA biogenesis in plants differs from animal biogenesis mainly in the steps of nuclear processing and export (Figure 2) (Millar and Waterhouse, 2005). All maturation steps of plant miRNAs are processed by Dicer-like proteins (Jones-Rhoades *et al*., 2006). In plants, miRNAs seem to be fully matured into a single stranded miRNA before being exported to the cytoplasm by a homologue of Exp5 termed HASTY (HST) and integrated into the silencing complex (Park *et al*., 2005; Bartel, 2004). The enzymes for miRNA biogenesis are under feedback regulation by miRNAs (Jones-Rhoades *et al*., 2006) and this feedback regulatory mechanism is deeply conserved among diverse plant species (Xie *et al*., 2010).

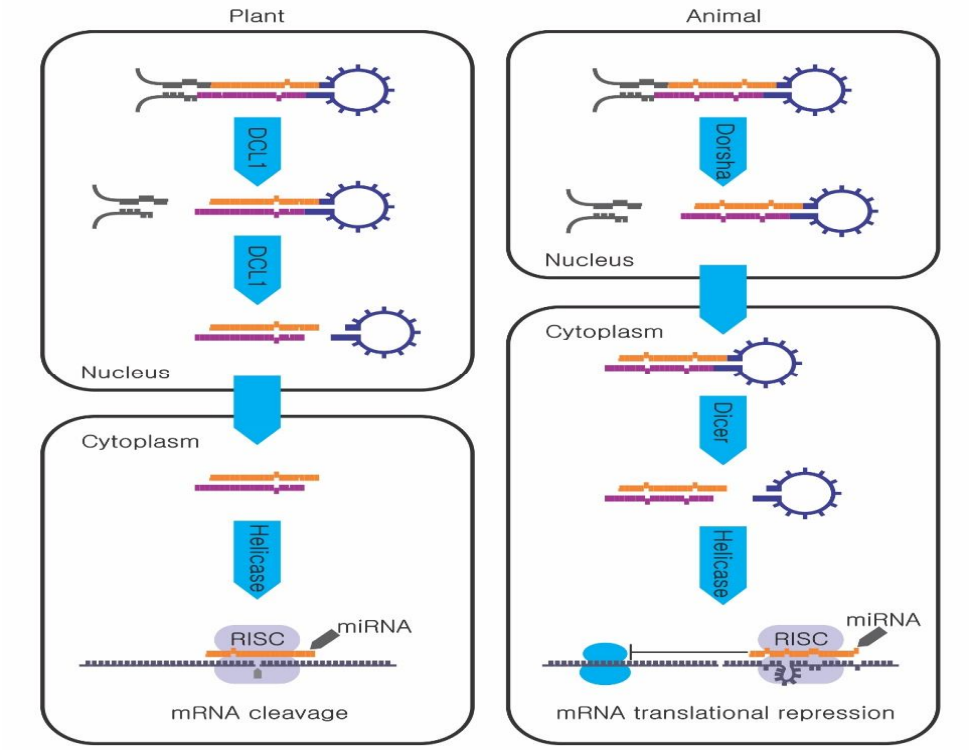

Figure 2. A comparative view of miRNA biogenesis and action in plant and animal. The Drosha gene that is responsible for processing of pri-miRNA to pre-miRNA in animals is absent from plant genomes; this function is performed by the plant Dicer-like 1 (DCL1). In animals, miRNA/miRNA* duplex is formed in the cytoplasm by Dicer endonucleolytic activity. In contrast, all maturation steps of plant miRNAs occur in the nucleus. Depending on the level of miRNA-mRNA complementarity, miRNA in animals acts as translational repressor whereas plant miRNA is considered for its mRNA decay activity.

miRNAs Function

In an Overall view, miRNAs regulate gene expression by inhibiting mRNA translation and/or facilitating mRNA degradation (Voinnet, 2009). Post-transcriptional control of gene silencing by miRNAs is a ribonucleoprotein-driven process, which involves specific RNA binding proteins, miRNAs and their mRNA targets (Cava *et al*., 2014). To this end, mature miRNA assembles into RISC, activating the complex to target mRNA specified by the miRNA (Pratt and MacRae, 2009). Members of the Argonaute (AGO) protein family are central to RISC function (Pratt and MacRae, 2009). A key component in the miRNA pathway is AGO1, which predominately binds mature miRNAs to cleave the target mRNA or represses translation depending on the level of miRNA-mRNA complementarity (Okamura *et al*., 2004). AGOs contain four characteristic domains: the N-terminal domain; the PAZ domain, which binds the 2nt overhang of the 3′ end of the mature miRNA; the MID domain, which provides a binding pocket for the 5′ phosphate of mature miRNAs; the PIWI domain, which adopts an RNase H fold and has endonucleolytic activity in

some, but not all, AGOs (Ma *et al*., 2005; Parker *et al*., 2005).

miRNAs and their targets seem to constitute remarkably complex regulatory networks since a single miRNA can bind to and regulate many different mRNA targets and, conversely, several different miRNAs can bind to and cooperatively control a single mRNA target (Lewis *et al*., 2003). In animals, miRNAs are considered to act mainly as translational repressors by their partially complementary binding to specific 3′-UTR regulatory elements on target mRNAs (Lai, 2002), although target sites in the coding region and 5′-UTR can also be functional (Lytle *et al*., 2007; Kloosterman *et al*., 2004). On the other hand, plant miRNAs frequently cleave and thus induce immediate degradation of the target mRNAs and are often almost perfectly complementary to sites in the coding region (Ehrenreich and Purugganan, 2008), as well as in the 3′-UTR (Sunkar and Zhu, 2004), and even in the 5′-UTR (Millar and Waterhouse, 2005). The functions of plant miRNAs are highly diverse and have essential roles in regulating plant growth, organogenesis, pattern formation, organ polarity, and hormone homeostasis (Voinnet, 2009).

miRNAs involvement in plant immunity

In dealing with pathogens, host plants can establish defense responses against pathogens which involve rapid changes in gene expression, hormone and metabolite levels (Sunkar *et al*., 2012). Plant small RNAs have been demonstrated as critical regulators in gene expression reprogramming during both PTI and ETI establishment (Padmanabhan *et al*., 2009; Voinnet, 2008). In *Arabidopsis*, the first reported miRNA contributing to antibacterial resistance was miR393 which plays a role in PTI response by regulating the auxin signaling pathway (Navarro *et al*., 2006). It has been shown that bacterial PAMP flg22 rapidly induces the miR393 expression which targets receptors of auxin (AFBs receptors) (Figure 3) (Navarro *et al*., 2006; Jones-Rhoades and Bartel, 2004). Perception of auxin by AFBs leads the degradation of the AUX/IAA protein, and subsequently activates auxin response genes by derepressing the auxin-response factor (ARF) transcription factors (Figure 3) (Chapman and Estelle, 2009). Fahlgren *et al*., (2007) reported that miR393 can be significantly induced at 3h post-inoculation (hpi) by nonpathogenic *Pst* DC3000 *hrcC*, a strain responsible for the induction of initiate immunity. In addition, miR160 and miR167 up-regulated by *Pst* DC3000 *hrcC* at 3-hpi rather than mir393 (Rhoades *et al*. 2002). mir160 and mir167 target the members of Auxin-responsive factor (ARF) family that are involved in auxin signaling pathway (Figure 3) (Li Y *et al*., 2010). Thus, three bacteria-responsive miRNAs (mir160, mir167 and mir393) suppress the auxin signaling and contribute to the PTI in plants. Auxin is a plant hormone which has growth-promoting role and is antagonistic to SA-mediated resistance (Wang *et al*., 2007). Upon perceiving the pathogen PAMPs, these miRNAs are induced to rapidly repress the auxin signaling and

shift the energy from plant growth to defense responses.

In *Arabidopsis-pseudomonas* interaction model, some miRNAs are induced which target negative defense response regulators and a group of miRNAs targeting positive regulators (e.g. resistance genes) are repressed upon bacterial infection (Ruiz-Ferrer and Voinnet, 2009). miR398 is down-regulated in response to avirulent strains of *Pst* DC3000 (*avrRpm1*) or *Pst* DC3000 (*avrRpt2*) at 12-hpi and continued until 24-hpi (Jagadeeswaran *et al*., 2009). The targets of miR398 are Cu/Zn superoxide dismutases 1 and 2 (CSD_1 and CSD_2) (Figure 3) (Bonnet *et al*., 2004). These enzymes decrease superoxide (as a form of ROS) levels by converting it to H_2O_2 and O_2 (Figure 3) (Draper, 1997). It has been found that miR398 negatively regulated PAMP induced callose deposition (Li *et al*., 2010).

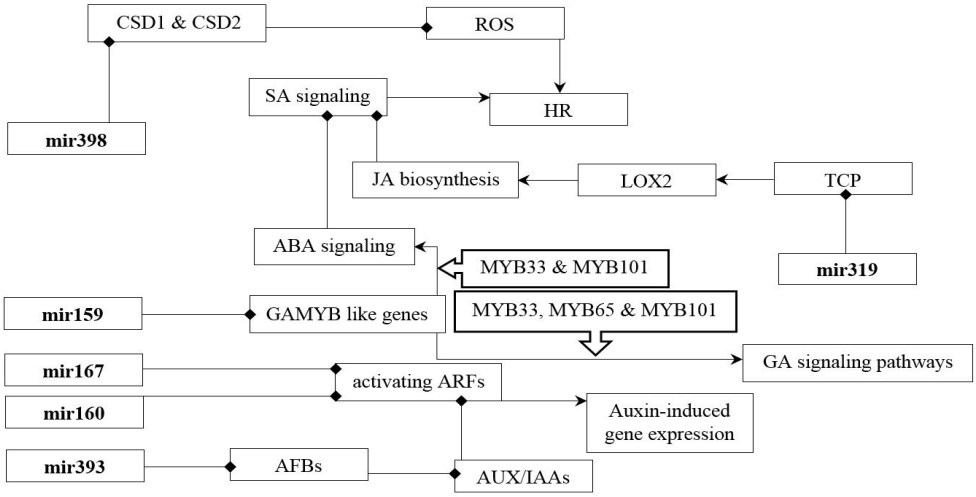

Figure 3. The regulatory role of responsive miRNAs in Arabidopsis-pseudomonas interaction. Arrows indicate positive regulations and diamond arrows indicate inhibitions. It has been shown that microRNAs inhibit protein production of their target genes and consequently lead plant biosystem toward regulation of hormone signaling and HR.

The repertoire of known bacterial-responsive miRNAs has increased and includes several families, such as miR159 involved in ABA signaling and miR319 (Zhang *et al*., 2011; Fahlgren *et al*., 2007). miR159 is down-regulated by *Pst* DC3000 (*EV*) and *Pst* DC3000 (*avrRpt2*) at 6-hpi, but up-regulated by *Pst* DC3000 (*avrRpt2*) at 14-hpi (Zhang *et al*., 2011). miR159 targets transcription factors MYB33, MYB65 and MYB101, the homologous genes of the barley GAMYB that activates Gibberellin (GA)-signaling pathways (Figure 3) (Reyes and Chua, 2007; Millar and Gubler, 2005). MYB33 and MYB101 act as positive regulators of ABA signaling pathways in *Arabidopsis* (Figure 3) (Reyes and Chua, 2007).

Northern blot analysis showed that mir319 is induced by *Pst* DC3000 *hrcC* and *Pst* DC3000 (*avrRpt2*) at 14-hpi (Zhang *et al*., 2011). miR319 targets TCP (*TEOSINTE BRANCHED/ CYCLOIDEA/PCF*) transcription factor family genes which directly regulate *LIPOXYGENASE2* (*LOX2*) (Figure 3) (Schommer *et al*., 2008). *LOX2* encodes a chloroplast-localized enzyme that is responsible for the first step in the JA biosynthesis pathway. JA signaling is usually antagonistic to SA signaling (Overmyer *et al*., 2003), while SA signaling is important for plant defense against biotrophic pathogens, including *Pst.*

A case study on miRNA time-dependent expression in lemon-*Xanthomonas* interaction

Xanthomonas citri subsp. *citri* strain A (Xc) with a broad host range is causal agent of citrus canker disease and is considered as one of the most devastating biotic stresses affecting the citrus industry (Brunings and Gabriel, 2003). Citrus canker is characterized by pustule-like lesions that raise on both surfaces of the leaf and which later become corky and surrounded by a watersoaked margin with a yellow halo (Schubert *et al*., 2001). Canker lesions can also develop on stems and fruits (Schubert *et al*., 2001) and are thought to be the result of intense cell division (hyperplasia) and expansion (hyper-trophy) that occurs in the host tissues after pathogen infection (Brunings and Gabriel, 2003). *Xanthomonas fuscans* subsp. *aurantifolii* strain C (XaC) has a narrower range of citrus hosts which are restricted to some citrus-producing areas in South America (Schubert *et al*., 2001). In addition, XaC induces HR in various citrus species including *Citrus* × *Limon* (lemon) (Brunings and Gabriel, 2003).

Expression analysis of conserved miRNAs including mir159 involved in gibberellin and ABA signaling, mir167 involved in auxin signaling and mir398 involved in detoxification of ROS demonstrates a time-dependent expression regulation during seven hours (0.5, 3, 6, 12, 24, 48 and 72) after lemon leaves infection by Xc and XaC (Alizadeh *et al*., 2014). It seems that the expression patterns of the miRNAs follow a rather zigzag model in lemon-*Xhanthomonas* interaction. According to the results, all three miRNAs are significantly induced at 6-hpi (Table 1). mir159 and mir167 gene expression follow a similar pattern upon both strains infection. After induction at 6-hpi, the high levels of mir159 and mir167 expression are reduced upon Xc infection whereas abundance of transcripts maintained at high levels in response to XaC.

The expression patterns in response to Xc suggest that mir159 and mir167 may contribute to inhibition of disease development through their down-regulatory roles in gibberellin and auxin signaling, respectively. Upon XaC infection, the expression patterns of mir159 and mir167 suggest probable roles in HR induction for both miRNAs. On the other hand, a stable level is observed after 6-hpi induction for mir398 gene expression in response to both strains. Opposite regulation patterns of mir398 gene expression in this study

compared to previous studies mention different strategy in mir398 regulation in various plant-pathogen interaction systems. The study eventually concludes that mir159 and mir167 can be investigated in future as major nods in lemon gene regulation network in order to develop the resistance to citrus canker and also proposes 6-hpi as a critical time for future studies to develop a model of gene expression regulatory network in lemon-*Xanthomonas* interaction.

Table 1. The fold change (FC) values of selected miRNAs after three post inoculation times in lemon-*Xanthomonas* strains interaction. The FC value was reported as Log_2 (Ratio). The Ratio was calculated using efficiency-based mathematical model (Pfaffl, 2001).

Strain	Xc			XaC		
Time (h)	6	12	48	6	12	48
mir159	6.985327	-3.44701	-0.27441	1.819573	2.463257	3.065113
mir167	5.628179	-0.18154	-0.26342	1.460915	1.94406	1.965262
mir398	3.183962	-1.71882	1.546557	2.724107	2.803568	0.266999

Future perspectives

In recent years, Identification and characterization of plant miRNAs and their targets in biotic stresses have demonstrated the importance of small RNAs machinery in plant immunity. miRNAs have central roles in gene expression reprogramming and balancing the host immune responses and fitness costs during host-microbial interaction. Despite the many experimental methods and computational approaches developed in order to solve the mystery of miRNAs involved networks, there is a need for a global and comprehensive understanding of the functions of miRNAs to provide adequate insights for conferring plant resistance to pathogens.

References

Alizadeh, M., Askari, H., Najafabadi, M.S., Rajaei, E. 2014. Time-dependent regulation of conserved putative micro-RNAs in response of *Citrus* × *Limon* to *Xanthomonas citri* subsp. *citri* and *Xanthomonas fuscans* subsp. *aurantifolii*. *Mol. Biol. Rep.*, in press.

Atkinson, N.J. and Urwin, P.E. 2012. The interaction of plant biotic and abiotic stresses: from genes to the field. *J. Exp. Bot*, 63:3523-3543.

Audenaert, K., De Meyer, G.B. and Höfte, M.M. 2002. Abscisic acid determines basal susceptibility of tomato to *Botrytis cinerea* and suppresses salicylic acid-dependent signaling mechanisms. *Plant Physiol*, 128:491-501.

Ausubel, F.M. 2005. Are innate immune signaling pathways in plants and animals conserved? *Nat. Immunol*, 6:973-979.

Bari, R. and Jones, J.D.G. 2009. Role of plant hormones in plant defence responses. *Plant Mol. Biol*, 69:473-488.

Bartel, D.P. 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*, 116:281-297.

Baulcombe, D. 2004. RNA silencing in

plants. *Nature*, 431:356-363.

Beckers, G.J.M. and Spoel, S.H. 2006. Fine-Tuning Plant Defence Signalling: Salicylate versus Jasmonate. *Plant Biol*, 8:1-10.

Board, J.E. and Kahlon, C.S. 2011. Soybean yield formation: what controls it and how it can be improved. *Soybean physiology and biochemistry. Intech Publ. InTech Open Access, Rijeka, Croatia*, 1-36.

Bonnet, E., Wuyts, J., Rouzé, P. and Van de Peer, Y. 2004. Detection of 91 potential conserved plant microRNAs in *Arabidopsis thaliana* and *Oryza sativa* identifies important target genes. *Proc. Natl. Acad. Sci. U. S. A*, 101:11511-11516.

Brodersen, P., Malinovsky, F.G., Hématy, K., Newman, M.A. and Mundy, J. 2005. The role of salicylic acid in the induction of cell death in Arabidopsis *acd11*. *Plant Physiol*, 138:1037-1045.

Brunings, A.M. and Gabriel, D.W. 2003. *Xanthomonas citri*: breaking the surface. *Mol. Plant Pathol*, 4:141-157.

Cai, X., Hagedorn, C.H. and Cullen, B.R. 2004. Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. *RNA*, 10:1957-1966.

Carrington, J.C. and Ambros, V. 2003. Role of microRNAs in plant and animal development. *Science*, 301:336-338.

Cava, C., Bertoli, G., Ripamonti, M., Mauri, G., Zoppis, I., Della Rosa, P.A., Gilardi, M.C. and Castiglioni, I. 2014. Integration of mRNA Expression Profile, Copy Number Alterations, and microRNA Expression Levels in Breast Cancer to Improve Grade Definition. *PloS One*, 9:e97681.

Cernadas, R.A. and Benedetti, C.E. 2009. Role of auxin and gibberellin in citrus canker development and in the transcriptional control of cell-wall remodeling genes modulated by *Xanthomonas axonopodis* pv. *citri*. *Plant Sci*, 177:190-195.

Chandra-Shekara, A.C., Gupte, M., Navarre, D., Raina, S., Raina, R., Klessig, D. and Kachroo, P. 2006. Light-dependent hypersensitive response and resistance signaling against Turnip Crinkle Virus in *Arabidopsis*. *Plant J*, 45:320-334.

Chapman, E.J. and Estelle, M. 2009. Mechanism of auxin-regulated gene expression in plants. *Annu. Rev. Genet*, 43:265-285.

Chen, Z., Agnew, J.L., Cohen, J.D., He, P., Shan, L., Sheen, J. and Kunkel, B.N. 2007. *Pseudomonas syringae* type III effector AvrRpt2 alters *Arabidopsis thaliana* auxin physiology. *Proc. Natl. Acad. Sci. U. S. A*, 104:20131-20136.

Chisholm, S.T., Coaker, G., Day, B. and Staskawicz, B.J. 2006. Host-microbe interactions: shaping the evolution of the plant immune response. *Cell*, 124:803-814.

Dangl, J.L., Horvath, D.M. and Staskawicz, B.J. 2013. Pivoting the plant immune system from dissection to deployment. *Science*, 341:746-751.

Dangl, J.L. and Jones, J.D.G. 2001. Plant pathogens and integrated defence responses to infection. *Nature*, 411:826-833.

De Torres-Zabala, M., Truman, W., Bennett, M.H., Lafforgue, G., Mansfield, J.W., Rodriguez Egea, P., Bögre, L. and Grant, M. 2007. *Pseudomonas syringae* pv. *tomato* hijacks the *Arabidopsis* abscisic acid signalling pathway to cause disease. *EMBO J*, 26:1434-1443.

Delledonne, M., Xia, Y., Dixon, R.A. and Lamb, C. 1998. Nitric oxide functions as a signal in plant disease resistance. *Nature*, 394:585-588.

Depuydt, S. and Hardtke, C.S. 2011. Hormone signalling crosstalk in plant growth regulation. *Curr. Biol*, 21:365-373.

Dodds, P.N. and Rathjen, J.P. 2010. Plant immunity: towards an integrated view of plant-pathogen interactions. *Nat. Rev. Genet*, 11:539-548.

Draper, J. 1997. Salicylate, superoxide synthesis and cell suicide in plant defence. *Trends Plant Sci*, 2:162-165.

Dugas, D.V. and Bartel, B. 2004. MicroRNA regulation of gene expression in plants. *Curr. Opin. Plant Biol*, 7:512-520.

Ehrenreich, I.M. and Purugganan, M. 2008. MicroRNAs in plants. *Plant Signaling Behav,* 3:829-830.

Fahlgren, N., Howell, M.D., Kasschau, K.D., Chapman, E.J., Sullivan, C.M., Cumbie, J.S., Givan, S.A., Law, T.F., Grant, S.R. and Dangl, J.L. 2007. High-throughput sequencing of *Arabidopsis* microRNAs: evidence for frequent birth and death of *MIRNA* genes. *PloS One*, 2:e219.

García, A.V. and Hirt, H. 2014. *Salmonella enterica* induces and subverts the plant immune system. *Front. Microbiol,* 5:141.

Grant, M. and Lamb, C. 2006. Systemic immunity. *Curr. Opin. Plant Biol*, 9:414-420.

Hasabi, V., Askari, H., Alavi, S.M., Goodarzi, T., Najafabadi, M.S., Zamanizadeh, H. 2014a. Inhibitory impact of plant nutritional compounds on Xanthomonas citri subsp. citri, the causal agent of bacterial canker of citrus. *J. Plant Pathol.*, 96:369-375.

Hasabi, V., Askari, H., Alavi, S.M., Najafabadi, M.S. 2014b. In Vitro and In Vivo Antibacterial Activity of Some Organic and Inorganic Salts against Asiatic Citrus Canker Agent *Xanthomonas Citri* Subsp. *Citri*. *Turkish. J Sci. Food Agr*, 2:296-300.

Hasabi, V., Askari, H., Alavi, S.M., Zamanizadeh, H. 2014c. Effect of amino acid application on induced resistance against citrus canker disease in lime plants. *J. Plant Prot. Res.*, 54:144-149.

Heidrich, K., Blanvillain-Baufumé, S. and Parker, J.E. 2012. Molecular and spatial constraints on NB-LRR receptor signaling. *Curr. Opin. Plant Biol*, 15:385-391.

Hirayama, T. and Shinozaki, K. 2010. Research on plant abiotic stress responses in the post-genome era: past, present and future. *Plant J*, 61:1041-1052.

Hutvágner, G.r. and Zamore, P.D. 2002. A microRNA in a multiple-turnover RNAi enzyme complex. *Science*, 297:2056-2060.

Jagadeeswaran, G., Saini, A. and Sunkar, R. 2009. Biotic and abiotic stress down-regulate miR398 expression in *Arabidopsis*. *Planta*, 229:1009-1014.

Jiang, C.J., Shimono, M., Sugano, S., Kojima, M., Yazawa, K., Yoshida, R., Inoue, H., Hayashi, N., Sakakibara, H. and Takatsuji, H. 2010. Abscisic acid interacts antagonistically with salicylic acid signaling pathway in rice-*Magnaporthe grisea* interaction. *Mol. Plant-Microbe Interact*, 23:791-798.

Jones, J.D.G. and Dangl, J.L. 2006. The plant immune system. *Nature*, 444:323-329.

Jones-Rhoades, M.W. and Bartel, D.P. 2004. Computational identification of plant microRNAs and their targets, including a stress-induced miRNA. *Mol Cell*, 14:787-799.

Jones-Rhoades, M.W., Bartel, D.P. and Bartel, B. 2006. MicroRNAs and their regulatory roles in plants. *Annu. Rev. Plant Biol,* 57:19-53.

Kazan, K. and Manners, J.M. 2009. Linking development to defense: auxin in plant-pathogen interactions. *Trends Plant Sci*, 14:373-382.

Kloosterman, W.P., Wienholds, E., Ketting, R.F. and Plasterk, R.H.A. 2004. Substrate requirements for *let-7* function in the developing zebrafish embryo. *Nucleic*

Acids Res, 32:6284-6291.

Kunkel, B.N. and Brooks, D.M. 2002. Cross talk between signaling pathways in pathogen defense. *Curr. Opin. Plant Biol*, 5:325-331.

Lai, E.C. 2002. Micro RNAs are complementary to 3' UTR sequence motifs that mediate negative post-transcriptional regulation. *Nat. Genet*, 30:363.

Lau, N.C., Lim, L.P., Weinstein, E.G. and Bartel, D.P. 2001. An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science*, 294:858-862.

Lee, R.C. and Ambros, V. 2001. An extensive class of small RNAs in *Caenorhabditis elegans*. *Science*, 294:862-864.

Lee, R.C., Feinbaum, R.L. and Ambros, V. 1993. The C. elegans heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell*, 75:843-854.

Lee, Y., Ahn, C., Han, J., Choi, H., Kim, J., Yim, J., Lee, J., Provost, P., Rådmark, O. and Kim, S. 2003. The nuclear RNase III Drosha initiates microRNA processing. *Nature*, 425:415-419.

Lee, Y., Jeon, K., Lee, J.T., Kim, S. and Kim, V.N. 2002. MicroRNA maturation: stepwise processing and subcellular localization. *EMBO J*, 21:4663-4670.

Lee, Y., Kim, M., Han, J., Yeom, K.H., Lee, S., Baek, S.H. and Kim, V.N. 2004. MicroRNA genes are transcribed by RNA polymerase II. *EMBO J*, 23:4051-4060.

Lewis, B.P., Shih, I.h., Jones-Rhoades, M.W., Bartel, D.P. and Burge, C.B. 2003. Prediction of mammalian microRNA targets. *Cell*, 115:787-798.

Li, Y., Zhang, Q., Zhang, J., Wu, L., Qi, Y. and Zhou, J.M. 2010. Identification of microRNAs involved in pathogen-associated molecular pattern-triggered plant innate immunity. *Plant Physiol*, 152:2222-2231.

Lim, L.P., Lau, N.C., Weinstein, E.G., Abdelhakim, A., Yekta, S., Rhoades, M.W., Burge, C.B. and Bartel, D.P. 2003. The microRNAs of *Caenorhabditis elegans*. *Genes Dev*, 17:991-1008.

Lund, E., Güttinger, S., Calado, A., Dahlberg, J.E. and Kutay, U. 2004. Nuclear export of microRNA precursors. *Science*, 303:95-98.

Lytle, J.R., Yario, T.A. and Steitz, J.A. 2007. Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5' UTR as in the 3' UTR. *Proc. Natl. Acad. Sci. U. S. A*, 104:9667-9672.

Ma, J.B., Yuan, Y.R., Meister, G., Pei, Y., Tuschl, T. and Patel, D.J. 2005. Structural basis for 5'-end-specific recognition of guide RNA by the *A. fulgidus* Piwi protein. *Nature*, 434:666-670.

Medzhitov, R. and Janeway, C.A. 1997. Innate immunity: the virtues of a nonclonal system of recognition. *Cell*, 91:295-298.

Millar, A.A. and Gubler, F. 2005. The *Arabidopsis GAMYB-like* Genes, *MYB33* and *MYB65*, are microRNA-regulated genes that redundantly facilitate anther development. *Plant Cell*, 17:705-721.

Millar, A.A. and Waterhouse, P.M. 2005. Plant and animal microRNAs: similarities and differences. *Funct. Integr. Genomics*, 5:129-135.

Moss, E.G. 2000. Non-coding RNAs: lightning strikes twice. *Curr. Biol*, 10:436-439.

Morel, J.B. and Dangl, J.L. 1997. The hypersensitive response and the induction of cell death in plants. *Cell Death Differ*, 4:671-683.

Mur, L.A.J., Kenton, P., Atzorn, R., Miersch, O. and Wasternack, C. 2006. The outcomes of concentration-specific interactions between salicylate and

jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. *Plant Physiol*, 140:249-262.

Navarro, L., Dunoyer, P., Jay, F., Arnold, B., Dharmasiri, N., Estelle, M., Voinnet, O. and Jones, J.D.G. 2006. A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. *Science*, 312:436-439.

Navarro, L., Zipfel, C., Rowland, O., Keller, I., Robatzek, S., Boller, T. and Jones, J.D.G. 2004. The transcriptional innate immune response to flg22. Interplay and overlap with Avr gene-dependent defense responses and bacterial pathogenesis. *Plant physiol*, 135:1113-1128.

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*, 150:1638-1647.

Okamura, K., Ishizuka, A., Siomi, H. and Siomi, M.C. 2004. Distinct roles for Argonaute proteins in small RNA-directed RNA cleavage pathways. *Genes Dev*, 18:1655-1666.

Overmyer, K., Brosché, M. and Kangasjärvi, J. 2003. Reactive oxygen species and hormonal control of cell death. *Trends Plant Sci*, 8:335-342.

Padmanabhan, C., Zhang, X. and Jin, H. 2009. Host small RNAs are big contributors to plant innate immunity. *Curr. Opin. Plant Biol*, 12:465-472.

Palatnik, J.F., Allen, E., Wu, X., Schommer, C., Schwab, R., Carrington, J.C. and Weigel, D. 2003. Control of leaf morphogenesis by microRNAs. *Nature*, 425:257-263.

Park, M.Y., Wu, G., Gonzalez-Sulser, A., Vaucheret, H. and Poethig, R.S. 2005. Nuclear processing and export of microRNAs in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A*, 102:3691-3696.

Parker, J.S., Roe, S.M. and Barford, D. 2005. Structural insights into mRNA recognition from a PIWI domain-siRNA guide complex. *Nature*, 434:663-666.

Pfaffl, M.W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res*, 29: e45.

Pieterse, C.M.J., Leon-Reyes, A., Van der Ent, S. and Van Wees, S.C.M. 2009. Networking by small-molecule hormones in plant immunity. *Nat. Chem. Biol*, 5:308-316.

Pratt, A.J. and MacRae, I.J. 2009. The RNA-induced silencing complex: a versatile gene-silencing machine. *J. Biol. Chem*, 284:17897-17901.

Reyes, J.L. and Chua, N.H. 2007. ABA induction of miR159 controls transcript levels of two MYB factors during Arabidopsis seed germination. *Plant J*, 49:592-606.

Rhoades, M.W., Reinhart, B.J., Lim, L.P., Burge, C.B., Bartel, B. and Bartel, D.P. 2002. Prediction of plant microRNA targets. *Cell*, 110:513-520.

Robert-Seilaniantz, A., Grant, M. and Jones, J.D.G. 2011. Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. *Annu. Rev. Phytopathol*, 49:317-343.

Rodriguez, A., Griffiths-Jones, S., Ashurst, J.L. and Bradley, A. 2004. Identification of mammalian microRNA host genes and transcription units. *Genome Res*, 14:1902-1910.

Ruiz-Ferrer, V. and Voinnet, O. 2009. Roles of plant small RNAs in biotic stress responses. *Annu. Rev. Plant Biol*, 60:485-510.

Schommer, C., Palatnik, J.F., Aggarwal, P., Chételat, A., Cubas, P., Farmer, E.E., Nath, U. and Weigel, D. 2008. Control of jasmonate biosynthesis and senescence by miR319 targets. *PLoS Biol*, 6:e230.

Schubert, T.S., Rizvi, S.A., Sun, X., Gottwald, T.R., Graham, J.H. and Dixon,

W.N. 2001. Meeting the challenge of eradicating citrus canker in Florida-Again. *Plant Dis*, 85:340-356.

Smalheiser, N.R. and Torvik, V.I. 2005. Mammalian microRNAs derived from genomic repeats. *Trends Genet*, 21:322-326.

Sunkar, R., Li, Y.F. and Jagadeeswaran, G. 2012. Functions of microRNAs in plant stress responses. *Trends Plant Sci*, 17:196-203.

Sunkar, R. and Zhu, J.K. 2004. Novel and stress-regulated microRNAs and other small RNAs from *Arabidopsis*. *Plant Cell*, 16:2001-2019.

Tao, Y., Xie, Z., Chen, W., Glazebrook, J., Chang, H.S., Han, B., Zhu, T., Zou, G. and Katagiri, F. 2003. Quantitative nature of Arabidopsis responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. *Plant Cell*, 15:317-330.

Thaler, J.S. and Bostock, R.M. 2004. Interactions between abscisic-acid-mediated responses and plant resistance to pathogens and insects. *Ecology*, 85:48-58.

Tomari, Y., Matranga, C., Haley, B., Martinez, N. and Zamore, P.D. 2004. A protein sensor for siRNA asymmetry. *Science*, 306:1377-1380.

Ton, J., Flors, V. and Mauch-Mani, B. 2009. The multifaceted role of ABA in disease resistance. *Trends Plant Sci*, 14:310-317.

Tsuda, K., Sato, M., Stoddard, T., Glazebrook, J. and Katagiri, F. 2009. Network properties of robust immunity in plants. *PLoS Genet*, 5:e1000772.

Unver, T., Bakar, M., Shearman, R.C. and Budak, H. 2010. Genome-wide profiling and analysis of *Festuca arundinacea* miRNAs and transcriptomes in response to foliar glyphosate application. *Mol. Genet. Genomics*, 283:397-413.

Vlot, A.C., Dempsey, D.A. and Klessig, D.F. 2009. Salicylic acid, a multifaceted hormone to combat disease. *Annu. Rev. Phytopathol*, 47:177-206.

Vlot, A.C., Klessig, D.F. and Park, S.W. 2008. Systemic acquired resistance: the elusive signal (s). *Curr. Opin. Plant Biol*, 11:436-442.

Voinnet, O. 2008. Post-transcriptional RNA silencing in plant-microbe interactions: a touch of robustness and versatility. *Curr. Opin. Plant Biol*, 11:464-470.

Voinnet, O. 2009. Origin, biogenesis, and activity of plant microRNAs. *Cell*, 136:669-687.

Wang, D., Pajerowska-Mukhtar, K., Culler, A.H. and Dong, X. 2007. Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. *Curr. Biol*, 17:1784-1790.

Wasilewska, A., Vlad, F., Sirichandra, C., Redko, Y., Jammes, F., Valon, C., Frei dit Frey, N. and Leung, J. 2008. An update on abscisic acid signaling in plants and more... *Mol. Plant*, 1:198-217.

Xie, Z., Khanna, K. and Ruan, S. 2010. Expression of microRNAs and its regulation in plants. *Semin Cell Dev Biol*, 21:790-797.

Zeier, J., Delledonne, M., Mishina, T., Severi, E., Sonoda, M., Lamb, C. 2004. Genetic elucidation of nitric oxide signaling in incompatible plant-pathogen interactions. *Plant Physiol*, 136:2875-2886.

Zhang, W., Gao, S., Zhou, X., Chellappan, P., Chen, Z., Zhou, X., Zhang, X., Fromuth, N., Coutino, G. and Coffey, M. 2011. Bacteria-responsive microRNAs regulate plant innate immunity by modulating plant hormone networks. *Plant Mol. Biol*, 75:93-105.

Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J.D.G., Boller, T. and Felix, G. 2006. Perception of the Bacterial PAMP EF-Tu by the Receptor EFR Restricts *Agrobacterium*-Mediated Transformation. *Cell*, 125:749-760.

نقش ریز آر ان آ ها و هورمون‌های گیاهی در سیستم ایمنی گیاه

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چکیده

برهم‌کنش گیاه و پاتوژن یک فرآیند چند عاملی است که ممکن است منجر به ایجاد پاسخ حساسیت یا مقاومت گیاه میزبان به پاتوژن مهاجم شود. در طی این مبارزه، تغییرات مختلفی همچون پیام‌رسانی گونه‌های اکسیژن واکنش‌گر، فعالیت هورمونی و برنامه‌ریزی مجدد بیان ژن در گیاه آغاز می‌شود. در گیاهان ریز آر ان آها تنظیم‌کنندگان کلیدی بیان ژن در سطح پس از رونویسی محسوب می‌شوند و در فرآیندهای متعدد سلولی همچون پاسخ به تنش‌های محیطی درگیر هستند. در سال‌های اخیر متخصصان بیماری‌های گیاهی یک الگوی منطقی را برای سیستم ایمنی گیاه با عنوان مدل زیگزاگ ارائه کرده‌اند که شامل دو فاز ایمنی به نام ایمنی حاصل از شناسایی PAMP و ایمنی حاصل از شناسایی effector است و مولکول‌های ریز آر ان آ دارای نقشی تعیین‌کننده در تنظیم این دو فاز هستند. در این مطالعه، کلی از زیست‌شناسی ریز آر ان آها، توضیح مختصری از سیستم‌های ایمنی گیاه بر اساس مدل زیگزاگ، نقش هورمون‌های گیاهی و ریز آر ان آها در ایمنی گیاه با تاکید بر مدل برهم‌کنش *Arabidopsis-Pseudomonas* و در نهایت نتایج کلی مطالعه‌ی انجام شده را بر روی بیان ریز آر ان آهای کاندیدا در برهم‌کنش لیمو و استرین‌های *Xanthomonas* ارائه شد.

کلمات کلیدی: ایمنی حاصل از شناسایی PAMP، ایمنی حاصل از شناسایی effector، سیستم‌های ایمنی گیاه، ریز آر ان آ.