E-ISSN: 2788-9297
P-ISSN: 2788-9289
https://www.agrijournal.org
SAJAS 2024; 4(1): 222-226
Received: 02-04-2024
Accepted: 07-05-2024

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Roles of phytohormones in regulation of plant immunity against pathogens

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DOI: https://doi.org/10.22271/27889289.2024.v4.i1c.132

Abstract
In nature, plants are subjected to multiple pathogens that can be subdivided into biotrophs, necrotrophs and hemibiotrophs. Biotrophic pathogens derive nutrients from living tissues via developing haustoria without disrupting the host plasma membrane. In contrast, necrotrophic pathogens kill the host tissues for nutrient access, using secreted phenolic- and proteic- phytotoxins and many extracellular proteins such as cell-wall degrading enzymes (CWDEs). Intermediary pathogens, called hemibiotrophs, are biotrophs in early infection stages and turn necrotrophs at later stages of infection. To resist to pathogens, arsenal of both constitutive and inducible defenses have been evolved by the host plant. This review focusses mainly on the role of Salicylic acid, Ethylene (ET), Jasmonic acid (JA) and Abscisic acid (ABA) in regulation of plant immunity against different life styles of pathogens.

Keywords: Plant immunity, phytohormone, pathogens, signalling and regulation

Introduction

Pathogen perception and triggered immunity in plants

Plant defenses are activated depending on the perception of both pathogen-associated molecular patterns (PAMPs) and pathogen effectors, that respectively bind to patterns recognition receptors (PRRs) or intracellular receptors (R proteins). Consequently, different lines of plant defenses have been established as a "zigzag" model (Fig. 1).

1.1 PAMP recognition and triggered immunity

The first line of plant defense is triggered by recognition of PAMPs by trans-membrane PRRs, resulting in PAMP-triggered immunity (PTI) [1]. PRRs are commonly plasma membrane-localized receptor-like kinases (RLKs) or receptor-like proteins (RLPs) with extracellular domains [2]. Similarly, the molecules derived from damages of plant patterns or DAMPs (Plant-derived damage-associated patterns) are also recognized by PRRs during infection and may also trigger the PTI. Once activated, immune responses includes stomatal closure, generation of reactive oxygen species or ROS, reinforcement of cell walls, transcription of defense-related genes and production of antimicrobial metabolites. Although PTI potentially suppresses a wide range of microbial pathogens, some highly aggressive pathogens carry a type III secreting system that secretes virulent effectors (or toxins) directly into the host cells without any PRRs recognition, thus suppressing PTI and stimulating diseases. This effector-triggered susceptibility (ETS) conduced the host plants to evolve a second line of defense to protect themselves [3].

1.2 Effector-triggered immunity

Effector-triggered immunity (ETI), known as secondary immune response, is activated through the perception of pathogen effectors by resistance (R) proteins (Fig. 1; Jones and Dangl, 2006) [1]. In some plant cultivars, R proteins can detect directly or indirectly the pathogen-specific effectors. A large class of R proteins is well-characterized by its nucleotide binding site (NB) and leucine-rich repeat domain (LRR), the so-called NB-LRRs proteins. PTI and ETI share a common regulatory signaling network and trigger common plant immune responses. However, defense responses during ETI are more prolonged and robust than during PTI, leading to a highly effective resistance against pathogens. Moreover, ETI is often associated with a hypersensitive response (HR), a form of program cell death (PCD) that limits availability of nutrients to potential pathogens [4]. This event might thus contribute to enhance a plant resistance against biotrophs, but not against pathogens already in a necrotrophic stage.

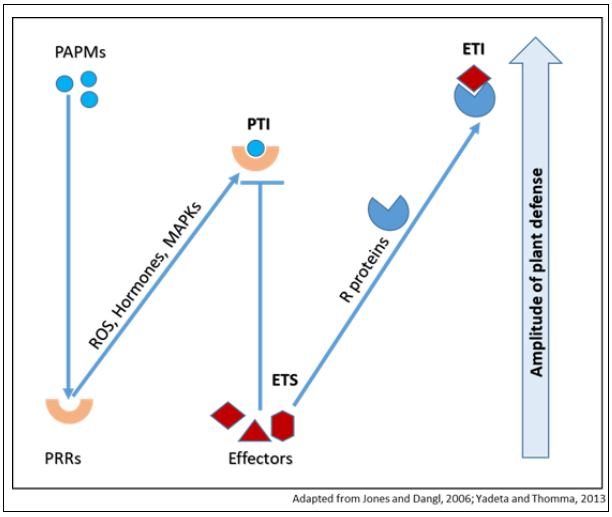

Fig 1: Schematic representation of plant innate immune response during plant–pathogen interactions.

Plants recognize Pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) to trigger PAMP-triggered immunity (PTI). Successful pathogens deliver effectors that interfere with PTI, leading to susceptibility (ETS). In turn, plants have evolved resistance (R) proteins to recognize the given effectors, resulting in effector-triggered immunity (ETI), which accelerates and amplifies PTI response. Abbreviations: ROS, reactive oxygen species; MAPKs, mitogen-activated protein kinases, ETS: Effector-triggered susceptibility.

2. Phytohormones regulate plant immunity

Plant hormones or phytohormones play crucial roles in the regulation of plant growth and immunity. As salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are mainly required to regulate plant defenses (Fig. 2; Pieterse *et al*., 2012) [10]. Others phytohormones, including abscisic acid (ABA), gibberellins (GAs), auxins, cytokinins (CKs) and brassinosteroids also take part in mediating the host immunity, via interaction with SA and/or JA/ET pathways (Fig. 3). To date, JA/ET signaling pathways are mainly described to positively regulate plant defenses against pathogens in a necrotrophic stage, while SA signaling is predominantly required to activate plant resistance against fully biotrophs. However, many exceptions exist. For instance, plant resistance against the necrotrophs B. cinerea or S. sclerotiorum is also dependent on SA signaling, whereas exogenous application of methyl jasmonate (MeJA) enhanced the protection of susceptible wheat cultivars to the biotroph powdery mildew. Similarly, JA induced a rice resistance to the biotrophic bacterium *Xanthomonas oryzae*, and to the hemibiotrophic fungal *Magnaporthe oryzae* [5]. This highlights that plant immunity was fine-tune regulated by a complex hormonal regulatory network, in which distinct hormones can individually or collectively function to optimize the plant defense responses to pathogens.

2.1 SA-induced responses

SA is a phenolic compound synthesized through two distinct enzymatic pathways, isochorismate synthase (ICS/SID2) and phenylalanine ammonia lyase (PAL) (Fig. 2). Defensive role of SA was first highlighted in tobacco showing resistance to TOBACCO MOSAIC VIRUS (TMV), and induction of SA-dependent pathogenesis-related proteins or PR proteins. Exogenous SA applications also reduced expansion of the hemibiotroph Pseudomonas syringae with upregulation of PR1, PR3 (chitinase), PR5 (thaumatin-like) and PR9 (peroxidase) transcription in barley. Similarly, foliar applications of SA induced plant resistance to the necrotrophic fungus Fusarium oxysporum f. sp. phaseoli and increased host activities of PAL and peroxidases. SA roles in plant resistance against pathogens were then functionally confirmed with mutants [6]. For instance, NahG transgenic plants (i.e. Tobacco and Arabidopsis) possess the nahG transgene encoding for hydroxylase converting SA to catechol. Failing to accumulate SA, NahG plants are most susceptible to different pathogens. However, several other mutant plants need to be used to confirm and fine-tune the roles of SA in plant immunity (i.e. sid2 that fails to produce SA, etc.). During PTI and ETI, SA accumulation requires the lipase-like enhanced disease susceptibility1 (EDS1) and the Phytoalexin Defficient4 (PAD4), whereas SA signaling transduction requires EDS5. Corresponding mutants unable to produce/transduce SA (i.e. eds1, eds5 and pad4) display an highest susceptibility to pathogens [7].

SA-inducible defenses require activation of the regulatory protein NPR1 (NONEXPRESSOR OF PR-GENES 1) since NPR1 mutant fails to induce SA-responsive PR genes. Upon SA, monomeric NPR1 translocates into nucleus to interact with transcription factors (TGA family) then induce the expression of the SA marker gene PR1 (Fig. 2). The full expression of PR1 additionally requires WRKY70 activation, but no direct interaction between WRKY70 and NPR1 has yet been reported. Activation of NPR1 is mediated by its paralogs NPR3 and NPR4 through differential affinity for SA. At high SA level, NPR3 binds to SA and degrade NPR1; while at low SA level, NPR4 stabilizes NPR1 and leads to an SA-dependent PR gene expression. NPR3 and NPR4 are thus identified as endogenous receptors mediating distinct events of SA signaling cascades (Fig. 2; Moreau *et al*., 2012)

2.2 JA/ET -induced responses

JA and derivatives originate from lipid compounds and might be involved in a variety of plant development- and resistance- processes (Pieterse *et al*., 2012) [10]. Defensive roles of JA are highlighted by an over-production of the defense-related proteins defensins and thionins upon JA- or derivatives- exogenous applications. In Arabidopsis, mutants impaired in JA -signaling and -synthesis were most susceptible to necrotrophic pathogens and insect herbivores. JA synthesis is initiated by an activation of the Lipoxygenase (LOX) enzymes that convert?-linolenic acid into JA (Fig. 2). Upon wounding or pathogen attack, LOX genes expression is upregulated. Rapidly, JA is conjugated to isoleucine by the JA Amido Synthetase (JAR1) and forms the (+)-7-iso-jasmonoyl- l-isoleucine (JA-Ile). JA can also be converted into the plant volatile methyl-jasmonate (MeJA), through methylation by the JA Carboxyl Methyltransferase (JMT). In response to the necrotrophic fungi B. cinerea, JMT transcription and JA-responsive genes are over-expressed (i.e. the Plant Defensin PDF1.2 and the Vegetative Storage Protein VSP2), and associated to an increased plant resistance to this fungal pathogen. MeJA acts thus too as a signaling molecule of plant immunity against necrotrophic pathogens [8].

JA signaling pathway is regulated by the E3-ligase SCF F-box protein Coronatine Insensitive1 (SCFCOI1), as JA

receptor, and with the Jasmonate ZIM-domain (JAZ) transcriptional repressor proteins (Fig. 2; Sheard *et al*., 2010; Yan *et al*., 2009).

In the absence of JA, JAZ proteins act as transcriptional repressor of JA-responsive genes. In contrast, upon pathogen infection or wounding, JA signaling is induced after binding of the complex JA-Ile-SCFCOI1 to JAZ proteins. This complex enable to address the JAZ repressive molecules to proteasome for degradation, activating thus the JA-responsive genes such as Vegetative Storage Protein2 (VSP2).

In Arabidopsis, another JA/ET signaling pathway is possibly induced upon challenge with necrotrophic pathogens, regulated by distinct transcription factors (TFs), such as ERF1 and ORA59 (Ethylene Response Factor and Octadecanoid-Responsive Arabidopsis 59, respectively), all members of the AP2/ERF family (AP2 meaning APETALA2). Activation of ERF1/ORA59 induces the expression of the JA/ET-responsive Plant Defensin1.2 (PDF1.2) gene. Depending on the attackers, plants may switch the activation of MYC- or ERF1- branches. Plants prioritize activation of ERF1 branch upon challenge with necrotrophic pathogens, while the MYC branch is activated in response to wounding and herbivory insects [9].

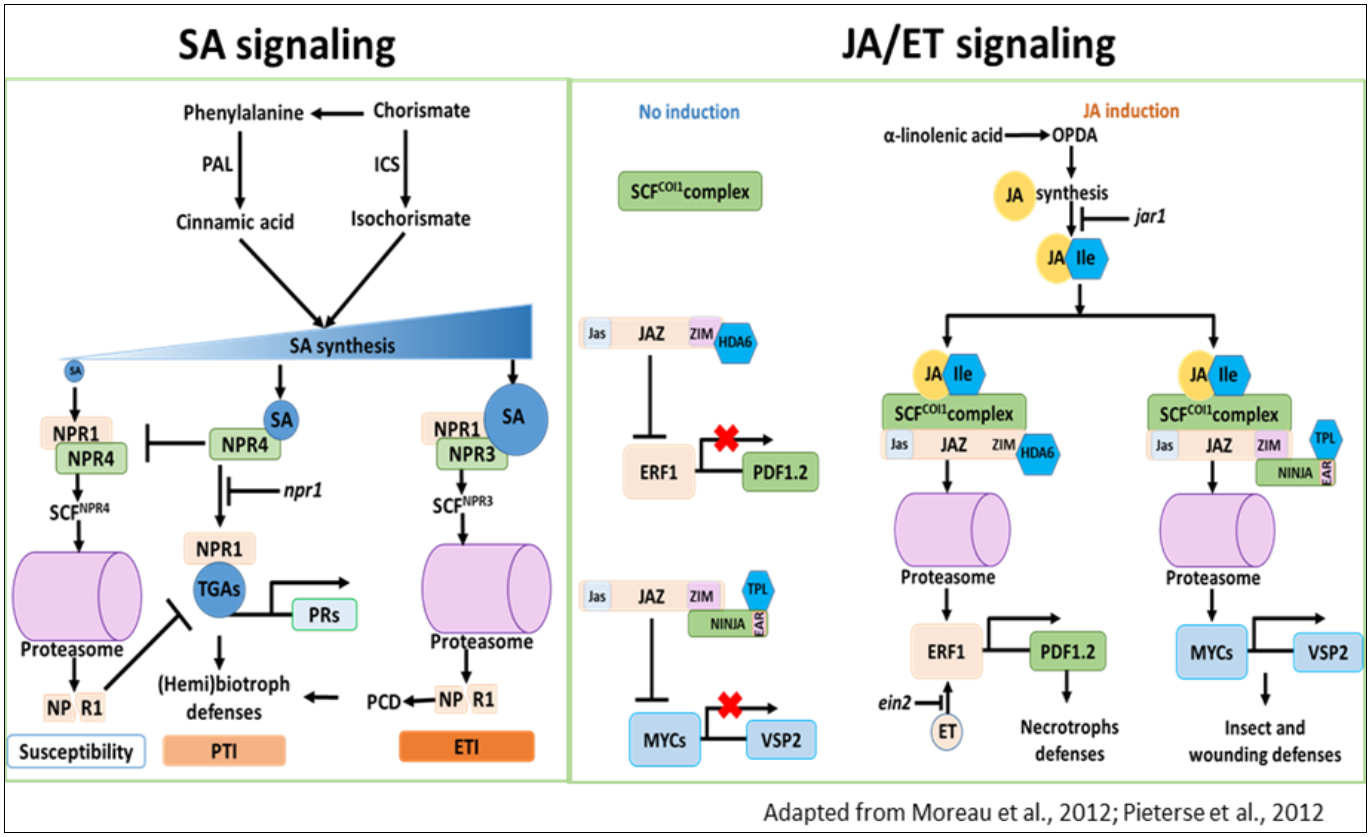

Fig 2: Plant defense regulated by SA and JA/ET signaling against different attackers

SA concentration play important role in shaping outcome of defenses to (hemi)biotrophs. At extremely low level of SA, nucleus-located *NPR1* is targeted by *NPR4* for degradation in proteasome, resulting in inactivating of SA-responsive defenses. Once plants perceive the pathogens and trigger basal resistance, SA accumulation is induced. At medium level of SA (PTI), SA binds to *NPR4* and disrupt interaction between *NPR4* and *NPR1*, leading to activation of SA-dependent responses. At higher SA concentration (ETI), *NPR3* expresses a highly affinity for SA and promotes its binding to *NPR1* for the degradation in proteasome, resulting in program cell death that suppresses the pathogen expanding. Similarly, in absence of JA signaling, *JAZ* proteins act as transcriptional repressor of JA-responsive genes, resulting in inhibiting plant immunity. JA synthesis is induced after necrotrophs, insect or wounded stresses. JA is then conjugated with isoleucine to form biological activity compound JA-Ile though activation of *JAR1* enzyme. The binding of JA-Ile to COI1 in the E3 ubiquitin-ligase SKP1-Cullin-F-box complex SCFCOI1 recruits JAZ repressor proteins for degradation in proteasome, resulting in release of activation of JA-induced transcription factors (TFs) and consequent expression of JA-dependent response. PAL: phenylalanine, ICS: Isochorismate synthease, OPDA: 12-oxo-phytodienoic acid, PTI: PAMP-triggered immunity, ETI: Effector-triggered immunity, SA: salicylic acid, NPR-: Non-expressor of PR gene, TGAs: Transcription factors belonging to TGA family, PRs: Pathogenesis- related proteins, PCD: Program Cell Death; JA/ET: Jasmonic acid/Ethylene; PDF1.2: PLANT DEFENSIN1.2; VSP2: VEGETATIVE STORAGE PROTEIN2; ERF1: ETHYLENE RESPONSE FACTOR1, *sid2*, *NahG* and *npr1:* SA synthesis and signaling deficient transgenic/mutant plants; *ein2* and *jar1:* ET- and JA-insensitive mutants. Arrows indicate induction, T bars present suppression.

2.3 SA-JA/ET crosstalk

Although SA and JA may regulate different defense responses depending on pathogen lifestyles, cross communication may also occurred within the signaling events of these two hormones, probably as part of a plant

strategy to fine-tune its defense responses [10, 11]. The first evidence of SA-JA crosstalk was reported in tomato, then in various plant species. Infection of Arabidopsis with the SA-inducing hemibiotrophic bacterium Pst DC3000 suppressed JA/ET-responsive genes, leading to susceptibility to necrotrophic fungal *Alternaria brassicicola*.

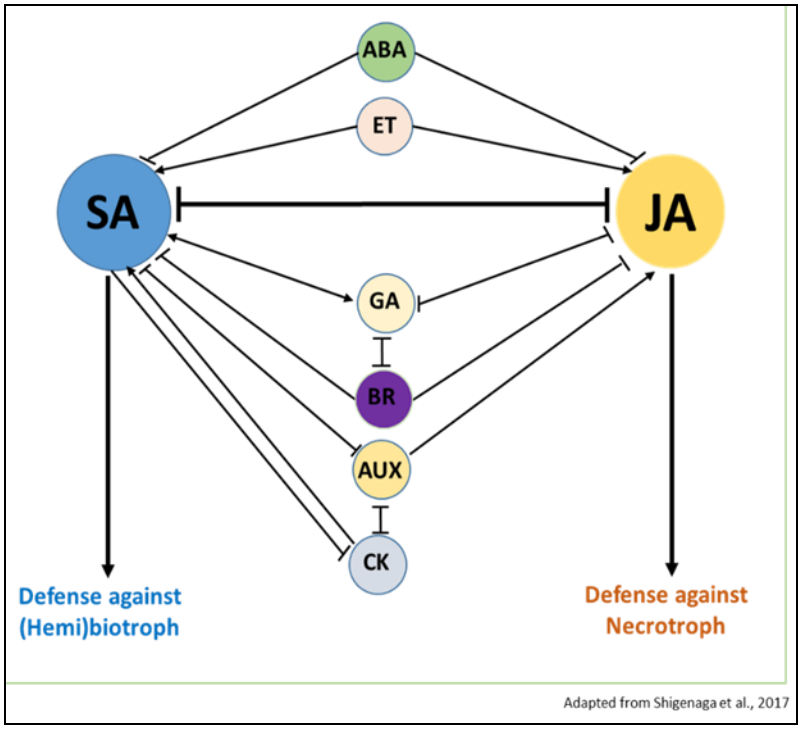

Fig 3: Crosstalk among phytohormones in regulating plant defenses against pathogens with different lifestyles.

The phytohormones, such as ABA, ET, GA, BR, AUX and CK might be involved in plant defense though their integration of SA-JA crosstalk). SA: salicylic acid, JA: jasmonic acid, ET: ethylene, ABA: abscisic acid, GA: gibberellins, AUX: auxins, CK: cytokinins, BR: brassinosteroids. Bars indicate two-way antagonism, bars represent for one way antagonism or inhibition, arrows indicate the induction.

The SA-mediated suppression of JA signaling is also shown in lima bean, tobacco, cucumber, rice, cotton, etc. Likewise, JA signaling is reduced after plant challenge with the fully biotroph *Hyaloperonospora arabidopsidis*. In turn, JA signaling might also antagonize the SA-dependent defenses as observed upon Arabidopsis-*P. syringae* interaction. Indeed, *P. syringae* produces the virulence factor coronatine (COR) that structurally mimics JA-Ile, then induces JA signaling that repress the SA-responsive defenses. This antagonisms was confirmed by the use of COR-deficient mutant of *P. syringae*, which induces SA- rather than JA-responsive genes. Conversely, synergistic interactions between SA-JA signaling pathways have also been reported. With an appropriate concentration, SA- and JA-responsive genes were coincidently induced in tomato [7]. Similarly in Arabidopsis, when both SA- and JA- inducible genes were upregulated upon challenge with biotrophic pathogens. Furthermore, even JA-deficient mutants may show a reduction of SA-dependent defenses. Besides that, the synergistic interactions between SA and JA have been reported in resistance against insect herbivores and nematodes as well.

SA-JA cross talk is predominantly regulated by NPR1, as a crucial transcriptional co-activator of many SA-responsive genes [12]. While no nuclear functions are yet identified for NPR1, its cytosolic localization is required for the SA-mediated suppression of JA signaling. Indeed, overexpression of the cytosolic OsNPR1 suppress some host JA-responsive gene transcriptions, and reduce the rice *(Oryza sativa*) resistance towards herbivory insects. Interestingly, NPR1-dependent SA-mediated suppression of JA signaling was dismissed in presence of ET or its precursor ACC in Arabidopsis. Moreover, an early activation of the JA/ET signaling pathways prevent the SA negative effects on JA-responsive defenses. The Glutaredoxin GRX480 expression also negatively affects the JA-responsive PDF1.2 expression in Arabidopsis. The GRX480 negative effects can result from its physical interaction with the SA-regulating transcription factors TGAs. By contrast, MAPK4 (mitogen-activated protein kinases4) suppresses SA- but induces JA- responsive defenses (Brodersen *et al*., 2006). Other regulators controlling SA-JA crosstalk have already been well-documented [10, 11].

2.4 Roles of Abscisic acid (ABA)

ABA is well-characterized as a crucial phytohormone associated with plant tolerance to abiotic stresses. However, its roles in regulating resistance against biotic stressed also retain attention [13]. Positively, upon pathogen infection, ABA induces stomatal closure and callose deposition to prevent pathogen entrance. Profitable roles of ABA for plant defenses have also been reported during the plant-beneficial microbe interactions upon pathogen infection. In contrast, ABA is also shown to repress the plant SA- and JA/ET-dependent defenses (Fig. 3) at the later stages of pathogen infection. Exogenous ABA application increases Arabidopsis susceptibility to SA upon challenge with the hemibiotrophic bacterium *P. syringae DC 3000*, or to JA/ET upon challenge with the necrotrophic fungus *Fusarium*

oxysporum. Another work highlighted that the enhanced *B. cinerea* 2100 resistance is dependent on WRKY33 activation that suppresses of ABA synthesis [14].

3. Conclusion

In conclusion, the manuscript delves into the pivotal role of phytohormones in orchestrating plant immunity against pathogens, highlighting the intricate molecular mechanisms underlying this regulatory process. Through a comprehensive exploration of the interplay between phytohormones such as salicylic acid, jasmonic acid, and ethylene, the manuscript elucidates their synergistic and antagonistic interactions, shaping the plant's defense responses. From perception of pathogen signals to activation of defense genes, the intricate crosstalk among these phytohormones finely tunes the plant's immune system, enabling precise and efficient defense against diverse pathogens. Understanding these molecular mechanisms not only sheds light on fundamental aspects of plant-pathogen interactions but also holds immense potential for developing novel strategies to enhance crop resistance and mitigate agricultural losses in the face of emerging pathogens and environmental stresses.

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