Plant in Challenging Environments 2

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# Hormones and Plant Response

Springer

# Plant in Challenging Environments

Volume 2

**Series Editors**

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# Preface

Higher plants possess several groups of molecules which can exert regulatory actions on almost all physiological processes from seed germination, organ development, the formation of flowers and fruit ripening as well as they participate in the mechanisms of response against adverse environmental conditions including biotic and abiotic factors. Among these molecules are included plant classical hormones also designated as phytohormones auxins (AUXs), cytokinins (CKs), gibberellins (GAs), ethylene (ET), brassinosteroids (BRs), jasmonic acids (JA), salicylic acid (SA), polyamines (PA), and strigolactones, as well as other molecules with, can also regulate plant functions such as nitric oxide (NO), hydrogen peroxide ($H_2O_2$), hydrogen sulfide ($H_2S$), gamma-aminobutyric acid (GABA) or melatonin. All these molecules have a complex net of interactions which make a puzzle of positive and negative connections that many times, it is not easy to decipher.

This book "Hormones and Plant Response" includes 12 chapters and has the main goal that covers key features of plant hormones in different processes. **Chapter 1** analyzes the involvement of SA, JA and ET in the rapid activation of the plant's innate immune system against diverse pathogens where NO and reactive oxygen species (ROS) are also implicated. Moreover, it is examined how the application of CRISPR/Cas9 editing has become a powerful tool for future enhancement of agronomic traits in crops. **Chapter 2** summarizes the progress in understanding the interactions of different hormones like auxin and ethylene with other signaling molecules (nitric oxide, glutathione, sucrose, peptides and microRNAs) in the regulation of the main nutrient deficiency responses. **Chapter 3** offers an overview of the interaction of several hormones (ABA, GA and ET) with ROS metabolism during seed germination where nitric oxide and plasma membrane H+-ATPases are also involved. **Chapter 4** provides an update on the light and dark-responsive changes during seedling development essentially involve differently regulated cell division and elongation in hypocotyls and cotyledons where behind cytokinins, they are other hormones such as ET, GAs, BRs, ABA, JA and strigolactones. **Chapter 5** explores complementary analyses of the regulatory networks between photoreceptor-mediated light perception and the biosynthesis, conjugation and degradation of several hormones including AUXs, GAs, ABA, CKs, ET and BRs. Thus, deeper

knowledge in these complex interactions could be very useful for improving crops under different perspectives such as stress resistance or nutritional quality. **Chapter 6** provides a comprehensive overview of how hormones can modulate the functioning of flowering induction pathways in plants with different photoperiod sensitivity as well as it discusses s whether photoperiod regulates the level of endogenous hormones and their signaling pathways during flower induction. **Chapter 7** provides recent insights into how auxin participates in the regulation of root growth and architecture during adverse environmental conditions. **Chapter 8** deals with the function of ABA during fruit ripening with a special focus on grapevine acclimation since ABA triggers biochemical changes which increase the content with antioxidant properties. **Chapter 9** presents an overview of the biosynthesis and the mode of action of BRs using forward and reverse genetic studies. **Chapter 10** highlights promising new aspects of the melatonin and its regulatory interactions with the main hormone including AUX, GBs, CKs, ABA, ET, BRs, JA and SA as well as polyamines. **Chapter 11** provides complementary information about tryptophan and derived molecules including serotonin and melatonin. It is also remarked the implication of melatonin in different physiological processes such as seed germination, root development, stomatal movements or fruit ripening. And how the exogenous application of melatonin can palliate different injuries associated with different stresses. Finally, **Chap. 12** summarizes and analyzes the current knowledge of GABA and proline accumulation in response to environmental stresses. Furthermore, it is discussed how these molecules can be used as functional markers of stress tolerance to select tolerant genotypes in breeding programs.

All these collected contributions from various laboratories throughout the globe studying plant hormone and other growth regulators provide an updated overview of the contemporary challenges and possibilities in different areas where these compounds are involved. We do hope that this book will raise interest in the field of higher plant hormones and will serve as a valuable reference material.

We would like to express our gratitude to all the authors/contributors and reviewers who contributed for this volume.

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# Chapter 1
# Plant Hormones and Plant Defense Response Against Pathogens


**Virginia Borrelli, Alessandra Lanubile, and Adriano Marocco**

**Abstract** Biotic stresses are responsible for 20 to 40% losses of global agricultural productivity. Higher plants interact continuously with virus, fungi and bacteria, some of which lead to plant response firstly in the cell wall and cuticle acting as a physical barrier. However, successful resistance comes from a rapid switch on of the plant's innate immune system, which involves the phytohormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET), main players in signal transduction. Strategies have been developed by pathogens to manipulate plant hormonal pathways and modify the immune signaling for their own resistance enhancement in the host. Nitric oxide (NO) participates in this challenging signaling pathway shared with reactive oxygen species during plant-pathogen interaction, playing a decisive role from both adversaries. The complex crosstalk between pathogen and plant will be discussed considering the main categories of pathogens and the genetic constitution of the host. Moreover, the phytohormones signaling and their network regulation along with the involvement of NO and reactive oxygen intermediates will be revised according the recent efforts in plant biotechnology. Now, a primary challenge is to identify and characterize the host genes underlying the proteins targeted by effector molecules and to design targets for future genome editing approaches. Among the New Breeding Techniques (NBT), the application of CRISPR/Cas9 editing has become an effective tool for future reinforcement of disease resistance in crops.

**Keywords** Plant defense · Pathogens · Phytohormones · Signaling · Genome editing

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## 1.1 Perception and Signal Transduction: The Apoplastic Crosstalk

Plant surface is composed of a wide diversification in microorganisms and substances, which create a heterogeneous space of interactions between the plant and the external world. Phyllospher and ryzosphre adaptations by bacteria and fungi are examples of survival and growth in plant ecosystem, where microorganisms, pathogens or providers of nutrients and shelter arrive by different vehicles as rain, insects and wind and microbes as part of the environment start their life on plant surfaces. The plant microbiome is a unique association of microorganism given by multiple factors coming from plant genotype and the environment. When different plant genotypes grow in the same environment each specie keeps different types and concentration of microorganisms, giving rise to a unique microbiome specific for each surface. Different experimental approaches are used in order to identify how the microbiome is generated and find the genetic factors associated to microbiome specificity. Transcriptome and quantitative trait loci studies put in evidence that plant genes are involved in microbial communities' selection and the genes are object of new breeding techniques (NBT).

In plant ecosystem the first space in which the interaction starts are the apoplast, which involves the cell wall and extracellular space. The cell wall forms a dense and complex network out of the cell with protection, structure and metabolic functions. Pathogens as necrotrophs synthesize cell wall-degrading enzymes to pass through the plant cell (Laluk and Mengiste 2010). The actively cell wall reinforcement is site specific and well liked to plant-pathogen crosstalk (Underwood 2012). In the conventional resistance gene model, avirulence (avr) genes are defined as genes of the pathogen that govern its specific recognition by particular plant genotypes. Recognition depends upon the presence of a pair of matching genes, an avr gene in the pathogen and a resistance (R) gene in the plant. Effectors (avr genes) are proteins secreted by microbial pathogens, which can either trigger or compromise immunity associated with specific R genes. Plant microbes start the colonization process by secreting effector proteins into the apoplast, the first interaction between plant and microbes.

The first layer of communication is called Microbe-associated molecular pattern Triggered Immunity (MTI) also referred as PTI (Pathogen-associated molecular pattern Triggered Immunity). MAMPs (Microbe-Associated Molecular Patterns), also referred as PAMPs (Pathogen-associated Molecular Patterns), and DAMPs (Damage-Associated Molecular Patterns) are microbial- or damage-associated molecular patterns proteins recognized by the plant immunosystem. Examples of DAMPs are damaged systemin or oligogalacturonides, which act as plant infection signals. Many MAMPs/PAMPs have been identified. MAMPs synthetized by *Trichoderma spp.* include the cerato-platanin protein Sm1 (Djonovic et al. 2007) and the ethylene-inducing xylanase (EIX) (Ron and Avni 2004). The EIX is involved in plant colonization both by lytic enzyme activity and systemic resistance. Bacterial flagellin as flg22 and the elongation factor elf18 have been widely studied as

MAMPs (Felix and Boller 2003; Trdá et al. 2013). Many other MAMPs have been discovered as lipopolysaccharides (Newman et al. 2002) and chitin oligomers (Miya et al. 2007). Pathogen can suppress MTI by secreting effector proteins that act by suppress MAMPs interactions.

The recognition of the effectors results in the effector-triggered immunity (ETI). ETI is stronger than MTI and it includes a more vigorous response that involves localized cell death as hypersensitive response (HR). HR can be activated by elicitors, foreign molecules which can switch on plant defense. They differ from effector in their source of delivery: the effector is produced by the pathogen, while elicitors can have different origin but in any case the chemical structure triggers the HR. Both elicitors and effectors differ from hormones because they are produced out of the plant in which they are triggering a response. Commonly, elicitors like chitosan are used to activate chemical defense. The elicitor Sm1 is induced during *Trichoderma virens*-plant interaction and promotes the expression of pathogenesis-related (PR) genes (Djonovic et al. 2007). Other effectors are transcription activator-like effector (TALE), as PthA4 which suppress plant basal defense promoting pathogen growth and cankers development (Jia et al. 2016). PthA4, contains a nuclear localization signal for host plant gene expression, which activate susceptibility genes and sustains *Xanthomonas* sp. infection process. The bacterial type III TAL (Transcription Activation-Like) effector PthXo1 from the *Xanthomonas* sp. bounds to the Xa13/Os*SWEET11* promoter (Chen et al. 2010). TAL effectors are prokaryotic transcription factors that bind to sequence-specific effector-binding elements (*EBEs*) of the eukaryotic host (Boch et al. 2009). TAL effectors were found to target different promoter regions of *SWEET* loci associated with host susceptibility (Zhou et al. 2015). The characterization of *xa13*, the associated *OsSWEET11* locus, and the PthXo1 TAL effector confirm the classical gene-for-gene interaction (R-avr). Promoter mutations prevents binding of the TAL effectors which leads to a recessive 'gain of function' resistance (Blanvillain-Baufume et al. 2017). Another case of pathogen gene regulation is *CsLOB1*, a disease susceptibility gene involved in *Xanthomonas campestris pv. campestris* infection process. Recently, experimental evidences identified the *CsLOB1* expression dependent from PthA4 delivery. In *CsLOB1* promoter region has been identified the PthA4 effector binding elements ($EBE_{PthA4}$), which activates its gene expression (Hu et al. 2014). Other examples of secreted effectors are Ecp6, which sequestrate chitin oligosaccharides of *Cladosporium fulvum* (de Jonge et al. 2010), and the toxin-like TOXB, which is secreted into the apoplast by the *Pyrenophora tritici-repentis,* a necrotrophic fungus of wheat. Those examples of gene regulation in plant-pathogen interaction suggest that plant-associated microbes and the host plant are part of a continuous apoplastic protein secretion, which mainly uses Golgi-endoplasmic reticulum pathway by N-terminal signal peptide. Apoplastic proteins (APs) can be delivered by an alternative pathway called leaderless secretory pathways (LSPs) (Delaunois et al. 2014).

The active players in perception and regulation during plant-microbe interactions need to be studied in deep by using proteomic, gene expression analysis and genome editing tools to understand how the colonization, infection and defense process take place in different models and finally enhance plant resistance.

## 1.2 Cell Signaling: Perception of Danger Signal

Cell signaling is required to coordinate a number of functions: the reception, the transduction and finally the response to the signal. In this paragraph we focus on MTI and ETI which are both responses to the perception of danger signal coming from the pathogen. The host answer to this signal can arise into different forms as the recognition of MAMPs, DAMPs, or effectors.

### *1.2.1 Effectors and Receptors*

When the pathogen starts the attack, the signal arrives by intercellular pathways and it takes place when MAMPs are recognized by the cell receptor systems. As described in the previous paragraph, the recognition of the pathogen occurs in the apoplast where the reception step starts. When damage molecules, as flagellin and chitin, reach the cell wall, a large number of receptors are ready to recognize and reinforce the host defense. Some examples of receptors involved in pathogen attack are: pattern recognition receptors (PRRs) specific for MAMPs as cysteine-rich receptor-like kinase (CRRK), leucine-rich receptor-like kinase (LRRK), serine threonine kinase (STK) and BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 (BAK1) (Lanubile et al. 2014; Wang et al. 2016a). FLAGELLIN-SENSING 2 (FLS2) is an example of a receptor kinase LRRK highly conserved across *Brassicaceae* (Gomez-Gomez and Boller 2000). Plant PRRs shows different affinity for glucan, chitin and xylanase. Protein studies in rice evidence that chitin, a classic fungal MAMPs, has affinity for a cell wall receptor with extracellular lysin motif (LysM) domains similar to CERK1 (Kaku et al. 2006; Zhang et al. 2007). Harpins are elicitors of hypersensitive response and are secreted by the type III patway (Kim et al. 2004). Pep13 is a conserved epitope typical of oomycetes, potent elicitor in parsley; the peptide has been reported also to activate defense responses in potato (Brunner et al. 2002). Another class of oomycete MAMPs includes the so-called elicitins, sterol-binding proteins that act as potent inducers of the HR in tobacco (Osman et al. 2001). Fatty acids and arachidonic acid are important elicitors in potatoes while ergosterol plays an important role in tobacco and grapevine defense response but its perception and receptor system is still to characterize (Lochman and Mikes 2006). N-glycosylated yeast peptides, peptidoglycan are important source of MAMPs. As fungal chitin, peptidoglycans are the backbone of bacterial cell wall and they act as MAMPs in the host. Peptidoglycans are structurally similar to chitin, and the perception is mediated by LysM-domain receptors (Zhang et al. 2007).

In gram-negative bacteria MAMPs are represented by lipooligosaccharides and their recognition is influenced by phosphorylation and acylation (Silipo et al. 2008). Pathogen can also produce lytic enzyme which can acts as DAMPs or MAMPs, as the case of cutinases produced by fungi and cutin monomers releasing (Lotze et al.

2007). An example of elicitors is the 18-amino-acid peptide systemin, also named the first peptide hormone in plants which triggers host defense response in tomato plants (Lotze et al. 2007). The receptor for systemin is probably a LRRK (Gomez-Gomez and Boller 2000) but the mechanism of its reception system has been defined "in vitro". In *Arabidopsis* the systemic orthodox is the 23-amino-acid peptide PEP1, it corresponds with the C-terminal part of a small protein translated in wounding condition. PEP1 has been indicated as endogenous signal released after injury, similar to the elicitor systemin in potatoes. Some evidences have shown the chemical link between PEP1 and the receptor (Shiu and Bleecker 2003). Successful bacterial pathogens have developed how to overcome MTI by delivering effectors directly into the plant cytoplasm. In ETI, these effectors belong to the family of nucleotide binding-site LRR (NB-LRR) and are recognized by the products of R genes (Caplan et al. 2008).

### *1.2.2 Signal Transduction Pathways*

The earliest physiological response to MAMPs and DAMPs occur in few seconds and the signal transduction consist of calcium ($Ca^{2+}$) signaling, ABA release, GTP-binding proteins and phytohormones as regulators of plant immunity. MAMPs are known to activate an influx of $Ca^{2+}$ from the apoplast and to increase cytoplasmic $Ca^{2+}$ concentrations. Calcium-protein kinases and specific channel activation are dependent to $Ca^{2+}$ influx, which answer to MAMPs arrives as second messenger (Brunner et al. 2002; Ku et al. 2018). Calcium receptor system is composed of calmodulins (CaMs) or calmodulin-like proteins (CMLs), calcineurin B-like proteins (CBLs), calcium-dependent protein kinases (CPKs), and calcium/calmodulin-dependent protein kinases (CCaMKs) (Tuteja and Mahajan 2007; Liese and Romeis 2013). In the transgenic tomato *SlCaM2* enhanced expression of *CaM2* led to *Botrytis cinerea* resistance after infection. The overexpression of *CBL1* in *Arabidopsi*s improved tolerance in abiotic stress condition, while *CBL10* silencing in tomato was found to be associated with improved pathogen resistance (Batistic and Kudla 2009; Chen et al. 2012). CPK proteins bind to $Ca^{2+}$and they can then phosphorylate their specific targets as ion channel, ABA responsive element binding factors and calcium ATPase. The overexpression of *OsCPK12* enhance tolerance of salt stress and increase susceptibility to blast fungus (*Magnaporthe grisea*) by reducing the accumulation of reactive oxygen species (ROS) (Asano et al. 2012). In *Arabidopsis* the expression of *AtCPK3* was induced by different stresses as cold, salt, heat, hydrogen peroxide ($H_2O_2$) and flagellin (Mehlmer et al. 2019). CCaMKs have been identified in different species as soybean, tomato and wheat (Yang et al. 2010, 2011). $Ca^{2+}$ influx and receptors here described confirm that calcium sensors are regulator of ABA. Another phytohormones involved in $Ca^{2+}$influx is Jasmonic acid (JA) involved in both abiotic and biotic stresses (Ahmad et al. 2016) because it activates gene expression of plant defense response (Yan and Xie 2015). Some example of responsive genes encodes for PR proteins as chitinases, PR3, PR10,

Lipoxygenase 3 (LOX3) and their importance in plaint-pathogen interaction has recently been reviewed as reported in (Borrego and Kolomiets 2016; Lanubile et al. 2017; Lim et al. 2017), because of the complex network behind the responsive gene activation and phythormones, the crosstalk will be further discussing in next paragraphs. Back on cell signaling transduction, the other player involved in defense activation after MAMPs and DAMPs recognition are G-proteins and Obg superfamily. G-proteins are activated after their hydrolysis of GTP in GDP active form, their modulation is due to the bound of downstream targets and their major involvement belong to the regulation of signal after biotic and abiotic stresses. Some examples of G-proteins showing involvement in pathogen resistance are: GPA1, AGB1 and XLG2. In *Arabidopsis* GPA1 has important role in bacterial pathogen defense where the *gpa1* mutant plants are significantly more susceptible to *Pseudomonas syringae* (Liu et al. 2013). Moreover, the Gα subunit of GPA1 confers plant resistance to bacterial pathogen also in rice *Os*GAP1-transgenic line (Komatsu et al. 2004). Another G-protein is AGB1 which modulates defense against necrotrophic pathogens *Fusarium oxysporum f. sp. conglutinans* and *Alternaria brassicicola* in *Arabidopsis* (Trusov et al. 2009). The *agb1* mutant exhibits the suppression of PR genes, JA and ABA after the infection confirming the important role of AGB1 in pathogen resistance. XLGs is involved in tomato *Pseudomonas syringae* resistance and gives origin to similar phenotypes with *agb1* in increasing susceptibility to the pathogen. Evidences show that XLG2 interacts with AGB1 and regulates the SA pathway after infection (Zhu et al. 2009). The last important group of players which occurs in signal transduction is the Obg superfamily, a big glass of GTPases, showing important role in basic cellular process from signal spread to translation. Some Obg protein linked to pathogen resistance are OsGAP1, OsYchF1 and OsRAC1. OsGAP1 and OsYchF1 are interacting proteins translated during tissue injury in rice bacterial blight-resistance. OsGAP1 interacts and regulate OsYchF1 according the subcellular localization and the substrate specificity (Cheung et al. 2010). The last example of Obg protein is OsRAC1 involved in rice elicitor (sphingolipid)-triggered responses against *M. grisea. OsRAC1* increases ROS production by NADPH oxidase positive regulation (Lemichez et al. 2001).

Once the pathogen and the plant start to interact, it's clear that a huge of signal network occurs in plant cell. Intracellular receptors, $Ca^{2+}$sensors, phytohormones, G-proteins and Obg proteins are responsible for decoding the pathogen signals and then transduce through the appropriate pathways to reach the physiological proper responses. Moreover, ABA and JA phytohormones are linked in pathogen signaling events and their behavior.

## 1.3 Nitric Oxide, Hydrogen Peroxide and Melatonin as Mediators for Defense Responses

Nitric oxide (NO) represents a signaling molecule involved during MTI/PTI and ETI, and in cooperation with hydrogen peroxide ($H_2O_2$) it participates in the pathogen-induced HR (Bellin et al. 2013; Trapet et al. 2015). Several physiological

plant processes are influenced by NO, such as root and pollen tube growth, flowering, stoma closure, iron uptake and sequestration, as well as hormonal signaling (Besson-Bard et al. 2009; Simontacchi et al. 2013). However, its pivotal role as mediator for plant immunity received particular attention and was deeply explored. The redox nature and the lipophilic properties of NO are of crucial importance for its signaling functions. Nitric oxide and its derivatives, including the radical NO, the nitrosium ion ($NO^+$), nitroxyl ions ($NO^-$) and the products of reaction between the ·NO and ROS, can react with superoxide ($O_2^{\bullet -}$), resulting in the generation of peroxynitrite ($ONOO^-$) and higher oxides of nitrogen, like $NO_2$ and $N_2O_3$. These compounds can in turn react with thiolate and tyrosine, evidencing the complexity of interactions between NO and several target proteins (Ferrer-Sueta and Radi 2009; Martínez-Ruiz et al. 2013).

The production of NO takes place by oxidative and reductive enzymatic routes. Despite the absence of a plant homolog of the animal nitric oxide synthase (NOS), a NOS-like activity exists in plants, requiring the same cofactors as animal NOS, such as NAPDH, calcium, calmodulin, flavin adenine dinucleotide (FAD), flavin mononucleotide, and tetrahydrobiopterin $BH_4$ (Asai et al. 2010; Corpas et al. 2009). This route represents the major source of NO production during plant-pathogen interactions. Moreover, a copper amine oxidase was described in *Arabidopsis* and identified as a further candidate of NO synthesis from polyamines induced by abscisic acid (Wimalasekera et al. 2011).

Nitric oxide can be also produced from nitrite by reductive enzymes. Mitochondrial electron transport chain is the main NO-generating mechanisms under anoxic conditions (Gupta et al. 2011). An alternative reductive enzyme was reported in tobacco roots using nitrite as substrate (NiNOR; Stohr et al. 2001) and involved in the regulation of root infection issued by mycorrhizal fungi (Moche et al. 2010). Furthermore, the enzyme nitrate reductase (NR) can also produce NO under normoxic conditions, catalyzing the reduction of nitrate to nitrite, and this last one to NO (Bright et al. 2006). It was observed that NR determines the accumulation of NO during defense responses against necrotrophic fungi (Asai et al. 2010; Perchepied et al. 2010), bacteria (Modolo et al. 2006; Oliveira et al. 2009) and chemical elicitors from PAMPs (Rasul et al. 2012).

Protein modifications dependent from NO act as redox signaling, which regulate protein structure functions, such as cysteine S-nitrosation, consisting in the covalent addition of a NO moiety to the sulfhydryl group of cysteine residues in target proteins and forming S-nitrosothiol (SNO; Lamotte et al. 2015; Gupta et al. 2020). The level of SNO is controlled by both NO content and S-nitrosoglutathione (GSNO), which participates in protein trans-nitrosation and as stable NO reservoir (Corpas et al. 2013). The intracellular levels of GSNO are in turn regulated by the GSNO reductase (GSNOR), a NADH-dependent enzyme belonging to the alcohol dehydrogenase family (Liu et al. 2001). Changes in SNO homeostasis controlled by GSNOR are fundamental for plant responses to pathogens and cell death, as well as in regulating SA signaling and cross-talk with other hormones (Malik et al. 2011). Increased levels of SNO and reduced GSNOR activity resulted in threatened resistance in *A. thaliana* infected with bacteria (*P. syringae* pv. tomato DC3000),

powdery mildew and downy mildew (Feechan et al. 2005; Tada et al. 2008), and in sunflower after the infection with the oomycete *Plasmopara halstedii* (Chaki et al. 2009). Recently, the activity of GSNOR was observed differentially modulated in susceptible and resistant *Lactuca* spp. plants dealing with downy and powdery mildew infection (Tichá et al. 2018). Furthermore, GSNOR-mediated reduced levels of S-nitrosothiols were found in response to mildew infection, bringing new insights into the role of oxidative and nitrosative processes involving NO and ROS during plant-pathogen interactions (Tichá et al. 2018).

Several proteins undergo S-nitrosation process in plants and among them, enzymes, transcription factors and co-activators involved in plant immunity and HR were described (Gupta et al. 2020). Wu and co-workers (2012) showed that the nonexpressor of NPR1 (pathogenesis-related 1) is a receptor of SA and its activity is subjected to redox regulation and S-nitrosation. Following redox changes after pathogen inoculation and SA accumulation, as well as after GSNO treatment, NPR1 is reduced and translocated to the nucleus, where it binds elements of the PR1 promoter, upregulating the expression of defense-related genes (Tada et al. 2008; Lindermayr et al. 2010). Similarly, to NPR1, the enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) undergoes redox regulation and the processes of oxidation, S-nitrosation and nitration determine the inhibition of its activity. It was reported that in tobacco cells the S-nitrosation of two isoforms of NtGAPCa and NtGAPCb stimulates the translocation into the nucleus of these enzymes in response to salinity stress (Wawer et al. 2010).

The reactivity of ROS, ROS producers and scavengers can be also modulated by NO through S-nitrosation. In *Arabidopsis*, the NADPH oxidase AtRBOHD represents the key enzyme for ROS production after pathogen infection (Torres et al. 2005). Yun et al. (2011) observed the S-nitrosylation at Cys-890 residue of AtRBOHD during HR elicited by *P. syringae* pv. *tomato* DC3000. This process is thought to be implicated in the disruption of the side chain position of Phe-921 responsible of FAD binding. The diminished FAD binding decreases NADPH oxidase activity and limits ROS production and cell death, as well.

A further protein modification mediated by NO and its intermediates is tyrosine nitration. A strong correlation between $ONOO^-$ accumulation and higher levels of tyrosine-nitrated proteins during HR was previously reported (Romero-Puertas et al. 2007; Vandelle and Delledonne 2011). $ONOO^-$ is a strong oxidant agent that brings to cell death in animal cells, but not in plants, where it acts indeed as primary reactive oxygen intermediate inducing glutathione S-transferase in a cellular protectant response (Delledonne et al. 2001). Accumulation of $ONOO^-$ was described during biotic interaction in *Arabidopsis* leaves inoculated with *P. syringae* pv. *tomato* (Gaupels et al. 2011) and the identification of several candidate target proteins modified by tyrosine nitration suggest a signaling function (Lozano-Juste et al. 2011).

The signaling role of NO during plant-pathogen interactions has been analyzed not only by the host side, but also within pathogenic microorganisms (Arasimowicz-Jelonek and Floryszak-Wieczorek 2016). In fungi, the role of this molecule has been deeply examined. The same oxidative and reductive NO pathways observed in

plants were found in both kingdoms. These pathogens use NO in various developmental processes including sporulation, spore germination and fruiting body formation (Gong et al. 2007; Prats et al. 2008; Baidya et al. 2011). Moreover, NO may be responsible of mycotoxin production and formation of several infection structures of the pathogens during host tissue colonization (Arasimowicz-Jelonek and Floryszak-Wieczorek 2014). A strong accumulation of NO in plant cells favors necrotic death and disease development under attack by necrotrophic fungal pathogens, such as *B. cinerea* and *B. elliptica* in grapevine cells and lily, respectively (van Baarlen et al. 2004; Vandelle et al. 2006).

Similar findings were reported for pathogen having hemi−/biotrophic life strategies. The accumulation of NO during the infection by the rice blast fungus *M. oryzae* resulted fundamental for the formation of the aspersorium and the final successful host invasion (Samalova et al. 2013). Zhang et al. (2015) showed that *M. oryzae* gene *MoSFA1* coding the S-(hydroxymethyl)-glutathione dehydrogenase is involved in NO metabolism through the reduction of GSNO and increased levels of SNO. *MoSFA1* mutants were compromised in their full virulence on rice cultivar CO-39 and showed a reduced condition and aspersorium turgor pressure (Zhang et al. 2015).

Mycotoxin production can be also being influenced by the synthesis of NO. The deletion of *fhbA*, a gene coding the flavohaemoglobin (Fhb) protein responsible for the reduction and detoxification of NO, determined a shortening of sterigmatocystin in *Aspergillus nidulans* mutants (Baidya et al. 2011). Furthermore, a decreased expression of the gene *aflR*, involved in the activation of the sterigmatocystin gene cluster, and a lower production of mycotoxins was observed as well. After the supplementation with a nitric oxide-releasing compound, Δ*fhbA* strain mutants increased the levels of *aflR* gene expression and recovered mycotoxin biosynthesis (Baidya et al. 2011).

Several studies drew attention on the mediating role of NO in the melatonin response, mainly in its auxin-like and plant immune strategies. Melatonin is a multiregulatory molecule and represents an important gene expression modulator associated to plant hormones, like auxin, cytokinin, gibberellins, abscisic acid, ethylene, jasmonic acid and salicylic acid (Arnao and Hernández-Ruiz 2018). The growth-promoting and rooting activity of melatonin represents one of its specific auxin-like function. It was observed that melatonin increases NO level through the upregulation of the enzyme NR in tomato seedlings (Wen et al. 2016). Interestingly, NO can in turn increase the level of melatonin, suggesting a possible feedback mechanism. Therefore, melatonin induces the expression of several auxin efflux genes (*PIN1*, *PIN3* and *PIN7*) and signaling transduction genes (*IAA19* and *IAA24*), promoting shoot and root generation and tropic processes as well (Wang et al. 2016b).

Also, melatonin plays a crucial role in the plant-pathogen interaction in cooperation with NO, SA and JA. It was described that the application of melatonin in *Arabidopsis* and tobacco induced pathogenesis-related, SA- and ethylene-dependent genes, along with a reduced susceptibility to the a virulent strain *P. syringae* Rpt2 (Lee et al. 2014, 2015). Moreover, the signaling cascade mediated by the mitogen-activated protein kinase (MAPKKK3) and the oxidative signal-inducible1 kinase

(OXI1) is required to induce melatonin-mediated plant innate immunity (Lee and Back 2016, 2017). Interestingly, the occurrence of elevated levels of melatonin in endophytic microbes of *Vitis*, such as *Bacillus amyloliquefaciens*, *B. thuringiensis*, *B. cereus*, *Agrobacterium tumefaciens* and *Pseudomonas fluorescens*, in concertation with ROS decrease due to the activation of scavenging enzymes, provides evidence of new communication strategies between beneficial symbiotic organisms and host plants by melatonin action (Jiao et al. 2016; Ma et al. 2017).

Recently, the role of melatonin in heavy metal detoxification strategies was demonstrated in safflower plants grown in soils contaminated with Pb (Namdjoyan et al. 2020). Exposure to Pb treatment determined limited biomass production and increased the content of oxidative damage biomarkers, as malondialdehyde and $H_2O_2$. This negative effect was alleviated by the application of melatonin to Pb-threatened plants that diminished Pb uptake and its translocation from root to shoot, stimulating antioxidant defense mechanisms, reducing the glutathione content and increasing the activity of enzymes involved in the glyoxalase system (Namdjoyan et al. 2020).

## 1.4 Phytohormones in Pathogen Resistance: Roles and Network

Plant pathogens are divided into biotrophs and necrotrophs according their behavior. Biotrophs derive energy from living host tissue whereas necrotrophs obtain nutrients from killed plant tissues and they live saprotrophically on dead host. Although the lack of plant mutants which defect in specific defense effectors, there is a large group of mutants in various signaling pathways and species. Evidences show that before pathogen attack enzymes of Reactive Oxygen Species (ROS) pathway as ascorbate peroxidase (APX), superoxide dismutase (SOD) are in active form to prevent cellular oxidation due to biotic infection (Campo et al. 2004; Lanubile et al. 2017). These prevention systems, highly conserved in resistant species (Maschietto et al. 2016) are ruled by phytohormones which can activate the proper signaling transduction pathway as the infection occurs. The closely linked hormones involved are SA, ABA and JA and pathway interactions are well described, as reported in (Lee et al. 2018). Most of this crosstalk between hormones consists of mutual repression even if some genes can be induced both by exogenous hormones (Glazebrook et al. 2003). The negative crosstalk between JA and SA is not well understood while the interactions occurs in known multiple point as described in *NahG* trangene expression where the SA suppression lead to JA responsive genes expression (Abreu and Munné-Bosch 2009). Considering the width and complexity of pathogens and phytohormones as topic, this paragraph provides a summary of the most exemplary phytohormones interactions involved in biotrophic and necrophic pathogens resistance. A scheme of the interaction described below is shown in Fig. 1.1.

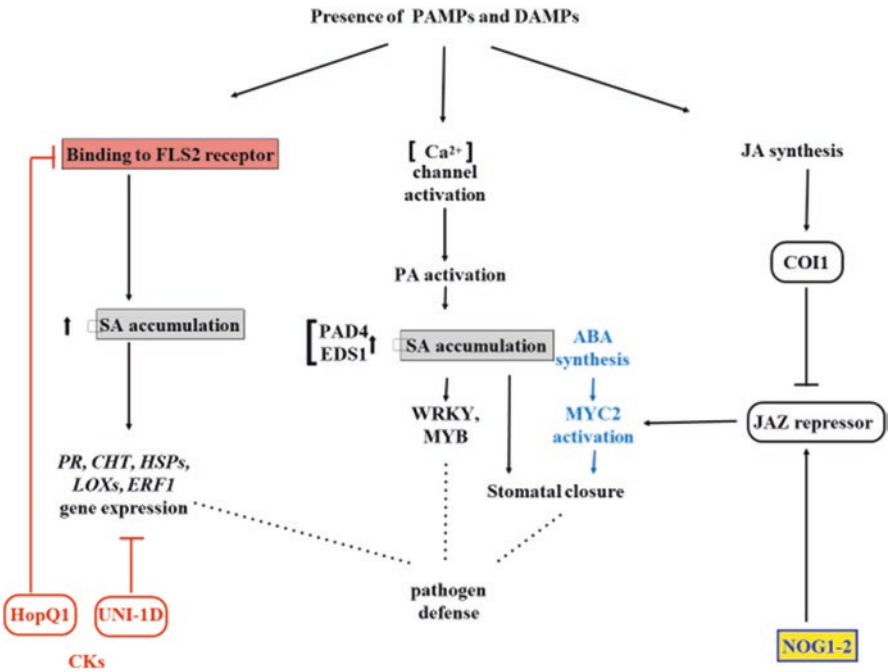


**Fig. 1.1** The interactions between salicylic acid (SA), jasmonic acid (JA), abscissic acid (ABA) and cytokinins (CK) under biotic stress. The response involves different pathways, with interaction points. The presence of MAMPs/DAMPs activate apoplastic receptor as FLS2, calcium ($Ca^{2+}$) channel and JA synthesis. The FLS2 receptor trigger SA accumulation and subsequent activation of PR genes. CKs interact with SA through HopQ1 and UNI-1D. HopQ1 and UNI-1D reduce the expression of FLS2 receptor for defence activation. ABA and SA biosynthesis and signaling are required for full stomatal closure. ABA interactions involve SA and JA pathway by NOG1-2. The increase in PA production leads to rapid SA accumulation and induction of WRKY and MYB transcription factors. PAD4 and EDS1 are required for SA activation. JA is perceived by the COI1 which mediated degradation of JAZ repressor and liberate a group of MYC for transcription reprogramming. JAZ also interacts with NOG1-1 and prevent JAZ degradation. NOG1-2 regulates guard cell signaling through jasmonic acid (JA)- and abscisic acid (ABA)-mediated pathways. JAZ9 interacts with NOG1-2 for the regulation of stomatal closure. Binding of NOG1-2 with JAZ9 affects MYC2 mediated signaling required for and stomatal closure

### *1.4.1 Salicylic Acid (SA)*

SA plays a role in defense against biotrophs through Fatty Acids (FAs) recruitment. Phospholipase enzyme are activated in order to enhance the cell wall based defense by increasing phosphatic acid (PA) production in *Arabidopsis* in case of bacterial and fungal attack (Wang et al. 2006; Pinosa et al. 2013; Hyodo et al. 2015). PA is activated after MAMPs arrive, this signal causes a rapid SA accumulation which lasts 2 h after the pathogen attack and corresponds to the first peak of SA

accumulation. SA continuous recruitment lead to a second activation of SA after 10 h, this second peak regulates the ETI during the infection. The gene network resulting after hormone-signaling pathways has been found to be composed of multiple transcriptional factor families, some important examples are WRKY and MYB (Fig. 1.1). *WRKY* are involved *PR* genes activation as *PR1* while *MYB* are involved in flavonol-specific gene activation in phenilpropanoid biosynthesis (Campos-Bermudez et al. 2013; Lanubile et al. 2014). The initial biotrophic growth phase of the *Fusarium verticillioides* has been found to be involved in the activation of plant heat shock proteins (HSPs), glucanases (GLUC) and PR proteins (PR5 and PR6). Other genes as *PHYTOALEXIN DEFICIENT 4 (PAD4), ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1)* are required for the activation of SA (Fig. 1.1). *PAD4* and *EDS1* encode proteins similar to triacyl-glycerol lipases needed for SA biosynthesis. The importance of SA results from the involvement of the *NahG* transgene, encoding a bacterial salicylate hydroxylase that destroys SA by chatecol conversion. *NahG* transgenic plants highlighted that SA is required for defense effector genes and for SAR resistance (Abreu and Munné-Bosch 2009). Another case of hormone activation is given by SA-ABA crosstalk as reported for the FLS2 receptor, involved in the PAMP response of *P. syringae* (Schulze-Lefert and Robatzek 2006). The FLS2 receptor triggers SA and ABA response by counteracting pathogen entry through stomata closing.

### *1.4.2 Jasmonates (JA), Ethylene (ET) and Polyamines*

Role of JA in defense against necrotrophs has been revised in plant kingdoms as reported in *Arabidopsis* against the fungi *B. cinerea* and *F. verticillioides* (Lanubile et al. 2014; Borrego and Kolomiets 2016; Chauvin et al. 2016). Many efforts are elucidating the importance of this hormones but its network is still quite complex because JA-regulated genes are also regulated by ET (Norman-Setterblad et al. 2000). QTL and gene expression approaches have been used to examine genotypes before and after pathogen attack in order to elucidate resistance gene expression patterns and the correlated gene networks (Maschietto et al. 2017). Out of last studies, JA signaling pathway has been found active in down-stream responsive genes, such as *PR3, CHIT, LOXs* genes. *LOXs* gene family have been object of disease response in different kingdoms, from animal to fungi and plants (Christensen and Kolomiets 2011; Lanubile et al. 2017).

*LOXs* encodes for dioxygenase enzymes that catalyze polyunsaturated fatty acids (PUFAs) oxygenation. The canalization process lead to JA and other metabolites production as green leaf volatiles (GLVs). In *Arabidopsis,* genome wide association mapping reported some genes associated with differential response to JA and hormones crosstalk. The genes involved encode for the nuclear localized type B response regulators (RRB), also called type B ARR in *Arabidopsis*, which are transcription factors regulating the expression of CK response genes (Zhang et al. 2017). JA has a key role in systemic wound signalling and its volatile compounds

are inter- or intraspecies warnings as methyl-JA (Schilmiller and Howe 2005; Yan and Xie 2015). In *Arabidopsis* the bioactive form of JA, (+)-7-iso-jasmonoyl-L-isoleucine (JA-Ile), has been found to be accumulated in distal leaves after 5 min form pathogen attack (Koo et al. 2009). JA-Ile is perceveid by coronatine-insensitive 1 (COI1), which mediates 26S proteasome-dependent degradation of JAZ (JA ZIM-domain) family proteins that acts as transcriptional repressor in JA signaling. As result, a group of MYC transcription factors are released starting then transcription reprogramming. Many studies report JA and the related metabolites oxylipins as long distance signaling molecules (Farmer et al. 2003; Taylor et al. 2004), and how MAMPS and DAMPs are interconnected to JA and oxylipins delivery (Choi et al. 2016). Surprisingly, the long-distance signalling seems to involves fast system as ROS and $Ca^{2+}$channel (Choi et al. 2018); new evidences suggest JA and oxylipins as a new source of elicitors as reported in Tripathi et al. (2019) and Ramirez-Prado et al. (2018). Another case of JA regulation is given by the GTPase protein NOG1-2 which stimulates stomatal opening in pathogen attack by preventing JAZ9 tag for degradation (Lee et al. 2018). JAZ 9 also interacts with NOG1-2 through this same binding domain, and by binding to JAZ9, NOG1-2 interrupts the interaction between JAZ9 and COI1 and prevents JAZ9 degradation. The JAZ9-COI1 system is an example of ETI interaction where the effector inhibits wounding response by stomatal re-opening in bacterial infection. NOG1-2 and JAZ9 are known to be induced in ABA response to drought but NGO1-2 has been discovered to be a crosstalk point between ABA and JA signaling pathway, moreover a study confirms this crosstalk and reports the inducing of stomatal closure through JA active form during pathogen infection (Lee et al. 2018). The transcription factors ERF1and JIN1 are all inducible by JA and are involved in JA and ET interaction signals. The overexpression of ERF1 results in the activation of many defense-related genes (Lorenzo et al. 2004). MYC2 transcriptional activator regulates JA-mediated suppression of *isochorismate synthase 1* (*ICS1*), a key enzyme of the isochorismate (IC) pathway, and leads to the induction of genes for SA metabolism through transcriptional regulation of *SNAC-A* transcription factors (Boter et al. 2004).

In *Brassicaceae,* during MTI, MYC2 regulates negatively the expression of *phytoalexin deficient 4* (*PAD4*) and positively the expression of *enhanced disease susceptibility 5* (*EDS5*), thereby contributing to SA accumulation.

MYC2 suppresses the transcription of the transcription factor *ORA59*, the core component in ET-mediated immunity in *A. thaliana* (Zhai et al. 2013). In addition, MYC2 antagonizes the transcription factor ethylene insensitive 3 (EIN3). Moreover, MYC2 modulates JA – ABA cross-talk through interaction with the ABA receptor PYL6. MYC2 function is also suppressed by DELLAs through physical interaction. Therefore, MYC2-regulated processes are enterely activated only when both JA and GA are present (Berens et al. 2017).

Interference with the functions of ET in plant defence is also associated with polyamine accumulation and decreased resistance to necrotrophic fungi, such as *B. cinerea* (Nambeesan et al. 2012). However, polyamines accumulate in leaves infected with obligately biotrophic fungal pathogens, such as rusts and powdery mildews (Walters 2015). It is suggested that the accumulation of polyamines in

diseased leaves is related to the well-known senescence retarding effects of polyamines, associated with reduced activity of lipoxygenase and greatly reduced ET evolution (Coghlan and Walters 1990). Increased polyamine biosynthesis and polyamine levels is observed in tobacco exhibiting HR to infection with Tobacco Mosaic Virus (TMV) and in the HR of barley to the powdery mildew fungus, *Blumeria graminis* f.sp. *hordei*. But also is the breakdown of polyamines by the activity of enzymes diamine oxidase (DAO) and polyamine oxidase (PAO), that leads to the formation of hydrogen peroxide as signals responsible for triggering the HR (Walters 2003; Gonzalez et al. 2011).

### *1.4.3 Cytokinins (CK)*

Another class of phytohormon, cytokinin (CK), originally discovered as main player of cell division and plant growth development, have diverse functions in defence to biotic and abiotic stresses. In biotic stresses, CK represents the host phytohormones involved in neoplastic growth of pathogen which activates CK synthesis and permits its growth in the infected cell (Sakakibara et al. 2005). Many studies report CK involvement in fungi infection where the pathogen causes an increase of host CK levels and the consequent cell division and increase availability of local resources (Baliji et al. 2010; Giron et al. 2013). One important example that confirm CK role in plant-pathogen interaction is shown in *Claviceps purpurea* where the mutated biographic fungi strains for CK inducing genes shows reduced virulence (Hinsch et al. 2016; Kind et al. 2018). The same strategy has been confirmed also in *Brassicaceae* against the vascular biographic fungus *Verticillium longisporum* (Reusche et al. 2013). Moreover, a number of detailed studies ubiquitously suggest that CK expression in the host is the main target to boost the fungi or viral infection. The elaborated strategies to pathogen attack response are often the action of different hormone as the case of CK and SA pathway. An important study case is *uni-1D* mutant of *Arabidopsis* which the mutation causes the constitutive activation of the disease protein PR1, CK and SA pathway suggesting the hormone crosstalk between the two hormones and their relevance for plant defense (Igari et al. 2008). Another confirm about CK-SA link arises from *Arabidospis Pst* effector protein HopQ1 (Hann et al. 2014) where the induction of HopQ1 effector protein causes the CK concentration increasing and reduce expression of *FLS2* receptor gene for defense activation. Recent evidences in necrotrophic pathogen as *X. campestris* show the pathogen ability in recognize plant-CK and enhance its protection against plant defense. A different number of studies report a CK and JA common network in plant defense activation. CK and JA have been found to be required in *Arabidopsis* defense to the necrotrophic fungus *A. brassicicola* where the expression of JA genes was decreased in CK mutants, indicating a possible network between the two hormones (Argueso et al. 2012).

### *1.4.4 Auxin*

Auxin plays important roles in many aspects of plant growth and development, including apical dominance, embryogenesis and cell division, expansion and differentiation (Hodson and Bryant 2012) by means of expression of three groups of genes: the *Aux/IAA* family, the *GH3* family, and the *SAUR* (small auxin-up RNA) family. In plants, most auxin occurs conjugated to amino acids, in reversible reactions catalysed by IAA-amido synthetases coded by *GH3* genes, and this mechanism is important to settle the concentration of active indole acetic acid (IAA). Auxin is known to promote disease caused by various bacteria, including *Agrobacterium tumefaciens, Pseudomonas savastanoi* and *P. syringae* pv. *tomato* DC3000 (Chen et al. 2007), suggesting that auxin reduces defence responses and that, if auxin responses are blocked, disease resistance can be increased (Wang et al. 2007). Auxin levels were found to increase in *Arabidopsi*s infected with the hemibiotrophic pathogen *Pst*. The injection of type III effector (T3E) proteins into host cells act to suppress both ETI and pathogen-associated molecular patter (PAMP)-triggered immunity (PTI) (Block and Alfano 2011). *Pst* infection also induced expression of genes involved in auxin biosynthesis but repressed genes belonging to the *Aux/IAA* family and auxin transporters. Chen et al. (2007) suggested that T3Es might be among the virulence factors used by *P. syringae* that modulate host auxin physiology in order to promote disease. Similar findings were reported for *Arabidopsis* infected with the root-infecting hemibiotrophic fungus *Fusarium oxysporum* and with the oomycete pathogen *Phytophthora parasitica* (Walters 2015). This suggests that promotion of auxin signaling raises disease susceptibility, while and repression results in enhanced resistance in plants. It has been suggested that auxin may suppress SA-mediated host defence (Mutka et al. 2013). Navarro et al. (2006) showed that down regulation of auxin receptor genes by over expression of a micro RNA (miR393) increased resistance to bacterial disease. In contrast, enhanced susceptibility was observed with the over expression of an auxin receptor that is recalcitrant to miR393-mediated transcript cleavage.

In contrast, in plants infected with necrotrophic fungal patogens, such as *A. brassicicola*, *B. cinerea* and *Plectosphaerella cucumerina,* auxin interacts positively with JA leading to an enhanced auxin response in the host (Qi et al. 2012).

Auxins are also involved in relation to clubroot disease of brassicas caused by the obligate biotrophic pathogen *Plasmodiophora brassicae.* The formation of galls (or clubs) in the roots is a consequence of pathogen-driven re-programming of existing host meristematic activity (Malinowski et al. 2012).

While cytokinin production by *P. brassicae* plasmodia take places during early stages of infection, elevated auxin levels occur during the later stages of infection. Increases affect both free and conjugated IAA levels suggesting that plasmodia were accumulating IAA in a sink-dependent manner.

In conclusion, the works described previously suggest that auxin signalling is required for resistance against necrotrophs but imparts susceptibility against

biotrophs. It also appears clear that some pathogens target host auxin physiology as part of their infection strategy.

### *1.4.5 Brassinosteroids (BRs)*

Brassinosteroids, the polyhydroxylated steroid of plants, influence plant responsiveness to the local environmental signals and regulate many aspects of plant development (Belkhadir and Jaillais 2015). Brassinosteroids are perceived by a small family of plasma membrane localized leucine-rich repeat receptor kinases (LRR-RKs) called BRASSINOSTEROID INSENSITIVE 1 (BRI1) (Cano-Delgado et al. 2004). A phosphorylation-dephosphorylation cascade involving the GSK3-like kinase BRASSINOSTEROID INSENSITIVE 2 (BIN2) transduces the BR signals to the downstream transcription factors BRASSINAZOLE-RESISTANT1 (BRZ1) and BR-INSENSITIVE-EMS-SUPPRESSOR1 (BES1) (Wang et al. 2014a). BZR1/BES1 integrate BR signals on the promoter of several genes involved in other signaling pathways. BZR1 coordinates transcriptional networks involved in defenses to a range of pathogens, such as *M. grisea*, *X. oryzae*, *Oidium* sp., *P. syringae* and *Phytophthora infestans*, apparently independently of SA-mediated defence signaling. It has been proposed that biotrophic pathogens have evolved virulence mechanisms based on modifying BR biosynthesis or signalling (Belkhadir et al. 2012; Belkhadir and Jaillais 2015). Elevated BR signaling downstream of BIN2 mediates, in part, the suppression of MTI through BZR1 (Lozano-Duran et al. 2013). BZR1 acts by increasing the expression of several WRKY transcription factors that negatively regulate early MAMP-related defenses. A further bHLH, called HOMOLOG OF BRASSINOSTEROID ENHANCED EXPRESSION2 INTERACTING WITH IBH1 (HBI1), operates as a negative regulator of MTI.

### *1.4.6 Gibberellins (GAs)*

Gibberellins are a group of isoprenoid hormones involved in regulating many aspects of plant growth and development, including seed germination, stem elongation by stimulating breakdown of negative regulators of growth known as DELLA proteins. In *Arabidopsis*, DELLA stabilization upon perception via the receptor-like kinase FLS2, contributes to flg22-inhibition of growth and enhanced antibacterial resistance. In addition, DELLAs promote susceptibility to virulent biotrophs *P. syringae p.v. tomato* DC3000 and *Hyaloperonospora arabidopsidis* and resistance to necrotrophs *B. cinerea* and *A. brassicicola*, by altering the relative stability of SA and JA signaling (Navarro et al. 2008). In *Arabidopsis*, DELLA mutants were insensitive to gene induction by MeJA. On the contrary, the constitutively active dominant DELLA mutant gai was responsive to JA-gene induction, implicating DELLAs in JA-signaling and/or perception. Taken together, these results suggest

that DELLA proteins promote resistance to necrotrophic pathogens by activating JA/ET-mediated defences but increase susceptibility to biotrophs by repressing SA-mediated defences. These findings suggest that the necrotrophic fungus *G. fujikuroi*, causal agent of the foolish-seedling disease of rice, secretes GA as a virulence factor to degrade DELLAs and disables both JA-mediated necrotroph resistance and DELLA-mediated growth contrain.

In rice, it is suggested that GAs play a negative role in basal disease resistance. Altering GA levels by manipulating the activities of GA deactivating enzymes can influence defence responses. One such enzyme is 'Elongated Uppermost Internode' (EUI) and loss of function *eui* rice mutants accumulate high levels of GA and exhibit susceptibility, while EUI overexpressors accumulate low GA levels and show enhanced resistance to the rice pathogens *X. oryzae* pv. *oryzae* and *M. grisea* (Yang et al. 2008). Mutants defective in GA perception, such as the *gid 1* mutant, have also been shown to express altered defence responses. The *gid 1* mutant accumulates GA and is more resistant to *M. grisea* than wild-type plants (Tanaka et al. 2006).

## 1.5 Genome Editing Tools: CRISPR/Cas Technology as New Approach to Improve Crop Resistance

The study of the phytohormones network in pathogen resistance contributes to reduce the impact of disease on crop development and yield, thereby can overcome several problems from population feeding to sustainable crop production. Classical breeding strategies for disease resistance are lengthy processes and require the knowledge of resistance loci or genes. Advances in genome editing tools, such as the CRISPR/Cas system opened new ways for improvement of biotic stress resistance in crops (Arora and Narula 2017; Borrelli et al. 2018; Doll et al. 2019). The application of CRISPR/Cas tools are exploring pathogens resistance in crops but few examples are reporting pathogen resistance improvement through relevant genes involved in phytohormone pathways (Table 1.1). The CRISPR/Cas genome editing approach has given rise to the following edited genes *OsERF22*, *OsSEC3A*, *VvWRKY52* and *TcNPR3.* The interaction points with phytohormones in pathogen disease will be discussed in this paragraph.

The first cases of edited genes involved in phytohormones pathway are *OsERF922* and *OsSEC3A,* edited for *M. oryzae* resistance in rice, a biographic fungus causing rice blast. *ERF922* encodes an ethylene responsive factor, a subfamily of the APETALA2/ethylene response factor (AP2/ERF) transcription factor family in plants, implicated in multiple stress responses. *ERF922* overexpression in tobacco has led to the susceptibility increasing against the biographic bacteria *P. syringae,* while the silencing of the same gene causes the enhancement to the pathogen resistance (Wang et al. 2016c). *OsSEC3A* is a subunit of the exocyst complex that recruit's other subunits from cytosol to plasma membrane: the mutant studied have showed enhanced immunity including SA content, up-regulation of defense-related

**Table 1.1** CRISPR/Cas9 applications for fungal and bacterial resistance

| Plant species | Pathogen | Target gene | References |
|---|---|---|---|
| *Triticum aestivum* | Powdery mildew (*Blumeria graminis* f. sp. *tritici*) | MLO-A1 | Wang et al. (2014b) |
| *Solanum lycopersicum* | Powdery mildew (*Oidium neolycopersici*) | MLO1 | Nekrasov et al. (2017) |
| *Vitis vinifera* | Powdery mildew (*Erysiphe necator*) | MLO-7 | Malnoy et al. (2016) |
| *Vitis vinifera* | Grey mold (*Botrytis cinerea*) | WRKY52 | Wang et al. (2018) |
| *Theobroma cacao* | Black pod disease (*Phytophthora tropicalis*) | NPR3 | Fister et al. (2018) |
| *Oryza sativa* L. *japonica* | Rice blast disease (*Magnaporthe oryzae*) | SEC3A | Ma et al. (2018) |
| *Oryza sativa* L. *japonica* | Rice blast disease (*Magnaporthe oryzae*) | ERF922 | Wang et al. (2016c) |
| *Oryza sativa* | Bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) | SWEET13 | Zhou et al. (2015) |
| *Citrus paradisi* | Citrus canker (*Xanthomonas citri* subspecies *citric*) | LOB1 | Jia et al. (2016) |
| *Citrus sinensis* Osbeck | Citrus canker (*Xanthomonas citri* subspecies *citric*) | LOB1 | Peng et al. (2017) |
| *Malus domestica* | Fire blight (*Erwinia amylovora*) | DIPM-1 | Malnoy et al. (2016) |
| | | DIPM-2 | |
| | | DIPM-4 | |

*MLO* Mildew Resistant Locus, *WRKY52* transcription factor, *NPR3* Non-Expressor of Pathogenesis-Related 3, *SEC3* subunit of the exocyst complex, *ERF* Ethylene Responsive Factor 922, *SWEET13* sugar transporter, *LOB1* Lateral Organ Boundaries, *DIPM* DspE-Interacting Proteins of Malus. Examples of relevant genes involved in phytohormone pathways and pathogen resistance improvement are reported in bold

genes and enhanced resistance against *M. oryzae* (Ma et al. 2018). The second case of gene editing involving phytohormons network corresponds to *VvWRKY52* from the grape (Wang et al. 2018). Four specific targets were designed in the first exon of *VvWRKY52*, and 22 independent mutant strains were generated, of which 15 were homozygous. Knock-out of *VvWRKY52* increase resistance to botrytis bunch rot (*Botrytis cinerea*). The last example entails *NPR3,* a suppressor of the immune system, involved in SA and JA pathway. The transient transformation of cacao leaves increased resistance to the biotroph fungus *Phytophthora tropicalis* (Fister et al. 2018).

CRISPR/Cas9 technology is readily applicable to pathogen resistance because disease resistance can be achieved by the modification of a single gene and the inactivation of susceptibility genes leads to protection.

Continuous efforts are necessary to identify and characterize genes involved in phytohormone interactions. Hundreds of effector molecules were discovered in the

past decades. In the future, a major challenge will be to identify the host genes underlying the proteins targeted by these effectors and to design genome editing approaches to enlarge the gene pool of a crop species beyond all the available natural variability. Novel knowledge will be exploited from the crop species of interest and model species (Wang et al. 2014b).

## 1.6 Conclusion

Plant response to pathogens are mediated by SA or JA/ET, depending on the nature of the attacker, and are likely influenced by changes in other hormones, such as ABA, CK, GA, BR, auxin and polyamines. Our understanding of molecular components that mediate hormone cross talk have significantly advanced with the model plant *A. thaliana*. There are six main components that are fundamental in hormone signaling networks: (1) NO and ROS regulate transcription factors, co-activators and enzymes involved in plant immunity and HR; (2) SA perception is explained by functions of three NPR proteins, which allow transcriptional regulation**.** NPRs mediate SA-regulated suppression of JA and ABA signaling using WRKY transcription factors. Thus, NPRs are an important core in the signaling between SA and other hormones. (3) JA signaling is mediated by the transcription factor MYC2 by means of suppression of *ICS1*and *PAD4* and induction of genes for SA metabolism through transcriptional regulation of *SNAC-A*. In addition, MYC2 suppresses ET-mediated responses through repression of the transcription factor *ORA59*, which is the main component in ET-mediated immunity. Moreover, MYC2-regulated processes are activated when both JA and GA are present. (4) DELLAs – JAZs interaction is a conserved hub that controls defense and growth. DELLA degradation upon GA perception releases JAZs that, hence, suppress JA-mediated responses, resulting in reduction of immunity against necrotrophic pathogens and enhancement of immunity against biotrophic and hemibiotrophic pathogens. (5) Auxin signaling occurs through reduction of the auxin receptor transcripts, which is mediated by the miR393, leading to increases resistance against biotrophic and hemibiotrophic pathogens. (6) GH3 family modulate immunity through changing the balance of active and conjugate phytohormones JA, SA and auxin.

Additional genetic approaches need to be adopted in order to link hormonal response to oxidative response and $Ca^{2+}$channel activation. Moreover, their findings put in evidence how important is the study of plant innate immunity and the defense mechanism in a challenging area of intense agriculture where the plant response to stresses is fundamental for the development of new plant varieties.

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